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DIVISIÓN DE INVESTIGACIÓN



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PROCEEDINGS OF THE 13TH WORLD BUFFALO CONGRESS 2023

THEME:

For a neutral
**CARBON FOOTPRINT
LIVESTOCK** 🌱

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EDITORIAL

Esteemed readers,

The 13th World Buffalo Congress in Venezuela 2023 brought together scientists, academicians, researchers, farmers, breeders, policymakers, and stakeholders to discuss the importance of buffalo farming committed to achieving a neutral carbon footprint of livestock for a sustainable world. Our scientific and Congress program had enclosed a complete agenda that combined academia, scientific exchange, and the business and industry arena, but also included a buffalo exhibition and judgment and social and cultural activities to integrate the Buffalo family from all over the world. After the Congress ended, the traditional Buffalo Tour took place, allowing nationals and foreigners to visit emblematic livestock and buffalo industry from the country.

We witnessed an entire program to discuss different areas to understand better and release new information and knowledge about buffalo Management, Animal Welfare, Feed and Nutrition, Animal and One Health, Breeding and genetics, Reproduction, Biotechnology, Buffalo's products, industry and marketing, Precision Technologies, Sustainability, and Socioeconomics. Additionally, several practical workshops allowed the participants to learn by their hands how to transform Buffalo's values into high-quality and nutritious products, as well as witness the application of modern reproductive biotechnologies and understand the approaches to warrant animal welfare and the tools for sustainable livestock farming. In addition, we had three scenarios in the format of forums for discussion and networking building. The Academician and Scientific Forum allowed the participants to strengthen ties and explore opportunities for scientific-academic exchange, studies, financing, and scholarships available. Similarly, the forum for Breeders and Associations allowed breeders to enhance collaboration and alliance with America's Buffalo Breeders Associations. Finally, the discussion for Professionals and Entrepreneurs was an extraordinary showcase to explore business opportunities and empower success.

Our scientific call received over 150 abstract proposals for poster and short oral communication presentations worldwide. Moreover, twenty-two lectures by internationally recognized scientists paved the way for an enriched discussion and nutritious feedback. The information released in the Congress is compiled into this volume of our internationally recognized scientific journal from the College of Veterinary Medicine of the University of Zulia. Herein, the abstracts accepted for publication and invited lecture manuscripts are presented in English and Spanish to allow a broad divulgation. The knowledge, experiences, and vision authors share in this compilation will contribute to advancing buffalo farming, addressing the challenges, and creating a better world for us and future generations.

It is a great honor to have had the opportunity to contribute to this legacy, and I hope it will be part of your digital library and contribute to your knowledge on this noble species.

Sincerely,

Prof. Dr. Juan Carlos Gutiérrez-Añez, DVM, MS, PhD

Full Professor College of Veterinary Medicine, University of Zulia
President of the 13th World Buffalo Congress Scientific Committee
Editor



EDITORIAL

Estimados lectores,

El 13^{er} Congreso Mundial del Búfalo en Venezuela 2023 reunió a científicos, académicos, investigadores, ganaderos, criadores, formuladores de políticas y partes interesadas para discutir la importancia de la cría de búfalos comprometida con lograr una ganadería con huella de carbono neutra para un mundo sostenible. Nuestro programa científico y de congreso incluyó una agenda completa que combinó la academia, el intercambio científico, pero además el ámbito empresarial e industrial, así como una exhibición y juzgamiento de búfalos, además de actividades sociales y culturales para integrar a la familia bufalera de todo el mundo. Después de finalizar el congreso, se realizó el tradicional búfalo tour, que permitió a los nacionales y extranjeros conocer emblemáticas ganaderías e industrias del búfalo del país.

Fuimos testigos de un programa completo donde se discutió diferentes áreas para comprender mejor y divulgar información nueva y conocimientos sobre manejo del búfalo, bienestar animal, alimentación y nutrición, salud animal y una salud, cría y genética, reproducción, biotecnología, productos, industria y marketing de búfalos, tecnologías de precisión, sostenibilidad y socioeconomía. Además, varios talleres prácticos permitieron a los participantes aprender cómo transformar los valores del búfalo (leche y carne) en productos nutritivos y de alta calidad, así como ser testigos de la aplicación de biotecnologías reproductivas modernas y comprender los enfoques para garantizar el bienestar animal y las herramientas para una ganadería sostenible. Además, contamos con tres escenarios en formato de foros de discusión y creación de networking. El Foro académico y científico permitió a los participantes estrechar lazos y explorar oportunidades de intercambio científico-académico, posibilidades de estudios, financiamiento y becas disponibles. De manera similar, el foro para criadores y asociaciones permitió a los criadores mejorar la colaboración y alianza de todas las asociaciones de criadores de búfalos de las Américas. Finalmente, el conversatorio para profesionales y emprendedores fue un escenario extraordinario para explorar oportunidades de negocio y potenciar el éxito empresarial.

Nuestra convocatoria científica recibió más de 150 propuestas de resúmenes para presentaciones de carteles y comunicaciones orales breves en todo el mundo. Además, veintidós conferencias impartidas por científicos reconocidos internacionalmente allanaron el camino para una discusión enriquecida y una retroalimentación nutritiva. La información difundida en el Congreso está recopilada en este volumen de nuestra revista científica reconocida internacionalmente de la Facultad de Ciencias Veterinaria de la Universidad del Zulia. En este documento, los resúmenes aceptados para publicación y los manuscritos de conferencias invitadas se presentan en inglés y español para permitir una amplia divulgación. El conocimiento, las experiencias y la visión que los autores comparten en esta compilación contribuirán a promover la cría de búfalos, abordar los desafíos y crear un mundo mejor para nosotros y las generaciones futuras.

Es un gran honor haber tenido la oportunidad de contribuir a este legado y espero que el mismo forme parte de su biblioteca digital y contribuya con el conocimiento que usted tiene sobre esta noble especie.

Atentamente,

Prof. Dr. Juan Carlos Gutiérrez-Añez, M.V., M.Sci. Ph.D.

Profesor Titular Facultad de Ciencias Veterinarias, Universidad del Zulia
Presidente Académico y Científico del 13^{er} Congreso Mundial de Búfalos
Editor





**13TH WORLD
BUFFALO CONGRESS**

VENEZUELA - 2023

Lectures
Conferencias

SUSTAINABILITY OF BUFFALO FARMING IN DIFFERENT ENVIRONMENTS IN THE WORLD

Sostenibilidad de la cría de búfalos en diferentes entornos del mundo

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ABSTRACT

The domestic buffalo (*Bubalus bubalis*) plays a strategic role in the world for the economy and society. 203.9 million heads on the planet represent this species and produce 137 million tons of milk and 4.3 million tons of meat (+18.7% and +8.2% increase from 2015 to 2021, respectively). Buffalo products are essential for the human requirements of proteins of high nutritional value. These requirements will increase rapidly in the future with the growth of the human population. Therefore, the buffalo has and will have more of a strategic role in Governments' economic programs in many countries. The buffalo is a long-living ruminant with a high capacity to convert fiber into energy and adapt in complex areas such as marshlands, Iraq, or Bangladesh, or in hot and humid climates where other ruminants cannot survive. Buffalo is a social and family animal in small villages in Southeast Asia, where it is used mainly in the rice fields as a draught animal producing milk essential for the children's nutrition, meat, leather, manure, horns, and bones. Thanks to its great adaptability, buffalo can be bred both in intensive and extensive systems, as in South American countries where natural pastures are highly available. In this paper, the contributions of local breeds and cosmopolitan dairy breeds to sustain protein production, maintain the land's biodiversity, and sustain the livelihood of populations are reported according to the different farming systems.

Keywords: Buffalo population, milk, meat, farming systems, sustainability.

RESUMEN

El búfalo doméstico (*Bubalus bubalis*) juega un papel estratégico en el mundo para la economía y la sociedad. 203,9 millones de cabezas en el planeta representan esta especie y producen 137 millones de toneladas de leche y 4,3 millones de toneladas de carne (aumento de +18,7% y +8,2% de 2015 a 2021, respectivamente). Los productos de búfalo son esenciales para los requerimientos humanos de proteínas de alto valor nutricional. Estos requisitos aumentarán rápidamente en el futuro con el crecimiento de la población humana. Por lo tanto, el búfalo tiene y tendrá un papel más estratégico en los programas económicos de los gobiernos de muchos países. El búfalo es un rumiante longevo con una alta capacidad para convertir la fibra en energía y adaptarse en zonas complejas como las marismas, Irak o Bangladesh, o en climas cálidos y húmedos donde otros rumiantes no pueden sobrevivir. El búfalo es un animal social y familiar en pequeñas aldeas del Sudeste Asiático, donde se utiliza principalmente en los campos de arroz como animal de tiro, produciendo leche esencial para la nutrición de los niños, carne, cuero, estiércol, cuernos y huesos. Gracias a su gran adaptabilidad, el búfalo puede criarse tanto en sistemas intensivos como extensivos, como en países sudamericanos donde los pastos naturales tienen alta disponibilidad. En este artículo, se informan las contribuciones de las razas locales y las razas lecheras cosmopolitas para sostener la producción de proteínas, mantener la biodiversidad de la tierra y sostener los medios de vida de las poblaciones de acuerdo con los diferentes sistemas agrícolas.

Palabras clave: Población de búfalos, leche, carne, sistemas agrícolas, sostenibilidad.

INTRODUCTION

The buffalo (*Bubalus bubalis*) was domesticated ~6,300 yr Before the Present (BP) in India, probably along the Indo River [1]. The buffalo is a water animal that withstands adverse weather conditions, thrives on rough food, and keeps riverbeds and wetlands clean, conditioned its diffusion in Southeast Asia countries linked to rice cultivation [2]. The buffalo can enter and work in the rice fields, particularly in the terraced areas, where mechanization cannot be applied, or cattle cannot survive.

So, buffalo spread out along the other Asian rivers, Nili and Ravi of Punjab, Gange until its delta marshlands in Bangladesh, Ping River in Thailand, Mekong in Vietnam, and Chang in China, going to East Asia in the first centuries. Buffalo moved also to the West, reaching the rivers Tigris and Euphrates and the Nile in Egypt.

In the Middle Ages, buffalo moved to Europe, South Italy along the Sele and Volturno rivers and Pontine marshlands, and the Balkan region along the Danube River.

In America, buffalo was introduced only two centuries ago in Trinidad and Tobago at the time of the English colonial empire in the wake of the Indian people. The Buffalypso breed was then created by crossing the different Indian breeds as dual-purpose animals (draught power and meat) and spread in the Caribbean area to be used in sugar cane plantation.

The richness of lands and natural pastures of the South American countries created a particular farming environment where buffalo have had an economic role in meat and milk production. Different breeds (Murrah, Italian Mediterranean, Jaffarabadi) imported mainly from Europe and Asia constituted the actual population.

In Australia, the Northern Territory was the original entry point for the Swamp and Riverine buffalo from Asia as a food supplier into the new settlements established by the British in the 1800s. Domesticated buffalo were released free-range and spread to most floodplain areas.

The buffalo population in the Americas is increasing at a 13% rate per year. South America was the first, with Brazil having around 85% of the herd and the most advanced buffalo industry, followed by Venezuela, Colombia, and Argentina as the most representative countries.

Buffalo has been in North America for many years in small numbers, but only in the last 15 years, the dairy industry has expanded.

Now, the buffalo species is represented by 203.9 million heads worldwide, 97% of which is in Asia, and plays a strategic role in the economy and society [2], producing 137 mil T of milk and 4.3 mil T of meat (TABLE I). Productions increased remarkably both for population increase and genetic improvement of herds, leading to a milk yield increase of +18.7% and meat + 8.2% from 2015 to 2021. For this reason, buffalo is part of many governmental development programs to increase the

availability of high-value proteins essential for human nutrition, considering that the world population will reach more than 9 billion in 2050 [3].

Compared to other ruminants, buffalo offers advantages such as longevity - with a productive life of often more than eight lactations - a high ability to convert fiber into energy, and adaptation to challenging environments from cold to sweltering and humid climates where other ruminants cannot thrive [4,5].

Moreover, buffalo need water for thermoregulation, and wallowing represents the natural species-specific behavior.

Due to these characteristics, buffaloes can adapt to different farming systems: a) extensive management (natural pastures, i.e., in South American countries, Indonesia, Australia); b) marshlands (i.e., Iraq, Bangladesh, Amazonia); c) family farms (i.e., India, Pakistan, China, and other Asian countries); d) intensive management (i.e., Italy).

Nevertheless, adopting the best sustainable farming systems is often complex as they could result in different outcomes, favoring, in some cases, biodiversity conservation and carbon sequestration, or in some others, privileging production [9]. For example, systems based on grazing may show higher environmental performances because of the lower inputs needed for production, albeit requiring more land. However, when land use options are included in the assessment methodology, results on environmental impacts may change [10].

EXTENSIVE MANAGEMENT

The extensive farming system is based on grazing pasture. A pasture is a system characterized by having a mixed botanical composition, including plant communities in which grasses (Poaceae) are the dominant species, with dicotyledonous herbaceous species (Magnoliopsida) present in various quantities. Moreover, in tropical zones, there are associations with legumes, weeds, trees, and shrubs, in line with the agroecological conditions of the climate, soil fertility, grazing pressure, pests, and livestock needs. Improved pastures are considered to be those that have been sown from selected species [11]. For Casimir and Rao [12], a pasture is sustainable if there is a balance between the first trophic level (pastures), the consumers of the second trophic level (herbivores), and the consumers of the third trophic level (humans), where the populations densities of both plants and herbivores remain relatively constant. The consumption does not exceed the primary productivity of plants. Similarly, [13] states that a sustainable pasture should consider practices that balance production objectives with social values and ecological needs. Therefore, the last two authors approach the concept more holistically and appropriately to sustainability.

Extensive farming practices may reduce both environmental impact and costs while promoting animal welfare and quality product differentiation [14]. These practices may be

TABLE I
BUFFALO POPULATION AND PRODUCTION IN THE MOST REPRESENTATIVE COUNTRIES IN 2021 [6]

	Head (mil)	Meat (T)	Milk (T)
Asia			
India	111.786.188	1.635.507	94.383.692
Pakistan	42.416.000	1.151.000	36.445
China	27.020.479	658.163	2.905.807
Nepal	5.159.931	188.172	1.419.412
Philippines	2.849.006	64.672	36.444.850
Viet Nam	2.264.700	91.223	26.622
Myanmar	1.980.000	26.898	176.137
Bangladesh	1.500.000	6.878	35.714
Laos	1.243.959	21.843	---
Indonesia	1.189.260	20.972	91.426
Thailand	748.484	15.459	6.674
Africa			
Egypt	3.445.172	166.745	1.508.000
Americas			
Brazil	3.000.000*	---	---
Venezuela	2.800.000*	---	---
Colombia	451.783	8.265	23
Europe			
Italy	409.410	---	257.460
Bulgaria	21.690	16.580	---
Romania	18.500	---	---
Germany	11.220	---	---
Oceania			
Australia	180.000**	---	---
World	203.939.158	4.322.190	137.761.643

* Patiño et al. (2023); ** estimated by Casanova (2019)

conveniently used for species well adapted to the environment, such as buffaloes, and non-productive animal categories, such as heifers [9].

The conduction of buffalo farming on natural pasture is possible, and it also allows the reduction of several sources of pollution while reducing the production costs, hence the economic sustainability, both in terms of fixed (the barn becomes unnecessary) and variable costs (part of the feed is directly gathered from natural pasture by the animals). In addition, permanent grasslands have an essential role in climate stability, as they store nearly as much carbon as forests [15], with 0.01 - 0.3 gross tons of volume C/ year [16]. With their manure, grazing animals avoid the risk of desertification, improving soil functionality in terms of structure, organic matter content, and resilience to erosion by wind or water. When deposited directly on pastures and fields, manure does not significantly increase the amount of methane [17].

Although this system requires greater land use, it increases animal welfare and livestock sustainability and promotes landscape ecology and biodiversity conservation.

However, grazing should be streamlined appropriately to avoid excessive ecological damage caused by overgrazing, trampling, soil compaction, and excessive exploitation of water sources [18]. The causes of these impacts can be ascribed to buffalo thermoregulation habits, including wallowing in the mud, especially in periods and areas characterized by hot climates [19]. Sabia et al. [9] found that heifer free-ranging reduced water consumption and most polluting agents in the atmosphere and in water. At the category level, they observed a reduction of the impact of climate change (9%), terrestrial acidification (10%), marine eutrophication (6%), and water depletion (11%), whereas agriculture land occupation was 7% higher.

The availability of excellent land extension, rich in pasture, in some areas in Asia, Australia, and much more in Cen-

tral and South America, represented an excellent opportunity to realize a new economy based on grazing.

The extensive system for meat purposes is a historical priority in South American countries, and the richness of pasture increased the number of buffaloes bred (FIG. 1).

Brazil has the highest numbers (around 85% of the Americas' herd) and the most advanced buffalo industry. The main breeds are Murrah, Italian Mediterranean, Carabao, and Jaffarabadi. Typically, beef production has basic foodstuff from native or cultivated tropical pastures. The nutritional requirements are satisfied with the pasture rotation or through concentrates supplementation.

Silvopastoral systems are also an alternative for tropical agriculture and ranching in Amazonas to optimize land use and integrate livestock, forage species, and trees [20]. When these systems are associated with intensively rotated pastures, they have a higher productive index with a stocking rate of up to 3.2

livestock units/ha/year, providing animal shading. Moreover, the meat from silvopastoral systems tended to have a lower $\omega 6:\omega 3$ ratio, which is more beneficial to human health [21].

Buffalo farms in Venezuela are often large properties (from 1,000 to 10,000 hectares). However, it is also expected to observe on a small scale of raising (< 100 hectares) with a different number of animals depending upon the farm size (from 100 or less up to 22,000 heads). The management and feeding are mainly entirely grazing-based. However, it is increasingly common to observe more sophisticated feeding systems with strategic supplementation based on fodder, silage, and specialized feeding according to the animal's nutritional requirements [22]. The main advantage of producing buffalo meat under tropical conditions is the shorter time to reach the ideal slaughtering weight (425-550 kg at 18-28 months old) compared to cattle.

The four breeds in the country are Italian Mediterranean, Murrah, Nili-Ravi, and Jaffarabadi [23].



FIGURE 1. Extensive system in South America. 1a. Buffaloes on pasture in Paraguay (Moss photo, 2022); 1b. Dairy buffaloes Mediterranean × Murrah grazing in the South Region of Maracaibo Lake, Venezuela (Gutierrez-Anez photo 2015); 1c. Buffalo reared in an extensive system in Vista Hermosa farm, Guanabacoa, Cuba (Chiariotti photo, 2022); 1d. Murrah × Buffalypso on pasture in Puchino farm, Costa Rica (Borghese photo, 2014)

Colombia is South America's second-largest exporter of buffalo dairy products and the third in population. The buffalo producers have found buffalo calf fattening a productive market, slaughtering buffalo at younger ages with an average weight of 450 kg. Buffalo plays a vital role in fruit recollection at oil palm plantations, making the recollection cost only 3% of the production cost. Since 2020, the Group of Rural and Agricultural Planification (UPRA) has found 27 million hectares, representing 71.3% of the national agricultural area, apt to produce grazing buffaloes [24].

The Northeast region of **Argentina** (NEA) concentrates 85% of the total buffalo population due to pasture characteristics, availability, and the convenience of rearing buffalo compared to cattle. They quickly reach 220 kg in 8 months, 480 kg in 24 months, and 550 kg in 27-30 months, reaching the slaughtering weight one year earlier than cattle and improving the carcass quality. The suckling (or baby) buffalo of 11 months with 250 – 300 kg of weight has good market niches in the medium cities of NEA [25].

In **Bolivia**, the buffalo was introduced recently, particularly in the Cochabamba region, to produce food (meat and milk) for the local market [26]. The breed comprises 70% Italian Mediterranean and 30% of its cross with Murrah. The University of San Simon in Cochabamba began a project for conservation and preservation with an agroecological approach to a grass and pasture rotation system. At 18 months, the average live weight for males is 390 kg, and for females is 383 kg. The most docile water buffalo cows are selected for milking. They milk 2 Liters daily with the calf present and leave the rest for the calf. Small farms are encouraged to implement water buffalo projects in areas since this species is adaptable to the rustic environment and natural grassland, producing meat and milk [27].

Mexico started to import buffalo in 1990; most of the Mexican population is found in the tropics and subtropics in the south of the country (Veracruz, Tabasco, Campeche, Chiapas), with almost permanent average temperatures of 34 to 36°C and rainfall of 1,800 to 2,000 mm annual. In this region (in the natural field), an average daily gain of 600 grams in live weight is achieved, reaching 450 kilos at 28 months of age. In addition, an average production of 5-6 liters of milk per day is obtained [28].

In **Paraguay**, buffalo were introduced almost 70 years ago, utilizing large pastures. In extensive conditions, herds often had little human contact without proper management, so the animals became feral, and this explains the relatively small herd despite all its benefits. Of the 267 small, medium, and large producers dedicated to raising buffalo in the country, 80% are doing so, mostly in marginal, challenging low, and wetlands. Their grazing in set-aside areas helps reduce fuel load and fire intensity [29].

In Central American countries such as Costa Rica, Trinidad and Tobago, and Cuba, the buffalo is reared on pastures, and the most representative breed is Buffalypso.

In **Cuba**, the Cuban Buffalypso performs better than the Carabao and the local cattle. The feeding is based on autochthonous species in natural meadows, such as paraná, jiribilla, perilla, moringa, and small areas of introduced grasses, such as Angola and coastal bermudagrass. In the less rainy season, the buffalo are fed king grass forage. Feeding with unimproved natural pastures and inefficient supplementation cause poor milk and meat production. The future perspective is to increase production volumes by having more milking buffaloes organized in small herds, with better care in all animal husbandry areas and related economic systems [30].

In Asia, particularly in **India** and the **Southeast**, even if the majority is family farming, extensive management can be found (FIG. 2). It is characterized by small farms, with buffaloes maintained on natural grasses on community lands, supplemented with agricultural by-products using family labor and traditional technology. The silvopastoral is the most economical management system, provided adequate fodder resources are available for grazing, and it ensures the possibility for animals to move around and graze on a wide variety of plant species. However, only some of the pastures provide the necessary nutritional requirements. Forage production during the dry season could be low and scarce, causing animal feed competition. Due to a lack of space for adequate grazing, farmers tend to take their small herd of animals to the open farmland or orchard and tie them with a long rope to facilitate spot grazing for a short period [31].

In **Sri Lanka**, more than 70% of the buffaloes are reared in extensive systems in the country's dry and dry intermediate zones. The local Sri Lankan breeds produce 1-2 liters of milk per day in a short lactation period of 3–5 months due to the poor quality of fodder [32]. Buffaloes are primarily used as a source of draught power and to obtain an income from selling fresh and processed milk as curd. A semi-intensive system is practiced in the wet intermediate zone, wet zone, and some parts of the coconut triangle [33].

In **Australia**, the only native pasture with reasonable carrying capacity in the native state is the coastal floodplain system, where there is a satisfactory phosphorus level in the soil and where there are species, such as Native Hymenachne, with very high protein content. Pasture-feeding livestock in the wet season is usually fine due to the abundant rainfall and good pasture growth. The main requirement is the use of fertilizer and improved pasture species. Besides, native pastures are generally of much lower feed quality and have much lower carrying capacity. Grasses and legumes both have an integral place [34]. Nowadays, the buffalo in southern Australia are Riverine bred for dairy purposes, in 12 dairy and 3000 heads [35]. Australian milk yields are variable; a purebred could produce an average between 5 and 15 liters/day (with twice daily milking) over the entire lactation [34,36]. In the more southern regions of Australia, it is essential to shelter the animals from the cold, particularly for the Swamp buffalo, as they are less



FIGURE 2. Extensive system in Southeast Asia. 2a. Bangladeshi buffaloes in Jamalpur District in an extensive system during the dry season (Borghese photo, 2012); 2b. Swamp buffaloes on pasture in West Sumatra (Borghese photo, 2008); 2c. Local buffalo in the extensive system in Sri Lanka (Borghese photo, 2000); 2d swamp buffalo in Thailand (Chiariotti photo, 2014)

tolerant to cold compared to the Riverine, which has a heavier coat of hair that grows with exposure to cold [34].

According to Motta et al. [11] definition, “a sustainable pasture is an anthropized ecosystem that must provide livelihoods, produce benefits to the soil and plants, as well as well-being to animals and to the family, from the use and rational management of the soil to allow nutrient recycling and avoid its biological degradation, for the supply of plant and animal products in stable volumes over time with appropriate management practices (Voisin rational grazing) and, at the same time, conserve or improve natural resources for present and future generations, ensuring the livelihood and well-being of the family”.

BUFFALO FARMING IN MARSHLANDS

The world has an estimated 7 to 9 million km² of marshlands which are characterized by their hydrology, physiochemical environment, and biota [37].

They play a key role in the development of life on Earth and have been critical to the survival of human communities throughout history. These ecosystems have important ecological functions such as water supply, flood control, groundwater restoration, sediment retention, climate change mitigation, and water purification [38]. However, large amounts of senescent vegetation and dry biomass accumulate without productive use, causing problems, and the nearby rural population experiences poverty and limited human development.

Thanks to its anatomical and physiological characteristics, the water buffalo can be used for grazing in marshlands and managing vegetation in tropical and subtropical wetlands. It can move easily in humid and muddy terrain, where cows and other livestock will not. It removes the biomass and cleans the riverbeds, allowing the regeneration of plants, seeds, and tiny organisms, food sources for aquatic birds. So, buffalo helps significantly increase biodiversity and species abundance in the wetlands. In these environmental conditions, buffalo may produce milk and meat of high nutritional value, making the



FIGURE 3. Buffalo farming in the marshlands. 3a. Papangan buffaloes in the marshes of South Sumatra (Borghese photo, 2008); 3b 3c. Mesopotamian buffalo in the Iraqi Marshlands (Borghese photo, 2019); 3d. Buffaloes living in Colombia lagoons (Roldan photo, 2006)

marshland farming system an exciting habitat conservation model and a strategy against climate change [39].

This type of buffalo farming is present in different regions of the world. The **Iraqi** marshlands are the most significant wetland ecosystem of Western Eurasia, a rare example of aquatic landscape in the desert, a unique habitat for endemic buffalo, and endangered animal and vegetal species. Buffaloes are bred in the marshes along the Tigris and Euphrates rivers, particularly in the Marshi region up to the confluence with the Persian Gulf. Buffaloes of the Mesopotamian breed have been living there since the second millennium B.C., as testified by archaeological remains discovered in Ur [40]. From that time, Mesopotamian buffaloes swam for feed, eating papyrus, reeds, and other autochthonous plants. When the flood is high, these plants are collected by breeders to feed buffaloes on platforms. Buffalo is the fundament for food security and livelihood for the Maadan population living on the little isles in the Iraqi marshlands in primitive reed huts. Women play a pivotal role in producing, processing, and marketing dairy products.

Another wide marshland area in Asia is located in the Gange River delta in **Bangladesh** in the Bathan saline coastal

region. The coastal region covers about 20% of the country, representing over one-third of the cultivable lands. About half of the coastal areas are affected by salinity, which causes unfavorable environments and hydrological situations that restrict normal crop production throughout the year. Agricultural land use in these areas is very poor, much lower than a country's average cropping intensity. Here, Swamp and River buffalo live in the same situation as Iraqi marshlands.

Coastal wetlands in **Indonesia** have a high potential for global climate change mitigation, for they have considerable capacities to store carbon compared to similar ecosystems globally. However, coastal wetlands in Indonesia potentially sustain significant loss due to inundation following the upcoming rapid sea level rise and land use change and conversion following rapid population growth in the coastal areas [41]. Shrimp farms have cleared large tracts of mangroves and coral reefs along the coastal and marine environment. These areas, such as around the North Coast of Java, are vulnerable to storms, tidal waves, and coastal erosion. This vulnerability will only increase with a changing climate, leading to storm surges and sea-level rise. In addition to aquaculture, urban development, pollution, over-harvesting of wood in the coastal forests, and

destructive fishing contribute to the destruction and degradation of mangrove forests [41].

In the province of South Sumatra (Papangan Subdistrict), there is a marshland coastal area with very hot and wet weather, with high dampness during the humid season, where Kerbau Papangan, a local Swamp breed, survives grazing autochthonous grass (kumpai copper, kumpai paddy, bento creep, parum). The dairy buffalo cows come to the islands to be hand-milked once daily.

Another example of marshland farming is in **Amazonia**, a wide area attractive, particularly Brazil, Colombia, and Venezuela, covered by immense rivers: Rio Amazonas, Rio Negro, Orinoco, Japurá, and others.

On Marajo Island of Rio Amazonas, there were about 500,000 buffaloes living free in the marshland area, flooded during the rainy season and very rich in water plants during the dry season, creating a unique resource for feeding. Nowadays, following the progressive decrease of the buffalo population, there is a need for governmental actions to protect the environment, taking into account the socioeconomic impact of this type of buffalo farming system for the indigenous population [42]

In **Colombia**, Buffalypso was the first buffalo breed introduced from Trinidad and Tobago and used mainly for draught power in marshland areas where cattle could not be reared. Buffalo is suitable for work in plantations due to its broad articulation in the hoofs, especially during the rainy period, when the soil becomes muddy, causing difficult mobility for other species. For this characteristic, the buffalo became the best draught power animal option for oil palm harvest in the tropics [43]. Later, numerous Murrah and Italian Mediterranean buffaloes were imported from Venezuela and Brazil for semi-intensive management to exploit the meat and milk production sector [24].

In **Paraguay**, there is about 23% of marshland areas. The largest are the Pantanal Paraguay, the Lagunas del Chaco Central, the Lagunas del Chaco Húmedo, the wetlands at the foot of the Cerrados, those belonging to the Arroyos y Esteros wetlands, those of the Atlantic Forest, the Ñeembucú wetland [38]. Of the 267 small, medium, and large buffalo farms, 80% operate in marginal, challenging wetland areas [44]. Due to the relationship between wetlands and buffalo, the latter can be considered a tool for regenerative and sustainable livestock farming in Paraguay. The remarkable capacity of the buffalo to convert fiber into energy is very important in this area, where in the estuaries, there is an abundant supply of crude fiber, such as reeds and grasslands. These forages, which cattle do not consume, allow the buffalo to obtain astonishing rates of daily gain. The buffalo is an exciting alternative for the wetlands of the Paraná Delta [29].

The Lower Paraná Delta in northern **Argentina** is a vast, forested marshland and an estuary with great natural values. Fish and birds have their breeding grounds around the many islands and wetlands. At the same time, deforestation is oc-

curing on a large scale, and urban development is spreading uncontrollably. The buffalo species are adapted to live in wetlands, and by adjusting the loads and managing the receptivity of the environment, it is possible to achieve sustainable buffalo production. Its rusticity, combined with rational management systems, alternating trampling with rest periods, stimulates plant and soil regeneration, promoting biodiversity and carbon sequestration [45]. In the summer, buffaloes are constantly submerged in the estuaries, feeding on water hyacinths and other aquatic plants. During the winter, with the low temperatures, the animals generally seek higher areas, sheltered from the cold and grazing on the natural vegetation of the hills.

In the marshlands, there are livestock management recommendations for the maintenance of water quality that include avoiding the accumulation of excreta and feces in bodies of water and no use of herbicides in bodies of water to eliminate plants. Also, reducing landscape modifications that affect the hydrological regime and water quality, such as the construction of dams or canals, is important, as well as rotating livestock distribution areas to minimize nutrient input and use veterinary products rationally, given their impact on the environment and water quality.

FAMILY FARM

According to the International Steering Committee for the 2014 IYFF, "Family Farming (which includes all family-based agricultural activities) is a means of organizing agricultural, forestry, fisheries, pastoral and aquaculture production which is managed and operated by a family and predominantly reliant on family labor, including both women's and men's. The family and the farm are linked, co-evolve, and combine economic, environmental, social, and cultural functions" [46].

Family farms, therefore, affect sustainable development, people, and the planet. An individual or a family runs more than 90% of farms, and women provide almost 50% of farm labor, holding less than 15% of farmland. Family farms produce more than 80% of the food in the world, managing around 70-80% of farmland worldwide. In short, they create employment, help expand rural economies, prevent depopulation and support the area, preserve biodiversity and ecosystems, and help reduce climate change risks [47].

There are some general problems in the family farming husbandry practices in many developing countries, such as no housing system, no artificial insemination system, no routine vaccination program, and no animal identification and recording system. In smallholder production, it is important to integrate genetic improvement programs with other livestock development activities such as health cover, nutrition, management, and access to output markets.

In **Iran** the buffalo farming system is based on smallholders (99%), and most buffaloes are raised in traditional rural farms with a herd size of 10 to 30 buffaloes. In spring and



FIGURE 4. Family farms. 4 a,b. Family buffalo in a rural village in Haryana, India (Chiariotti photo, 2018); 4c. Family buffalo in Chitwan, Nepal (Moioli photo, 2017); 4d Woman preparing buffalo dung cake (Pathee) in a small village in Haryana, India (Chiariotti photo, 2018)

summer, simultaneous with rice cultivation, buffaloes are transferred for their nutrition to fenced and exclusion rangelands and summer grazing areas, which are covered with hircany forests. In autumn and winter, they are kept in closed housing and are hand-fed with roughages such as rice stem, rice bran, and wheat bran [48]. The feeding stuffs are straw, alfalfa, and sugar cane pulp. The desert is irrigated and produces sugar cane and dates.

In **Bangladesh**, 95% of dairy buffaloes are reared under the transhumant system. Production is usually based on a small herd of mixed ages and sexes at the rural level, generally for draught and breeding purposes. Buffaloes are sheltered at night and graze for roughage together with other animals. Grazing and browsing ranges over practically all village lands during the dry season but is restricted to upland non-cropped areas during the rainy season. Sometimes, they graze on roads and other aquatic herbage [33].

In **Nepal**, most households raise at least one buffalo for milk and manure production and sell male calves or mature

males for family income. In road-accessible areas, buffaloes are the main contributor to urban milk supply. Nepal's indigenous buffalo River breeds are Terai, Gaddi, Lime, and Parkote, and exotic breeds are Murrah and other Indian breeds. Native breed productions are inferior; however, their adaptability is better [49]. Lime and Parkote live on the country's hills and mountains, producing about 900 kg of milk yield per lactation. These purebreds will be reduced as crossbreeding with Murrah is applied to increase the milk yield, reaching 1500 kg per lactation [50]. Fodder tree leaves in mountains and hills, grasses, legumes, straw, stover, and other agro byproducts are the primary feeding resources in Nepal.

In **Sri Lanka**, the buffalo population is integral to mixed farming enterprises and rural life. More than 90% of the local indigenous breed and their crosses are found in small holdings (0.8 ha in extent). A limited number of Murrah, Surti, and Nili-Ravi breeds are maintained in large farms to serve as breeding stock and improve local animals. The average herd size of the country is approximately 20-25 animals but can

vary with different agroecological zones, feeding resources, climate cropping patterns, and production systems. Buffaloes are allowed to graze on the naturally available forage during the daytime and paddock housed in the nighttime. Buffaloes are primarily used as a source of draught power and to obtain an income from selling fresh or processed milk as curd. In Sri Lanka, raising buffaloes for meat is not practiced [32]

China has the third-largest buffalo population in the world. Chinese buffalo belong to the Swamp type, with 18 local breeds. They are strong animals mainly used for draught in the marshlands, particularly in rice fields, as a family animal, similar to **Thailand, Vietnam, Laos, Malaysia,** and the **Philippines**. They are also a source of meat, but their milk production is deficient (500-700 kg milk yield for lactation on average) and used in family and local economy. Murrah buffalo arrived in China from India in the late 1950s, while Nili-Ravi arrived from Pakistan twenty years later.

In **India** and **Pakistan**, most dairy farms are small holdings practicing subsistence farming; however, several larger farms are expanding and generating growth in the dairy sector, which is highly significant in the country's economy.

Employment generation for rural people, particularly women; nutritional security for the majority vegetarian population with high-quality milk and resistance to the prevalent infectious diseases make buffalo a livestock species of national importance in **India**. The production systems are based, for the most part, on small farms that maintain 1 to 3 buffaloes with crop residues and limited yields and tie their buffaloes at night in small shelters, which are made of mud or thatched walls and thatched or tin roofs for protection from rain and wind [31].

Under the rural subsistence smallholder production system, which is most common in **Pakistan**, milk is produced for the family at minimal cost. The Nili-Ravi produces 1000-3000 kg of milk and swamp-type 600 kg [51]. Grazing provides over 60% of the animals' feed requirements. Some roughages and a small quantity of concentrate are given only to milking animals. This traditional system is heavily labor-consuming.

Egypt is the leading African country with an economy based on buffalo farming of 3.9 million head [52], the highest population in the Mediterranean area. Egyptian buffalo is not only considered a vital genetic resource with great potential for milk and meat production but also the main dairy animal raised by small Egyptian farmers [53]. Buffalo milk represents 45-50% of Egyptian milk, with an average milk yield of up to 2,500 kg in 305 days of lactation [54]. 80% of all farms have less than two feddans (0.420), and small Egyptian farmers prefer buffalo since they can use lower quality and less digestible feeds. The locally available feeds include clover, corn silage, rice straw, clover hay, concentrates feed mixtures, and other plant residues. A notable decrease in the buffalo population in the lower and middle regions was due to the drought and desertification in rural and semi-rural areas [55]. The supply of milk increas-

es in winter due to the availability of green fodders. More than 69% of farms tend to practice meat production along with milk production. Women share important activities such as milking, calf rearing, and interrelated activities [56].

INTENSIVE FARMING

Agricultural intensification is the increased production of agricultural commodities per unit of inputs, such as labor, land use, fertilizer, time, feed, animals, or cash [57]. As land is the ultimate limiting input for agricultural production, agricultural intensification is often defined as increased production per unit of land area [58].

The main drivers for livestock intensification are population growth, an increase in gross domestic product (GDP), urbanization, and the globalization of the markets, which increased demand for animal products. This caused the development of the livestock sector towards product intensification and changes in branch industries and transportation, mainly in developed countries. Intensification per cow was primarily due to a better feed conversion ratio, while intensification per ha was due to increased production per cow, lower culling and replacement rates of cows, and increased forage and crop yields per ha [59]. Dairy system intensification invariably leads to higher impacts per ha of land occupied due to the higher proportion of maize silage and concentrate feed in the diet, which entails increased use of inputs, so combining environmental impacts and productivity is difficult [60].

Buffaloes reared in intensive systems are handled according to the same or comparable management routines of cattle (FIG. 5). However, these conditions cause animals new stressors generated by new technologies such as artificial rearing of calves and reduced space. Space restriction can adversely affect health, social behavior, and heat dissipation, particularly without access to pasture and water for wallowing [14].

For this reason, ensuring the buffalo's welfare in intensive systems is a pivotal goal, providing the possibility to express species-specific behavior and might positively affect product quality. In addition, the sustainability and welfare of the animals may also affect consumer liking [61].

Some advanced techniques are being developed to increase the sustainability of intensive management, such as intelligent farming (FIG. 6). The smart farming system is a new concept in the agricultural field and refers to managing farms using technologies like sensors, software, data analysis, communication systems, that enable farmers to give plants/animals the precise treatment that they need. Regarding livestock, most of these technologies are utilized in the dairy cattle sector, where the use of automated and robotic devices is increasing rapidly. In buffalo ear tags, sensor systems for recording activity, resting, rumination, and temperature at ear level are tested to get information on health, nutrition, and reproduction [62].



FIGURE 5. Intensive systems in Asia. 5a, intensive farm of ICAR, Haryana, India (Chiariotti photo, 2018); 5b. Traditional intensive system in India with tie-stall (Chiariotti photo, 2018); 5c, Intensive system in Egypt (Barile photo, 2019); 5d. Intensive system in CREA Research center, Roma, Italy (Barile photo, 2023)

In **Italy**, buffalo farming has been conducted for centuries in extensive conditions based on marshland environments. In the last forty years, the increasing demand for mozzarella cheese induced a proportional increase in the number of buffaloes, mainly located in central-southern Italy. Therefore, buffalo farming has shifted to a more intensive farming system based on three different feeding regimens corresponding to the three main buffalo productive stages (lactating cows, dry cows, and growing heifers [63] with limited access to pasture and water for wallowing. Such management changes concerned all animal categories (including juvenile and heifers) that do not need milking facilities and feeding supplementation.

Due to the high commercial value of buffalo milk, calves are separated from the dams at birth and reared artificially. Calves are weaned at 90/100 kg, around three months of age. Lactating buffalo cows are housed in paddocks. They are fed a total mixed ration (composed of maize or sorghum silage, concentrates, hay, straw, and sometimes agricultural by-pro-

ducts). The average milk production is over 2,350 kg for lactation up to over 5,600 kg milk/270 days of lactation and 8.32% fat and 4.63% protein [64]. Male buffaloes are reared as young bulls in intensive systems for the meat market. Usually, the live weight at slaughter is 400-440 kg obtained at 15-16 months of age with 800-1000 g of daily gain [5]. According to the Italian market requirements, young bulls have good quality carcasses and medium fattening (57% dressing percentage, 62% meat on carcass) [65]. A good pasture system could be adopted for reared heifers, reducing the production costs (i.e., management and feedstuffs) and increasing the sustainability of the breeding system. In Italy, numerous farms produce renewable energy from manure and sludge in co-digestion with agricultural waste such as cheese whey and tomato pomace in biogas plants (FIG. 7). The process residue, so-called digestate, is used as organic fertilizer.

In **Asia**, an intensive management system is practiced in the urban, peri-urban areas where the demand for milk and



FIGURE 6. Italian Mediterranean buffaloes in an intensive system with modern technologies for animal welfare. 6a. Wide space, rubber mats, and brushes in Vannulo farm, Salerno, Italy (Barile photo, 2023); 6b. Milking robot unit in Vannulo farm, Salerno, Italy (Barile photo, 2023); 6 c, d. Calf management in an intensive system in Garofalo Farm, Caserta, Italy (Chiariotti photo, 2023)

dairy products is high and constantly growing due to the increasing urbanization.

Indian farmers who own high-yielding buffaloes try to grow or buy fodder to ensure higher production. Fodder cultivation can be more remunerative than many cash crops if the cows and buffaloes fed are high yielders. India is highly advanced in buffalo nutrition, production, reproduction, biotechnologies and genetic improvement, and scientific and technological development. The country possesses 13 recognized breeds, plus 20 different River and Swamp types populations. The main River dairy breeds are Nili-Ravi, Bhadawari, Pandharpuri, Surti, Jaffarabadi, and particularly Murrah, which is the most widespread breed in India and the world (2500 kg per lactation on average) [66]

The herd has up to 100 buffaloes in the intensive system, mainly in Haryana, Punjab, Uttar Pradesh, Rajasthan, Gujarat, and peri-urban areas. There is also a semi-intensive system

of 3 to 5 buffaloes maintained with fodder produced under irrigation, crop by-products, concentrates, improved housing, and care.

Pakistan is the second country in the world after India for buffalo heads. In Pakistan, only River buffaloes are found, mainly dairy, of the Nili-Ravi breed, with an average milk yield of 2300 and a maximum of 3500 kg/lactation in the rice-wheat zone of Punjab, and the Kundhi breed, having a smaller body size, with an average milk yield of 2100 kg/lactation up to 2600 in the wealthiest areas [67]. Therefore, the buffalo's role in Pakistan's milk production system and food availability is pivotal. Peri-urban commercial dairy farms are around all big cities, the largest being at the Landhi Cattle Colony, located in the suburbs of Karachi, where more than 400,000 milking animals (around 95% are buffaloes) are kept. It has about 1,500 farms spread around 650 ha, with a daily yield of about four million liters of milk and 7,200 T of dung, making it the world's largest



FIGURE 7. Biogas plant for green energy production at Roana farm, Latina, Italy (Photo Barile, 2023)

dairy colony. Individual farmers often own up to 200 animals each [68]

FUTURE PERSPECTIVES

Sustainable livestock farming must consider various economic and social factors, including food security, poverty alleviation, mitigation strategies, and social and cultural value preservation. This could only be achieved through policy initiatives, enabling discussion with all stakeholders involved in the supply chain. Climate change impacts will need special attention through adopting sustainable agricultural practices, better utilization of local feed resources, and enrichment of its nutritional values, considering land and water shortage.

Buffalo, with its genetic diversity and adaptability to different environments, from cold climates to hot, humid ones and wetlands, where other ruminants could not thrive, could help achieve some Agenda 2030 Sustainable Development Goals [69].

Pastures are anthropogenic ecosystems that offer low-cost food resources mainly for feeding ruminants, buffalo in particular, being the ground for production and livelihoods in many rural areas worldwide. More intensive buffalo meat and milk production on pasture has proven to be sustainable if rational grazing is applied. In environmental terms, it can help reduce the pressure of deforestation to increase new areas for

cultivation, water depletion, fertilization, and pesticide inputs to fulfill plants' needs. Crop-livestock-forest integration systems, including silvopastoral ones, will gain relevance in the future and help reclaim agricultural areas altered by inappropriate previous use. It could add value to the land, enabling better nutrient cycling or animal welfare due to natural shading or to the environment because the presence of trees also acts as a carbon sink.

Thanks to its anatomical and physiological characteristics, the buffalo is the only ruminant that can be reared in wetlands and produce milk and meat of high nutritional value, making the marshland farming system an exciting model for habitat conservation. So, it helps increase biodiversity and the livelihood of the local population.

The family farming system spread particularly in South-east Asia, has a pivotal role in sustaining people's livelihood due to the local buffalo breeds, some of which are endangered. Those should be maintained because they are the most adapted to low-input production systems and a specific environment, producing food with unique properties.

Future advancements in smart technologies hold great promise, especially in intensive production systems; adapting to individual animals' needs will help reduce the inputs and improve animal welfare.

The livestock production systems are strictly linked to sustainability, welfare, and quality of products, and new tech-

nologies and education will play a pivotal role in facing the challenges of climate change.

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REFERENCES

- [1] Minervino AHH, Zava M, Vecchio D, Borghese A. Bubalus bubalis: A short story. *Frontiers in Veterinary Science*. 2020; 7. <https://doi.org/10.3389/fvets.2020.570413>.
- [2] Borghese A, Chiariotti A, Barile VL. Chapter entitled "Buffalo in the World: Situation and Perspectives in: Springer's edited book Manmohan Singh Chauhan and Naresh Selokar (Eds): Biotechnological Applications in Buffalo Research. 2022; 16:978-981. ISBN, 978-981-16-7531-7.
- [3] United Nations – Department of Economic and Social Affairs. The Sustainable Development Goals Report 2018.
- [4] Borghese A Buffalo Production and Research. FAO Ed. REU, Roma, Italy. Technical Series 2005; 67:1-315.
- [5] Borghese A Buffalo Livestock and Products. A. Borghese and CRA (Council of Research in Agriculture) (Eds), Rome, Italy 2013; 1-511
- [6] FAOSTAT, 2023. <https://www.fao.org/faostat/en/#data>.
- [7] Patiño EM, Crudeli GA, Coronel Sicairos I. Buffaloes in America: Current Status and Future Challenges of Buffalo Production Post-centenary of its Introduction in Venezuela. *Buffalo Newsletter*. 2017; 39:16-21.
- [8] Casanova S. Top End Buffalo Population estimates. Paper presented to 2019 Territory NRM conference. 2019. <https://www.abc.net.au/news/rural/2019-11-21/nt-buffalo-population-on-the-rise/11713352>
- [9] Sabia E, Napolitano F, Claps S, De Rosa G, Barile VL, Braghieri A, et al. Environmental impact of dairy buffalo heifers kept on pasture or in confinement. *Elsevier Agricultural System*. 2018; 159(c):42-49. <https://doi.org/10.1016/j.agsy.2017.10.010>.
- [10] Thomassen MA, Van Calker K.J, Smits MCJ, Iepema GL, De Boer IJM. Life cycle assessment of conventional and organic milk production in The Netherlands. *Agric. Syst* 2008; 96(1-3):95-107. <https://doi.org/10.1016/j.agsy.2007.06.001>.
- [11] Motta-Delgado PA, Ocaña Martínez HE, Rojas-Vargas EP. Indicators associated to pastures sustainability: a review. *Ciencia y Tecnología Agropecuaria*. 2019 August; 20(2):387-430.
- [12] Casimir M, Rao A. Sustainable herd management and the tragedy of no Man's Land: an analysis of west Himalayan pastures using remote sensing techniques. *Human Ecology*. 1998; 26(1):113-134. doi:10.1023/A:1018701001793.
- [13] Sherren K, Fischer J, Clayton H, Schirmer J, Dovers S. Integration by case, place and process: transdisciplinary research for sustainable grazing in the Lachlan River catchment, Australia. *Landscape Ecology*. 2010; 25(8): 1219-1230. doi:10.1007/s10980-010-9494-x.
- [14] Napolitano F, Pacelli C, Grasso F, Braghieri A, De Rosa G, 2013. The behaviour and welfare of buffaloes (*Bubalus bubalis*) in modern dairy enterprises. *Animal*. 2013; 7:1704-1713.
- [15] EIP-AGRI Focus Group Profitability of permanent grassland. How to manage permanent grassland in a way that combines profitability, carbon sequestration, and biodiversity? Starting Paper Koldo Osoro. 2014 28th May.
- [16] Lal R. Soil carbon sequestration impacts on global climate change and food security. *Science*. 2004; 304:1623-1627.
- [17] Bernués A, Ruiz R, Olaizola A, Villalba D, Casasús I. Sustainability of pasture-based livestock farming systems in the European Mediterranean context: Synergies and Trade-offs. *Livestock Science*. 2011; 139:44-57.
- [18] Hoogesteijn R, Hoogesteijn A. Conflicts between cattle ranching and large predators in Venezuela: Could use of water buffalo facilitate felid conservation? *Oryx*. 2008; 42:132-8. <https://doi.org/10.1017/S0030605308001105>
- [19] Mora-Medina P, Napolitano F, Mota-Rojas D, Berdugo Gutiérrez Ruiz-Buitrago J, Guerrero-Legarreta I. Imprinting, sucking and allosucking behaviors in buffalo calves. *J Buffalo Sci* 2018; 7: 49-57. <https://doi.org/10.6000/1927-520X.2018.07.03.3>
- [20] da Silva JA, Garcia AR, de Almeida AM, Bezerra AS, de Brito Lourenco Junior J. Water buffalo production in the Brazilian Amazon Basin: A review. *Tropical Animal Health and Production*. 2021 Jul; 53(3):343
- [21] Joele MR, Lourenço LF, Lourenço Júnior JB, Araújo GS, Budel JC, Garcia AR. Meat quality of buffaloes finished in traditional or silvopastoral system in the Brazilian Eastern Amazon. *Journal of the Science of Food and Agriculture*. 2017 Apr; 97(6):1740-5.
- [22] Gutiérrez-Añez JC. Current status and future challenges of buffalo production post-centenary of its introduction in Venezuela. *Buffalo Newsletter*. 2019; 35:29-36.
- [23] Cuéllar Yáñez. Buffalo in Venezuela. *Buffalo Newsletter*. 2021; 37:28-30.
- [24] Suarez A L. Buffalo in Colombia. *Buffalo Newsletter*. 2021; 37:27-28.

- [25] Zava M. Present situation and future perspective of buffalo production in America. *The J of Anim and Plant Sci.* 2012; 22(3):262-269. ISSN/ISBN: 1018-7081.
- [26] Chacón Condori E. Buffalo's production in Bolivia. *Buffalo Newsletter.* 2019; 35:10-13.
- [27] Skorc L. Buffalo in Bolivia. *Buffalo Newsletter.* 2021; 37:24-26.
- [28] Zava M. Búfalos: México será sede de un encuentro global de productores. Sitio Argentino de Producción Animal. 2017 https://www.produccion-animal.com.ar/informacion_tecnica/razas_de_bufalos/126-Bufalos-Mexico.pdf
- [29] Moss Ferreira R, Patiño EM. Buffaloes in Paraguay: Past, Present and Future. *Buffalo Newsletter.* 2022; 38:3-9.
- [30] Mitat VA. Cuban Buffalo Production. *Buffalo Newsletter.* 2022; 39:25-32.
- [31] Hegde NG. Buffalo husbandry for sustainable development of small farmers in India and other developing countries. *Asian Journal of Research in Animal and Veterinary Sciences.* 2019 Mar 18; 3(1):1-20.
- [32] Seresinhe Thakshala. Buffalo production systems and the role of buffalo in Sri Lanka. *Buffalo Newsletter.* 2018; 33:13-14.
- [33] Habib KA, Kim CG, Oh J, Neogi AK, Lee YH. Aquatic Biodiversity of Sundarbans, Bangladesh. *Korea Institute of Ocean Science and Technology (KIOST).* 2017; 394.
- [34] Lemke B. The Australian water buffalo manual. Department of Primary Industry and Resources. 2017:43-50.
- [35] Agrifutures 2021-2025. <https://agrifutures.com.au/wp-content/uploads/2021/03/21-004.pdf>.
- [36] Suarez A L. Buffalo in Colombia. *Buffalo Newsletter.* 2021; 37:27-28
- [37] Mitsch WJ. Applying science to conservation and restoration of the world's wetlands. *Water Science and Technology.* 2005 Apr 1st; 51(8):13-26.
- [38] Secretaría del Ambiente (SEAM) y Programa Marco para la Gestión Sostenible de los Recursos Hídricos de la Cuenca del Plata en relación con los efectos de la variabilidad y el cambio climático (PMCIC-PLATA. Inventario de humedales del Paraguay. OEA Contrato. 2015; 1^o Edición N° 366: 127.
- [39] Barboza Jiménez G. El búfalo en la rehabilitación de humedales: finca las delicias en Guanacaste, pacifico norte de Costa Rica." *Revista CES Medicina Veterinaria y Zootecnia.* 2016; 11(3):124.
- [40] Al-Fartosi K, Al-Saedy JK. Mesopotamian buffalo: home-tract and challenges. *Proceed. Intern. Buffalo Congress, Lahore, Pakistan. J. Vet. Animal Sci.* 2019; 6:1-13.
- [41] Hapsari KA, Biagioni S, Jennerjahn TC, Reimer P, Saad A, Sabiham S, Behling H. Resilience of a peatland in Central Sumatra, Indonesia to past anthropogenic disturbance: Improving conservation and restoration designs using palaeoecology. *Journal of Ecology.* 2018 Nov; 106(6):2473-90.
- [42] Vale WG, Ribeiro HFL, Barbosa LAL, Rolim Filho ST, Neves KAL, Silva AOA, Lima WF. Forty years fo buffalo artificial insemination in the Amazon Valley, Brazil – An Historical Retrospective. *Buffalo Newsletter.* 2022; 38:9-20.
- [43] Cortés RH. El uso del búfalo en la cosecha de palma africana. *IIIrd American Buffalo Symposium, Medellín, Colombia.* 2006 September 6th-8th.
- [44] Frutos, L. (2021) Producción de búfalos crece 25% al año (criadores están comercializando carne, hamburguesas, embutidos, quesos y leche). *Infonegocios*<https://infonegocios.com.py/infoganaderia/produccion-de-bufalos-crece-25-al-ano-criadores-estan-comercializando-carne-hamburguesas-embutidos-quesos-y-leche>
- [45] Travaini, A.; Astrada, E.; Cadoppi, A. Búfalos de agua (*Bubalus bubalis*) en el Delta del Rio Paraná. *Serie Ganadera en Humedales. Programa Corredor Azul. Fundación Humedales./Wetlands International. Bs. As. Argentina. Rubén D. Quintana Editor.* 2019:1-28. ISBN 978-987-29811-7-4.
- [46] FAO 2013. <https://www.fao.org/news/archive/news-by-date/2013/it/>.
- [47] World Family Forum. 2019. <https://www.fao.org/news-room/detail/global-forum-of-the-un-decade-of-family-farming-2019-2028-begins/en>
- [48] Derisavi F. Introduction of excellent Nikookar Buffalo Farm in Gilan province. *Buffalo Newsletter.* 2021; 37:9-12.
- [49] Devkota B (2018) Overview of Buffalo Development in Nepal. *Buffalo Newsletter.* 2018; 33:11-13.
- [50] Nirmal BK, Devkota B, Shrestha S et al. Nepal country report on buffalo production system. In: *Book of abstract 9th Asian Buffalo Congress Hisar, India.* 2018 February 1st-4th February.
- [51] Farooqi A. Buffalo Farming: Its Significance in Economy of Pakistan. 2022 March 4th.
- [52] FAOSTAT, 2019. <https://www.fao.org/faostat/en/#data>.
- [53] Eid L. Insight on the status of Egyptian buffalo. *Buffalo Newsletter.* 2019; 35:14-19.
- [54] Shafik Basant MN, El-Bayomi KMC Abo-Salem MES, et al. Environmental factors affecting some productive and reproductive traits in Egyptian Buffaloes. *Benha Veterinary Medical Journal.* 2017; 32(1):153-159.

- [55] Hassanein MK, Abolmaaty SM, Khalil AA, Taqi MO, Essa YH, Shawki HH. Geographical Distribution and Developmental Pattern of Buffalo in Egypt. *World Rural Observations*. 2013; 5(4):14-9.
- [56] Fahim NH, Abdel-Salam SA, Mekki W, Ismael A, Abo Bakr S, El Sayed M, Ibrahim MA. Delta and upper egypt buffalo farming systems: asurvey comparison. *Egyptian Journal of Animal Production*. 2018 May 1; 55(2):95-106.
- [57] Kenmore PE, Stannard C, Thompson PB. The ethics of sustainable agricultural intensification. *FAO*. 2004; 3.
- [58] Donald PF, Green RE, Heath MF. Agricultural intensification and the collapse of Europe's farmland bird populations. *Proceedings of the Royal Society of London. Series B: Biological Sciences*. 2001 Jan 7th; 268(1462):9-25.
- [59] Crosson P, Shalloo L, O'Brien D, Lanigan GJ, Foley PA, Boland TM, Kenny DA. A review of whole farm systems models of greenhouse gas emissions from beef and dairy cattle production systems. *Animal Feed Sci. Technol*. 2011; 166 and 167:29-45.
- [60] Salou T, Le Mouél C, Van der Werf HM. Environmental impacts of dairy system intensification: the functional unit matters!. *Journal of Cleaner Production*. 2017 Jan 1; 140:445-54.
- [61] Mota-Rojas D, De Rosa G, Mora-Medina P, Braghieri A, Guerrero-Legarreta I, Napolitano F. Invited review: Dairy buffalo behaviour and welfare from calving to milking. *CAB Rev* 2019; 14: 1-12. <https://doi.org/10.1079/PAVS-NNR201914035>
- [62] Meo Zilio D, Steri R, Iacurto M, Catillo G, Barile VL, Chiarriotti A, Cenci F, La Mantia MC, Buttazzoni L. Precision Livestock Farming for Mediterranean Water Buffalo: Some Applications and Opportunities from the Agridigit Project. In: Biocca M., Cavallo E., Cecchini M., Failla S., Romano E. (eds) *Safety, Health and Welfare in Agriculture and Agro-food Systems*. SHWA 2020. *Lecture Notes in Civil Engineering*. SpringerNature. 2022; 252:41-50. https://doi.org/10.1007/978-3-030-98092-4_5.
- [63] Sabia E, Napolitano F, Claps S, Braghieri A, Piazzolla N, Pacelli C. Feeding, nutrition and sustainability in dairy enterprises: the case of Mediterranean buffaloes (*Bubalus bubalis*). In: Vastola, A. (Ed.), *The Sustainability of Agro-food and Natural Resource Systems in the Mediterranean Basin*. Springer Open. 2015a:57-64.
- [64] AIA 2023. (available at: <https://aia.it>).
- [65] Failla S et al., see Borghese A. Buffalo Livestock and Products. A. Borghese and CRA (Council of Research in Agriculture) (Eds), Rome, Italy. 2013a:1-511.
- [66] Singh I. Buffalo – the harbinger of prosperity in rural India. In: book of abstract 9th Asian Buffalo Congress Hisar, India. 2018 February 1st-4th.
- [67] Aujla KM, Hussain A. Economics of milk production of major dairy buffalo breeds by agroecological zones in Pakistan. *Pakistan J. Agric. Res*. 2015; 28(2):179-191. <https://popularinpakistan.com/buffalo-farming/>
- [68] Afzal M, Naqvi AN. Livestock resources of Pakistan: present status and future trends. *Quarterly Science Vision*. 2004; 9(1):1-2.
- [69] United Nations – Department of Economic and Social Affairs – Sustainable Development. *Transforming our World: The 2030 Agenda for Sustainable Development*. <https://sdgs.un.org/2030agenda>

MAXIMIZING THE POTENTIAL OF WATER BUFFALO MILKABILITY: BEST PRACTICES AND LESSONS LEARNED

Maximizando el potencial de la ordeñabilidad de los búfalos de agua: mejores prácticas y lecciones aprendidas

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ABSTRACT

Milk yield and flow profiles are essential parameters to record and evaluate. Buffaloes are characterized by longer teats and teat canals and, in particular, stronger muscular resistance of the teat wall than in cows; it is necessary to have a high vacuum level to open the teat canal and begin milk ejection. The milk stored in the buffalo udder can be divided into two fractions: the cisternal fraction, which has already been transferred from the alveoli to the cistern during the interval between milkings and is immediately available, and the alveolar fraction, which can only be removed from the udder by the action of oxytocin. In buffalo milking management, the milking machine is a critical point, and the characteristics of the milking vacuum and the pulsation rate are closely related to milk flow observations; in Italy, the most commonly used vacuum levels are 44–46 kPa (range 40–53 kPa). The data on the milkability traits of the Mediterranean Italian breed made it possible to classify eight different types of milk flow curves due to anatomical, physiological, and management differences. The most represented milk flow curve was type 3 (with a similar time between PPT and DPT, 27.32%), followed by type 6 (17.79%), characterized by a very long plateau phase. The least represented curve was type 1 (4.41%), characterized by a long lag time and low peak flow rate. Buffaloes with curves of type 5 (10.62%) and 6 (17.79%) were characterized by the highest milk yield at milking, the lowest somatic cell score, and the shortest milking time. The analysis of the milk emission profiles showed an excessive duration of DPT and overmilking (BT); these results suggest the detachment of the milking cluster to reduce the BT with the following advantages: a) reduction of the total milking time and consequently of the worker's time, b) improvement of the farmer's income and milk quality thought the mastitis incidence decreases. Concerning the automation of milking technologies, a recent study ana-

lyzed the main milk flow traits and milk yield recorded in Mediterranean Italian buffaloes milked with the Automatic Milking System (AMS). The results showed a considerable variation in milk ejection and, consequently, in the milk flow curves of the buffaloes milked in AMS with a forced system compared to the conventional one. The differences were the following: better pre-stimulation allowing a positive endogenous release of oxytocin, with reduced lag time; independent milk ejection for each teat, with optimal milking of all quarters, with the reduction of BT; improvement of milking hygiene during milking, limiting the incidence of mastitis and with a low value of somatic cell score; adverse effects due to failed or incomplete milking (17% of the total milkings), limiting the potential capacity and efficiency of AMS; frequent leakage of airflow, which could cause alteration of milk composition. In conclusion, continuous monitoring of milk ability will help to optimize milking practices by reducing labor time and increasing farmers' income through better milk quality and fewer udder issues. In addition, the identification of buffaloes with desirable types of milk flow curves could be helpful for buffalo breeders' associations to address farmer management and also to define potential new breeding objectives.

Keywords: dairy buffalo, milkability, milk flow curves, milking time.

RESUMEN

Los perfiles de producción y flujo de leche son parámetros esenciales para registrar y evaluar. Los búfalos se caracterizan por tener pezones y canales pezones más largos y, en particular, una mayor resistencia muscular de la pared del pezón que en las vacas; es necesario tener un nivel de vacío alto para abrir el canal del pezón y comenzar la eyección de la leche. La leche almacenada en la ubre de búfala se puede dividir en dos

fracciones: la fracción cisternal, que ya ha sido transferida de los alvéolos a la cisterna durante el intervalo entre ordeños y está inmediatamente disponible, y la fracción alveolar, que sólo puede extraerse de la ubre por la acción de la oxitocina. En el manejo del ordeño de búfalas, la máquina de ordeño es un punto crítico, y las características del vacío de ordeño y la tasa de pulsación están estrechamente relacionadas con las observaciones del flujo de leche. En Italia, los niveles de vacío más utilizados son de 44 a 46 kPa (rango de 40 a 53 kPa). Los datos sobre las características de ordeñabilidad de la raza mediterránea italiana permitieron clasificar ocho tipos diferentes de curvas de flujo de leche debido a diferencias anatómicas, fisiológicas y de manejo. La curva de flujo de leche más representada fue la tipo 3 (con un tiempo similar entre PPT y DPT, 27,32%), seguida de la tipo 6 (17,79%), caracterizada por una fase de meseta muy larga. La curva menos representada fue la tipo 1 (4,41%), caracterizada por un largo tiempo de desfase y un bajo caudal máximo. Los búfalos con curvas de tipo 5 (10,62%) y 6 (17,79%) se caracterizaron por la mayor producción de leche en el momento del ordeño, el menor puntaje de células somáticas y el menor tiempo de ordeño. El análisis de los perfiles de emisión de leche mostró una duración excesiva del DPT y del sobreordeño (BT); Estos resultados sugieren el desprendimiento del grupo de ordeño para reducir el BT con las siguientes ventajas: a) reducción del tiempo total de ordeño y consecuentemente del tiempo del trabajador, b) mejora de los ingresos del productor y de la calidad de la leche al disminuir la incidencia de mastitis. En cuanto a la automatización de las tecnologías de ordeño, un estudio reciente analizó las principales características del flujo de leche y la producción de leche registrada en búfalas italianas del Mediterráneo ordeñadas con el Sistema de Ordeño Automático (AMS). Los resultados mostraron una variación considerable en la eyección de leche y, en consecuencia, en las curvas de flujo de leche de las búfalas ordeñadas en AMS con sistema forzado respecto al convencional. Las diferencias fueron las siguientes: mejor preestimulación que permite una liberación endógena positiva de oxitocina, con un tiempo de retardo reducido; eyección de leche independiente para cada pezón, con ordeño óptimo de todos los cuartos, con reducción de BT; mejora de la higiene del ordeño durante el ordeño, limitando la incidencia de mastitis y con un bajo valor de puntuación de células somáticas; efectos adversos por ordeño fallido o incompleto (17% del total de ordeños), limitando la capacidad potencial y eficiencia del AMS; Fugas frecuentes de flujo de aire, que podrían provocar alteraciones en la composición de la leche. En conclusión, el monitoreo continuo de la capacidad lechera ayudará a optimizar las prácticas de ordeño al reducir el tiempo de trabajo y aumentar los ingresos de los ganaderos a través de una mejor calidad de la leche y menos problemas con la ubre. Además, la identificación de búfalas con tipos deseables de curvas de flujo de leche podría ser útil para que las asociaciones de criadores de búfalas aborden la gestión de los ganaderos y también para definir posibles nuevos objetivos de cría.

Palabras clave: búfala lechera, ordeñabilidad, curvas de flujo de leche, tiempo de ordeño.

INTRODUCTION

The buffalo (*Bubalus bubalis*) is a species of worldwide importance, reared for the production of milk, meat, hides, and other by-products and often used as a working animal in marginal rural areas [1, 2]. Although the milk produced by buffaloes is an irreplaceable source of nutrients and energy, especially in some countries or confined environments, its extraction from the mammary gland is not always easy. Milk removal is essential both for production purposes and to ensure the health of the organ; it can be done in two ways: a) natural extraction by sucking the calf, b) artificial extraction by manual or mechanical milking. In dairy species, milking is the foremost essential operation affecting production efficiency. A general definition of “milkability” is ‘the ability of an animal to provide regular, complete and rapid milk secretion from the mammary gland in response to correct milking technique.’ The primary method of assessing “milkability” is based on the analysis of the milk flow curve; in this way, the electronic milk meter (LactoCorder® device) records milk yield in the whole milking, electrical conductivity, and the main parameters of the milk flow curve, including the Total Milking Time (TMT). Since 1999, the Milk Quality laboratory of the “Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri” (Rome, Italy) has been engaged in the study of milk emission kinetics by recording milk flow curves in the main dairy species (buffalo, cow, goat, sheep, and donkey).

CLASSIFICATION OF THE TYPES OF MILK FLOW CURVES

A graphical representation of milk ejection can be displayed through milk flow curves, which differ according to the dairy species [3, 4]. The milk flow curves are subdivided into three main phases and a fourth one. The first phase is the “Lag Time” (LT), represented by the time elapsed between the attachment of the milking clusters and the time until there is a constant milk flow. The second is the “Plateau Phase” (PPT), where the milk flow is constant. The third phase is the “Decreasing Phase” (DPT), which represents the time from the PPT until milk flow drops below 0.20 kg/min. The fourth phase may be the “Blind Phase” (BT). The BT (milk flow below 0.2 kg/min) occurs between the end of the DPT and the detachment of the milking cluster [5, 6]. Usually, the detachment is not performed promptly to collect the small amount of milk, the residual fraction, by stripping (often obtained by manual traction of the milking cluster by a milker) followed by further overmilking before detachment of the milking cluster.

Milk letdown is influenced by several factors: anatomical, physiological, sanitary, and environmental [7, 8, 9]. Many studies performed in different countries have shown that buffalo are challenging to milk because there is a delay in milk ejection. Because the udder anatomy and arrangement of the mammary tissue, a cisternal fraction of milk, and teat canal length are

pretty different in buffaloes compared to dairy cattle [10, 11, 12]. Although each animal has its anatomical and physiological characteristics, the milking machine can affect the milkability and machine times. Buffaloes are characterized by a longer teat with a longer teat canal and stronger muscular resistance of the teat wall [11, 7, 10]. Therefore, a higher vacuum level is needed to open the teat canal and start milk ejection in this species.

In buffalo milking management, milking machine set-up is critical, and the milking vacuum levels and pulsation rate are strictly connected with milk flow observations. For Mediterranean Italian (MI) breeds, a working vacuum level of up to 45 kPa in buffalo is generally ineffective unless alveolar milk ejection has occurred. Generally, different studies conducted in different parts of the world in which the vacuum level varies in the range of 45–68 kPa for buffalo, while in Italy, the most used vacuum levels are 44–46 kPa (range 40–53 kPa) [8, 9, 13].

Data on milkability traits available in the literature can be traced back to the MI breed. In a recent study of 2,419 MI buffaloes reared on 187 farms in central Italy, Boselli et al. [14] classified eight types of milk flow curves based on anatomical, physiological and management differences. The classification of curves was based on visual inspection of curve shape, milk yield, and milk flow parameters. Only the 2,288 milk flow curves have been classified into eight different types. The most represented curve was type 3 (similar time between PPT and DPT, 27.32%), followed by type 6 (17.79%), characterized by a very long plateau phase. The least represented curve was type 1 (4.41%), characterized by a long lag and low peak flow rate.

According to the analysis of variance, the milk yield ranged from 2.21 to 5.22 kg per milking for types 1 and 6, respectively, while the peak flow rate was minimum (0.50 kg/min) and maximum (1.73 kg/min) for types 1 and 4 respectively. Concerning the main milkability parameters, the results show that the TMT averaged 11.29 min; the lag time and the milk emission time averaged 2.19 min and 4.30 min, respectively. The 12.5% of the total curves were classified as bimodal (two different peaks of milk flow are evident; the first peak is due to the removal of the cisternal milk fraction, while the second peak is due to the action of oxytocin, which allows the fraction of alveolar milk), and 60 of these were found to be of type 4. Based on the literature, type 4 curves are representative of very short teat canals and very high milk flow. The average somatic cell score was 3.63 units, with a maximum value found for type 1 and a minimum for type 6. The highest milk yield at milking, the lowest somatic cell score, and the shortest milking time characterized buffaloes showing curves of type 5 and 6.

The results of this study showed that such traits could be used as indicators to improve udder health and milkability in dairy buffaloes. The classification proposed in our field study shows significant differences among the milk flow curves, which could impact milk production and udder health. The results showed a high prevalence of overmilking, which may be re-

sponsible for adopting a higher TMT in buffaloes than in cows. Appropriate pre-milking udder stimulation should be used to reduce LT, increase Average Flow Rate (AFR), and limit TMT. In addition, proper milking practices would result in reduced labor time and improved farmer income due to better milk quality and fewer udder diseases. The results of this study allowed the identification of optimal milk flow curve types for the MI breed in terms of milk production and udder health; these results could be helpful to buffalo breeders' associations in herd management and in defining potential new breeding goals.

FUTURE PERSPECTIVES

Further research is needed to investigate the variability of these phenotypes at the population level and whether they can be used as indicator traits for breeding purposes. Finally, a brief mention is made of the Automatic Milking System (AMS), which has been available since 1992 and used as a method of voluntary milking in cattle and, more recently, in dairy buffaloes. Only a few authors [15, 16, 17, 18] report data on the adaptability of buffaloes to AMS. Boselli et al. [15] measured data on buffalo with a portable milometer LactoCorder installed after the tubes connecting the milking cups. The TMT measured between the beginning of the cleaning of the first teat and the detachment of the last teat ranged from 8.80 min [15] to 8.3 min [16].

Regarding the number of milkings per day, Faugno et al. [17] reported 2.3 milkings per buffalo per day, Sannino et al. [18] and Boselli et al. [15] reported 2.5 milkings per buffalo per day. Concerning the introduction of AMS in dairy buffaloes, the preliminary results of the recent study show considerable differences in milk ejection and milk flow curves for buffaloes milked in AMS with a forced system compared to the conventional one. The differences are as follows: better prestimulation, allowing a positive endogenous release of oxytocin, with a reduced milk letdown phase; independent milk ejection for each teat, with optimal milking of all quarters, with a reduction in overmilking; better milking hygiene during the milking routine, limiting the incidence of mastitis and with a low somatic cell score; adverse effects due to failed or incomplete milkings (17% of the total milkings), which limit the potential performance and efficiency of AMS; frequent air leakage, which could cause alterations in milk composition. AMS is suitable for buffaloes and opens up a new strategy for recording and managing many milk ability traits in dairy buffalo milking.

CONCLUSIONS

In conclusion, it is possible to know the milkability of the herd by monitoring milk flow curves. Continuous milkability monitoring will help optimize milking practices by reducing labor time and increasing farmers' income through better milk quality and fewer udder diseases. In addition, identifying buffaloes with desirable types of milk flow curves could be helpful for

buffalo breeders' associations to address farmer management and define potential new breeding objectives. However, further research is needed to investigate the variability of these phenotypes at the population level and to understand whether they can be used as trait indicators for reproductive purposes.

REFERENCES

- [1] Borghese A. Buffalo livestock and products in Europe. *Buffalo Bulletin*. 2013 Oct 31;32(1):50-74.
- [2] Minervino AH, Zava M, Vecchio D, Borghese A. *Bubalus bubalis*: A short story. *Frontiers in Veterinary Science*. 2020 Dec 1;7:570413..
- [3] Boselli, C., M. Mazzi, C.S. Thomas and A. Borghese. Milkability of Buffalo (milk flow curves). In: "Milking Management of Dairy Buffaloes". *Bulletin of the International Dairy Federation* n. 426/2008. Editors: Rasmussen M.,-Thomas C.S. and Borghese A.: 37-41
- [4] Borghese A, Boselli C, Rosati R. Lactation curve and milk flow. *Buffalo Bulletin*. 2013 Jan 1;32(1):334-50.
- [5] Boselli C., R. Rosati, G. Giangolini, S. Arcuri, A. Fagiolo, S. Ballico and A. Borghese. Milk flow measurements in buffalo herds. *Proc. of the Seventh World Buffalo Congress Manila, Philippines*, 20-23 Oct. 2004, 244-246.
- [6] Boselli C, Mazzi M, Borghese A, Terzano GM, Giangolini G, Filippetti F, Amatiste S, Rosati R. Milk flow curve and teat anatomy in Mediterranean Italian buffalo cows. *Rev. Vet.* 2010 Apr 1;21(1):576-81.
- [7] Boselli C, Campagna MC, Amatiste S, Rosati R, Borghese A. Pre-Stimulation Effects on Teat Anatomy and. *Journal of Animal and Veterinary Advances*. 2014;13(15):912-6.
- [8] Caria M, Boselli C, Murgia L, Rosati R, Pazzona A. Effect of vacuum level on milk flow traits in Mediterranean Italian buffalo cow. *Italian Journal of Animal Science*. 2012 Jan 1;11(1):e25.
- [9] Caria M, Boselli C, Murgia L, Rosati R, Pazzona A. Influence of low vacuum levels on milking characteristics of sheep, goat and buffalo. *Journal of Agricultural Engineering*. 2013 Sep 8;44(s2).
- [10] Thomas, C.S.; Svennersten-Sjaunja, K.; Bhosrekar, M.R.; Bruckmaier, R.M. Mammary cisternal size, cisternal milk and milk ejection in Murrah buffaloes. *Journal of Dairy Research* 2004, 71, 162–168.
- [11] Ambord S, Thomas CS, Borghese A, Mazzi M, Boselli C, Bruckmaier RM. Teat anatomy, vacuum to open the teat canal, and fractionized milk composition in Italian buffaloes. *Milchwissenschaft*. 2009;64(4):351-3.
- [12] Costa A, De Marchi M, Visentin G, Campagna MC, Borghese A, Boselli C. The effect of pre-milking stimulation on teat morphological parameters and milk traits in the Italian Water Buffalo. *Frontiers in Veterinary Science*. 2020 Dec 8;7:572422.
- [13] Thomas CS. Efficient dairy buffalo production. *DeLaval International AB, Tumba, Sweden*. 2008.
- [14] Boselli C, De Marchi M, Costa A, Borghese A. Study of milkability and its relation with milk yield and somatic cell in Mediterranean Italian water buffalo. *Frontiers in Veterinary Science*. 2020 Aug 11;7:432.
- [15] Boselli C., Mazzi M., Borghese A., Terzano G.M., Coletta A., Diruppo M., Giangolini G., Filippetti F., Rosati R., Amatiste S. Automatic milking system in Mediterranean Italian Buffalo cows: a field study on milk flow curve. *Proceedings of VI Buffalo Symposium of Americas, Havana, Cuba*, 21-26 Nov 2011.
- [16] Caria M, Tangorra FM, Leonardi S, Bronzo V, Murgia L, Pazzona A. Evaluation of the performance of the first automatic milking system for buffaloes. *Journal of Dairy Science*. 2014 Mar 1;97(3):1491-8.
- [17] Faugno S, Pindozi S, Okello C, Sannino M. Testing the application of an automatic milking system on buffalo (*Bubalus bubalis*). *Journal of Agricultural Engineering*. 2015 Apr 21;46(1):13-8.
- [18] Sannino M, Faugno S, Crimaldi M, Di Francia A, Ardito L, Serrapica F, Masucci F. Effects of an automatic milking system on milk yield and quality of Mediterranean buffaloes. *Journal of Dairy Science*. 2018 Sep 1;101(9):8308-12.

NUTRITIONAL STRATEGIES FOR MAXIMIZING BUFFALO PRODUCTION EFFICIENCY

Estrategias nutricionales para maximizar la eficiencia de la producción de búfalos

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INTRODUCTION

Since more than 5000 years ago, buffalo have played a significant role in Asian livestock farming, providing milk, meat, skins, and draught power. Buffaloes, described as the “Black Gold”, are the favourite multipurpose animals of farmers and are, in fact, the “bank on hooves” with massive potential for social and economic changes for the agrarian community. Asia is home to more than 90% of the world’s buffalo population, with 77.9% living in South Asian nations. The 20th livestock census between 2012 and 2019 shows that the buffalo population increased by 1.06%. India is the greatest producer and exporter of buffalo meat and the country with the highest population of buffaloes in the world (57% of the total). The leading states in buffalo rearing are Uttar Pradesh, Rajasthan, Gujarat, Madhya Pradesh, Bihar, Andhra Pradesh, Maharashtra, Haryana, Telangana, and Punjab. In these states, raising buffaloes generates between 54-85% of the nation’s milk production, a significant income source for rural households.

According to predictions made by Opio et al. [31], the demand for animal products is likely to rise further by 70% by 2050 due to industrialization and rising population, both of which may put significant strain on the planet’s natural resources. Because there is increased demand for limited resources nowadays, it is necessary to increase the efficiency of dairy animals, making improvements in feeding management a crucial strategy. Generally, it is a well-known statistic that feeding accounts for around 70% of the ongoing costs associated with starting a dairy operation. Currently, the farming community is one of many involved in feeding management. The purpose of feeding management is also evolving as time goes on. Also, with the increasing livestock population and farming sectors, ruminants are blamed for global greenhouse gas production. Animal production makes for around 37% of the world’s CH₄ emissions and 14.5% of the world’s anthropogenic greenhouse gas emissions within the agricultural sector [31].

OPTIMUM FEEDING PRACTISES TO INCREASE PRODUCTION PERFORMANCE

Feeding frequency

It may be possible to better understand the underlying processes of dry matter intake by tracking circadian (virtually 24-h) feed intake trends. More recently, increasing feeding frequency from once to twice or twice to four times daily increased average feeding time per cow in group-fed lactating cows [10]. Over the course of the 24-hour period, the cows fed more often consumed their food more evenly. Additionally, the more frequent feeding resulted in less feed sorting. This might increase fiber digestibility, which the frequent feeding may have decreased.

Improving the availability of feed and fodder and advising on ration balancing

The genetic potential of animals could be harnessed by providing balanced nutrition and keeping them healthy. Animals of high genetic potential are maintained with high-quality feeds, which include fodder crops/silage, grains, and protein concentrates, often in the form of a total mixed ration. Like many affluent nations, livestock nutrition in India often consists of one or two concentrate feed components that are locally accessible, grasses, and forages, which might result in imbalanced feeding owing to either too high or too low amounts of energy and protein. Because the milk production capacity of dairy animals is only partially used, imbalanced feeding harms the health and productivity of animals in numerous ways. It also lowers the net daily revenue to milk producers from dairying. The Ration Balancing Programme was established to inform farmers about the effects of unbalanced feeding and the significance of providing animals with a balanced diet. With benefits for both farmers and the environment, “ration balancing” has been put into place in 18 states under the National Dairy Plan-I, a project supported by the World Bank and the government of India.

The NDDDB created this innovative initiative to balance the ratio of animals being picked up using simple software that can be used by committed and knowledgeable local resource personnel assigned to the area. By visiting milk producers and registering their animals in the NDDDB's animal identification system, local village resource people who have been educated in animal nutrition are employed by this scheme. The local village resource people then create the optimized rations using the ration balancing software based on body weight, milk output, and milk fat yield.

Garg et al. [14] studied the effect of ration balancing on twenty-six lactating buffaloes. They reported that the average increase in milk yield, fat (%), 6% FCM yield (kg/day), and microbial nitrogen supply in lactating buffaloes were higher ($p < 0.01$) than before the implementation of the program. Similarly, methane emission in terms of g/kg DM intake, g/kg OM intake, and g/kg milk yield were also reduced ($p < 0.01$). The gross energy lost as methane was reduced (@ 18.13%; $p < 0.01$) in buffaloes after balancing the ration. The ration balancing program has reduced the cost of feed per liter of milk for cows in Gujarat and Punjab by roughly 18–19% and for buffaloes in Gujarat by about 2.6%, according to field-level statistics by Sirohi et al. [40]. Tomar et al. [41] conducted a field survey to assess the ration balancing program undertaken by ICAR-NDRI on the extent of productivity increase and cost reduction on the adoption of balanced animal ration. Results revealed that the average daily milk yield of Murrah, HF, and Jersey crossbreds was significantly increased by 25.7%, 18.8%, and 37%, respectively. Fat% and SNF% were improved significantly ($p < 0.01$) only in Murrah buffaloes fed with a balanced ration. However, the feed cost per kg of milk of the RBP animals was around 15%, statistically significant ($p < 0.01$) lower than the non-RBP animals.

BENEFITS OF RBP

1. An increase in milk output that contains more fat and fat-free solids
2. Increased fertility efficiency
3. Reduction in the inter-calving period, thereby increasing the productive life of animals
4. A decrease in the emissions of methane.
5. Farmers in various parts of the nation who provide balanced animal rations have seen a rise in their net daily revenue of between Rs.15-25 per animal.

UREA MOLASSES BLOCKS

Although the major base feed for buffaloes is cellulosic material, seasonal shortages sometimes happen during weather extremes, droughts, and flood conditions, negatively impacting the buffalo output. Solid blocks containing a mix of urea, molasses, and minerals have been created and fed to

buffaloes. These blocks are chosen over ammoniating crop leftovers as a source of fermentable carbohydrates and nitrogen because they are simple to export and secure for usage by farmers. Urea molasses block supplementation to buffaloes enhanced growth and milk production when given with straw-based diets [35].

PRECISION NUTRITION

Providing an animal with feed that satisfies its nutritional needs for maximum production efficiency to create better-quality animal products is referred to as precision feeding. It is a tool for effectively utilizing available feed resources to maximize the animal's response to nutrients for economic production. Precision nutrition aims to precisely meet the nutritional needs of livestock, increase production efficiency, reduce environmental pollution, improve nutrient utilization, lower feeding costs, decrease feed refusal by livestock, reduce nutrient excretion in feces and urine, lower labor costs, and increase overall profit.

Tools to achieve precision animal nutrition

Processing methods for food: It consists of methods like grinding and pelleting. The feed should be ground to a homogeneous particle size of 600 μm since this enhances nutrient uptake. Enhancing digestibility, improving feed conversion efficiency, and accelerating calf growth are all made possible by particle size reduction. According to one study, feeding pellets reduced feed waste by up to 5% and decreased DM excretion by 23%, N excretion by 22%, and feed efficiency by 6.6%.

Strategic nutrient supplementation: Some nutrients, particularly mineral combination and vitamin premix, are needed in extremely small doses. However, their inclusion in the diet is necessary for optimal production. Specific nutrient supplementation (e.g., AA, iodine, lipids, vitamins, minerals) has altered important performance variables, including lactation, reproduction, offspring survival, and growth at crucial developmental time windows. However, occasionally, the diet may include enough levels of nutrients, but the animal may have a deficiency condition and be unable to use them effectively. This might be caused by: a) The animal not having the enzyme necessary to break down the meal and use that specific nutrient, such as lignified cellulose or phytic phosphorus, b) Antagonistic interaction of nutrients among themselves, e.g., Copper – Zinc mineral interaction (bioavailability decreases) c) Parasitic infestation also decreases voluntary feed intake, absorption of nutrients, e.g., stomach worms, liver flukes, and intestinal parasites.

Precise ration formulation

Many nutritionists suggested that developing a low-cost, adequate feed is key to the dairy industry's profitability. Concerns about the unjustified use of hormones and antibiotics in food animals to increase output and their harmful effects on consumers owing to their residues have been raised in recent

years. Therefore, a modern animal nutritionist must provide diets devoid of antibiotics, hormones, meat and bone meals, and other unpleasant feed components, which harm both animal and human health and result in significant financial loss. Even though it might be difficult for animal nutritionists to develop feeds that generate animal products that consumers would accept, do not cause environmental pollution, preserve animal health, and stay commercially viable. Ration must be balanced and palatable for the animals.

METABOLIC AND FERMENTATION MODIFIERS

In general, substances given, injected, or implanted into animals are called metabolic modifiers. These compounds enhance nutrient utilization, feed efficiency, growth rate, milk supply, and composition. Products called fermentation modifiers are added to feed to control rumen fermentation and improve feed utilization. Conjugated linoleic acid, chromium, carnitine, magnesium, niacin, manganese, selenium betaine, and vitamins A, D, and E are among the metabolic and fermentation modifiers widely used in dairy and beef processing. Other fermentation modifiers include methane inhibitors, proteolysis and deamination inhibitors, defaunation agents, microbial enzymes, buffer agents, ionophores, yeast cultures, and mold. Animals given bST injections had an average milk output of 25% higher than control animals. No adverse effects of bST injection on the buffaloes' reproductive response were documented by Usmani and Athar [46]. The amount of feed consumed and milk produced by buffaloes getting bST injection was noticeably higher than in controls. Niacin is produced in ruminants by rumen microbes, and its production has been deemed sufficient for ruminant performance. Niacin addition in buffalo diets increased rumen fermentation by lowering ammonia-N concentration and raising protein synthesis [23].

NUTRITIONAL MANAGEMENT OF BUFFALO CALVES

An effective calf-feeding method is essential for having good dairy replacement stock since it influences the enterprise's future profitability and sustainability. The buffalo husbandry and research area that has received the least attention is buffalo calf nutrition and feeding management. According to Wynn et al. [52], inadequate colostrum and calves' improper feeding practices contributed to increased mortality and morbidity losses in buffalo calves. Although calves in South Asia are often taken away from their mothers at birth, they are permitted to drink a small amount of milk for a few weeks directly from the dam's teat at each milking. The calves are then typically milk-weaned between the ages of 4 and 12 weeks. The cost of milk, the dam's behavior when the calf is being weaned off of milk, and the calf's gender are the primary factors in buffalo calf milk weaning. Therefore, it is crucial to create and employ appropriate scientific language based on the physiology, body

mass, and age of buffalo calves in the first place. Then, for calves raised for two different goals, namely milk and meat production, considerable coordinated research efforts are needed to design pre-weaning and post-weaning nutritional regimens and feeding management systems.

PROBIOTICS

The concept of direct-fed microbials is different from the term probiotics. Probiotics are live beneficial bacteria that, when administered in adequate amounts, confer a health benefit on the host [19], often by colonizing the gastrointestinal tract and supporting the native microflora that is already established in the animal's digestive system. The DFM has a narrower definition than probiotics as it is defined as a source of life, naturally occurring microorganisms alive, that improve the digestive function of livestock. The emergence and manifestation of antibiotic resistance in the food chain have prompted a search for alternatives to antibiotics that have growth-promoting effects on livestock. Since the diversity of the rumen microbiome is closely related to the animal's ability to acquire and assimilate nutrients, ideal growth promoters would have only a negligible impact on the animal's natural microbiome while enhancing the animal's growth, well-being and reproduction [8, 29].

Numerous studies on animals have demonstrated the benefits of probiotics in terms of feed efficiency [28], growth performance [42], nitrogen retention [36], and decreased risk of intestinal infections [38]. When symbiotic formulation was given as a supplement to Murrah buffalo calves, improved digestibility, average daily gain, and lower incidence of diarrhea and fecal scores were seen. Similar outcomes were noted when *Limosilactobacillus reuteri* BF-E7 and *Ligilactobacillus salivarius* BF-17 were given to Murrah buffalo calves [47, 48]. Previous research [1, 5, 25] has found similar increases in growth performance, demonstrating the benefits of probiotics, notably LAB, for preruminant calves. Other studies found contradictory results, such as no appreciable differences in DMI, ADG, or nutritional digestibility when feeding LAB to calves [53, 22]. The variation in results might be related to the host's age, health, management, nutritional state, type, or changed probiotic effect with different doses, feeding schedules, bacterial strains, or other factors [51]. Feeding probiotics, prebiotics, and synbiotics to Murrah buffalo calves significantly enhanced structural development and performance, according to recent experiments conducted in our lab [37, 39].

NUTRITIONAL STRATEGIES IN REDUCING ENTERIC METHANE PRODUCTION

The ruminal microbiome is extraordinarily diverse, with bacteria (10^{10} - 10^{11} cfu/mL, >200 species), protozoans (10^4 - 10^6 cfu/mL, 25 genera), fungi (10^3 - 10^5 cfu/mL, 6 genera), and methanogens (10^6 cfu/mL) making up the majority of its constituents.

A group of microorganisms known as methanogens are found in the Archaea domain, with certain cyanobacteria and marine microbes also contributing in trace amounts [26]. In 2020, cattle and dairy cows were the main sources of CH₄ emissions, making for 72% of all sector emissions [12]. According to Opio et al. (2013), 8.7% of CH₄ emissions are caused by buffalos. Methane has a lifespan of 12.4 years in the atmosphere and is 84 times more powerful on a 20-year timeline than carbon dioxide (CO₂) in terms of potential for global warming [32]. Additionally, ruminants lose up to 12% of their total energy intake due to methane generation [43]. In order to reduce CH₄ emissions from animals, especially ruminants, breeders and researchers from all over the world have been concentrating on both raising animal output and doing so. Culling nonproductive and low-producing animals in wealthy nations is advised to lower CH₄ emissions. Due to economic, cultural, and religious differences in emerging nations, this is both impracticable and challenging. Various farming techniques, dietary changes, feed additives, chemical methanogenesis modulators, probiotics, vaccination against the rumen microbiota, selective breeding, and genetic methods are now employed to minimize CH₄ emissions in ruminants. These tactics heavily rely on the direct and indirect suppression of methanogens by feed additives [9, 17]. Between 1960 and 2018, the literature contained about 9000 studies on rumen methanogenesis [7].

Leitanthem et al. [24] observed a reduction in CH₄ (%) and CH₄ (mL/100 mg dDM) with increased supplementation of *Moringa olifera* leaves (0%, 5%, 10%, 15%, 20%, 30%, and 40%) in *in vitro*. Gupta et al. [18] reported that daily enteric methane production was reduced by 12.61% numerically when sodium monensin (0.6 mg/Kg body wt) was fed to Murrah buffalo heifers. Dixit et al. [11] found that CH₄ emissions decreased from 34.48 to 12.73 g/Kg DMI when murrah buffalo calves were fed graded levels of dietary crude protein (5, 7.5, 10, 12.5 and 15%). Feeding practices like precision feeding and changing grazing management practices can decrease CH₄ emissions [6, 49]. Medium-chain fatty acids found in coconut oil and palm kernel oil have been shown to suppress methanogenesis by poisoning protozoa. According to Zhang et al. [54], methanogens may reduce the amount of dissolved hydrogen in the rumen by hydrogenating unsaturated fatty acids in corn oil. Because medium-chain fatty acids are poisonous to methanogens and protozoa, they can stop the processes that produce CH₄. A moderate dietary lipid percentage of less than 6% can reduce 24-hour CH₄ emissions by up to 20% with enhanced feed efficiency, according to Beauchemin et al. [7]. Similar to this, ionophores like monensin reduce CH₄ emissions by inhibiting the action of bacteria that break down cellulose. Ionophores have the ability to reduce the population of gram-positive bacteria such as cocci bacteria, which effectively inhibits the formation of acetate and CH₄ [3]. The impact of probiotics on CH₄ mitigation has also been gradually reported by several researchers [33, 50] by adding several probiotics like *Ruminococcus flavefaciens*, *Propionibacterium* and *Lactobacillus rhamnosus* and *Enterococcus* into diets of sheep. A final dose of 6*10¹⁰ cfu/animal/day of *Lactoba-*

cillus pentosus D31 results in a decrease of methane of around 13% [20]. Similar to this, methanogen cell lysis had been accomplished by carbon nanoparticle application [13].

DEVELOPMENT OF RUMINANT DERIVED FUNCTIONAL FOODS

As animal-derived foods are the major contributors of saturated fatty acids (SFA) and trans fatty acids due to biohydrogenation, people are increasingly skeptical about the adverse health effects of consuming dairy-origin foods. In the long run, a high intake of SFA has been associated with increased blood-low-density lipoprotein cholesterol, leading to atherosclerosis and other cardiovascular-related diseases [16]. Most food regulatory bodies recommend that total SFA consumption should not exceed 10-11% of total daily energy intake [45]. Nutritionists are relentlessly working on dietary strategies to reduce SFA levels in animal-derived food through rumen manipulation techniques. Such initiatives have increased conjugated linoleic acid (CLA) and other polyunsaturated fatty acids (PUFAs) in milk and its byproducts.

The fatty acid metabolism can be modulated either by increasing the fatty acids source/ content in the feed or by restricting the biohydrogenation process in the rumen. Research on the modulation of milk FA composition has developed specific nutritional strategies based on these two points. These strategies rely, in particular, on feeding pasture [44], supplementing vegetable oils [21], oilseeds, fish oils [30], and addition of phytoadditive or plant secondary metabolites in the ruminant diet. Banakar et al. [4] reported increased concentrations of PUFA such as vaccenic acid, linoleic acid and decreased concentration of saturated fatty acids when *Aloe vera* extract (2 and 4% of DMI) was fed to cross-breed (Alpine × Beetal) lactating goats. Alipanahi et al. [2] found higher milk vaccenic acid (trans-11 C18:1) and CLA (cis-9, trans-11 C18:2) concentrations in goats fed the oak acorn (9.1 g/Kg DM tannins) with or without polyethylene glycol supplementation compared to control group. Miri et al. [27] reported that dietary supplementation of (*Cuminum cyminum*) seed extract in goats increased the ruminal concentration of CLA by 34.8% and VA by 11.4% in the lower supplemented group as compared to CSE free diet. Rana et al. [34] reported that the concentration of stearic acid was decreased whereas rumenic acid and CLA were increased in the *Longissimus dorsi* muscle of young goats treated with *Terminalia chebula* compared to the control. Gesteira et al. [15] noticed a drop in the oleic acid and MUFA levels in the salted and sun-dried meat from young Nellore bulls treated with condensed tannin extract (0, 10, 30, or 50 g/kg of dry matter) from *Acacia mearnsii*.

CONCLUSION

Ruminant cattle do not directly compete with the human population because they can create high-quality human food,

especially high-quality protein, from feedstuffs that have little or no value for human food. By offsetting maintenance and other nutritional expenses, strategies that boost output per animal are an excellent way to improve the sustainability of ruminant production. Dairy output is greatly enhanced by feeding practices such as ration balancing, precision nutrition, and methane mitigation techniques. Probiotics are fed to dairy calves at a very young age to aid in better colonization, increased performance, decreased mortality, and calf scours. The challenge for scientists in many developing countries is how best to combine in a diet for dairy animals the available green forage, crop residues, and agro-industrial by-products with the available protein resources and molasses/urea block to increase the performance of buffaloes. Think of your buffalo as a family for maximum productivity and success. You will be rewarded with lots of nourishing milk if you take the time to attend to all of their needs.

REFERENCES

- [1] Agazzi A, Tirloni E, Stella S, Marocco S, Ripamonti B, Bersani C, Caputo JM, Dell'Orto V, Rota N, Savoini G. (2014). Effects of species-specific probiotic addition to milk replacer on calf health and performance during the first month of life. *Annals of Animal Science*. 14:101–115.
- [2] Alipanahi Z, Fatahnia F, Jafari H, Taasoli G, Mirzaei-Alamouti H, Barrett D, Pormalekshahi, AJLS. (2019). Effect of oak acorn with or without polyethylene glycol in diets containing extruded soybean on milk fatty acid profile, ruminal fermentation and plasma metabolites of lactating goats. *Livestock Science*, 221, 57–62.
- [3] Appuhamy JADRN, Strathe AB, Jayasundara S, Wagner-Riddle C, Dijkstra J, France J, Kebreab E. (2013). Anti-methanogenic effects of monensin in dairy and beef cattle: a meta-analysis. *Journal of Dairy Science*, 96(8): 5161–5173.
- [4] Banakar PS, Kumar S, Varada VV, Dixit S, Tyagi N, Tyagi AK. (2022). Dietary supplementation of Aloe vera extract modulates rumen microbes and improves the functional food value of milk by altering phenolic content, antioxidant capacity, and fatty acid profile in lactating goats. *Animal Biotechnology*, 1–12.
- [5] Bayatkouhsar J, Tahmasebi AM, Naserian AA, Mokarram RR, Valizadeh R. (2013). Effects of supplementation of lactic acid bacteria on growth performance, blood metabolites and fecal coliform and lactobacilli of young dairy calves. *Anim Feed Sci Technol* 186:1–11.
- [6] Beauchemin KA, Ungerfeld EM, Abdalla AL, Alvarez C, Arndt C, Becquet P, Benchaar C, Berndt A, Mauricio RM, McAllister TA, Oyhançabal W, Salami SA, Shalloo L, Sun Y, Tricarico J, Uwizye A, De Camillis C, Bernoux M, Robinson T, Kebreab E. (2022). Invited review: Current enteric methane mitigation options. *Journal of Dairy Science*, 105(12): 9297–9326.
- [7] Beauchemin KA, Ungerfeld EM, Eckard RJ, Wang M. (2020). Review: Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. *Animal*, 14, s2–s16.
- [8] Breves G, Walter C, Burmester M, Schröder B. (2000). In vitro studies on the effects of *Saccharomyces boulardii* and *Bacillus cereus* var. *toyoi* on nutrient transport in pig jejunum. *Journal of Animal Physiology and Animal Nutrition*. 84, 9–20.
- [9] Buddle BM, Denis M, Attwood GT, Altermann E, Janssen PH, Ronimus RS, Pinares-Patiño CS, Muetzel S, Neil Wedlock D. (2011). Strategies to reduce methane emissions from farmed ruminants grazing on pasture. *The Veterinary Journal*. 188, 11–17.
- [10] DeVries TJ, Von Keyserlingk MAG, Beauchemin KA. (2005). Frequency of feed delivery affects the behavior of lactating dairy cows. *Journal of dairy science*, 88(10), 3553–3562.
- [11] Dixit S, Keshri A, Vinay VV, Kundu SS. (2021). Effect of graded levels of dietary crude protein on nutrient utilization and enteric methane emissions in growing Murrah buffalo calves. *Indian Journal of Dairy Science*. 74(6), 550–553.
- [12] FAO. FAOSTAT Database Collections. Available online: <http://faostat.fao.org/> (accessed on 10 December 2022).
- [13] Fujinawa K, Nagoya M, Kouzuma A, Watanabe K. (2019). Conductive carbon nanoparticles inhibit methanogens and stabilize hydrogen production in microbial electrolysis cells. *Applied Microbiology and Biotechnology*.
- [14] Garg MR, Kannan A, Phondba BT, Shelke SK, Sherasia PL. (2012). A study on the effect of ration balancing for improving milk production and reducing methane emission in lactating buffaloes under field conditions. *Indian Journal of Dairy Science*, 65(3), 250–255.
- [15] Gesteira SM, Oliveira RL, Trajano JD, Ribeiro CVDM, Costa, EIDS, Ribeiro, RDX, Bezerra LR. (2019). Fatty acid profile, physicochemical composition and sensorial attributes of salted and sun-dried meat from young Nelore bulls supplemented with condensed tannins. *Plos one*, 14(4), e0216047.
- [16] Givens DI. (2022). Saturated fats, dairy foods and cardiovascular health: No longer a curious paradox?. *Nutrition Bulletin*, 47(4), 407–422.
- [17] Greening C, Geier R, Wang C, Woods LC, Morales SE, McDonald MJ, Rushton-Green R, Morgan, XC, Koike, S, Leahy, SC. (2019). Diverse hydrogen production and consumption pathways influence methane production in ruminants. *ISME J*. 13, 2617–2632.

- [18] Gupta S, Mohini M, Malla BA, Mondal G, Pandita S. (2019). Effects of monensin feeding on performance, nutrient utilisation and enteric methane production in growing buffalo heifers. *Tropical animal health and production*, 51(4), 859–866.
- [19] Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot, B. (2014). Expert consensus document. The international scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology*. 11, 506–514.
- [20] Jeyanathan J, Martin C, Morgavi DP. (2016). Screening of bacterial direct-fed microbials for their antimethanogenic potential in vitro and assessment of their effect on ruminal fermentation and microbial profiles in sheep. *Journal Animal Science*. 94: 739–50.
- [21] Katirvelen C, Tyagi AK. (2009). Conjugated linoleic acid content of milk from buffaloes fed a mustard oil-based diet. *International Journal of Dairy Technology*. 62, 141–146.
- [22] Kumar M, Kala A, Chaudhary LC, Agarwal N, Kochewad SA. (2021a). Microencapsulated and lyophilized *Lactobacillus acidophilus* improved gut health and immune status of preruminant calves. *Probiotics and Antimicrobial Proteins*. 1–12.
- [23] Kumar R, Dass RS. (2006). Effect of niacin supplementation on rumen metabolites in Murrah buffaloes (*Bubalus bubalis*). *Asian-Australasian Journal of Animal Sciences*, 18(1), 38–41.
- [24] Leitanthem VK, Chaudhary P, Bhakat M, Mohini M, Mondal G. (2022). Impact of *Moringa oleifera* on rumen fermentation and methane emission under in vitro condition. *AMB Express*, 12(1), 1-10.
- [25] Maldonado NC, Nader MEF. (2016). Production of fermented milk with autochthonous lactobacilli for newborn calves and resistance to the dairy farm conditions. *Journal of Bioprocessing and Biotechnology*. 6:278.
- [26] Mauerhofer LM, Zwirtmayr S, Pappenreiter P, Bernacchi, S, Seifert, AH, Reischl, B, Schmider, T, Taubner, RS, Paulik, C, Rittmann, SKMR. (2021). Hyperthermophilic methanogenic archaea act as high-pressure CH₄ cell factories. *Communications Biology*. 4, 289.
- [27] Miri VH, Ebrahimi SH, Tyagi AK. (2015). The effect of cumin (*Cuminum cyminum*) seed extract on the inhibition of PUFA biohydrogenation in the rumen of lactating goats via changes in the activity of rumen bacteria and linoleate isomerase enzyme. *Small ruminant research*, 125, 56–63.
- [28] Moreira GM, Meneses JAM, Ribeiro CV, de Melo Faria A, Arantes HG, da Luz MH. (2019). Performance and feed efficiency of beef cattle fed high energy diet with probiotic consortium technology. *Revista Brasileira de Saude e Producao Animal*. 20, 1–13.
- [29] Mostafa TH, Elsayed FA, Ahmed, MA, Elkholy MA. (2014). Effect of using some feed additives (tw-probiotics) in dairy cow rations on production and reproductive performance. *Egypt. Journal of Animal Production*. 51, 1–11.
- [30] Nguyen QV, Malau-Aduli BS, John C, Nichols PD, Malau-Aduli EO. (2019). Enhancing Omega-3 Long-Chain Polyunsaturated Human Consumption. *Nutrients* 743, 1–23.
- [31] Opio C, Gerber P, Mottet A, Faluccci A, Tempio G, MacLeod M, Vellinga, T, Henderson, B, Steinfeld, H. *Greenhouse Gas Emissions from Ruminant Supply Chains—A Global Life Cycle Assessment*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2013.
- [32] Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; IPCC: Geneva, Switzerland, 2014.
- [33] Philippeau C, Lettat A, Martin C, Silberberg M, Morgavi D P, Ferlay A, Berger C, Nozière P. (2017). Effects of bacterial direct-fed microbials on ruminal characteristics, methane emission, and milk fatty acid composition in cows fed high- or low-starch diets. *Journal of Dairy Science*, 100(4): 2637–2650.
- [34] Rana MS, Tyagi A, Hossain SA, Tyagi AK. (2012). Effect of tanniferous *Terminalia chebula* extract on rumen biohydrogenation, Δ 9-desaturase activity, CLA content and fatty acid composition in longissimus dorsi muscle of kids. *Meat Science*, 90(3), 558–563.
- [35] Sahoo B, Vishwanath CB, Kwatra J, Agarwal A. (2009). Effect of Urea Molasses Mineral Block Supplementation on milk production of cows (*Bos indicus*) in mid Hills of Uttarakhand. *Animal Nutrition and Feed Technology*, 9(2), 171–178.
- [36] Schofield BJ, Lachner N, Le OT, McNeill DM, Dart P, Ouwerkerk D. (2018). Beneficial changes in rumen bacterial community profile in sheep and dairy calves as a result of feeding the probiotic *Bacillus amyloliquefaciens* H57. *Journal of Applied Microbiology*. 124, 855–866.
- [37] Sharma AN, Kumar S, Tyagi AK. (2018). Effects of mannan-oligosaccharides and *Lactobacillus acidophilus* supplementation on growth performance, nutrient utilization and faecal characteristics in Murrah buffalo calves. *Journal of Animal Physiology and Animal Nutrition*. 102:679–689.
- [38] Signorini ML, Soto LP, Zbrun MV, Sequeira GJ, Rosmini MR, and Frizzo LS. (2012). Impact of probiotic adminis-

- tration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. *Research in Veterinary Science*. 93, 250–258.
- [39] Singh A, Kumar S, Vinay VV, Tyagi B, Choudhury PK, Rashmi HM, Banakar PS, Tyagi N, Tyagi AK. (2021b). Autochthonous *Lactobacillus* spp. isolated from Murrah buffalo calves show potential application as probiotic. *Current Research in Biotechnology*. 3:109–119.
- [40] Sirohi S, Sridhar V, Srivastava AK, Kalamkar SS, Sharma D, Boyal V. (2017). Ration balancing: promising option for doubling income from dairying. *Agricultural Economics Research Review*, 30.
- [41] Tomar DS, Lathwal SS, Singh P. Devi I. (2023). Impact of Ration Balancing on Productive Performance and Economics of Milk Production in Field Conditions. *Indian Journal of Veterinary Sciences & Biotechnology*, 19(3).
- [42] Tripathi MK, Karim SA. (2010). Effect of individual and mixed live yeast culture feeding on growth performance, nutrient utilization and microbial crude protein synthesis in lambs. *Animal Feed Science and Technology*. 155, 163–171.
- [43] Tseten T, Sanjorjo RA, Kwon M, Kim S. (2022). Strategies to Mitigate Enteric Methane Emissions from Ruminant Animals. *Journal of Microbiology and Biotechnology*. 32, 269–277.
- [44] Tyagi A, Kewalramani N, Kaur H, Singhal KK. (2008). Effect of Green Fodder Feeding on Conjugated Linoleic Acid in Milk and Ghee (Clarified Butter Oil) of Cows and Buffaloes. 45, 342–352.
- [45] U. S. Department of Agriculture and U. S. Department of Health and Human Services. *Dietary Guidelines for Americans 2010*. Washington D. C. 2010 December 2010.
- [46] Usmani RH, Athar IH. (1997). Effect of short term treatment with BST on ovarian activity and fertility of dairy buffaloes. In *Proceedings 5th World Buffalo Congress, Royal Palace, Caserta, Italy*, 13-16 October, 1997 (pp. 728–731).
- [47] Varada VV, Kumar S, Chhotaray S, Tyagi AK. (2022). Host-specific probiotics feeding influence growth, gut microbiota, and fecal biomarkers in buffalo calves. *AMB Express*, 12(1), 118.
- [48] Varada VV, Tyagi AK, Banakar PS, Das A, Tyagi N, Mallapa RH, Kumar S. (2022). Autochthonous *Limosilactobacillus reuteri* BFE7 and *Ligilactobacillus salivarius* BF17 probiotics consortium supplementation improves performance, immunity, and selected gut health indices in Murrah buffalo calves. *Veterinary Research Communications*, 46(3), 757–767.
- [49] Vargas J, Ungerfeld E, Muñoz C, DiLorenzo N. (2022). Feeding strategies to mitigate enteric methane emission from ruminants in grassland systems. *Animals*. 12(9): 1132.
- [50] Villar ML, Hegarty RS, Nolan JV, Godwin IR, McPhee M. (2020). The effect of dietary nitrate and canola oil alone or in combination on fermentation, digesta kinetics and methane emissions from cattle. *Animal Feed Science and Technology*. 259: 114294.
- [51] Vlasova AN, Kandasamy S, Chattha KS, Rajashekara G, Saif LJ. (2016). Comparison of probiotic lactobacilli and bifidobacteria effects, immune responses and rotavirus vaccines and infection in different host species. *Veterinary Immunology and Immunopathology*. 172:72.
- [52] Wynn PC, Warriach HM, Morgan A, McGill DM, Hanif S, Sarwar M, Bush RD. (2009). Perinatal nutrition of the calf and its consequences for lifelong productivity. *Asian-Australasian Journal of Animal Sciences*. 22(5), 756–764.
- [53] Zhang R, Zhou M, Tu Y, Zhang NF, Deng KD, Ma T, Diao QY. (2016). Effect of oral administration of probiotics on growth performance, apparent nutrient digestibility and stress-related indicators in Holstein calves. *Journal of Animal Physiology and Animal Nutrition*. 100:33–38.
- [54] Zhang, Q., Wang, M., Ma, X., Gao, Q., Wang, T, Shi, X., Zhou, J., Zuo, J., Yang, Y. 2019. High variations of methanogenic microorganisms drive full-scale anaerobic digestion process. *Environment International*: 126:543–551.

BUFFALO FEED EFFICIENCY: OPTIMIZING THE USE OF FIBROUS BIOMASS CARBON TO ENHANCE PRODUCTION WHILE MITIGATING RUMEN METHANE EMISSION

Eficiencia alimenticia para búfalos: optimización del uso de carbono de biomasa fibrosa para mejorar la producción y al mismo tiempo mitigar la emisión de metano en el rumen

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ABSTRACT

Buffaloes are one of the important ruminants contributing to food security in the form of meat and milk, as well as their by-products such as skin, horns, and manure. The buffalo's meat, milk, and their products have been revealed to have high nutritious values, especially the protein and amino acids profiles and some essential bioactive compounds for health and well-being. Above all, the buffaloes are the smart ruminants in converting the high fibrous feeds, in particular, the agricultural biomass and industrial by-products, by enhancing the rumen fermentation end-products via the anaerobic Embden-Meyerhof-Parnas pathway while mitigating rumen methane emission. Concerning the rumen microbiomes of buffaloes, it has been reported that they were highly diversified and relatively higher-abundant compared to cattle, leading to enhanced total volatile fatty acids (VFAs) and lower methane production, the biogenic methane. The current advanced analysis using Next-Generation Sequencing (NGS) of rumen microbiomes offered a more insightful understanding of their ability to degrade more fibrous feeds, especially agricultural crop residues, low-quality roughages, and some plastic materials. Relative abundances of the predominant bacterial population have been found under the phyla; Firmicutes and Bacteroidetes such as *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* and *Prevotella ruminicola*. Enormous agricultural biomass has been commonly available globally as a result of crop cultivation, especially those of straws, stovers, vines, and tops, as well as industrial by-products such as pineapple peels, cassava pulp, sugarcane bagasse, sorghum residues. Despite their abundance, their nutritive values contain a low crude protein level, and high lignocellulosic is additionally encountered. Hence, simple and practical pre-treatment

such as the urea-lime (2+2% U-lime) has been successfully exploited to enhance the crude protein content and digestibility of nutrients while mitigating the rumen methane production. The U-lime treated rice straw and supplementation of phytonutrient fruit-peel pellets (MARABAC) have significantly improved rumen fermentation in the ruminants. Therefore, dietary manipulation based on the pre-treatment of the fibrous feeds and supplementation of phytonutrient pellets should be more intervened. Furthermore, the feeding interventions based on the use of locally available resources should be extensively exploited, focusing on the hands-on establishment (E), development (D), utilization (U), and sustainability (S) (EDUS) for all engaged stakeholders of farm level up to the industrial platform, ultimately, the buffalo production scenario would be economically viable and environmentally friendly system.

Keywords: Ruminant, smart converter, net-zero, biomass, animal protein-based food, biogenic methane.

RESUMEN

Los búfalos son uno de los rumiantes importantes que contribuyen a la seguridad alimentaria en forma de carne y leche, así como sus subproductos como piel, cuernos y estiércol. Se ha revelado que la carne, la leche y sus productos de búfala tienen altos valores nutritivos, especialmente los perfiles de proteínas y aminoácidos y algunos compuestos bioactivos esenciales para la salud y el bienestar. Por encima de todo, los búfalos son rumiantes eficientes a la hora de convertir los alimentos ricos en fibra, en particular la biomasa agrícola y los subproductos industriales, al mejorar los productos finales de la fermentación ruminal a través de la vía anaeróbica Embden-Meyerhof-Parnas y al mismo tiempo mitigar la emisión de

metano ruminal. Con respecto a los microbiomas del rumen de los búfalos, se ha reportado que están altamente diversificados y relativamente más abundantes en comparación con el ganado vacuno, lo que lleva a un aumento de los ácidos grasos volátiles totales (AGV) y una menor producción de metano, el metano biogénico. El análisis avanzado actual que utiliza la secuenciación de próxima generación (NGS) de los microbiomas del rumen ofreció una comprensión más profunda de su capacidad para degradar alimentos más fibrosos, especialmente residuos de cultivos agrícolas, forrajes de baja calidad y algunos materiales plásticos. Se han encontrado abundancias relativas de la población bacteriana predominante bajo los filos; Firmicutes y Bacteroidetes como *Ruminococcus albus*, *Ruminococcus flavefaciens* y *Fibrobacter succinogenes* y *Prevotella rumicola*. Una enorme biomasa agrícola ha estado comúnmente disponible en todo el mundo como resultado de cosecha de cultivos, especialmente de paja, rastrojos, enredaderas y copas, así como de subproductos industriales como cáscaras de piña, pulpa de yuca, bagazo de caña de azúcar y residuos de sorgo. A pesar de su abundancia, sus valores nutritivos contienen un bajo nivel de proteína cruda y, además, se encuentra un alto nivel de lignocelulósico. Por lo tanto, un pretratamiento

simple y práctico como el de urea-cal (2+2% U-cal) se ha aprovechado con éxito para mejorar el contenido de proteína cruda y la digestibilidad de los nutrientes al tiempo que mitiga la producción de metano en el rumen. La paja de arroz tratada con cal U y la suplementación con pellets de cáscara de fruta con fitonutrientes (MARABAC) han mejorado significativamente la fermentación ruminal en los rumiantes. Por lo tanto, debería intervenir más la manipulación dietética basada en el pretratamiento de los piensos fibrosos y la suplementación con pellets de fitonutrientes. Además, las intervenciones de alimentación basadas en el uso de recursos disponibles localmente deben explotarse ampliamente, centrándose en el establecimiento práctico (E), el desarrollo (D), la utilización (U) y la sostenibilidad (S) (EDUS) para todas las partes involucradas e interesadas desde el nivel de granja hasta la plataforma industrial, en última instancia, el escenario de producción de búfalos sería un sistema económicamente viable y amigable con el medio ambiente.

Palabras clave: rumiantes, convertidor inteligente, cero emisiones netas, biomasa, alimentos a base de proteínas animales, metano biogénico.

OUR EXPERIENCE OF BUFFALO IMPROVEMENT IN INDIA: LAST 50 YEARS JOURNEY

Nuestra experiencia de mejora del búfalo en India: viaje de los últimos 50 años

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ABSTRACT

Man has always been searching for an Elixir to free him from all suffering. Though unsure of the existence of such an elixir, India's ancient scriptures have identified it as 'Milk'. India's story as the largest producer of milk is eventful. There was a phase when the country's milk production hit an all-time low, a mere 17 million Tonnes (mT) in 1950. In 1970, with Operation Flood under the leadership of Verghese Kurien, India embarked on a journey to create history by replicating the dairy cooperatives, ushering in a transformation in the sector popularly known as the 'white revolution.' In 2022, India produced a record of 221 mT of milk. In India, 31.58% of the total milk production is contributed by Indigenous Buffaloes, and non-descript buffaloes contribute to 13.49% of the total milk production in the country, making it a total of 45.07% by buffaloes alone. It is well documented that buffaloes are efficient milk producers and the most preferred animal over cattle in many parts of the country owing to their superior quality of milk, disease resistance, longer productive life, and higher milk productivity. India holds the world's largest buffalo population, with 109.85 million headcounts, constituting 20.45% of the livestock population. India has a rich genetic diversity of buffaloes with 20 registered breeds, with Murrah as the most prominent breed (42% of the total buffalo population). However, it faces challenges concerning low artificial insemination (AI) coverage (~40% of breedable females), demand gap of frozen semen doses, realized genetic gain, scarce fodder availability, and market linkages. With the small-holder farming system (≤ 10 cows & buffaloes/unit) being the most popular in India, the joint efforts by the Government of India, ICAR institutes such as CIRB, National Dairy institutes, and various research organizations have led to the science-driven transformation of the dairy sector. Research at ICAR-CIRB continuously emphasizes essential dimensions like genetic improvement of prominent breeds of buffalo, dissemination of high genetic merit germplasm to farmers, use of advanced reproduction biotechnologies for faster multiplication

of superior germplasm, developing improved feeding modules for efficient production while reducing the methane emission and capacity enhancement of buffalo farmers, young entrepreneurs, and researchers. Alongside the dairy sector, buffaloes have a significant role in India's meat production and export system. Total Buffalo meat production in the country is 1584.65 thousand Tonnes, which is being exported to more than 70 countries in the world. Though there are particular challenges for the overall development of buffalo production in India, the present-day progress and the future opportunities for scientific interventions through collaborative efforts have the potential to open new avenues for buffalo development and make this species the most economical food animal.

Keywords: Murrah, genetic improvement, buffaloes, milk, India.

RESUMEN

El hombre siempre ha estado buscando un Elixir que lo libere de todo sufrimiento. Aunque no están seguros de la existencia de tal elixir, las antiguas escrituras de la India lo han identificado como "Leche". La historia de la India como mayor productor de leche está llena de acontecimientos. Hubo una fase en la que la producción de leche del país alcanzó un mínimo histórico, apenas 17 millones de toneladas en 1950. En 1970, con la Operación Flood bajo el liderazgo de Verghese Kurien, la India se embarcó en un viaje para crear historia replicando las cooperativas lácteas, marcando el comienzo de una transformación en el sector conocida popularmente como la 'revolución blanca'. En 2022, India produjo un récord de 221 toneladas de leche. En la India, el 31,58% de la producción total de leche la aportan los búfalos nativos (caracterizados), y los búfalos sin caracterizar contribuyen al 13,49% de la producción total de leche del país, lo que hace un total del 45,07% solo de búfalos. Está bien documentado que los búfalos son productores eficientes de leche y el animal preferido sobre el ganado en

muchas partes del país debido a su calidad superior de leche, resistencia a enfermedades, vida productiva más larga y mayor productividad de la leche. India tiene la población de búfalos más grande del mundo, con 109,85 millones de cabezas, lo que constituye el 20,45% de la población ganadera. India tiene una rica diversidad genética de búfalos con 20 razas registradas, siendo Murrah la raza más destacada (42% de la población total de búfalos). Sin embargo, enfrenta desafíos relacionados con la baja cobertura de inseminación artificial (IA) (~40% de las hembras reproducibles), la brecha en la demanda de dosis de semen congelado, la ganancia genética obtenida, la escasa disponibilidad de forraje y los vínculos con el mercado. Dado que el sistema agrícola de pequeños propietarios (≤ 10 vacas y búfalos/unidad) es el más popular en la India, los esfuerzos conjuntos del Gobierno de la India, los institutos ICAR como el CIRB, los Institutos Nacionales de Lechería y varias organizaciones de investigación han llevado a la transformación del sector lácteo impulsada por la ciencia. La investigación en ICAR-CIRB enfatiza continuamente dimensiones esenciales

como la mejora genética de razas prominentes de búfalos, la diseminación de germoplasma de alto valor genético entre los ganaderos, el uso de biotecnologías de reproducción avanzadas para una multiplicación más rápida de germoplasma superior, el desarrollo de módulos de alimentación mejorados para una producción eficiente y al mismo tiempo reducir las emisiones de metano y mejorar la capacidad de gestión los criadores de búfalos, jóvenes empresarios e investigadores. Además del sector lácteo, los búfalos desempeñan un papel importante en el sistema de producción y exportación de carne de la India. La producción total de carne de búfalo en el país es de 1.584,65 mil toneladas, que se exporta a más de 70 países del mundo. Aunque existen desafíos particulares para el desarrollo general de la producción de búfalos en la India, el progreso actual y las oportunidades futuras para intervenciones científicas a través de esfuerzos de colaboración tienen el potencial de abrir nuevas vías para el desarrollo del búfalo y hacer de esta especie el animal de consumo más económico.

Palabras clave: Murrah, mejoramiento genético, búfalos, leche, India.

GENETIC, HEREDITARY AND CONGENITAL DISEASES IN BUFFALOES: ARE THEY A LIMITING FACTOR FOR THE BUFFALO DEVELOPMENT IN LATIN AMERICA?

Enfermedades genéticas, hereditarias y congénitas en búfalos: ¿son un factor limitante para el desarrollo del búfalo en América Latina?

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ABSTRACT

Along with the exponential growth of the buffalo population in Latin America (12 percent annually), for a population of 6 million head, the problems derived from inbreeding have increased as the national herd, estimated at 3.0 million heads by the Brazilian Association of Buffalo Breeders, come from just over 200 imported animals. Importing animals from the Asian continent was suspended in 1956 for sanitary reasons (rinderpest). 1962, this prohibition was broken, and some buffaloes were brought from India. After that date, there was no official importation of genetic material from Asia; it was only in 1989 that some animals of the Mediterranean, Murrah, and Jafarabadi breeds were imported from Italy and Bulgaria, and currently, semen of Murrah and Jafarabadi breeds have not been imported from any country. Genetic, hereditary, and congenital disabilities, complete or partial, are characterized by abnormalities in the structure and/or function of tracts or organs, complete system or parts of them. These problems have been identified in Brazil and other countries of Latin America (Vale, 1998; Damé, 2013), where more than 40 cases of different semi-lethal and lethal cases have been diagnosed, which were for the first time scientifically studied and published describing the presence of undesirable genes in buffaloes due to the inbreeding existing in the buffalo Brazilian population. On the existence of inbreeding problems in buffalo herds in Brazil, many authors claimed the leading cause of the reduced number of animals that gave the origin of the different herds (the founder element) of the different breeds was composed of a reduced number of animals imported from the origin countries. Therefore, proof of genetic origin is necessary since most hereditary diseases are transmitted by recessive genes, which must be identified due to the extreme importance of animal production in every domes-

tic species. While it becomes difficult to identify and eliminate animals carrying these genes due to the high cost resulting from the laboratory for diagnosis and animal elimination, the problem has been neglected and is continuously spreading and increasing. Once such problems occur in any animal species, those abnormalities/diseases must be identified since it is essential to determine the occurrence, prevalence, epidemiology, clinical symptoms, pathology, and distribution of the genes observed among the population, which must be screened in order to subsequently identified of kinship and set up a development control strategies through the use of breeding animals proven to be free of these undesirable genes, in order to control and eliminate such kind of diseases.

Keywords: buffalo, genetic, hereditary problems, inbreeding.

RESUMEN

Junto con el crecimiento exponencial de la población de búfalos en América Latina (12 por ciento anual), para una población de 6 millones de cabezas, los problemas derivados de la endogamia han aumentado ya que el rebaño nacional, estimado en 3,0 millones de cabezas por la Asociación Brasileña de Criadores de Búfalos, proviene de poco más de 200 animales importados. La importación de animales del continente asiático se suspendió en 1956 por razones sanitarias (peste bovina). En 1962 se rompió esta prohibición y se trajeron algunos búfalos de la India. Después de esa fecha, no hubo importación oficial de material genético de Asia; Recién en 1989 se importaron algunos animales de las razas Mediterránea, Murrah y Jafarabadi de Italia y Bulgaria, y actualmente no se ha importado semen de las razas Murrah y Jafarabadi de ningún país. Las discapacidades genéticas, hereditarias y congénitas, comple-

tas o parciales, se caracterizan por anomalías en la estructura y/o función de tractos u órganos, sistema completo o partes de ellos. Estos problemas han sido identificados en Brasil y otros países de América Latina (Vale, 1998; Damé, 2013), donde se han diagnosticado más de 40 casos de diferentes casos similares y letales, que por primera vez fueron estudiados científicamente y publicados describiendo la presencia de genes indeseables en búfalos debido a la endogamia existente en la población de búfalos brasileños. Sobre la existencia de problemas de endogamia en los rebaños de búfalos en Brasil, muchos autores afirmaron que la causa principal del reducido número de animales que dio origen a los diferentes rebaños (el elemento fundador) de las diferentes razas estaba compuesto por un reducido número de animales importados de los países de origen. Por tanto, es necesaria la prueba del origen genético, ya que la mayoría de las enfermedades hereditarias se transmiten por genes recesivos, que deben identificarse debido a la extrema importancia de la producción animal en todas

las especies domésticas. Si bien se vuelve difícil identificar y eliminar los animales portadores de estos genes debido al alto costo que resulta en análisis de laboratorio para el diagnóstico y la eliminación de los animales, el problema se ha descuidado y se está extendiendo y aumentando continuamente. Una vez que tales problemas ocurren en cualquier especie animal, esas anomalías/enfermedades deben ser identificadas ya que es esencial determinar la ocurrencia, prevalencia, epidemiología, síntomas clínicos, patología y distribución de los genes observados entre la población, los cuales deben ser examinados para posteriormente se pueda identificar el parentesco y establecer estrategias de control del desarrollo mediante el uso de animales reproductores comprobados libres de estos genes indeseables, con el fin de controlar y eliminar este tipo de enfermedades.

Palabras clave: búfalo, genético, problemas hereditarios, endogamia.

EXPANSION OF THE BUFFALO AGRI-SYSTEM AND INDUSTRY FOLLOWING THE ONE HEALTH APPROACH

Expansión del sistema agrícola y la industria del búfalo siguiendo el enfoque One Health

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ABSTRACT

One Health approach is looking for conceiving health as a consequence of interaction in the interphase of the human/animal/environment complex as an interlinked whole, targeting to change how human beings have traditionally managed health. Lessons learned with the recent Covid 19 pandemic and the silent pandemic of antimicrobial resistance (AMR), among others known as potential pandemics, have put on top the need to change how to tackle health threats. The world is constantly challenged by zoonotic diseases, the emergence of new diseases promoted by human ecosystem invasion, climate change, loss of biodiversity, and the inability of nature to restore exhausted ecosystems due to human activity. The increasing world population demands sufficient, safe, and nutritious food, but food production also needs good practices and fair trade. Otherwise, food can become an essential source of illness, disseminating pathogens and AMR, leading to an unsustainable economy. Links between food and health are currently well recognized. Risk assessment and traceability during food production and distribution are crucial to ensure food safety, security, and sustainability. As growing food producers and suppliers, Buffalo Agri-systems and industry have the opportunity to positively impact the development of sustainable economic systems. Expansion of buffalo Agri-systems and industry will require to be in line with the six areas of action of the One Health Joint Plan of Action 2022-2026 (OH JPA) through incorporation of good production practices, decreasing ecosystem negative impact and processing and distribution of safe food in agreement with national and international standards and guidelines.

Keywords: One Health, Buffalo, Sustainability, AMR.

RESUMEN

El enfoque One Health busca concebir la salud como consecuencia de la interacción en la interfaz del complejo humano/animal/ambiente como un todo interrelacionado, con el objetivo de cambiar la forma en que los seres humanos han gestionado tradicionalmente la salud. Las lecciones aprendidas con la reciente pandemia de Covid 19 y la pandemia silenciosa de resistencia a los antimicrobianos (RAM), entre otras conocidas como pandemias potenciales, han puesto de relieve la necesidad de cambiar la forma de abordar las amenazas a la salud. El mundo se enfrenta constantemente al desafío de las enfermedades zoonóticas, la aparición de nuevas enfermedades promovidas por la invasión de los ecosistemas humanos, el cambio climático, la pérdida de biodiversidad y la incapacidad de la naturaleza para restaurar ecosistemas agotados debido a la actividad humana. La creciente población mundial exige alimentos suficientes, inocuos y nutritivos, pero la producción de alimentos también necesita buenas prácticas y un comercio justo. De lo contrario, los alimentos pueden convertirse en una fuente esencial de enfermedades, diseminando patógenos y resistencia a los antimicrobianos, lo que conduciría a una economía insostenible. Actualmente, los vínculos entre alimentación y salud son bien reconocidos. La evaluación de riesgos y la trazabilidad durante la producción y distribución de alimentos son cruciales para garantizar la inocuidad, la protección y la sostenibilidad de los alimentos. Los agrosistemas bufalinos y la industria, como productores y proveedores de alimentos en crecimiento, tienen la oportunidad de impactar positivamente el desarrollo de sistemas económicos sostenibles. La expansión de los sistemas agrícolas y la industria del búfalo deberá estar en línea con las seis áreas de acción del Plan de Acción Conjunto Una Salud 2022-2026 (OH JPA) mediante la incorporación de buenas prácticas de producción, la disminución

del impacto negativo en el ecosistema y el procesamiento y distribución. de alimentos seguros de acuerdo con normas y directrices nacionales e internacionales.

Palabras clave: One Health, búfalos, Sostenibilidad, RAM.

INTRODUCTION

Buffalo husbandry has progressively developed worldwide from its origin lands in Asia and Africa to the Americas, especially in tropical and sub-tropical countries. In the last decades, it has emerged as an economic alternative due to its adaptation capacity, high-quality meat and milk, and resistance to infectious diseases, among other vital traits [1, 2]. The increasing Buffalo production can bring a significant contribution to achieving United Nations-Sustainable Development Goals (SDGs) by supplying a demanding market of animal protein, promoting at the same time employment generation and human well-being improvements.

Water buffaloes can better resist climate change than other food-producing livestock types; the husbandry can be profitable and sustainable in small and large production systems [2]. However, buffalo breeding practices impact the food production environment (environment and ecosystem shared with humans, other food-producing animals, and wildlife) and could also affect public health. One Health approach and SDGs seek a positive balance in the complex interlink of animal husbandry/environment/health/economy development. The SDGs 2023 report has stated the urgent need to implement policies that support a shift towards sustainable practices and new resource-independent indexes to measure economic growth. The establishment of an exemplary structure of governance is critical to generating appropriate policies supporting legal and financial development or improvements of food production systems focused on sustainable development. Sustainability reports at the company level have been increasing since 2016, showing a rise in industries' awareness to prioritize sustainable practices [3]. As a growing industry, expansion of buffalo Agri-systems and industry will require to promote assessment of the current husbandry and trade practices and then, gradually implement the required adaptations to guarantee a sustainable food production system.

Scientific research and epidemiological analysis have brought to light unquestionable evidence of how human behavior and uncontrolled risk factors have led to cumulative effects generating negative consequences. Depicted by climate change, modified natural ecosystems, diminished biodiversity, emergence and re-emergence of infectious diseases, and increased zoonotic potential of some microorganisms, these changes seriously challenge efforts to guarantee global health and food supply [4].

The primary products of the buffalo industry, meat, and dairy, pose a great responsibility for producing and supplying nutritious and safe food. The complexity of the food chain (from

farm to fork), which includes primary production at the farm level, processing, packaging, transportation, and points of sale to reach the end consumer, implies vulnerabilities at any point of this chain, increasing the probability of contamination of food with potential risk to human health. This is why the traceability of produce represents a key factor for risk analysis: identification of potential risk factors, risk assessment, risk control measures, and ability to measure the impact of risk management. Beyond that, it is also essential to design preparedness strategies for further potential risks [5]. Expansion of buffalo Agri-systems and industry will depend on good food production practices, processing, distribution, and trade, and should be in sound with established national and international standards and regulations.

ONE HEALTH OVERVIEW

Throughout modern history, human health has mostly been considered an isolated area, merely the competency and responsibility of human physicians, and in a narrow point of view, it has to be that way due to areas of specialization needed to treat the variety nature of human diseases. Such a perspective also prevailed for animal health, where animal issues, especially those linked with animal husbandry for food production, are just of veterinary services and producers' competency. Zoonotic diseases are well recognized as an essential link between humans and animals, profoundly impacting human health and wellness; these two branches of health usually work apart from each other, even though both are looking for population development and well-being. Also, for a long time, environmental consequences of the impact of human activities and animal breeding (food-producing animals) were ignored. This narrow perspective of health has paved unprecedented global consequences due to neglected risk factors and critical points at different areas of health on earth.

Moving toward sustainability of field productive activities that guarantee the well-being of human beings, animals, and ecosystems in an equilibrated process of gain and loss, the concept of One Health emerged as the most suitable and efficient approach to face health challenges to achieve everyone's positive balance.

Leader health organizations: World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organization for Animal Health (WOAH) have jointly worked for decades. Nevertheless, it was not until 2010 that the Tripartite commitment surged as a strategic alignment and a global governance structure to face public and animal health and environmental risks under the One Health perspective [6]. Special attention to health hazards associated with zoonoses and animal diseases, food safety, and antimicrobial resistance was settled. In February of 2021, a fourth partnership, the United Nations Environment Program (UNEP), joined Tripartite, currently named Quadripartite, reinforced the crucial role of the environment and ecosystem.

The Quadripartite currently defines One Health as “an integrated, unifying approach that aims to sustainably balance and optimize the health of humans, animals, plants and ecosystems. It recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent” [4]*.

This collaborative work has also resulted in the development of the first Global Action Plan on Antimicrobial Resistance (GAP) as a leading global guidance to tackle this silent pandemic under the One Health perspective [8, 9]. Antimicrobial resistance (AMR) is recognized as an increasingly serious threat to public health and sustainable food production, and participation of all sectors of government and society is a primary requirement to generate an adequate response (39th Conference of FAO, June 2015). In Latin America and the Caribbean, essential achievements have been made by the technical support of regional organizations such as the Inter-American Institute for Cooperation on Agriculture (IICA), joined with academia represented by Ohio State University. Their efforts in capacity building and designing programs have enabled nearly ten Latin American and Caribbean countries to develop and implement monitoring and surveillance programs on AMR under the One Health perspective.

Having faced the COVID-19 pandemic, the main lesson is the need for a joint vision of One Health implementation, intersectoral collaboration, and political will as the only way to prevent future pandemics. Besides the One Health holistic concept, the functional consideration for the implementation of the One Health approach necessarily requires collaborative, multisectoral, and trans-disciplinary work with active participation at different levels of society, from policymakers to the public general, to achieve the primary goal of optimal health outcomes, always recognizing the interconnection between humans, animals, plants and their shared environment [5]. Collaborative efforts on tackling health and environmental threats mainly aim to contribute to sustainable development, looking for balance and integration of the social, environmental, and economic dimensions. This is the operational definition of One Health.

In summary, the “One Health” approach recognizes that people’s health is connected to the health of animals and the environment [8]. It acknowledges the indivisible interlink between the three focal points of health and how they impact each other.

The One Health Joint Plan of Action (OH JPA) 2022-2026 is guided by the theory of change and focuses on six action areas:

1. Enhancing One Health capacities to strengthen health systems
2. Reducing the risks from emerging and re-emerging zoonotic epidemics and pandemics.
3. Controlling and eliminating zoonotic, neglected tropical and vector-borne diseases.
4. Strengthening the assessment, management and communication of food safety risks.
5. Curbing the silent pandemic of Antimicrobial Resistance (AMR)
6. Integrating the Environment into One Health. [4]



SUSTAINABLE DEVELOPMENT GOALS OF THE UNITED NATIONS 2030 AGENDA

The Sustainable Development Goals (SDG) are a call for action by all countries independently of their wealth index, looking for economic growth addressing social needs. At the same time, protect the planet.10 There have been established 17 SDGs constituting the focal point of the 2030 Agenda adopted by the United Nations General Assembly (UNGA) in 2015 (see annex 1). All United Nations Member States adopted the SDGs to end poverty, reduce inequality, and build more peaceful, prosperous societies by 2030, seeking economic development, promoting environmental sustainability, and guaranteeing social inclusion [10, 11].

Sustainable development is “development that meets the needs of the present without compromising the ability of future generations to meet their own needs” [12, 13].

According to this, efforts must focus on reversing the triple planetary climate change crisis, nature depletion, biodiversity loss, and pollution and residues [10].

FOOD PRODUCTION CHALLENGES

The world population is expected to climb to 8.5 billion souls by 2030. With an increase of 2 billion in the next thirty years, it has been estimated to be 9.7 billion by 2050 and nearly 10.9 billion by 2100 [14]. With such growth, food demand increases as fast as the population. Thus, several of the seventeen SDGs are directly related to food production (see annex 1), and it is essential to highlight that this increasing world population requires higher food production levels and massive food distribution, and both rely necessarily on agriculture development and food safety systems.

* **Note:** In this definition, the environment is considered a whole, including ecosystems. From a more specific and operational point of view, environment refers to abiotic components (e.g., water, soil, air, chemicals), and ecosystems refer to biotic components (fauna, flora, microorganisms) of a geographic area [7].

Globalization and the growing world population, among other causes, have pushed massive movements of people worldwide and changed consumption behavior. High food production levels to supply world requirements have then induced increased movements of live animals and food products across borders, facilitating the emergence of new pathogens and faster dissemination of zoonosis and antimicrobial resistance.

The world is constantly challenged by zoonotic diseases, food safety, antimicrobial resistance (AMR), disruption of ecosystems, availability of food and water, and economic well-being, among others. These challenges are especially important in communities where agriculture is the main economic activity or when agriculture significantly contributes to the country's wealth, but also in distant communities due to food demand. It is necessary to remember that the advancement or evolution of humanity will always rely on our capacity to protect and ensure safe food systems [15].

Even though buffaloes are more resistant to infectious diseases and can adapt better than cattle, they can be an important source of disseminating pathogens and antimicrobial resistance. Moreover, microorganisms from food-producing animals could alter the composition of the human microbiome, and both human and animal microbiomes can coevolve due to their close interdependence [16]. This coevolution increases the probability of susceptibility to the same pathogens and facilitates both senses sharing of microorganisms (animal to human and human to animal). Then, good practices in buffalo husbandry, appropriate animal health programs (e.g., timely vaccination, parasites, and vector control), and rational use of antimicrobials are crucial to reduce the possibilities of exchanging microorganisms between humans and buffaloes and avoid significant microbiome variations.

Food-producing animals represent a crucial intersection between humans, animals, and the environment and ecosystem in the primary production sector. On one hand, food safety starts on the farm, and it depends on animal and ecosystem health. Food from buffalo origin will enter the food chain to reach humans as final consumers, and deviations from good practices from farm to table can impact human health in various ways. In addition, food of buffalo origin is as susceptible to contamination as any other food type. On the other hand, if buffalo farming is driven at the expense of the ecosystem, environmental health, and/or animal welfare, tremendous pressure will be placed on natural resources with a rapid utilization rate, and nature will not be able to keep up the pace to replenish resources [4]. Thus, the production of food of buffalo origin requires implementing the One Health approach to be safe, efficiently produced, economically profitable, and sustainable.

SAFE FOOD FROM BUFFALO ORIGIN AND TRADE

Access to safe food is a human right. However, over 420,000 people die after consumption of contaminated food

(whatever the cause be, microorganisms, chemical residues, or toxins), and nearly 600 million people currently get ill due to consumption of food lacking minimum quality standards. Foodborne hazards are different and can be present or enter at any point in the food chain. They also cause different natures of acute or chronic diseases, from digestive tract infections to cancer [15].

In order to assure food safety during production, processing, distribution, and trade, the food-producing buffalo industry should meet the international food standards established by FAO/WHO Codex Alimentarius, and in those cases of exporting should comply with World Trade Organization (WTO) standards. In all cases, following the World Organization of Animal Health (WOAH, founded as OIE) guidelines to meet food-producing animal health requirements is necessary [17].

International trade of food from buffalo origin will require to meet these standards in order to get an appropriate registration of the products according to international trade regulations and commercial partner's legislations, e.g., [Food and Drug Administration](#) (FDA), [Food Safety and Inspection Service](#) (FSIS. USDA), [Canadian Food Inspection Agency](#), [European Food Safety Authority](#) (EFSA), and [Food Standards Australia New Zealand](#) (FSANZ) [18-22]. Following food safety standards will facilitate fair practices in the international food trade. Buffalo health, food safety, and fair food trade go alongside the generation of healthy, nutritious, and sufficient food, reinforcing at the same time opportunities for buffalo products and byproducts marketing and food industry expansion.

Non-compliance with food safety standards could lead to the withdrawal of food products from the market and legal action against the involved food enterprise. Most important, a single foodborne disease outbreak could cause lives toll and loss of businesses. Main food safety issues are associated with contamination with microorganisms altering nutritional and microbiology characteristics of food that could lead to foodborne diseases and reduce the market life span of produce [23]. Contaminating microorganisms could be pathogens or not, but harboring antimicrobial resistance that efficiently disseminates through food is possible. Any of these cases, foodborne outbreaks, AMR, or reduced produce life span, represent a severe threat to public health and impact the economy.

Some remarkable features of buffalo are the innate resistance to some bacteria, parasites, and even ticks and the extensive nature of husbandry in most countries where buffalo breeding has emerged [23]. Both imply less use of antibiotics, antiparasitics, and other biocides, providing benefits beyond the breeding and production. Less use of antimicrobials results in lower selective pressure on resistant microorganisms, reducing the chances of disseminating antimicrobial resistance and antimicrobial residues to the environment. This feature represents a significant advantage, positioning food of buffalo origin as a source of safer food for human consumption and improving trading opportunities.

The advantages of buffalo nature could be reduced or lost due to some human practices. For example, to solve farming issues regarding scarcity or lack of fresh water, in some countries, buffaloes are bathed with sewage. This practice increases the risk of diseases such as clinical mastitis, foot and mouth disease, and tick infestation (increasing vector-borne disease likelihood), reducing at the same time milk production and slaughter value and impacting the economy, productivity, animal welfare, and undoubtedly quality of food from buffalo origin [25]. Although buffaloes have shown to be more resistant than cattle, they still bear susceptibility to a variety of infectious diseases such as leptospirosis, brucellosis, tuberculosis, and bovine viral diarrhea, and parasitic diseases like fasciolosis and schistosomiasis, all impacting animal and/or public health [26]. Thus, it is crucial to assess farming practices that could reduce the advantages traits that buffalo naturally express and mitigate them to ensure food quality and better trade opportunities.

IMPLEMENTING ONE HEALTH APPROACH IN THE AGRIFOOD CHAIN

More buffalo farmers than we can think to have some One Health practices without even knowing. If they are part of a community dedicated to buffalo husbandry or farmers' organizations with standard plans, or if good practices for livestock rearing and food production are considered, and ecosystem preservation is of interest, they have started on the right foot. However, if it is not, the best way is to start simply by integrating into working groups and inducing simple changes with what farmer has available regarding resources and installed capacity. To move toward sustainability, it is crucial to remember that excellent infrastructure investment is optional as long as feasible objectives are established and gradually scale up to integrating humans, animals, and ecosystem wellbeing. Implementing the One Health approach can only be achieved with integrative, collaborative, multisectoral, and transdisciplinary actions. However, good governance is required to build legal basement and regulatory policies.

The best example of implementing the One Health approach in the agrifood chain is the monitoring and surveillance programs on antimicrobial resistance (AMR). Since 2015, a Global Action Plan on AMR has been ongoing, and specific directives and objectives have been established [9]. Although developed countries like the United States of America, Canada, Denmark, Sweden, and Korea, among others, have been working on such programs long ago, many countries worldwide have started designing and implementing their own national action plan according to their resources and capacities. Harmonized methodologies have been established and updated to assess critical points along the human and animal health systems related to the agrifood chain from farm to table. Different animal production systems (mainly poultry, cattle, and swine) are monitored to identify phenotype-resistant bacteria in various sample types and critical points throughout the food chain.

In Latin America and the Caribbean, the InterAmerican Institute for Cooperation on Agriculture (IICA), joint with the Veterinary Preventive Medicine Department of Ohio State University (OSU), has been providing technical support on the designing and implementation of such plans under One Health perspective for several countries in the region. This collaborative work has yielded National monitoring and surveillance programs for AMR already in implementation or the designing phase (e.g., Paraguay, Ecuador, Costa Rica, Belize, Chile, Brazil, Jamaica, and Colombia). Multisectoral and transdisciplinary teamwork has been constituted with the active participation of Ministries of human and animal health, agriculture and environment, academies, reference laboratories, producers' associations, private industry, and retailers, among other stakeholders called to participate.

In addition, pilot projects have been successfully designed and executed; the CISARA project involved seven poultry-producing Caribbean countries [27]; last year, pilot projects for Honduras, El Salvador, and Nicaragua were designed and are ahead to be implemented (IICA et al. of Pilot Plan for Monitoring Antimicrobial Resistance in the Agricultural sector in Honduras, El Salvador, and Nicaragua, financially supported by USDA). As a result of the IICA-OSU collaboration, a systematic design process has been developed, establishing harmonized methodologies, and following the One Health perspective. The technical support and guidance IICA-OSU is bringing to countries in Latin America and the Caribbean is crucial for strengthening the monitoring and surveillance systems of AMR under the One Health approach.

On a global scale, many countries worldwide have been collecting AMR and antimicrobial use (AMU) information in the food and agriculture sectors. However, unexpectedly, the data obtained has yet to be thoroughly analyzed, generating gaps in epidemiological data. Thus, FAO aims to promote and facilitate AMR stewardship by developing the International FAO AMR Monitoring (InFARM) System and IT platform as a global epidemiological information system to host, analyze, interpret, and leverage AMR data generated by countries, enhancing the availability and quality of data in a harmonized way [28]. InFARM will generate integrated global data on AMR of the food and agriculture sectors following the One Health perspective. Additionally, the WOAHA (founded as OIE) ANIMAL antimicrobial USE global database (ANIMUSE) has collected information on the amounts and reasons for animal antimicrobial use since 2015. Free access database allows interactive display of reports on the global and regional trends [29, 30].

In the Buffalo production system, we can mention a few examples. A One Health integrated project has been developed in Thailand and Vietnam. It aims to assess buffalo care practices based on the herder's knowledge and environmental assessment of the impact of extensive farming at the village paddies field and within the forest grazing area. Also, to study the buffalo microbiota for AMR gene detection and microorganisms' distribution in the ecosystem [31]. In other countries, studies

on mitigation of the environmental impact of buffalo production through genetic improvement and mitigation alternatives of the negative environmental impacts of the introduction of buffalo breeding have been done.

The global buffalo population has reached nearly 200 million; 97% are reared in Asia, where many depend on this species for livelihood. Buffalo breeding has roughly grown 2% per year in the last two decades and is still growing in the rest of the world, and currently, more than sixty countries account for buffalo husbandry [2, 32, 23]. The products and byproducts from the buffalo Agri-system and industry are spreading worldwide due to features such as milk and meat nutritional quality. For example, buffalo milk ranks second after cow milk, with a significant share of global milk production [2]. Thus, buffalo farming has gained a worldwide competitive position, generating essential responsibilities for safe and sustainable food production.

ECONOMIC GROWTH UNDER ONE HEALTH APPROACH

Besides food production, the development of the food production system allows society to benefit through employment generation, income increases, infrastructure improvements, industry expansion, and economic growth. Traditionally, the economic indicator Gross Domestic Product (GDP) has determined progress assessment considering the total value of goods produced and services provided by a country, which translates into national wealth [11, 14].

Health world leaders have realized that such indicators exclude accountability for environmental effects and the cost it harbors. In that sense, a new indicator has been proposed named the Inclusive Wealth Index (IWI), Green Gross Domestic Product, or Green National Income (GNI) to weigh environmental sustainability and equity, leading to measuring economic sustainability and well-being. This new indicator aims to assure sustainability and the long-term well-being of the population. This new perspective considers the impact of human productive activity on the ecosystem where such activities are performed. The food production and processing environment will represent the food and agriculture sectors [34, 35].

The Inclusive Wealth Index (IWI) was created by the United Nations University- International Human Dimensions Programme on Global Environmental Change (UNU-IHDP) and the United Nations Environment Programme (UNEP) [34]. In order to achieve an actual measure of changes in the wealth of nations, this index includes assets of a country employing its human, natural, social, and physical capital, moving estimation of nation wealth beyond traditional measurements and updating the way of wealth assessment. This broad approach is expected to facilitate heads of governance better management of the economy, being aware of wealth assets, and understanding promoter factors of their increases and/or depletion [35].

In general, most countries have significantly exhausted their natural capital in efforts to achieve growth, although some have accumulated human and productive capital with relatively low loss of natural capital. According to the Inclusive Wealth Report (IWR) 2023, Latin America and the Caribbean have a higher loss of natural capital than any other region globally. Some of its countries have lost more than half of their natural capital since 1990, which means that economic performance has been made at the expense of natural capital. However, the most significant loss between 1990 and 2019 occurred in Japan, with a 70% loss of natural capital at the expense of fisheries and forest overexploitation. Globally, changes in the estimation of wealth urge order to promote an increase in renewable natural capital and reverse this depletion trend focused on building socially just and environmentally sustainable development pathways [35].

CONCLUSIONS

- One Health approach could seem a utopic health perspective due to its holistic meaning and our usual way to account for development. However, based on the health challenges we are experiencing, the unstoppable increasing demand for food, and the exhaustion of natural resources and biodiversity, the only way to achieve sustainability is to establish practices rendering a positive balance between the triad human/animals/environment. Suitable governance structures are crucial.
- It is imperative to know that the only way to implement the One Health approach is through integrative, multisectoral, and transdisciplinary collaboration.
- Implementing the One Health approach does not necessarily imply great investment; it just requires the establishment of feasible objectives based on existing resources and capacities. Start simple with achievable small goals, progressively scaling up for more significant outcomes according to developed capacities during the process.

RECOMMENDATIONS

- Producers should strengthen their associations and look for new partnerships like academics, researchers, and international organizations to develop systematic ways to embrace changes and challenges based on scientific evidence.
- Identifying risk factors for introducing buffaloes in new areas should account for animal health and welfare and sustainability of the ecosystem and production system.
- Perform a current situation analysis, which is highly recommended to identify the baseline and determine the start point.

- Feedback and suggestions from all interested parties or stakeholders are essential to advance sustainability. Thus, sharing relevant data obtained from the buffalo agri-system and industry assessment will promote capacity building and will allow better and more efficient use of resources.

FUTURE PERSPECTIVES

- Buffalo industry has excellent chances to expand beyond borders if products and byproducts meet the international trade standards of food safety and quality and ensure traceability and security of the food produced.
- Expansion of the buffalo Agri-system, as with any other food-producing system, will require enrolling in a self-assessment of production, processing, distribution, and trade practices to understand how profound the impact on society, environment, and economy is. Production of food from buffalo origin could positively impact achieving the SDGs.
- It is necessary to change in the way we usually think food production should be and the pathway we usually use to measure wealthiness.

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REFERENCES

- [7] Wahid H, Rosnina Y. Management of Dairy Animals: Buffalo: Asia [Internet]. 2nd ed. Encyclopedia of Dairy Sciences. Elsevier Inc.; 2016 [cited 2023 Nov 1]. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7157481/>
- [8] El Debaky H, Kutchy NA, Ul-Husna A, Indriastuti R, Akhter S, Purwantara B, et al. REVIEW: Potential of water buffalo in world agriculture: Challenges and opportunities. ARPAS, editor. Applied Animal Science [Internet]. 2019 Apr;35(2):255–68. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S2590286519300370>
- [9] United Nations. Department of Economic and Social Affairs. The Sustainable Development Goals Report 2023: Special Edition [Internet]. UN DESA Publications. 2023 [cited 2023 Nov 1]. Available from: <https://desa-publications.un.org/publications/sustainable-development-goals-report-2023-special-edition>
- [10] One Health Joint Plan of Action, 2022–2026 [Internet]. WHO. FAO; UNEP; WHO; World Organisation for Animal Health (WOAH) (founded as OIE); 2022 [cited 2023 Nov 1]. Available from: <https://www.who.int/publications/item/9789240059139>
- [11] GUIDELINES ON INTEGRATED MONITORING AND SURVEILLANCE OF FOODBORNE ANTIMICROBIAL RESISTANCE CXG 94-2021 Adopted in 2021 [Internet]. FAO; 2021 [cited 2023 Oct 31]. Available from: <https://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/es>
- [12] FAO-OIE-WHO. The FAO-OIE-WHO Collaboration Sharing responsibilities and coordinating global activities to address health risks at the animal-human-ecosystems interfaces A Tripartite Concept Note [Internet]. 2010 [cited 2023 Oct 31]. Available from: <http://www.fao.org/3/ak736e/ak736e00.pdf>
- [13] Kahn LH. Developing a One Health approach by using a multi-dimensional matrix. One Health [Internet]. 2021 Jul 9 [cited 2021 Oct 30];13:100289. Available from: <https://www.sciencedirect.com/science/article/pii/S2352771421000793?via%3Dihub>
- [14] FAO. The Tripartite's Commitment Providing multi-sectoral, collaborative leadership in addressing health challenges [Internet]. 2017 Oct [cited 2023 Nov 1]. Available from: <https://www.fao.org/3/i7377e/i7377e.pdf>
- [15] WHO. Global action plan on antimicrobial resistance [Internet]. www.who.int. WHO; 2016 [cited 2023 Nov 1]. Available from: <https://www.who.int/publications/item/9789241509763>
- [16] UNEP. Sustainable Development Goals [Internet]. UNEP - UN Environment Programme. 2022. Available from: <https://www.unep.org/explore-topics/sustainable-development-goals>
- [17] United Nations Environment Programme. Inclusive Wealth Report 2023: Measuring Sustainability and Equity [Internet]. Unep.org. United Nations Environment Programme; 2023 [cited 2023 Oct 1]. Available from: <https://wedocs.unep.org/handle/20.500.11822/43131#:~:text=The%20Inclusive%20Wealth%20Report%202023>
- [18] United Nations. United Nations Sustainable Development [Internet]. United Nations Sustainable Development. United Nations; 2023. Available from: <https://www.un.org/sustainabledevelopment/>
- [19] Sustainable Development [Internet]. International Institute for Sustainable Development. Available from: <https://www.iisd.org/mission-and-goals/sustainable-development#:~:text=What%20is%20sustainable%20development%3F>

- [20] Department of Economic and Social Affairs Population Division. Global Population Growth and Sustainable Development | Population Division [Internet]. Vol. 2, www.un.org. [cited 2023 Nov 1]. Available from: <https://www.un.org/development/desa/pd/content/global-population-growth>
- [21] FAO. The Future of Food Safety [Internet]. <https://www.fao.org>: FAO; 2019 [cited 2023 Nov 1]. Available from: <https://www.fao.org/3/ca4289en/ca4289en.pdf>
- [22] Qian J, Wu Z, Zhu Y, Liu C. One Health: a holistic approach for food safety in livestock. *Science in One Health* [Internet]. 2023 May 10 [cited 2023 Nov 1];1:100015. Available from: <https://www.sciencedirect.com/science/article/pii/S2949704323000094?via%3Dihub>
- [23] Food and Agriculture Organization of the United Nations and the World Trade Organization. TRADE AND FOOD STANDARDS [Internet]. <https://www.wto.org/>: FAO-WTO; 2017 [cited 2020 May 1]. Available from: https://www.wto.org/english/res_e/booksp_e/tradefoodfao17_e.pdf
- [24] U.S. Food and Drug Administration. U S Food and Drug Administration [Internet]. [Fda.gov](https://www.fda.gov). 2021 [cited 2023 Nov 1]. Available from: <https://www.fda.gov/>
- [25] U.S. Department of Agriculture. USDA Food Safety and Inspection Service [Internet]. [Usda.gov](https://www.fsis.usda.gov/). 2018 [cited 2023 Nov 1]. Available from: <https://www.fsis.usda.gov/>
- [26] Government of Canada. Canadian Food Inspection Agency [Internet]. inspection.canada.ca. Canadian Food Inspection Agency. Government of Canada; 2015 [cited 2023 Nov 1]. Available from: <https://inspection.canada.ca/eng/1297964599443/1297965645317>
- [27] European Union. European Food Safety Authority [Internet]. European Food Safety Authority. [cited 2023 Nov 1]. Available from: <https://www.efsa.europa.eu/en>
- [28] Food Standards Australia New Zealand. Food Standards Australia New Zealand [Internet]. [Foodstandards.gov.au](https://www.foodstandards.gov.au). 2016 [cited 2023 Nov 1]. Available from: <https://www.foodstandards.gov.au/Pages/default.aspx>
- [29] Warmate D, Onarinde BA. Food safety incidents in the red meat industry: A review of foodborne disease outbreaks linked to the consumption of red meat and its products, 1991 to 2021. *International Journal of Food Microbiology* [Internet]. 2023 May 3 [cited 2023 Nov 1];398(398):110240–0. Available from: <https://www.sciencedirect.com/science/article/pii/S0168160523001563?via%3Dihub>
- [30] Chanu KV, Thakuria D, Kumar S. Antimicrobial peptides of buffalo and their role in host defenses. *Veterinary World* [Internet]. 2018 Feb 15 [cited 2020 Oct 9];11(2):192–200. Available from: www.veterinaryworld.org/Vol.11/February-2018/17.pdf
- [31] Elahi E, Abid M, Zhang H, Cui W, Shabeh ul Hasson. Domestic water buffaloes: Access to surface water, disease prevalence and associated economic losses. *Preventive Veterinary Medicine* [Internet]. 2018 Jun 1 [cited 2023 Nov 1];154:102–12. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S016758771730675X>
- [32] Villanueva MA, Mingala CN, Tubalinal GA, Gaban PB, Nakajima C, Suzuki Y. Emerging Infectious Diseases in Water Buffalo: An Economic and Public Health Concern. In: *Emerging Infectious Diseases in Water Buffalo - An Economic and Public Health Concern* [Internet]. Intech; 2018 [cited 2023 Nov 1]. p. 1–55. Available from: <https://www.intechopen.com/chapters/59095>
- [33] Caribbean Animal Health Network. CISARA Pilot Projects / News - Caribbean animal health network [Internet]. [Caribvet.net](http://caribvet.net). 2016 [cited 2023 Nov 1]. Available from: <https://www.caribvet.net/news/cisara-pilot-projects>
- [34] FAO. The International FAO Antimicrobial Resistance Monitoring (InFARM) System | Resistencia a los antimicrobianos | Organización de las Naciones Unidas para la Alimentación y la Agricultura [Internet]. www.fao.org. FAO; 2022 [cited 2023 Nov 1]. Available from: <https://www.fao.org/antimicrobial-resistance/resources/database/infarm/es/>
- [35] World Organization for Animal Health (Founded as OIE). ANIMUSE: Monitoring antimicrobial use in animals [Internet]. WOA - World Organisation for Animal Health. 2015 [cited 2023 Nov 1]. Available from: <https://www.woah.org/en/article/animuse-monitoring-antimicrobial-use-in-animals/>
- [36] World Organisation for Animal Health. Seventh Annual Report on Antimicrobial Agents Intended for Use in Animals [Internet]. WOA - World Organisation for Animal Health. 2022 [cited 2023 Nov 1]. Available from: <https://www.woah.org/en/document/seventh-annual-report-on-antimicrobial-agents-intended-for-use-in-animals-2/>
- [37] Lainé N, Prasongmaneerut T, Janyasuthiwong S, Foyontpanich K. Beyond Data Source: Engaging Herders' Knowledge and Perspectives within the BuffFarm Research Process. *One Health Cases* [Internet]. 2023 Jul 11 [cited 2023 Oct 31];2023. Available from: <https://www.cabidigitallibrary.org/doi/10.1079/onehealthcases.2023.0023>
- [38] Zhang Y, Colli L, Barker JS. Asian water buffalo: domestication, history and genetics. *Animal Genetics* [Internet]. 2020 Jan 22 [cited 2023 Nov 1];51(2):177–91. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/age.12911>
- [39] Minervino AH, Zava M, Vecchio D, Borghese A. *Bubalus bubalis*: A short story. *Frontiers in Veterinary Science* [Internet]. 2020 Dec 1 [cited 2023 Nov 1];7(570413):1–

15. Available from: <https://www.frontiersin.org/articles/10.3389/fvets.2020.570413/full>
- [40] United Nations. Inclusive Wealth Report 2012: Measuring Progress Towards Sustainability. Uneporg [Internet]. 2022 [cited 2023 Oct 31]; Available from: <https://wedocs.unep.org/handle/20.500.11822/32228>
- [41] Inclusive Wealth Report 2023: Measuring Sustainability and Equity. United Nations Environment Programme. Year: 2023 Container: Unep.org Publisher: United Nations Environment Programme DOI: <https://doi.org/10.59117/20.500.11822/43131> ISBN: 978-92-807-4051-6 URL: <https://wedocs.unep.org/handle/20.500.11822/43131>

ANNEX

Annex 1. United Nations Sustainable development goals



SUSTAINABLE DEVELOPMENT GOALS (SDGs)

Goal 1	End poverty in all its forms everywhere
Goal 2	End hunger, achieve food security and improved nutrition and promote sustainable agriculture
Goal 3	Ensure healthy lives and promote well-being for all at all ages
Goal 4	Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all
Goal 5	Achieve gender equality and empower all women and girls
Goal 6	Ensure availability and sustainable management of water and sanitation for all
Goal 7	Ensure access to affordable, reliable, sustainable and modern energy for all
Goal 8	Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all
Goal 9	Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation
Goal 10	Reduce inequality within and among countries
Goal 11	Make cities and human settlements inclusive, safe, resilient and sustainable
Goal 12	Ensure sustainable consumption and production patterns
Goal 13	Take urgent action to combat climate change and its impacts
Goal 14	Conserve and sustainably use the oceans, seas and marine resources for sustainable development
Goal 15	Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss
Goal 16	Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels
Goal 17	Strengthen the means of implementation and revitalize the Global Partnership for Sustainable Development

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SDGs logo use: https://www.un.org/sustainabledevelopment/wp-content/uploads/2019/01/SDG_Guidelines_AUG_2019_Final.pdf

BUILDING RESILIENT CATTLE THROUGH BETTER MANAGEMENT OF BUFFALO HEALTH

Creando ganado resiliente mediante una mejor gestión de la salud de los búfalos

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ABSTRACT

Livestock has been involved among the causes of environmental deterioration due to the felling of forests, soil degradation, the affectation of water resources, and greenhouse gas (GHG) emissions. The high proportion of damage attributed to the livestock sector is debatable compared to other sectors inherent to human development. However, it is unquestionable that immediate action is required to address the global environmental situation. The most effective way to stop it is to reduce methane, but it is not the only way. This refers to producing food through more sustainable, friendly models, less dependent on external inputs, with low energy consumption and reduced GHG emissions, but at the same time with better yields (productive and reproductive) and high efficiency. It is necessary to implement sustainable buffalo production systems through a planning process, which includes efficient technologies that interact favorably with the environment. The execution of management programs (vegetable, animal, genetic, advanced reproduction, health, well-being, and profitability) must focus on new concepts and strategies that are friendly to the environment. In addition to sustainability and food production, there is a need to maximize the capacities of the buffalo and establish sanitary programs based on and guaranteed a healthy herd and safe production (on the postulate of "one health"). It is necessary to know in depth about the immunobiology of the buffalo and its strategies for controlling pathogens. In particular, the immune system's effectiveness in recognizing the progression and pathogenic action of some agents, from which the buffalo is not exempt from becoming infected, but which do not thrive or induce clinical manifestations or functionality variations. On the contrary, other acute diseases (clostridial, for example) or those caused by intracellular bacteria may have a reserved prognosis, developing complex pictures. This unique set defines that the buffalo species is not resistant to these diseases. But, instead, resistant to many. This condition should be under-

stood as the ability to adapt efficiently and recover quickly from stress, adversity, or infection. This resilience must be strengthened through handling strategies and by implementing specific and strategic health plans built on solid foundations of diagnostic structures to promote prevention and contribute to more ethical and rational treatments, stimulating a unique association (environment - pathogen - buffalo). Therefore, interventions do not necessarily have to be done through drugs. Resilience development involves maximizing immunity, based on genetic programs and selection for individual health and product; In health management strategies that strengthen mother/child interaction from birth; In which the colostrum is guaranteed in time and form; In which anthelmintic treatments respond to a rational use on populations with parasitic pathological loads, and that have a minimum environmental impact (on dung insects, grassland ecosystems and edaphic factors), or on the dynamics of the ecosystems; Where the (restricted and cautious) use of antimicrobials prevents the continued proliferation of resistance, nor compromises human health. Build it through genetic improvement for a genotype/environment interaction favorable to the development of optimal immunity; Through the comfort and guarantee of animal welfare; Through rational grazing with low environmental impact, in polyphytic pastures, with shade and minimal use of pesticides. Strengthen the population's health through strict controls to eradicate diseases such as Brucellosis and Tuberculosis, which will positively impact public health. In a short time, we will have multiple possibilities generated by molecular bioengineering and molecular genetics for identifying and multiplying actual resistance genes to these diseases and others of economic importance that compromise buffaloes. In conclusion, buffalo production and the tropics represent elements with extraordinary potential to generate positive synergies and promote agroecological models that lead to climate change mitigation by promoting carbon storage in the soil and reducing GHG emissions, neutralizing the adverse effects on soils and vegetation and, ultimately, contributing to

the regeneration of the ecosystem. However, for it to work, it is necessary to generate a deep knowledge of the animal species, the ecosystem, and the ecological principles of grazing, Capacities that producers must develop.

Keywords: resilient, buffalo health, sustainability, One Health, greenhouse gas emissions.

RESUMEN

La ganadería ha estado involucrada entre las causas del deterioro ambiental por la tala de bosques, la degradación de suelos, la afectación de los recursos hídricos y las emisiones de gases de efecto invernadero (GEI). La alta proporción de daños atribuidos al sector ganadero es discutible en comparación con otros sectores inherentes al desarrollo humano. Sin embargo, es incuestionable que se requieren acciones inmediatas para abordar la situación ambiental global. La forma más eficaz de detenerlo es reducir las emisiones de metano, pero no es la única. Esto se refiere a producir alimentos a través de modelos más sostenibles, amigables, menos dependientes de insumos externos, con bajo consumo de energía y reducidas emisiones de GEI, pero al mismo tiempo con mejores rendimientos (productivos y reproductivos) y alta eficiencia. Es necesario implementar sistemas sustentables de producción bufalina a través de un proceso de planificación, que incluya tecnologías eficientes que interactúen favorablemente con el medio ambiente. La ejecución de programas de manejo (vegetal, animal, genético, reproducción avanzada, salud, bienestar y rentabilidad) debe enfocarse en nuevos conceptos y estrategias amigables con el medio ambiente. Además de la sostenibilidad y la producción de alimentos, es necesario maximizar las capacidades de los búfalos y establecer programas sanitarios basados y garantizados en un rebaño sano y una producción segura (sobre el postulado de "Una Salud"). Es necesario conocer en profundidad sobre la inmuno-biología del búfalo y sus estrategias para el control de patógenos. En particular, la eficacia del sistema inmunológico para reconocer la progresión y la acción patogénica de algunos agentes, de los cuales el búfalo no está exento de infectarse, pero que no prosperan ni inducen manifestaciones clínicas o variaciones de funcionalidad. Por el contrario, otras enfermedades agudas (clostridiales, por ejemplo) o las causadas por bacterias intracelulares pueden tener un pronóstico reservado, desarrollándose cuadros complejos. Este conjunto único define que la especie de búfalo no es resistente a estas enfermedades, sino que, en cambio, es resistente a muchas. Esta condición debe entenderse como la capacidad de

adaptarse eficientemente y recuperarse rápidamente del estrés, la adversidad o la infección. Esta resiliencia debe fortalecerse mediante estrategias de manejo y la implementación de planes de salud específicos y estratégicos contruidos sobre bases sólidas de estructuras de diagnóstico para promover la prevención y contribuir a tratamientos más éticos y racionales, estimulando una asociación única (medio ambiente - patógeno - búfalo). Por lo tanto, las intervenciones no necesariamente tienen que hacerse a través de medicamentos. El desarrollo de la resiliencia implica maximizar la inmunidad, basándose en programas genéticos y en la selección de la salud y el producto individual; En estrategias de gestión de la salud que fortalezcan la interacción madre/hijo desde el nacimiento; En el que se garantice el calostro en tiempo y forma; En los que los tratamientos antihelmínticos respondan a un uso racional sobre poblaciones con cargas patológicas parasitarias, y que tengan un mínimo impacto ambiental (sobre insectos estiércol, ecosistemas de pastizales y factores edáficos), o sobre la dinámica de los ecosistemas; Donde el uso (restringido y cauteloso) de antimicrobianos impida la proliferación continua de resistencias, ni comprometa la salud humana. Construirlo mediante el mejoramiento genético para una interacción genotipo/ambiente favorable al desarrollo de una inmunidad óptima; A través del confort y garantía del bienestar animal; mediante pastoreo racional y de bajo impacto ambiental, en pastos polifitos, con sombra y mínimo uso de pesticidas. Fortalecer la salud de la población a través de controles estrictos para erradicar enfermedades como la Brucelosis y la Tuberculosis, lo que impactará positivamente en la salud pública. En poco tiempo tendremos múltiples posibilidades generadas por la bioingeniería molecular y la genética molecular para identificar y multiplicar genes reales de resistencia a estas enfermedades y otras de importancia económica que comprometen a los búfalos. En conclusión, la producción bufalina y el trópico representan elementos con extraordinario potencial para generar sinergias positivas y promover modelos agroecológicos que conduzcan a la mitigación del cambio climático al promover el almacenamiento de carbono en el suelo y reducir las emisiones de GEI, neutralizando los efectos adversos sobre los suelos y la vegetación y, en última instancia, contribuyendo a la regeneración del ecosistema. Sin embargo, para que funcione es necesario generar un conocimiento profundo de las especies animales, del ecosistema y de los principios ecológicos del pastoreo, capacidades que los productores deben desarrollar.

Palabras clave: resiliente, salud de los búfalos, sostenibilidad, una sola salud, emisiones de gases de efecto invernadero.

PREGNANCY-ASSOCIATED GLYCOPROTEINS IN BUFFALO: ORIGINS, FUNCTIONS AND CLINICAL APPLICATION FOR PREGNANCY FOLLOW-UP

Glicoproteínas asociadas a la preñez en búfala: orígenes, funciones y aplicación clínica para el seguimiento de la preñez

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ABSTRACT

Reproductive efficiency represents an essential aspect of farm management as the productivity and profitability of a farm depend on it, particularly in dairy farming. Early detection of pregnancy and identification of non-pregnant buffalo cows is fundamental in the management of reproduction because it allows an early resynchronization and rebreeding of the non-pregnant animals, shortening the calving-conception interval. Different pregnancy diagnostic tools are currently available; among these, the utilization of pregnancy-associated glycoproteins (PAGs) contributed significantly to the improvement of reproductive management. PAGs are secreted by the placenta of several ruminant species, including buffalo, and can be detected in the maternal blood. In addition to their utilization as diagnostic tools to identify pregnant or non-pregnant animals, PAGs help identify the presence of vital embryos and predict future embryo mortality during early gestation. This paper presents an overview of the PAGs origins, function, pregnancy trend, and their use to improve reproductive management in buffalo farming.

Keywords: pregnancy-associated glycoproteins, buffalo, pregnancy, reproductive management.

RESUMEN

La eficiencia reproductiva representa un aspecto esencial de la gestión ganadera ya que de ella dependen la productividad y la rentabilidad de una explotación, especialmente en la producción lechera. La detección temprana de la gestación y la identificación de búfalas no gestantes es fundamental en

el manejo de la reproducción porque permite una resincronización temprana y una nueva reproducción de los animales no gestantes, acortando el intervalo parto-concepción. Actualmente se encuentran disponibles diferentes herramientas de diagnóstico de la preñez; entre ellos, la utilización de glicoproteínas asociadas a la preñez (PAGs) la cual contribuye significativamente a la mejora del manejo reproductivo. Las PAGs son secretados por la placenta de varias especies de rumiantes, incluida la búfala, y pueden detectarse en la sangre materna. Además de su utilización como herramientas de diagnóstico para identificar animales preñados o no preñados, las PAGs ayudan a identificar la presencia de embriones viables y predecir la mortalidad futura de los embriones durante la gestación temprana. Este artículo presenta una descripción general de los orígenes, la función, la tendencia de preñez y su uso de los PAG para mejorar el manejo reproductivo en la cría de búfalos.

Palabras clave: glicoproteínas asociadas al embarazo, búfalo, el embarazo, manejo reproductivo.

INTRODUCTION

In dairy production, reproductive performances and profitability are strongly associated. Declining fertility is a worldwide recognized problem that represents a major source of economic loss and culling of large ruminants [1]. Many factors contribute to the decline in reproductive efficiency; among these, embryonic mortality (EM) represents the major cause of reproductive failure. The EM following day 28 of pregnancy has a greater economic impact on dairy herd management because the delay in resynchronization and re-breeding of animals pro-

longs their intercalving. Identification of early embryonic mortality improves reproductive efficiency and pregnancy rate by decreasing the interval between AI services, thereby increasing the AI service rate [2]. So, identifying EM can help in rescheduling new inseminations of non-pregnant animals, reducing the environmental impact of the cow's unproductive period.

In the last decades, new technological approach in diagnostic methods has contributed to the development of the earlier diagnosis of pregnancy in cattle, including buffalo [3,4].

Pregnancy can be monitored using various methods, including direct methods such as rectal palpation or ultrasonography or indirect methods using biochemical markers that can be detected in the maternal blood of pregnant animals. Among these, pregnancy-associated glycoproteins (PAGs) have become a valuable tool for early pregnancy diagnosis and predicting embryonic mortality [4].

PAGS ORIGINS AND FUCTIONS

PAGs are a family of glycoproteins belonging to the subclass of aspartic proteases [5,6], proteolytic enzymes with an acid pH. They are characterized by the presence of aspartic acid residues surrounding the recognition sites and are similar to pepsin, renin, cathepsin D and E, and chymosin [7]. These proteins have a sequence that is 50% like pepsin [8], but a substitution of amino acids in the active site renders them enzymatically inactive [9]. Other authors successively showed that PAGs conserved features are typically found in functionally aspartic proteins; therefore, some possess proteolytic activity [10].

PAGs are expressed in the outer epithelial layer (chorion/trophectoderm) of the placenta in the eutherian species of Cetartiodactyla order (even-toed ungulate) [11]. They are synthesized by the mononucleate and binucleate trophoblastic cells, some of which are secreted into the maternal blood when the conceptus becomes more closely attached to the uterine wall and placentome formation begins [12,13]. From a phylogenetic point of view, PAGs can be grouped as "ancient" (originated about 87 million years ago), expressed in both mono- and binucleate trophoblastic cells (PAG-2 group), and "modern" (originated about 52 million years ago), expressed only in binucleate cells (PAG-1 group) [14]. Bovine PAG-2 coexists with Bovine PAG-1 in the trophoctoderm [15]; while PAG-1 is produced in binucleate cells of both inter cotyledonary and cotyledonary chorion, PAG-2 molecules are produced in both mononucleate and binucleate trophoblastic cells [13]. In ruminants, the PAGs gene family is particularly large and complex. It was estimated that cattle, sheep, and other pecoran mammals possess 100 or more PAG genes [16]. To date, 74 different complementary DNA (cDNA) of PAG genes (differing by at least 5% in nucleotide sequence) have been identified in species with a synepitheliochorial placenta. In bovine species, 22 PAG genes (boPAG-1 to boPAG-22) have been cloned and fully sequenced

[11,17], and many also in water buffalo species (wtPAG-1 [18]; wtPAG-2 to wtPAG-19 [15]).

Not all PAGs are present in the same stage of gestation, as some appear earlier and others later. Green et al. [11] have highlighted the existence of their spatially and temporally distinct expression patterns during pregnancy. Some PAGs are entirely expressed in the trophoctoderm, while others are localized mainly in binuclear cells: among the latter, some are present from mid to late pregnancy, while others already appear at day 25 but are absent in the advanced stages [11]. The different PAGs temporal expressions opened the way for their practical use and speculation of their potential physiological role.

Given that the peculiar characteristic of PAGs is the constant increase along the course of gestation, it is reasonable to think that this is related to their biological function. Since PAGs and PSPBs were identified, several authors have searched for a relationship between these molecules or their concentration profile and a local or systemic immunological function. Telogu et al. [10,19] having highlighted that some PAGs possess proteolytic activity, it seems possible that proteolytically active PAGs present at the placenta-uterine interface could process latent growth factors that are known to be present at that location [20]. Another possibility proposed by Wooding et al. [21] is that PAGs accumulating at the placenta-uterine interface might play a role in adhesion by acting as bridging molecules. Several works correlate the high concentrations of PAGs with the decrease in the activity of polymorphonuclear neutrophils [22], suggesting that the trophoblast PAGs production, influencing the maternal immunological status, could be a mechanism by which the conceptus protects itself from rejection. Austin et al. [23] attribute a hormonal role to PAGs in inducing the release of granulocyte chemotactic protein-2 (GCP-2), an alpha chemokine whose synthesis is induced by interferon-tau (IFN-tau) in early pregnancy. In support of a possible luteotropic role of the PAGs, the studies by Del Vecchio et al. [24] and Weems et al. [25] showed how these glycoproteins induce the release of prostaglandin (PG) E2 and progesterone from luteal cells and PGE2 from endometrial cells grown in vitro. For this reason, it was considered to represent one of the luteotropic factors of the placenta of ruminants [9].

Many authors have highlighted the relationship between PAGs synthesis and fetal well-being [7,26]. The relationship between PAGs and fetal well-being is clearly understood if we consider that these glycoproteins are secreted by the mono- and binucleate cells of the trophoctoderm, which migrate from the fetal to uterine tissue and fuse with maternal uterine epithelial cells to form the hybrid feto-maternal trinucleate cells, which are responsible for the releasing of glycoproteins in the maternal organism. This active process presupposes the presence of healthy trophoblastic tissue and, therefore, of a healthy embryo. If this condition fails, the source of production of the proteins themselves is missing. Furthermore, thanks to this "active migration", PAGs would play an essential role in remodeling fetal membranes during pregnancy [7].

The use of the PAGs is helpful in advancing a pregnancy diagnosis and obtaining information on embryonic and/or fetal well-being. The concentration decline in maternal circulation is linked to a reduction in the activity of trophoblast cells. Therefore, PAGs can be considered a marker of placental function and a direct tool to monitor the vitality of the conceptus.

PAGS IN REPRODUCTIVE MANAGEMENT

In bovine, PAGs have been detected in maternal blood as early as Days 15 to 22 after fecundation [27]. The detection results are more accurate from Days 28 to 30 onwards [28]. Their concentrations then triple, more precisely between 20 and 10 days prepartum, up to their maximum increase, which occurs about 5 - 10 days before calving [12]. Thus, detecting PAGs in the maternal circulation can be a helpful tool for early pregnancy diagnosis. After calving, the PAGs concentrations begin to disappear from maternal blood to return to the baseline levels but persist in appreciable concentration for 80-100 days [12,29]. Therefore, to diagnose pregnancy using the PAGs test in the early stage of the postpartum in cows, the interval between calving and AI should be at least 60 days [29]. In this case, post-calving interference with the residual PAGs in the peripheral circulation during the postpartum period can be minimal.

Recently, many authors have shown the relationship between PAGs level and embryonic losses [30-32], suggesting that determining PAGs concentration besides pregnancy diagnosis can also help to determine embryonic or fetal mortalities.

Unlike the bovine, buffalo species are characterized by reproductive seasonality that affects the efficiency of breeding programs, particularly during the spring and summer seasons with the daylight lengthening period, which corresponds to the low breeding period for buffalo [33,34]. It was observed a high-

er incidence of embryo loss (20 to 40%) in buffaloes that conceive during the daylight lengthening period, whereas a lower incidence (7%) was observed during decreasing daylight length [35,36].

PAGs determination in maternal blood represents one strategy for improving reproductive performance aimed at diagnosing pregnancy and shortening the calving-conception interval by rapidly identifying embryo losses and rebreeding non-pregnant buffalo cows. The first RIA system adopted for detecting PAGs molecules in buffalo was RIA-706, which uses antisera raised against caprine. This system was proved to be accurate for detecting pregnant buffaloes from days 28 and after mating [37,38]. More recently, the isolation and purification of PAGs from buffalo placenta allowed for developing a specific RIA system for buffalo (RIA 860) [39-42]. This system described buffalo plasma PAGs profiles during pregnancy and postpartum (FIG. 1) [41]. During gestation, PAGs concentration increased to day 105 and remained constant until parturition. PAGs concentrations decrease rapidly in the postpartum period, reaching minimum values (<1 ng/mL) on day 30. The rapid decrease in PAGs concentration during the postpartum is essential when using PAGs as an appropriate marker of pregnancy just after calving. Moreover, unlike bovines, they do not require a cut-off limit in postpartum animals to detect a new pregnancy.

As demonstrated by different authors [42-46], PAGs assay can be a reliable biomarker for early pregnancy detection and EM in buffalo (FIG. 2). Recently, Barile et al. [4] have shown that PAGs permitted the discrimination between buffalo that experienced embryonic mortality and those that maintained pregnancy starting from 25 days of gestation, defining the optimal cut-off value for predicting mortality in 1,1 ng/mL and 2.2 ng/mL at day 25 and 28 respectively, post artificial insemination (AI). Since PAGs reflect embryo well-being, the re-

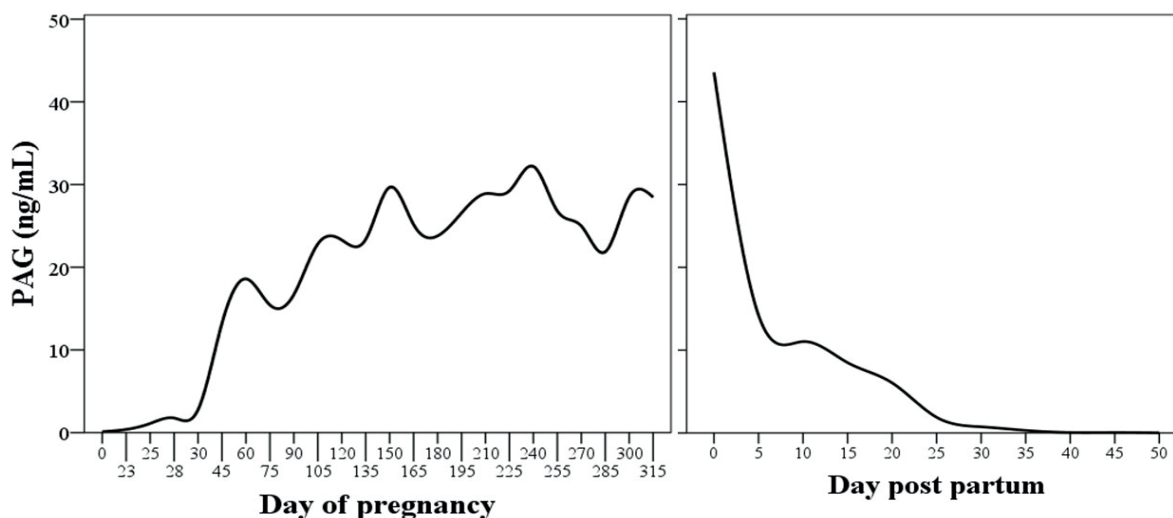


FIGURE 1. Pregnancy-associated glycoprotein (PAG) plasma profile during pregnancy and post-partum period in buffalo cows; adapted from Barbato et al [41].

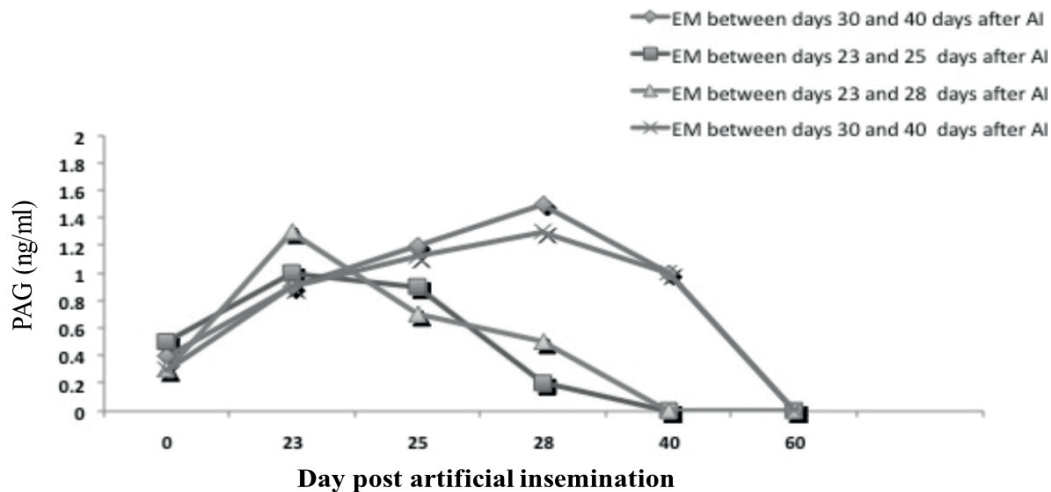


FIGURE 2. Pregnancy-associated glycoprotein (PAG) plasma concentrations in buffalo cows experienced embryonic mortality; adapted from Barbato and Barile [45]

duction of its circulating concentrations is a prognostic sign of pregnancy failure.

Therefore, PAGs could be utilized as a diagnostic tool to improve farm reproductive management through pharmacological intervention for animals at risk for embryo loss. In this case, a single injection of GnRH at day 35 after AI increased PAGs concentration and recovery of embryo well-being, allowing a reduction of pregnancy loss in the early pregnancy in buffalo at risk of EM [47]. GnRH treatment enhances embryo survival rates by delaying the luteolytic mechanism [48] that could occur due to failure in maternal recognition of pregnancy.

LATEST RESEARCH ON EARLY PREGNANCY MARKERS EXPRESSION

The peri-implantation period is an extremely delicate time in the establishment of pregnancy in which various mechanisms are involved in maintaining the corpus luteum (CL). One of the first molecules involved in early maternal recognition in ruminants is the interferon tau (*IFN τ*) [49]. This protein is secreted by the trophoctodermal cells of the blastocysts around days 16-25 in buffalo [50] and increases with the elongation of the conceptus. *IFN τ* prevents secretion of PGF2 α by uterine epithelium, prolonging CL life span and maintaining the progesterone production for the maternal recognition of pregnancy. *IFN- τ* enters circulation, triggering the expression of a group of molecules known as Interferon Stimulated Genes (*ISGs*). The members of *ISGs*, such as interferon-stimulated protein 15 (*ISG15*), myxovirus resistance protein 2 (*MX2*), and 2'-5'oligoadenylate synthetase (*OAS1*), are hypothesized to regulate endometrial receptivity to implantation as well as survival, growth, and development of the conceptus [51]. In addition to *IFN τ* , it is hypothesized that PAGs participate in immune modulation at the conceptus-maternal interface during

early pregnancy [23]. The correlation between *ISGs* and *IFN τ* expression and PAGs plasma concentration during peri-implantation and early pregnancy in buffalo cows [52] supports the possible immunomodulatory role for these glycoproteins at the maternal-fetal level, necessary for the establishment and the maintenance of the maternal-fetal unit histocompatibility, and to prevent luteolysis by inhibiting PGF2 α release, resulting in the maintenance CL function. When PAG-1, *IFN τ* and *ISGs* expression were used as diagnostic and prognostic markers of maternal-fetal cellular interaction in buffalo cows, *ISGs* proved to be a more reliable peripheral biomarker for the prediction of pregnancy and embryonic mortality in buffalo during the peri-implantation period [53]. The results of this study showed that *MX2* and *MX1* were the best genes to discriminate pregnancy or embryonic mortality, respectively, at 19 days post-AI.

Studies on PAG expression in the maternal subset of blood leukocytes at the peri-implantation period in buffalo showed that the quantification of PAG-2 mRNA could be used to differentiate pregnant and non-pregnant buffaloes starting from the second-week post-AI [54,55].

At the moment, PAG-2, as well as *ISGs* expression, could be a valuable marker for studies on early pregnancy and embryonic mortality but not for use in the field because of the single analysis's cost and procedure.

CONCLUSIONS

In veterinary practice, the PAGs measurement is a feasible method used for early pregnancy diagnosis, pregnancy confirmation, and follow-up of the embryo vitality. The first aspect can help breeders manage reproduction, allowing an early resynchronization and rebreeding of the non-pregnant animals, aiming to shorten the calving-conception interval.

The identification of subjects at risk of embryonic mortality can influence management decisions for the recovery of those animals that would experience pregnancy loss, i.e., through pharmacologic intervention strategies for the maintenance of pregnancy.

Finally, quantifying PAG mRNA expression could be a helpful tool to understand better the mechanisms involved in embryonic mortality, supporting studies on embryonic implantation and pregnancy failures.

REFERENCES

- [1] Deka, R.P.; Magnusson, U.; Grace, D.; Randolph, T.F.; Shome, R.; Lindahl, J.F. Estimates of the economic cost caused by five major reproductive problems in dairy. *Animals* **2021**, *11*, 3116.
- [2] Fricke, P.M. Scanning the future – ultrasonography as a reproductive management tool for dairy cattle. *J Dairy Sci* **2002**, *85*, 1918–26.
- [3] Fricke, P.M.; Ricci, A.; Giordano, J.O.; Carvalho, P.D. Methods for and implementation of pregnancy diagnosis in dairy cows. *Vet. Clin. Food Anim. Pract.* **2016**, *32*, 165–180.
- [4] Barile, V.L.; Menchetti, L.; Casano, A, B.; Brecchia, G.; Sousa, N.M.; Zelli, R.; Canali, C.; Beckers, S.F.; Barbato, O. Approaches to Identify Pregnancy Failure in Buffalo Cows. *Animals* **2021**, *1*, 481.
- [5] Xie, S.C.; Low, B.G.; Nagel, R.J.; Kramer, K.K.; Anthony, R.V., Zoli, A.P.; Beckers, J.F. & Roberts, R.M. Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of the aspartic proteinase family. *PNAS* **1991**, *88*, 10247–10251.
- [6] Green, J.A.; Xie, S.; Roberts, R.M. Pepsin-related molecules secreted by trophoblast. *Reviews of Reproduction* **1998**, *3*, 62–69.
- [7] Beckers, J.F.; Drion, P.V.; Garbayo, J.M., Perenyi Z.; Zarrrouk, A. Sulon, J.; Remy, B.; Szenci, O. Pregnancy-associated glycoproteins in ruminantes: inactive members of the aspartic proteinase family. *Acta Veterinaria Hungarica* **1999**, *47*, 461-469.
- [8] Roberts, R.M.; Xie, S.; Mathialagan, N. Maternal recognition of pregnancy. *Biology of Reproduction* **1996**, *54*, 294-302.
- [9] Xie, S.; Low, B.G.; Nagel, R.J.; Beckers, J.F.; Roberts, R.M. A novel glycoprotein of the aspartic proteinase gene family expressed in bovine placental trophectoderm. *Biol Reprod* **1994**, *51*, 1145-1153.
- [10] Telugu, B.P.; Palmier, M.O.; Van Doren, S.R.; Green, J.A. An examination of the proteolytic activity for bovine pregnancy-associated glycoproteins 2 and 12. *Biological Chemistry* **2010**, *391*, 259–270.
- [11] Green, J.A.; Xie, S.; Quan, X.; Bao, B.; Mathialagan, N.; Beckers, J.F.; Roberts, R.M. Pregnancy-associated bovine and ovine glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy. *Biol Reprod* **2000**, *62*, 1624-1631.
- [12] Zoli, A.P.; Guilbault, L.A.; Delahaut, P.; Ortiz, W.B.; Beckers, J.F. Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: its application for pregnancy diagnosis. *Biology of Reproduction* **1992**, *46*, 83–92.
- [13] Touzard, E.; Reinaud, P.; Dubois, O.; Guyader-Joly, C.; Humblot, P.; Ponsart, C.; Charpigny, G. Specific expression patterns and cell distribution of ancient and modern PAG in bovine placenta during pregnancy. *Reproduction* **2013**, *146*, 347–362.
- [14] Hughes, A.L.; Green, J.A.; Garbayo, J.M.; Roberts, R.M. Adaptive diversification within a large family of recently duplicated, placentally-expressed genes. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3319–3323.
- [15] Wallace, R.M.; Pohler, K.G.; Smith, M.F.; Green, J.A. Placental PAGs: Gene origins, expression patterns, and use as markers of pregnancy. *Reproduction* **2015**, *149*, R115–R126
- [16] Xie, S.; Green, J.A.; Bao, B.; Beckers, J.F.; Valdez, K.E.; Hakami, L.; Roberts, R.M. Multiple pregnancy-associated glycoproteins are secreted by day 100 ovine placental tissue. *Biol Reprod* **1997**, *57*, 1384–1393.
- [17] Garbayo, J.M.; Serrano, B.; Lopez-Gatius, F. Identification of novel pregnancy-associated glycoproteins (PAG) expressed by the perimplantation conceptus of domestic ruminants. *Anim Reprod Sci* **2008**, *103*, 120–134
- [18] Jerome, A.; Singh, S.K.; Agarwal, S.K.; Mohini, S.; Raut A. Characterization and In Silico analysis of pregnancy-associated glycoprotein-1 gene of buffalo (*Bubalus bubalis*). *Genet Res Int* **2011**, 436138.
- [19] Telugu, B.P.; Green, J.A. Characterization of the peptidase activity of recombinant porcine pregnancy-associated glycoprotein-2. *Journal of Biochemistry* **2008**, *144*, 725–732.
- [20] Moussad, E.E.; Rageh, M.A.; Wilson, A.K.; Geisert, R.D.; Brigstock, D.R. Temporal and spatial expression of connective tissue growth factor (CCN2; CTGF) and transforming growth factor beta type 1 (TGF- β 1) at the utero-placental interface during early pregnancy in the pig. *Journal of Clinical Pathology: Molecular Pathology* **2002**, *55*, 186-192
- [21] Wooding, F.B.; Roberts, R.M.; Green, J.A. Light and electron microscope immunocytochemical studies of

- the distribution of pregnancy-associated glycoproteins (PAGs) throughout pregnancy in the cow: possible functional implications. *Placenta*, **2005**, *26*, 807–827.
- [22] Dosogne, H.; Burvenich, C.; Freeman, A.E.; Kehrl Jr, M.E.; Detilleux, J.C.; Sulon, J.; Beckers, J.F.; Hoeben, D. Pregnancy-associated glycoprotein and decreased polymorphonuclear leukocyte function in early post-partum dairy cows. *Vet Immunology and immunopathology* **1999**, *67*, 47-54.
- [23] Austin, K.J.; King, C.P.; Vierk, J.E.; Sasser, R.G.; Hansen, T.R. Pregnancy-specific protein B induces release of an alpha chemokine in bovine endometrium. *Endocrinology* **1999**, *140*, 542–545.
- [24] Del Vecchio, R.P.; Sutherland, W.D.; Sasser, R.G. Bovine luteal cell production in vitro of prostaglandin E2, oxytocin and progesterone in response to pregnancy-specific protein B and prostaglandin F2a. *Journal of Reproduction and Fertility* **1996**, *107*, 131–136.
- [25] Weems, Y.S.; Lammoglia, M.A.; Vera-Avila, H.R.; Randel, R.D.; King, C.; Sasser, R.G.; Weems, C.W. Effect of luteinizing hormone (LH), PGE2, 8-Epi- PGE1, 8-EPI-PGE2, trichosanthin, and pregnancy specific protein B on secretion of progesterone in vitro by corpora lutea (CL) from nonpregnant and pregnant cows. *Prostaglandins & Other Lipid Mediators* **1998**, *55*, 27–42.
- [26] Kornmatitsuk, B.; Veronesi, M.C.; Madej, A.; Dahl, E.; Ropstad, E.; Beckers, J.F.; Forsberg, M.; Gustalsson, H.; Kindhal, H. Hormonal measurements in late pregnancy and parturition in dairy cows-possible tools to monitor fetal well being. *Anim Reprod Sci* **2002**, *72*, 153-164.
- [27] Giordano, J.O.; Guenther, J.N.; Lopes, G. Jr; Fricke, P.M. Changes in serum pregnancy-associated glycoprotein, pregnancy-specific protein B, and progesterone concentrations before and after induction of pregnancy loss in lactating dairy cows. *Journal of Dairy Science* **2012**, *95*, 683–697.
- [28] Karen, A.; De Sousa, N.M.; Beckers, J.F.; Bajcsy, A.C.; Tibold, J.; Madl, I.; Szenci, O. Comparison of a commercial bovine pregnancy-associated glycoprotein ELISA test and pregnancy-associated glycoprotein radioimmunoassay test for early pregnancy diagnosis in dairy cattle. *Anim Reprod Sci* **2015**, *159*, 31-37.
- [29] Haugejorden, G.; Waage, S.; Dahl, E.; Karlberg, K.; Beckers, J.F.; Ropstad, E. Pregnancy associated glycoproteins (PAG) in postpartum cows, ewes, goats and their offspring. *Theriogenology* **2006**, *66*, 1976-1984.
- [30] Franco, G.A.; Peres, R.F.G.; Martins, C.F.G.; Reese, S.T.; Jose Luiz Moraes Vasconcelos, J.L.M.; Pohler, K.J. Sire contribution to pregnancy loss and pregnancy-associated glycoprotein production in Nelore cows. *J. Anim. Sci.* **2018**, *96*, 632–640.
- [31] Reese, S.T., Geary, T.W.; Franco, G.A.; Moares, J.G.N.; Spencer, T.E.; Pohler, K.G. Pregnancy associated glycoproteins (PAGs) and pregnancy loss in high vs sub fertility heifers. *Theriogenology* **2019**, *135*, 7-12.
- [32] Pohler, K.G.; Reese, S.T.; Franco, G.A.; Oliveira, R.V.; Paiva, R.; Fernandez, L.; de Melo, G.; Vasconcelos, J.L.M.; Cooke, R.; Poole, R.K. New approaches to diagnose and target reproductive failure in cattle. *Anim. Reprod.* **2020**, *17*. [
- [33] Barile, V.L. Improving reproductive efficiency in female buffaloes. (Review). *Liv. Prod. Science*, **2005**, *92*, 183-194.
- [34] Barile, V.; Terzano, G.; Pacelli, C.; Todini, L.; Malfatti, A.; Barbato, O. LH peak and ovulation after two different estrus synchronization treatments in buffalo cows in the daylight-lengthening period. *Theriogenology* **2015**, *84*, 286–293.
- [35] Baruselli, P.; Visintin, J.; Barnabe, V.; Barnabe, R.; Amaral, R.; Souza, A. Early pregnancy ultrasonography and embryonic mortality occurrence in buffalo. In Proceedings of the V World Buffalo Congress, **1997**. Caserta, Italy, 13–18 October 1997; pp. 13–16.
- [36] Campanile, G.; Neglia, G.; D’Occhio, M.J. Embryonic and fetal mortality in river buffalo (*Bubalus bubalis*). *Theriogenology*, **2016**, *86*, 207-213.
- [37] Karen, A.; Darwish, S.; Ramoun, A.; Tawfeek, K.; Van Hanh, N.; De Sousa, N.; Sulon, J.; Szenci, O.; Beckers, J.-F. Accuracy of ultrasonography and pregnancy-associated glycoprotein test for pregnancy diagnosis in buffaloes. *Theriogenology* **2007**, *68*, 1150–1155.
- [38] El-Battawy, K.A.; Sousa, N.M.; Szenci, O.; Beckers, J.F. Pregnancy-associated glycoprotein profile during the first trimester of pregnancy in Egyptian buffalo cows. *Reprod Domest Anim Zuchthyg* **2009**, *44*, 161–166.
- [39] Barbato, O.; Sousa, N.M.; Klisch, K.; Clerget, E.; Debenedetti, A.; Barile, V.L.; Malfatti, A.; Beckers, J.F. Isolation of new pregnancy-associated glycoproteins from water buffalo (*Bubalus bubalis*) placenta by Vicia villosa affinity chromatography. *Res Vet Sci* **2008**, *85*, 457–466.
- [40] Barbato, O.; Melo de Sousa, N.; Barile, V.L.; Canali, C.; Beckers, J.-F. Purification of pregnancy-associated glycoproteins from late-pregnancy *Bubalus bubalis* placentas and development of a radioimmunoassay for pregnancy diagnosis in water buffalo females. *BMC Vet Res* **2013**, *9*, 89.
- [41] Barbato, O.; Menchetti, L.; Sousa, N.M.; Malfatti, A.; Brecchia, G.; Canali, C.; Beckers, J.F.; Barile, V.L. Pregnancy-associated glycoproteins (PAGs) concentrations in water buffaloes (*Bubalus bubalis*) during gestation and the postpartum period. *Theriogenology* **2017**, *97*, 73–77.

- [42] Barbato, O.; Menchetti, L.; Sousa, N.M.; Brecchia, G.; Malfatti, A.; Canali, C.; Beckers J.F.; Barile, V.L. Correlation of two radioimmunoassay systems for measuring plasma pregnancy-associated glycoprotein concentrations during early pregnancy and postpartum periods in water buffalo. *Reprod Domest Anim* **2018**, *53*, 1483-149.
- [43] Karen. A.; Darwish, S.; Ramoun, A.; Tawfeek, K.; Van Hanh, N.; Sousa, N.M.; Sulon. J.; Szenci, O.; Beckers, J.F. Accuracy of ultrasonography and pregnancy-associated glycoprotein test for pregnancy diagnosis in buffaloes. *Theriogenology* **2007**, *68*, 1150–1155.
- [44] Nguyen, V.H.; Barbato, O.; Bui, X.N.; Beckers, J.-F.; de Sousa, N.M. Assessment of pregnancy-associated glycoprotein (PAG) concentrations in swamp buffalo samples from fetal and maternal origins by using interspecies antisera. *Anim Sci J* **2012**, *83*, 683–689.
- [45] Barbato, O.; Barile, V.L. The pregnancy diagnosis in buffalo species: laboratory methods. *J Buffalo Sci* **2012**, *1*, 157-162.
- [46] Barbato, O.; Chiaradia, E.; Barile, V.L.; Pierri, F.; Sousa, M.N.; Terracina, L.; Canali, C.; Avellini, L.; Investigation into omocysteine, vitamin E and malondialdehyde as indicators of successful artificial insemination in synchronized buffalo cows (*Bubalus bubalis*). *Research in Veterinary Science* **2016**, *104*, 100-105.
- [47] Pacelli, C.; Barile, V.L.; Sabia, E.; Casano, A.B.; Braghieri, A.; Martina, V.; Barbato, O. Use of GnRH Treatment Based on Pregnancy-Associated Glyco-Proteins (PAGs) Levels as a Strategy for the Maintenance of Pregnancy in Buffalo Cows: A Field Study. *Animals* **2022**, *12*, 2822.
- [48] Mann, G.E.; Lamming, G.E.; Fray, M.D. Plasma oestradiol and progesterone during early pregnancy in the cow and the effects of treatment with buserelin. *Anim. Reprod. Sci.* **1995**, *37*, 121–131.
- [49] Thatcher, W.W.; Meyer, M.D.; Danet-Desnoyers, G. Maternal Recognition of Pregnancy. *J. Reprod. Fertil.-Suppl.* **1995**, *49*, 15–28.
- [50] Saugandhika, S.; Sharma, V.; Malik, H.; Saini, S.; Bag, S.; Kumar, S.; Singh, N.K.; Mohanty, A.K.; Malakar, D. Expression and Purification of Buffalo Interferon-Tau and Efficacy of Recombinant Buffalo Interferon-Tau for in Vitro Embryo Development. *Cytokine* **2015**, *75*, 186–196.
- [51] Rocha CC, Silveira JC, Forde N, Binelli M, Pugliesi G. Conceptus-modulated innate immune function during early pregnancy in ruminants : a review. *Anim Reprod.* **2021**,*18*(1) :e20200048.
- [52] Casano, A.B; Barile, V.L.; Menchetti, L.; Guelfi, G.; Brecchia, G.; Agradi, S.; De Matteis, G.; Scatà, M.C.; Grandoni, F.; Barbato, O. Interferon Tau (IFNt) and Interferon-Stimulated Genes (ISGs) Expression in Peripheral Blood Leukocytes and Correlation with Circulating Pregnancy-Associated Glycoproteins (PAGs) during Peri-Implantation and Early Pregnancy in Buffalo Cows. *Animals*, **2022**, *12*, 3068.
- [53] Casano A.B. Menchetti L., Trabalza-Marinucci M., Riva F, De Matteis G., Brecchia G., Inglesi A, Rossi E., Signorelli F., Barile V.L., Barbato O. Gene expression of pregnancy-associated glycoproteins-1 (PAG-1), interferon-tau (IFNt) and interferon stimulated genes (ISGs) as diagnostic and prognostic markers of maternal-fetal cellular interaction in buffalo cows. *Theriogenology*, **2023**, *209*, 89-97.
- [54] Barbato O, Guelfi G, Barile VL, Menchetti L, Tortiello C, Canali C, Brecchia G, Traina G, Beckers JF, Melo de Sousa N. 2017. Using real-time PCR to identify pregnancy-associated glycoprotein 2 (PAG-2) in water buffalo (*bubalus bubalis*) blood in early pregnancy. *Theriogenology* **2017**, *89*, 106-113.
- [55] Barbato, O.; Guelfi, G.; Menchetti, L.; Brecchia, G.; Sousa, M.N.; Canali, C.; Grandoni, F.; Scatà, M.C.; De Matteis, G.; Casano, A.B.; Beckers, J.F.; Barile, V.L. Investigation of PAG2 mRNA Expression in Water Buffalo Peripheral Blood Mononuclear Cells and Polymorphonuclear Leukocytes from Maternal Blood at the Peri-Implantation Period. *Veterinary Sciences* **2019**, *6*, 8.

BREEDING FOR SUSTAINABILITY: HOW REPRODUCTIVE BIOTECHNOLOGIES CAN HELP BUFFALO FARMERS COMBAT CLIMATE CHANGE

Reproduciendo para la sostenibilidad: cómo las biotecnologías reproductivas pueden ayudar a los criadores de búfalos a combatir el cambio climático

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ABSTRACT

The global attention on enteric CH₄ production in ruminants requires a response that involves collaboration between researchers and industry. Future generations of buffaloes will be characterized by better efficiency and fertility, which may reduce CH₄ emission intensity. This goal will result from balanced multi-trait selection and the introduction of efficient reproductive and productive management. Currently, efficient reproductive programs using assisted reproductive technologies (ARTs) are available on buffalo farms. Our expanding knowledge of ovarian function during the buffalo estrous cycle has given new approaches for precisely synchronizing follicular development and ovulation to apply ARTs consistently. Synchronization protocols are designed to control both luteal and follicular function and permit fixed-time AI with high pregnancy rates during the breeding (autumn-winter) and non-breeding (spring-summer) seasons. Additionally, it allows the initiation of superstimulatory treatments at a self-appointed time, providing opportunities to superstimulate buffalo donors associated with ovum pick-up (OPU) and *in vitro* embryo production (IVEP). Furthermore, it allows fixed-time embryo transfer in recipients, with high efficiency and no need for estrus detection. Thus, ARTs, such as AI and ET, are applied for buffalo's targeted multiplication and dispersal with defined production and environmental credentials. Also, the urgency in moving to the next generation of buffaloes will increase the production of embryos from genomically defined prepubertal heifers. Using these biotechnologies will reduce generation interval and accelerate the rate

of genetic improvement to buffalo, defined by better efficiency and fertility and lower CH₄ emission. The challenge remains to communicate the importance of buffaloes for food security and the environment.

Keywords: enteric methane, efficiency, fertility, assisted reproductive technology.

RESUMEN

La atención mundial sobre la producción de CH₄ entérico en rumiantes requiere una respuesta que implique la colaboración entre investigadores y la industria. Las generaciones futuras de búfalos se caracterizarán por una mayor eficiencia y fertilidad, lo que puede reducir la intensidad de las emisiones de CH₄. Este objetivo será el resultado de una selección equilibrada de múltiples rasgos y la introducción de un manejo reproductivo y productivo eficiente. Actualmente, las granjas de búfalos cuentan con programas reproductivos eficientes que utilizan tecnologías de reproducción asistida (ART). Nuestro creciente conocimiento sobre la función ovárica durante el ciclo estral de las búfalas ha brindado nuevos enfoques para sincronizar con precisión el desarrollo folicular y la ovulación para aplicar las ART de manera consistente. Los protocolos de sincronización están diseñados para controlar la función lútea y folicular y permitir la IA a tiempo fijo (IATF) con altas tasas de preñez durante las temporadas de reproducción (otoño-invierno) y no reproductiva (primavera-verano). Además, permite el inicio de tratamientos de superestimulación en el momento que usted

elija, brindando oportunidades para superestimular a los donantes de búfalos asociados con la recogida de óvulos (OPU) y la producción de embriones *in vitro* (IVEP). Además, permite la transferencia de embriones a tiempo fijo (TETF) en las receptoras, con alta eficiencia y sin necesidad de detección de estro. Por lo tanto, las ART, como la IA y la ET, se aplican para la multiplicación y dispersión selectiva del búfalo con credenciales ambientales y de producción definidas. Además, la urgencia de pasar a la próxima generación de búfalos aumentará la producción de embriones a partir de novillas prepúberes genómicamente definidas. El uso de estas biotecnologías reducirá el intervalo generacional y acelerará la tasa de mejora genética del búfalo, definida por una mayor eficiencia y fertilidad y una menor emisión de CH₄. El desafío sigue siendo comunicar la importancia de los búfalos para la seguridad alimentaria y el medio ambiente.

Palabras clave: metano entérico, eficiencia, fertilidad, tecnología de reproducción asistida.

INTRODUCTION

The world's population is projected to increase by 24% by 2050, potentially reaching 9.7 billion people [1]. Food production must increase by 49% to sustain this population explosion [2]. In this scenario, urbanization, and growing concerns about the environmental impact of livestock farming demand a long-term global strategy for more sustainable ruminant production. Buffalo, therefore, will continue to have a significant role in future global food security. The global buffalo population is approximately 202 million head [3], compared to 1.5 billion cattle [4].

Buffalo milk and meat products can meet human needs for high-quality protein. They excel over cattle exploiting low-quality feed typical of many rearing areas and demonstrate great adaptability to various management and temperature conditions [5]. Furthermore, most buffalo production is carried out extensively in pastures and savannas suited for low-input and low-cost animal production. In South Asia, the River buffalo is a primary source of milk and meat and has a crucial role in food security. The riverine buffalo also supports high-value, differentiated food production in Europe and the Americas. The Swamp buffalo is a vital draft animal and a source of food in Southeast Asia and East Asia.

However, the environmental impact of ruminant production has gained significant attention worldwide [6, 7, 8]. Cattle contribute around 4.5-5.0% of global anthropogenic methane [9]. Enteric fermentation, with an annual emission of 87-97 Tg (i.e., 1012g), is one of the agricultural sector's significant methane sources [10]. The global contribution of cattle and buffalo to annual enteric methane emissions is 77% and 13%, respectively [11]. The primary source of methane in ruminants originates from the enteric fermentation process, where complex carbohydrates are converted into simple sugars by methano-

genic protozoa [12]. Extensive reviews have comprehensively covered the biology and function of the rumen [13, 14]. The quantity of methane an animal produces is significantly influenced by the relative abundance of ruminal methanogenic and non-methanogenic microbes [12]. Microbial gene abundance analysis advancements allow for determining ruminal microbe populations [15]. In addition to enteric methane (CH₄) produced by the rumen, beef, and dairy production also contributes carbon dioxide (CO₂; feed), nitrous oxide (N₂O; feed production, manure), and other CH₄ (manure) to the total greenhouse gas (GHG) budget of the production systems.

Malik et al. [16] compared the enteric methane yield between cattle and buffaloes under the same nutritional management. Enteric methane emissions (g/d) depended on dry matter intake (kg/d). However, the methane yield (g/kg dry matter intake; DMI) did not differ between species when fed on the same diet (Cattle=13.4 g/kg DMI vs. Buffaloes=13.5 g/kg DMI). This result confirms that methane yield depends on the diet rather than the species compared. Thus, methane mitigation strategies developed in one of the species can be effective in the other.

In this scenario, the use of assisted reproductive technologies can have a significant impact on improving efficiency in buffalo production systems. Reproductive technology has been progressively refined in buffaloes, and today, the success of artificial insemination and embryo transfer is comparable to cattle. Artificial insemination (AI), combined with estrus synchronization, is a potent strategy of assisted reproduction technology to improve reproductive efficiency and expedite genetic gain in buffaloes [16]. Furthermore, embryo transfer (ET) enables the multiplication of high maternal and paternal genetic value, playing a more significant role in the genetic enhancement of this species [17]. This review seeks to demonstrate how assisted reproductive technologies (ARTs) can improve reproductive efficiency and harvest the next generation of buffaloes that produce more milk and meat to combat climate change.

REPRODUCTIVE EFFICIENCY IN BUFFALO AND APPLICATION OF ARTIFICIAL INSEMINATION TO IMPROVE PRODUCTION AND REDUCE METHANE EMISSION

The cow-calf operation system utilizes approximately 70% of resources. Therefore, selection for reproductive efficiency significantly affects farm efficiency, profitability, and sustainability. With high reproductive efficiency, fewer cows are required to produce the next generation of calves, reducing resource requirements, herd methane production, and costs [18]. Furthermore, assisted reproduction technologies can also be used to manipulate reproduction in buffalo. This includes synchronization of the breeding time, influencing the age at first breeding, the interval between the calving, and improving the breeding during seasonal anestrus [16].

Artificial insemination can be incorporated into buffalo breeding programs to further improve reproductive efficiency and genetic gain, collaborating to reduce CO₂-eq emission intensity. However, the traditional AI program efficiency needs to be improved by low estrous detection. Buffalo presents a poor manifestation of estrus symptoms, implying operational difficulties in detecting estrus [19].

Furthermore, the success of reproductive programs is closely related to the buffalo reproductive seasonality. Buffalo is a seasonal reproductive species and becomes sexually active in response to a decreasing day length (short days) in late summer to early autumn [20, 21]. During the non-breeding season, buffalo often exhibit anestrus, which extends the anovulatory period and reduces reproductive performance [22].

Nowadays, timed artificial insemination (TAI) can be applied routinely in farm reproductive programs. TAI protocols are designed to control both luteal and follicular function, permitting the AI without estrus detection and during the anestrous period with high reproductive efficiency during the breeding and non-breeding season [19, 23, 24]. Several studies demonstrate that it is possible to establish an effective AI program in buffaloes throughout the year, collaborating to increase the number of pregnant buffaloes during the non-breeding season and distributing calving and milk production throughout the year. Using reproductive programs with TAI followed by resynchronization,

it is possible to obtain high reproductive efficiency (>80% pregnancy rate after 3 FTAI) with inter-calving intervals close to 12 months (FIG. 1; adapted from Baruselli et al. [25]).

The efficiency of TAI in buffalo demonstrates that it is possible to introduce efficient artificial insemination programs on farms that collaborate to increase the reproductive and genetic efficiency of the herds.

EMBRYO TECHNOLOGY TO MITIGATE METHANE EMISSION

In vivo, (superovulation; SOV) and *in vitro* (ovum pick-up and *in vitro* embryo production; OPU/IVEP) embryo productions are reproductive biotechnologies used worldwide in beef and dairy operations to disseminate the genetic material of superior animals. Selection and genetic gain are essential to improve efficiency, product quality, and sustainability [16]. When comparing both biotechnologies in buffalo, OPU/IVEP demonstrates higher efficiency and greater commercial applicability than SOV. However, there are some limitations to using OPU/IVEP, such as seasonality, the low number of antral follicles, and the low quantity and quality of the recovered oocytes [17].

Experiments have been conducted to enhance OPU/IVEP efficiency. In one study, Sá Filho et al. [26] demonstrated

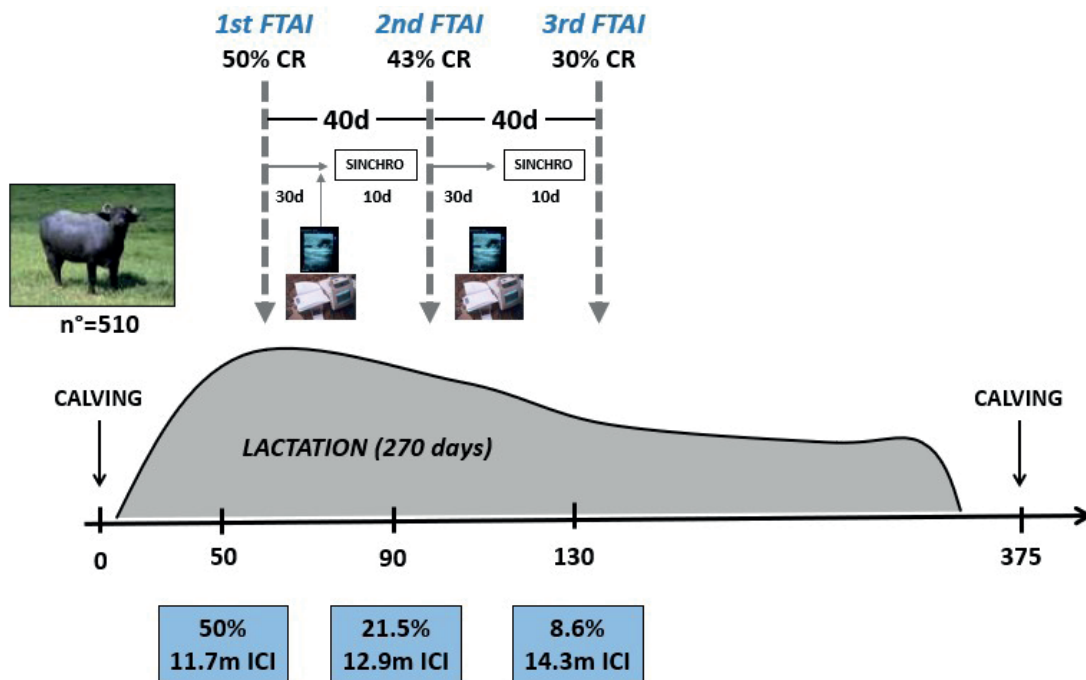


FIGURE 1. Conception rate (P/AI) of lactating buffalo (n=510) submitted to fixed time AI following resynchronization in non-pregnant cows. Ultrasonography evaluation was performed to detect non-pregnant buffaloes 30 days after AI for re-synchronization. Pregnant buffaloes from the 1st FTAI with 50 days postpartum presented a 50% conception rate (CR) and 11.7 months of inter-calving interval (ICI). Pregnant buffaloes from the 2nd FTAI with 90 days postpartum presented a 43% CR and 12.9 months of ICI. Pregnant buffaloes from the 3rd FTAI with 130 days postpartum presented a 30% CR and 14.3 months of ICI. After 3 FTAI, buffaloes presented an 80.1% pregnancy rate with a mean of 12.3 months of ICI (adapted from Baruselli et al., 2003)

that using bST increased the number of small antral follicles at OPU. Additionally, bST tended to increase the number of recovered oocytes and improved the percentage of high-quality oocytes. However, bST showed no effect on cleavage and blastocyst production rates. In another study, Carvalho et al. [27] showed that FSH treatment for superstimulation before aspiration improved the outcomes of OPU/IVEP. FSH treatment increased the proportion of large and medium follicles at OPU and enhanced the viable oocyte rate, blastocyst rate, and number of embryos produced per OPU session.

The use of OPU/IVEP in females before puberty, apart from the genetic gain inherent in this biotechnology, also reduces the generation intervals, further accelerating genetic improvement. This technology can be employed in prepubertal buffalo heifers, where ovaries have established follicular waves and respond to superstimulation, or in buffalo calves, where OPU is performed via laparoscopy [LOPU; 28, 29, 30]. LOPU permits the recovery of oocytes from calves of two months of age and the *in vitro* production of embryos that will be transferred to recipients. This technology allows a donor animal to produce offspring before it reaches sexual maturity. The use of young donors has two main key points that make this alternative interesting: the first one is the larger follicular population, and the number of cumulus-oocyte complexes (COCs) recovered, and the second is the shorter generation interval, increasing genetic gain [30, 31].

In a study conducted by our group, we compared embryo production in buffalo calves (2-4 months of age), prepubertal buffalo heifers (13-15 months of age), and lactating buffalo cows [28]. The treatment for calves involved using a sheep intravaginal P4 device on day 0 of the protocol, and for stimulating follicular growth, 140 mg of FSH was administered in four decreasing doses every 12 hours on days 5 and 6. On day 7, oocytes were recovered by LOPU in calves and through OPU on a random day of the estrous cycle in prepubertal heifers and lactating cows. The results showed that calves had a lower blastocyst production rate, but the number of embryos produced was similar between calves and lactating cows. Embryos produced from calves ($n=8$) resulted in three pregnancies (3/8; 38%), which led to the birth of three healthy calves [28]. This study demonstrated the feasibility of IVEP in young animals to reduce generation interval and significantly accelerate genetic progress in buffaloes. However, calves were less efficient in embryo production than prepubertal heifers and cows, and further research is needed to optimize IVEP in young buffalo [30].

Regarding the impact of assisted reproductive techniques on methane emissions in cattle operations, IVEP of oocytes retrieved from young animals presents a viable approach to achieving genetic gain and reducing generation intervals [8]. Although the efficiency of IVEP in young animals is relatively lower due to hormonal and metabolic differences, its integration with genomic selection offers a powerful strategy to enhance genetic gain, efficiency, and fertility, as well as mitigate methane emissions in buffalo operations [32].

BALANCING FEED EFFICIENCY IN MEAT AND MILK PRODUCTION WITH FERTILITY AND LOW CO₂-EQ EMISSION

Ruminants are crucial in maintaining sustainable agricultural systems due to their distinctive capacity to transform forages into high-quality meat and dairy products [33]. The link between feed efficiency, methane production, and sustainability has been known for over 20 years [34, 35, 5]. The relatively high heritability of growth and feed efficiency in cattle was recognized some 70 years ago and subsequently confirmed [6, 36, 37, 38].

Furthermore, in tropical and subtropical regions, the conjunction of elevated temperatures and humidity during the summer months leads to the onset of reproductive problems, decreasing milk and meat production in buffaloes [39, 40, 41, 42]. Implementing management techniques, such as active cooling, is imperative to alleviate these stressors and uphold a certain level of productivity. Additionally, the summer season decreases feed quantity and quality, compounding the nutritional challenges that impact reproductive capabilities [39, 42]. Beyond photoperiod, it is essential to address external influences that detrimentally affect reproduction and production to fully capitalize on the potential afforded by the worldwide demand for buffalo food items. Methods encompass targeted nutritional enrichment, assisted reproductive technologies (ARTs) application, and managerial tactics (such as cooling techniques and ample resting areas) to enhance buffalo welfare within naturally endowed and non-endowed production setups.

Buffalo farming has transitioned to a more intensive model, utilizing a feeding system structured around three distinct rations corresponding to the primary buffalo production stages: lactating cows, dry cows, and growing heifers. Their diet primarily comprises maize silage and ryegrass hay, with additional concentrates reserved solely for lactating buffalo cows [43]. These farming conditions developed for buffalo production in Italy entail the absence of pasture access and wallowing water.

Recent studies suggest that incorporating more digestible forages into ruminant diets may mitigate CO₂ emissions, even within intensive systems [44]. Despite this, the cumulative emissions of free-ranging (FR) animals exceeded those of confined (C) systems by approximately 662 kg CO₂-eq. This discrepancy stemmed from the animals in the FR system consuming a greater volume of fibrous feed than the C heifers. At puberty, the heifers reached a weight of 402±3 and 382±3 kg in systems C and FR, respectively. Differences between groups were significant ($p<0.05$) due to the higher feeding regimen of group C, the higher physical activity performed while grazing by group FR, and the lower environmental temperature of the hilly area where this group was located.

Nevertheless, these animals reached puberty at an age not significantly different from that observed in group C ($p>0.05$; [45]). This finding has been attributed to the fact that grazing

animals used the available resources (pasture and feeding supplementation) efficiently. In contrast, confined heifers used spare nutrients only to increase their body mass after fulfilling their requirements for development [46].

In temperate regions, buffalo experience a distinct seasonal reproductive pattern influenced by photoperiod and melatonin secretion, as indicated by previous research [20, 47, 21]. Optimal conditions lead to a resumption of anoestrus in buffalo within 30–90 days postpartum. However, factors including inadequate nutrition and poor body condition [48], suckling management [49], and climate [50] can significantly delay this process. For instance, buffalo in Sri Lanka under free grazing with limited calf access to dams for suckling once a day resumed estrous cycles within 30–60 days, whereas those exposed to harsher conditions and free calf suckling remained in anestrus for 150–200 days [51]. Buffalo's postpartum LH secretion remains low initially, with detectable episodic pulses a few weeks before ovarian activity starts. Improved nutrition and controlled suckling prompt LH release earlier than those with poor nutrition or free suckling [52, 53]. There are recommended methods to overcome extended postpartum anestrus in buffalo, including ensuring proper nutrition before and after calving, regulating calf suckling, and alleviating heat stress through activities like wallowing or using water sprinklers [54], improving the reproductive and productive efficiency.

Limited research has been conducted on evaluating the environmental repercussions of dairy buffalo farms on environmental sustainability. In a study, Pirlo et al. [55] found that the ecological footprint of dairy buffalo farms, quantified in terms of global warming potential, amounted to 5.07 kg of CO₂ equivalent per 1 kg of standardized buffalo milk. This figure is nearly fivefold greater than that generated by dairy cow farms [56]. This disparity could be attributed to the similarity in energy inputs and raw material acquisition between dairy buffalo and cow farms, coupled with comparatively lower milk production from buffalo.

According to Chirone et al. [57], buffaloes' milk productivity varies from farm to farm and is a key factor determining environmental performance. The remaining differences are explained by a combination of the type of feed (including the portion cultivated in-house and purchased) and the strategy for managing manure. These findings reinforce the importance of increasing the genetic capacity of buffaloes to produce milk and meat more efficiently.

Buffaloes exhibit notable feed conversion efficiency and sustain productivity even when subjected to diets limiting for cattle [58]. In a previous investigation, metabolic condition and reproductive performance were observed in Murrah buffalo heifers fed either a high-energy (HE) or low-energy (LE) diet [58]. Heifers following the HE diet displayed elevated plasma levels of insulin, leptin, and T3, along with increased concentrations of IGF-1 in follicular fluid and a higher oocyte quality index. These outcomes highlight the positive effect of the nu-

trition improving the reproduction performance and production in buffaloes.

Recently, the currently used methods of estimating the carbon footprint of processed animal products and dairy products should consider the subtraction of carbon emissions and sequestration. According to De Vivo et al. [59], considering carbon sequestration and implementing this calculation method would demonstrate sustainability regarding the carbon footprint of agricultural products of animal origin, such as buffalo dairy products (Mozzarella cheese) and meat products.

ENTERIC METHANE IN PRODUCTION SYSTEM: LIFE CYCLE ASSESSMENT (LCA)

Enteric methane forms part of beef and dairy production systems' broader greenhouse gas (GHG) budget [60]. The broader GHG budget includes methane, nitrous oxide (N₂O), and CO₂ emissions from manure, feed production, vehicles and transport, and other plants and equipment. The total GHG budget of a production system is determined by life cycle assessment (LCA) methodology, standardized by ISO 14040 [61] and ISO 14044 [62] ([63, 64]).

Recently, studies were carried out to evaluate the impact of buffalo production on greenhouse gas emissions [65, 66, 57, 67].

In collaboration with Embrapa Research Institute and Cargill, our research group has studied the LCA of a buffalo milk production farm in Brazil. The data showed that enteric methane produced by buffaloes is the most relevant source of GHG production. One estimate of LCA for buffalo milk production was 63.4% for enteric methane (CH₄), 33.9% for feed production (CO₂, N₂O), and 1.92% for manure (FIG. 2).

As enteric methane has the most significant impact on the production of CO₂ equivalent, the reduction in production cycles (reduction in age at first calving and the interval between calving) and an increase in individual production (milk and meat) contribute significantly to the farm's sustainability. Furthermore, new technologies can potentially manipulate the rumen microbiome through genetic selection and various dietary intervention strategies to reduce CH₄ emissions. According to Yusuf et al. [68], methane reduction strategies have been grouped into three crucial factors: management, nutrition, and the use of advanced biotechnology. Manipulation of the rumen in reducing methane using chemicals, feed additives, roughage, concentrate utilization, and plants containing secondary compound oils has been reported [5]. Using technologies to reduce methane emissions from these crucial factors in the production chain will considerably impact the sustainability of buffalo farming).

Studies in Italy found that, despite the methane production on buffalo farms, the amount of greenhouse gases converted into CO₂-eq emitted during the buffalo dairy pro-

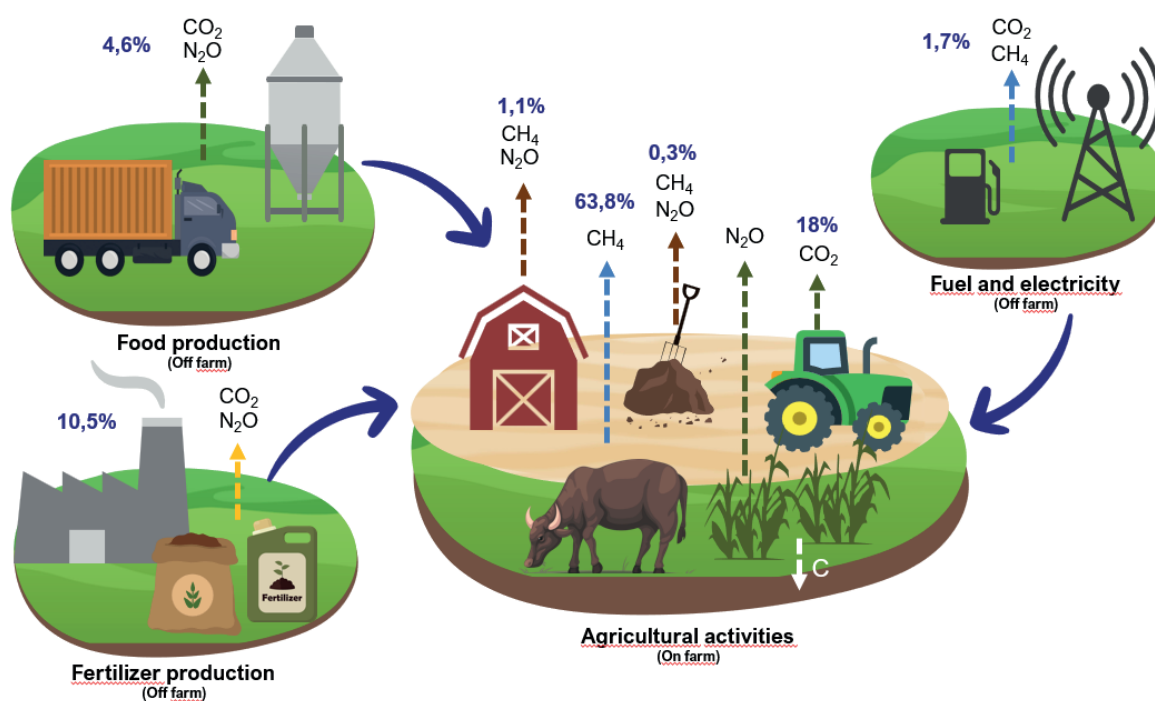


FIGURE 2. Main emission pathways related to a buffalo dairy farm in Brazil, quantified by life cycle assessment (LCA) methodology [Methodology standardized by ISO 14040 (ISO, 2006a) and ISO 14044 (ISO, 2006b); de Vries et al., 2015; Kyttä et al., 2022]. On-farm emissions represent 83.2% (agriculture activities), and off-farm emissions represent 16.8% (food production, fertilizer production, and fuel and electricity; Adapted from Abreu et al., 2023; data not yet published)

duction system is lower than the CO_2 -eq removed from the atmosphere [59]. The authors found that for every kg of buffalo Mozzarella cheese produced, 52 kg of CO_2 -eq is subtracted from the atmosphere (differences between CO_2 -eq emissions from the production system and CO_2 -eq removal from the atmosphere).

This information demonstrates that it is possible to produce beef and buffalo meat in balance with the environment, if appropriate technologies based on scientific information are used.

CONCLUSIONS AND FUTURE DIRECTION

The global attention on enteric CH_4 production in buffaloes requires a response that involves collaboration between researchers and industry. Future generations of buffaloes will be characterized by better efficiency and fertility, which may reduce CH_4 emission intensity. This will result from a balanced multi-trait selection and improved management. Artificial insemination can be incorporated into buffalo breeding programs to further improve reproductive efficiency and genetic gain, collaborating to reduce CO_2 -eq emission intensity. The urgency in moving to the next generation of buffaloes will increase the production of embryos from genomically defined prepubertal heifers. This will reduce generation interval and accelerate the rate of genetic improvement to buffaloes defined by better efficiency

and fertility and lower CH_4 emission. The growing importance of buffaloes in the world requires that they undergo an accelerated rate of genetic gain for efficiency of production, product quality, and sustainability. The challenge remains to develop integrated sustainable meat and milk production systems and communicate the importance of buffaloes for food security and the environment.

REFERENCES

- [1] United Nations Department of Economic and Social Affairs, Population Division. World Population Prospects 2022: Summary of Results. UN DESA/POP/2022/TR/NO. 3.
- [2] FAO. Sustainable Development Goals (SDG). End hunger, achieve food security, and improved nutrition, and promote sustainable agriculture. 2019. Available: <http://www.fao.org/sustainable-development-goals/news/detail-news/en/c/424259/>
- [3] Zhang Y, Colli L, Barker JSF. Asian water buffalo: Domestication, history and genetics. *Animal Genetics*. 2020; 51(2): 177-191. <https://doi.org/10.1111/age.12911>
- [4] FAO. Crops and livestock products. License: CC BY-NC-SA 3.0 IGO. Extracted from: <https://www.fao.org/faostat/en/#data/QCL>. Data of Access: 27-09-2023.

- [5] Wanapat M, Kang SC. World buffalo production: Challenges in meat and milk production, and mitigation of methane emission. *Buffalo Bulletin*. 2013, 32(1): 1–21.
- [6] Knapp Jr B, Nordskog AW. Heritability of growth and efficiency in beef cattle. *Journal of Animal Science*. 1946; 5(1): 62-70. <https://doi.org/10.2527/jas1946.5162>
- [7] Faverdin P, Guyomard H, Puillet L, Forslund A. Animal board invited review: Specialising and intensifying cattle production for better efficiency and less global warming: contrasting results for milk and meat co-production at different scales. *Animal*. 2022; 16(1): 100431. <https://doi.org/10.1016/j.animal.2021.100431>
- [8] Baruselli PS, Abreu LA, Paula VR, Carvalho B, Gricio EA, Mori FK, Rebeis LM, Albertini S, Souza AH, D’Occhio MJ. Applying assisted reproductive technology and reproductive management to reduce CO₂-equivalent emission in dairy and beef cattle: a review. *Animal Reproduction*. 2023; 20(2): e20230060. <https://doi.org/10.1590/1984-3143-AR2023-0060>
- [9] Galyean ML, Hales KE. Feeding management strategies to mitigate methane and improve production efficiency in feedlot cattle. *Animals*. 2023; 13(4): 758. <https://doi.org/10.3390/ani13040758>
- [10] Chang J, Peng S, Ciais P, Saunois M, Dangal SRS, Herrero M, Havlík P, Tian H, Bousquet P. Revisiting enteric methane emissions from domestic ruminants and their $\delta^{13}\text{CCH}_4$ source signature. *Nature Communications*. 2019; 10: 3420. <https://doi.org/10.1038/s41467-019-11066-3>
- [11] FAO. Reducing enteric methane for improving food security and livelihoods. 2021. Available: <http://www.fao.org/in-action/enteric-methane/background/why-is-enteric-methane-important/en/>
- [12] Bowen JM, Cormican P, Lister SJ, McCabe MS, Duthie CA, Roehe R, Dewhurst RJ. Links between the rumen microbiota, methane emissions and feed efficiency of finishing steers offered dietary lipid and nitrate supplementation. *PLoS One*. 2020; 15(4): e0231759. <https://doi.org/10.1371/journal.pone.0231759>
- [13] Ross EM, Moate PJ, Maret L, Cocks BG, Hayes BJ. Investigating the effect of two methane-mitigating diets on the rumen microbiome using massively parallel sequencing. *Journal of Dairy Science*. 2013; 96(9): 6030-6046. <https://doi.org/10.3168/jds.2013-6766>
- [14] Knapp JR, Laur GL, Vadas PA, Weiss WP, Tricarico JM. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. *Journal of Dairy Science*. 2014; 97(6): 3231-3261. <https://doi.org/10.3168/jds.2013-7234>
- [15] Roehe R, Dewhurst RJ, Duthie CA, Rooke JA, McKain N, Ross DW, Hyslop JJ, Waterhouse A, Freeman TC, Watson M, Wallace RJ. Bovine host genetic variation influences rumen microbial methane production with best selection criterion for low methane emitting and efficiently feed converting hosts based on metagenomic gene abundance. *PLoS Genetics*. 2016; 12(2): e1005846. <https://doi.org/10.1371/journal.pgen.1005846>
- [16] Baruselli PS, Carvalho NAT, Gasparrini B, Campanile G, D’Occhio MJ. Review: Development, adoption, and impact of assisted reproduction in domestic buffaloes. *Animal*. 2023; 17(S1): 100764. <https://doi.org/10.1016/j.animal.2023.100764>
- [17] Malik PK, Trivedi S, Mohapatra A, Kolte AP, Sejian V, Bhatta R, Rahman H. Comparison of enteric methane yield and diversity of ruminal methanogens in cattle and buffaloes fed on the same diet. *PLoS ONE*. 2021; 16(8): e0256048. <https://doi.org/10.1371/journal.pone.0256048>
- [18] Baruselli, PS, Carvalho JGS, Elliff FM, Da Silva JCB, Chello D, Carvalho NAT. Embryo transfer in buffalo (*Bubalus bubalis*). *Theriogenology*. 2020; 150: 221-228. <https://doi.org/10.1016/j.theriogenology.2020.01.037>
- [19] Hegarty RS, McEwan JC. Genetic opportunities to reduce enteric methane emissions from ruminant livestock. In ‘Proceedings of the Ninth World Congress on Genetics Applied to Livestock Production’; 2010; Leipzig, Germany. Leipzig: German Society for Animal Science. 2010. pp 181-186.
- [20] Baruselli PS, Carvalho NAT, Gimenes LU, Crepaldi GA. Fixed-time artificial insemination in buffalo. *Italian Journal of Animal Science*. 2007; 6(S2): 107-118. <https://doi.org/10.4081/ijas.2007.s2.107>
- [21] Zicarelli L. Reproductive seasonality in buffalo. *Bubalus Bubalis*. 1997; 4(29): 52–54.
- [22] D’Occhio MJ, Ghuman SS, Neglia G, Della Valle G, Baruselli PS, Zicarelli L, Visintin JA, Sarkar M, Campanile G. Exogenous and endogenous factors in seasonality of reproduction in buffalo: A review. *Theriogenology*. 2020; 150: 186-192. <https://doi.org/10.1016/j.theriogenology.2020.01.044>
- [23] Zicarelli L. Can we consider buffalo a non precocious and hypofertile species? *Italian Journal of Animal Science*. 2007; 6(S2): 143-154. <https://doi.org/10.4081/ijas.2007.s2.143>
- [24] Carvalho NAT, Soares JG, Porto Filho RM, Gimenes LU, Souza DC, Nichi M, Sales JS, Baruselli PS. Equine chorionic gonadotropin improves the efficacy of a timed artificial insemination protocol in buffalo during the non-breeding season. *Theriogenology*. 2013; 79(3): 423-428. <https://doi.org/10.1016/j.theriogenology.2012.10.013>
- [25] Monteiro BM, Souza DC, Vasconcellos GSFM, Carvalho NAT, Baruselli PS. Effect of season on dairy buffalo reproductive performance when using P4/E2/eCG-based fixed-time artificial insemination management. *Therio-*

- genology. 2018; 119: 275-281. <https://doi.org/10.1016/j.theriogenology.2018.07.004>
- [26] Baruselli PS, Madureira EH, Barnabe VH, Barnabe RC, Berber RCA. Evaluation of synchronization of ovulation for fixed timed insemination in buffalo (*Bubalus bubalis*). *Brazilian Journal of Veterinary Research and Animal Science*. 2003; 40(6): 431-442. <https://doi.org/10.1590/S1413-95962003000600007>
- [27] Sá Filho MF, Carvalho NAT, Gimenes LU, Torres-Júnior JR, Nasser LFT, Tonhati H, Garcia JM, Gasparini B, Zicarelli L, Baruselli, PS. Effect of recombinant bovine somatotropin (bST) on follicular population and on in vitro buffalo embryo production. *Animal Reproduction Science*. 2009; 113(1-4): 51-59. <https://doi.org/10.1016/j.anireprosci.2008.06.008>
- [28] Carvalho JGS, Carvalho NAT, Bayeux BM, Watanabe YF, Watanabe OY, Mingoti RD, Baruselli PS. Superstimulation prior to the ovum pick-up improves the in vitro embryo production in nulliparous, primiparous and multiparous buffalo (*Bubalus bubalis*) donors. *Theriogenology*. 2019; 138: 164-168. <https://doi.org/10.1016/j.theriogenology.2019.07.003>
- [29] Silva JCB, Rezende RG, Colli MHA, Bayeux, BM, Mingoti RD, Ojeda-Rojas OA, Basso AC, Naves JR, Baruselli PS. In vitro embryo production in buffalo: Comparison between calves, prepubertal heifers and lactating cows. *Animal Reproduction*. 2017; 14(3): 766.
- [30] Baldassarre H, Bordignon V. Laparoscopic ovum pick-up for in vitro embryo production from dairy bovine and buffalo calves. *Animal Reproduction*. 2018; 15(3): 191-196. <https://doi.org/10.21451/1984-3143-AR2018-0057>
- [31] Baruselli PS, Soares JG, Bayeux BM, Da Silva JCB, Mingoti RD, Carvalho NAT. Assisted reproductive technologies (ART) in water buffaloes. *Animal Reproduction*. 2018; 15(1): 971-983. <http://dx.doi.org/10.21451/1984-3143-AR2018-0043>
- [32] Currin L, Baldassarre H, Bordignon V. In vitro production of embryos from prepubertal Holstein cattle and Mediterranean water buffalo: Problems, progress and potential. *Animals*. 2021; 11(8): 2275. <https://doi.org/10.3390/ani11082275>
- [33] Baruselli PS, Rodrigues CA, Ferreira RM, Sales JNS, Elliff FM, Silva LG, Viziack MP, Factor L, D'Occhio MJ. Impact of oocyte donor age and breed on in vitro embryo production in cattle, and relationship of dairy and beef embryo recipients on pregnancy and the subsequent performance of offspring: A review. *Reproduction, Fertility and Development*. 2021; 34(2): 36-51. <https://doi.org/10.1071/RD21285>
- [34] Cammack KM, Austin KJ, Lamberson WR, Conant GC, Cunningham HC. Ruminant Nutrition Symposium: Tiny but mighty: The role of the rumen microbes in livestock production. *Journal of Animal Science*. 2018; 96(2): 752-770. <https://doi.org/10.1093/jas/skx053>
- [35] Johnson DE, Ward GM. Estimates of animal methane emissions. *Environmental Monitoring and Assessment*. 1996; 42: 133-141. <https://doi.org/10.1007/BF00394046>
- [36] Arthur PF, Herd RM. Efficiency of feed utilisation by livestock - Implications and benefits of genetic improvement. *Canadian Journal of Animal Science*. 2005; 85(3): 281-290. <https://doi.org/10.4141/A04-062>
- [37] Berry DP, Crowley JJ. Cell Biology Symposium: Genetics of feed efficiency in dairy and beef cattle. *Journal of Animal Science*. 2013; 91(4): 1594-1613. <https://doi.org/10.2527/jas2012-5862>
- [38] Gonzalez-Recio O, Pryce JE, Haile-Mariam M, Hayes BJ. Incorporating heifer feed efficiency in the Australian selection index using genomic selection. *Journal of Dairy Science*. 2014; 97(6): 3883-3893. <https://doi.org/10.3168/jds.2013-7515>
- [39] Sypniewski M, Strabel T, Pszczola M. Genetic variability of methane production and concentration measured in the breath of Polish Holstein-Friesian cattle. *Animals*. 2021; 11(11): 3175. <https://doi.org/10.3390/ani11113175>
- [40] Perera BMAO. Reproductive cycles of buffalo. *Animal Reproduction Science*. 2011; 124(3-4): 194-199. <https://doi.org/10.1016/j.anireprosci.2010.08.022>
- [41] Khan FA, Das GK, Pande M, Sarkar M, Mahapatra RK, Shankar U. Alterations in follicular fluid estradiol, progesterone and insulin concentrations during ovarian acyclicity in water buffalo (*Bubalus bubalis*). *Animal Reproduction Science*. 2012; 130(1-2): 27-32. <https://doi.org/10.1016/j.anireprosci.2011.12.020>
- [42] Abdoon AS, Gabler C, Holder C, Kandil OM, Einspanier R. Seasonal variations in developmental competence and relative abundance of gene transcripts in buffalo (*Bubalus bubalis*) oocytes. *Theriogenology*. 2014; 82(8): 1055-1067. <https://doi.org/10.1016/j.theriogenology.2014.07.008>
- [43] Phogat JB, Pandey AK, Singh I. Seasonality in buffaloes reproduction. *International Journal of Plant and Animal Sciences*. 2016; 6(2): 46-54.
- [44] Sabia E, Napolitano F, Claps S, Braghieri A, Piazzolla, N, Pacelli C. Feeding, nutrition and sustainability in dairy enterprises: the case of Mediterranean buffaloes (*Bubalus bubalis*). In: Vastola, A. (Ed.), *The Sustainability of Agro-food and Natural Resource Systems in the Mediterranean Basin*. 2015. Springer Open, pp. 57-64. https://doi.org/10.1007/978-3-319-16357-4_5
- [45] Sabia E, Claps S, Napolitano F, Annicchiarico G, Bruno A, Francaviglia R, Sepe L, Aleandri R. In vivo digestibility

- of two different forage species inoculated with arbuscular mycorrhiza in Mediterranean red goats. *Small Ruminant Research*. 2015; 123: 83-87. <https://doi.org/10.1016/j.smallrumres.2014.10.008>
- [46] Sabia E, Napolitano F, Salvatore C, De Rosa G, Barile VL, Braghieri A, Pacelli C. Environmental impact of dairy buffalo heifers kept on pasture or in confinement. *Agricultural Systems*. 2018; 159: 42-49. <https://doi.org/10.1016/j.agsy.2017.10.010>
- [47] Sabia E, Napolitano F, De Rosa G, Terzano GM, Barile VL, Braghieri A, Pacelli C. Efficiency to reach age of puberty and behaviour of buffalo heifers (*Bubalus bubalis*) kept on pasture or in confinement. *Animal*. 2014; 8(11): 1907-1916. <https://doi.org/10.1017/S1751731114001876>
- [48] Borghese A. Buffalo Production and Research. Technical Series 67. 2005. Rome, Italy: Food and Agriculture Organization of the United Nations.
- [49] Baruselli OS, Barnabe VH, Barnabe RC, Visintin JA, Molero-Filho JR, Porto R. Effect of body condition score at calving on postpartum reproductive performance in buffalo. *Buffalo Journal*. 2001; 1: 53-65.
- [50] Usmani RH, Dailey RA, Inskeep EK. Effects of limited suckling and varying prepartum nutrition on postpartum reproductive traits of milked buffaloes. *Journal of Dairy Science*. 1990; 73(6): 1564-1570. [https://doi.org/10.3168/jds.S0022-0302\(90\)78826-1](https://doi.org/10.3168/jds.S0022-0302(90)78826-1)
- [51] Nanda AS, Brar PS, Prabhakar S. Enhancing reproductive performance in dairy buffalo: Major constraints and achievements. *Reproduction Supplement*. 2003; 61: 27-36.
- [52] Perera BMAO, de Silva LNA, Kuruwita VY, Karunaratne AM. Postpartum ovarian activity, uterine involution and fertility in indigenous buffaloes at a selected village location in Sri Lanka. *Animal Reproduction Science*. 1987; 14(2): 115-127. [https://doi.org/10.1016/0378-4320\(87\)90091-1](https://doi.org/10.1016/0378-4320(87)90091-1)
- [53] Mohan V, Kuruwita VY, Perera BMAO, Abeygunawardena H. Effects of suckling on the resumption of postpartum ovarian activity in buffaloes. *Tropical Agricultural Research*. 1990; 2: 306-315.
- [54] Singh AK, Brar PS, Nanda AS, Prakash BS. Effect of suckling on basal and GnRH-induced LH release in postpartum dairy buffaloes. *Animal Reproduction Science*. 2006; 95(3-4): 244-250. <https://doi.org/10.1016/j.anireprosci.2005.10.004>
- [55] Perera BMAO, Abeygunawardena H, Vale WG, Chantalakhana C. Buffalo. In: *Livestock and Wealth Creation – Improving the Husbandry of Animals Kept by Poor People in Developing Countries*. Livestock Production Programme. Natural Resources International Limited. UK. 2005; pp 451–471.
- [56] Pirlo G, Caré S, Fantin V, Falconi F, Buttol P, Terzano GM, Masoni P, Pacelli C. Factors affecting life cycle assessment of milk produced on 6 Mediterranean buffalo farms. *Journal of Dairy Science*. 2014; 97(10): 6583-6593. <http://dx.doi.org/10.3168/jds.2014-8007>
- [57] O'Brien D, Shalloo L, Patton J, Buckley F, Grainger C, Wallace M. A life cycle assessment of seasonal grass-based and confinement dairy farms. *Agricultural Systems*. 2012; 107: 33-46. <https://doi.org/10.1016/j.agsy.2011.11.004>
- [58] Chirone R, Paulillo A, Salatino P, Salzano A, Cristofaro B, Cristiano T, Campanile G, Neglia G. Life cycle assessment of buffalo milk: A case study of three farms in southern Italy. *Journal of Cleaner Production*. 2022; 365: 132816. <https://doi.org/10.1016/j.jclepro.2022.132816>
- [59] Campanile G, Baruselli PS, Vecchio D, Prandi A, Neglia G, Carvalho NAT, Sales JNS, Gasparrini B, D'Occhio MJ. Growth, metabolic status and ovarian function in buffalo (*Bubalus bubalis*) heifers fed a low energy or high energy diet. *Animal Reproduction Science*. 2010; 22(1-2): 74-81. <https://doi.org/10.1016/j.anireprosci.2010.07.005>
- [60] De Vivo R, Zicarelli L, Napolano R, Zicarelli F. Calculation method of the carbon footprint of products of animal origin integrated with the physiological absorption of carbon dioxide: Calculation example of the CFP of mozzarella di Bufala Campana DPO. *Advances in Environmental and Engineering Research*. 2023; 4(3): 044. <https://doi.org/10.21926/aeer.2303044>
- [61] Ibdhi R, Calsamiglia S. Carbon footprint assessment of Spanish dairy cattle farms: Effectiveness of dietary and farm management practices as a mitigation strategy. *Animals*. 2020; 10(11): 2083. <https://doi.org/10.3390/ani10112083>
- [62] ISO 14040. Environmental management - life cycle assessment - principles and framework. 2006. Reference number ISO 14040:2006(E).
- [63] ISO 14044. Environmental management - life cycle assessment - requirements and guidelines. 2006. Reference number ISO 14044:2006(E).
- [64] de Vries M, van Middlelaar CE, de Boer IJM. Comparing environmental impacts of beef production systems: A review of life cycle assessments. *Livestock Science*. 2015; 178: 279-288. <https://doi.org/10.1016/j.livsci.2015.06.020>
- [65] Kyttä V, Roitto M, Aastapsev A, Saarinen M, Tuomisto HL. Review and expert survey of allocation methods used in life cycle assessment of milk and beef. *The International Journal of Life Cycle Assessment*. 2022; 27: 191-204. <https://doi.org/10.1007/s11367-021-02019-4>
- [66] Ijaz M, Goheer MA. Emission profile of Pakistan's agriculture: past trends and future projections. *Environment*,

- Development and Sustainability. 2021; 23: 1668-1687. <https://doi.org/10.1007/s10668-020-00645-w>
- [67] Romano E, De Palo P, Tidona F, Maggiolino A, Braggaglio A. Dairy buffalo Life Cycle Assessment (LCA) affected by a management choice: The production of wheat crop. Sustainability. 2021; 13(19): 11108. <https://doi.org/10.3390/su131911108>
- [68] Correddu F, Lunesu MF, Caratzu MF, Pulina G. Recalculating the global warming impact of italian livestock methane emissions with new metrics. Italian Journal of Animal Science. 2023; 22(1): 125-135. <https://doi.org/10.1080/1828051X.2023.2167616>
- [69] Yusuf RO, Noor ZZ, Abba AH, Abu Hassan MA, Mohd Din MF. Greenhouse gas emissions: Quantifying methane emissions from livestock. American Journal of Engineering and Applied Sciences. 2012; 5(1): 1-8. <https://doi.org/10.3844/ajeassp.2012.1.8>

INTEGRATED TECHNIQUE OF BUFFALO FERTILITY MANAGEMENT IN NEPAL

Técnica integrada de gestión de la fertilidad de los búfalos en Nepal

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ABSTRACT

Water buffalo (*Bubalus bubalis*) in Nepal contributes 57% of the total milk and 36% of the total meat production in the country. Even though the buffalo farming systems range from large-scale and semi-intensive, with herd sizes of more than 50 animals, to small-scale, intensive systems in which farmers keep 1 to 5 animals, while large-scale farms are very few, and medium and small-scale farms predominate in the country. The productive efficiency of Nepalese buffaloes is relatively low, due mainly to subfertility and infertility. Delayed puberty and prolonged inter-calving intervals, attributed mainly to anestrus due to silent cyclicity and ovarian acyclicity, are the major forms of infertility in Nepalese buffaloes. Moreover, buffaloes in Nepal show a distinct seasonal breeding pattern, with July to December as the active breeding season and April to June and January to March as the low and transitional breeding seasons, respectively. It is understood that acyclicity with inactive ovaries is a major problem during the transition and low breeding season; however, silent cyclicity is expected during the good seasons. Among several factors compromising the treatment response of anestrus buffaloes in terms of pregnancy outcome, poor body condition score, sub-normal levels of some blood metabolic parameters, and gastrointestinal parasitic infection are more common. Putting together all those information, we developed an integrated technique of buffalo fertility management that includes anthelmintic treatment at least one month prior to the initiation of specific timed artificial insemination (TAI) protocol and nutritional management by means of providing vitamin–mineral supplementation in addition to improving the roughage supply and energy-protein content in the ration soon after anthelmintic treatment until two weeks after AI. Specific TAI protocol includes Ovsynch during the good season and CIDR co-synch during the transition and low seasons, as silent ovulation during the good season and acyclicity during the other two seasons are the major causes of anestrus. The TAI is

important in medium and small-scale farms as the owner is responsible for heat detection, which is challenging in buffaloes. The integrated technique has succeeded in anestrus buffaloes, particularly during the low breeding season. The broader adoption of this technique as a package of practices could be a key to improving the reproductive efficiency of buffaloes in Nepal and other countries with similar farming systems.

Keywords: anestrus, fertility management, nutritional status, seasonality, water buffalo.

RESUMEN

El búfalo de agua (*Bubalus bubalis*) en Nepal aporta el 57% de la producción total de leche y el 36% de la producción total de carne del país. Generalmente, los sistemas de cría de búfalos varían desde sistemas a gran escala y semi-intensivos, con rebaños de más de 50 animales, hasta sistemas intensivos a pequeña escala en los que los agricultores mantienen de 1 a 5 animales, mientras que las granjas a gran escala son muy pocas, y en el país predominan las explotaciones agrícolas de mediana y pequeña escala. La eficiencia productiva de los búfalos nepaleses es relativamente baja, debido principalmente a la subfertilidad y la infertilidad. El retraso de la pubertad y los intervalos prolongados entre partos, atribuidos principalmente al anestro debido a la ciclicidad silenciosa y la aciclicidad ovárica, son las principales formas de infertilidad en las búfalas nepalesas. Además, los búfalos en Nepal muestran un patrón de reproducción estacional distinto, siendo de julio a diciembre la temporada de reproducción activa y de abril a junio y de enero a marzo las temporadas de reproducción baja y de transición, respectivamente. Se entiende que la aciclicidad con ovarios inactivos es un problema importante durante la transición y la temporada baja de reproducción; sin embargo, se espera un comportamiento cíclico silencioso durante las buenas temporadas. Entre varios factores que comprometen la respuesta al

tratamiento de los búfalos en anestro en términos de resultado del embarazo, los más comunes son la mala condición corporal, los niveles subnormales de algunos parámetros metabólicos sanguíneos y las infecciones parasitarias gastrointestinales. Reuniendo toda esa información, desarrollamos una técnica integrada de manejo de la fertilidad de las búfalas que incluye tratamiento antihelmíntico al menos un mes antes del inicio de un protocolo específico de inseminación artificial a tiempo fijo (IATF) y manejo nutricional mediante el suministro de suplementos de vitaminas y minerales además de mejorar el suministro de forraje y el contenido de energía y proteínas en la ración poco después del tratamiento antihelmíntico hasta dos semanas después de la IA. El protocolo IATF específico incluye Ovsynch durante la buena temporada y co-sincroniza-

ción con dispositivos de P4 (CIDR) durante la transición y las temporadas bajas, ya que la ovulación silenciosa durante la buena temporada y la aciclicidad durante las otras dos temporadas son las principales causas del anestro. La IATF es importante en granjas de mediana y pequeña escala, ya que el propietario es responsable de la detección del celo, lo cual es un desafío en las búfalas. La técnica integrada ha tenido éxito en búfalos anestro, particularmente durante la temporada baja de reproducción. La adopción más amplia de esta técnica como un paquete de prácticas podría ser clave para mejorar la eficiencia reproductiva de los búfalos en Nepal y otros países con sistemas agrícolas similares.

Palabras clave: anestro, manejo de la fertilidad, estados nutricionales, estacionalidad, búfalo de agua.

IMPLEMENTING GENOMIC SELECTION IN THE IMB: CHALLENGES AND OPPORTUNITIES

Implementando la selección genómica en el búfalo mediterráneo italiano: desafíos y oportunidades

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ABSTRACT

Single-step genomic best linear unbiased predictor (ssGBLUP) is a method for jointly estimating breeding values (BV) for genotyped and non-genotyped animals. Genomic information in the Italian Mediterranean Buffalo (IMB) is now available. Its inclusion in the genetic evaluation system could increase both the accuracy and genetic progress of the traits of interest of the breed. The study aimed to test the feasibility of ssGBLUP and show the first results of implementing a genomic evaluation for production and type traits in the IMB. Phenotypic information on production (270-day milk, mozzarella yield (MY), protein and fat kg and %, respectively) and morphology: feet and legs (FL) and mammary system (MS) were used for this study. Production records included 743,904 lactations from 276,451 buffalo cows born from 1984 to 2019. Morphological traits were from 91,966 buffalo cows from 2004 to 2022. Regarding the genotypes, 2,017 buffalo cows and 133 bulls were used. Data were analyzed fitting two multi-trait animal models, a 6-trait model for production data and a 2-trait model for morphology data. According to the relationship matrix used, two models were fitted: (i) the BLUP with the numerator relationship matrix (A) and (ii) the ssGBLUP where A and the genomic relationship matrix (G) are blended into H. BVs were estimated with BLUP and ssGBLUP models. The cutoff year used to create the partial data set was 2012. The correlation, accuracy, dispersion, and bias statistics were calculated (LR method). Both bulls (N=49) and cows (N=1288) were used for validations. On average, the correlation between EBVs from partial and whole datasets estimated with BLUP and ssGBLUP increased from 6 to 49% and from 14 to 17% for production and type traits, respectively. Among the traits analyzed, the most affected by the change

were protein/fat content, MY, and AM. The accuracy increase for these traits was above 20% when using the ssGBLUP. All LR statistics also improved for non-genotyped females. These results showed that implementing ssGBLUP in the breeding program can generate more accurate predictions for essential traits in dairy IMB than traditional BLUP.

Keywords: genomics, Italian Mediterranean buffalo, selection.

RESUMEN

El mejor predictor lineal insesgado genómico de un solo paso (ssGBLUP) es un método para estimar conjuntamente los valores genéticos (BV) para animales genotipados y no genotipados. La información genómica del búfalo mediterráneo italiano (IMB) ya está disponible. Su inclusión en el sistema de evaluación genética podría incrementar tanto la precisión como el progreso genético de los rasgos de interés de la raza. El estudio tuvo como objetivo probar la viabilidad de ssGBLUP y mostrar los primeros resultados de la implementación de una evaluación genómica para rasgos de producción y tipo en el IMB. Para este estudio se utilizó información fenotípica sobre producción (leche a 270 días, rendimiento de kg de mozzarella (MY), % y kg de proteína y grasa, respectivamente) y morfología: pies y piernas (FL) y sistema mamario (MS). Los registros de producción incluyeron 743.904 lactancias de 276.451 vacas búfalas nacidas de 1984 a 2019. Los rasgos morfológicos fueron de 91.966 vacas búfalas de 2004 a 2022. En cuanto a los genotipos, se utilizaron 2.017 vacas búfalas y 133 toros. Los datos se analizaron ajustando dos modelos animales con múltiples rasgos, un modelo de 6 rasgos para datos de producción y un modelo de 2 rasgos para datos de morfología. De acuerdo

con la matriz de relaciones utilizada, se ajustaron dos modelos: (i) el BLUP con la matriz de relaciones del numerador (A) y (ii) el ssGBLUP donde A y la matriz de relaciones genómicas (G) se mezclan en H. Los BV se estimaron con Modelos BLUP y ssGBLUP. El año de corte utilizado para crear el conjunto de datos parcial fue 2012. Se calcularon las estadísticas de correlación, precisión, dispersión y sesgo (método LR). Para las validaciones se utilizaron toros (N=49) y vacas (N=1288). En promedio, la correlación entre los EBV de conjuntos de datos parciales y completos estimados con BLUP y ssGBLUP aumentó del 6 al 49 % y del 14 al 17 % para los rasgos de producción y tipo, respectivamente. Entre los rasgos analizados, los más afectados por el cambio fueron el contenido de proteína/grasa, MY y AM. El aumento de precisión para estos rasgos fue superior al 20 % cuando se utilizó ssGBLUP. Todas las estadísticas de LR también mejoraron para las hembras no genotipadas. Estos resultados mostraron que la implementación de ssGBLUP en el programa de mejoramiento puede generar predicciones más precisas para rasgos esenciales en el IMB lechero que el BLUP tradicional.

Palabras clave: genómica, búfalo mediterráneo italiano, selección.

INTRODUCTION

The current breeding goal for the Italian Mediterranean Buffalo (IMB) centers on enhancing production and functional traits. Genetic selection in buffaloes has faced obstacles due to the absence of genealogical data, challenges in implementing data collection, and poor reproductive performance. Consequently, the full potential of the buffalo has yet to be actualized. Nevertheless, the Italian Mediterranean buffalo stands out as the sole breed globally, with a well-established genetic program that has been in operation for over two decades.

The advent of genomics has significantly transformed the genetic selection of livestock, although its implementation varies across species. This discrepancy arises from factors such as population structure, the extensive use of artificial insemination, and the accessibility of precise phenotypic data. Initially, methodological approaches restricted genomics application to large populations, but algorithms now cater to highly heterogeneous situations. Among these approaches is the Single-step Genomic BLUP (ssGBLUP) method. This method has replaced the multiphase process initially utilized for genomic evaluations in various farm animal species. The ssGBLUP method employs the inverse of a relationship matrix H^{-1} [1], merging the traditional additive relatedness matrix (A) with the genomic relatedness matrix (G). Despite earlier challenges related to unknown parental groups (UPG) and computational costs, recent studies have demonstrated the efficacy of this method in estimating Estimated Breeding Values (EBV) across diverse livestock species, including dairy animals [2, 3, 4], beef cattle [5], goats [6], sheep [7, 8], and buffaloes [9, 10].

The enhancement of buffalo genetics holds significant importance in Italian breeding. Italy ranks sixth globally in buffalo milk production and sixteenth in livestock numbers. Consequently, this study assesses the efficacy of genomic models in predicting breeding values for production traits in Italian Mediterranean buffalo.

MATERIALS AND METHODS

Ethics Statement

Animal welfare and use committee approval was unnecessary for this study as datasets were obtained from pre-existing databases based on routine animal recording procedures.

Data

The National Association of Italian Buffalo Breeders (ANASB) provided data for the present study and regarded productive, morphological and pedigree information. Production records included 743,904 lactations from 276,451 buffalo cows born from 1984 to 2019. Morphological traits were from 91,966 buffalo cows from 2004 to 2022. Regarding the genotypes, a total of 2,250 animals were used.

Analysis

Data were analysed fitting two multi-trait animal models, a 6-trait model for production data and a 2-trait model for morphology data. According to the relationship matrix used, two models were fitted: (i) the BLUP with the numerator relationship matrix (A) and (ii) the ssGBLUP where A and the genomic relationship matrix (G) are blended into H. Breeding values were estimated with BLUP and ssGBLUP models. The genetic values were estimated twice to evaluate the models: in the first test, the candidate animals had their phenotypes available (complete data), while in the second, they had their phenotypes masked (partial data). The cutoff year used to create the partial data set was set at 2012. Therefore, the reduced data set corresponded to buffaloes with fine calving in 2012. The following statistics were calculated to evaluate the models: dispersion, precision, correlation and bias.

RESULTS AND DISCUSSION

The correlation between the two methods was larger than 90% for production and between 87 and 91% for morphology. Regarding average reliability, the ssGBLUP method showed a higher value than to the classic method. As seen in TABLE I, the increase was between 6 and 41%, depending on the trait. However, differences are observed between traits, and this is because they have diverse heritabilities, and this causes the snps to have a different impact on each trait.

The validation of the results through the LR method [11] has the purpose of verifying the impact of the snp on the estimation and its predictive capacity when an animal does not have a phenotype. This method is widely used to validate the transition from genetics to genomics in different livestock species, such as cattle [5, 4], ovine [8], chicken [12] e buffalo [10, 13]. In theory, the dispersion, correlation and precision should be close to 1, and the results show how the ssGBLUP method tends to predict better than the classical method. While, the Bias indicates the error that we are making in the estimate. Overall, ssGBLUP results were better than BLUP's (TABLE II). There was a moderate increase (18%) in the minimum prediction accuracy with ssGBLUP. This result further confirms the benefits of including genomic information in the genetic evaluation for production and morphological traits.

When we talk about genetic selection, the precision of our estimate is essential to guarantee the genetic progress of our population. The data's reliability increase is achieved with the permanent inclusion of information, the validation of the pedigree, and the meticulous implementation of quality control measures [14]. Regarding genotypes, various studies have confirmed that it is beneficial to genotype females; in fact, including female genotypes is becoming more attractive lately [15]. In the IMB this aspect is fundamental, since it has a low number of bulls authorized for artificial fertilization. Therefore, the genotyping of females is essential. Current data show that of the total available genotypes, 10% correspond to males and 90% to females. Increasing precision with the inclusion of females has also been reported in small and large populations [16]. For example, it was concluded that a less biased and more reliable GEBV was obtained in Nordic Jersey cattle by including genotypes from unselected females [17].

The correlation between the total dataset and the partial dataset (Table II) indicated that the use of genomic information leads to greater stability of the evaluation for the selection candidates (ssGBLUP, 0.51-0.60), which means that the genomic model predicts better genetic values when the animal does not have its phenotype. Similar results were shown by Cesarani et al. [15] in the Italian Simmental cattle, with the ssGBLUP method obtaining an increase in the correlation of 14%.

With the ssGBLUP, we create a new matrix of combined relationships that turns out to be more efficient than what we had with the BLUP method and also perform an adjustment of the kinship relationships between genotyped and non-genotyped individuals. This adjustment is crucial role in reducing bias when calculating breeding values from animals with no phenotypic data available [18].

CONCLUSION

These results obtained by inserting the genotypes into the calculation of the genetic values move in the expected direction. These results indicate that ssGBLUP can be imple-

TABLE I
INCREASED ACCURACY, SSGBLUP VS BLUP

Trait	Method	Increased (%)
Milk	ssGBLUP vs BLUP	10.39
Fat %	ssGBLUP vs BLUP	11.84
Protein %	ssGBLUP vs BLUP	10.39
Fat Kg	ssGBLUP vs BLUP	10.67
Protein Kg	ssGBLUP vs BLUP	9.09
Yield	ssGBLUP vs BLUP	40.68
Feet and legs	ssGBLUP vs BLUP	6.56
Under teat	ssGBLUP vs BLUP	7.46

TABLE II
MINIMUM AND MAXIMUM VALUE OBTAINED BY THE BLUP AND SSGBLUP METHOD

Statistics LR	BLUP	ssGBLUP
Dispersion	0.64 - 1.15	0.80 - 1.06
Correlation	0.37 - 0.57	0.51 - 0.60
Accuracy	0.38 - 0.51	0.45 - 0.52
Bias	-0.57 - 0.06	-0.18 - 0.43

mented in the routine genetic evaluation of Italian Mediterranean Buffalo. This implementation will be beneficial, especially for those traits with low heritability. It is advisable to continue increasing the number of genotyped animals more significant to obtain greater precision increases.

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REFERENCES

- [1] Aguilar, I., Misztal, I., Johnson, D.L., Legarra, A., Tsuruta, S., and Lawlor, T.J. (2010). Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score1. *Journal of Dairy Science* 93(2), 743-752. doi: <https://doi.org/10.3168/jds.2009-2730>.

- [2] Cesarani, A., Biffani, S., Garcia, A., Lourenco, D., Bertolini, G., Neglia, G., et al. (2021a). Genomic investigation of milk production in Italian buffalo. *Italian Journal of Animal Science* 20(1), 539-547. doi: 10.1080/1828051X.2021.1902404.
- [3] Legarra, A., and Reverter, A. (2018). Semi-parametric estimates of population accuracy and bias of predictions of breeding values and future phenotypes using the LR method. *Genetics Selection Evolution* 50(1), 53. doi: 10.1186/s12711-018-0426-6.
- [4] Bermann, M., Lourenco, D., Breen, V., Hawken, R., Brito Lopes, F., and Misztal, I. (2021). Modeling genetic differences of combined broiler chicken populations in single-step GBLUP. *J Anim Sci* 99(4). doi: 10.1093/jas/skab056.
- [5] Herrera, J.R.V., Flores, E.B., Duijvesteijn, N., Moghadar, N., and van der Werf, J.H. (2021). Accuracy of Genomic Prediction for Milk Production Traits in Philippine Dairy Buffaloes. *Front Genet* 12, 682576. doi: 10.3389/fgene.2021.682576.
- [6] Christensen, O.F., Madsen, P., Nielsen, B., Ostensen, T., and Su, G. (2012). Single-step methods for genomic evaluation in pigs. *Animal* 6(10), 1565-1571.
- [7] Cesarani, A., Garcia, A., Hidalgo, J., Degano, L., Vicario, D., Macciotta, N.P.P., et al. (2021b). Genomic information allows for more accurate breeding values for milkability in dual-purpose Italian Simmental cattle. *J Dairy Sci* 104(5), 5719-5727. doi: 10.3168/jds.2020-19838.
- [8] Cooper, T.A., Wiggans, G.R., and VanRaden, P.M. (Year). "Including cow information in genomic prediction of Holstein dairy cattle in the US".
- [9] Gao, H., Madsen, P., Nielsen, U.S., Aamand, G.P., Su, G., Byskov, K., et al. (2015). Including different groups of genotyped females for genomic prediction in a Nordic Jersey population. *Journal of Dairy Science* 98(12), 9051-9059. doi: <https://doi.org/10.3168/jds.2015-9947>.
- [10] Amaya Martínez, A., Martínez Sarmiento, R., and Cerón-Muñoz, M. (2020). Genetic evaluations in cattle using the single-step genomic best linear unbiased predictor. *Ciencia y Tecnología Agropecuaria* 21(1), 19-31.
- [11] Himmelbauer, J., Schwarzenbacher, H., and Fuerst, C. (2021). Implementation of single-step evaluations for fitness traits in the German and Austrian Fleckvieh and Brown Swiss populations. *Interbull Bulletin* (56), 82-89.
- [12] Pimentel, E.d.C.G., Edel, C., Krogmeier, D., Emmerling, R., and Götz, K.-U. (2021). Effects of use of external information in Single-Step evaluations for linear type traits in Brown Swiss. *Interbull Bulletin* (56), 121-124.
- [13] Cesarani, A., Lourenco, D., Tsuruta, S., Legarra, A., Nicolazzi, E.L., VanRaden, P.M., et al. (2022). Multibreed genomic evaluation for production traits of dairy cattle in the United States using single-step genomic best linear unbiased predictor. *Journal of Dairy Science* 105(6), 5141-5152. doi: 10.3168/jds.2021-21505.
- [14] Lourenco, D.A., Tsuruta, S., Fragomeni, B.O., Masuda, Y., Aguilar, I., Legarra, A., et al. (2015). Genetic evaluation using single-step genomic best linear unbiased predictor in American Angus. *J Anim Sci* 93(6), 2653-2662. doi: 10.2527/jas.2014-8836.
- [15] Teissier, M., Larroque, H., and Robert-Granié, C. (2018). Weighted single-step genomic BLUP improves accuracy of genomic breeding values for protein content in French dairy goats: a quantitative trait influenced by a major gene. *Genetics Selection Evolution* 50(1), 31. doi: 10.1186/s12711-018-0400-3.
- [16] Cesarani, A., Gaspa, G., Correddu, F., Cellesi, M., DiMauro, C., and Macciotta, N.P.P. (2019). Genomic selection of milk fatty acid composition in Sarda dairy sheep: Effect of different phenotypes and relationship matrices on heritability and breeding value accuracy. *J Dairy Sci* 102(4), 3189-3203. doi: 10.3168/jds.2018-15333.
- [17] Macedo, F.L., Christensen, O.F., Astruc, J.-M., Aguilar, I., Masuda, Y., and Legarra, A. (2020). Bias and accuracy of dairy sheep evaluations using BLUP and SSGBLUP with metafounders and unknown parent groups. *Genetics Selection Evolution* 52(1), 47. doi: 10.1186/s12711-020-00567-1.
- [18] Aspilcueta-Borquis, R.R., Araujo Neto, F.R., Santos, D.J., Hurtado-Lugo, N.A., Silva, J.A., and Tonhati, H. (2015). Multiple-trait genomic evaluation for milk yield and milk quality traits using genomic and phenotypic data in buffalo in Brazil. *Genet Mol Res* 14(4), 18009-18017. doi: 10.4238/2015.December.22.27.

ANIMAL CLONING AND GENOME EDITING IN BUFFALO, WITH SPECIAL REFERENCE TO INDIA

Clonación de animales y edición del genoma del búfalo, con especial referencia a la India

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ABSTRACT

India owns the best buffalo breeds, particularly Murrah, which is famous worldwide for high milk production. India's white and pink revolution cannot be imagined without the contribution of buffalo, and to achieve this, the best productive animals need to be produced through scientific interventions. Animal cloning is a technique used to produce multiple copies of the best animals without normal reproduction. In India, buffalo cloning has already happened, and India's first cloned buffalo was produced in 2009. Later, several buffalo clones were produced, and attempts are ongoing to produce a stock of more elite animals. Buffalo cloning has made its way from scientific manuscripts to farmers' farms. Recently, genome editing has emerged as a powerful tool to manipulate the genomes of several animal species. Various livestock models have already been produced, and it can be foreseen that more model animals will be produced in the future using the CRISPR-Cas system. These success stories have opened multiple prospects for Indian researchers. In this article, we provide an overview of the progress of buffalo cloning in India and new initiatives on genome editing.

Keywords: Buffalo, Embryo, Cloning, genome editing, CRISPR.

RESUMEN

India posee las mejores razas de búfalos, en particular Murrah, famosa en todo el mundo por su alta producción de leche. La revolución blanca y rosa de la India no se puede imaginar sin la contribución del búfalo y, para lograrlo, es necesario producir los animales más productivos mediante intervenciones científicas. La clonación animal es una técnica utilizada para producir múltiples copias de los mejores animales sin una reproducción normal. En la India ya se ha clonado búfalos, y el primer búfalo clonado del país se produjo en 2009. Posteriormente, se pro-

dujeron varios clones de búfalos y se están realizando intentos para producir una población de más animales de élite. La clonación de búfalos ha llegado desde los manuscritos científicos hasta las granjas de los agricultores. Recientemente, la edición del genoma se ha convertido en una poderosa herramienta para manipular los genomas de varias especies animales. Ya se han producido varios modelos de ganado y se puede prever que en el futuro se producirán más animales modelo utilizando el sistema CRISPR-Cas. Estas historias de éxito han abierto múltiples perspectivas para los investigadores indios. En este artículo, ofrecemos una descripción general del progreso de la clonación de búfalos en la India y nuevas iniciativas sobre edición del genoma.

Palabras clave: Búfalo, Embrión, Clonación, edición del genoma, CRISPR.

BUFFALO CLONING JOURNEY

Ten years ago, on February 6, 2009, a team of scientists at the ICAR-National Dairy Research Institute (NDRI), Karnal, created history in the field of animal cloning research in India. They produced the world's first cloned riverine buffalo calf, Samrupa, using an inexpensive, simple somatic cell nuclear transfer (SCNT) technique called handmade cloning [1]. This simple technique is less demanding regarding equipment, skill, and time. It does not require sophisticated tools like micromanipulators and needle grinders to perform microscopic manipulation of oocytes and somatic cells [2]. The world's first cloned mammal, 'Dolly', the sheep, was born 13 years earlier at Scotland's Roslin Institute using SCNT. SCNT is an advanced assisted reproduction technique in which a somatic cell is transferred or fused with an enucleated oocyte. The reconstructed embryo, thus produced, develops to the blastocyst stage, at which it transplants into a surrogate mother, who carries the pregnancy and delivers the offspring. Worldwide, more than 20 animal species have been cloned using different SCNT methods, including handmade cloning. Currently, China is at

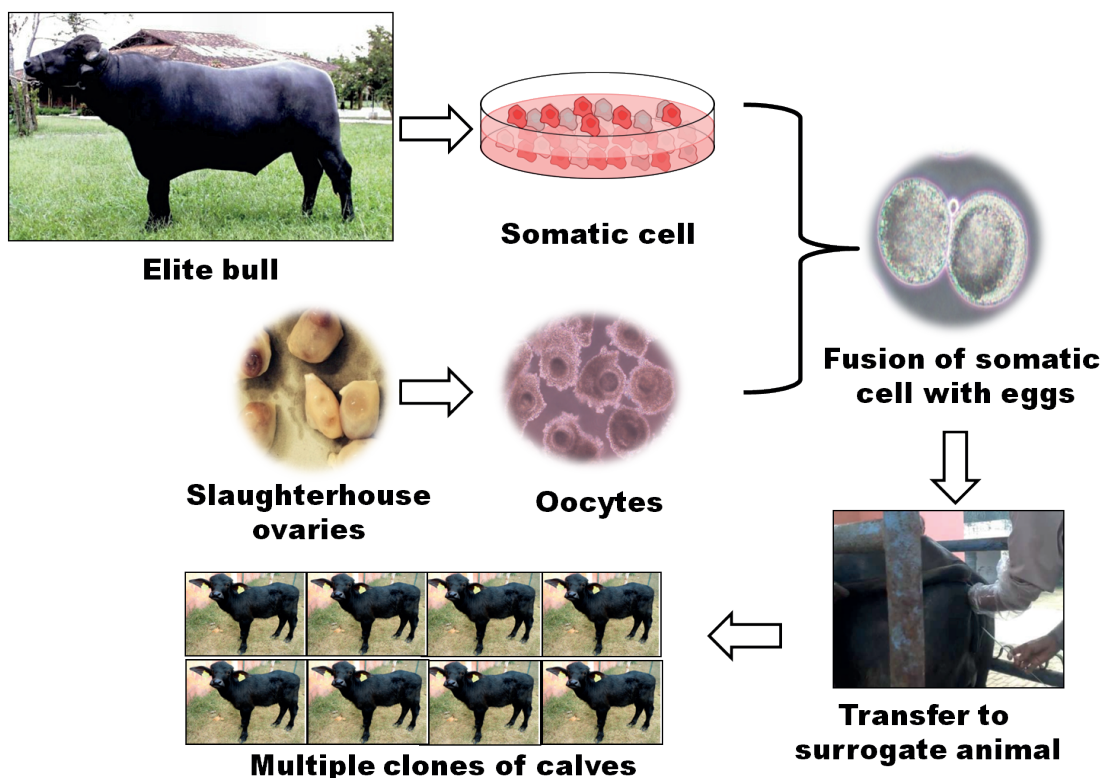
the forefront of cattle and pig cloning, whereas India is heading buffalo cloning and has made significant achievements.

In comparison to other farm animals, limited studies have been done in the field of buffalo cloning. Researchers at NDRI made the first attempt to clone buffaloes, Karnal, during 1994-97, in which cells from fertilized or *in vivo*-produced embryos were injected into enucleated oocytes to generate cloned embryos [3]. This method of nuclear transfer using micromanipulators was similar to a method that was used to produce the Dolly. Unfortunately, no blastocyst-stage embryo could be produced during that time. The possible reasons could be 1) lack of technical advances at that time, since highly skilled micromanipulation methods were used; 2) improper culture conditions for SCNT embryos; 3) not much research had been done in SCNT during the late nineties; therefore, the researchers did not have much information on SCNT. Later, in 2009, a simplified method of SCNT, named handmade cloning, was used for reprogramming differentiated somatic cells of adult animals. This method was used successfully at NDRI, Karnal, for producing blastocyst-stage embryos, and India's first cloned farm animal was born in 2009 [4]. Subsequently, many cloned buffaloes were produced [1]. Later, in 2017, several years after the birth of India's first cloned buffalo, another research institute, ICAR- Central Institute for Research on Buffalo (CIRB), Hisar, transferred the buffalo cloning technology to the field, away from the cloning laboratory [5]. CIRB also produced seven cloned copies from a single breeding bull.

METHOD OF BUFFALO CLONING

The method of producing cloned embryos appears to be quite simple and straightforward. In principle, the donor genome is fused with the cytoplasm of an enucleated ooplasm, followed by activation of the recombined ooplasm to stimulate embryonic development to the blastocyst stage, at which the embryos can be either transferred to suitably synchronized recipients or cryopreserved for future use. Two different versions of SCNT have been used to produce cloned embryos in buffalo [1]. The first is the traditional micromanipulation-based method of SCNT, which was used to produce the first cloned mammal, 'Dolly.' This method is well-established and has been extensively used in most cloning laboratories. To date, 23 animal species, including buffalo, have been successfully cloned using this method. An alternative method called Hand-made cloning (HMC), which was developed as a simplified alternative to micromanipulation-based SCNT [2], has been successfully used for the production of cloned offspring in many farm animal species such as cattle, pig, sheep, and goat, and buffalo. A significant advantage of HMC over the micromanipulation-based method is that there is no requirement for micromanipulators and their tools-making instruments for enucleation and fusion and for a highly skilled workforce to operate those instruments [2]. This significantly reduces the cost of establishing a cloning laboratory, so such laboratories can be set up in developing countries where the availability of funds and technical expertise

Steps used for production of cloned Buffaloes



are major constraints. In addition to the multiple advantages of HMC, one practical problem is that two oocytes are used for the reconstruction of a single embryo, which increases the requirements of oocytes to generate cloned embryos, and the use of two recipient oocytes to generate a single cloned embryo may be responsible for higher mitochondrial heteroplasmy. We adopted this simplified method of SCNT developed by Vajta et al. (2001) and incorporated several modifications in the basic procedures, resulting in efficient enucleation, fusion, and activation, leading to a high blastocyst development rate [1]. Published reports suggest that with a higher blastocyst production rate, HMC can be used as an alternative method of SCNT.

APPLICATION OF BUFFALO CLONING IN INDIA

Potential applications of buffalo cloning in India are 1) make multiple copies of elite buffaloes such as high milk-producing females or proven breeding bulls; 2) creation of transgenic buffaloes that harbor human genes in their genome and can serve as bioreactors to produce therapeutic proteins like insulin and blood clotting factors, preferably in milk and 3) creation of disease model buffaloes that are designed to express certain human diseases. Despite multiple applications, buffalo cloning needs improvement since, on average, less than 6-8 % of the transferred cloned embryos produce healthy offspring [6]. Faulty or improper reprogramming of differentiated somatic cells is considered a major problem behind the low success of cloning technology. Joint research efforts are going on at two laboratories in India, namely CIRB, Hisar, and NDRI, Karnal, to unravel the exact cause of faulty reprogramming and to improve buffalo cloning efficiency.

CURRENT STATUS OF BUFFALO CLONING RESEARCH

Since Dolly died at the age of 6 years, the fear has been spread that clones could not survive as long as their original donors due to premature aging and genetic abnormalities. Researchers who produced Dolly spent more than 15 years unraveling the issue of aging in cloned animals. They recently published a paper in the Nature Journal that proved premature aging in clones was a wrong perception and that four genomic copies of Dolly have normal growth, health, and aging processes [7]. This study tells us that premature aging in cloned animals has been exaggerated. The same aging principle can be applied to buffalo since the basic reprogramming mechanisms are similar across the species. In addition to the wrong perception of the premature aging of cloned animals, people are also worried about the safety of products such as milk, meat, and semen obtained from cloned animals. In 2006, the U.S. Food and Drug Administration (FDA) examined the results of extensive studies that were conducted in different countries such as the U.S., U.K., Japan, China, and New Zealand, and recommended that products obtained from cloned animals are



FIGURE 1. The cloned buffalo, named Garima, born at ICAR-National Dairy Research Institute, Karnal on 6th June, 2009 with the birth weight of 43 kg, has produced seven healthy and normal progeny. This cloned female has been growing well and does not have any physiological abnormalities

similar in terms of constituents and nutrients to the products of non-cloned animals [8]. Therefore, consumers are expected to benefit from consistent milk, meat, and semen from cloned animals, including buffalo.

During the last ten years, research and improvement in buffalo cloning techniques have produced several buffalo clones that are normal, healthy, and fertile [1,6]. These advances were made possible by significant improvements in blastocyst production rate and reduced health risks to born clones. Buffalo cloning can allow breeders and farmers to produce identical copies of their best animals, particularly proven breeding bulls, to exploit maximum production potential from them. By considering the potential utility of buffalo cloning in India, the Indian Council of Agriculture Research has been working on a mega research project to improve buffalo's production potential using semen of clones of elite breeding bulls, and our team is heading towards this goal.

METHODS USED TO MODIFY GENOME OF DOMESTIC ANIMALS

Conventionally, genetic changes in the livestock genome can be achieved by selective breeding, in which continual mating of favorable elite animals with unfavorable animals over many generations leads to the up-gradation of the specific alleles in the genome of the targeted animal population [9]. In dairy husbandry, semen from bulls of high milk-producing breeds (e.g., Holstein Friesian bulls) have been used to inseminate the low milk-producing breeds (e.g., Tharparkar cows) with the aim of producing upgraded female calves with im-

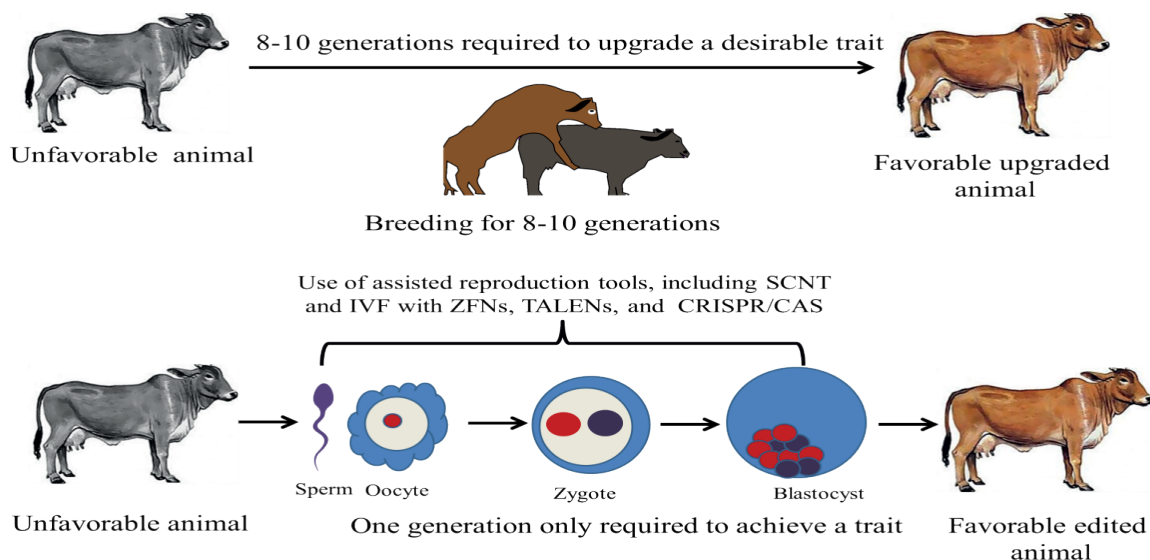
proved milk production trait. A well-known ‘Karan Fries’ breed of cattle was developed by the crossing of Holstein Friesian with Tharparkar at the National Dairy Research Institute, Karnal, using the principle of conventional breeding (http://www.ndri.res.in/ndri/Design/livestock_farm.html, accessed on September 10, 2021). With the selective breeding, milk production in India has increased from 55 million tonnes in 1991-92 to 187 million tonnes in 2018-19, almost triple (<http://www.nddb.org/information/stats/milkprodindia>, accessed on September 10, 2021), titled India becomes world’s largest producer of milk. It is perceived that selective breeding continues to be a vital approach to producing desirable animals. Moreover, breeding allows the genome modification of those genetic traits which are naturally available in the population.

The employment of engineered nucleases would save the eight to ten generations of back-crossing required in conventional breeding to modify animals and allow the addition of new genes or the deletion of unfavorable genes. In FIG. 2, we have schematically shown the comparison between traditional breeding and current genetic engineering methods; both can be used to produce desirable animals. Before the birth of the famed ‘Dolly’ (born in 1996), the world’s first genetically modified bovine, Herman the Bull, was produced by manipulation of the early embryo stage with the human lactoferrin gene [10]. Eighty-three

calves were produced from this modified bull, and all calves inherited the lactoferrin production gene [11]. This work has been opening unlimited promises to livestock research, such as manipulating genes that affect milk and meat production, the production of essential proteins in milk, and producing model animals for biomedical or veterinary applications. Multiple research success stories have already demonstrated that genetic engineering with reproductive technologies (e.g., SCNT and IVF) can be used to manipulate the livestock genome [12]. However, wide-scale applications have always been a challenge due to the extremely poor efficiency of these techniques.

CRISPR EDITING

Engineered nucleases, including zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein (Cas) system have been used for targeted modifications of the genomes of many organisms. Before engineered nucleases were developed, efficient genome manipulations, particularly knocking-out of genes, could only be achieved by using the inefficient homologous recombination (HR) in pluripotent embryonic stem (ES) cells. Thousands of mice lines were produced using the



Conventional breeding and new genome editing tools can use to manipulate the genome of domestic animals. In conventional breeding, unfavorable animals population (lower producers) were repeated inseminated with the semen of favorable animals (Bulls of high milk producers) for many generations (8-10) resulting in the up-gradation of the specific trait (milk production). In genome editing, new genes can add to a genome (e.g. lactoferrin in milk) or existing genes can delete (e.g. beta-lactoglobulin from milk) to a genome, possibly in one generation. Working mechanisms of molecular scissors are shown in fig 1 in details).

FIGURE 2. Conventional breeding and new genome editing as tools to manipulate the genome of domestic animals. In conventional breeding, unfavorable animal population (lower milk producers) inseminated with the semen of favorable animals (Bulls of high milk producers) for many generations (8-10), which resulting in the up-gradation of the specific trait (milk production). In genome editing, new genes can add to genome (e.g. lactoferrin in milk) or existing genes can delete (e.g. beta-lactoglobulin from milk) from genome, possibly in one generation

HR approaches; however, the translational to other species, including livestock, was limited by the absence of genuine ES cells in these species. In recent years, genome manipulation has gained tremendous momentum due to the discoveries of engineered nucleases, popularly named genome nucleases or scissors, namely ZFNs, TALENs, and CRISPR. In principle, these designer nucleases create double-strand breaks (DSBs) at specific desired sites in the genomic DNA. DSBs are repaired either by the non-homologous end joining (NHEJ) or the homologous directed repair (HDR) procedures, which have been exploited to manipulate the genome [13]. NHEJ is an error-prone procedure involving interrupted joining of the broken ends of DNA, creating insertions and deletions (indels) of nucleotides at cleavage sites, leading to disruption of gene function. HDR is a more accurate procedure, which requires homologous DNA sequences as templates for repair; therefore, a designed nucleotide sequence can be integrated into the genome by supplying it as an exogenous DNA template for HR events. FIG. 3 represents how designer nucleases work.

POSSIBLE OPPORTUNITIES TO INDIA

India possesses a large number of domestic animals: about 192.49 million cattle, 109.85 million buffaloes, 74.26 million sheep, and 148.88 million goats. Milk and milk products,

meat and meat products, eggs and eggs products are integral to the Indian diet. To satisfy the demand for food for the ever-growing population, the country needs the continuous and sustainable production of milk, meat, and other animal products. Climate change, emergence and re-emergence of disease pathogens, and non-availability of fodders are new challenges that might be overcome through scientific intervention for sustainable animal production. We believe that the CRISPR-Cas system and other sophisticated genome engineering techniques could be able to manage some of these concerns. A few futuristic examples are: 1) the production of bulls carrying disease resistance (e.g., FMD, rinderpest, and black quarter) or stress-tolerant (e.g., extreme heat or extreme cold) genes. The semen of such bulls would be valuable to produce tolerance offsprings. 2) The generation of bulls, which produce only spermatozoa with the X chromosome, produced sorted semen, producing female offspring. Male offspring are unprofitable to farmers due to the ban on cattle slaughter and mechanization of agriculture. 3) The production of veterinary vaccines (e.g., against FMD and rinderpest) or human therapeutic proteins (e.g., insulin) in the mammary glands of dairy animals for clinical uses.

According to existing knowledge and technologies available in India, the buffalo will be a preferred choice among domestic animals for exploring CRISPR-Cas applications. Ex-

Delivery of molecular scissors (ZFNs, TALENs, and CRISPRs) by microinjection

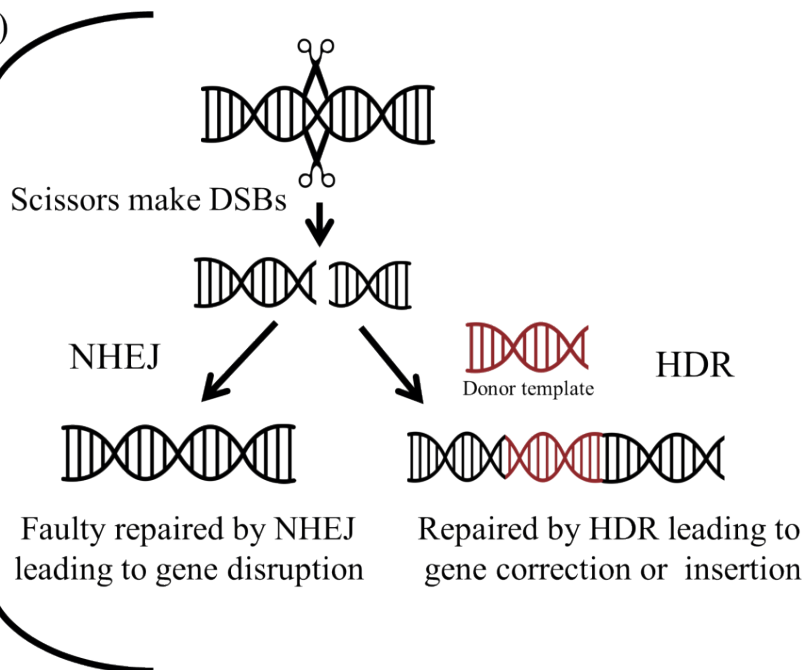
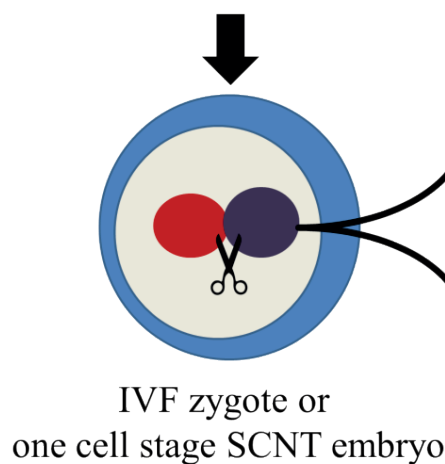


FIGURE 3. How molecular scissors/designer nucleases work in embryos: Molecular scissors are delivered in a one cell stage embryos, they specifically cleavage the target DNA causing a double-strand break (DSB). The DSB is subsequently repaired either by the error-prone non-homologous end joining pathway (NHEJ), or the homology-directed repair (HDR) processes. NHEJ leads to gene disruption due to insertions or deletions (indels) of nucleotides, whereas HDR leads to gene correction or insertion if provided with donor template

perimental working with buffalos has several advantageous aspects: 1) ample availability of oocytes for manipulations, 2) most assisted reproductive techniques, such as IVM, IVF, IVC, ET, and MOET, have been demonstrated, 3) SCNT has successfully been proved and is currently being used in the cloning of breeding bulls and elite females, and 4) scientific expertise and technical personnel are available, including staff for efficient manipulation of oocytes and embryo transfer. Our lab recently initiated a proof of principle study aiming to produce manipulated buffaloes expressing improved production traits and health. Two major limitations need to be addressed to reach this aim: 1) poor efficiency of buffalo cloning success rate, and 2) the complete annotation of the buffalo genome, which still needs to be fully accomplished. Basic and applied aspects of research are required to achieve successful gene editing in the buffalo species.

CONFLICT-OF-INTEREST STATEMENT

The authors have no conflicts of interest to declare.

ACKNOWLEDGMENT

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REFERENCES

- [1] Selokar N L, Saini M, Palta P, Chauhan M S, Manik R S *et al*, Cloning of buffalo, a highly valued livestock species of south and southeast Asia: Any achievements?, *Cell Reprogram.*, 20(2) (2018) 89-98.
- [2] Vajta G, Handmade cloning: the future way of nuclear transfer?, *Trends Biotechnol.*, 25 (2007) 250-253.
- [3] Singla S K, Manik R S, & Madan M L, Micromanipulation and cloning studies on buffalo oocytes and embryos using nucleus transfer, *Indian. J. Exp. Biol.*, 35 (1997) 1273-1283.
- [4] Singla S K, Selokar N L, Saini M, Palta P, Chauhan M S & Manik RS, Buffalo Cloning: What we have achieved so far. *Current Science*, 109 (4) (2015) 670-671.
- [5] Selokar N L, Sharma P, Kumar D, Sharma R K & Yadav P S, Sach-Gaurav: world's first cloned buffalo born in the field at an Indian dairy farm, *Current science*, 115 (2018) 198.
- [6] Saini M, Selokar N L, Palta P, Chauhan M S, Manik R S *et al*, An update: Reproductive handmade cloning of water buffalo (*Bubalus bubalis*), *Anim. Reprod. Sci.*, 197 (2018) 1-9.
- [7] Sinclair K D, Corr S A, Gutierrez C G, Fisher P A, Lee J H *et al*. Healthy ageing of cloned sheep, *Nat. Commun.*, 7 (2016) 12359.
- [8] Tanne J H, FDA approves use of cloned animals for food, *BMJ*, 336 (2008) 176.
- [9] Hale, E.B. Domestication and the evolution of behavior. In: Hafez ESE, editor., ed. *The Behavior of Domestic Animals*. Baltimore: Williams & Wilkins. PP 22–24.
- [10] Krimpenfort, P., et al., Generation of transgenic dairy cattle using 'in vitro' embryo production. *Biotechnology*, 9(9) (1969) 844-847.
- [11] Seltzer, R. First transgenic bull sires transgenic calves. *Chem. Eng. News*, 72 (7) (1994) 30.
- [12] Tan, W., et al., Gene targeting, genome editing: from Dolly to editors. *Transgenic Research*, 25(1) (2016): 102-103.
- [13] Gaj, T., et al., ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology*, 31(7) (2013) 397-405.

FROM DNA TO SUSTAINABILITY: THE GENOMICS APPROACH IN LIVESTOCK PRODUCTION

Del ADN a la sostenibilidad: el enfoque genómico en la producción ganadera

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ABSTRACT

The livestock industry has used genetic selection to improve phenotypic characteristics for decades. The identification of characteristics in the population, such as adaptation, disease resistance, production capacity, and specific phenotypic characteristics, combined with the use of directed and assisted reproduction techniques, have been the tools that have allowed us to reach current production levels in the livestock industry. Since the discovery of DNA and the advancement of molecular genetics, it is well known that phenotypic characteristics (milk production, meat production, and absence of horn, among many others) are conditioned and governed mainly by DNA. Thus, indirectly, genetic improvement programs based on the measurement of the characters of interest, selection, and control of reproduction to multiply the selected-desired character have as their principle the intervention and control of the DNA replicated in the next generations. On the other hand, genomic sequencing is the technology that has advanced the fastest in the last decade. Its applications and benefits for human development and improving the quality of life of current and future generations are innumerable and have tremendous implications. For instance, genomic selection has been proposed as a valuable tool to combat climate change. In this sense, genomic selection uses information from many genetic markers or single nucleotide polymorphisms (SNPs) in conjunction with measurements (phenotypes) of essential traits in livestock to improve selection accuracy, reduce the generation intervals, and increase genetic gains since the observation can be read and estimated with a DNA sequencing before the population express the character even before the animal is born. Therefore, genomic selection has generated significant buzz as it has the potential to revolutionize livestock production globally, allowing farmers to be more productive using fewer resources (feed, water) to fill the rapidly changing climates and human needs.

Keywords: sustainability, genomic selection, DNA, livestock.

RESUMEN

La industria ganadera ha utilizado la selección genética para mejorar las características fenotípicas durante décadas. La identificación de características en la población, como adaptación, resistencia a enfermedades, capacidad de producción y caracteres morfológicos fenotípicos específicos, combinadas con el uso de técnicas de reproducción dirigida y asistida, han sido las herramientas que nos han permitido alcanzar los niveles de producción actuales en la industria ganadera. Desde el descubrimiento del ADN y el avance de la genética molecular, es bien sabido que las características fenotípicas (producción de leche, producción de carne y ausencia de cuerno, entre muchas otras) están condicionadas y regidas principalmente por el ADN. Así, indirectamente, los programas de mejoramiento genético basados en la medición de los caracteres de interés, la selección y el control de la reproducción para multiplicar el carácter seleccionado-deseado tienen como principio la intervención y control del ADN replicado en las siguientes generaciones. Por otro lado, la secuenciación genómica es la tecnología que más rápido ha avanzado en la última década. Sus aplicaciones y beneficios para el desarrollo humano y la mejora de la calidad de vida de las generaciones actuales y futuras son innumerables y tienen enormes implicaciones. Por ejemplo, la selección genómica se ha propuesto como una herramienta valiosa para combatir el cambio climático. En este sentido, la selección genómica utiliza información de muchos marcadores genéticos o polimorfismos de un solo nucleótido (SNP) junto con mediciones (fenotipos) de rasgos esenciales en el ganado para mejorar la precisión de la selección, reducir los intervalos generacionales y aumentar las ganancias genéticas, ya que la observación puede ser leído y estimado con una secuenciación de ADN antes de que la población exprese el carácter incluso antes de nacer. Por lo tanto, la selección genómica ha generado un gran revuelo, ya que tiene el potencial de revolucionar la producción ganadera a nivel mundial, permitiendo a los agricultores ser más productivos utilizando menos recursos (piensos, agua) para satisfacer las necesidades humanas y climáticas que cambian rápidamente.

Palabras clave: sostenibilidad, selección genómica, ADN, ganadería.

BUFFALO MEAT QUALITY, PROCESSING, AND MARKETING: HARNESSING ITS BENEFITS AND NUTRACEUTICAL POTENTIAL

Calidad, procesamiento y comercialización de la carne de búfalo: aprovechando sus beneficios y potencial nutracéutico

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ABSTRACT

Buffalo meat production is growing in different countries also because buffalo products exhibit some positive characteristics for human health compared to red meats from other species. However, meat quality is also defined by organoleptic aspects. This review aims to highlight the distinctive characteristics of buffalo meat. The principal problems of buffalo meat production are related to the low yield and an abundant layer of subcutaneous fat deposition when the animal is older than 14 months. This last trait, which may seem negative, allows us to carry out prolonged aging time (PAT) without compromising the shelf life and improving meat tenderness, one of the organoleptic characteristic's consumers desires. Another organoleptic characteristic that guides consumer choice is color. This trait depends mainly on the amount and state of myoglobin, a species-specific sarcoplasmic heme protein, and buffalo presents a different molecule than beef. Furthermore, this bright red molecule in the oxidized state transforms into metmyoglobin or deoxy myoglobin in the absence of oxygen, giving a dark color to the meat, which consumers associate with poor quality from old animals. The presence or absence of oxygen shows advantages and disadvantages by acting on the one hand, on the bright meat color and, on the other, on the oxidation of lipids. The best compromise between the two situations is given by skin packaging, which preserves the meat in an anaerobic environment. Keeping the high nutritional quality over time is imperative to maintain the numerous nutritional properties of buffalo meat. In this regard, buffalo meat presents numerous distinctive characteristics among the different groups of fatty acids. Among the saturated one, the abundance of odd and branched fatty acids compared to the bovine breed should be highlighted; for the unsaturated ones, this meat is characterized by the abundance of biohydrogenation products by rumen bacteria such as trans vaccenic and conjugated linoleic acid (CLA), best known for its

anti-carcinogenic properties. In comparison, the endogenous pathway of fatty acid formation through the liver or adipose tissue allows us to enhance the elongation capacity of polyunsaturated n3 fatty acids, vital for human health as precursors of anti-inflammatory prostaglandins. Finally, buffalo meat is an essential source of N-acetylneuraminic sialic acid (Neu5Ac), a nine-carbon molecule located in the terminal ends of glycoproteins and glycolipids, an essential nutrient for brain development and function. This molecule can also counteract the intestinal absorption of N-glycolyl sialic acid (Neu5Gc) exogenous for humans, abundant in red meat, with high inflammatory action. In addition to being marketed as fresh meat, Buffalo meat can also be transformed into semi-processed or cured products with peculiar characteristics. Lean products could be well integrated into the modern diet, with clear advantages to consumers and breeders. The nutritional and technological potential of buffalo meat is considerable, and it is necessary to communicate this to the consumer, creating an efficient and dynamic market for buffalo meat-based products.

Keywords: buffalo meat, dry aging, oxymyoglobin, nutraceutical compounds, fatty acids.

RESUMEN

La producción de carne de búfalo está creciendo en diferentes países también porque los productos de búfalo presentan algunas características positivas para la salud humana en comparación con las carnes rojas de otras especies. Sin embargo, la calidad de la carne también viene definida por aspectos organolépticos. Esta revisión tiene como objetivo resaltar las características distintivas de la carne de búfalo. Los principales problemas de la producción de carne de búfalo están relacionados con el bajo rendimiento y una abundante capa de grasa subcutánea cuando el animal tiene más de 14 meses. Este últi-

mo rasgo, que puede parecer negativo, nos permite realizar un tiempo de envejecimiento prolongado (PAT) sin comprometer la vida útil y mejorando la terneza de la carne, una de las características organolépticas que desea el consumidor. Otra característica organoléptica que orienta la elección del consumidor es el color. Este rasgo depende principalmente de la cantidad y el estado de la mioglobina, una proteína hemo sarcoplásmica específica de cada especie, y el búfalo presenta una molécula diferente a la de la carne de res. Además, esta molécula de color rojo brillante en estado oxidado se transforma en metmioglobina o desoximioglobina en ausencia de oxígeno, dando un color oscuro a la carne, que los consumidores asocian con la mala calidad de los animales viejos. La presencia o ausencia de oxígeno presenta ventajas e inconvenientes al actuar, por un lado, sobre el color brillante de la carne y, por otro, sobre la oxidación de los lípidos. El mejor compromiso entre ambas situaciones lo proporciona el envasado tipo "skin", que conserva la carne en un ambiente anaeróbico. Mantener la alta calidad nutricional en el tiempo es imperativo para mantener las numerosas propiedades nutricionales de la carne de búfalo. En este sentido, la carne de búfalo presenta numerosas características distintivas entre los distintos grupos de ácidos grasos. Entre los saturados cabe destacar la abundancia de ácidos grasos impares y ramificados respecto a los vacunos; para las insaturadas, esta carne se caracteriza por la abundancia de productos de biohidrogenación por bacterias ruminales como el trans vaccénico y el ácido linoleico conjugado (CLA), más conocido por sus propiedades anticancerígenas. En comparación, la vía endógena de formación de ácidos grasos a través del hígado o el tejido adiposo nos permite potenciar la capacidad de elongación de los ácidos grasos poliinsaturados n3, vitales para la salud humana como precursores de las prostaglandinas antiinflamatorias. Finalmente, la carne de búfalo es una fuente esencial de ácido siálico N-acetilneuramínico (Neu5Ac), una molécula de nueve carbonos ubicada en los extremos terminales de las glicoproteínas y glicolípidos, un nutriente esencial para el desarrollo y la función del cerebro. Esta molécula también puede contrarrestar la absorción intestinal del ácido N-glicolil siálico (Neu5Gc) exógeno al ser humano, abundante en las carnes rojas, con elevada acción inflamatoria. Además de comercializarse como carne fresca, la carne de búfalo también puede transformarse en productos semielaborados o curados con características peculiares. Los productos magros podrían integrarse bien en la dieta moderna, con claras ventajas para los consumidores y los criadores. El potencial nutricional y tecnológico de la carne de búfalo es considerable y es necesario comunicarlo al consumidor, creando un mercado eficiente y dinámico para los productos a base de carne de búfalo.

Palabras clave: carne de búfalo, maduración en seco, oximioglobina, compuestos nutracéuticos, ácidos grasos.

INTRODUCTION

In the last decade, Buffalo (*Bubalus bubalis* L.) meat has reported an increase in population head livestock as in tonnes of meat production. According to FAO Stat data¹, the world buffalo population in 2021 was 203.940 million buffalo head, about more than 9.860 million head compared to 2010. TABLE I shows the buffalo animals slaughtered in 2021 and the quantity of meat produced. On average, each buffalo head produces 151kg of meat, which is low weight if we consider the slaughter of adults. This data highlights that even in 2021, many male calves are slaughtered very young. Furthermore, only around 14% of the world's buffalo population is slaughtered, and most of these are spent animals.

These data highlight consumer disaffection for this type of product, even though it is clear that buffalo meat has positive characteristics for human health. However, improving knowledge on meat quality and meat processing of buffaloes is slowly bringing about a consumption positive trend because buffalo meat could represent a considerable economic and nutritional source [2]. The principal problems of buffalo meat production are related to the low yield and an abundant layer of subcutaneous fat deposition when the animal is older than 14 months.

The different breeds, age, breeding, and pre-and post-slaughter technologies result in great variability in carcass and meat characteristics, leading to poor quantitative and qualitative performances [2, 3]. Meat quality is defined by different parameters concerning carcass, organoleptic, and nutritional characteristics of meat. For increasingly health-conscious consumers, the last two aspects cannot be separated to satisfy their needs.

ORGANOLEPTIC QUALITY OF BUFFALO MEAT

Color and tenderness are the principal organoleptic characteristics because they direct consumers' choices during buying and eating [4]. Both characteristics depend on pre- and post-mortem processes during slaughtering on meat aging and enzymatic proteolysis [5]. At the same time, color is also linked to oxidation processes that occur during aging but also retail and domestic storage [6]. The color of meat depends mainly on the amount and oxidation state of myoglobin (Mb), a sarcoplasmic protein with an iron molecule in the center surrounded by a tetrapyrrole ring called heme. Meat color for each species is typical because the chemistry of myoglobin is species-specific [7].

The molecular mass of buffalo Mb is 86.20 Da higher than the bovine Mb. This is confirmed by analyzing its primary structure. Comparing the amino acid sequences of both Mbs, we found three amino acid differences out of 153 amino acid residues [7]; only one of these substitutions is made by a threonyl amino acid that is a destabilizing β -branched residue. These observations indicate that the structural architecture

TABLE I
CONSISTENCY OF BUFFALO SLAUGHTERED AND TONNES OF MEAT PRODUCT IN 2021

2021	Buffalo heads	Buffalo slaughtered heads	Meat production (tonnes per year)	Meat yields pro head slaughtered
World	203,939,158	28,601,802	4,322,190.48	151.1
Asia	200,182,688	27,870,440	4,107,873.87	147.4
India	11,786,188	11,769,252	1,635,506.69	139.0
China	27,022,807	4,526,052	658,617.77	145.5
Africa	1,263,128	507,634	166,744.50	328.5
Americas	2,010,831	115,779	26,047.91	225.0
Europe	482,334	110,949	21,277.00	191.8
Italy	409,410	107,949	20,691.24	191.6

Data: FAO [1]

of the heme pockets in the two Mbs is similar, with the same functional properties, but with different behavior during oxidation, due also to higher myoglobin content in buffalo meat as reported by [8]. Even if the Mb quantity depends on age and muscle type, the range is very high, from 2.70 to 9.40 mg/g [9]. Moreover, buffalo meat is richer in iron (1.83 mg/100g) than other species like beef and sheep (1.53 mg/100g on average). The most immediate way to analyze meat color is given by the Cielab system (TABLE II), which considers lightness (L*), redness (a*), and yellowness (b*).

Buffalo meat increases in lightness during aging, while it decreases during oxidation. The old animals show different colors, particularly for major concentrations in Mb (+ an index) and intramuscular fat (+ b indexes), as found for grazing animals, which have meat richer in pigments [10]. However, the buffalo cannot accumulate large percentages of carotenoids in the adipose tissue; in fact, this tends to be white even in the carcass of adult animals [11].

In the function of heme oxidation, the myoglobin changes in color; the bright red molecule in the oxidized state (oxymyoglobin) transforms into metmyoglobin due to the oxidation of iron from ferrous to ferric state or into deoxy myoglobin in the absence of oxygen as in vacuum packaged meat, giving a dark color to the meat and consumers associate it with poor quality from old animals [16]. Different packaging technologies try to solve this very pronounced problem in buffalo meat due to the excessive presence of iron [17]. Among the technologies that improve the marketing and shelf life of buffalo meat, the following are highlighted: modified or protective atmosphere packaging (MAP), whose atmosphere blown inside consists mainly of 80% oxygen (O₂) and 20% carbon dioxide (CO₂); vacuum packaging and skin packaging, storage in the absence of oxygen and edible film. These types of packaging can be supported by using absorbent pads and films activated with antimicrobial and antioxidant substances [18, 5]. The presence or absence of oxygen shows advantages and disadvantages by acting on the one hand on oxymyoglobin formation and the bright meat color

TABLE II
COLOR (CIELAB PARAMETERS) OF MEAT BUFFALO IN DIFFERENT CONDITIONS

Type	Lightness L*	Redness a*	Yellowness b*
Muscles [12]			
LD	44.33 ^b	16.35 ^b	13.01 ^b
GB	49.45 ^a	17.32 ^{ab}	15.82 ^a
CLOTB	46.04 ^b	18.11 ^a	15.02 ^a
Aging period* [13]			
24 hours	36.5 ^c	17.12 ^a	13.27
48 hours	39.0 ^b	16.54 ^{ab}	13.34
168 hours	42.9 ^a	16.10 ^b	13.91
Aging period*[14]			
7 days	44.65	20.22 ^a	8.21 ^b
21 days	44.13	19.74 ^b	8.04 ^b
35 days	44.61	19.43 ^b	10.53 ^a
Storage time*[12]			
15 days	45.53 ^a	18.98 ^b	13.98 ^a
90 days	42.99 ^{ab}	17.36 ^c	10.03 ^c
180 days	41.12 ^b	20.03 ^a	11.69 ^b
Age at slaughter*[15]			
10 months	43.95 ^a	19.24 ^b	15.72
14 months	41.69 ^b	20.22 ^b	15.79
18 months	39.79 ^c	21.41 ^a	16.22
Feeding system*[10]			
Mais silage	46.81 ^a	18.10 ^b	14.53 ^a
Hay	42.46 ^b	19.65 ^a	12.60 ^{ab}
Pasture	41.10 ^b	19.92 ^a	10.93 ^b

LD=Longissimus dorsi; GB=Gluteus biceps; CLOTB=Caput longuum tricipite brachii; *LD. Different letters on column indicate significant differences per $p < 0.05$

on the other on the oxidation of lipids. The best compromise between the two situations is given by skin packaging, which preserves the meat in an anaerobic environment, producing all the positive effects of the vacuum. However, it does not allow the dripping liquids from the meat, significantly improving and extending the shelf life [19].

Nonetheless, this technology is still costly and is used for small portions of raw meat, grounded meat, or meat preparations, which could be purchased in retail. The main problem of meat packaging, besides the need to enhance efficiency in terms of shelf life, is to lower the production cost and limit plastic pollution. An answer to these problems could be given by edible film and coat using hydrocolloid and lipid components.

The buffalo slaughter produces many residues, such as skin, blood, bones, meat trimmings, and fatty tissues, which represent a risk to the environment because they are often discarded as waste without being used. These biowastes contain biopolymers and other compounds such as proteins, polysaccharides, and fat with good biological properties capable of producing sustainable food packaging (both active film or coating), which could be edible, biodegradable, and act as carriers of biobased active agents such as antimicrobials, antioxidants, and health-promoting compounds. The difference between films and coatings consists in their manufacture and application. Edible films are dried, preformed thin sheets, while edible coatings are applied as a liquid of varying viscosity onto the surface of muscle by spraying or dipping [20].

In addition to active and edible packaging, intelligent systems were tested on buffalo meat based on sensors with bromophenol blue sensitive to total volatile basic nitrogen released from buffalo meat during prolonged refrigerated storage [21].

However, preserving time for the high nutritional quality of buffalo meat is imperative in order not to lose the numerous nutritional properties like antioxidant compounds such as Q10, glutathione, vitamins, minerals such as iron, zinc, and selenium, essential amino acids in particular branch amino acids and polyunsaturated fatty acids.

If color is the first parameter consumers use for buying meat, tenderness is the first attribute that consumers use to

evaluate the organoleptic quality during eating. Generally, the buffalo meat obtained from young animals is tender compared to beef at the same age and breeding system. Meat tenderization depends on various factors that occur mainly in the period around slaughter and are linked both to the animal and to technological tenderization systems like variation in temperature/time of aging, electrical stimulation, different suspension methods, and use of exogenous enzymes. These factors affect the glycolytic and proteolytic enzymatic activity, acting on pH, ability to retain liquids, and myofibrillar degradation processes. Therefore, meat tenderization is a delicate multifactorial process during aging [22].

The effect of aging is time-dependent; some first-quality cuts, in particular loin, usually receive a prolonged aging time (PAT), but in order to avoid rancidity and microbial proliferation, various preservation techniques have been implemented. The most common ones are classified into: "dry aging" and "wet aging" [23].

Dry aging includes maturation in a controlled environment (+2°C of temperature and 78% of relative humidity) or protected maturation with films that ensure oxygen penetration and the losses of liquids and allow an antimicrobial barrier [24]. This process can last for weeks or even months. The anatomical cuts for prolonged aging time must generally be significant and have an abundant subcutaneous fat layer to prevent liquid loss and excessive oxygen penetration, blocking lipid oxidation. At the same time, proteolysis continues due to the enzymatic activity. Usually, buffalo loin has an abundant adipose panniculus that is well suited to this process [25], ensuring tenderness, flavor, and formation of bioactive peptides. The chunk of meat dries outside, making the "crust," after a few weeks, starts the fiber contraction, preventing the deterioration of meat.

The crust will be removed before consumption, with considerable product losses [26].

Among the wet aging processes, we have vacuum-packed meat maturation, or meat maturation with marinade, improving overall acceptability (TABLE III). The vacuum-packed meat remains in contact with liquids, giving blood and metal taste, but using calcium chloride marination (FIG. 1), the tenderness

TABLE III
SENSORY EVALUATION OF LONGISSIMUS THORACIS ET LUMBORUM OF BUFFALO DURING WET AGING [14]

Aging (Days)	Odor	Flavor Intensity	Overall Tenderness	Juiciness	Overall Acceptability
0	6.12 ± 0.45	5.78 ± 0.60	6.25 ± 0.73 ^f	5.48 ± 0.61	5.42 ± 0.62 ^c
7	6.08 ± 0.50	5.75 ± 0.53	6.66 ± 0.57 ^e	5.54 ± 0.59	5.44 ± 0.59 ^c
14	6.05 ± 0.19	6.69 ± 0.56	6.97 ± 0.65 ^d	5.59 ± 0.81	5.50 ± 0.81 ^c
21	6.00 ± 0.26	5.72 ± 0.72	7.32 ± 0.69 ^c	5.65 ± 0.73	5.67 ± 0.56 ^b
28	5.97 ± 0.35	5.76 ± 0.51	7.47 ± 0.67 ^b	5.64 ± 0.83	5.69 ± 0.28 ^b
35	5.99 ± 0.31	6.82 ± 0.42	7.86 ± 0.40 ^a	5.66 ± 0.52	5.79 ± 0.57 ^a

Hedonic scale on 8-point, where 8 = excellent, while 1 = extremely bad. Different letters on column indicate significant differences per $p < 0.05$.

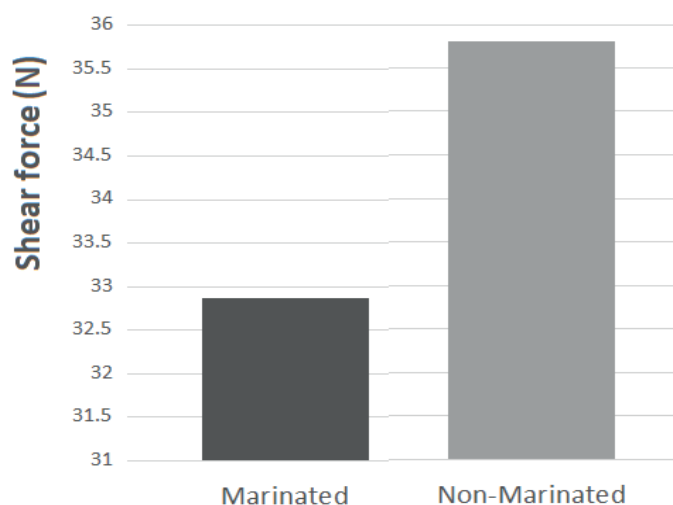


FIGURE 1. SHEAR FORCE OF *LONGISSIMUS THORACIS ET LUMBORUM* OF BUFFALO MARINATED WITH CaCl_2 [27]

improves during aging time [27]. The flavor can be improved with the addition of brines and spices, which also show an antibacterial and antioxidant function, catching iron and improving color [23, 14].

NUTRITIONAL QUALITY

The proximate composition of buffalo meat is not different from little marbled bovine meat if we consider both species have the same muscle, just as the percentage of collagen is not different to beef at the same age and breeding conditions. Buffalo meat has a lower percentage of cholesterol than beef, but this difference is related to the bovine breed used in comparison; in fact, Di Stasio and Brugiapaglia [3] consider only

eight bibliographic sources report cholesterol values that range from 32.20 to 123.79 mg/100g of buffalo meat.

Fatty acids are most important to define the nutritional quality of meat because the quantity and the structure of fatty acids impact human health. In this regard, buffalo meat presents numerous distinctive characteristics in the different groups of fatty acids (TABLE IV) if compared with other species fed and bred similarly. Among the saturated ones, the abundance of branched fatty acids compared to the bovine breed should be highlighted because these fatty acids have a nutraceutical function in the human diet [28]. For the monounsaturated ones, this meat enhances its characteristics thanks to the abundance of oleic acid and products of the biohydrogenation of rumen bacteria such as trans vaccenic (18:1 trans 11), trans palmitoleic (16:1 trans 9), and so on. Also, some polyunsaturated fatty acids come from the rumen activity as conjugated linoleic acids (CLA), best known for their anti-carcinogenic properties [29], are significantly more abundant in buffalo than beef and yak [30].

The endogenous pathway of fatty acid formation through the liver or adipose tissue allows us to enhance the elongation capacity of polyunsaturated n3 fatty acids, vital for human health as precursors of anti-inflammatory prostaglandins. Also, if the composition of n6 and n3 fatty acids is principally diet-dependent, the elongation capacity of PUFA is partly due to genetic effects [31]. The inability of humans to produce linoleic acid (C18:2n6) and linolenic acid (C18:3n3) means that their presence in the diet is vital. Although many vegetables help us overcome this deficiency, we need animal products to get long-chain n3 PUFAs such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and Docosapentaenoic acid (DPA). These are precursors of lipoxins, prostaglandins, and leukotrienes with anti-inflammatory action and are abundantly present in fish. If we consider DPA instead, with its numerous

**TABLE IV
FATTY ACID (% OF TOTAL FAME) ON *LONGISSIMUS THORACIS* OF DIFFERENT SPECIES**

	Buffalo	Beef	Yak	RMSE
\sum SFA	45.28 ^a	45.50 ^a	42.15 ^b	3.27
\sum MUFA	40.23	41.64	40.12	3.58
\sum PUFA	14.49 ^b	12.87 ^c	17.73 ^a	1.42
\sum CLA	0.71 ^a	0.53 ^b	0.64 ^{ab}	0.11
BCFA	1.25 ^a	1.04 ^b	1.17 ^{ab}	0.18
MUFA trans	1.35 ^a	1.37 ^a	0.85 ^b	0.36
\sum PUFA n6	12.10 ^b	11.07 ^c	13.91 ^a	0.99
\sum PUFA n3	2.39 ^b	1.80 ^c	3.82 ^a	0.57
\sum UFA/ \sum SFA	1.21	1.20	1.37	0.32
\sum n6/ \sum n3	5.06 ^{ab}	6.14 ^a	3.64 ^b	1.50

FAME = fatty acid methyl ester; SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids; UFA= unsaturated fatty acids. Different letters on row indicate significant differences per $p < 0.05$

Data: CREA not published

metabolic functions that influence the decrease in serum adiponectin and hepatic lipogenesis [32], this is highly present in meat (TABLE V).

Buffalo meat DPA showed an intermediate content if compared with beef and yak. Recently, numerous studies have highlighted the capability of DPA to be converted into EPA and DHA as needed and, therefore, can be considered a source of long-chain n3 storage thanks to its remarkable ability to resist oxidation. Furthermore, if we feed the buffaloes with a diet based on grass or added flaxseeds and algae, we can enrich the products of animal origin with DPA and functional elements for humans.

Finally, buffalo meat is an essential source of N-acetylneuraminic sialic acid (Neu5Ac), a nine-carbon molecule located in the terminal ends of glycoproteins and glycolipids, an essential nutrient for brain development and function [33]. This molecule can counteract the intestinal absorption of N-glycolilneuramic acid (Neu5Gc) exogenous sialic acid for humans, which is abundant in red meat, and it has high inflammatory action, also considered one of the leading causes of colon cancer. As reported in Kawanishi [34], a dietary intake of Neu5Ac five times higher than Neu5Gc was sufficient to eliminate the incorporation of Sialic acids (Sia) foreign forms in human cell membranes.

In this case, to reduce the inflammation caused by exogenous Sias, reducing red meat consumption would not be necessary, but increasing consumption of animal products with a higher quantity of Neu5Ac, as buffalo meat showed Neu5Ac 6.2 times greater than Neu5Gc. The sialic acid in buffalo meat can provide functional molecules and block the absorption of exogenous sialic acid that harms humans.

To avoid inflammation potentially caused by meat, switching to a vegetarian diet is inconsistent because it lacks iron, vitamin B12, essential amino acids, and long-chain essential fatty acids. A promising alternative is an increase in the weekly consumption of meat with higher amounts of Neu5Ac, which can prevent the accumulation of exogenous sialic acid in human cells.

SEMI-PROCESSED AND CURED PRODUCTS: A BRIEF REVIEW

In addition to being marketed as fresh meat suitably packaged to extend its shelf life, Buffalo meat can also be transformed into semi-processed products or various cured products [35, 36].

An exciting review was published by Maheswarappa [37] where grounded or emulsion buffalo meat products were listed: patties, sausages, burgers, restructured meat differently [38] also using sodium alginate and carrageenan [39], enrobed meat. Natural emulsifiers, conservatives, and polyphenols were used in these products to extend the shelf life and consumer

TABLE V
LONG CHAIN PUFA N3 FATTY ACIDS (% OF TOTAL FAME)
ON *LONGISSIMUS THORACIS* OF DIFFERENT SPECIES

	Buffalo	Beef	Yak	RMSE
C20:5n3 EPA	0.347 ^b	0.304 ^b	0.814 ^a	0.301
C22:5n3 DPA	0.942 ^b	0.557 ^c	1.425 ^a	0.323
C22:6n3 DHA	0.097	0.087	0.117	0.081

Different letters on row indicate significant differences per $p < 0.05$. Data: CREA not published

TABLE VI
CONTENT IN NEU5GC AND NEU5AC ON *LONGISSIMUS THORACIS* OF DIFFERENT SPECIES [33]

Species	Neu5Gc µg/g protein	Neu5Ac µg/g protein	Neu5Ac/ Neu5Gc
Beef	228.3 ^a	432.5 ^b	1.8 ^b
Buffalo	82.7 ^c	512.6 ^a	6.2 ^a
Yak	145.7 ^b	508.5 ^a	3.5 ^b
RMSE	51.4	103.5	2.3

Different letters on column indicate significant differences per $p < 0.05$

acceptability [40]. Shelf-stable, ready-to-eat, spiced pickle-type buffalo meat was also produced [41].

The addition of fat to these products brings an improvement in juiciness and palatability. Of all the fats, pork appears to respond better to the technological transformations of buffalo meat.

Buffalo meat can also be used for numerous preparations involved in the drying and salting processes. These cured products can be obtained with whole cuts of meat and derived from ground meat supplemented with pork fat. By way of example, we report some processed products obtained in Italy with buffalo meat:

Bresaola

Bresaola of buffalo meat, like beef meat GPI product (EEC 1263/96), a typical product of Valtellina (north Italy), is produced by salting and curing different cuts of hindquarters. A strict trimming process is essential to give a unique flavor.

Legs of beef are defatted and seasoned with a dry rub of salt and spices, such as juniper berries, cinnamon, and nutmeg. They are then left to cure for a few days. The drying period is between one and three months. The meat loses up to 40% of its original weight during aging.

Carne salada

Carne salada is obtained with the topside of adult animals. The cuts, cleaned of all fat and tendinous parts, are sprinkled with a mixture of salt and other ingredients and placed in a

container where they will remain from 2 to 5 weeks, depending on the size of the individual pieces. During the entire maturation period, the carne salada must be kept in a dark room at a maximum temperature of +12 °C and massaged at least every 2/3 days.

Sfilacci

Sfilacci is a typical product of the Veneto region, usually obtained with horse meat, but buffalo meat also has excellent qualitative performances. The strips of flaccid meat are prepared with very lean meat cut into thin slices along the fiber. It is put in brine for about 15 days, seasoned for about one month, and finally beaten. The meat fibers separate, forming dry filaments of a deep red color.

CONCLUSION

Buffalo meat has valuable organoleptic and nutritional characteristics. Scientific information must be disseminated, informing consumers that this product is lean and could be integrated into the modern diet, with obvious health advantages.

The nutritional and technological potentiality of buffalo meat is considerable; it is necessary to create an efficient and dynamic market for both fresh and cured products, bringing advantages to breeders who are in this way encouraged to support and spread a meat production chain just as the production of mozzarella and dairy products has spread in many countries.

REFERENCES

- [1] FAOSTAT. Available online: <http://www.fao.org/faost4at/en/#home> (accessed on 8 October 2023).
- [2] Naveena BM, Kiran M. Buffalo meat quality, composition, and processing characteristics: Contribution to the global economy and nutritional security. *Anim Front.* 2014; 4:18-24. Doi:10.2527/af.2014-0029
- [3] Di Stasio L, Brugiapaglia A. Current Knowledge on River Buffalo Meat: A Critical Analysis. *Animals.* 2021; 11:2111. Doi:10.3390/ani11072111
- [4] Santos D, Monteiro MJ, Voss HP, Komora N, Teixeira P, Pintado M. The most important attributes of beef sensory quality and production variables that can affect it: A review. *Liv. Sci.* 2021; 250:104573. Doi:10.1016/j.livsci.2021.104573
- [5] Jaspal MH, Badar IH, Usman Ghani M, Ijaz M, Yar MK, Manzoor A, Nasir J, Nauman K, Junaid Akhtar M, Rahman A, Hussnain F, Ahmad A. Effect of Packaging Type and Aging on the Meat Quality Characteristics of Water Buffalo Bulls. *Animals.* 2022; 12:130. Doi: 10.3390/ani12020130.
- [6] Tateo A, De Palo P, Quaglia NC, Centoducati P. Some qualitative and chromatic aspects of thawed buffalo (*Bubalus bubalis*) meat. *Meat Sci.* 2007; 76:352–358. Doi:10.1016/j.meatsci.2006.12.003
- [7] Dosi R, Di Maro A, Chambery A, Colonna G, Costantini S, Geraci G, Parente A. Characterization and kinetics studies of water buffalo (*Bubalus bubalis*) myoglobin. *Comp Biochem Physiol B Biochem.* 2006; 145:230-238. Doi:10.1016/j.cbpb.2006.07.006
- [8] Swartidyana FR, Yuliana ND, Adnyane IKM, Hermanianto J, Jaswir I. Differentiation of Beef, Buffalo, Pork, and Wild Boar Meats Using Colorimetric and Digital Image Analysis Coupled with Multivariate Data Analysis. *J. Teknol. dan Industri Pangan* 2022; 33:87-99. Doi: 10.6066/jtip.2022.33.1.87 J
- [9] Valin C, Pinkas A, Dragnev H, Boikovski S, Polikronov D. Comparative study of buffalo meat and beef. *Meat Sci.* 1984; 10:69-84. Doi: 10.1016/0309-1740(84)90032-9
- [10] Contò M, Cifuni GF, Iacurto M, Failla S. Effect of pasture and intensive feeding systems on the carcass and meat quality of buffalo. *Anim Biosci.* 2022; 35:105-114. Doi: 10.5713/ab.21.0141
- [11] Huang J, Liu X, Feng X, Zhang M, Qu K, Liu J, Wei, X., Huang B, Ma Y. Characterization of different adipose depots in fattened buffalo: histological features and expression profiling of adipocyte markers. *Arch Anim Breed.* 2020; 63: 61–67. Doi:10.5194/aab-63-61-2020
- [12] Settineri D, Failla S, Bisegna V, Di Giacomo A. Effect of different storage conditions on lipid oxidation in buffalo meat. Proc. "Meat consumption and culture", 44th ICoM-ST, Barcellona (Spagna). 1998; 666-667.
- [13] Failla S, Vincenti F, Saltarelli E, Contò M, Ballico S, Ficco A. Aging time effect on quality meat from buffaloes fed on two different diets. Proceedings of the 8th World Buffalo Congress, Caserta, It *J Anim Sci.* 2007; 6:2S 1195-1198
- [14] Jaspal MH, Badar IH, Amjad OB, Yar MK, Ijaz M, Manzoor A, Nasir J, Asghar B, Ali S, Nauman K, Effect of Wet Aging on Color Stability, Tenderness, and Sensory Attributes of Longissimus lumborum and Gluteus medius Muscles from Water Buffalo Bulls. *Animals.* 2021; 11:2248. Doi:10.3390/ani11082248
- [15] Failla S, Gigli S, Iacurto M, Di Giacomo A, Fioretti M. Meat quality in male buffaloes reared with two nutritive levels and slaughtered at 4 different ages. Proceeding of International Symposium on Buffalo Products, Paestum (SA), 1-4/12 1996 EAAP Publication
- [16] Ramanathan R, Suman SP, Faustman C. Biomolecular Interactions Governing Fresh 403 Meat Color in Post-Mortem Skeletal Muscle: A Review. *J Agric Food Chem.* 2020; 404 Doi:10.1021/acs.jafc.9b08098

- [17] Tamburrano A, Tavazzi B, Callà CAM, Amorini AM, Lazarino G, Zottola T, Campagna MC, Moscato U, Lauretti P. Biochemical and nutritional characteristics of buffalo meat and potential implications on human health for a personalized nutrition. *Int J Food Saf.* 2019; 8:8317. Doi:10.4081/ijfs.2019.8317
- [18] Sekar A, Dushyanthan K, Radhakrishnan KT, Narendra Babu R. Effect of modified atmosphere packaging on structural and physical changes in buffalo meat. *Meat Sci.* 2006; 72:211–215. Doi:10.1016/j.meatsci.2005.07.003
- [19] Stella S, Bernardi C, Tironi E. Influence of Skin Packaging on Raw Beef Quality: A Review. *J of Food Qual.* 2018; 1–9. Doi:10.1155/2018/7464578.
- [20] Hamed I, Jakobsen AN, Lerfall J. Sustainable edible packaging systems based on active compounds from food processing by-products: A review. *Compr Rev Food Sci Food Saf.* 2022; 21:198–226. Doi:10.1111/1541-4337.12870
- [21] Shukla V, Kandeepan G, Vishnuraj MR. Development of On-Package Indicator Sensor for Real-Time Monitoring of Buffalo Meat Quality During Refrigeration Storage. *Food Anal Meth.* 2015; 8:1591–1597. Doi:10.1007/s12161-014-0066-6
- [22] Bhat ZF, Morton JD, Mason SL, Bekhit A, El-Din A. Applied and emerging methods for meat tenderization: a comparative perspective. *Compr. Rev Food Sci Food Saf.* 2018; 17:841-859. Doi:10.1111/1541-4337.12356
- [23] Terjung N, Witte F, Heinz V. The dry aged beef paradox: Why dry aging is sometimes not better than wet aging. *Meat Sci.* 2021; 172:108355. Doi:10.1016/j.meatsci.2020.108355
- [24] Barragán-Hernández W, Leighton PLA, López-Campos O, Segura J, Aalhus JL, Prieto N. Effect of in-the-bag dry-ageing on meat palatability and volatile compounds of cull cows and youthful steers. *Meat Sci.* 2022; 188:108800. Doi:10.1016/j.meatsci.2022.108800
- [25] Salzano A, Cotticelli A, Marrone RJ, D’Occhio M, D’Onofrio N, Neglia G, Ambrosio RL, Balestrieri ML, Campanile G. Effect of Breeding Techniques and Prolonged Post Dry Aging Maturation Process on Biomolecule Levels in Raw Buffalo Meat. *Vet Sci.* 2021; 8:66. Doi:10.3390/vetsci8040066
- [26] Dashdorj D, Tripathi VK, Cho S, Kim YS, Inho H. Dry aging of beef; Review. *J Anim Sci Technol.* 2016; 58:20. Doi:10.1186/s40781-016-0101-9
- [27] Ijaz, M, Jaspal MH, Akram MU, Badar IH, Yar MK, Suleman R, Manzoor A, Farooq M, Ali S, Hussain Z. Effect of Animal Age, Postmortem Calcium Chloride Marination, and Storage Time on Meat Quality Characteristics of *M. longissimus thoracis et lumborum* of Buffalo Bulls. *Foods.* 2022; 11:3193. Doi:10.3390/foods11203193
- [28] Dąbrowski GI, Konopka I. Update on food sources and biological activity of odd-chain, branched and cyclic fatty acids. A review. *Trends Food Sci Technol.* 2022; 119:514-529. Doi: 10.1016/j.tifs.2021.12.019
- [29] National Research Council (NRC): Carcinogens and anticarcinogens in the human diet. 1996; National Academy Press, Washington DC.
- [30] Juárez ., López-Campos O, Prieto N, Galbraith JR, Fajlla S, Aalhus JL. Chapter 5- Carcass Characteristics and Meat Quality of Bison, Buffalo, and Yak. 2019. In: More than Beef, Pork and Chicken – The Production, Processing, and Quality Traits of Other Sources of Meat for Human Diet. Lorenzo J, Muneke P, Barba F, Toldrá F (eds) pp.95-117 ed. Springer, Cham. Doi:10.1007/978-3-030-05484-7_5, ISBN 978-3-030-05484-7
- [31] De Smet S, Raes K, Demeyer D. Meat fatty acid composition as affected by fatness and genetic factors: a review. *Anim. Res.* 2004; 53:81–98. Doi:10.1051/animres:2004003
- [32] Drouin G, Rioux V, Legrand P. The n-3 docosapentaenoic acid (DPA): A new player in the n-3 long chain polyunsaturated fatty acid family. *Biochimie.* 2019; 159:36-48. Doi:10.1016/j.biochi.2019.01.022
- [33] Failla S, Contò M, Miarelli M. Variability of sialic acids in meat from alternative species to beef and pork. *Anim Front.* 2003; (in press).
- [34] Kawanishi K, Coker JK, Grunddal KV, Dhar C, Hsiao J, Zengler K, Varki N, Varki A, Gordts PL. Dietary Neu5Ac Intervention Protects Against Atherosclerosis Associated With Human-Like Neu5Gc Loss-Brief Report. *Arterioscler Thromb Vasc Biol.* 2021; 41(11):2730-2739. doi: 10.1161/ATVBAHA.120.315280.
- [35] Paleari MA, Beretta G, Colombo F, Foschini S, Bertolo G, Camisasca S. Buffalo meat as a salted and cured product. 2000; 54:365–367. Doi:10.1016/s0309-1740(99)00111-4
- [36] Anjaneyulu ASR, Thomas R, Kondaiah N. Technologies for Value Added Buffalo meat products-a review. *Am J Food Technol.* 2007; 2:104–114 DOI:10.3923/ajft.2007.104.114
- [37] Maheswarappa NB, Muthupalani M, Mohan K, Banerjee R, Sen AR, Barbudde SB. Buffalo Meat Processing and Value Addition. 2022. In: *Asiatic Water Buffalo.* Springer, Singapore. Doi:10.1007/978-981-19-2619-8_8
- [38] Ahmad SR, Sharma BD, Irshad A, Ranjan RK, Malav OP, Suman T. Effect of aerobic storage conditions on the quality of functional restructured buffalo meat fillets enriched with natural sources of dietary fibers and antioxidant components. *J Food Proc Pres.* 2020; 45(3):1–8. Doi:10.1111/jfpp.15072

- [39] Suman SP, Sharma BD. Effect of grind size and fat levels on the physico-chemical and sensory characteristics of low-fat ground buffalo meat patties. *Meat Sci.* 2003; 65:973–976. Doi:10.1016/S0309-1740(02)00313-3
- [40] Ahamed ME, Anjaneyulu ASR, Sathu T, Thomas R. Effect of different binders on the quality of enrobed buffalo meat cutlets and their shelf-life at refrigeration storage ($4\pm 1^\circ\text{C}$). *Meat Sci.* (2007); 75(3):451–459. Doi: 10.1016/j.meatsci.2006.08.008
- [41] Malik AH, Sharma BD. Shelf life study of hurdle treated ready-to-eat spiced buffalo meat product stored at $30\pm 3^\circ\text{C}$ for 7 weeks under vacuum and aerobic packaging. *J Food Sci Technol* 2014; 51:832–844. Doi:10.1007/s13197-011-0592-9

EXPLORING THE BUFFALO WHEY: A LOOK AT NANOTECHNOLOGY IN SEARCH OF STRATEGIES FOR THE MODERN NUTRACEUTICAL INDUSTRY

Aprovechamiento del lactosuero de leche de búfala: una mirada a la nanotecnología en busca de estrategias para la industria nutracéutica moderna

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ABSTRACT

Buffalo whey is the most important by-product of the cheese industry. Whey proteins constitute the most exciting component from a technological and economic point of view. They present attractive, functional properties and, similarly to proteins of bovine milk whey, they could also be used as an ingredient (dairy beverages, nutritional supplements) and/or food additives (emulsifier, foaming agent, encapsulant) in novel applications with high-added value, such as nano or microencapsulation. However, buffalo whey has traditionally been used as a raw material for producing ricotta, as animal feed, or well discarded, constituting the main effluent in cheese-making establishments. The use of buffalo whey in novel applications poses a promising scenario, while research is still limited, which warns of endless possibilities.

Keywords: nanoencapsulation, bioactive compounds, bioaccessibility, functional foods.

RESUMEN

El suero de búfalo es el subproducto más importante de la industria quesera. Las proteínas del suero constituyen el componente más interesante desde el punto de vista tecnológico y económico. Presentan propiedades atractivas y funcionales y, al igual que las proteínas del suero de leche vacuna, también podrían usarse como ingrediente (bebidas lácteas, suplementos nutricionales) y/o aditivos alimentarios (emulsionante, espumante, encapsulante) en novedosas aplicaciones con alta valor añadido, como la nano o la microencapsulación. Sin embargo, el suero de búfalo se ha utilizado tradicionalmente como materia prima para la producción de ricotta, como alimento para

animales, o bien desechado, constituyendo el principal efluente en los establecimientos queseros. El uso del suero de búfala en aplicaciones novedosas plantea un escenario prometedor, mientras que la investigación es aún limitada, lo que advierte de infinitas posibilidades.

Palabras clave: nanoencapsulación, compuestos bioactivos, bioaccessibilidad, alimentos funcionales.

INTRODUCTION

In the last two decades, nanotechnology has demonstrated a powerful ability to solve problems in various fields such as medicine, cosmetics, agroindustry, and the environment [1]. Concerning the food sector, nanotechnology has made it possible to improve the stability and bioaccessibility of bioactive compounds, that is, with a potential beneficial effect on health [2]. The resultant products could be consumed as nutraceuticals (supplements) or used as ingredients in the formulation of functional foods. The synthesis of nanomaterials through green methods and under mild conditions constitutes an essential aspect of sustainable development.

Given their biocompatibility properties, biosafety, and wide availability, proteins constitute excellent alternatives for manufacturing structures on the nanometer size scale (10E-9). In this context, buffalo whey represents a promising, renewable, and feasible resource, especially in developing countries, where its use would enable the generation of added value with imminent economic benefits [3]. Due to the nutritional quality and functional aptitude of its proteins, cheese whey has gained a prominent position among agri-food by-products, given its great capacity for reuse. This conference plans to present some novel uses of bubaline whey proteins, particularly as raw

material in synthesizing functional nanostructures and as nano-carriers of physiologically active compounds.

BODY PARAGRAPH

A group of young researchers at the National University of Chaco Austral currently works on obtaining, characterizing, and studying the properties of buffalo milk whey in nanotechnological applications. Firstly, whey was obtained at a laboratory scale from raw buffalo milk following the methodology proposed by Bustos et al. [4]. Milk was obtained by milking buffaloes in good health and nutrition under hygienic conditions. Mainly, milked buffaloes (*Bubalus bubalis*) were from the Pedro A. Silva Farm (Paso Florentín, Corrientes, Argentina). The Milk was packaged in aseptic containers and stored at freezing temperature (-18 °C). The Milk was suitably pasteurized (65 °C, 30 min) and subjected to routine tests to verify its hygienic quality. Enzymatic coagulation was then carried out by adding commercial rennet (1 g/L) to milk previously enriched with calcium (CaCl_2 0.01% w/v) and acidified with lactic acid to pH 6 for 40 min at 35 °C.

After that, the curd was cut, gently heated, and filtered with muslin to separate the whey. Skimming, dialysis, and lyophilization were then carried out. The buffalo whey protein concentrate (BWPC) obtained presented 56 % of total proteins, comprising six main fractions: β -lactoglobulin (43.3 %), α -lactalbumin (28.6 %), bovine serum albumin (10.2 %), α -lactalbumin dimers (7.5 %), lactoferrin (2.5 %) and immunoglobulins (2.2%). From this, the formation of nanostructures was studied by the interaction between BWPC/polysaccharides and BWPC/vitamins [4].

In the presence of polysaccharides (PS), proteins (PR) can form structures at the nanoscale based on the self-assembly or self-organization of individual molecules by controlling experimental conditions [5]. In particular, by pH control, initially disordered molecules with opposite charges can form spontaneously ordered structures or patterns without an external direction. Therefore, intimate knowledge of the molecular properties of the substances involved is required to predict their behavior and functionality [6]. In turn, the study of PS and PR interactions constitutes a decisive aspect in building nanomaterials, mainly when using scarcely studied protein sources such as buffalo whey (*Bubalus bubalis*) is intended to be used.

Experimentally, the formation of complexes between bubaline whey proteins (PR) and gum arabic as a polysaccharide (PS) was monitored in a ratio of 2:1 and 0.15% w/v. The pH was adjusted to the range of 3 to 6 by post-mixing acidification. Through turbidimetric analysis, structures in the range 4 to 5 were formed, thus confirming the ability of BWPCs to create polymeric structures through electrostatic interactions. The molecular complexes presented an intermediate charge distribution between proteins (+) and polysaccharides (-), denoting excellent colloidal stability. Under these conditions, the

particles showed a monomodal size distribution centered at 250 nm. These structures are versatile and can be used as delivery vehicle systems for molecules of interest.

On the other hand, the ability of BWPC to form molecular complexes with bioactive compounds at pH 7 was also evaluated. Folic acid (FA), the synthetic form of vitamin B9, and tocopherol acetate, a variant of vitamin E, were used as a model of sensitive compounds. The formation of complexes was studied by spectrophotometric and fluorometric techniques as well as by in-silo molecular simulations. The results showed that non-covalent molecular interaction occurs, forming molecular complexes at the nanoscale. The complexes thus obtained showed the ability to retain and protect vitamins from harmful environmental factors and transport and release the bioactive compounds under simulated gastrointestinal conditions [4]. These results highlight the feasibility of using bubaline whey proteins as delivery and protection systems for sensitive hydrophilic (vitamin B9) and hydrophobic (vitamin E) compounds, laying the foundations for the design of new encapsulation/delivery systems.

CONCLUSIONS AND FUTURE TRENDS

Nanotechnology offers enormous opportunities regarding the use of buffalo whey. The possibility of generating innovative products with high-added value contributes not only to the economic benefit of dairy farms and industries but also to minimizing the polluting effect of this effluent. In this way, numerous possibilities are raised, which have been little explored in the science and technology of buffalo dairy products. In this way, it is expected to deepen the study of the potential uses of dairy components of bubaline origin, motivating the attention of researchers who place bubaline dairy derivatives at the center of cutting-edge research.

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REFERENCES

- [1] Santiago, L. G., & Castro, G. R. (2016). Novel technologies for the encapsulation of bioactive food compounds. *Current Opinion in Food Science*, 7, 78-85.
- [2] Zhang, Q., Zhou, Y., Yue, W., Qin, W., Dong, H., Vasanthan, T., (2021). Nanostructures of protein-polysacchari-

- de complexes or conjugates for encapsulation of bioactive compounds. *Trends in Food Science & Technology*.
- [3] Allahdad, Z., Varidi, M., Zadmand, R., Haertlé, T., Sawyer, L., & Saboury, A. A. (2020). Efficiency of milk proteins in eliminating practical limitations of β -carotene in hydrated polar solution. *Food chemistry*, 330, 127218.
- [4] Bustos, L F, Judis, M A, Vasile, F E and Pérez, O E (2022) Molecular interactions involved in the complexation process between buffalo whey proteins concentrate and folic acid. *Food chemistry* 396 133734.
- [5] Wei, Z., Huang, Q., (2019). Assembly of protein–polysaccharide complexes for delivery of bioactive ingredients: A perspective paper. *Journal of agricultural and food chemistry* 67(5), 1344-1352.
- [6] Hosseini SMH, Emam-Djomeh Z, Negahdarifar M, Sepeidnameh M, Razavi SH & Van der Meeren P (2016) Polysaccharide type and concentration affect nanocomplex formation in associative mixture with β -lactoglobulin. *International journal of biological macromolecules*. 93, 724-730.

SUSTAINABILITY AND LIVESTOCK: A DOABLE COMBINATION

Sostenibilidad y ganadería: una combinación factible

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ABSTRACT

Sustainable development means meeting the needs of the present while ensuring future generations can meet their own needs (European Commission). Rapid urbanization, increased purchasing power, and dietary change drive demand for richer diets and animal-origin proteins, leaving more than 868 million undernourished citizens worldwide and 850 million living in developing countries. Food security could be granted to large populations by reducing food waste, which accounts for 1.3 billion tons per year, or implementing livestock farming and promoting a sustainable food demand. With economic progress and the world's growing population, estimated to reach more than 9 billion people in 2050, animal proteins will increase as meat and milk demand. Nevertheless, ruminants produce methane, which accounts for most of the agricultural sector emissions (5.8% of the total anthropogenic), raising concerns about their production. If ruminant livestock increase, methane production increases, accelerating global warming inevitably. Depending on resource quality, environmental factors, and social and economic contexts, various types of livestock production systems may vary considerably in sustainability. These livestock systems include extensive grassland, intensive landless, mixed, and family farming systems. Massive worldwide research has investigated the effect of various mitigation strategies. Nonetheless, the under-representation of certain strategies, geographic regions, the calculation's robustness, and long-term studies are the main limitations in providing an accurate quantitative estimation of the respective mitigation potential under diverse animal production systems. Ruminant livestock is important not only for producing nutrient-dense meat and milk for human diets but also for providing hides, fiber, manure, and animal power for farming and transportation in many countries and contributing to biodiversity. To obtain this, they eat grass and legume plants that would be inedible to humans or live on land unsuitable for cultivation. Livestock also contributes to much-needed income for family farmers in developing countries. The buffalo (*Bubalus bubalis*), represented by a total of 204 million head (3.9 % increase in the last ten years), could

play a strategic role due to its peculiar characteristics: the high ability to convert fiber into energy, the longevity, and the adaptation in extreme areas with cold or hot-humid climate where other ruminants cannot thrive. Moreover, it contributes to the sustenance of many people living in rural areas. A multidisciplinary approach considering the environment, animal health and welfare, and social and economic contexts is requested to increase the sustainability of livestock.

Keywords: sustainability, buffalo, climate change, mitigation strategies.

RESUMEN

El desarrollo sostenible significa satisfacer las necesidades del presente y al mismo tiempo garantizar que las generaciones futuras puedan satisfacer sus propias necesidades (Comisión Europea). La rápida urbanización, el aumento del poder adquisitivo y los cambios en la dieta impulsan la demanda de dietas más ricas y proteínas de origen animal, lo que deja a más de 868 millones de ciudadanos desnutridos en todo el mundo y a 850 millones viviendo en países en desarrollo. Se podría garantizar la seguridad alimentaria a grandes poblaciones reduciendo el desperdicio de alimentos, que representa 1.300 millones de toneladas al año, o implementando la ganadería y promoviendo una demanda alimentaria sostenible. Con el progreso económico y la creciente población mundial, que se estima alcanzará los 9 mil millones de personas en 2050, las proteínas animales aumentarán a medida que la demanda de carne y leche. Sin embargo, los rumiantes producen metano, que representa la mayor parte de las emisiones del sector agrícola (5,8% del total antropogénico), lo que genera preocupación sobre su producción. Si aumenta el ganado rumiante, aumenta la producción de metano, acelerando inevitablemente el calentamiento global. Dependiendo de la calidad de los recursos, los factores ambientales y los contextos sociales y económicos, la sostenibilidad de varios tipos de sistemas de producción ganadera puede variar considerablemente. Estos sistemas ganaderos incluyen pastizales extensivos, sistemas

de agricultura intensiva sin tierra, mixtos y familiares. Una investigación masiva a nivel mundial ha investigado el efecto de varias estrategias de mitigación. No obstante, la subrepresentación de ciertas estrategias, regiones geográficas, la solidez de los cálculos y los estudios a largo plazo son las principales limitaciones para proporcionar una estimación cuantitativa precisa del potencial de mitigación respectivo en diversos sistemas de producción animal. El ganado rumiante es importante no sólo por producir carne y leche ricas en nutrientes para la dieta humana, sino también por proporcionar pieles, fibras, estiércol y energía animal para la agricultura y el transporte en muchos países y contribuir a la biodiversidad. Para obtenerlo, comen pastos y leguminosas que no serían comestibles para los humanos o viven en tierras no aptas para el cultivo. La ganadería también contribuye a unos ingresos muy necesarios para los agricultores familiares de los países en desarrollo. El búfalo (*Bubalus bubalis*), representado por un total de 204 millones de cabezas (un aumento del 3,9 % en los últimos diez años), podría desempeñar un papel estratégico por sus peculiares características: la alta capacidad de convertir la fibra en energía, la longevidad y la adaptación en zonas extremas con clima frío o cálido-húmedo donde otros rumiantes no pueden prosperar. Además, contribuye al sustento de muchas personas que viven en zonas rurales. Se requiere un enfoque multidisciplinario que considere el medio ambiente, la salud y el bienestar animal y los contextos sociales y económicos para aumentar la sostenibilidad de la ganadería.

Palabras clave: sostenibilidad, búfalo, cambio climático, estrategias de mitigación.

INTRODUCTION

The Global Agenda for Sustainable Livestock (GASL) defines sustainable livestock as follows: "To be sustainable, livestock sector growth needs to simultaneously address key environmental, social, and economic challenges: growing natural resources scarcity, climate change, widespread poverty, food insecurity and global threats to animal and human health and animal welfare". Sustainable livestock solutions are driven by two significant elements: the sector's diversity and the demand for livestock commodities [1].

According to FAO estimates, the livestock sector accounts for 40% of the agricultural gross domestic product in a significant part of South Asia and sub-Saharan Africa, occupying 33% of the world's land and supporting more than 1 billion people who depend on pastoralism for food and livelihood and providing more than 25% of the world's protein intake [2].

The world's growing population will reach more than 9 billion people in 2050, and an improved standard of living will inevitably increase demands for animal proteins (meat and milk). Nevertheless, ruminants produce methane, which accounts for most of the agricultural sector emissions (5.8% of the total anthropogenic), raising concerns about their production.

If ruminant livestock increase, methane production increases, accelerating global warming in the process.

To obtain a vast range of food and services, livestock use vegetable resources that would be inedible to humans and/or live on land unsuitable for cultivation. Moreover, rearing livestock also offers much-needed income for small-scale farmers in developing nations. Ruminants, especially when fed with feedstuff produced on land unsuitable for primary cropping or by-products from agro-industrial, can be a net contributor to procuring human edible food [3]. Moreover, they maintain and enhance protein and essential micronutrient supply (Zinc, calcium, Vit.B12, and riboflavin), often challenging to obtain from vegetable crops [4, 5].

The livestock sector faces numerous challenges, such as climate change, water depletion, desertification, and land erosion. Even though it may have contributed to enhancing some of these issues, it can contribute to the solution, operating within an agroecological and environmental framework while protecting biodiversity [6]. The livestock sector relates also to the importance of different ecosystem types, management methods, and local needs and traditions. In fact, livestock products and production systems are different, and they span from intensive to extensive, from cold to tropical, and from highly technological to local traditional. The most suitable approaches depend on the context and cannot be integrated into one global model [7].

Among ruminants, with a total of 204 million head (a 3.9 % increase in the last ten years), buffalo (*Bubalus bubalis*) could contribute to sustainability for its specie-specific characteristics: its high ability to convert raw fiber into energy, its rusticity, its ability to adapt to different climatic environments (cold, tropical, or swampy), and its longevity, which is always higher than cattle.

CONCERNS ABOUT LIVESTOCK

There is a growing concern that the demand for animal products, associated with population growth, prolonged lifespan, and improved economic welfare, particularly in developing countries, will put an unsustainable call on the environment [8].

It also must be considered that animal production yields highly heterogeneous categories of foods (i.e., dairy, meats, eggs), each produced differently, displaying its own biochemical and nutritional properties, produced in regions with different ecological contexts, and consumed by populations with specific nutritional, economic, and cultural needs. So, animal-source food intake substantially differs between geographical regions and socioeconomic categories.

In the general debate, the complexity of the food system is often neglected and reduced to three interconnected claims that consumption of animal-source foods causes harm to human health, to the planet, and the animal itself related to health



FIGURE 1. Graphical abstract

hazards, climate change, and animal welfare [5], forgetting that livestock sustains the livelihood of millions of people in the world (up to 12%), both in developing and developed countries.

CLIMATE CHANGE

Methane is a greenhouse gas (GHG) far less abundant than CO₂ but with a global warming potential 28 times more potent on a 100-year scale [9]. Methane derives from the balance between sources and sinks. Sources are biogenic (i.e., wetlands, agriculture, waste/landfill, permafrost), thermogenic (i.e., fossil fuel), pyrogenic (biomass and biofuel burning), or mixed sources, while the sink is mainly methane oxidation in soil [10].

Agriculture contributes with a percentage varying from 8 to 18% of total anthropogenic GHG emissions. Feed production, land use change, energy (not only as farm inputs and feed, but other activities such as animal housing and ventilation), and product processing are included in most global estimates. Livestock mainly contributes by enteric fermentation, manure as methane and nitrous oxide, and different manure management systems generate different emissions levels. Among ruminants-related direct emissions, cattle are responsible for 65% and buffaloes for 8% [11].

Climate change can increase extreme weather conditions that directly and indirectly affect livestock productivity (TABLE I). Due to the increase in temperatures, livestock production is experiencing reduced growth and reproductive efficiency, reduced milk and meat production, and animal health, making

them vulnerable to new diseases. Fodder and water supplies are also affected by climate extremes and seasonal variations.

Global food security is threatened by climate change and its adverse impact on livestock production.

HOW TO COPE WITH CLIMATE CHANGE

For a livestock production “climate-smart”, the two possible approaches are adapting to climatic changes and mitigating GHG [12]. Integrating these two aspects can exploit synergies and minimize trade-offs between mitigation and adaptation.

Adaptation approaches might include promoting resilient livestock production, modifying production and management systems, scientific and technological improvements, governance and policy changes, and changing farmers’ perceptions and adaptive capacity [13]. Adaptation measures should incorporate agroecological principles (e.g., improved circularity) while limiting feed-food competition. However, they should also remain respectful of the diversity of ecosystem contexts, the availability of resources, and the various social and economic needs of local populations [6].

Feed sources with increased drought-tolerant producing more biomass and being more resilient to environmental extremes, could be more sustainable. Moreover, genetic improvement can select livestock with greater heat tolerance and less energy requirements, which might help ensure their performances so production is less affected [14]. There are more than 40 species of farmed animal species and more than 8,800

local breeds adapted to specific contexts [15], and due to their greater ability to thrive in a stressful environment, indigenous breeds display higher resilience than exotic breeds.

MITIGATION STRATEGIES

Microbial fermentations in the rumen play an essential role in the ability of ruminants to utilize lignocellulosic materials to produce volatile fatty acids (VFAs) and to convert non-protein nitrogen into microbial protein, which is an essential source of energy and protein for the host. In contrast, the rumen provides the microbes with a suitable environment to thrive and grow [16]. Nevertheless, microbes also have potential environmental detrimental effects through methane emission and excessive nitrogen excretions in feces and urine. Rumen methane production also represents energy loss (from 2 to 12% of gross energy intake) for animal growth and production [17].

A massive worldwide research effort has been devoted to finding successful mitigation strategies that can be summarized into three categories (TABLE II): changes in animal and feed management, diet formulation, and rumen manipulation [18,19,20,21,22].

All of them potentially involve changes in the rumen microbiome [23], thus lowering methane emissions, which would benefit the environment and, eventually, the livestock production efficiency. Nevertheless, according to Arndt et al. (2022), methane yield is not the only relevant measure; other methane emissions and animal performance metrics should be considered to estimate the feasibility of mitigation strategies.

SUSTAINABLE MANAGEMENT

Well-managed livestock are an integral and productive part of agriculture. Among other ecological services, they can convert non-edible biomass from pasture systems and produce human food, recycle plant nutrients back into the soil, improve soil health, and sequester carbon [6].

Integrating crop and livestock farming is an effective strategy to reduce emissions associated with animal production [24]. Agroforestry systems (i.e., silvopastoral), where trees and meadows are combined, can reward farmers financially while improving yields and reducing the environmental burden. In addition, research has shown that pasture-based production systems are better for animal welfare and enhance biodiversity, as these systems allow for more natural animal behavior.

Grazing management and soil management practices include rotational grazing, cover cropping, and conservation tillage. Rotational grazing involves altering grazing patterns to ensure that the plants are not overgrazed and have time to regrow. In contrast, cover cropping involves the planting of specific species of crops after harvesting to add fertility to the soil while conserving soil moisture and reducing erosion. Con-

TABLE I
CLIMATIC CHANGE IMPACT ON LIVESTOCK PRODUCTION

Impact	Observed Impact	Causes
Direct	- Feed intake	
	- Milk and meat production	
	- Reproductive performance	+ Temperature (heat stress)
	- Immune functions	
	+ Mortality	
Indirect	- Crop yield	
	Change in pasture composition	> CO ₂ concentrations
	Change in forage quality	+ Temperature > CO ₂ concentrations
	Seasonal changes in resource supply	> Frequent extreme weather events
	- Water availability	
	+ Water consumption	+ Temperature
	+ Diseases, pests, and stress	+ Temperature change in rainfall frequency

Modified Cheng et al., 2022

TABLE II
MITIGATION STRATEGIES FOR THE REDUCTION OF METHANE IN RUMINANTS

Mitigation strategies		
Animal and feed management	Diet formulation	Rumen manipulation
Genomic selection	Forage quality	Vaccination
Rational grazing	Lipids	Defaunation
Agroforestry	Sea weeds	Direct-fed microbial
Animal health	Additives	
Manure management		

servation tillage involves minimal mechanical disturbance of the soil. It helps retain a large portion of the crop residues on the soil surface to be used as organic matter for soil nutrition. Practices such as rotational grazing and fodder banking can also increase the production efficiency of smallholder farms and prevent land degradation. These methods reduce methane emissions from the soil, along with reducing erosion and water pollution [25]. Nevertheless, adopting the best sustainable farming systems is often complex as they could result in different outcomes, favoring, in some cases, biodiversity conservation and carbon sequestration or, in some others, privileging

production [26]. For example, systems based on grazing may show higher environmental performances because of the lower inputs needed for production, albeit requiring more land.

Swapping traditional animal feeds with more carbon-friendly ones could help [27]. For instance, soybean meal and maize are incredibly carbon-intensive due to the large amounts of inputs needed to produce them. Alternatives such as barley, alfalfa, or sorghum are more sustainable. Furthermore, some livestock farms are now using by-products from agro-industrial residues that could help reduce waste and their disposal costs.

Knowledge about management and information sharing among farmers are also substantial interventions for sustainable livestock production. Access to accurate and timely information can increase farmers' capacity to manage their resources, leading to improved yields and reduced emissions [28].

INNOVATIVE TECHNOLOGIES

Research and governance have been exploring recent innovations in sustainable livestock production to respond to climate challenges, maintaining the environment and an efficient food system. Innovations in different fields can open new solutions, such as smart farming, genetics, robotics, environmental monitoring, and developing new business models [29]. Advances in informatics allowed the advancement of cameras, sensors, and environmental technologies. Moreover, networking and farm management software allow farmers to improve animal management on individual needs to make informed decisions. Through these techniques, for example, farmers can monitor soil fertility and reduce the input of fertilizers to maintain soil health.

Sustainable livestock production can utilize renewable energy sources to reduce carbon emissions and produce green energy for the farm, thus reducing reliance on fossil fuel sources [30]. Some farms are now utilizing solar power, wind turbines, and biogas digesters to power their operations to save on operational costs, thus reducing the emissions associated with farming [31].

BUFFALO IS A TOOL FOR SUSTAINABILITY

The buffalo (*Bubalus bubalis*), a species represented by more than 204 million heads worldwide, plays a strategic role in the world economy and society. One characteristic that makes the buffalo so widely used is its ability to convert fiber into energy. Numerous studies indicate the superiority of buffalo over cattle in food conversion and using fodder and agricultural by-products with low nutrient content [32]. In addition, from a recent molecular study, buffalo rumen, compared to bovine rumen, appears to have a greater potential for fiber degradation and less potential for gastroenteric methane production [33]. Other important characteristics of the buffalo are

its rusticity, ability to adapt to different climatic environments (from hot-humid to very cold), and longevity, which is always higher than that of the bovine. Buffalo is suitable for work in plantations or wetlands due to its broad articulation in the hoofs, especially during the rainy period, when the muddy soil causes difficult mobility for other species. For this characteristic, the buffalo became many countries' best draught power animal option.

It should be emphasized that this goes hand in hand with buffalo products of high quality. Buffalo meat has a lower calorie content, lower cholesterol, an unsaturated fatty acid/saturated fatty acid ratio >1 , a higher protein level, and a higher iron content ($>1.5\text{mg}/100\text{g}$) compared to beef [34]. Buffalo milk also plays a vital role in human nutrition, especially in developing countries. It is richer than cow's milk in all major constituents, such as fat (6.6-8.8%), lactose (4.5-5.2%), protein (3.8-4.5%), casein, and ash [35]. These chemical characteristics also allow for a cheese yield twice as high as that usually obtained with cow's milk. Furthermore, the presence of the A2 versus A1 variant of β -casein makes this milk more like human breast milk and, therefore, probably easier to digest [36].

CONCLUSIONS

A multidisciplinary approach embracing the more comprehensive and varied aspects of nutrition, landscapes, and culture considering the environment, livestock management, animal health and welfare, and social factors is requested to deal with the environmental issues of livestock. There is a considerable margin for correcting and improving livestock production that can substantially decrease the environmental burden and advances in animal welfare. The optimal quantity of animal-source foods in the diet of different populations will depend on health, environmental, and social factors as well as management methods that vary considerably and are challenging to bring down to simple metrics. In conclusion, when livestock production is done well, respecting local ecosystems and social contexts, it could improve public health and environmental resilience.

REFERENCES

- [1] Schneider F, Tarawali S. Sustainable Development Goals and livestock systems. *Revue Scientifique et Technique (International Office of Epizootics)*. 2021 Aug 1;40(2):585-95.
- [2] Food and Agriculture Organization of the United Nations (FAO). *Livestock and Landscapes: Sustainability Pathways*. Food and Agriculture Organizations of the United Nations. Available online: <https://www.fao.org/3/ar591e/ar591e.pdf> (accessed in November 2023).
- [3] Van Zanten HH, Herrero M, Van Hal O, Rööös E, Muller A, Garnett T, Gerber PJ, Schader C, De Boer IJ. Defining

- a land boundary for sustainable livestock consumption. *Global change biology*. 2018 Sep;24(9):4185-94.
- [4] Scollan ND, Hocquette JF, Richardson RI, Kim EJ. Raising the nutritional value of beef and beef products to add value in beef production. *Nutrition and climate change: major issues confronting the meat industry* (ed. JD Wood and C Rowlings). 2011 Apr 1:79-104.
- [5] Leroy F, Smith NW, Adesogan AT, Beal T, Iannotti L, Moughan PJ, Mann N. The role of meat in the human diet: evolutionary aspects and nutritional value. *Animal Frontiers*. 2023 Apr 1;13(2):11-8.
- [6] Thompson L, Rowntree J, Windisch W, Waters SM, Shalloo L, Manzano P. Ecosystem management using livestock: embracing diversity and respecting ecological principles. *Animal Frontiers*. 2023 Apr 1;13(2):28-34
- [7] Leroy F, Beal T, Gregorini P, McAuliffe GA, van Vliet S. Nutritionism in a food policy context: the case of 'animal protein'. *Animal Production Science*. 2022 Feb 21;62(8):712-20.
- [8] Salter AM. Improving the sustainability of global meat and milk production. *Proceedings of the Nutrition Society*. 2017 Feb;76(1):22-7.
- [9] Jackson RB, Saunio M, Bousquet P, Canadell JG, Poulter B, Stavert AR, Bergamaschi P, Niwa Y, Segers A, Tsuruta A. Increasing anthropogenic methane emissions arise equally from agricultural and fossil fuel sources. *Environmental Research Letters*. 2020 Jul 15;15(7):071002.
- [10] Saunio M, Jackson RB, Bousquet P, Poulter B, Canadell JG. The growing role of methane in anthropogenic climate change. *Environmental Research Letters*. 2016 Dec 12;11(12):120207.
- [11] Steinfeld H, Opio C, Chara J, Davis KF, Tomlin P, Gunter S. Overview paper: Livestock, Climate and Natural Resource Use. https://www.livestockdialogue.org/fileadmin/templates/res_livestock/docs/2019_Sept_Kansas/4_Climate_and_Natural_Resource_Use_-_Online_consultation.pdf
- [12] Rojas-Downing MM, Nejadhashemi AP, Harrigan T, Woznicki SA. Climate change and livestock: Impacts, adaptation, and mitigation. *Climate risk management*. 2017 Jan 1;16:145-63.
- [13] IFAD (International Fund for Agricultural Development) https://www.ifad.org/documents/38714170/40864504/CAR_2018_web.pdf/c88b3b3b-92a4-4a48-9536-ded3c-83fed87
- [14] Brito LF, Bedere N, Douhard F, Oliveira HR, Arnal M, Peñagaricano F, Schinckel AP, Baes CF, Miglior F. Genetic selection of high-yielding dairy cattle toward sustainable farming systems in a rapidly changing world. *Animal*. 2021 Dec 1;15:100292.
- [15] FAO. Animal Genetics. <http://www.fao.org/animal-genetics/background/why-is-ag-important/en/> [accessed November 2023].
- [16] Cammack KM, Austin KJ, Lamberson WR, Conant GC, Cunningham HC. Ruminant nutrition symposium: Tiny but mighty: The role of the rumen microbes in livestock production. *Journal of animal science*. 2018 Feb;96(2):752-70.
- [17] Johnson KA, Johnson DE. Methane emissions from cattle. *Journal of animal science*. 1995 Aug 1;73(8):2483-92.
- [18] Arndt C, Hristov AN, Price WJ, McClelland SC, Pelaez AM, Cueva SF, Oh J, Dijkstra J, Bannink A, Bayat AR, Crompton LA. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 C target by 2030 but not 2050. *Proceedings of the National Academy of Sciences*. 2022 May 17;119(20):e2111294119.
- [19] Hristov AN, Oh J, Firkins JL, Dijkstra J, Kebreab E, Waghorn G, Makkar HP, Adesogan AT, Yang W, Lee C, Gerber PJ. Special topics—Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *Journal of animal science*. 2013 Nov 1;91(11):5045-69.
- [20] Veneman JB, Saetnan ER, Clare AJ, Newbold CJ. MitiGate; an online meta-analysis database for quantification of mitigation strategies for enteric methane emissions. *Science of the Total Environment*. 2016 Dec 1;572:1166-74.
- [21] Tseten T, Sanjorjo RA, Kwon M, Kim SW. Strategies to mitigate enteric methane emissions from ruminant animals. *J. Microbiol. Biotechnol*. 2022 32(3):269-277.
- [22] Chiariotti A. Rumen environmental and nutritional strategies to mitigate emissions from livestock. *Cuban Journal of Agricultural Science*. 2023 Oct 16;57.
- [23] Tapio I, Snelling TJ, Strozzi F, Wallace RJ. The ruminal microbiome associated with methane emissions from ruminant livestock. *Journal of animal science and biotechnology*. 2017 Dec;8(1):1-1.
- [24] Thornton PK, van de Steeg J, Notenbaert A, Herrero M. The impacts of climate change on livestock and livestock systems in developing countries: A review of what we know and what we need to know. *Agricultural systems*. 2009 Jul 1;101(3):113-27.
- [25] Cheng M, McCarl B, Fei C. Climate change and livestock production: a literature review. *Atmosphere*. 2022 Jan 15;13(1):140.
- [26] Sabia E, Napolitano F, Claps S, De Rosa G, Barile VL, Braghieri A, et al. Environmental impact of dairy buffalo heifers kept on pasture or in confinement. *Elsevier Agricultural System*. 2018;159(c):42-49. <https://doi.10.1016/j.agsy.2017.10.010>.

- [27] Grossi G, Goglio P, Vitali A, Williams AG. Livestock and climate change: impact of livestock on climate and mitigation strategies. *Animal Frontiers*. 2019 Jan;9(1):69-76.
- [28] Monteiro A, Santos S, Gonçalves P. Precision agriculture for crop and livestock farming—Brief review. *Animals*. 2021 Aug 9;11(8):2345.
- [29] Tilman, D. and Clark, M., 2014. Global diets link environmental sustainability and human health. *Nature*, 515(7528), pp.518-522.
- [30] Twine, R., 2021. Emissions from animal agriculture—16.5% is the new minimum figure. *Sustainability*, 13(11), p.6276.
- [31] Hou D, Bolan NS, Tsang DC, Kirkham MB, O'Connor D. Sustainable soil use and management: An interdisciplinary and systematic approach. *Science of the Total Environment*. 2020 Aug 10;729:138961.
- [32] Terramoccia S, Bartocci S, Taticchi A, Di Giovanni S, Pauselli M, Mourvaki E, Urbani S, Servili M. Use of dried stoned olive pomace in the feeding of lactating buffaloes: Effect on the quantity and quality of the milk produced. *Asian-Australasian journal of animal sciences*. 2013 Jul;26(7):971.
- [33] Tong F, Wang T, Gao NL, Liu Z, Cui K, Duan Y, Wu S, Luo Y, Li Z, Yang C, Xu Y. The microbiome of the buffalo digestive tract. *Nature Communications*. 2022 Feb 10;13(1):823.
- [34] Contò M, Cifuni GF, Iacurto M, Failla S. Effect of pasture and intensive feeding systems on the carcass and meat quality of buffalo. *Animal Bioscience*. 2022 Jan;35(1):105.
- [35] Abd El-Salam MH, El-Shibiny S. A comprehensive review on the composition and properties of buffalo milk. *Dairy science & technology*. 2011 Nov;91:663-99.
- [36] de Oliveira LS, Alves JS, Bastos MS, da Cruz VA, Pinto LF, Tonhati H, Costa RB, de Camargo GM. Water buffaloes (*Bubalus bubalis*) only have A2A2 genotype for beta-casein. *Tropical Animal Health and Production*. 2021 Mar;53:1-4.

PRECISION LIVESTOCK FARMING IN BUFFALO SPECIES: A SUSTAINABLE APPROACH FOR THE FUTURE

Ganadería de precisión en búfalos: un enfoque sostenible para el futuro

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ABSTRACT

The growth of the world population that will occur in the next 30 years will be responsible for an increase in animal-derived food and proteins of animal origin. The livestock sector will be obliged to face new challenges, such as the reduction of environmental impact, the improvement of animal-derived food quality and safety, the reduction of antibiotics, and the increase in efficiency. One of the strategies that could be adopted is Precision Livestock Farming (PLF), recognized as the most sustainable tool to improve farm sustainability. It can be defined as *"the continuous, automated, and real-time monitoring of production, reproduction, health, and welfare through the application of advanced information and communication technologies (ICT)"*. In this new farm concept, animals, environment, machinery, and processes become "information objects" to enhance data; farm management and animals are defined as CITD systems: they are Complex, Individually different, Time-variant, and Dynamic. Several PLF technologies have been recently applied to buffalo species, improving some critical points of the farm, such as milking, nutrition, reproduction, and management. This short review reports some experiences carried out in buffalo species.

Keywords: Precision livestock farming, sustainability, buffaloes.

RESUMEN

El crecimiento de la población mundial que se producirá en los próximos 30 años será responsable de un aumento de los alimentos de origen animal y de las proteínas de origen animal. El sector ganadero se verá obligado a afrontar nuevos retos, como la reducción del impacto ambiental, la mejora de la calidad y seguridad de los alimentos de origen animal, la reducción de antibióticos y el aumento de la eficiencia. Una de las estrategias que podrían adoptarse es la Ganadería de Pre-

cisión (PLF), reconocida como la herramienta más sostenible para mejorar la sostenibilidad de las explotaciones agrícolas. Puede definirse como "el seguimiento continuo, automatizado y en tiempo real de la producción, la reproducción, la salud y el bienestar mediante la aplicación de tecnologías avanzadas de la información y la comunicación (TIC)". En este nuevo concepto de granja, los animales, el medio ambiente, la maquinaria y los procesos se convierten en "objetos de información" para mejorar los datos; La gestión agrícola y los animales se definen como sistemas CITD: son complejos, individualmente diferentes, variables en el tiempo y dinámicos. Recientemente se han aplicado varias tecnologías PLF a los búfalos, mejorando algunos puntos críticos de la granja, como el ordeño, la nutrición, la reproducción y el manejo. Esta breve reseña reporta algunas experiencias realizadas en búfalos.

Palabras clave: Ganadería de precisión, sostenibilidad, búfalos.

INTRODUCTION

It is known that the world's human population is actually about 8 billion, and it is estimated to reach 8.5 billion in 2030 and 9.7 billion in 2050. This sharp increase will occur mainly in developing countries, particularly Africa and Asia, where about 80% of the human population is distributed. This condition will cause an increase in the global demand for food, to 70% higher than in 2010 [1], for both plant and animal-derived food. One of this scenario's main limitations is the unavailability of further arable land. Simultaneously, the world is encountering a profound climate change [2] that is caused by the so-called "global warming," an increase of the global temperature that is expected to be about 3.5–5.5°C in 2080 [3]. Production, reproduction, and sensibility to pathogens or different environmental conditions are only some aspects that livestock will be (and are) obliged to face. The world's increasing demand for

animal-derived food requires new strategies to increase farm efficiency and sustainability. The impact of livestock farming on natural resources is under further pressure [4] from consumers, who demand high-quality products, animal welfare, and traceability information.

On the other hand, it is known that livestock is one of the most demanding sectors in terms of resources for several reasons, such as land use (for both grazing and feed production) [5], water [6], and energy [7] consumption. Furthermore, it is often accused of being one of the main ones responsible for environmental impact, for both poor manure management, mainly for nitrogen and phosphorus pollution [8], and greenhouse (GHG) emissions: Livestock accounts for 30% of GHG emissions of the agriculture sector, which is responsible for 14.0% of world GHG [9]. This led to a new environmental awareness, and animals are assumed to be a source of impact on the environment and public health.

NEW CHALLENGES OF THE LIVESTOCK SECTOR

In this complex scenario, the livestock sector must face new challenges: the reduction of environmental impact, the improvement of animal-derived food quality and safety, the reduction of antibiotics, and the increase of efficiency in one health view are some of these. Several solutions have been proposed in this sense, such as cultured meat (for review, see [10]) and edible insects [11]. The former has several advantages: it does not require animals, is highly efficient (one billion burgers can be produced from one biopsy in 45 days [12]), meets the favor of vegetarians and vegans, and does not produce GHG. However, there are also some negative aspects: its production is expensive, and growth factors and antibiotics are used during production. Insects are considered one of the most sustainable sources of nutrients because of their high protein, vitamins, minerals, and unsaturated fatty acids content [13]. However, one of the main limitations of entomophagy is from a cultural point of view.

THE PRECISION LIVESTOCK FARMING (PLF)

Precision Livestock Farming (PLF) is recognized as the most sustainable tool to improve these aspects [14]. It can be defined as “the continuous, automated, and real-time monitoring of production, reproduction, health, and welfare through the application of advanced information and communication technologies (ICT)” [15]. The PLF approach includes many technologies that aim to utilize the vast amount of data that can be collected daily on the farm and transform them into useful information. Basically, Industry 4.0 is based on the utilization of the IIoT (Industrial Internet of Things) to develop a new and personalized production model: the IoHAT (Internet of Animal Health Things) [16]. In this new farm concept, animals, environment, machinery, and processes become “information objects”

to enhance data farm management. One of the main differences between the traditional approach and that performed by PLF is the change in animal role. The latter has a central position in PLF systems since it is the main responsible for the information of the process. However, no animal is identical to another, and the same animal has different responses and behaviors according to its physiological or pathological condition.

Furthermore, it is more complicated than an electronic system, and its response can be different, variable, and dynamic based on different conditions. Indeed, in a PLF approach, the animals are defined as CIRD systems, where they are defined as Complex, Individually different, Time-variant, and Dynamic [15; 17]. The great revolution that derives from this vision of the animal is that if, in the traditional vision, a group of animals is considered as a “unicum”, through the PLF approach, the same group is considered as a “set of individualities”, where each individual contributes with its variability and differences in response to the average.

Sensors utilized in PLF can monitor animals, the environment, and products. Several PLF technologies have been recently applied to buffalo species, improving some critical points of the farm, such as milking, nutrition, reproduction, and management.

BUFFALO SPECIES & PLF

The buffalo (*Bubalus bubalis*) species is widespread worldwide, particularly in developing countries. According to FAO statistics [18], more than 203 million heads are actually present, and about 98% of the total population is concentrated in Asia. Only 0.2% of the world population is bred in Europe. However, the majority of European buffaloes are concentrated in Italy, where buffalo milk is almost totally utilized for mozzarella cheese production. Throughout the last 40 years, buffalo husbandry in Italy underwent a profound transformation, modifying the farming conditions closer and closer to those of dairy cows.

Furthermore, the physiological characteristics of the species, such as seasonality, caused a completely different methodological approach [19]. A hard work of selection has been carried out: although the national average milk yield is 2,350 kg in 270 days [20], with fat and protein percentages of 7.72% and 4.65%, respectively, it is not rare to find farms with an average milk yield that exceeds 3,000 kg of milk/lactation. These productive levels were achieved through proper selective criteria, improvements in rationing schemes, environmental farming conditions, and management in general. Therefore, a growing interest is deserved in the application of several PLF technologies. Although their utilization is still limited, some interesting experiences have been reported in several fields.

- Identification and localization systems are nowadays considered indispensable for a correct management of the herd [21]. The most commonly technologies used in

the buffalo are the radio frequency identification (RFID) technology [22]. RFID sensors are usually located in the rumen as boluses but can be also positioned as subcutaneous implants or ear tags and, as in cattle, sensors are developed using full duplex (FDX) and half duplex (HDX) technologies [23].

- Another field in which PLF has been applied in buffalo is the genomic prediction [24]. The technique consists in estimating the genetic value of thousands of markers (single nucleotide polymorphism - snps) distributed in the genetic heritage and associated with phenotypes of interest [25; 26; 27]. In 2013 the sequencing and assembly of the buffalo genome was completed (GCF_000471725.1; filed on NCBI in November 2013) and a new chip of a 90K SNP genotyping assay was designed and validated [28]. Quantitative Trait Loci (QTLs) associated with several features have been studied in buffalo, such as productive traits and lactation [29-34] reproduction [29; 35], welfare [36] and mastitis [37]. Through PLF technologies the collection of phenotypes can be performed with high precision and accuracy.
- Farm management can be improved in several ways. Milking has been improved through both the application of automated milking systems (AMS) and the adaptability to machine milking. The latest generation of milking robots is equipped with a digital camera and a laser triangulation sensor, utilizing a 3-D time-of-flight (Time-of-Flight-TOF) camera. Through AMS, animal welfare is increased, together with number of milkings/day, milk yield and milk quality [38-39]. The “milkability” has been study to recognize the capability of animals to release milk and identify those that can be adapted to machine milking by using lactocorder [40]. Another robotic technology is applied in calves: calf management can be improved through automatic milk feeder integrated with a robotic arm (Calf-rail®, Germany) for the administration of milk replacer.
- Animal welfare can also be monitored through machine vision and 3D vision for the simultaneous control of one or more variables (body condition, dimensions, weight, etc.). Through this approach it is possible to indirectly and non-invasively evaluate the biometry of Mediterranean buffalo calves, to estimate their growth, using depth cameras, as stereocamera or LIDAR [41-42] and to measure volume and weight of feed [43].
- Several Automated Estrus Detection (AED) technologies have been developed to monitor reproduction. A common problem of these tools is that behavioral and physiological changes are not typical of estrus: therefore, the warnings supplied by the AED technologies needs to be verified and confirmed through a gold standard (i.e. progesterone or a clinic exam by vets). For reproduction monitoring pedometers were used at the beginning of the 21st century [44] and sensitive telemetry devices (Heat Watch®, DDX Inc, Colorado, USA) were tested in Brazil [45]. Recently, also the infrared thermography (IRT) has been applied to the reproductive management of buffalo, in both female [46] and male [47].
- The monitoring of health is probably one of the most important advantages of PLF. Some physiological behaviors (feeding, rumination, lying, and standing) have been recently validated in buffaloes through NEDAP monitoring technology [48] and this can be used also for calving management [49]. Similarly, an algorithm for locomotion behavior by using 3-dimensional accelerometers (Rumi-Watch®) with high level of accuracy [50]. The health of the mammary gland has been largely studied, because of the high incidence of subclinical intramammary infections [51, 52]. For this reason, the SCC (Somatic Cell Count) or SCS (Somatic Cell Score) that represents its log-transformed value [53] is largely used to identify infections in buffaloes [52]. Furthermore, in the last couple of years, also other techniques have been studied in buffalo, such as Differential Somatic Cell Count (DSCC) [54, 55] together with the electric conductivity (EC) of milk [56] and IRT [46].
- The environmental influences were studied evaluating the effects of the bioclimatic index THI (Temperature Humidity index) on milk yield and characteristics. Several studies suggested that buffaloes are sensitive to heat [57, 58] and cold [59]. stress, suggesting the importance of this monitoring. Further environmental monitoring were carried out regarding methane emissions through Laser Methane Detector or LMD [60, 61] and pasture management [62].
- Product quality is studied through Infrared Spectroscopy (IRS), that allows the construction of prediction models and the detection of phenotypic traits that are not easily detectable, such as freezing point, pH, antioxidant power of milk, mineral composition, as well as coagulation characteristics, acidity and GHG emissions. Furthermore, the presence of buffalo milk in mixture with other milks can also be performed [63].

CONCLUSIONS

The increase in sustainability is one of the main aims requested by the livestock sector, including buffalo. To this aim, buffalo species will be obliged to face new challenges in the next few years, and this could occur only through the application of new technologies in order to enhance the grade of innovation. The PLF is probably the most applicable solution to reach these aims, allowing real-time, continuous, and automated monitoring of the main processes of the farm (such as welfare, health, production, and reproduction), the environment, and the quality of the productions. Although few studies have

been carried out in buffalo in this field, an increased interest has been recently developed. One of the main problems that must be faced is the need for more specific algorithms and prediction models for this species; therefore, all these techniques must be validated in buffalo to obtain reliable results. For this reason, further studies should be carried out in the future to increase the knowledge in this field.

REFERENCES

- [1] Godfray HC, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C. Food security: the challenge of feeding 9 billion people. *Science*. 2010; 327(5967):812-8. doi: <https://doi.org/10.1126/science.1185383>.
- [2] Intergovernmental Panel on Climate Change (IPCC), 2023. Summary for policymakers. In: Core Writing Team, Lee, H., Romero, J. (Eds.), *Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Geneva, Switzerland, pp. 1–34. <https://doi.org/10.59327/IPCC/AR6-9789291691647.001>.
- [3] Upadhyay RC, Singh SV, Kumar A, Gupta SK. 2007. Impact of climate change on milk production of Murrah buffaloes. *Ital J Anim Sci*. 2007; 6(Suppl 2):1329–1332. doi: <http://dx.doi.org/10.4081/ijas.2007.s2.1329>.
- [4] MacLeod MJ, Vellinga T, Opio C, Falcucci A, Tempio G, Henderson B, Makkar H, Mottet A, Robinson T, Steinfeld H, Gerber PJ. Invited review: A position on the Global Livestock Environmental Assessment Model (GLEAM). *Animal*. 2018; 12(2):383-397. doi: <https://doi.org/10.1017/S1751731117001847>.
- [5] Phelps LN, Kaplan JO. Land use for animal production in global change studies: Defining and characterizing a framework. *Global Change Biology* 2017; 23(11):4457–4471. doi: <https://doi.org/10.1111/gcb.13732>.
- [6] Potopová V, Musiolková M, Gaviria JA, Trnka M, Havlík P, Boere E, Trifan T, Muntean N, Chawdhery MRA. Water Consumption by Livestock Systems from 2002–2020 and Predictions for 2030–2050 under Climate Changes in the Czech Republic. *Agriculture*. 2023; 13(7):1291. doi: <https://doi.org/10.3390/agriculture13071291>.
- [7] Oz O, Sahin M, Akar O. Modeling of an HPS for the electric power demand of the cattle farm using genetic algorithm. *Heliyon*. 2023 Jun 13; 9(6):e17237. doi: <https://doi.org/10.1016/j.heliyon.2023.e17237>.
- [8] Kronberg SL, Provenza FD, van Vliet S, Young SN. Review: Closing nutrient cycles for animal production - Current and future agroecological and socio-economic issues. *Animal*. 2021; 15(Suppl 1):100285. doi: <https://doi.org/10.1016/j.animal.2021.100285>.
- [9] Bertoni G. Human, Animal and Planet Health for Complete Sustainability. *Animals (Basel)*. 2021; 11(5):1301. doi: <https://doi.org/10.3390/ani11051301>.
- [10] Pawar D, Lo Presti D, Silvestri S, Schena E, Massaroni C. Current and future technologies for monitoring cultured meat: A review. *Food Res Int*. 2023; 173(Pt.2):113464. doi: <https://doi.org/10.1016/j.foodres.2023.113464>.
- [11] Olivadese M, Dindo ML. Edible Insects: A Historical and Cultural Perspective on Entomophagy with a Focus on Western Societies. *Insects* 2023; 14(8):690. doi: <https://doi.org/10.3390/insects14080690>.
- [12] Tomiyama AJ, Kawecki NS, Rosenfeld DL, Jay JA, Rajagopal D, Rowat AC. Bridging the gap between the science of cultured meat and public perceptions. *Trends in Food Science and Technology* 2020; 104:144-152. doi: <https://doi.org/10.1016/j.tifs.2020.07.019>.
- [13] Tanga CM, Ekesi S. Dietary and Therapeutic Benefits of Edible Insects: A Global Perspective. *Annu Rev Entomol*. 2023. <https://doi.org/10.1146/annurev-ento-020123-013621>.
- [14] Lovarelli D, Bacenetti J, Guarino, M., 2020. A review on dairy cattle farming: is precision livestock farming the compromise for an environmental, economic and social sustainable production? *J. Clean. Prod*. 2020; 262:121409. doi: <https://doi.org/10.1016/j.jclepro.2020.121409>.
- [15] Berckmans D.. General introduction to precision livestock farming, *Animal Frontiers*; 2017; 7(1):6–11. <https://doi.org/10.2527/af.2017.0102>.
- [16] Norton T, Chen C, Larsen MLV, Berckmans D. Review: Precision livestock farming: building ‘digital representations’ to bring the animals closer to the farmer. *Animal* 2019; 13(12):3009-3017. doi: <https://doi.org/10.1017/S175173111900199X>.
- [17] Berckmans D, Aerts JM. Integration of biological responses in the management of bioprocesses. Master Course in the Masters of BioSystems and of Human Health Engineering at KU Leuven 2016.
- [18] Food and Agriculture Organization of the United Nations (FAO). 2019. How to Feed the World in 2050. Editor(s): P. Ferranti, E.M. Berry, J.R. Anderson (Eds). Elsevier, 2019: 481-487 (ISBN 9780128126882)https://www.fao.org/fileadmin/templates/wfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf <https://www.fao.org/faostat/en/#data/QCL>.
- [19] Neglia G, De Nicola D, Esposito L, D’Occhio MJ, Fatone G. Reproductive management in buffalo by artificial

- insemination. *Theriogenology* 2020; 150:166–172. doi: <https://doi.org/10.1016/j.theriogenology.2020.01.016>.
- [20] Associazione Nazionale Allevatori Specie Bufalina (ANASB), 2023. <https://www.anasb.it/statistiche/>.
- [21] Garcia AR, Barros DV, de Oliveira Junior MCM, Barioni Junior W, da Silva JAR, Lourenço Junior JDB, dos Santos Pessoa J. 2020. Innovative use and efficiency test of subcutaneous transponders for electronic identification of water buffaloes. *Trop Anim Health Prod.* 2020; 52:3725-3733. doi: <https://doi.org/10.1007/s11250-020-02410-7>.
- [22] Zhang J, Tian GY, Marindra AMJ, Sunny AI, Zhao AB. A Review of Passive RFID Tag Antenna-Based Sensors and Systems for Structural Health Monitoring Applications. *Sensors.* 2017; 17(2):265. doi: <https://doi.org/10.3390/s17020265>.
- [23] Stewart SC, Rapnicki P, Lewis JR, Perala M. Detection of low frequency external electronic identification devices using commercial panel readers. *J Dairy Sci.* 2007 Sep; 90(9):4478-82. doi: <https://doi.org/10.3168/jds.2007-0033>.
- [24] Cesarani A, Biffani S, Garcia A, Lourenco D, Bertolini G, Neglia G, Misztal I, Macciotta N. Genomic investigation of milk production in Italian Buffalo. *Italian Journal of Animal Science* 2021; 20:539-547. <https://doi.org/10.1080/1828051X.2021.1902404>.
- [25] Brito LF, Bedere N, Douhard F, Oliveira HR, Arnal M, Peñagaricano F, Schinckel AP, Baes CF, Miglior F. Review: Genetic selection of high-yielding dairy cattle toward sustainable farming systems in a rapidly changing world. *Animal* 2021; 15(1):100292. <https://doi.org/10.1016/j.animal.2021.100292>.
- [26] Bickhart DM, McClure JC, Schnabel RD, Rosen BD, Medrano JF, Smith TPL. Symposium review: Advances in sequencing technology herald a new frontier in cattle genomics and genome-enabled selection. *J. Dairy Sci.* 2020; 103(6):5278-5290. doi: <https://doi.org/10.3168/jds.2019-17693>.
- [27] Strandén I, Kantanen J, Lidauer MH, Mehtiö T, Negussie E. Animal board invited review: Genomic-based improvement of cattle in response to climate change. *Animal* 2022; 16(12):100673. doi: <https://doi.org/10.1016/j.animal.2022.100673>.
- [28] Iamartino D, Nicolazzi EL, Van Tassell CP, Reecy JM, Fritz-Waters ER, Koltes JE, Biffani S, Sonstegard TS, Schroeder SG, Ajmone-Marsan P, Negrini R, Pasquariello R, Ramelli P, Coletta A, Garcia JF, Ali A, Ramunno L, Cosenza G, de Oliveira DAA, Drummond MG, Bastianetto E, Davassi A, Pirani A, Brew F, Williams JL. Design and validation of a 90K SNP genotyping assay for the water buffalo (*Bubalus bubalis*). *PLoS One.* 2017; 12(10):e0185220. doi: <https://doi.org/10.1371/journal.pone.0185220>.
- [29] Ravi Kumar D, Nandhini PB, Joel Devadasan M, Sivalingam J, Mengistu DW, Verma A, Gupta ID, Niranjan SK, Kataria RS, Tantia MS. Genome-wide association study revealed suggestive QTLs for production and reproduction traits in Indian Murrah buffalo. *3 Biotech.* 2023; 13(3):100. doi: <https://doi.org/10.1007/s13205-023-03505-2>.
- [30] Silva AA, Brito LF, Silva DA, Lazaro SF, Silveira KR, Stefani G, Tonhati H. Random regression models using B-splines functions provide more accurate genomic breeding values for milk yield and lactation persistence in Murrah buffaloes. *J. Anim. Breed. Genet.* 2023; 140(2):167-184. doi: <https://doi.org/10.1111/jbg.12746>.
- [31] Lázaro SF, Tonhati H, Oliveira HR, Silva AA, Nascimento AV, Santos DJA, Stefani G, Brito LF. Genomic studies of milk-related traits in water buffalo (*Bubalus bubalis*) based on single-step genomic best linear unbiased prediction and random regression models. *J Dairy Sci.* 2021; 104(5):5768-5793. doi: <https://doi.org/10.3168/jds.2020-19534>.
- [32] Deng T, Liang A, Liang S, Pang C, Ma X, Lu X, Duan A, Pang C, Hua G, Liu S, Campanile G, Salzano A, Gasparrini B, Neglia G, Liang X, Yang L. Integrative Analysis of Transcriptome and GWAS Data to Identify the Hub Genes Associated with Milk Yield Trait in Buffalo. *Frontiers in Genetics* 2019; 10:36. doi: <https://doi.org/10.3389/fgen.2019.00036>.
- [33] Li J, Liu S, Li Z, Zhang S, Hua G, Salzano A, Campanile G, Gasparrini B, Liang A, Yang L. DGAT1 polymorphism in Riverine buffalo, Swamp buffalo and crossbred buffalo. *J Dairy Res.* 2018; 85(4):412-415. doi: <https://doi.org/10.1017/S0022029918000468>.
- [34] Liu JJ, Liang AX, Campanile G, Plastow G, Zhang C, Wang Z, Salzano A, Gasparrini B, Cassandro M, Yang LG. Genome-wide association studies to identify quantitative trait loci affecting milk production traits in water buffalo. *J Dairy Sci.* 2018; 101(1):433-444. doi: <https://doi.org/10.3168/jds.2017-13246>.
- [35] de Araujo Neto FR, Takada L, Dos Santos DJA, Aspilcueta-Borquis RR, Cardoso DF, do Nascimento AV, Leão KM, de Oliveira HN, Tonhati H. Identification of genomic regions related to age at first calving and first calving interval in water buffalo using single-step GBLUP. *Reprod Domest Anim.* 2020; 55(11):1565-1572. doi: <https://doi.org/10.1111/rda.13811>.
- [36] de Araujo Neto FR, Dos Santos JCG, da Silva Arce CD, Borquis RRA, Dos Santos DJA, Guimarães KC, do Nascimento AV, de Oliveira HN, Tonhati H. Genomic study of the resilience of buffalo cows to a negative energy balan-

- ce. *J Appl Genet.* 2022; 63(2):379-388. doi: <https://doi.org/10.1007/s13353-021-00680-x>.
- [37] Jaiswal S, Jagannadham J, Kumari J, Iquebal MA, Gurjar AKS, Nayan V, Angadi UB, Kumar S, Kumar R, Datta TK, Rai A, Kumar D. Genome Wide Prediction, Mapping and Development of Genomic Resources of Mastitis Associated Genes in Water Buffalo. *Front Vet Sci.* 2021; 8:593871. doi: <https://doi.org/10.3389/fvets.2021.593871>.
- [38] Faugno S, Pindozi S, Okello C, Sannino M. Testing the application of an automatic milking system on buffalo (*Bubalus bubalis*). *J Agric Eng.* 2015; 46:13–18. doi: <https://doi.org/10.4081/jae.2015.437>.
- [39] Verde MT, Matera R, Bonavolonta F, Angrisani A, Fezza C, Borzacchiello L, Cotticelli A, Neglia G. Comparative Performance Analysis Between Two Different Generations of Automatic Milking System. *Acta Imeko* 2023; (work accepted, in press).
- [40] Boselli C, De Marchi M, Costa A, Borghese A. Study of Milkability and Its Relation With Milk Yield and Somatic Cell in Mediterranean Italian Water Buffalo. *Front Vet Sci.* 2020; 7:432. doi: <https://doi.org/10.3389/fvets.2020.00432>.
- [41] Matera R, Angrisani L, Neglia G, Salzano A, Bonavolontà F, Verde MT, Piscopo N, Vistocco D, Tamburis O. Reliable use of smart cameras for monitoring biometric parameters in Buffalo Precision Livestock Farming. *Acta Imeko*; 2023 (Work accepted, in press).
- [42] Tamburis O, Matera R, Salzano A, Calamo A, Vistocco D, Neglia G. 'Smart' Buffalo Weight Estimation via Digital Technologies: Experiences from South Italy. *Stud Health Technol Inform.* 2023; 302:895-896. doi: <https://doi.org/10.3233/shti230298>.
- [43] Cotticelli A, Verde MT, Liccardo A, de Alteriis G, Matera R, Neglia G, Peric T, Prandi A, Bonavolontà F. On the Use of 3D Camera to Accurately Measure Volume and Weight of Dairy Cow Feed. *Acta Imeko* 2023; (Work accepted, in press).
- [44] Di Palo R, Campanile G, Zicarelli L. Tecnologie utilizzate per la rilevazione dei calori e inseminazione strumentale nella specie bufalina. *Proceedings of the 1st Congresso Nazionale sull'Allevamento del Bufalo*; 2001 Oct 03-05; Eboli, Italy. P. 100-113.
- [45] Porto-Filho RM, Gimenes LU, Monteiro BM, Carvalho NAT, Ghuman SPS, Madureira EH, Baruselli PS. Detection of estrous behavior in buffalo heifers by radiotelemetry following PGF2 α administration during the early or late luteal phase. *Animal Reproduction Science* 2014; 144(3-4):90–94. doi: <https://doi.org/10.1016/j.anireprosci.2013.12.006>.
- [46] Neglia G, Matera R, Cotticelli A, Salzano A, Campanile G. Chapter 23 – PLF e allevamento bufalino. In: *Zootecnia di Precisione e Tecnologie Innovative in Allevamento*. Eds: Abeni F, Nannoni E, Sandrucci A. 2024; *Le Point Veterinarie Italie* (in press).
- [47] Yadav SK, Singh P, Kumar P, Singh SV, Singh A, Kumar S. 2019. Scrotal infrared thermography and testicular biometry: Indicator of semen quality in Murrah buffalo bulls. *Anim Reprod Sci.* 209: 106145. doi: <https://doi.org/10.1016/j.anireprosci.2019.106145>.
- [48] Quddus RA, Ahmad N, Khalique A, Bhatti JA. Validation of NEDAP Monitoring Technology for Measurements of Feeding, Rumination, Lying, and Standing Behaviors, and Comparison with Visual Observation and Video Recording in Buffaloes. *Animals (Basel)* 2022; 12(5):578. doi: <https://doi.org/10.3390/ani12050578>.
- [49] Quddus RA, Ahmad N, Khalique A, Bhatti JA. Evaluation of automated monitoring calving prediction in dairy buffaloes a new tool for calving management. *Braz. J. Biol.* 2022; 84:e257884. doi: <https://doi.org/10.1590/1519-6984.257884>.
- [50] D'Andrea L, Guccione J, Alsaad M, Deiss R, Di Loria A, Steiner A, Ciaramella P. Validation of a pedometer algorithm as a tool for evaluation of locomotor behaviour in dairy Mediterranean buffalo. *J Dairy Res.* 2017; 84(4):391-394. doi: <https://doi.org/10.1017/S0022029917000668>.
- [51] Costa A, De Marchi M, Neglia G, Campanile G, Penasa M. Milk somatic cell count-derived traits as new indicators to monitor udder health in dairy buffaloes. *It. J. Anim. Sci.* 2021; 20:548–558. Doi: <https://doi.org/10.1080/1828051X.2021.1899856>.
- [52] Costa A, Neglia G, Negrini R, Campanile G, De Marchi M. (2020). Milk somatic cells and their relationships with milk yield and quality traits in a large population of Water Buffaloes. *J. Dairy Sci.* 2020; 103:5485–5494. Doi: <https://doi.org/10.3168/jds.2019-18009>.
- [53] Ali AKA, Shook GE. An optimum transformation for somatic cell concentration in milk. *J. Dairy Sci.* 1980; 63:487–490. doi: [https://doi.org/10.3168/jds.S0022-0302\(80\)82959-6](https://doi.org/10.3168/jds.S0022-0302(80)82959-6).
- [54] Bobbo T, Matera R, Biffani S, Gomez M, Cimmino R, Pedota G, Neglia G. Exploring the sources of variation of electrical conductivity, total and differential somatic cell count in the Italian Mediterranean Buffaloes. *J. Dairy Sci.* 2023; S0022-0302(23)00659-8. Doi: <https://doi.org/10.3168/jds.2023-23629>
- [55] Bobbo T, Matera R, Pedota G, Manunza A, Cotticelli A, Neglia G, Biffani S. Exploiting machine learning methods with monthly routine milk recording data and climatic information to predict subclinical mastitis in the Italian Mediterranean Buffaloes. *J. Dairy Sci.* 2023; 106:1942–1952. Doi: DOI number: <https://doi.org/10.3168/jds.2022-22292>.

- [56] Matera R., Di Vuolo G., Cotticelli A., Salzano A., Neglia G., Cimmino R., D'Angelo D., Biffani S. Relationship among milk conductivity, production traits and somatic cell score in the Italian Mediterranean Buffalo. *Animals* 2022; 12:2225. doi: <https://doi.org/10.3390/ani12172225>.
- [57] Koga A, Sugiyama M, Del Barrio AN, Lapitan RM, Arendá BR, Robles AY, Cruz LC, Kanai Y. 2004. Comparison of the thermoregulatory response of buffaloes and tropical cattle, using fluctuations in rectal temperature, skin temperature and haematocrit as an index. *J Agric Sci.* 2004; 142(3): 351–355. doi: <https://doi.org/10.1017/S0021859604004216>.
- [58] Marai IFM, Haebe AAM. 2010. Buffalo's biological functions as affected by heat stress — a review. *Livest Sci.* 2010; 127(2-3):89–109. doi: <https://doi.org/10.1016/j.livsci.2009.08.001>.
- [59] Matera R, Cotticelli A, Gómez Carpio M, Biffani S, Iannaccone F, Salzano A, Neglia G. Relationship among production traits, somatic cell score and THI in the Italian Mediterranean Buffalo. *It. J. Anim. Sci.* 2022; 21:551–561. doi: <https://doi.org/10.1080/1828051X.2022.2042407>.
- [60] Chagunda MGG, Ross D, Rooke J, Yan T, Douglas JL, Poret L, McEwan NR, Teeranavattanakul P, Roberts DJ. Measurement of enteric methane from ruminants using a hand-held laser methane detector. *Acta Agric. Scand. A Anim. Sci.* 2013;63:68–75. Doi: <https://doi.org/10.1080/09064702.2013.797487>.
- [61] Meo Zilio D, Steri R, Iacurto M, Catillo G, Barile V, Chiarriotti A, Cenci F, La Mantia MC, Buttazzoni L. (2022). Precision Livestock Farming for Mediterranean Water Buffalo: Some Applications and Opportunities from the Agridigit Project. In: Biocca, M., Cavallo, E., Cecchini, M., Failla, S., Romano, E. (eds) *Safety, Health and Welfare in Agriculture and Agro-food Systems*. SHWA 2020. Lecture Notes in Civil Engineering, vol 252. Springer, Cham. https://doi.org/10.1007/978-3-030-98092-4_5.
- [62] Valente GF, Ferraz GAeS, Santana LS, Ferraz PFP, Mariano DdC, dos Santos CM, Okumura RS, Simonini S, Barbari M, Rossi G. Mapping Soil and Pasture Attributes for Buffalo Management through Remote Sensing and Geostatistics in Amazon Biome. *Animals.* 2022; 12(18):2374. <https://doi.org/10.3390/ani12182374>.
- [63] Spina AA, Ceniti C, Piras C, Tilocca B, Britti D, Morittu VM. 2022. Mid-infrared (Mir) spectroscopy for the detection of cow's milk in buffalo milk. *J Anim Sci Technol.* 64(3): 531–538.



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Growth performance and gut health of Murrah buffalo calves fed with developed novel synbiotic formulation

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ABSTRACT

The present study aimed to develop a novel synbiotic formulation by screening different prebiotics as a substrate for autochthonous *Lactobacillus* spp. and studying their effects on the gut health and performance of buffalo calves. *Ligilactobacillus salivarius* BF17 (NCBI GenBank Accession No-MG966326), a potential probiotic previously isolated from Murrah buffalo calves, was chosen and evaluated for the best prebiotic substrate by screening five different prebiotics (fructo oligosaccharides, mannan oligosaccharides, inulin, corn dextrin, and wheat dextrin). Then, 23 neonatal buffalo calves were selected for 90 days and randomly divided into four groups, with five animals in the control group and six each in the treatment group. The groups were as follows: 1) control (CON) fed basal diet alone; 2) prebiotic group (PRE) supplemented with chicory powder (a natural source of inulin) at eight g/d/calf; 3) probiotic group (PRO), supplemented with milk fermented with *L. salivarius* BF17 having 108 CFU/g/d/calf and 4) synbiotic group (SYN) supplemented with both prebiotic and probiotic. All the calves had *ad libitum* access to a basal diet, having calf starter (from the second week onwards), green forage (maize and sorghum), and fresh, clean water throughout the experimental period. The calf starter (concentrate mixture) was formulated using quality ingredients comprising maize (28%), bajra (5%), groundnut (10%), soybean meal (15%), mustard oil cake (13%), wheat bran (15%), rice polish (11%), vitamin and mineral premix (2%), salt (1%). The body weight and structural measurements (body length, wither height, heart girth) were recorded fortnightly. Feed intake and residue were also measured during a digestion trial to determine the digestibility of various nutrients. Fecal samples were collected at fort-

Rendimiento del crecimiento y salud intestinal de terneros de búfalo Murrah alimentados con una nueva formulación sinbiótica desarrollada

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RESUMEN

El presente estudio tuvo como objetivo desarrollar una nueva formulación sinbiótica mediante la selección de diferentes prebióticos como sustrato para *Lactobacillus* spp autóctonos. y estudiar sus efectos sobre la salud intestinal y el rendimiento de los terneros de búfalo. *Ligilactobacillus salivarius* BF17 (NCBI GenBank Accession No-MG966326), un probiótico potencial previamente aislado de terneros Murrah, fue elegido y evaluado como el mejor sustrato prebiótico mediante la selección de cinco prebióticos diferentes (fructooligosacáridos, mananoligosacáridos, inulina, dextrina de maíz y dextrina de trigo). Luego, se seleccionaron 23 búfalos neonatos durante 90 días y se dividieron aleatoriamente en cuatro grupos, con cinco animales en el grupo de control y seis en cada grupo de tratamiento. Los grupos fueron los siguientes: 1) control (CON) alimentado solo con dieta basal; 2) grupo prebiótico (PRE) suplementado con achicoria en polvo (una fuente natural de inulina) a ocho g/día/ternero; 3) grupo probiótico (PRO), suplementado con leche fermentada con *L. salivarius* BF17 con 108 UFC/g/d/ternero y 4) grupo sinbiótico (SYN) suplementado tanto con prebiótico como con probiótico. Todos los terneros tuvieron acceso *ad libitum* a una dieta basal, contando con iniciador (a partir de la segunda semana), forraje verde (maíz y sorgo) y agua fresca y limpia durante todo el período experimental. El iniciador para terneros (mezcla concentrada) se formuló utilizando ingredientes de calidad que comprenden maíz (28%), mijo perla (5%), maní (10%), harina de soja (15%), torta de aceite de mostaza (13%), salvado de trigo (15%), pulimento de arroz (11%), premezcla de vitaminas y minerales (2%) y sal (1%). El peso corporal y las medidas estructurales

nightly intervals to analyze fecal pH, lactate, ammonia, and short-chain VFA. Microbiota enumeration of health-promoting (lactobacilli and bifidobacterial) and health-negative (coliform and clostridium) was enumerated by colony-forming method to assess gut health. Results indicated that inulin showed the highest ($p<0.05$) prebiotic index and prebiotic activity score by FOS, MOS, and dextrans; hence, inulin was chosen for synbiotic formulation with *L. salivarius* BF17. Chicory root powder was taken as a natural and cheaper source of inulin for animal feeding. The inulin content of chicory powder was estimated using the spectrophotometric method and found to be 57.5%. The final body weight of buffalo calves was higher ($p<0.05$) in SYN, followed by PRE and PRO, compared to CON. Structural growth measurements were also higher ($p<0.05$) in all the supplemented groups compared to the control group. A simultaneous increase ($p<0.05$) in Lactobacillus and Bifidobacterium populations and a decrease ($p<0.05$) in coliforms were observed in the synbiotic and probiotic-supplemented groups. The fecal biomarkers altered favorably, significantly in all the supplemented groups compared to the control group. Overall, it was concluded that inulin was found to be the best prebiotic for synbiotic formulation with *Ligilactobacillus salivarius* BF17, and supplementation of prebiotic, probiotic, and synbiotic improved the growth performance and gut health parameters in Murrah buffalo calves, however, the results were found to be better in the synbiotic group.

Keywords: calves, probiotics, synbiotic, gut health, Murrah buffalo calves.

(longitud corporal, altura a la cruz, circunferencia del corazón) se registraron quincenalmente. También se midieron el consumo de alimento y los residuos durante una prueba de digestión para determinar la digestibilidad de varios nutrientes. Se recolectaron muestras fecales a intervalos quincenales para analizar el pH fecal, el lactato, el amoníaco y los AGV de cadena corta. La enumeración de la microbiota promotora de la salud (lactobacilos y bifidobacterias) y negativa para la salud (coliformes y clostridium) se enumeró mediante el método de formación de colonias para evaluar la salud intestinal. Los resultados indicaron que la inulina mostró el índice prebiótico y la puntuación de actividad prebiótica más altos ($p<0,05$) por FOS, MOS y dextrinas; por lo tanto, se eligió la inulina para la formulación sinbiótica con *L. salivarius* BF17. El polvo de raíz de achicoria se consideró una fuente natural y más económica de inulina para la alimentación animal. El contenido de inulina del polvo de achicoria se estimó mediante el método espectrofotométrico y resultó ser del 57,5%. El peso corporal final de las terneras bufalinas fue mayor ($p<0,05$) en SYN, seguido de PRE y PRO, en comparación con CON. Las mediciones de crecimiento estructural también fueron mayores ($p<0,05$) en todos los grupos suplementados en comparación con el grupo control. Se observó un aumento simultáneo ($p<0,05$) en las poblaciones de lactobacillus y bifidobacterium y una disminución ($p<0,05$) en los coliformes en los grupos suplementados con sinbióticos y probióticos. Los biomarcadores fecales se alteraron favorablemente de manera significativa en todos los grupos suplementados en comparación con el grupo control. En general, se concluyó que la inulina era el mejor prebiótico para la formulación sinbiótica con *Ligilactobacillus salivarius* BF17, y la suplementación de prebióticos, probióticos y sinbióticos mejoró el rendimiento del crecimiento y los parámetros de salud intestinal en terneros Murrah; sin embargo, los resultados fueron encontrados ser mejor en el grupo sinbiótico.

Palabras clave: probióticos, sinbióticos, salud intestinal, terneros de búfalos Murrah.

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Meat characteristics of buffaloes (*Bubalus bubalis*) in two production systems and two slaughter weights

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ABSTRACT

Under two production systems, this study evaluated carcass and meat characteristics of finished male buffaloes at 400 and 600 kg live weight (LW). Twenty-eight male buffaloes uncastrated of 216 ± 21.9 kg LW were used and were randomly distributed into four treatments (T): T1 and T2, grazing buffaloes (GB), slaughtered at 400 and 600 kg LW, respectively; T3 and T4, buffaloes in intensive system (IS), slaughtered at 400 and 600 kg LW, respectively. GB groups remained in a *Brachiaria humidicola* meadow (6.8% CP, 66.4% NDF, and 2 Mcal EM kg⁻¹ DM) and were supplemented (32.02% CP, 25.19% NDF, and 2.8 Mcal ME kg⁻¹ MS) at 1% of LW. The diet provided in the IS contained 14.22% CP, 44.25% NDF, and 2.7 Mcal ME kg⁻¹ MS. The supplement's nutrients for GB and the diet provided in IS were adjusted according to the change in the animals' LW. The time to reach slaughter weight was 301, 368, 228, and 304 d for T1 to T4, respectively. After slaughter, the empty live weight (ELW= The filled green viscera were weighed, and later, they were washed with running water to remove the contents and determine the empty weight) and cold carcass yield (CCY= Cold carcass weight/slaughter weight) were evaluated. Color (A Minolta colorimeter was used to record the values of *L, *a, and *b,) and pH was measured on the *Longissimus dorsi* muscle (24 h, and 7, 14, and 21 d); in addition to performing the proximal chemical analysis (moisture, crude protein, fat, and collagen), resistance to cutting and water retention capacity. A

Características de la carne de búfalos (*Bubalus bubalis*) en dos sistemas de producción y dos pesos de sacrificio

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RESUMEN

Bajo dos sistemas de producción, este estudio evaluó las características de canal y la carne de búfalos machos terminados de 400 y 600 kg de peso vivo (PV). Se utilizaron veintiocho búfalos machos sin castrar de 216 ± 21.9 kg PV, distribuidos aleatoriamente en cuatro tratamientos (T): T1 y T2, búfalos en pastoreo (GB), sacrificados a 400 y 600 kg PV, respectivamente; T3 y T4, búfalos en sistema intensivo (SI), sacrificados a 400 y 600 kg PV, respectivamente. Los grupos de GB permanecieron en una pradera de *Brachiaria humidicola* (6.8% PB, 66.4% FND y 2 Mcal EM kg⁻¹ MS) y fueron suplementados (32.02% PB, 25.19% FND y 2.8 Mcal EM kg⁻¹ MS) al 1% de LW. La dieta proporcionada en el IS contuvo 14,22% PB, 44,25% FND y 2,7 Mcal EM kg⁻¹ MS. Los nutrientes del suplemento para GB y la dieta proporcionada en IS se ajustaron de acuerdo con el cambio en el PV de los animales. El tiempo para alcanzar el peso al sacrificio fue de 301, 368, 228 y 304 días para T1 a T4, respectivamente. Después del sacrificio, se pesó el peso vivo vacío (ELW=se pesaron las vísceras verdes llenas y posteriormente se lavaron con agua corriente para retirar el contenido y determinar el peso vacío) y el rendimiento de la canal fría (CCY= peso de la canal fría/peso al sacrificio) fueron evaluados. El color (se utilizó un colorímetro Minolta para registrar los valores de *L, *a y *b) y el pH se midieron en el músculo *Longissimus dorsi* (24 h, y 7, 14 y 21 d), además de realizar el análisis químico proximal (humedad, proteína cruda,

completely randomized design with a 2 x 2 factorial arrangement was used. The ELW of the buffaloes slaughtered at 400 kg did not vary ($p > 0.05$) between the GB or IS, nor did it vary between the groups slaughtered at 600 kg. However, the CCY of the animals sacrificed at 600 kg was higher in the IS than in the GB (54.06^b vs 57.97^a). Meat from T4 had the highest pH values ($p < 0.001$; 5.81^c, 6.04^b, 6.08^b, 6.24^a at 24 h, and 5.72^b, 5.93^b, 5.72^b and 6.13^a at 7 days, T1 to T4, respectively). No differences ($p > 0.05$) were observed between moisture and protein content treatments. Nevertheless, fat content was higher ($p < 0.001$) in T2 and lower ($p < 0.001$) in T3 (2.28^a vs 0.86^c), and collagen was higher ($p < 0.01$) in samples from GB (T1 and T2). The shear force was similar ($p > 0.05$) in the groups evaluated. Water retention capacity was higher ($p < 0.05$) in buffaloes' meat in IS (T3 and T4), and the lowest water retention was determined in animals kept grazing and sacrificed at 600 kg LW (T2). In conclusion, meat from buffaloes fed concentrates has lower ($p < 0.05$) collagen and fat content, better color characteristics, pH, and water retention capacity, related to better meat quality. Due to the time needed to reach slaughter weight, it is recommended to slaughter buffaloes at 400 kg LW when fed in intensive systems to obtain better carcass and meat characteristics.

Keywords: Buffalo, production system, slaughter weight, carcass yield, meat quality.

grasa y colágeno), resistencia al corte y capacidad de retención de agua. Se utilizó un diseño completamente al azar con arreglo factorial 2 x 2. El ELW de los búfalos sacrificados a 400 kg no varió ($p > 0.05$) entre el GB o IS, ni tampoco varió entre los grupos sacrificados a 600 kg. Sin embargo, el CCY de los animales sacrificados a 600 kg fue mayor en el IS que en el GB (54,06^b vs 57,97^a). La carne del T4 tuvo los valores de pH más altos ($p < 0.001$; 5.81^c, 6.04^b, 6.08^b, 6.24^a a las 24 h, y 5.72^b, 5.93^b, 5.72^b y 6.13^a a los 7 días, T1 a T4, respectivamente). No se observaron diferencias ($p > 0.05$) entre los tratamientos de humedad y contenido de proteína. Sin embargo, el contenido de grasa fue mayor ($p < 0,001$) en T2 y menor ($p < 0,001$) en T3 (2,28^a vs 0,86^c), y el colágeno fue mayor ($p < 0,01$) en las muestras de GB (T1 y T2). La fuerza cortante fue similar ($p > 0,05$) en los grupos evaluados. La capacidad de retención de agua fue mayor ($p < 0,05$) en la carne de búfalo en el IS (T3 y T4), y la menor retención de agua se determinó en los animales mantenidos en pastoreo y sacrificados a 600 kg PV (T2). En conclusión, la carne de búfalos alimentados con concentrados tiene menor ($p < 0,05$) contenido de colágeno y grasa, mejores características de color, pH y capacidad de retención de agua, relacionados con una mejor calidad de la carne. Debido al tiempo necesario para alcanzar el peso de sacrificio, se recomienda sacrificar búfalos a 400 kg PV cuando se alimentan en sistemas intensivos para obtener mejores características de la canal y la carne.

Palabras clave: búfalo, sistema de producción, peso al sacrificio, rendimiento de la canal, calidad de la carne.

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Productive performance of buffaloes (*Bubalus bubalis*) in two production systems and two slaughter weights

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ABSTRACT

The objective of this study was to evaluate productive performance in finished male buffaloes at 400 and 600 kg live weight (LW) under two production systems. Twenty-eight male uncastrated buffaloes of 216 ± 21.9 kg LW were used and were randomly distributed into four treatments (T): T1 and T2, grazing buffaloes + 1% live weight supplement (31% soybean paste, 53% ground corn, 10% wheat bran, 1.4% urea and 3.2% minerals, with 30% crude protein and 25% neutral detergent fiber), slaughtered at 400 and 600 kg LW, respectively; T3 and T4, buffaloes in intensive system (10% soybean paste, 15% ground corn, 10% wheat bran, 50% corn stover, 23% poultry litter and 2% minerals, with 12% crude protein and 44% neutral detergent fiber), slaughtered at 400 and 600 kg LW, respectively. Analysis of variance was conducted (PROC GLM; SAS®), considering weight and daily weight gain and fattening period as dependent variables and treatments as independent variables. When numerical differences were detected, a multiple comparison Tukey test was utilized ($p < 0.05$). Results indicated that buffaloes in T4 showed significantly higher values of daily weight gain ($p < 0.001$) compared to T3 and T4, and finally, T5 (1.10, 0.92, 0.95 y 0.62 kg/day, respectively). Likewise, it was observed that buffaloes that were raised with grazing equaled gains of the animals raised in intensive systems and slaughtered at 400 kg ($p > 0.05$). Finally, it was observed that the fattening period was more extended in T2 compared to T1, T3, T4, and T1 (368, 228, 304, and 301 days; $p < 0.001$, respectively). In conclusion, buffaloes raised in intensive systems show better daily weight gain. Feeding buffaloes with concentrated diets and slaughtering them at 600 kg LW is related to better weight

Comportamiento productivo de búfalos (*Bubalus bubalis*) en dos sistemas de producción y dos pesos de sacrificio

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RESUMEN

El objetivo de este estudio fue evaluar el comportamiento productivo en búfalos machos terminados de 400 y 600 kg de peso vivo (PV) bajo dos sistemas de producción. Se utilizaron veintiocho búfalos machos sin castrar de 216 ± 21.9 kg PV, distribuidos aleatoriamente en cuatro tratamientos (T): T1 y T2, búfalos en pastoreo + 1% suplemento de peso vivo (31% pasta de soya, 53% maíz molido, 10% salvado de trigo, 1.4% urea y 3.2% minerales, con 30% proteína cruda y 25% fibra detergente neutra), sacrificados a 400 y 600 kg PV, respectivamente; T3 y T4, búfalos en sistema intensivo (10% pasta de soja, 15% maíz molido, 10% salvado de trigo, 50% rastrojo de maíz, 23% gallinaza y 2% minerales, con 12% proteína cruda y 44% fibra detergente neutra), sacrificados con 400 y 600 kg de PV, respectivamente. Se realizó análisis de varianza (PROC GLM; SAS®), considerando el peso y la ganancia diaria de peso y el período de engorde como variables dependientes y los tratamientos como variables independientes. Cuando se detectaron diferencias numéricas, se utilizó una prueba de Tukey de comparación múltiple ($p < 0,05$). Los resultados indicaron que los búfalos en T4 mostraron valores significativamente mayores de ganancia de peso diaria ($p < 0.001$) en comparación con T3 y T4, y finalmente, T5 (1.10, 0.92, 0.95 y 0.62 kg/día, respectivamente). Asimismo, se observó que los búfalos criados bajo pastoreo igualaron ganancias de los animales criados en sistemas intensivos y sacrificados a 400 kg ($p > 0.05$). Finalmente, se observó que el período de engorde fue más prolongado en el T2 respecto al T1, T3, T4 y T1 (368, 228, 304 y 301 días; $p < 0.001$, respectivamente). En conclusión, los búfalos criados en sistemas intensivos muestran una mejor ganancia de peso

gains, but the time to reach this weight is longer than 300 days. Therefore, it is recommended to slaughter at 400 kg LW.

Keywords: intensive systems, grazing, weight gain, water buffalo.

diaria. Alimentar a los búfalos con dietas concentradas y sacrificarlos a 600 kg PV se relaciona con mejores ganancias de peso, pero el tiempo para alcanzar este peso es superior a los 300 días. Por lo tanto, se recomienda sacrificar a los 400 kg PV.

Palabras clave: sistemas intensivos, pastoreo, aumento de peso, búfalo de agua.

GENETICS & ANIMAL BREEDING

Mejoramiento y Genética

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Buffalo genetic improvement program in Venezuela

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Programa de mejoramiento genético del búfalo en Venezuela

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ABSTRACT

The water buffalo (*Bubalus bubalis*) has become one of the most important livestock species locally and worldwide, contributing significantly to people's food security and being a source of milk, meat, leather, manure, horns, and traction power. During the last decades, its inventory has doubled, with a notable improvement in genetics, management, and nutrition practices, coupled with advanced breeding programs, allowing the dissemination and acquisition of improved germplasm. The implementation of genetic evaluations in the water buffalo through Best Linear Unbiased Prediction (BLUP) through the animal model has increased over the last two decades across several countries. This methodology allows the prediction of breeding values (EBVs). It is an integral part of most breeding programs based on two fundamental pillars: phenotypic data (milk production, fat, protein, or morphological traits) and genealogical information (through the availability of a pedigree). Since many of the selection plans in this species have been directed to production traits (milk, solids in milk, and meat), reproduction needs to be included to avoid a reduction in fertility since poor reproductive ability, long age at first calving, calving intervals, and a low conception rate, constituting these elements as one of the main drawbacks of buffalo livestock. Therefore, this research aimed to evaluate the outcome of sixteen years of an Artificial Insemination (AI) program and develop a selection index that included multiparous females' milk

RESUMEN

El búfalo de agua (*Bubalus bubalis*) se ha convertido en una de las especies ganaderas más importantes a nivel local y mundial, contribuyendo significativamente a la seguridad alimentaria de las personas y siendo fuente de leche, carne, cuero, estiércol, cuernos y fuerza de tracción. Durante las últimas décadas, su inventario se ha duplicado, con una notable mejora en las prácticas genéticas, de manejo y nutrición, sumado a programas avanzados de mejoramiento, que permiten la difusión y adquisición de germoplasma mejorado. La implementación de evaluaciones genéticas en el búfalo de agua mediante Mejor Predicción Lineal Insesgada (BLUP) a través del modelo animal ha aumentado en las últimas dos décadas en varios países. Esta metodología permite la predicción de valores genéticos (EBV). Es parte integral de la mayoría de los programas de mejoramiento genético basados en dos pilares fundamentales: datos fenotípicos (producción de leche, grasa, proteína o rasgos morfológicos) e información genealógica (a través de la disponibilidad de un pedigrí). Dado que muchos de los planes de selección en esta especie se han dirigido a características de producción (leche, sólidos en la leche y carne), es necesario incluir la reproducción para evitar una reducción en la fertilidad debido a la baja capacidad reproductiva, la larga edad al primer parto, los intervalos entre partos, los cuales constituyen uno de los principales inconvenientes de la ganadería bufalina. Por lo tanto, esta investigación tuvo como

yield (MY) and reproduction (calving intervals: CI) (60% MY 40% CI) while in the heifers, milk ability and age at first service. This index allows for jointly selecting both important characteristics in buffalo production systems. Moreover, in the American tropics, the crossing of Murrah with the Mediterranean buffalo breed should be avoided since both breeds have a good adaptation to the environment and excellent production levels, which is why an absorption program for the pure breed is recommended in programs for individuals of each breed. The results obtained from the implementation of Artificial Insemination (AI) from 2007 and this index from 2016 to 2023 have allowed us to go from 1445 ± 430 to 1597 ± 415 kg of milk adjusted to 244d and an CI from 420 ± 65.4 to 413 ± 60.7 days. Moreover, the age at the first service of the buffaloes improved from 32.7 ± 10 to 29.7 ± 9.91 months, respectively. The average genetic values of the herd for MY increased from 2.94 to 57.35 kg/lactation, representing a significant increase, and the genetic values for CI improved from -0.02 to -1.12 days, giving indications that it is possible under a selection index, improving both characteristics in buffalo herds in the tropics. Similarly, using proven bulls through Artificial Insemination (AI) for 16 years has shown average improvement values in LA, LA1, LA2, and LA3 animals of 1368.3, 1677.3, and 2083.1 kg/lactation, respectively. In conclusion, genetic improvement programs based on the selection index and the massive use of reproductive biotechnologies are potent tools that must simultaneously accelerate buffalo herds' genetic progress.

Keywords: genetic improvement, Mediterranean breed, milk production, calving interval, precocity.

objetivo evaluar el resultado de dieciséis años de un programa de Inseminación Artificial (IA) y desarrollar un índice de selección que incluyó la producción de leche (MY) y la reproducción (intervalos entre partos: CI) de hembras multíparas (60% MY 40% CI) mientras que, en las novillas, capacidad de producción y edad al primer servicio. Este índice permite seleccionar de forma conjunta ambas características importantes en los sistemas de producción bufalina. Además, en el trópico americano se debe evitar el cruce de Murrah con la raza Mediterránea ya que ambas razas tienen una buena adaptación al medio y excelentes niveles de producción, por lo que se recomienda un programa de absorción de pureza para cada raza. Los resultados obtenidos de la implementación de la Inseminación Artificial (IA) desde el año 2007 al y de este índice desde el 2016 al 2023 nos han permitido pasar de 1445 ± 430 a 1597 ± 415 kg de leche ajustados a 244d y un CI de $420 \pm 65,4$ a $413 \pm 60,7$ días. Además, la edad al primer servicio de los búfalos mejoró de $32,7 \pm 10$ a $29,7 \pm 9,91$ meses, respectivamente. Los valores genéticos promedio del rebaño para la producción de leche aumentaron de 2,94 a 57,35 kg/lactancia, lo que representa un aumento significativo, y los valores genéticos para CI mejoraron de -0,02 a -1,12 días, dando indicios de que es posible bajo un índice de selección, mejorando ambas características en rebaños de búfalos en los trópicos. De manera similar, el uso de toros probados mediante inseminación artificial durante 16 años ha mostrado valores promedio de mejora en animales LA, LA1, LA2 y LA3 de 1368.3, 1677.3 y 2083.1 kg/lactancia, respectivamente. En conclusión, los programas de mejoramiento genético basados en el índice de selección y el uso combinado con biotecnologías reproductivas son potentes herramientas que aceleran simultáneamente el progreso genético de los rebaños bufalinos.

Palabras clave: mejoramiento genético, raza mediterránea, producción de leche, intervalo entre partos, precocidad.

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Evaluation of the impact of the environment on the genetic improvement of the buffalo species

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ABSTRACT

In two farms located in Zulia and Mérida in Venezuela, 1804 records were analyzed to evaluate the effect of environment on birth weight (BW), calving conception interval (CCI), calving interval (CI), and milk production (MP). Statistical analyses were performed with the statistical package SAS version 9.1, using analysis of variance (ANOVA) and the GLM procedure. The effects evaluated on BW were ecological zone (EZ), year of birth (YB), season of birth (SB), sex (S), origin (O): natural mating (NM), artificial insemination (AI) and embryo transfer (ET), number of calvings (NC) and breed (B): Murrah or Mediterranean. The results showed effects on BW ($p < 0.05$), while the breed factor had no effect ($p > 0.05$). The BW averages were 35.24 kg. For CCI, the following effects were studied: ecological zone (EZ), breed (Murrah and Mediterranean), number of calvings (NC) of the animal (NC), age in years (AY), and age at first calving (AFC). The variables NC and AY affected CCI ($p < 0.05$), and EZ, B, and AFC had no effect ($p > 0.05$). The CCI averaged 114.58 days. A total of 557 calving intervals (CI) reproductive records were analyzed to evaluate the effect of ecological zone (EZ), breed (B), number of calvings of the animal (NC), age of the animal in years (AY), age at first calving (AFC), NC and AY having an effect ($p < 0.05$); the factors EZ, B and AFC had no effect ($p > 0.05$). The average CI was 420.42 days. On 5 May 2004, a buffalo farm was started in Finca Florida, in Zulia state's arid tropical forest zone (DTFZ). Furthermore, on 9 May 2012, the herd was transferred to Finca Miraflores, located in a premontane rainforest zone (PRZ) in Mérida state. To evaluate the environmental effects, 574 records of total milk production (MP) of the Florida and Miraflores farms were analyzed by PROC GLM, using sums of squares type III (SSIII): EZ, breed: Murrah and Mediterranean, number of calvings of the animal (NC), lactation duration (LD), dry days (DD) and maternal milk production (MMP). The factors EZ, NC, LD, DD, and MMP were significant ($p < 0.05$). The breed factor was not significant ($p > 0.05$). MP, LD, and DD average were 1596.64 kg,

Efecto del medio ambiente sobre el mejoramiento genético de la especie bufalina

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RESUMEN

En dos sistemas de producción ubicadas una en el estado Zulia y Mérida en Venezuela, de donde se obtuvieron los registros de 1804 datos para evaluar el efecto del ambiente sobre: peso al nacer (BW), intervalo parto-concepción (CCI), intervalo entre partos (CI) y producción de leche (MP). Los análisis estadísticos se realizaron con el paquete estadístico SAS versión 9.1, utilizando análisis de varianza (ANOVA) y el procedimiento GLM. Para la variable BW los efectos evaluados correspondieron a: zona ecológica (EZ), año de nacimiento (BY), época de nacimiento (SB), sexo (S), origen (O): apareamiento natural (NM), inseminación artificial (AI) y embrión. transferencia (ET), número de partos (NC) y raza (B): Murrah o Mediterránea. Los resultados mostraron efectos sobre el BW ($p < 0.05$), el factor raza no tuvo efecto ($p > 0.05$). El promedio del peso al nacer correspondió a 35,24 kg. Para CCI se estudiaron los siguientes efectos: zona ecológica (EZ), raza (Murrah vs Mediterráneo), número de partos del animal (CN), edad en años (YA) y edad al primer parto (AFC). CN y YA tuvieron efecto sobre CCI ($p < 0,05$), EZ, B y AFC no tuvieron efecto ($p > 0,05$). El CCI promedió 114,58 días. Se analizaron un total de 557 registros reproductivos de intervalo entre partos (IC) para evaluar el efecto de la zona ecológica (EZ), raza (B), número de partos del animal (NC), edad del animal en años (AY), edad al primer parto (AFC). NC y AY presentaron efecto ($p < 0.05$); mientras que los factores EZ, B y AFC no tuvieron efecto ($p > 0.05$). El IC promedio fue de 420,42 días. El 5 de mayo de 2004 se inició la explotación de búfalos en la Finca Florida, ubicada en una zona de bosque tropical muy seco (DTFZ) en el estado Zulia, y el 9 de mayo de 2012 se trasladó el rebaño a la Finca Miraflores, ubicada en una zona de bosque húmedo premontano (PRZ) en el estado Mérida. Para evaluar los efectos ambientales, se analizaron mediante PROC GLM, 574 registros de producción total de leche (MP) de las granjas Florida y Miraflores, utilizando sumas de cuadrados tipo III (SSIII). Se consideraron los efectos de la EZ, la raza (B): Murrah y Mediterránea, nú-

285.48 days, and 127.05 days, respectively. At the end of 2022, 13% of the herd (195 buffaloes) exceed 3,000 kg per lactation. The variable MP is influenced by the management system implemented in the herd in both production units.

Keywords: buffaloes, dairy production, genetic factors, non-genetic factors.

mero de partos del animal (NC), duración de la lactancia (LD), días secos (DD) y producción de leche materna (MMP). Los factores EZ, NC, LD, DD y MMP fueron significativos ($p < 0,05$). El factor raza no fue significativo ($p > 0,05$). Los promedios para MP, LD y DD fueron: 1596,64 kg, 285,48 días y 127,05 días respectivamente. Al cierre del año 2022, el 13% del rebaño (195 búfalos) supera los 3.000 kg por lactancia. La variable MP está influenciada por el sistema de manejo implementado en el rebaño en ambas unidades de producción.

Palabras clave: búfalos, producción de leche, factores genéticos, factores no genéticos.

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Evaluation of genetic trend in milk production of buffalo cattle in Colombia

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ABSTRACT

Assessing genetic trends in livestock is pivotal for comprehending how populations have evolved over time. This provides valuable insights into the direction and magnitude of genetic change, which is crucial for evaluating the outcomes of selection processes within herds. This study aimed to evaluate the genetic trend in milk production of buffalo cattle in Colombia. The study used the productive database of purebred Murrah Buffaloes recorded by the Colombian Buffalo Breeders Association (ACB), encompassing productive and genealogical records of 774 animals born between 2008 and 2017. A GAMS model was applied to assess genetic values, considering the birth year of the animal. The mean genetic value in the evaluated population was 45.42 ± 85.31 kg, with minimum and maximum values of -160.54 and 377.34 kg, respectively. When analyzing genetic values by year, the highest value was observed in 2014 (56.26 kg), while the lowest was recorded in 2017 (32.65 kg). The GAMS model estimated an intercept of 45.42 kg ($p < 0.001$), with an explained variance of 63%. The genetic trend showed stable values from 2008 to 2011, but from that point onwards, a decline was evident, becoming more pronounced from 2014 onward. The results indicate a decrease

Evaluación de tendencia genética en la producción de leche de ganado bufalino en Colombia

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RESUMEN

Evaluar las tendencias genéticas en el ganado es fundamental para comprender cómo han evolucionado las poblaciones a lo largo del tiempo. Esto proporciona información valiosa sobre la dirección y magnitud del cambio genético, lo cual es crucial para evaluar los resultados de los procesos de selección dentro de los rebaños. Este estudio tuvo como objetivo evaluar la tendencia genética en la producción de leche del ganado bufalino en Colombia. El estudio utilizó la base de datos productiva de Búfalos Murrah de pura raza registrada por la Asociación Colombiana de Criadores de Búfalos (ACB), abarcando registros productivos y genealógicos de 774 animales nacidos entre 2008 y 2017. Se aplicó un modelo GAMS para evaluar los valores genéticos, teniendo en cuenta el año DE nacimiento del animal. El valor genético medio en la población evaluada fue de $45,42 \pm 85,31$ kg, con valores mínimo y máximo de -160,54 y 377,34 kg, respectivamente. Al analizar los valores genéticos por año, el valor más alto se observó en 2014 (56,26 kg), mientras que el más bajo se registró en 2017 (32,65 kg). El modelo GAMS estimó un intercepto de 45,42 kg ($p < 0,001$), con una varianza explicada del 63%. La tendencia genética mostró valores estables de 2008 a 2011, pero a partir

in genetic values for the milk production trait in the evaluated population. This decrease is attributed to the limited use of high-genetic-value sires in biotechnological programs and low selection intensity. To achieve favorable genetic progress, it is important to conduct selection processes based on genetic evaluations undertaken in the country, increase selection intensity by the widespread use of sires with positive genetic values, and restrict the use of animals with negative genetic values. In 2015, Agudelo et al. also estimated similar genetic trends without significant increases in the genetic trend for milk production. Strategies should be developed to allow the introduction of new animals or genetic lines through the purchase or exchange of sires with positive genetic values. Those strategies can enhance genetic diversity and provide new opportunities for improvement by expanding the range of selection possibilities. Additionally, the use of biotechnological tools such as artificial insemination and embryo transfer should be promoted to expedite the dissemination of genetically superior animals.

Keywords: genetic evaluation, genetic improvement, progress, selection.

de ese momento se evidenció un descenso, haciéndose más pronunciado a partir de 2014. Los resultados indican una disminución en los valores genéticos del rasgo de producción de leche en la población evaluada. Esta disminución se atribuye al uso limitado de toros de alto valor genético en programas biotecnológicos y a la baja intensidad de selección. Para lograr un progreso genético favorable, es importante realizar procesos de selección basados en evaluaciones genéticas realizadas en el país, aumentar la intensidad de la selección mediante el uso generalizado de toros con valores genéticos positivos y restringir el uso de animales con valores genéticos negativos. En 2015, Agudelo et al. también estimó tendencias genéticas similares sin aumentos significativos en la tendencia genética para la producción de leche. Se deben desarrollar estrategias que permitan la introducción de nuevos animales o líneas genéticas mediante la compra o intercambio de toros con valores genéticos positivos. Esas estrategias pueden mejorar la diversidad genética y brindar nuevas oportunidades de mejora al ampliar la gama de posibilidades de selección. Además, se debe promover el uso de herramientas biotecnológicas como la inseminación artificial y la transferencia de embriones para acelerar la difusión de animales genéticamente superiores.

Palabras clave: evaluación genética, mejoramiento genético, progreso, selección.

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Relationship between body volume and rib eye area in Colombian buffalo cattle

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ABSTRACT

In genetic improvement programs, one of the key aspects is the selection of animals with high genetic potential for economically important traits. Rib eye area (REA) is important due to its positive relationship with carcass yield. REA is traditionally measured on the animal's carcass or using a real-time ultrasound tool. Therefore, searching for alternatives that facilitate REA estimation is crucial for livestock producers. This

Relación entre el volumen corporal y el área del ojo del lomo en ganado bufalino colombiano

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RESUMEN

En los programas de mejoramiento genético, uno de los aspectos clave es la selección de animales con alto potencial genético por rasgos económicamente importantes. El área del ojo del lomo (REA) es importante debido a su relación positiva con el rendimiento en canal. El REA se mide tradicionalmente en el cadáver del animal o utilizando la herramienta de ultrasonido en tiempo real. Por lo tanto, la búsqueda de alternati-

study aimed to estimate the relationship between body volume and REA in Murrah buffalo. Data from 24 Murrah buffaloes participating in the VI performance test were used. The test lasted for 252 days, including an initial adaptation period. Morphometric and ultrasound measurements were taken every 28 days to assess the animals' development using a zoometric cane and an Esaote MyLab ultrasound scanner device equipped with a linear transducer (3.5-MHz; 18 cm). Analysis of variance was conducted to identify the effect on REA and correlations between significant variables and REA were estimated. A regression model with significant variables from the ANOVA was used to identify the proportion of each variable's effect on REA. To estimate body volume (BV), a cone trunk formula was applied using the following measurements: thoracic perimeter (TP), abdominal perimeter (AP), inguinal perimeter, and sacrum-croup length. Analysis of variance was performed to determine the effect of morphometric measurements on REA. A highly significant effect was found for weight, height, and BV. Although BV was highly significant, the independent variables included in the calculation were not significant. The correlation between weight, height, and BV with REA was 0.77, 0.50, and 0.67, respectively. The regression analysis indicated that for every unit change in weight, height, and BV, REA increases by 0.07, 0.05, and 42.37 cm³, respectively. A regression model can be used to estimate REA and assist in the selection process of buffaloes for future breeding when real-time ultrasound is unavailable.

Keywords: morphometry, genetic improvement, selection; ultrasound.

vas que faciliten la estimación del REA es de gran importancia para los productores ganaderos. El objetivo de este estudio fue estimar la relación entre el volumen corporal y el REA en búfalos Murrah. Se utilizaron datos de 24 búfalos Murrah que participaron en la prueba de desempeño VI. La prueba duró 252 días, incluido un período inicial de adaptación. Se tomaron medidas morfométricas y ecográficas cada 28 días para evaluar el desarrollo de los animales utilizando un bastón zoométrico y un dispositivo de ultrasonido Esaote MyLab equipado con un transductor lineal (3,5 MHz; 18 cm). Se realizó un análisis de varianza para identificar el efecto sobre el REA y se estimaron las correlaciones entre las variables significativas y el REA. Se utilizó un modelo de regresión con variables significativas del ANOVA para identificar la proporción del efecto de cada variable sobre el REA. Para estimar el volumen corporal (BV), se aplicó una fórmula de cono-tronco utilizando las siguientes medidas: perímetro torácico (TP), perímetro abdominal (AP), perímetro inguinal y longitud sacro-grupa. Se realizó un análisis de varianza para determinar el efecto de las mediciones morfométricas en REA. Se encontró un efecto muy significativo para el peso, la altura y el BV. Aunque el BV fue muy significativo, las variables incluidas en el cálculo de forma independiente no fueron significativas. La correlación entre peso, talla y BV con REA fue de 0,77, 0,50 y 0,67, respectivamente. El análisis de regresión indicó que, por cada cambio unitario en peso, talla y VB, el REA aumenta en 0,07, 0,05 y 42,37 cm³, respectivamente. Se puede utilizar un modelo de regresión para estimar el REA y ayudar en el proceso de selección de búfalos para reproducción futura cuando no se dispone de ultrasonido en tiempo real.

Palabras clave: morfometría, mejoramiento genético, selección, ultrasonido.

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Morphological evaluations in buffaloes (*Bubalus bubalis*) in Venezuela

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ABSTRACT

The buffalo herds in Venezuela are characterized by a high phenotypic polymorphism, showing high diversity among individuals. The leading cause is the great mixture of breeds

Evaluaciones morfológicas en búfalos (*Bubalus bubalis*) en Venezuela

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RESUMEN

Los rebaños de búfalos en Venezuela, se caracterizan por un alto polimorfismo fenotípico, mostrando alta diversidad entre individuos. La causa principal es la gran mezcla de razas

involved in the crossbreeding programs, such as Murrah, Mediterranean, Nili Ravi, Jafarabadi, and Carabao. Morphometric measurements assist in racial characterization by comparing genetic groups (within and between) and establishing the relationship between the conformation and functional traits, eliminating undesirable characters, and helping in the selection process for the identification of morphologically superior animals. The morphometry study consists of the search for a functional animal. These traits also contribute to the selection process in identifying the morphologically superior animals and eliminating the unwanted characters. This phenotypic characterization of buffaloes comprised quantitative and qualitative traits of 157 female buffaloes aged 24 to 48 months and 21 males aged 18 to 36 months. There were measurements on 24 traits, 18 quantitative (cm) variables considered as morphometric: (i) wither height (HW), (ii) rump length (RL), (iii) rump angle (RA), (iv) hip length (HL), (v) rump width (RW), (vi) hip width (WH), (vii) heel depth (HD), (viii) shoulder width (SW), (ix) chest depth (CD), (x) heart girth (HG), (xi) body length (BL), (xii) tail length (TL) and six qualitative traits, a) loin strength (LS), b) hock angle (HA), c) foot angle (FA) and d) dairy form (DF); and only for females (xiii) fore udder attachment (FUA), (xiv) rear udder height (RUH), (xv) rear udder width (UW), (xvi) udder depth (UD), (xvii) udder cleft (UC), (xviii) teat length (LT) and e) Nipple direction (ND) and f) Nipple position (NP). The data were subjected to descriptive and ANOVA analysis for breed predominance and sex. No difference was found between the predominant breed ($p>0.05$) means \pm SE were: for HW (132.41 \pm 0.82), RL (44.86 \pm 0.67), RA (10.68 \pm 1.45), HL (56.26 \pm 0.98), RW (26.76 \pm 0.80), WH (50.5 \pm 0.97), HD (2.8 \pm 0.10), HG (201.42 \pm 2.60), TL (88.09 \pm 1.50); except SW 41.69 \pm 0.73 vs 44.31 \pm 0.70 ($p<0.002$); CD 72.68 \pm 1.45 vs 76.03 \pm 1.40 ($p<0.01$) and BL 136.7 \pm 2.06 vs 142.02 \pm 1.98 cm ($p<0.04$) for Mediterranean vs Murrah predominance, respectively. The sex affects only the variables for rump RL, HL, RW, 45.5 \pm 0.42 vs. 42.7 \pm 1.05 ($p<0.01$), 59.42 \pm 0.59 vs. 53.42 \pm 1.49 ($p<0.001$), and 28.99 \pm 0.48 vs. 22.97 \pm 1.20 cm, ($p<0.001$), for females and males, respectively. On all qualitative measurements, there were no significant differences. The results could suggest that the body morphometric traits could be incorporated in designing selection programs for buffalo breeding in Venezuela. This information would include the genetic evaluation using the BLUP methodology and the morphological characterization of the breeds present in Venezuela, constituting a starting point in the genetic programs.

Keywords: morphometry, dairy wedge, type, Murrah, Mediterranean, genetics.

involucradas en los programas de cruce, como son, Murrah, Mediterránea, Nili Ravi, Jafarabadi y Carabao, principalmente. Las mediciones morfométricas ayudan en la caracterización racial comparando grupos genéticos (dentro y entre ellas) y estableciendo la relación entre la conformación y los rasgos funcionales, eliminando caracteres indeseables y ayudando en el proceso de selección para la identificación de animales morfológicamente superiores. El estudio de morfometría consiste en la búsqueda del animal funcional. Estos rasgos también contribuyen al proceso de selección, identificando los animales morfológicamente superiores. Esta caracterización fenotípica de los búfalos estuvo compuesta por rasgos cuantitativos y cualitativos de 157 búfalas con edades de 24 a 48 meses y 21 machos con edades de 18 a 36 meses. Se midieron 24 rasgos, 18 variables cuantitativas (cm) consideradas como morfométricas: (i) altura a la cruz (HW), (ii) largo de grupa (RL), (iii) ángulo de grupa (RA), (iv) largo de cadera (HL), (v) ancho de grupa (RW), (vi) ancho de cadera (WH), (vii) profundidad del talón (HD), (viii) ancho de hombros (SW), (ix) profundidad del pecho (CD), (x) circunferencia del corazón (HG), (xi) longitud del cuerpo (BL), (xii) longitud de la cola (TL) y 6 rasgos cualitativos, a) fuerza del lomo (LS), b) ángulo del corvejón (HA), c) ángulo del pie (FA) y d) tipo lechero (DF); y sólo para hembras (xiii) inserción de la ubre anterior (FUA), (xiv) altura de la ubre trasera (RUH), (xv) ancho de la ubre trasera (UW), (xvi) profundidad de la ubre (UD), (xvii) inserción de la ubre (UC), (xviii) longitud del pezón (LT) y e) dirección del pezón (ND) y f) Posición del pezón (NP). Los datos fueron sometidos a análisis descriptivo y ANOVA para predominio de raza y sexo. No se encontró diferencia entre las razas predominantes ($p>0.05$) las medias \pm SE fueron: para HW (132.41 \pm 0.82), RL (44.86 \pm 0.67), RA (10.68 \pm 1.45), HL (56.26 \pm 0.98), RW (26.76 \pm 0.80), WH (50.5 \pm 0.97), HD (2.8 \pm 0.10), HG (201.42 \pm 2.60), TL (88.09 \pm 1.50); excepto SW 41.69 \pm 0.73 vs 44.31 \pm 0.70 ($p<0.002$); CD 72.68 \pm 1.45 vs 76.03 \pm 1.40 ($p<0.01$) y BL 136.7 \pm 2.06 vs 142.02 \pm 1.98 cm ($p<0.04$) para predominio Mediterráneo vs Murrah, respectivamente. El sexo afectó sólo las variables para grupa RL, HL, RW, 45.5 \pm 0.42 vs 42.7 \pm 1.05 ($p<0.01$), 59.42 \pm 0.59 vs 53.42 \pm 1.49 ($p<0.001$) y 28.99 \pm 0.48 vs 22.97 \pm 1.20 cm, ($p<0.001$), para hembras y machos, respectivamente. En todas las mediciones cualitativas no hubo diferencias significativas. Los resultados podrían sugerir que los rasgos morfométricos corporales podrían incorporarse en el diseño de programas de selección para la cría de búfalos en Venezuela. Esta información permitiría incluir la evaluación genética mediante la metodología BLUP, así como la caracterización morfológica de las razas presentes en Venezuela, constituyendo un punto de partida en los programas de mejora genética.

Palabras clave: morfometría, cuña lechera, tipo, Murrah, Mediterráneo, genética.

GAB-246 Rev. Cientif. FCV-LUZ, XXXIII, SE, 146-147, 2023, <https://doi.org/10.52973/rcfcv-wbc028>**Influence of type traits on stayability in the Italian Mediterranean buffalo****Damiano Altieri¹, Stefano Biffani², Mayra Gómez³, Yuri Gombia³, Dario Rossi³, Luca Gubitosi³, Nadia Piscopo¹, Francesca Carnovale⁴, Rossella Di Palo¹**¹Dept. Veterinary Medicine and Animal Production-Federico II University of Naples, Italy.²Institute of Agricultural Biology and Biotechnology, National Research Council, Milano, Italy.³Italian Mediterranean Buffalo National Breeder Association (ANASB), Caserta, Italy.⁴Department of Agronomy - Federico II University – Naples, Italy.*Corresponding author: Rossella Di Palo (dipalo@unina.it).**ABSTRACT**

The selection schemes adopted over the past 20 years for the Italian Mediterranean Buffalo, together with the marked improvement in farm management techniques and the systematic application of AI in an increasing number of subjects, have resulted in a sharp improvement in the quantities of milk produced per lactation. Experiences from dairy cows suggest that such an improvement could reduce the average productive life span of breeding females and, therefore, their longevity. Animal longevity has a multifactorial etiology where physiology and morphology, in close correlation with each other, cross their effects to enable animals to continue to produce efficiently on the farm. In the past, the selection of the Italian Mediterranean buffalo has mainly focused on increasing milk production, which has had an unfavorable impact on the average farm life of the IMB. This trend needs to be reversed as this inevitably leads to an increase in production costs due to the increase in the replacement rate. There are many ways to assess longevity, both quantitatively, such as age at culling and parity at culling, and qualitatively, such as in the case of stayability, where it is evaluated as a binary variable where 1 and 0 indicate the ability to stay or be culled at a given time, respectively. In a recent study, we evaluated the genetic aspects of stayability in IMB, and the results suggest the possibility of using it in the selection process. This study aimed to correlate stayability with the linear type traits measured by the ANASB for IMB and with the merit score values for body conformation, limbs, feet, and udder. Data from 276.415 buffalo cows registered in the ANASB stud-book starting from 1989 were extracted and merged with the official milk test-day recording datasets from the Italian Breeder's Association (AIA). The stayability from parity one up to 10 was assessed assigning a value of 1 if a calving date was present or 0 otherwise. Investigating the relationship between some morphological traits and longevity would give them the proper em-

Influencia de los características de tipo sobre la vida productiva del búfalo mediterráneo italiano

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Los programas de selección adoptados durante los últimos 20 años en el búfalo mediterráneo italiano, junto con la notable mejora de las técnicas de gestión de las explotaciones y la aplicación sistemática de la IA en un número cada vez mayor de individuos, han dado lugar a una fuerte mejora de las cantidades de leche producida por lactancia. Las experiencias de las vacas lecheras sugieren que tal mejora podría conducir a una reducción en el promedio de vida productiva de las hembras reproductoras y, por lo tanto, en su longevidad. La longevidad animal tiene una etiología multifactorial donde la fisiología y la morfología, en estrecha correlación entre sí, cruzan sus efectos para permitir que los animales continúen produciendo eficientemente en la granja. En el pasado, la selección del búfalo mediterráneo italiano se centraba principalmente en aumentar la producción de leche, lo que tenía un impacto desfavorable en la vida media de las granjas de las IMB. Es necesario revertir esta tendencia, ya que inevitablemente conduce a un aumento de los costos de producción debido al aumento de la tasa de reemplazo. Hay muchas maneras de evaluar la longevidad, tanto cuantitativamente, como la edad en el momento del sacrificio, la paridad en el momento del sacrificio, entre otros, como cualitativamente, como en el caso de la permanencia, donde se evalúa como una variable binaria donde 1 y 0 indican la capacidad. quedarse o ser sacrificado en un momento dado, respectivamente. En un estudio reciente se evaluó los aspectos genéticos de la permanencia en IMB y los resultados sugieren la posibilidad de utilizarlo en el proceso de selección. El objetivo de este estudio fue correlacionar la permanencia con los rasgos de tipo lineal medidos por la ANASB para IMB y con los valores de puntuación de mérito para la conformación corporal, extremidades, patas y ubres. Los datos de 276.415 vacas de búfala registradas en el libro genealógico de ANASB a partir de 1989 se extrajeron y fusionaron con

phasis in formulating genetic indexes or creating specific aggregated indexes to make the selection process more efficient.

Keywords: buffalo, IMB, stayability, type trait, selection.

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los conjuntos de datos oficiales de registro del día de prueba de leche de la Asociación Italiana de Criadores (AIA). La permanencia desde el parto 1 hasta el 10 se evaluó asignando un valor de 1 si había fecha de parto presente o 0 en caso contrario. Investigar la relación entre algunos rasgos morfológicos y la longevidad permitiría darles el énfasis adecuado en la formulación de índices genéticos o crear índices agregados específicos para hacer más eficiente el proceso de selección.

Palabras clave: búfalo, IMB, longevidad, tipo lechero, selección.

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ANIMAL HEALTH & ONE HEALTH

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Risk factors for *Toxoplasma gondii* seropositivity in buffaloes in a Mediterranean area

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ABSTRACT

Toxoplasmosis is a worldwide widespread zoonotic infection caused by the intracellular protozoan *Toxoplasma gondii*. This infection is considered one of the most important food-borne parasitic zoonoses globally. In addition to its public health impact, toxoplasmosis also has important veterinary implications, as it causes abortions or congenital malformations with negative economic impacts. Several serological studies on toxoplasmosis in water buffaloes (*Bubalus bubalis*) are fragmentary, dated, consider a low number of animals compared to the present population and do not relate the epidemiological update in an area of the Mediterranean. For this reason, the objectives of this study were to define the seroprevalence of *T. gondii* infection in water buffaloes and to assess the risk factors associated with infection in water buffalo farms. A total of 184 dairy buffalo farms were randomly selected upon arrival of blood samples collected during state prophylaxis and analyzed with an indirect ELISA kit (ID Screen®, Indirect Toxoplasmosis Multi-Species, IDVET, France), according to the manufacturers instructions. The random selection of farms allowed us to consider different areas of the Campania region where the highest concentration of buffalo herds exists. For the risk assessment study, a specially designed questionnaire was administered that considered several factors (number of animals,

Factores de riesgo de seropositividad para *Toxoplasma gondii* en búfalos de una zona mediterránea

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RESUMEN

La toxoplasmosis es una infección zoonótica extendida a nivel mundial causada por el protozoo intracelular *Toxoplasma gondii*. Esta infección es considerada una de las zoonosis parasitarias transmitidas por alimentos más importantes a nivel mundial. Además de su impacto en la salud pública, la toxoplasmosis también tiene importantes implicaciones veterinarias, ya que provoca abortos o malformaciones congénitas con impactos económicos negativos. Varios estudios serológicos sobre toxoplasmosis en búfalos de agua (*Bubalus bubalis*) son fragmentarios, anticuados, consideran un número bajo de animales respecto a la población presente y no relacionan la actualización epidemiológica en una zona del Mediterráneo. Por esta razón, los objetivos de este estudio fueron definir la seroprevalencia de la infección por *T. gondii* en búfalos de agua y evaluar los factores de riesgo asociados con la infección en granjas de búfalos de agua. Se seleccionaron aleatoriamente un total de 184 granjas de búfalas lecheras a la llegada de muestras de sangre recolectadas durante la profilaxis estatal y analizadas con un kit ELISA indirecto (ID Screen®, Indirect Toxoplasmosis Multi-Species, IDVET, Francia), según las instrucciones del fabricante. La selección aleatoria de granjas nos permitió considerar diferentes áreas de la región de Campa-

presence of cats and rodent control measures) as well as presence of clinical signs such as abortions in adult buffaloes. Of a total of 13,649 animals analyzed, 2,693 tested positive for *T. gondii* (19.7%; 95% Confidence Interval [CI] = 19.7-24.6%), and out of 184 buffalo farms 179 tested positive (97.3%; 95% CI = 93.8- 98.8%). Of the *T. gondii* positive farms, 97% had cats on the farm and 85% did not implement rodent control measures. These findings indicate that these two risk factors are the most prevalent and need to be monitored for infection control on buffalo farms.

Keywords: risk factors, *Toxoplasma gondii*, seroprevalence, water buffalo (*Bubalus bubalis*).

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nia donde existe la mayor concentración de manadas de búfalos. Para el estudio de evaluación de riesgos, se administró un cuestionario especialmente diseñado que tuvo en cuenta varios factores (número de animales, presencia de gatos y medidas de control de roedores), así como la presencia de signos clínicos como abortos en búfalos adultos. De un total de 13.649 animales analizados, 2.693 dieron positivo a *T. gondii* (19,7%; intervalo de confianza [IC] del 95% = 19,7-24,6%), y de 184 granjas de búfalos 179 dieron positivo (97,3%; IC del 95% = 93,8-98,8%). De las granjas positivas a *T. gondii*, el 97% tenía gatos en la granja y el 85% no implementó medidas de control de roedores. Estos hallazgos indican que estos dos factores de riesgo son los más prevalentes y deben ser monitoreados para controlar la infección en las granjas de búfalos.

Palabras clave: factores de riesgo, *Toxoplasma gondii*, seroprevalencia, búfalo de agua (*Bubalus bubalis*).

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Reproductive problems in water buffalo cows associated with non-diagnosed genital leptospirosis. Preliminary results

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ABSTRACT

Leptospirosis in female buffaloes can cause abortions, fetal death, premature calving, and the birth of weak and/or underweight calves; however, sub-fertility and early embryonic death have been little studied. In addition, it is not completely understood whether these reproductive problems are associated with the persistence of the bacteria in the reproductive tract. The main goal of this research is to demonstrate the presence of the bacterium *Leptospira* spp. in the genitourinary tract of *Bubalus bubalis* females. For this, samples of 25 females from 4 farms with poor reproductive performance were brought to a slaughterhouse, and samples of uteri, oviducts, ovaries, and kidneys were collected. Fresh tissue samples were cultured in the liquid Ellinghausen–McCullough–Johnson–Harris medium (EMJH), a selective medium for *Leptospira*. In addition, tissue samples were prepared for histological analysis by fixation in a 10% buffered formaldehyde solution and stained with hematoxylin and eosin. PCR was performed on nine culture samples for identification of *Leptospira* spp., by using two pairs of primer for the markers, G1-G2 derived from a sequence from the genomic library of *L. interrogans* serovar *icterohaemorrhagiae*, strain

Problemas reproductivos en vacas búfalo de agua asociados a leptospirosis genital no diagnosticada. Resultados preliminares

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RESUMEN

La leptospirosis en búfalas puede provocar abortos, muerte fetal, partos prematuros y nacimiento de terneros débiles y/o de bajo peso; sin embargo, la subfertilidad y la muerte embrionaria temprana han sido poco estudiadas. Además, no se comprende del todo si estos problemas reproductivos están asociados con la persistencia de las bacterias en el tracto reproductivo. El objetivo principal de esta investigación es demostrar la presencia de la bacteria *Leptospira* spp. en el tracto genitourinario de las hembras de *Bubalus bubalis*. Para ello, se llevaron a un matadero muestras de 25 hembras de 4 granjas con bajo rendimiento reproductivo, y se recolectaron muestras de útero, oviductos, ovarios y riñones. Se cultivaron muestras de tejido fresco en el medio líquido Ellinghausen-McCullough-Johnson-Harris (EMJH), un medio selectivo para *Leptospira*. Además, se prepararon muestras de tejido para análisis histológico mediante fijación en una solución tamponada de formaldehído al 10% y se tiñeron con hematoxilina y eosina. Se realizó PCR en nueve muestras de cultivo para la identificación de *Leptospira* spp., utilizando dos pares de cebadores para los marcadores, G1-G2 derivados de una secuencia de

RGA, which was described by Gravekamp et al. (1993) and Internal 1 and Internal 2 derived from the sequence of the gene encoding the LipL32 protein and specific to identify pathogenic *Leptospira*, described by Haake et al. (2000). The reproductive efficiency of the farms was characterized by prolonged days open and calving intervals (average of 307 and 587 days, respectively). In addition, the abortion rate averaged 40%, and the occurrence of hemoglobinuria, jaundiced vaginal mucosa, and congestive vaginal mucosa were 32%, 16%, and 24% respectively. The histological examination revealed uterine fluid (68%), uterine fibrosis (48%), chronic non-suppurative endometritis in 68%, severe glandular atrophy in 44%, and hemorrhage in the perimetrium in 60%. In oviducts, fibroplasia was observed in 52% of samples and salpingitis in 32%, while in kidneys, chronic interstitial nephritis was observed in 56% of samples. At the ovary level, 16% of follicular cysts, 20% of atrophy, and persistent corpus luteum in 28% were observed. The tissue culture was positive for *Leptospira* spp. in the following percentages: uterus 76%, ovary 84%, oviduct 88%, and kidney 96%. PCR using the marker G1-G2 revealed the presence of *Leptospira* DNA in 55.5% of uterus, ovary, and oviduct samples, and 44.4% of kidneys; and using the primer Internal 1 and 2 identified pathogenic *Leptospira* spp. in 55.5% of the uterus and kidney samples, 33.3% in ovaries and 66.6% oviduct. These results show reproductive problems in female buffaloes associated with genital leptospirosis. These findings suggest the need to develop new approaches for diagnosing this pathology using molecular techniques and bacterial cultures, as well as the evaluation of new prophylactic and therapeutic alternatives to avoid the colonization of the reproductive tract.

Keywords: sub-fertility, genital tract, jaundice, histologic & pathologic, PCR.

la biblioteca genómica de *L. interrogans* serovar *icterohaemorrhagiae*, cepa RGA, que fue descrita por Gravekamp. et al. (1993) e Internal 1 e Internal 2 derivados de la secuencia del gen que codifica la proteína LipL32 y específico para identificar *leptospirosis* patógenas, descrito por Haake et al. (2000). La eficiencia reproductiva de las granjas se caracterizó por días abiertos prolongados e intervalos entre partos elevados (promedio de 307 y 587 días, respectivamente). Además, la tasa de aborto promedió el 40% y la aparición de hemoglobinuria, mucosa vaginal con ictericia y mucosa vaginal congestiva fue del 32%, 16% y 24% respectivamente. El examen histológico reveló líquido uterino (68%), fibrosis uterina (48%), endometritis crónica no supurativa en 68%, atrofia glandular severa en 44% y hemorragia en el perimetrio en 60%. En oviductos se observó fibroplasia en el 52% de las muestras y salpingitis en el 32%, mientras que en riñones se observó nefritis intersticial crónica en el 56% de las muestras. A nivel de ovario se observó un 16% de quistes foliculares, un 20% de atrofia y cuerpo lúteo persistente en un 28%. El cultivo de tejido fue positivo para *Leptospira* spp. en los siguientes porcentajes: útero 76%, ovario 84%, oviducto 88% y riñón 96%. La PCR utilizando el marcador G1-G2 reveló la presencia de ADN de *Leptospira* en el 55,5% de las muestras de útero, ovario y oviducto, y en el 44,4% de los riñones; y mediante el cebador Interno 1 y 2 se identificaron *Leptospira* spp patógenas, en el 55,5% de las muestras de útero y riñón, el 33,3% en ovarios y el 66,6% de oviducto. Estos resultados muestran problemas reproductivos en búfalas hembras asociados con leptospirosis genital. Estos hallazgos sugieren la necesidad de desarrollar nuevos enfoques para el diagnóstico de esta patología mediante técnicas moleculares y cultivos bacterianos, así como la evaluación de nuevas alternativas profilácticas y terapéuticas para evitar la colonización del tracto reproductivo.

Palabras clave: subfertilidad, tracto genital, ictericia, histológico y patológico, PCR.

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Gastrointestinal parasites in buffalo calves in a farming context in Colon county, Zulia state, Venezuela

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ABSTRACT

The buffalo (*Bubalus bubalis*) is an animal species of great importance for producing milk and meat. Buffaloes have been considered rustic and resistant to many pathologies. However, they are susceptible to parasitic infections. Gastrointestinal parasites (GIP) decrease production, cause growth retardation and death of animals. Furthermore, buffalo calves are the most affected animals. To evaluate the prevalence (%) and mean intensity of infection of gastrointestinal parasites in buffalo calves and their association with age and sex, a non-experimental field study was carried out in the Agropecuaria Rosa Elena (a dual-purpose buffalo system) using crossbreed animals (Murrah and Mediterranean breeds). The buffalo farm is located in the municipality of Colón, Zulia, Venezuela. Eighty-three out of 106 buffaloes were randomly selected, stratified by age (months): A (≤ 1 m); B ($>1 \leq 2$ m); C ($>2 \leq 3$ m); and D ($> 3 \leq 4$ m), and sex. Fecal samples were taken directly from the rectum and processed by McMaster's technique using saturated saline as flotation medium. The eggs or oocysts per gram of feces (EPG/OPG) were determined. The data was analyzed to determine the prevalence using contingency tables and Chi²; for the intensity variable, the mean of EPG and OPG was calculated, and an ANOVA and comparison of Tukey means was performed. The overall prevalence was 94%, with polyparasitism (37.8%, 32.9%, and 3.7% in two, three, and four GIP species, respectively). Therefore, it was possible to identify these parasites: strongylids (34%), *Strongyloides* sp. (44%); *Toxocara vitulorum* (37%) and *Eimeria* spp. (83%). In the age groups the overall prevalence was higher in C (100%), for strongylids (77.8%) and *Strongyloides* sp. (72.2%) in C, *T. vitulorum* (66.7%) in B, with significant association ($p < 0.05$); *Eimeria* spp. was the most prevalent GIP in all age groups. The variation in intensity of infection of strongylids increased from A (666.7 EPG) to C (978.6 EPG); in contrast, *Strongyloides*

Parásitos gastrointestinales en becerros en un contexto ganadero en el cantón Colón, estado Zulia, Venezuela

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RESUMEN

El búfalo (*Bubalus bubalis*) es una especie animal de gran importancia para la producción de leche y carne. Los búfalos han sido considerados rústicos y resistentes a muchas patologías. Sin embargo, son susceptibles a las infecciones parasitarias. Los parásitos gastrointestinales (GIP) disminuyen la producción, provocan retraso en el crecimiento y muerte de los animales. Además, las crías de búfalo son los animales más afectados. Para evaluar la prevalencia (%) e intensidad media de infección de parásitos gastrointestinales en bucerros y su asociación con la edad y el sexo, se realizó un estudio de campo no experimental en la Agropecuaria Rosa Elena (sistema bufalino de doble propósito) utilizando cruces animales (razas Murrah y mediterráneas). La granja bufalina está ubicada en el municipio de Colón, Zulia, Venezuela. Se seleccionaron al azar 83 de 106 búfalos, estratificados por edad (meses): A (≤ 1 m); B ($>1 \leq 2$ m); C ($>2 \leq 3$ m); y D ($> 3 \leq 4$ m), y sexo. Se tomaron muestras fecales directamente del recto y se procesaron mediante la técnica de McMaster utilizando solución salina saturada como medio de flotación. Se determinaron los huevos u oocistos por gramo de heces (EPG/OPG). Los datos fueron analizados para determinar la prevalencia mediante tablas de contingencia y Chi²; para la variable intensidad se calculó la media de EPG y OPG, y se realizó ANOVA y comparación de medias de Tukey. La prevalencia global fue del 94%, con poliparasitismo (37,8%, 32,9% y 3,7% en dos, tres y cuatro especies de GIP, respectivamente). Por lo tanto, fue posible identificar estos parásitos: estrongílicos (34%), *Strongyloides* sp. (44%); *Toxocara vitulorum* (37%) y *Eimeria* spp. (83%). En los grupos de edad la prevalencia global fue mayor en C (100%), para estrongílicos (77,8%) y *Strongyloides* sp. (72,2%) en C, *T. vitulorum* (66,7%) en B, con asociación significativa

des sp. decreased with age, being higher in A (442.3 EPG). *T. vitulorum* increased from A (2250 EPG) to C (9225 EPG). However, these variations did not show significance ($p>0.05$). *Eimeria* spp. presented the higher mean intensity of infection in A (102243.18 OPG), with significant statistical differences ($p<0.05$) in all groups. No significant statistical differences were found in the prevalence and mean intensity associated with sex. It is concluded that GIP are affecting buffalo calves with a high general prevalence, being *Eimeria* spp. the most significant. It is also shown that the prevalence is associated with the age of the animals, which is of great importance for the design of control programs.

Keywords: buffalo calves, gastrointestinal parasites, mean intensity of infection, prevalence.

($p<0,05$); *Eimeria* spp. fue el PIB más prevalente en todos los grupos de edad. La variación en la intensidad de la infección de estrogilidos aumentó de A (666,7 EPG) a C (978,6 EPG); en cambio, *Strongyloides* sp. disminuyó con la edad, siendo mayor en A (442,3 EPG). *T. vitulorum* aumentó de A (2250 EPG) a C (9225 EPG). Sin embargo, estas variaciones no mostraron significancia ($p>0,05$). *Eimeria* spp. presentó la mayor intensidad media de infección en A (102243,18 OPG), con diferencias estadísticas significativas ($p<0,05$) en todos los grupos. No se encontraron diferencias estadísticas significativas en la prevalencia e intensidad media asociadas al sexo. Se concluye que las GIP están afectando a las becerras con una alta prevalencia general, siendo *Eimeria* spp la más significativa. También se demuestra que la prevalencia está asociada a la edad de los animales, lo cual es de gran importancia para el diseño de programas de control.

Palabras clave: becerros, parásitos gastrointestinales, intensidad media de infección, prevalencia.

REPRODUCTION

Reproducción

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Pregnancy rate following an estradiol-free fixed-TAI protocol during breeding and non-breeding season in water buffaloes under tropical conditions

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Tasa de preñez tras un protocolo de IATF libre de estradiol durante la temporada reproductiva y no reproductiva en búfalas de agua bajo condiciones tropicales

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ABSTRACT

Fixed-timed artificial insemination (FTAI) protocols combining estradiol (E2) and progesterone (P4) are commonly used in the tropics, with adequate pregnancy rates throughout the year in buffaloes. However, the application of E2 and its related esters in food-producing animals is banned in the European Union and by the FDA-USA. This study aimed to evaluate the pregnancy rate (PR) after FTAI in buffalo cows (*Bubalus bubalis*) treated during the breeding (September-January) and non-breeding season (February-June) using a protocol based on P4 intravaginal devices (IVD) combined with GnRH and PGF2 α . The trial was performed on a commercial buffalo farm in Zulia State, Venezuela. Two hundred forty-seven buffalo cows (1-14 calvings) with a body condition score (BCS) of 3.15 ± 0.39 were selected. Cows were synchronized on a random day of the estrus cycle. To synchronize the ovulation on day 0, a dose of 10.5 μ g GnRH-analog (Buserelin benzoate acetate, Gestar[®]) was injected intramuscularly (IM) plus the insertion of an IVD maintained for nine days [TRIU B[®]: 1.0 gr P4, (n=139); CIDR[®]: 1.38 gr P4 (n=77); or PREGNAHEAT-E[®]: 250 mg of medroxyprogesterone acetate (MPA), (n= 18)]. On day 9, IVD was removed, and 0.50 mg of Cloprostenol (Estrumate[®], Intervet, MSD Animal Health) plus 500 IU of eCG (Folligon[®], Intervet, MSD Animal Health) were given IM. On day 11, a dose of 10.5 μ g GnRH-analog IM was used, and 8-12 hours later, they were artificially inseminated using frozen-thawed semen of bulls with proven fertility. The pregnancy rate (PR) was determined 30-32 days after AI by ultrasound. The data

RESUMEN

Los protocolos de inseminación artificial de tiempo fijo (IAFT) que combinan estradiol (E2) y progesterona (P4) se utilizan comúnmente en los trópicos con tasas de preñez adecuadas durante todo el año en búfalas. Sin embargo, la aplicación de E2 y sus ésteres relacionados en animales productores de alimentos está prohibida en la Unión Europea y por la FDA-EE. UU. Este estudio tuvo como objetivo evaluar la tasa de preñez (RP) después de la IATF en búfalas (*Bubalus bubalis*) tratadas durante la época reproductiva (septiembre-enero) y no reproductiva (febrero-junio) mediante un protocolo basado en dispositivos intravaginales (DIV) de P4 combinado con GnRH y PGF2 α . El ensayo se realizó en un rebaño comercial de búfalos en el estado Zulia, Venezuela. Se seleccionaron un total de 247 vacas búfala (1-14 partos), con un puntaje de condición corporal (BCS) de $3,15 \pm 0,39$. Las búfalas se sincronizaron en un día aleatorio del ciclo estrual. Para sincronizar la ovulación el día 0, se inyectó por vía intramuscular (*im*) una dosis de 10,5 μ g de análogo de GnRH (benzoato acetato de buserelina, Gestar[®]) más la inserción de un DIV mantenido durante nueve días [TRIU B[®]: 1,0 gr P4, (n=139); CIDR[®]: 1,38 gr P4 (n=77); o PREGNAHEAT-E[®]: 250 mg de medroxiacetato de progesterona (MPA), (n= 18)]. El día 9, se retiró el DIV y se administró 0,50 mg de cloprostenol *im* (Estrumate[®], Intervet, MSD Animal Health) más 500 UI de eCG (Folligon[®], Intervet, MSD Animal Health). El día 11 se aplicó una dosis de 10,5 μ g de análogo de GnRH *im* para inducir la ovulación y 8-12 horas después se in-

was analyzed by logistic regression through the Proc Logistic, and continuous and linear character variables were analyzed through the Proc GLM of the SAS® package. The overall PR was 52.2% (n = 129/247). Evaluation of independent variables, such as the number of calving and BCS, did not affect the PR ($p>0.05$). The PR was similar ($p>0.05$) amongst the reproductive and non-reproductive seasons [52.2%: (66/129) vs. 53.4% (63/118), respectively]. IVD-type did not affect the PR ($p>0.05$) [TRIU-B®: 52.6% (80/152), CIDR®: 53.3% (41/77) and PREGNAHEAT-E®: 44.4% (8/18)]. In conclusion, this FTAI protocol based on P4 and GnRH and PGF2 α produced adequate pregnancy rates during breeding and non-breeding seasons. These findings suggest that GnRH could be considered to substitute E2 esters in the P4-based FTAI protocols in water buffaloes under tropical conditions.

Keywords: water buffaloes, AI, synchronization, TAI, pregnancy.

seminaron artificialmente utilizando semen congelado-descongelado de toros con fertilidad comprobada. La tasa de preñez (TP) se determinó entre 30 y 32 días después de la IA mediante ecografía. Los datos fueron analizados mediante regresión logística a través del paquete Proc Logistic y las variables de carácter continuo y lineal a través del paquete Proc GLM de SAS®. La TP global fue del 52,2% (n = 129/247). La evaluación de variables independientes, como número de partos, BCS no afectó el TP ($P>0.05$). La TP fue similar ($p>0.05$) entre la temporada reproductiva y no reproductiva [52.2%: (66/129) vs. 53.4% (63/118) respectivamente]. El tipo de DIV no afectó la TR ($p>0,05$) [TRIU-B®: 52,6% (80/152), CIDR®: 53,3% (41/77) y PREGNAHEAT-E®: 44,4% (8/18)]. En conclusión, este protocolo de IATF basado en P4, combinado con GnRH y PGF2 α , produjo tasas de preñez adecuadas durante las temporadas reproductiva y no reproductiva. Estos hallazgos sugieren que se podría considerar que la GnRH puede reemplazar los ésteres de estradiol en los protocolos de IATF basados en P4 en búfalos de agua bajo condiciones tropicales.

Palabras clave: búfalos de agua, IA, sincronización, TAI, preñez.

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Poly(ADP-ribose) (PAR) levels in Peripheral Blood Mononuclear Cells (PBMC) during peri-implantation and early pregnancy in buffalo cows

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ABSTRACT

Poly(ADP-ribosyl)ation (PAR) is a reversible post-translational protein modification catalyzed by enzyme members of the poly(ADP-ribose) polymerases (PARPs) family. PAR is receiving growing attention due to its involvement in various biological processes. Despite many studies on humans, very little data on PAR in livestock are reported in the literature. Since its involvement in the blastocyst implantation has been hypothesized and considered the importance of markers for predicting

Niveles de poli(ADP-ribosa) (PAR) en células mononucleares de sangre periférica (PBMC) durante la periimplantación y la gestación temprana en vacas búfala

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RESUMEN

La poli(ADP-ribosil)ación (PAR) es una modificación postraduccional reversible catalizada por enzimas miembros de la familia de las poli(ADP-ribosil) polimerasas (PARP). La PAR está recibiendo una atención cada vez mayor debido a su implicación en diversos procesos biológicos. A pesar de muchos estudios en humanos, en la literatura se informan muy pocos datos sobre PAR en ganado. Dado que se ha planteado

pregnancy success, with this study, we wanted to evaluate dynamic changes in PAR levels during peri-implantation and early pregnancy in peripheral blood mononuclear cells (PBMCs) in buffalo species. Blood samples from 12 buffalo cows were collected on days 14, 19, and 28 after artificial insemination (AI), and PBMCs were isolated. Buffalo cows were divided *ex post* into pregnant (n=7) and non-pregnant (n=5) groups on Pregnancy-associated glycoproteins (PAGs) plasma concentrations and transrectal ultrasonography. The concentration of PAR levels (pg/mL) was determined in PBMC cellular extracts using the HT PARP *in vivo* Pharmacodynamic Assay II (Trevigen). The repeated measures ANOVA tested differences in mean values across time points and between experimental groups. This is the first study that evaluates PAR levels in bubaline PBMC during peri-implantation and early pregnancy. Results showed a different trend between the two groups. On day 14 after AI, the pregnant animals showed an increase of PAR compared to the non-pregnant group (23.12 ± 18.96 vs 15.30 ± 12.11 pg/mL, respectively), although the difference was insignificant. Interestingly, in the subsequent sampling period, the pregnant animals showed a constant decrease of PAR levels (19.64 ± 8.81 and 3.49 ± 4.71 pg/mL on days 19 and 28 after AI; $p=0.019$), reaching significantly lower levels compared to non-pregnant animals on day 28 after AI (3.49 ± 4.71 vs. 18.75 ± 5.76 pg/mL, respectively; $p=0.001$). The non-pregnant group showed a fluctuating trend of PAR (15.30 ± 12.11 , 29.09 ± 17.30 , and 18.75 ± 5.76 , on days 14, 19 and 28, respectively). In conclusion, although it can be considered an exploratory and preliminary study, results provide new evidence that PAR could be involved in mediating pregnancy success in dairy buffalo. However, further studies are needed to clarify the role of PAR modification during the establishment and maintenance of pregnancy in livestock.

Keywords: PAR polymers, PBMC, pregnancy, water buffalo.

la hipótesis de su participación en la implantación de blastocistos y se ha considerado la importancia de los marcadores para predecir el éxito de la preñez, con este estudio quisimos evaluar los cambios dinámicos en los niveles de PAR durante el periodo peri-implantacional y la preñez temprana en células mononucleares de sangre periférica (PBMC) en especie búfalina. Se recogieron muestras de sangre de 12 búfalas adultas los días 14, 19 y 28 después de la inseminación artificial (IA), y se aislaron de PBMC. Las búfalas se dividieron *ex post* en grupos preñadas (n=7) y no preñadas (n=5) según las concentraciones plasmáticas de glicoproteínas asociadas a la preñez (PAG) y la ecografía transrectal. La concentración de los niveles de PAR (pg/ml) se determinó en extractos celulares de PBMC utilizando el ensayo farmacodinámico II HT PARP *in vivo* (Trevigen). El ANOVA de medidas repetidas probó diferencias en los valores medios entre puntos temporales y entre grupos experimentales. Este es el primer estudio que evalúa los niveles de PAR en PBMC búfalinas durante la peri-implantación y el embarazo temprano. Los resultados mostraron una tendencia diferente entre los dos grupos. El día 14 después de la IA, los animales preñados mostraron un aumento de PAR en comparación con el grupo no preñado ($23,12 \pm 18,96$ vs $15,30 \pm 12,11$ pg/mL, respectivamente), aunque la diferencia fue insignificante. Curiosamente, en el período de muestreo posterior, los animales preñados mostraron una disminución constante de los niveles de PAR ($19,64 \pm 8,81$ y $3,49 \pm 4,71$ pg/mL en los días 19 y 28 después de la IA; $p=0,019$), alcanzando niveles significativamente más bajos en comparación con los animales no preñados el día 28 después de la IA ($3,49 \pm 4,71$ vs $18,75 \pm 5,76$ pg/mL, respectivamente; $p=0,001$). El grupo de no preñadas mostró una tendencia fluctuante de PAR ($15,30 \pm 12,11$, $29,09 \pm 17,30$ y $18,75 \pm 5,76$, en los días 14, 19 y 28, respectivamente). En conclusión, aunque puede considerarse un estudio exploratorio y preliminar, los resultados proporcionan nueva evidencia de que la PAR podría estar implicada en la mediación del éxito de la preñez en búfalas lecheras. Sin embargo, se necesitan más estudios para aclarar el papel de la modificación de PAR durante el establecimiento y mantenimiento de la preñez en el ganado.

Palabras clave: polímeros PAR, PBMC, preñez, búfalo de agua.

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Oocyte yield of nulliparous and pluriparous buffaloes submitted to synchronization prior to follicular aspiration

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ABSTRACT

This study aimed to analyze the quantity and quality of oocytes obtained from buffaloes submitted to follicular aspiration (OPU) after synchronization of the follicular wave emergency. So, 10 nulliparous and 10 pluriparous buffaloes were submitted to follicular aspirations. The data showed no differences in the number of oocytes obtained when the aspirations were done on days 4 or 5 after the follicular wave emergency. Although, when considering the buffaloes category, nulliparous buffaloes presented a higher mean number of retrieved oocytes/OPU (25.00 ± 3.36 vs. 13.40 ± 1.14), viable oocytes/OPU (6.22 ± 1.62 vs. 3.60 ± 1.27) and a total of recovered oocytes (250 vs. 134). On the other hand, considering the day of aspirations, there was no difference when OPU was done on day four or day 5. According to the results, it can be concluded that nulliparous buffaloes yield twice as many oocytes as pluriparous buffaloes, making them a good choice of category to be used to improve embryo production by IVF in the species.

Keywords: *Bubalus bubalis*, heifer, IVF, reproductive biotechnology.

Rendimiento de ovocitos de búfalas nulíparas y pluríparas sometidas a sincronización antes de la aspiración folicular

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RESUMEN

Este estudio tuvo como objetivo analizar la cantidad y calidad de los ovocitos obtenidos de búfalas sometidas a aspiración folicular después de la sincronización de la emergencia de la onda folicular. Así, 10 búfalas nulíparas y 10 pluríparas fueron sometidas a aspiraciones foliculares. Los datos obtenidos no mostraron diferencias en cuanto al número de ovocitos obtenidos cuando las aspiraciones se realizaron a los 4 ó 5 días después de la emergencia de la onda folicular. Aunque, al considerar la categoría de búfalas, las búfalas nulíparas presentaron una mayor media de ovocitos recuperados/UPP ($25,00 \pm 3,36$ vs $13,40 \pm 1,14$), ovocitos viables/OPU ($6,22 \pm 1,62$ vs $3,60 \pm 1,27$) y total de ovocitos recuperados (250 frente a 134). Por otro lado, considerando el día de las aspiraciones, no hubo diferencia si las aspiraciones se realizaron el día 4 o el día 5. De acuerdo con los resultados, se puede concluir que las búfalas nulíparas producen el doble de ovocitos que las búfalas pluríparas, lo que las convierte en una buena elección de la categoría que se utilizará para mejorar la producción de embriones por FIV en la especie.

Palabras clave: *Bubalus bubalis*, novilla, FIV, biotecnología reproductiva.

BIOTECHNOLOGY & OMICS TECHNOLOGIES

Biotecnología y Tecnologías Ómicas

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Performance of the Illumina *GGP Bovine 100K* SNP array for buffalo populations genotyping in Colombia

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ABSTRACT

The selection of buffaloes in Colombia is primarily based on the assessment of genetic merit or performance testing. Despite the availability of buffalo-specific genotyping tools, their cost limits routine implementation for genomic selection in the country. Therefore, we conducted an evaluation of single nucleotide polymorphism (SNP) arrays originally designed for cattle to determine their potential applicability in genetic evaluation of Colombian buffalo populations. Eight DNA samples from buffalo individuals belonging to the Murrah, Mediterranean, and crossbred genetic groups were genotyped using the Illumina Bovine HD770K Array. SNPs that overlapped with the GGP-HD150K and GGP-LD30K microarrays (Illumina) were specifically selected considering commercial chip availability. Subsequently, 24 additional samples were analyzed using the GGP Bovine 100K array. Allele segregation, heterozygosity, genetic structure, and genomic association to weight at 25 months were calculated. Out of the 734,240 identified SNPs, 86,521 (11.7%) exhibited uniform segregation in buffaloes across chromosomes ranging from 10.8% for Chr11 to 12.5% in Chr 12. By extracting segregating SNPs in HD770K from the GGP-HD150K and GGP-LD30K chips, we identified 15,169 and 3,311 markers, respectively. Analysis using the *GGP Bovine 100K* revealed 5,995 segregating SNPs, of which 42.01% had $H_o \leq 0.5$. On average, the observed heterozygosity in buffaloes was higher than cattle. Genetic structure among the three included genetic groups was successfully detected using 2,519 SNPs ($H_o \leq 0.5$). Moreover, 6 and 20 SNPs with FDR 0.05 and

Rendimiento del sistema Illumina *GGP Bovine 100K* SNP para el genotipado de poblaciones de búfalos en Colombia

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RESUMEN

La selección de búfalos en Colombia se basa principalmente en la evaluación del mérito genético o pruebas de desempeño. A pesar de la disponibilidad de herramientas de genotipado específicas para búfalos, su costo limita la implementación rutinaria de la selección genómica en el país. Por lo tanto, llevamos a cabo una evaluación de matrices de polimorfismos de un solo nucleótido (SNP) diseñadas originalmente para ganado vacuno para determinar su potencial aplicabilidad en la evaluación genética de poblaciones de búfalos colombianos. Se genotiparon ocho muestras de ADN de individuos de búfalo pertenecientes a los grupos genéticos Murrah, Mediterráneo y mestizos utilizando el Illumina Bovine HD770K Array. Los SNP que se superpusieron con los microarrays GGP-HD150K y GGP-LD30K (Illumina) se seleccionaron específicamente considerando la disponibilidad de chips comerciales. Posteriormente, se analizaron 24 muestras adicionales utilizando la matriz GGP Bovine 100K. Se calculó la segregación de alelos, la heterocigosidad, la estructura genética y la asociación genómica con el peso a los 25 meses. De los 734.240 SNP identificados, 86.521 (11,7%) exhibieron una segregación uniforme en búfalos en todos los cromosomas que oscilaban entre el 10,8% para Chr11 y el 12,5% en Chr 12. Al extraer los SNP segregantes en HD770K de los chips GGP-HD150K y GGP-LD30K, identificaron 15.169 y 3.311 marcadores, respectivamente. El análisis utilizando GGP Bovine 100K reveló 5.995 SNP segregantes, de los cuales el 42,01% tenía $H_o \leq 0,5$. En promedio, la heterocigosidad observada en los búfalos

0.1 respectively, were associated with weight at 25 months. Notably, some of these SNPs located in genomic regions harboring genes such as SLC24A4 or RPUSD4 were previously reported associated with conformation traits or feed efficiency in cattle. Considering the current scenario, the cost-benefit ratio analysis favors use of the *GGP Bovine 100K* array, as an initial strategy to support genetic structure of the populations, parentage testing and future genomic evaluation trials in buffaloes.

Keywords: cost-benefit, genomic selection, heterozygosity, segregation.

fue mayor que en el ganado vacuno. La estructura genética entre los tres grupos genéticos incluidos se detectó con éxito utilizando 2519 SNP ($H_o \leq 0,5$). Además, 6 y 20 SNP con FDR 0,05 y 0,1 respectivamente, se asociaron con el peso a los 25 meses. En particular, algunos de estos SNP fueron ubicados en regiones genómicas que albergan genes como SLC24A4 o RPUSD4, previamente reportados asociados con rasgos de conformación o eficiencia alimenticia en ganado vacuno. Considerando el escenario actual, el análisis de relación costo-beneficio favorece el uso del array *GGP Bovine 100K* como estrategia inicial para sustentar la estructura genética de las poblaciones, pruebas de paternidad y futuros ensayos de evaluación genómica en búfalos.

Palabras clave: costo-beneficio, selección genómica, heterocigosidad, segregación.

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Metagenomic analysis post thermal stress in buffalo sludge and tomato pomace anaerobic digestion: a case study

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ABSTRACT

The tomato processing industry is one of the most important sectors in the agri-food industry, and annually, more than 39.2 million tons of tomatoes are processed globally. Italy is the first producer of processed tomatoes in the Mediterranean area and the third-largest producer in the world. Buffalo farming represents a relevant sector within Italian agriculture, and one of its main issues is manure management. Both economic activities generate a substantial amount of waste (buffalo sludge - BS and tomato pomace - TP, peels plus seeds) that can significantly impact the disposal costs and, hence, their economic and environmental sustainability. The case study followed the evolution of microflora composition during anaerobic co-digestion of BS and TP for biogas production (Inoculum/Substrate ratio 0.5 according to volatile solid content) under mesophilic conditions (39°C) using an Automatic Methane Potential Test System, before and after a thermal stress (three-day period at 50 °C) occurred. BS was used as inoculum and control. The bacterial and archaeal communities were an-

Análisis metagenómico post estrés térmico en digestión anaeróbica de lodos de búfalo y orujo de tomate: un estudio de caso

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RESUMEN

La industria procesadora de tomate es uno de los sectores más importantes de la industria agroalimentaria y anualmente se procesan más de 39,2 millones de toneladas de tomates en todo el mundo. Italia es el primer productor de tomates procesados de la zona del Mediterráneo y el tercer productor del mundo. La cría de búfalos representa un sector relevante dentro de la agricultura italiana y una de sus principales cuestiones es la gestión del estiércol. Ambas actividades económicas generan una cantidad sustancial de residuos (lodos de búfalo - BS y orujo de tomate - TP, cáscaras más semillas) que pueden impactar en gran medida los costos de eliminación y, por tanto, su sostenibilidad económica y ambiental. El estudio de caso siguió la evolución de la composición de la microflora durante la codigestión anaeróbica de BS y TP para la producción de biogás (relación inóculo/sustrato 0,5 según el contenido de sólidos volátiles) en condiciones mesófilas (39 °C) utilizando un Sistema Automático de Prueba de Potencial de Metano, antes y después de que se ocurrió un estrés térmico (período de

alyzed using next-generation sequencing of 16S rRNA gene amplicons. After seven days of fermentation, due to the high volatile solid content in TP (77 g/L), the pH dropped to a value of 5, and the methane production reached a plateau (860 Nml of cumulative CH₄ production). After the thermal stress, there was a shift in microbial populations, the pH increased back to neutrality, and the TP batch was able to resume biogas production, reaching 3048 Nml of cumulative CH₄ production at the end of fermentation (45 days). A maximum methane peak at 78.5% in BS/TP biogas was recorded compared to 63.5% in BS. Taxonomic classification showed that the bacteria phyla belonging to Firmicutes (51.7%), of whom 47.4% were Clostridia, Bacteroidetes (29.9%) mostly Bacteroidia (29.0%), Tenericutes (4.1%) and Proteobacteria (3.2%), accounted for 88.9% of total OTUs. Among the Clostridiaceae, *Fonticella* (4.3% on average) was the most represented genus. The latter was absent in the BS sample and increased to 17.1% in the BS/TP sample when methane production was restored, while in the archaeal community predominated the phylum Euryarcheota, with *Methanocorpusculum* being the most represented and *Metanosarcina* the most correlated to methane production. Brief thermal stress significantly changed bacterial and archaeal populations crucial to increasing methane production at the conditions tested.

Keywords: biogas, agricultural waste, anaerobic digestion, tomato pomace, buffalo slurry.

tres días a 50 °C). Se utilizó BS como inóculo y control. Las comunidades bacterianas y arqueales se analizaron mediante secuenciación de próxima generación de amplicones del gen 16S rRNA. Después de 7 días de fermentación debido al alto contenido de sólidos volátiles en TP (77 g/L), el pH cayó a un valor de 5 y la producción de metano alcanzó una meseta (860 Nml de producción acumulada de CH₄). Después del estrés térmico, hubo un cambio en las poblaciones microbianas, el pH volvió a ser neutral y el lote TP pudo reanudar la producción de biogás alcanzando 3048 Nml de producción acumulada de CH₄ al final de la fermentación (45 días). Se registró un pico máximo de metano del 78,5% en el biogás BS/TP en comparación con el 63,5% en BS. La clasificación taxonómica mostró que los filos de bacterias pertenecientes a Firmicutes (51,7%), de los cuales el 47,4% eran Clostridia, Bacteroidetes (29,9%), principalmente Bacteroidia (29,0%), Tenericutes (4,1%) y Proteobacteria (3,2%), representaron el 88,9% del total de los OTU. Entre las Clostridiaceae, *Fonticella* (4,3% en promedio) fue el género más representado. Este último estuvo ausente en la muestra de BS y aumentó hasta un 17,1% en la muestra de BS/TP cuando se restableció la producción de metano. En la comunidad de arqueas predominó el filo Euryarcheota, siendo *Methanocorpusculum* el más representado y *Metanosarcina* el más correlacionado con la producción de metano. Un breve estrés térmico produjo un cambio significativo en las poblaciones de bacterias y arqueas, crucial para aumentar la producción de metano en las condiciones probadas.

Palabras clave: biogás, residuos agrícolas, digestión anaeróbica, orujo de tomate, purín de búfalo.

BUFFALO'S PRODUCTS & INDUSTRY

Productos e Industria de Búfalos

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Comparison of sialic acid content in bovine and buffalo milk and meat

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Comparación del contenido de ácido siálico en leche y carne de bovino y búfala

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ABSTRACT

Animal-origin food, such as meat and milk, are important sources of sialic acids (SIAs) with relevant implications for human health. Two significant SIAs in mammals are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Neu5Ac is the major form of SIAs in all mammalian tissues, and it is an essential nutrient for the development and function of the brain. Neu5Ac differs from Neu5Gc by a single oxygen atom, but humans cannot synthesize the Neu5Gc due to a genetic mutation. Thus, Neu5Gc causes a human antibody response, which promotes the inflammatory processes. On the other hand, animal products are excellent sources of Neu5Ac, which has a high nutritional value. A diet with Neu5Ac/Neu5Gc ratio higher than 5 is recommended to inhibit the absorption of Neu5Gc. This ratio is rarely reached in red meat, so it is considered potentially carcinogenic. This study aimed to determine the concentrations of Neu5Ac and Neu5Gc in the milk and meat of buffalo and cattle and to evaluate the ratio of Neu5Ac/Neu5Gc. The study was conducted on 12 bulk milk and 12 samples of *Longissimus thoracis* for each species. Samples were collected from Friesian bovine and buffalos raised in farms located in Central Italy. The concentration of the two types of SIAs was determined by the reverse phase (RP) high-performance liquid chromatography (HPLC) procedure of SIAs released from glycoconjugates through acidic hydrolysis and derivatized with 1,2-diamino-4,5-methylenedioxy-benzene (DMB). Data was analyzed by ANOVA using a bifactorial model with interactions (products and species). Both products and the species influenced the content of Neu5Ac ($p < 0.001$ and $p < 0.014$, respectively) and Neu5Gc ($p < 0.001$, for both). Neu5Ac content was significantly higher in buffalo milk than in cow milk (1045 vs. 906 $\mu\text{g/g}$ protein). In contrast, Neu5Gc content was significantly lower in buffalo milk than in cow milk (58 vs. 106 $\mu\text{g/g}$ protein).

RESUMEN

Los alimentos de origen animal, como la carne y la leche, son fuentes importantes de ácidos siálicos (SIA) con implicaciones relevantes para la salud humana. Dos SIA importantes en los mamíferos son el ácido N-acetilneuramínico (Neu5Ac) y el ácido N-glicolilneuramínico (Neu5Gc). Neu5Ac es la forma principal de SIA en todos los tejidos de los mamíferos y es un nutriente esencial para el desarrollo y la función del cerebro. Neu5Ac se diferencia de Neu5Gc por un solo átomo de oxígeno, pero los humanos no pueden sintetizar Neu5Gc debido a una mutación genética. Por tanto, Neu5Gc provoca una respuesta de anticuerpos humanos que promueve los procesos inflamatorios. Por otro lado, los productos animales son excelentes fuentes de Neu5Ac, que tiene un alto valor nutricional. Se recomienda una dieta con una relación Neu5Ac/Neu5Gc superior a 5 para inhibir la absorción de Neu5Gc. En la carne roja rara vez se alcanza esta proporción, por lo que es considerada potencialmente carcinogénica. El objetivo de este estudio fue determinar las concentraciones de Neu5Ac y Neu5Gc en leche y carne de búfala y vacuno y evaluar la relación Neu5Ac/Neu5Gc. El estudio se realizó con 12 muestras de leche a granel y 12 muestras de *Longissimus thoracis* para cada especie. Se recolectaron muestras de bovinos frisonos y búfalos criados en granjas ubicadas en el centro de Italia. La concentración de los dos tipos de SIA se determinó mediante el procedimiento de cromatografía líquida de alto rendimiento (HPLC) en fase inversa (RP) de SIA liberados de glicoconjugados mediante hidrólisis ácida y derivatizados con 1,2-diamino-4,5-metilenedioxi-benceno. (DMB). Los datos fueron analizados mediante ANOVA utilizando un modelo bifactorial con interacciones (productos y especies). Tanto los productos como las especies influyeron en el contenido de Neu5Ac

On the contrary, there was no significant difference in Neu5Ac meat content in the two species (473 µg/g protein, on average). In contrast, Neu5Gc content was significantly higher in cattle meat than in buffalo meat (228 vs. 83 µg/g protein $p < 0.001$). Buffalo products showed a Neu5Ac/Neu5Gc ratio greater than 5 (18.5 for milk and 6.4 for meat), while beef meat had a ratio of 2.2 (less than 5). In conclusion, due to the high amount of Neu5Ac and the limited presence of Neu5Gc, buffalo products could have an exciting effect on human health.

Keywords: N-acetylneuraminic acid, N-glycolylneuraminic acid, buffalo, bovine, milk, meat.

($p < 0,001$ y $p < 0,014$, respectivamente) y Neu5Gc ($p < 0,001$, para ambos). El contenido de Neu5Ac fue significativamente mayor en la leche de búfala que en la leche de vaca (1045 frente a 906 µg/g de proteína). Por el contrario, el contenido de Neu5Gc fue significativamente menor en la leche de búfala que en la leche de vaca (58 frente a 106 µg/g de proteína). Por el contrario, no hubo diferencias significativas en el contenido de Neu5Ac en la carne de las dos especies diferentes (473 µg/g de proteína, en promedio). Por el contrario, el contenido de Neu5Gc fue significativamente mayor en la carne de vacuno que en la de búfalo (228 vs. 83 µg/g de proteína $p < 0,001$). Los productos de búfalo mostraron una relación Neu5Ac/Neu5Gc superior a 5 (18,5 para la leche y 6,4 para la carne), mientras que la carne de vacuno tuvo una relación de 2,2 (inferior a 5). En conclusión, debido a la alta cantidad de Neu5Ac y la presencia limitada de Neu5Gc, los productos de búfalo podrían tener un efecto interesante en la salud humana.

Palabras clave: ácido N-acetilneuramínico, ácido N-glicolilneuramínico, búfala, bovino, leche, carne.

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Buffalo whey proteins performance at the oil/water interface: rheology characterization of the interfacial films

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ABSTRACT

Whey proteins can form thin layers at the interface of non-miscible liquids like oil drops and water by emulsifying. The interfacial activity and the rheology of the interfacial films constitute a crucial aspect in assessing ingredients' ability to form and stabilize emulsions. Although cow-origin whey protein concentrates (WPC) have been extensively used in the food industry, the WPC from buffalo (BWPC) still needs to be studied. In this context, the objective of this work was to explore the interfacial activity and the viscoelastic properties of BWPC at the oil-in-water (O/W) interfaces. WPC was used for comparison. With this purpose, BWPC and WPC were dispersed at 2.6×10^{-3} – 1.6% w/v in 10 mM phosphate buffer (pH 7) and equilibrated

Rendimiento de las proteínas del suero de búfalo en la interfaz aceite/agua: caracterización reológica de las películas interfaciales

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RESUMEN

Las proteínas del suero pueden formar capas delgadas en la interfaz de líquidos no miscibles como gotas de aceite y agua al emulsionarse. La actividad interfacial y la reología de las películas interfaciales constituyen un aspecto crucial en la evaluación de la capacidad de los ingredientes para formar y estabilizar emulsiones. Aunque los concentrados de proteína de suero de vaca (WPC) se han utilizado ampliamente en la industria alimentaria, el WPC de búfalo (BWPC) aún necesita ser estudiado. En este contexto, el objetivo de este trabajo fue explorar la actividad interfacial y las propiedades viscoelásticas del BWPC en las interfaces aceite en agua (O/W). Se utilizó

ed at four °C for 24 h. Then, the equilibrium interfacial pressure (π_{eq}) was registered through the Wilhelmy plate method. Additionally, adsorption dynamics at the O/W interface at protein concentrations of 0.5 and 1% were obtained over three h with a drop tensiometer. Subsequently, the rheological dilatational behavior (10% deformation amplitude and 0,1 Hz of angular frequency) was evaluated. Plate tensiometry showed that π_{eq} of BWPC and WPC solutions increased from 9.9 to 16.7 mN/m and 11.5 to 15.0 mN/m, respectively, and conform protein concentration increased. An intersection between π – concentration isotherms was observed, indicating that below protein concentration of 1.3x10⁻²%, the π_{eq} of WPC was higher than BWPC. This observation could be attributed to compositional differences and the possibility of generating different structural patterns in the interface. The drop tensiometer displayed that π increased with time for both protein concentrates, indicating the protein adsorption at the O/W interface. Interfacial rheology results indicated a gradual increase in the solid or elastic character (E_d) over time for both types of proteins, indicating more cohesive and packed structures. E_d of BWPC was higher than WPC at the lowest protein concentration, while the opposite also occurred. This behavior can be explained by considering that the higher the protein concentration, the stronger interactions among BPWC proteins occur. In conclusion, BWPC increased the interfacial pressure to a greater extent than WPC and concomitantly formed films with a higher solid character. These findings contribute to considering the use of BWPC as an efficient emulsifier agent with the benefits of adding value and minimizing the environmental impact of this byproduct.

Keywords: buffalo whey protein, oil-water interface, surface pressure, rheology.

WPC para comparación. Con este propósito, BWPC y WPC se dispersaron a 2,6x10⁻³ – 1,6% p/v en tampón fosfato 10 mM (pH 7) y se equilibraron a cuatro °C durante 24 h. Luego, se registró la presión interfacial de equilibrio (π_{eq}) mediante el método de placas de Wilhelmy. Además, se obtuvo la dinámica de adsorción en la interfaz O/W a concentraciones de proteína de 0,5 y 1% durante tres horas con un tensiómetro de gota. Posteriormente se evaluó el comportamiento reológico dilatacional (10% de amplitud de deformación y 0,1 Hz de frecuencia angular). La tensiometría con placa mostró que la π_{eq} de las soluciones de BWPC y WPC aumentó de 9,9 a 16,7 mN/m y de 11,5 a 15,0 mN/m, respectivamente, y aumentó la concentración de proteína conformada. Se observó una intersección entre las isotermas de concentración π , lo que indica que por debajo de una concentración de proteína de 1,3x10⁻²%, la π_{eq} de WPC fue mayor que la de BWPC. Esta observación podría atribuirse a diferencias compositivas y a la posibilidad de generar diferentes patrones estructurales en la interfaz. El tensiómetro de gota mostró que π aumentó con el tiempo para ambos concentrados de proteínas, lo que indica la adsorción de proteínas en la interfaz O/W. Los resultados de la reología interfacial indicaron un aumento gradual en el carácter sólido o elástico (E_d) a lo largo del tiempo para ambos tipos de proteínas, lo que indica estructuras más cohesivas y empaquetadas. La E_d de BWPC fue mayor que la de WPC en la concentración de proteína más baja, mientras que también ocurrió lo contrario. Este comportamiento puede explicarse considerando que cuanto mayor es la concentración de proteínas, más fuertes se producen las interacciones entre las proteínas BPWC. En conclusión, BWPC fue capaz de aumentar la presión interfacial en mayor medida que WPC y al mismo tiempo formar películas con un mayor carácter sólido. Estos hallazgos contribuyen a considerar el uso de BWPC como un agente emulsionante eficiente con los beneficios de agregar valor y minimizar el impacto ambiental de este subproducto.

Palabras clave: proteína de suero de búfala, interfaz aceite-agua, presión superficial, reología.

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How do consumers evaluate buffalo meat?

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ABSTRACT

Consumers have been the target of studies since they constitute the focal point of production chains. The objective of this study was to analyze the opinions of a given population and the consumers' perception regarding industrialized and commercialized buffalo meat in the metropolitan region of Recife, in the state of Pernambuco, located in the Northeast region of Brazil. A questionnaire was applied in August and September 2022, and the technique used for the research was the survey. The research filter was the affirmative answer to the question about the purchase and consumption of buffalo meat products. The test was applied electronically, structured in closed, open, and dichotomous questions, to obtain qualitative and quantitative data from 178 consumers of buffalo meat. The questionnaire addressed the following aspects: age, sex, family income, the most consumed meat cut, the main aspects observed in fresh and ready-to-eat buffalo meat and the determinants in the choice, degree of satisfaction with the product, prior knowledge of buffalo meat and the degree of satisfaction with the consumption of hamburgers and sausages. Data were tabulated and subjected to descriptive statistical analysis using the Excel 2013 Office version program. The proportion of male and female consumers was 48.0% and 52.0%, respectively. The average age observed was 37 years. The evaluated consumers have an average family income of more than US\$ 550,00. The most consumed meat cuts are those considered "day-to-day", emphasizing the topside, knuckle, eye round, flat, minced meat, and sun-dried meat, making up 67.8% of the choices. Consumers reported choosing meat primarily considering cost accessibility, product quality, and sensory characteristics, highlighting flavor and tenderness. The degree of satisfaction was between satisfied and very satisfied for 92.0% of the assessed

¿Cómo valoran los consumidores la carne de búfalo?

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RESUMEN

Los consumidores han sido objeto de estudios ya que constituyen el punto focal de las cadenas productivas. El objetivo de este estudio fue analizar las opiniones de una población determinada y la percepción del consumidor sobre la carne de búfalo industrializada y comercializada en la región metropolitana de Recife, en el estado de Pernambuco, ubicado en la región Nordeste de Brasil. Se aplicó un cuestionario en agosto y septiembre de 2022, y la técnica utilizada para la investigación fue la encuesta. El filtro de la investigación fue la respuesta afirmativa a la pregunta sobre la compra y consumo de productos cárnicos de búfalo. La prueba se aplicó de forma electrónica, estructurada en preguntas cerradas, abiertas y dicotómicas, para obtener datos cualitativos y cuantitativos de 178 consumidores de carne de búfalo. El cuestionario abordó los siguientes aspectos: edad, sexo, ingresos familiares, corte de carne más consumido, principales aspectos observados en la carne de búfala fresca y lista para el consumo y los determinantes en la elección, grado de satisfacción con el producto, conocimientos previos de carne de búfalo y el grado de satisfacción con el consumo de hamburguesas y embutidos. Los datos fueron tabulados y sometidos a análisis estadístico descriptivo utilizando el programa Excel versión Office 2013. La proporción de consumidores masculinos y femeninos fue del 48,0 y 52,0%, respectivamente. La edad promedio observada fue de 37 años. Los consumidores evaluados tienen un ingreso familiar promedio superior a US\$ 550,00. Los cortes de carne más consumidos son los considerados "del día a día", con énfasis en la parte superior, codillo, ojo redondo, plano, picada y carne secada al sol, conformando un total del 67,8% de las opciones. Los consumidores informaron que eligieron la carne considerando principalmente la accesibilidad de los costos, la

consumers. 81.0% of consumers said they had never consumed buffalo meat or meat products. The buffalo meat burger was rated good by 35.0% of consumers and very good by 62.0%. It is observed that the commercial availability of buffalo meat is a determining factor for consumption since the degree of rejection of buffalo meat products is minimal.

Keywords: *Bubalus bubalis*, consumers, meat.

calidad del producto y las características sensoriales, destacando el sabor y la ternura. El grado de satisfacción estuvo entre satisfecho y muy satisfecho para el 92,0% de los consumidores evaluados. El 81,0% de los consumidores afirmó nunca haber consumido carne de búfalo o productos cárnicos. La hamburguesa de carne de búfalo fue valorada como buena por el 35,0% de los consumidores y como muy buena por el 62,0%. Se observa que la disponibilidad comercial de la carne de búfalo es un factor determinante para su consumo ya que el grado de rechazo de los productos cárnicos de búfalo es mínimo.

Palabras clave: *Bubalus bubalis*, consumidores, carne.

SUSTAINABILITY & SOCIOECONOMICS

Sustentabilidad y Socioeconomía

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Methodological aspects in gastroenteric methane evaluation trial in water buffalo

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ABSTRACT

Methane (CH₄) is a potent greenhouse gas, and ruminants constitute a significant source of agricultural contributions. It has been hypothesized that the host's genome ultimately controls rumen microbial communities, but numerous samples are needed to achieve robust results. The ease and representativeness of the sampling method and the cost-efficiency of large-scale sequencing will, therefore, be major factors in ensuring the feasibility of a research project. The rationale of this work was to verify if non-invasive samples could be a proxy for ruminal digesta and to validate the concentration registered by a laser methane detector (LMD) at the nostrils by measuring the actual concentration of CH₄ at the rumen cannula immediately after opening. The LMD is a remote CH₄ monitoring system for air used in other ruminants, but studies on buffalo are scarce. The laser beam is pointed toward the source to acquire the data. The trial aimed to compare an easy sampling (1' during a milking session), with a longer and labor-intensive one (5' animals captured in a crush). In five (n=5) cannulated buffalo cows, various matrices (whole rumen content, feces, and buccal swabs) were tested for DNA recovery. Two different Buccal swabs were tested. Frozen solid and liquid rumen fractions, feces as-is, pelleted, or in preserving glycerol solution at room temperature were tested. Different protocols for DNA extraction (WUR protocol; Kit Maxwell® Promega; Kit Quick Extract™ Lucigen) and various sampling methods were compared. Saliva was a difficult matrix to process and did not yield satisfactory DNA, so it is unsuitable as a proxy. Feces showed no difference in preservation methods, and DNA recovery was like the rumen. Nevertheless, metagenomic analysis must be carried out to verify whether the species composition is

Aspectos metodológicos en el ensayo de evaluación de metano gastroentérico en búfalos de agua

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RESUMEN

El metano (CH₄) es un potente gas de efecto invernadero y los rumiantes son una fuente importante de aportes agrícolas. Se ha planteado la hipótesis de que las comunidades microbianas del rumen están controladas en última instancia por el genoma del huésped, pero se necesitan numerosas muestras para lograr resultados sólidos. Por lo tanto, la facilidad y representatividad del método de muestreo y la rentabilidad de la secuenciación a gran escala serán factores importantes para garantizar la viabilidad de un proyecto de investigación en este campo. El objetivo de este trabajo fue verificar si las muestras no invasivas podrían ser un indicador de la digesta ruminal y validar la concentración registrada por un detector láser de metano (LMD) en las fosas nasales midiendo la concentración real de CH₄ en la cánula ruminal inmediatamente después de su apertura. El LMD es un sistema de monitoreo remoto de CH₄ en el aire, utilizado en otros rumiantes, pero los estudios en búfalos son escasos. El rayo láser apunta hacia la fuente para adquirir los datos. El ensayo tuvo como objetivo comparar un muestreo fácil (1' durante una sesión de ordeño), con uno más largo y laborioso (5' animales capturados en un muestreo). En cinco (5) búfalas canuladas, se analizaron varias matrices (contenido total del rumen, heces e hisopos bucales) para la recuperación de ADN. Se probaron dos hisopos bucales diferentes. Se analizaron fracciones de rumen sólidas y líquidas congeladas, heces tal cual, granuladas o en solución de glicerol conservante a temperatura ambiente. Se compararon diferentes protocolos para la extracción de ADN (protocolo WUR; Kit Maxwell® Promega; Kit Quick Extract™ Lucigen) y varios métodos de muestreo. La saliva era una matriz difícil

comparable. All DNA extraction methods were satisfying: Quick Extract was the easiest to perform, WUR resulted in the highest amount of DNA, and Kit Maxwell was the one that gave the overall best performance. As for the CH₄ measure, there was a significant correlation between the rumen's highest pick value of emission at the cannula and the intensity and duration of breath emission, calculated as breath + eructation. Moreover, we found a highly significant difference in emissions patterns when animals were subjected to distress (confinement, manipulation). LMD showed different emission trends under different sampling conditions, which seems suitable for CH₄ measurement in the buffalo species.

Keywords: Cannulated buffalo, rumen microbiome, DNA extraction, methane emission, laser methane detector.

de procesar y no produjo ADN satisfactorio, por lo que no es adecuada como sustituto. Las heces no mostraron diferencia en cuanto a los métodos de conservación y la recuperación de ADN fue similar al rumen; sin embargo, se debe realizar análisis metagenómicos para verificar si la composición de especies también es comparable. Todos los métodos de extracción de ADN fueron satisfactorios: Quick Extract fue el más fácil de realizar, WUR dio como resultado la mayor cantidad de ADN y Kit Maxwell fue el que brindó el mejor rendimiento general. En cuanto a la medida de CH₄, hubo una correlación significativa entre el valor más alto de emisión del rumen en la cánula y la intensidad y duración de la emisión de aire, calculada como aliento + eructos. Además, encontramos una diferencia muy significativa en los patrones de emisiones cuando los animales fueron sometidos a angustia (confinamiento, manipulación, etc.). LMD mostró diferentes tendencias de emisión bajo diferentes condiciones de muestreo, por lo que parece adecuado para la medición de CH₄ en la especie de búfalo.

Palabras clave: búfalo canulado, microbioma ruminal, extracción de ADN, emisión de metano, detector láser de metano.

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Productivity and socioeconomic sustainability of *Bubalus bubalis* in the western lowlands of Venezuela

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ABSTRACT

The productivity and socioeconomic sustainability of *Bubalus bubalis* was compared to that of *Bos indicus* (as a reference), using an econometric livestock simulator in the lowlands of Barinas, Venezuela. Located at 7° 59' 57.57" north latitude and 68° 59' 00.13" west longitude, and from there to a radius of 50 kilometers. The simulator, built with Microsoft Excel, used the methodology of formulation and evaluation of agricultural investment projects. Given the importance of making prospective evaluations as a way of demonstrating sustainability, it was corroborated the future behavior of the family's assets every time through the projected balance sheet: it was inquire, using a "Focus Group" with 80 breeders, the following variables: Number of hectares; carrying capacity; milk production per day; milk/cheese conversion; animal unit; projected income; projected health costs, pasture maintenance costs, feed costs and projected financial costs; depreciation; interest rate; inflation; investments; mortality rate; calving percentage, among others. The central motivation of the study was to show

Productividad y sostenibilidad socioeconómica de *Bubalus bubalis* en las tierras bajas occidentales de Venezuela

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RESUMEN

Se comparó la productividad y sostenibilidad socioeconómica de *Bubalus bubalis* con la de *Bos indicus* (como referencia), utilizando un simulador econométrico de ganadería en las tierras bajas de Barinas, Venezuela. Ubicado a los 7° 59' 57.57" de latitud norte y 68° 59' 00.13" de longitud oeste, y desde allí en un radio de 50 kilómetros. El simulador, construido con Microsoft Excel, utilizó la metodología de formulación y evaluación de proyectos de inversión agrícola. Dada la importancia de realizar evaluaciones prospectivas como forma de demostrar la sostenibilidad, se corroboró en cada ocasión el comportamiento futuro del patrimonio de la familia a través del balance proyectado: se indagó, mediante un "Grupo focal" con 80 ganaderos, las siguientes variables: Número de hectáreas; capacidad de carga; producción de leche por día; conversión de leche/queso; unidad animal; ingresos proyectados; costos de salud proyectados, costos de mantenimiento de pastos, costos de alimentación y costos financieros proyectados; depreciación; tasa de interés; inflación; inversiones; tasa

breeder families a different alternative to traditional cattle farming, in which the study area displaces a lot of forestry due to the expansion of agricultural lands. In contrast, buffalo farming requires forests. However, to convince farmers to apply this type of livestock; it was necessary to demonstrate its greater profitability. Upon inputting random values, internal rates of return (IRR) were obtained for a ten-year projection horizon and, comparing both type of livestock herds: the bubaline herd was more productive, as shown by a higher IRR in the year 2022 (IRR=35%), than the cattle herd (IRR=10%) during the same period. After statistically processing those resulted with a positive sign, by comparing means through Student's t-tests for independent samples, using the SPSS statistical package: it was verified that the annual positive IRR results obtained are statistically and probabilistically valid with bilateral significance levels $p < 0.05$ and $p < 0.01$ of admissible errors respectively, indicating that there are significant differences between the means. It was concluded that, in the western lowlands of Venezuela, *Bubalus bubalis* is both productive and socioeconomically sustainable. The latter could be corroborated by using Linear Regression in the projected financial statements (Income Statement and Balance Sheet) measuring the evolution of the family patrimony, with the livestock family being the fundamental socioeconomic unit and its central object of study. In 2023, the projections of the present work have been verified through the statistics provided by the Agrifood Network of Venezuela.

Keywords: productivity, socioeconomic sustainability, livestock family, wetlands.

de mortalidad; porcentaje de parto, entre otros. La motivación central del estudio fue mostrar a las familias de ganaderos una alternativa diferente a la ganadería tradicional, en la que la zona de estudio desplaza gran parte de la forestación debido a la expansión de las tierras agrícolas. Por el contrario, la cría de búfalos requiere bosques. Sin embargo, convencer a los ganaderos para que apliquen este tipo de ganadería; era necesario demostrar su mayor rentabilidad. Al ingresar valores aleatorios, se obtuvieron tasas internas de retorno (TIR) para una proyección futura de diez años y, comparando ambos tipos de rebaños ganaderos: el rebaño bubalino fue más productivo, como lo demuestra una TIR mayor en el año 2022 (TIR=35%), que el hato bovino (TIR=10%) durante el mismo período. Luego de procesar estadísticamente los resultados con signo positivo, mediante comparación de medias mediante la prueba t de Student para muestras independientes, utilizando el paquete estadístico SPSS: se verificó que los resultados de TIR positivos anuales obtenidos son estadística y probabilísticamente válidos con niveles de significancia bilateral $p < 0,05$. y $p < 0,01$ de errores admisibles respectivamente, lo que indica que existen diferencias significativas entre las medias. Se concluyó que, en las tierras bajas occidentales de Venezuela, *Bubalus bubalis* es productivo y socioeconómicamente sostenible. Esto último podría corroborarse mediante el uso de Regresión Lineal en los estados financieros proyectados (Estado de Resultados y Balance) midiendo la evolución del patrimonio familiar, siendo la familia ganadera la unidad socioeconómica fundamental y su objeto central de estudio. En el año 2023, las proyecciones del presente trabajo han sido verificadas a través de las estadísticas proporcionadas por la Red Agroalimentaria de Venezuela.

Palabras clave: productividad, sostenibilidad socioeconómica, familia ganadera, humedales.

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The carbon footprint of pasture-based buffalo milk production in Colombia

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ABSTRACT

Colombia's water buffalo dairy market has experienced significant growth in recent years, emerging as the industry's leading sector in milk quality and favorable prices. Buffaloes are renowned for their early maturity and ability to produce high-quality meat. Two key characteristics stand out when

La huella de carbono de la producción de leche de búfala a base de pastos en Colombia

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RESUMEN

El mercado lácteo de búfala de agua de Colombia ha experimentado un crecimiento significativo en los últimos años, emergiendo como el sector líder de la industria en calidad de leche y precios favorables. Los búfalos son famosos por su madurez temprana y su capacidad para producir carne de alta

considering animals for dual-purpose systems: excellent milk quality suitable for sale, calf rearing and strong maternal abilities. A yield gap analysis was conducted to assess the potential for greenhouse gas emissions mitigation in Colombian buffalo systems to identify achievable buffalo milk productivity. This study is adapted to the standards for life cycle assessment, focusing specifically on greenhouse gas emissions from the International Organization for Standardization. Two functional units were used for measurement: tons of CO₂-equivalents (CO_{2e}) per hectare (ha) and kilograms of CO_{2e} per kilogram of fat-and-protein-corrected milk (FPCM). Greenhouse gas emissions were calculated using the 2019 Refinement to the 2006 IPCC guidelines, with emission factors sourced from databases. Emissions were categorized into those associated with animals, feces, and soils, with the corresponding sequestration or assimilation accounted in soils, forage biomass, trees, shrubs, and milk production. The simulations were conducted for 550 kg live-weight buffaloes, with a dry matter intake of 11 kg per day alongside 2 kg of balanced feed, under two milk production scenarios: 1500 liters and 3000 liters per lactation. Herd gross energy (GE) intake was calculated using IPCC Tier 2 equations, considering daily gross energy intake per animal category, diet digestibility, and daily net energy requirements for pregnancy, lactation, growth, activity and maintenance. Dry matter intake (DMI) was determined by dividing gross energy intake values by 18.45 MJ of metabolizable energy per kg dry matter, representing the feed's energy density. These greenhouse gas emissions encompassed methane (CH₄) from enteric fermentation and excretions left in paddocks, nitrous oxide (N₂O) from excretions deposited on pastures and fertilizer application, and carbon dioxide (CO₂) from lime and urea application, as well as the burning of fossil fuels. The global warming potential indices GWP100 and GWP* were employed to calculate the impact of emissions. Milk carbon footprints (CFs) ranged from 0.7 to 1.41 kgCO₂-eq kg FPCM-1, but when calculated with the GWP* index, they decreased by nearly 35% for both scenarios.

Keywords: life cycle assessment, milk, production, buffalo, pasture-based.

calidad. Dos características clave se destacan al considerar animales para sistemas de doble propósito: excelente calidad de leche apta para la venta, crianza de terneros y fuertes habilidades maternas. Se realizó un análisis de la brecha de rendimiento para evaluar el potencial de mitigación de las emisiones de gases de efecto invernadero en los sistemas búfalos colombianos para identificar la productividad alcanzable de las búfalas lecheras. Este estudio está adaptado a los estándares de evaluación del ciclo de vida, centrándose específicamente en las emisiones de gases de efecto invernadero de la Organización Internacional de Normalización. Se utilizaron dos unidades funcionales para la medición: toneladas de equivalentes de CO₂ (CO_{2e}) por hectárea (ha) y kilogramos de CO_{2e} por kilogramo de leche corregida con grasa y proteína (FPCM). Las emisiones de gases de efecto invernadero se calcularon utilizando el Refinamiento de 2019 de las directrices del IPCC de 2006, con factores de emisión obtenidos de bases de datos. Las emisiones se categorizaron en aquellas asociadas a animales, heces y suelos, contabilizándose el correspondiente secuestro o asimilación en suelos, biomasa forrajera, árboles, arbustos y producción de leche. Las simulaciones se realizaron para búfalas de 550 kg de peso vivo, con un consumo de materia seca de 11 kg por día junto con 2 kg de alimento balanceado, bajo dos escenarios de producción de leche: 1500 litros y 3000 litros por lactancia. La ingesta de energía bruta (GE) del rebaño se calculó utilizando las ecuaciones de Nivel 2 del IPCC, considerando la ingesta de energía bruta diaria por categoría animal, la digestibilidad de la dieta y los requerimientos energéticos netos diarios para la gestación, la lactancia, el crecimiento, la actividad y el mantenimiento. El consumo de materia seca (CMS) se determinó dividiendo los valores de consumo bruto de energía por 18,45 MJ de energía metabolizable por kg de materia seca, que representa la densidad energética del alimento. Estas emisiones de gases de efecto invernadero abarcaron el metano (CH₄) procedente de la fermentación entérica y las excreciones dejadas en los potreros, el óxido nitroso (N₂O) procedente de las excreciones depositadas en los pastos y la aplicación de fertilizantes, y el dióxido de carbono (CO₂) procedente de la aplicación de cal y urea, así como de la quema de combustibles fósiles. Para calcular el impacto de las emisiones se utilizaron los índices de potencial de calentamiento global GWP100 y GWP*. Las huellas de carbono de la leche oscilaron entre 0,7 y 1,41 kgCO₂-eq kg FPCM-1, pero cuando se calcularon con el índice GWP*, disminuyeron casi un 35% en ambos escenarios.

Palabras clave: evaluación del ciclo de vida, leche, producción, búfala, basada en pastos.

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Bubaludicus: games and playfulness for the buffalo promotion

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ABSTRACT

Information on any issue is currently available with just one click, which determines the need to rethink how to teach. Creating an effective learning environment is not easy. Innovative methods attract more interest from people due to their needs. This paper describes the application of an innovative strategy (*Bubaludicus*) through the use of the games for promotion of knowledge about buffalo (*Bubalus bubalis*) breeding and production. The strategy is based on the belief of the existence of a relationship between game and learning. A game compatible with android phones was developed. In the game, the user needs to fit geometric pieces together to achieve the ultimate goal of completing the shape. At the end of play, information about *Bubalus bubalis* (species, production, products, importance, among others) is displayed on the screen. Puzzles and memory games (physical games) were also developed with the same objectives. 45 children and 32 adults were selected to evaluate the effectiveness of the strategy. It was observed that the challenge of the game resulted in memorization of information about the domestic buffalo. An important feature associated with the memory game was the competitor's familiarization with the "figure" of the buffalo, distinguishing it from *Bison bison* and *Cyncerus caffer*. This research highlighted the use of the games as a knowledge-diffusion strategy. It is possible to consider "*Bubaludicus*" as a powerful and playful tool for the *Bubalus bubalis* promotion.

Keywords: *Bubalus bubalis*, kids, playful.

Bubaludicus: juego y alegría para la promoción del búfalo

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RESUMEN

Actualmente la información sobre cualquier tema está disponible con solo un clic, lo que determina la necesidad de repensar la forma de enseñar. Crear un entorno de aprendizaje eficaz no es fácil. Los métodos innovadores atraen más interés de la gente por sus necesidades. El presente artículo describe la aplicación de una estrategia innovadora (*Bubaludicus*) mediante el uso de juegos para la promoción del conocimiento sobre la cría y producción del búfalo (*Bubalus bubalis*). La estrategia se basa en la creencia de la existencia de una relación entre juego y aprendizaje. Se desarrolló un juego compatible con teléfonos Android. En el juego, el usuario necesita unir piezas geométricas para lograr el objetivo final de completar la forma. Al finalizar el paso se muestra en pantalla información sobre *Bubalus bubalis* (especie, producción, productos, importancia, entre otros). También se desarrollaron rompecabezas y juegos de memoria (juegos físicos) con los mismos objetivos. Se seleccionaron 45 niños y 32 adultos para evaluar la efectividad de la estrategia. Se observó que el desafío del juego resultó en la memorización de información sobre el búfalo doméstico. Una característica importante asociada con el juego de memoria fue la familiarización del competidor con la "figura" del búfalo, distinguiéndolo del *Bison bison* y *Cyncerus caffer*. Esta investigación destacó el uso de los juegos como estrategia de difusión de conocimiento. Es posible considerar al "*Bubaludicus*" como una herramienta poderosa y lúdica para la promoción del *Bubalus bubalis*.

Palabras clave: *Bubalus bubalis*, niños, jugueteón.



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Effect of natural accelerated growth in the performance of buffalo calves in a dual-purpose production system

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ABSTRACT

The objective of this work was to evaluate the effect of implementing a system of naturally accelerated growth in crossbreed buffalo calves in a dual-purpose production system in Hato Los Olivos, Barinas, Venezuela. The crossbreed buffaloes in the trial descend mainly from Murrah, Mediterranean and Buffalypso. The dual-purpose system is based on selling weaning calves by using milk to raise the calves and help to cover daily expenses. A total of 79 female and 67 male buffalo calves born between November and January were randomly divided into two groups to apply different treatments. The first group (T1) went under the farm's traditional management system (the buffalo cow and the calf enter the milking parlour 15 days after calf birth, leaving a milk quarter for the calf to feed). The other calves underwent a system of management (T2) which we call "growth flare", that proposes natural accelerated growth in buffalo calves: the buffalo cow and the calf remain together without going to the milking parlour until the thoracic perimeter of the calf reaches 100 cm. From that moment, the buffalo calf goes to the milking parlour to stimulate the milk let-down reflex. Results showed that the percentage of mortality for T1 was 13%, whereas for T2, it was 2%. The weaning average weight in T1 was 134 Kg for females and 141 Kg for males. In T2, females reached 192 Kg and males 206 Kg. The heifer's first breeding was, on average, 36 months for T1 and 24 months for T2. The average age at slaughter was 46 months for T1 and 27 months for T2. The animals from T2 showed more than 60 Kg in average weaning weight gain compared with those of the traditional treatment (T1) and reduced mortality in the milking parlour of 2%. This impacts a heifer's first breeding and age at slaughter, optimizing the buffalo's dual-purpose breeding in the western plains of Venezuela. The system T2 required less use of medicines, which may help to

Efecto del crecimiento natural acelerado en el desempeño de terneros bufalinos en un sistema de producción de doble propósito

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RESUMEN

El objetivo de este trabajo fue evaluar el efecto de implementar un sistema de crecimiento naturalmente acelerado en terneros bufalinos mestizos en un sistema de producción de doble propósito en Hato Los Olivos, Barinas, Venezuela. Los búfalos cruzados del ensayo descienden principalmente de Murrah, Mediterranean y Buffalypso. El sistema de doble propósito se basa en la venta de terneros destetados utilizando leche para criarlos y ayudar a cubrir los gastos diarios. Un total de 79 crías de búfalo hembras y 67 machos nacidos entre noviembre y enero se dividieron aleatoriamente en dos grupos para aplicar diferentes tratamientos. El primer grupo (T1) estuvo bajo el sistema de manejo tradicional de la finca: la búfala y el ternero ingresan a la sala de ordeño 15 días después del nacimiento del ternero, dejando un cuarto de leche para la alimentación del ternero. Los demás terneros pasaron a un sistema de manejo (T2) que llamamos "llamarada de crecimiento", que tiene como objetivo el crecimiento acelerado natural de los búfalos: la búfala y el ternero permanecen juntos sin pasar por la sala de ordeño hasta que el perímetro torácico del ternero alcanza los 100 cm. A partir de ese momento el ternero pasa a la sala de ordeño para estimular el reflejo de bajada de la leche de la búfala. Los resultados mostraron que el porcentaje de mortalidad para T1 fue del 13%, mientras que para T2 fue del 2%. El peso promedio al destete en T1 fue de 134 Kg para las hembras y de 141 Kg para los machos. En el T2 las hembras alcanzaron los 192 Kg y los machos los 206 Kg. La primera monta de la novilla ocurrió, en promedio, a 36 meses para T1 y a 24 meses para T2. La edad promedio al sacrificio fue de 46 meses para el T1 y de 27 meses para el T2. Los animales del T2 presentaron más de 60 Kg de ganancia de peso promedio al destete respecto a los del tratamiento tradicional (T1) y una reducción de la mortalidad en sala de ordeño del 2%. Esto

reach a more sustainable development system. It is an unfinished trial that has been going on over the last four years, and we are still evaluating the results. However, accelerated growth in calves is an excellent alternative to reduce costs, resulting in a mortality decrease; the weaning weight gain compensates for the cost of the milk consumed by the calf in the first two months of lactation. Developing a buffalo weight measuring tape (weigh band) is also highly recommended to simplify weight measuring in the fieldwork.

Keywords: buffalo calves, accelerated natural growth, dual purpose production system.

impacta la primera crianza de las novillas y la edad al sacrificio, optimizando la crianza de doble propósito de la búfala en los llanos occidentales de Venezuela. El sistema T2 requirió un menor uso de medicamentos, lo que puede ayudar a alcanzar un sistema de desarrollo más sostenible. Es un ensayo inacabado que se ha estado realizando durante los últimos cuatro años y todavía estamos evaluando los resultados. Sin embargo, el crecimiento acelerado de los terneros es una excelente alternativa para reducir costos, traduciéndose en una disminución de la mortalidad, y el aumento de peso al destete compensa el costo de la leche consumida por el ternero en los dos primeros meses de lactancia. También se recomienda encarecidamente desarrollar una cinta métrica de peso para búfalos (banda de pesaje) para simplificar la medición del peso en el trabajo de campo.

Palabras clave: terneros bufalinos, crecimiento natural acelerado, sistema de producción de doble propósito.

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Natural reproduction in tropical buffalo herds

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ABSTRACT

Reproduction is one of the most important aspects of farm management that, combined with genetics, allows the productive system to develop and meet minimum productivity standards for sustainability and market demands. Despite the close phylogenetic relationship to cows, the buffalo has many differences in estrus expression, follicle diameter size at ovulation, and progesterone profiles (Bertoni et al., 2020). Seasonality is very marked in buffaloes. Knowledge of natural reproduction is the first step for the successful application of reproductive biotechnologies. This study analyzes the reproductive parameters during 2023 of two Colombian buffalo herds located in different regions: Uraba (7.7580555,-76.6552777) and Puerto Boyaca (5.9387942,-74.577977,1). Animals were maintained in low tropical rainforest with a temperature of 23–32°C, annual rainfall (2300 mm³ and 570 mm³), altitude (2 – 190 masl) for Uraba and Puerto Boyaca, kept in natural and improved pastures (*Brachiaria humidicola* or *B. decumbens*), with water and minerals *ad libitum*. Pregnancies were obtained by natural mating (1/55 male-female). A reproductive check-up was done by

Reproducción natural en manadas de búfalos tropicales

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RESUMEN

La reproducción es uno de los aspectos más importantes del manejo agrícola que, combinado con la genética, permite que el sistema productivo se desarrolle y cumpla con estándares mínimos de productividad para la sostenibilidad y las demandas del mercado. A pesar de la estrecha relación filogenética con las vacas, la búfala tiene muchas diferencias en la expresión del estro, el tamaño del diámetro del folículo en el momento de la ovulación y los perfiles de progesterona (Bertoni et al., 2020). La estacionalidad es muy marcada en los búfalos. El conocimiento de la reproducción natural es el primer paso para la aplicación exitosa de las biotecnologías reproductivas. Este estudio analiza los parámetros reproductivos durante el año 2023 de dos manadas de búfalos colombianos ubicados en diferentes regiones: Urabá (7.7580555,-76.6552777) y Puerto Boyacá (5.9387942,-74.577977,1). Los animales se mantuvieron en selva tropical baja con una temperatura de 23–32°C, precipitaciones anuales (2300 mm³ y 570 mm³), altitud (2 – 190 msnm) para Urabá y Puerto Boyacá, mantenidos en pastos

rectal palpation every three months. Data were managed using specialized software for herd management. Descriptive statistics of parameters are presented, and comparisons were performed using the Mann-Whitney test. Data from 624 and 254 were analyzed, average age 4.80 (2.8-14.0) and 8,98 years (3,5-19), birth rate 79.38% and 82.01%, inter calving period 435 and 452 days, calving-conception 133 and 126, age to first calving 40.1 and 37.0 for the herds located in Uraba and Puerto Boyaca, respectively; 91.1% and 87.6% of the dry buffaloes are pregnant. There are no statistical differences in the parameters within the herds. As expected, the reproductive parameters of the two buffalo herds are better than the average for cows of the same region. The large number of dry-pregnant females is evidence of the effect of seasonality since, at the time of writing this summary (June 2023), we are in an unfavorable reproductive season in Colombia. The two farms in the tropical region, 504 km away, showed comparable reproductive parameters. First calving is low compared to cows. Rapid onset of ovarian activity allows breeders to apply reproductive biotechnologies in early postpartum to improve reproductive outcomes.

Keywords: natural reproduction, water buffalo, Colombia, tropics.

Acknowledgements: Bufaleras el Delirio and San Felipe.

naturales y mejorados (*Brachiararia humidicola* o *B. decumbens*), con agua y minerales *ad libitum*, las preñeces se obtuvieron por apareamiento natural (1/55 macho-hembra). Se realizó un control reproductivo mediante palpación rectal cada tres meses. Los datos se gestionaron mediante software especializado para el manejo del rebaño. Se presentan estadísticas descriptivas de los parámetros y las comparaciones se realizaron mediante la prueba de Mann-Whitney. Se analizaron datos de 624 y 254, edad promedio 4,80 (2,8-14,0) y 8,98 años (3,5-19), tasa de natalidad 79,38% y 82,01%, período interparto 435 y 452 días, parto-concepción 133 y 126, edad al primer parto 40,1 y 37,0 para los rebaños ubicados en Urabá y Puerto Boyacá, respectivamente; el 91,1% y el 87,6% de las búfalas secas se encuentran preñadas. No existen diferencias estadísticas en los parámetros dentro de los rebaños. Como era de esperar, los parámetros reproductivos de los dos rebaños de búfalos son mejores que el promedio de las vacas de la misma región. Un gran número de hembras preñadas secas es evidencia del efecto de la estacionalidad ya que, al momento de escribir este resumen (junio de 2023), nos encontramos en una temporada reproductiva desfavorable en Colombia. Las dos granjas en la región tropical, a 504 km de distancia, mostraron parámetros reproductivos comparables. El primer parto es bajo en comparación con las vacas. El rápido inicio de la actividad ovárica permite a los criadores aplicar biotecnologías reproductivas en el posparto temprano para mejorar los resultados reproductivos.

Palabras clave: reproducción natural, búfalo de agua, Colombia, trópico.

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Effect of sex on the weight gain of calves (*Bubalus bubalis*) reared in silvopastoral systems during the dry season

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ABSTRACT

This study aimed to evaluate the effect of sex on the weight gain of calves raised in silvopastoral systems. Be-

Efecto del sexo en la ganancia de peso de terneros (*Bubalus bubalis*) criados en sistemas silvopastoriles durante la época seca

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RESUMEN

Este estudio tuvo como objetivo evaluar el efecto del sexo en la ganancia de peso de terneros criados en sistemas silvo-

tween 2019 and 2020, 30 calves divided into two groups according to sex (females and males) were evaluated. At birth, the calves were identified and weighed within the first hours after birth. Afterward, a weight measurement was performed monthly in the morning time until 18 months of age. Likewise, daily weight gains (kg) and final weight (kg) were calculated. From birth to weaning, the calves were kept in restricted suckling systems (after milking, they were held with the mother and fed with one-quarter of the udder and the residual milk). After weaning, the animals were abruptly separated and placed in 15 38-hectare paddocks with silvopastoral systems and rotational grazing in pastures with native and introduced grasses and natural shade. The data was analyzed by comparing means using Tukey's test with a significance level of $p < 0.05$. No effects of sex were observed on birth weight (37.66 ± 0.24 and 37.46 ± 0.24 ; $p = 0.5673$), weaning weight (161.93 ± 37.46 and 163.60 ± 33.75 ; $p = 0.8991$) and final weight (380.93 ± 12.03 and 381.66 ± 12.03 ; $p = 0.9659$) between females and males, respectively. Weight gains from birth to 6 months of age, from 6 months to 12 months, and from 12 months to 18 months were not significant between females and males (0.69 ± 0.05 and 0.69 ± 0.04 ; $p = 0.9653$, 0.64 ± 0.03 and 0.62 ± 0.02 ; $p = 0.06229$, 0.55 ± 0.03 and 0.59 ± 0.03 ; $p = 0.06229$). Average daily gains between females and males (0.60 ± 0.02 kg/day and 0.63 ± 0.05 , respectively) were not significant because of sex ($p = 0.9653$). It is concluded that females and males reared in restricted suckling systems and later, in silvopastoral systems, show good productive performance under dry season conditions. It is necessary to carry out more studies in which physiological, hormonal, and behavioural variables are related to improve the understanding of the productive performance of water buffalo in silvopastoral systems.

Keywords: daily weight gain, silvopastoral system, water buffalo.

pastoriles. Entre 2019 y 2020 se evaluaron 30 terneros divididos en dos grupos según sexo (hembras y machos). Al nacer, las crías fueron identificadas y pesadas dentro de las primeras horas de vida. Posteriormente se realizó una medición de peso mensualmente en horario de la mañana hasta los 18 meses de edad. Asimismo, se calcularon las ganancias de peso diarias (kg) y el peso final (kg). Desde el nacimiento hasta el destete, los terneros se mantuvieron en sistemas de lactancia restringidos (después del ordeño, se los mantuvo con la madre y se los alimentó con una cuarta parte de la ubre y la leche residual). Luego del destete, los animales fueron separados abruptamente y ubicados en 15 potreros de 38 hectáreas con sistemas silvopastoriles y pastoreo rotacional en potreros con pastos nativos e introducidos y sombra natural. Los datos se analizaron comparando medias mediante la prueba de Tukey con un nivel de significancia de $p < 0,05$. No se observaron efectos del sexo sobre el peso al nacer ($37,66 \pm 0,24$ y $37,46 \pm 0,24$; $p = 0,5673$), peso al destete ($161,93 \pm 37,46$ y $163,60 \pm 33,75$; $p = 0,8991$) y peso final ($380,93 \pm 12,03$ y $381,66 \pm 12,03$; $p = 0,9659$) entre mujeres y hombres, respectivamente. Las ganancias de peso desde el nacimiento hasta los 6 meses de edad, desde los 6 meses hasta los 12 meses y desde los 12 meses hasta los 18 meses no fueron significativas entre mujeres y hombres ($0,69 \pm 0,05$ y $0,69 \pm 0,04$; $p = 0,9653$, $0,64 \pm 0,03$ y $0,62 \pm 0,02$; $p = 0,06229$, $0,55 \pm 0,03$ y $0,59 \pm 0,03$; $p = 0,06229$). Las ganancias medias diarias entre mujeres y hombres ($0,60 \pm 0,02$ kg/día y $0,63 \pm 0,05$, respectivamente) no fueron significativas por el efecto del sexo ($p = 0,9653$). Se concluye que hembras y machos criados en sistemas de lactancia restringidos y posteriormente en sistemas silvopastoriles muestran buen comportamiento productivo en condiciones de estación seca. Es necesario realizar más estudios en los que se relacionen variables fisiológicas, hormonales y de comportamiento para mejorar el conocimiento del desempeño productivo del búfalo de agua en sistemas silvopastoriles.

Palabras clave: ganancia diaria de peso, sistema silvopastoril, búfalo de agua.

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Identification of most motivating resource for estimation of social hierarchy in water buffaloes

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ABSTRACT

Buffaloes are social animals and live in herds in natural settings. Under husbandry conditions, they are loosely housed

Identificación del recurso más motivador para la estimación de la jerarquía social en búfalos de agua

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RESUMEN

Los búfalos son animales sociales y viven en manadas en entornos naturales. En condiciones de cría, se les aloja en

in groups to allow them to express social behavior. The construction of social hierarchy and knowledge of its determinants is warranted for determining the optimum group size and formulating strategies for shifting animals from one group to another. Social hierarchy is typically estimated by recording agonistic interactions among all animals for possession of limited resources. This study aimed to find the most limiting resource for estimating social hierarchy in riverine buffaloes. The study was conducted on 63 loose-housed (covered area=250m²; open area=500m²) with lactating Murrah buffaloes (BCS=3.25±0.16; 1-6 parity) maintained at ICAR-National Dairy Research Institute, Karnal, Haryana, India. Three limited resources considered for observing agonistic interactions were: i) seasonal green fodders, ii) concentrate mixture, and iii) possession of space under the foggers during the summer season (11:00, 07:00, and 14:00 hours, respectively). The manger length was restricted to allow 10% (length x width: 4.0 x 0.6 m) of animals to eat simultaneously, and 10% of foggers (3.7 m² space for two foggers) were operated to invite maximum aggression. Each restriction was carried out once daily in the same group. Access to resources was allowed from 10 m to ensure equal opportunity for possession. Agonistic interactions for each resource were recorded manually and by Closed Circuit Television recordings five times weekly by continuous sampling for 1 hour. A socio-metric matrix of all agonistic interactions was prepared, and pooled data was analyzed using one-way ANOVA in SPSS version 22. Participation of buffaloes for possession was higher ($p<0.01$) for the concentrate mixture (40.32±0.86%) compared to green fodders (25.08±0.66%) and foggers (12.38±1.80%). Several agonistic interactions were statically significant ($p<0.01$) for possession of concentrate (25.4±0.54), followed by green fodders (15.80±0.42) and foggers (7.80±1.13). The number of physical (fighting, bunting, pushing) and non-physical (threatening, avoiding) interactions were higher ($p<0.01$) for concentrate (439.4±13.49, 368.8±12.16) than green fodder (126.6±9.49, 147.8±90) and foggers (36.90±7.22, 83.5±6.61). We concluded that concentrate restriction motivates the expression of agonistic interactions for resource possession in socially stable lactating buffaloes.

Keywords: agonistic interactions, buffaloes, hierarchy, limited resource.

grupos para permitirles expresar un comportamiento social. La construcción de una jerarquía social y el conocimiento de sus determinantes está justificada para determinar el tamaño óptimo del grupo y formular estrategias para cambiar los animales de un grupo a otro. La jerarquía social generalmente se estima registrando interacciones agonísticas entre todos los animales por la posesión de recursos limitados. Este estudio tuvo como objetivo encontrar el recurso más limitante para estimar la jerarquía social en búfalos de río. El estudio se llevó a cabo en 63 alojamientos sueltos (área cubierta = 250 m²; área abierta = 500 m²) con búfalos Murrah lactantes (BCS = 3,25 ± 0,16; paridad 1-6) mantenidas en el Instituto Nacional de Investigación Láctea ICAR, Karnal, Haryana, India. Tres recursos limitados considerados para observar interacciones agonísticas fueron: i) forrajes verdes de la estación, ii) mezcla de alimento concentrado y iii) posesión de espacio debajo de los nebulizadores durante la temporada de verano (11:00, 07:00 y 14:00 horas, respectivamente). La longitud del establo se restringió para permitir que el 10% (largo x ancho: 4,0 x 0,6 m) de los animales comieran simultáneamente, y el 10% de los nebulizadores (3,7 m² de espacio para dos nebulizadores) se operaron para invitar a la máxima agresión. Cada restricción se llevó a cabo una vez al día en el mismo grupo. Se permitió el acceso a los recursos desde 10 m para garantizar la igualdad de oportunidades de posesión. Las interacciones agonísticas para cada recurso se registraron manualmente y mediante grabaciones de circuito cerrado de televisión cinco veces por semana mediante muestreo continuo durante 1 hora. Se preparó una matriz sociométrica de todas las interacciones agonísticas y los datos agrupados se analizaron mediante ANOVA unidireccional en SPSS versión 22. La participación de los búfalos en la posesión fue mayor ($p<0,01$) para la mezcla concentrada (40,32±0,86%) que para forrajes verdes (25,08±0,66%) y nebulizadores (12,38±1,80%). Varias interacciones agonísticas fueron significativas ($p<0.01$) para posesión de concentrado (25.4±0.54), seguido de forrajes verdes (15.80±0.42) y nebulizadores (7.80±1.13). El número de interacciones físicas (pelear, tocar, empujar) y no físicas (amenazar, evitar) fue mayor ($p<0,01$) para el concentrado (439,4±13,49, 368,8±12,16) que para el forraje verde (126,6±9,49, 147,8±90) y nebulizadores (36,90±7,22, 83,5±6,61). Concluimos que la restricción de concentrado motiva la expresión de interacciones agonísticas por la posesión de recursos en búfalos lactantes socialmente estables.

Palabras clave: interacciones agonísticas, búfalos, jerarquía, recursos limitados.

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Effect of the season on blood changes of oxidative stress index (OSi) in the Italian Mediterranean Buffalo (*Bubalis bubalis*)

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ABSTRACT

The oxidative stress index (OSi) has recently been used as a valid tool for providing an in-depth picture of redox status and oxidative stress. Previous studies in cattle showed that high temperatures increase the production of reactive oxygen species (ROS), causing an imbalance between ROS and the ability of antioxidant systems to detoxify and remove the reactive intermediates. As such studies remain limited in buffalo, the effect of temperature on oxidative stress was investigated through the OSi by combining the results of derivatives of reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) in an Italian buffalo herd. Blood samples were collected from 40 buffaloes over 12 time points distributed in two years (2021, 2022). Samples were taken during hot and cold/mild seasons monthly. Plasma free oxygen radicals were determined using a d-ROMs test (Diacron, Italy) modified for a microplate procedure, and results were expressed in arbitrary Carratelli Units (U CARR). Plasma antioxidants were determined using the BAP test (Diacron) in a dedicated spectrophotometer (Carpe Diem Free, Diacron). The OSi parameter was calculated as $d-ROMs/BAP \times 100$. Temperature and humidity were recorded daily during the trial to calculate the Temperature Humidity Index (THI). The formula used was $THI = (1.8 \times T - ((1 - Ur/100) \times (T - 14.3)) + 32)$, with T=temperature (°C) and Ur=relative humidity (%). The model included year and season and their interaction for statistical analysis. Results of this study showed for the first time the effect of the season on the oxidative stress in buffalo. The minimum and maximum THI values for the cold and hot seasons recorded during the experimental period were 29.6-74.5 and 54-85 in 2021 and 31.6-66.3 and 47.9-86.2 in 2022, respectively. Levels of d-ROMs and BAP were affected by season (133.0 ± 3.67 vs 145.1 ± 3.54 UCARR, $p=0.0189$, and 2489.19 ± 32.51 vs 2392.43 ± 31.33 mmol/L, $p=0.033$, in hot and cold/mild season, respectively). Significant year*season interaction was found for d-ROMs and BAP ($p=0.06$ and $p<0.0001$, respectively). Moreover, OSi was affected by season, showing a growing trend from hot to cold season (5.35 ± 0.15 vs 6.17 ± 0.14 , $p<0.0001$), but, interestingly,

Efecto de la estación sobre los cambios sanguíneos del índice de estrés oxidativo (OSi) en el búfalo mediterráneo italiano (*Bubalis bubalis*)

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RESUMEN

El índice de estrés oxidativo (OSi) se ha utilizado recientemente como una herramienta válida para proporcionar una imagen detallada del estado redox y del estrés oxidativo. Estudios anteriores en ganado demostraron que las altas temperaturas aumentan la producción de especies reactivas de oxígeno (ROS), provocando un desequilibrio entre las ROS y la capacidad de los sistemas antioxidantes para desintoxicar y eliminar los intermediarios reactivos. Como estos estudios siguen siendo limitados en búfalos, se investigó el efecto de la temperatura sobre el estrés oxidativo a través del OSi combinando los resultados de los derivados de los metabolitos reactivos del oxígeno (d-ROM) y el potencial biológico antioxidante (BAP) en una manada de búfalos italianos. Se recolectaron muestras de sangre de 40 búfalos en 12 momentos distribuidos en dos años (2021, 2022). Las muestras se tomaron mensualmente durante las estaciones cálida y fría/templada. Los radicales libres de oxígeno en plasma se determinaron mediante una prueba de d-ROM (Diacron, Italia) modificada para un procedimiento de microplaca, y los resultados se expresaron en Unidades Carratelli arbitrarias (U CARR). Los antioxidantes plasmáticos se determinaron mediante la prueba BAP (Diacron) en un espectrofotómetro dedicado (Carpe Diem Free, Diacron). El parámetro OSi se calculó como $d-ROM/BAP \times 100$. La temperatura y la humedad se registraron diariamente durante la prueba para calcular el índice de temperatura y humedad (THI). La fórmula utilizada fue $THI = (1.8 \times T - ((1 - Ur/100) \times (T - 14.3)) + 32)$, siendo T=temperatura (°C) y Ur=humedad relativa (%). El modelo incluyó año y estación y su interacción para el análisis estadístico. Los resultados de este estudio mostraron por primera vez el efecto de la estación sobre el estrés oxidativo en búfalos. Los valores mínimo y máximo de THI para las estaciones fría y cálida registrados durante el período experimental fueron 29,6-74,5 y 54-85 en 2021 y 31,6-66,3 y 47,9-86,2 en 2022, respectivamente. Los niveles de d-ROM y BAP se vieron afectados por la estación ($133,0 \pm 3,67$ vs $145,1 \pm 3,54$ UCARR, $p=0,0189$, y $2489,19 \pm 32,51$ vs $2392,43 \pm 31,33$ mmol/L, $p=0,033$, en temporada cálida y fría/templada, respectivamente). Se encontró

it was unaffected by annual variation. In conclusion, this field study showed that OSi could be considered a better and independent marker of oxidative status in buffalo concerning the evaluation of single determinations of d-ROMs and BAP.

Keywords: d-ROMs, BAP, OSi, buffalo, heat stress, oxidative stress.

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una interacción significativa año*estación para d-ROM y BAP ($p=0,06$ y $p<0,0001$, respectivamente). Además, OSi se vio afectado por la estación, mostrando una tendencia creciente de la estación cálida a la fría (5.35 ± 0.15 vs 6.17 ± 0.14 , $p<0.0001$), pero, curiosamente, no se vio afectado por la variación anual. En conclusión, este estudio de campo demostró que OSi podría considerarse un marcador mejor e independiente del estado oxidativo en búfalos con respecto a la evaluación de determinaciones únicas de d-ROM y BAP.

Palabras clave: d-ROM, BAP, OSi, búfalo, estrés por calor, estrés oxidativo.

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Work performance, physiological and hemato-biochemical responses in working buffalo with different load weights

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ABSTRACT

The objective of the present study was to evaluate the effect of the weight of different loads on work performance and

Desempeño, respuestas fisiológicas y hematobioquímicas en búfalos de trabajo con diferentes pesos de carga

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RESUMEN

El objetivo del presente estudio fue evaluar el efecto del peso de diferentes cargas sobre el desempeño laboral y las

physiological, biochemical, and hematological responses in working buffaloes. The study was carried out in the province of Esmeraldas, Cantón Quinindé, Ecuador. During the study, the area maintained an average temperature of 24.4 °C and a humidity of 91.7%. Twelve male working buffaloes 7 to 10 years old (782.16±21.62 kg) were evaluated while transporting African oil palm fruits in metal baskets placed on the back. The weights of the loads were 200, 300, and 500 kg. The work was carried out from 7:00 to 15:00 h. Buffaloes had five years of previous experience performing the same activities in the production unit. The animals always had access to water and natural shade from the African palm. Different variables related to work performance, physiological indicators, and biochemical and hematological responses were recorded 30 minutes before the start of work, immediately after work, and in the morning on the rest day, except for work performance variables, since it was measured immediately after work. All variables were compared with a mixed model for repeated measures, considering the factors time and load weight and Time x Load weight interactions as fixed effects, with the animal in each group as a random effect. The Tukey test with a probability level set at $p < 0.05$ was used to make multiple comparisons. Results indicated that buffaloes that transported loads of 500, followed by 350 and 200 kg moved slower (3.40, 3.45, 3.52 km/h, $p = 0.0164$), worked less time (2.39, 2.55, 2.79 h; $p < 0.0001$), traveled less distance (7.29, 8.71, 9.75 km, $p < 0.0001$) and made fewer trips (8.27, 10.94, 16.61, $p < 0.0001$), respectively. However, the total weight of the load was greater (4,138.88, 3,830.55, 3,322.22 kg, $p = 0.0281$), and they were stopped longer (5.59, 5.44, 5.20 h, $p < 0.0001$), respectively. Physiological (respiratory rate, pulse, heart rate, and rectal temperature) and biochemical (glucose, lactate dehydrogenase, creatine kinase, and total protein) parameters, as well as blood cell count (white blood cell, neutrophils, lymphocytes, monocytes, eosinophils, and basophils), increased after work regardless of weight load ($p < 0.001$). In contrast, red blood cell counts, hemoglobin, and hematocrit were decreased after work ($p < 0.001$). Likewise, these changes were more pronounced when the load was 500 kg compared to 200 kg ($p < 0.05$). However, all changes remained within the physiological ranges reported for the buffaloes. Therefore, it is concluded that heavier loads increase work efficiency but generate greater physiological and metabolic demand. Further studies are needed to understand the long-term effects on the welfare of working buffaloes destined for rural activities.

Keywords: working buffalo, physiological response, animal welfare.

respuestas fisiológicas, bioquímicas y hematológicas en búfalos de trabajo. El estudio se realizó en la provincia de Esmeraldas, Cantón Quinindé, Ecuador. Durante el estudio, la zona mantuvo una temperatura promedio de 24,4 °C y una humedad de 91,7%. Se evaluaron doce búfalos de trabajo machos de 7 a 10 años (782,16±21,62 kg) mientras transportaban frutos de palma africana en cestas metálicas colocadas en la espalda. Los pesos de las cargas fueron 200, 300 y 500 kg. Los trabajos se realizaron de 7:00 a 15:00 h. los búfalos tenían cinco años de experiencia previa desempeñando las mismas actividades en la unidad de producción. Los animales siempre tuvieron acceso al agua y a la sombra natural de la palma africana. Se registraron diferentes variables relacionadas con el desempeño laboral, indicadores fisiológicos y respuestas bioquímicas y hematológicas 30 minutos antes del inicio del trabajo, inmediatamente después del trabajo y en la mañana del día de descanso, excepto las variables de desempeño laboral, ya que se midieron inmediatamente después de trabajar. Todas las variables se compararon con un modelo mixto para medidas repetidas, considerando los factores tiempo y peso de carga y las interacciones Tiempo x Peso de carga como efectos fijos, siendo el animal de cada grupo como efecto aleatorio. Para realizar comparaciones múltiples se utilizó la prueba de Tukey con un nivel de probabilidad establecido en $p < 0,05$. Los resultados indicaron que los búfalos que transportaron cargas de 500, seguidos de 350 y 200 kg se movieron más lento (3.40, 3.45, 3.52 km/h, $p = 0.0164$), trabajaron menos tiempo (2.39, 2.55, 2.79 h; $p < 0.0001$), viajaron menos distancia (7.29, 8.71, 9.75 km, $p < 0,0001$) y realizaron menos viajes (8,27, 10,94, 16,61, $p < 0,0001$), respectivamente. Sin embargo, el peso total de la carga fue mayor (4.138,88, 3.830,55, 3.322,22 kg, $p = 0,0281$), estuvieron detenidos por más tiempo (5,59, 5,44, 5,20 h, $p < 0,0001$), respectivamente. Parámetros fisiológicos (frecuencia respiratoria, pulso, frecuencia cardíaca y temperatura rectal) y bioquímicos (glucosa, lactato deshidrogenasa, creatina quinasa y proteínas totales), así como el recuento de células sanguíneas (glóbulos blancos, neutrófilos, linfocitos, monocitos, eosinófilos, y basófilos), aumentó después del trabajo independientemente de la carga de peso ($p < 0,001$). En contraste, los recuentos de glóbulos rojos, hemoglobina y hematocrito disminuyeron después del trabajo ($p < 0,001$). Asimismo, estos cambios fueron más pronunciados cuando la carga fue de 500 kg en comparación con 200 kg ($p < 0,05$). Sin embargo, todos los cambios se mantuvieron dentro de los rangos fisiológicos informados para los búfalos. Por lo tanto, se concluye que cargas más pesadas aumentan la eficiencia del trabajo, pero generan mayor demanda fisiológica y metabólica. Se necesitan más estudios para comprender los efectos a largo plazo sobre el bienestar de los búfalos de trabajo destinados a actividades rurales.

Palabras clave: búfalo de trabajo, respuesta fisiológica, bienestar animal.

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Animal welfare and biosecurity correlation in buffalo farm evaluated by ClassyFarm system

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ABSTRACT

ClassyFarm is an integrated system for categorizing farms according to the risk assessment (RA) methodology. It is an Italian innovation aimed at improving the synergy between breeders and competent authorities to improve the safety and quality of food of animal origin. ClassyFarm gathers and processes data referred to in the following areas: biosecurity, animal welfare, health, and antimicrobial usage. It can be applied to livestock species, including water buffaloes. Upon request of the Italian Ministry of Health (IMH), the National Reference Centre on Water Buffalo Farming and Production Hygiene and Technologies (CReNbuf), in collaboration with the Italian Reference Centre for Animal Welfare (CReNBA), developed a RA-based checklist (CL) for the on-farm assessment of buffalo welfare and farm biosecurity level, included in the ClassyFarm system. The multiple-choice CL consists of 79 items. Each item is scored according to 3 categories: “insufficient”, “acceptable” and “excellent”. The assessment system for animal welfare includes non-animal-based (N-ABMs) and animal-based measures (ABMs). N-ABMs are divided into two macro-areas: Area A (32 items): “Management factors” and Area B (31 items): “Housing factors”. ABMs are assessed in Area C (17 items). Biosecurity was assessed using 15 indicators. The CL has been tested in 102 farms, with an average size of 412 heads. On average, the overall welfare value was 61.55% (on a scale from 0 to 100%), and the average biosecurity score was 43.31%. The statistical analysis was performed by Spearman Rank correlation coefficient using GraphPad Prism 8.0.1. (GraphPad Software, San Diego, CA, USA). The two variables were positively correlated (Spearman's $Rho=0.501$; $p<0.001$). The average welfare values of the specific areas were: A, 61.76%; B, 42.17%; C, 70.43%. At least one potential legislative non-compliance was recorded in 39.80% of the farms. The highest correlations between the different areas of well-being and biose-

Correlación entre bienestar animal y bioseguridad en granja de búfalos evaluada por el sistema ClassyFarm

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RESUMEN

ClassyFarm es un sistema integrado para categorizar granjas según la metodología de evaluación de riesgos (RA). Se trata de una innovación italiana destinada a mejorar la sinergia entre los criadores y las autoridades competentes para mejorar la seguridad y la calidad de los alimentos de origen animal. ClassyFarm recopila y procesa datos referidos en las siguientes áreas: bioseguridad, bienestar animal, salud y uso de antimicrobianos. Puede aplicarse a especies ganaderas, incluidos los búfalos de agua. A petición del Ministerio de Salud italiano (IMH), el Centro Nacional de Referencia sobre Higiene y Tecnologías en la Agricultura y la Producción de Búfalos de Agua (CReNbuf), en colaboración con el Centro de Referencia Italiano para el Bienestar Animal (CReNBA), desarrolló una lista de verificación basada en RA (CL) para la evaluación en granja del bienestar de los búfalos y el nivel de bioseguridad de la granja, incluido en el sistema ClassyFarm. El CL de opción múltiple consta de 79 ítems. Cada ítem se califica según 3 categorías: “insuficiente”, “aceptable” y “excelente”. El sistema de evaluación del bienestar animal incluye medidas no basadas en animales (N-ABM) y medidas basadas en animales (ABM). Las N-ABM se dividen en dos macroáreas: Área A (32 ítems): “Factores de gestión” y Área B (31 ítems): “Factores de vivienda”. Los ABM se evalúan en el Área C (17 ítems). La bioseguridad se evaluó utilizando 15 indicadores. El CL ha sido probado en 102 explotaciones, con un tamaño medio de 412 cabezas. En promedio, el valor de bienestar general fue de 61,55% (en una escala de 0 a 100%) y el puntaje promedio de bioseguridad fue de 43,31%. El análisis estadístico se realizó mediante el coeficiente de correlación de Spearman Rank utilizando GraphPad Prism 8.0.1. (GraphPad Software, San Diego, CA, EE. UU.). Las dos variables se correlacionaron positivamente (Rho de Spearman= $0,501$; $p<0,001$). Los valores medios de bien-

curity are found for the management areas, highlighting how management is essential to ensure both the levels of well-being and those of biosecurity. These CLs represent a functional and effective tool to assign animal welfare and biosecurity indexes to farms, improving farm management and housing conditions, giving answers to consumers, and adding value to farmers' good practices. The IMH is promoting the application of this system at European and international levels.

Keywords: animal welfare, biosecurity, buffalo, Classyfarm.

estar de las zonas específicas fueron: A, 61,76%; B, 42,17%; C, 70,43%. En el 39,80% de las explotaciones se registró al menos un posible incumplimiento legislativo. Las correlaciones más altas entre las diferentes áreas de bienestar y bioseguridad se encuentran para las áreas de gestión, destacando cómo la gestión es fundamental para garantizar tanto los niveles de bienestar como los de bioseguridad. Estas CL representan una herramienta funcional y efectiva para asignar índices de bienestar animal y bioseguridad a las granjas, mejorar la gestión de las granjas y las condiciones de alojamiento, dar respuestas a los consumidores y agregar valor a las buenas prácticas de los agricultores. El IMH está impulsando la aplicación de este sistema a nivel europeo e internacional.

Palabras clave: bienestar animal, bioseguridad, búfalo, ClassyFarm.

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Monitoring of environmental gases and calf health in Italian Mediterranean buffalo herd

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ABSTRACT

In buffalo breeding, calves require special attention and housing systems that provide thermal comfort, good air quality, brightness, and adequate space. Overcrowding, poor ventilation, high temperatures, or improper management of animals can result in increased concentrations of environmental gases such as ammonia (NH₃), carbon dioxide (CO₂), and hydrogen sulfide (H₂S), altered microclimate parameters, and worsened air quality. The increase in these gases can be problematic for the environment and the calves, whose stress levels can increase and adversely affect the buffalo calves' nutrition, health, and physiological parameters. During the late spring and early summer season, gas monitoring was carried out in 7 buffalo calf housing spaces in the Campania region, and special measuring instruments were used to measure CO₂ (Portable indoor air quality CO₂ meter, Extech instruments), H₂S and NH₃ (Pro gas badge, Industrial scientific) produced by calves. The recording of perceived data was performed continuously and over 15 minutes. Measurements were taken early in the morning before the morning milk feed, and the instruments were positioned at the height of the calves. The calves analysed are all housed in completely closed structures equipped with doors and windows, usually left open in the summer, and closed in the winter. Furthermore, the age range of the animals housed ranges from 0 to 90 days of life, during which the calf mainly

Seguimiento de los gases ambientales y la salud de los terneros en la manada de búfalos del Mediterráneo italiano

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RESUMEN

En la cría de búfalos, los terneros requieren atención especial y sistemas de alojamiento que les proporcionen confort térmico, buena calidad del aire, luminosidad y espacio adecuado. El hacinamiento, la mala ventilación, las altas temperaturas o el manejo inadecuado de los animales pueden resultar en mayores concentraciones de gases ambientales como amoníaco (NH₃), dióxido de carbono (CO₂) y sulfuro de hidrógeno (H₂S), parámetros microclimáticos alterados y empeoramiento de la calidad del aire. El aumento de estos gases puede ser problemático para el medio ambiente y para los terneros, cuyos niveles de estrés pueden aumentar y afectar negativamente a la nutrición, la salud y los parámetros fisiológicos del búfalo. A finales de la primavera y principios del verano, se llevó a cabo un seguimiento de los gases en 7 alojamientos para terneros de búfalo en la región de Campania y se utilizaron instrumentos de medición especiales para medir el CO₂ (medidor portátil de CO₂ para la calidad del aire interior, Industrial scientific), H₂S y NH₃ (Insignia de gas profesional, Industrial scientific) producida por terneros. El registro de los datos percibidos se realizó de forma continua y durante 15 minutos. Las mediciones se tomaron temprano en la mañana, antes de la toma de leche de la mañana, y los instrumentos se colocaron a la altura de los terneros. Todos los terneros analizados estuvieron alojados en

takes on reconstituted milk and then is sent towards weaning in the last few weeks. The m² available per calf varies on each farm, and all the calves are housed individually. On average, the available space is approximately 100 cm x 1.40 cm. In all structures, the litter used is straw, mandatory for the first two weeks of the calf's life. In addition, the "Health score" of 300 calves was also evaluated. Score 0 indicated no cough; normal serous discharge or a small amount of unilateral turbid discharge; normal eyes or a small amount of ocular discharge; normal ear, flick ear, or head shake; normal or semi-formed feces; forelimbs entirely covered by bedding when calf lying down. Score 1 indicates coughs on movement; bilateral, excessive, or mucopurulent mucus discharge; moderate or heavy ocular discharge; head tilt or unilateral/bilateral droop; loose or watery feces sifts through the bedding; partially visible or visible forelimbs when the calf is lying down. Each parameter has its score and is considered independent of the other. Finally, as regards the state of cleanliness of the litter, rather than in the calf score, the state of cleanliness of the litters was considered in the data collection regarding the management of the calf house, expressing a judgment such as: "The litter is: kept in good condition; fairly well kept; it is not kept in good condition". The data collected show that in all calf housing spaces, the estimated NH₃ concentrations were in the range of 0 ppm. Only in one particularly overcrowded, the maximum concentration was 1 ppm. For CO₂ levels, the values recorded also never exceeded 1,000 ppm, ranging from a minimum of 678 ppm to a maximum of 946 ppm, and the H₂S values were 0 ppm in each one. In addition, all buffalo calves evaluated with the calf health score had a score of 0, indicating optimal health conditions. The monitored seven buffalo farms showed adequate attention to environmental conditions, and all the raised buffalo calves showed optimal health status.

Keywords: microclimatic parameters, Italian Mediterranean buffalo, buffalo calves, health, air quality, environment.

estructuras completamente cerradas equipadas con puertas y ventanas, generalmente abiertas en verano y cerradas en invierno. Además, el rango de edad de los animales alojados osciló entre los 0 y los 90 días de vida, durante los cuales el ternero tomó principalmente leche reconstituida y luego pasó al destete en las últimas semanas. Los m² disponibles por ternero variaron en cada explotación, y todos los terneros se alojan individualmente. De media, el espacio disponible fue de aproximadamente 100 cm x 1,40 cm. En todas las estructuras, el lecho utilizado es paja, obligatoria durante las dos primeras semanas de vida del ternero. Además, también se evaluó la "puntuación de salud" de 300 terneros. La puntuación 0 indicó que no había tos; secreción serosa normal o una pequeña cantidad de secreción turbia unilateral; ojos normales o una pequeña cantidad de secreción ocular; oído normal, movimiento de oído o movimiento de cabeza; heces normales o semiformadas; extremidades anteriores completamente cubiertas por la paja cuando el ternero está acostada. La puntuación 1 indica tos al moverse; secreción mucosa bilateral, excesiva o mucopurulenta; secreción ocular moderada o abundante; inclinación de la cabeza o caída unilateral/bilateral; las heces sueltas o acuosas se filtran a través de la ropa de cama; extremidades anteriores parcialmente visibles o visibles cuando el ternero está acostado. Cada parámetro tiene su puntuación y se considera independiente del otro. Finalmente, en cuanto al estado de limpieza de la camada, más que en la puntuación de los terneros, se consideró el estado de limpieza de las camadas en la recogida de datos sobre el manejo del galpón de terneros, expresando un juicio como: "La camada está: conservado en buen estado; bastante bien conservado; no se conserva en buen estado". Los datos recopilados muestran que, en todos los espacios de alojamiento de terneros, las concentraciones estimadas de NH₃ estuvieron en el rango de 0 ppm. Sólo en uno especialmente superpoblado la concentración máxima fue de 1 ppm. Para los niveles de CO₂, los valores registrados tampoco superaron nunca los 1.000 ppm, oscilando desde un mínimo de 678 ppm hasta un máximo de 946 ppm, y los valores de H₂S fueron de 0 ppm en cada uno. Además, todos los terneros de búfalo evaluados con el puntaje de salud del ternero tuvieron un puntaje de 0, lo que indica condiciones de salud óptimas. Las siete granjas de búfalos monitoreadas mostraron una atención adecuada a las condiciones ambientales y todas las crías de búfalo criadas mostraron un estado de salud óptimo.

Palabras clave: parámetros microclimáticos, búfalo Mediterráneo italiano, búfalos terneros, salud, calidad del aire, medio ambiente.

FEED & NUTRITION

Alimentación y Nutrición

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Dietary supplementation of composite feed additive reduces enteric methane production and enhances feed efficiency, milk production and immunity in Murrah buffalo (*Bubalus bubalis*)

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ABSTRACT

Buffalo plays a fundamental role in the livelihood of people of Asian countries by providing milk, meat, draught power, and dung. Asia is home to more than 90% of the world's buffalo population, out of which South Asian countries harbor about 78% of buffaloes. India ranks first in the world's total milk production, where about 50% of milk is contributed by 108.9 million total buffaloes, 57% of the global buffalo population. At the same time, buffaloes are one of the country's most significant contributors to greenhouse gases. Therefore, the objective of the present study was to develop a rumen-balanced composite feed additive (RESMI) for reducing enteric methane production and improving health and production performances in buffaloes. The present research involved a series of *in vitro*, *in sacco*, and *in vivo* experiments in growing (n=24) and milking buffaloes (n=18). The feed supplement was developed at the institute, composed of an ideal combination of methane inhibitors, alternate hydrogen sinks, and some rumen-stimulating agents (Indian Patent Grant No. 388717 dated 08.02.2022) and its efficacy examined through a series of *in vitro* experiments. A completely randomized design was used to analyze the experimental data. Supplementation of RESMI (@ 2.5% of feed intake.) was found to reduce methane emission ($p<0.05$) by reducing the number of methanogenic archaea in the rumen

La suplementación dietética con aditivos alimentarios compuestos reduce la producción de metano entérico y mejora la eficiencia alimenticia, la producción de leche y la inmunidad en la búfala Murrah (*Bubalus bubalis*)

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RESUMEN

El búfalo desempeña un papel fundamental en el sustento de la población de los países asiáticos al proporcionar leche, carne, fuerza de tiro y estiércol. Asia alberga a más del 90% de la población mundial de búfalos, de los cuales los países del sur de Asia albergan alrededor del 78% de los búfalos. India ocupa el primer lugar en la producción total de leche del mundo, donde alrededor del 50% de la leche proviene de 108,9 millones de búfalos en total, el 57% de la población mundial de búfalos. Al mismo tiempo, los búfalos son uno de los contribuyentes más importantes a los gases de efecto invernadero del país. Por lo tanto, el objetivo del presente estudio fue desarrollar un aditivo alimentario compuesto equilibrado en el rumen (RESMI) para reducir la producción de metano entérico y mejorar la salud y el rendimiento productivo en búfalos. La presente investigación involucró una serie de experimentos *in vitro*, *in sacco* e *in vivo* en búfalas en crecimiento (n=24) y búfalas en ordeño (n=18). El complemento alimenticio se desarrolló en el instituto y está compuesto por una combinación ideal de inhibidores de metano, sumideros de hidrógeno alternativos y algunos agentes estimulantes del rumen (Patente de la India n.º 388717, del 8 de febrero de 2022) y se examinó su eficacia mediante una serie de experimentos *in vitro*. Se utilizó un

and promoting the growth of alternate hydrogen utilizers like sulfate-reducing bacteria. The feed additive stimulated the rumen ecosystem by increasing microbial fibrolytic activity. It thus increased ($p<0.05$) fiber digestion, reducing the rate of ammonia production in the rumen with concomitant improvement in feed utilization and performance of buffaloes, resulting in an increase in the growth rate by 10%, feed conversion efficiency by 15% and milk production by 10.4%. The results revealed increased digestibility of nutrients, especially crude fiber, with decreased ($p<0.05$) methane concentration in the exhaled air of lactating buffaloes. The health status regarding cell-mediated and humoral immune response was also enhanced ($p<0.05$) in supplemented buffaloes. The feed supplement RESMI significantly reduced enteric methane production from buffaloes and lowered environmental pollution from ruminant production systems. A discernible positive impact on growth, milk production, and immunity status was evident in supplemented buffaloes.

Keywords: buffalo, feed supplement, methanogenesis, growth, milk production, immunity.

diseño completamente al azar para analizar los datos experimentales. Se encontró que la suplementación de RESMI (@ 2,5% de la ingesta de alimento) reduce la emisión de metano ($p<0,05$) al reducir la cantidad de arqueas metanogénicas en el rumen y promover el crecimiento de usuarios alternativos de hidrógeno, como las bacterias reductoras de sulfato. El aditivo alimentario estimuló el ecosistema ruminal al aumentar la actividad fibrolítica microbiana. Por lo tanto, aumentó ($p<0,05$) la digestión de la fibra, reduciendo la tasa de producción de amoníaco en el rumen con una mejora concomitante en la utilización del alimento y el rendimiento de los búfalos, lo que resultó en un aumento en la tasa de crecimiento en un 10%, la eficiencia de conversión alimenticia en un 15% y la producción de leche en un 10,4%. Los resultados revelaron una mayor digestibilidad de los nutrientes, especialmente la fibra cruda, con una disminución ($p<0,05$) de la concentración de metano en el aire exhalado de las búfalas lactantes. El estado de salud con respecto a la respuesta inmune humoral y mediada por células también mejoró ($p<0,05$) en los búfalos suplementados. El complemento alimenticio RESMI redujo significativamente la producción de metano entérico de los búfalos y redujo la contaminación ambiental de los sistemas de producción de rumiantes. Un impacto positivo discernible en el crecimiento, la producción de leche y el estado de inmunidad fue evidente en las búfalas suplementadas.

Palabras clave: búfalo, complemento alimenticio, metanogénesis, crecimiento, producción de leche, inmunidad.

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Impact of propylene glycol supplementation during early postpartum period on serum metabolites and reproductive performance in Nili-Ravi buffalo

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ABSTRACT

The present study investigated the effect of propylene glycol supplementation during the early postpartum period on serum metabolites in buffalo. Nili Ravi buffalo (n=25) were randomly assigned to one of two groups, i.e., Treatment Group (n=15) and Control Group (n=10). In the experimental group, propylene glycol (500 ml/ animal/ day) supplementation started

Impacto de la suplementación con propilenglicol durante el período posparto temprano sobre los metabolitos séricos y el rendimiento reproductivo en búfalas Nili-Ravi

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RESUMEN

El presente estudio investigó el efecto de la suplementación con propilenglicol durante el período posparto temprano sobre los metabolitos séricos de búfalas. Los búfalos Nili Ravi (n=25) fueron asignado aleatoriamente a uno de los dos grupos, es decir, grupo de tratamiento (n=15) y grupo de control (n=10). En el grupo experimental, la suplementación con pro-

from day 7 to day 50 postpartum. These animals were under observation for weekly measurements from 3 weeks pre-partum to 10 weeks postpartum. Blood sampling was done weekly for glucose, insulin, and beta-hydroxybutyric acid analysis. Propylene glycol supplementation did not affect body weight and milk yield ($p > 0.05$). Serum glucose and insulin concentration were significantly increased ($p < 0.05$), and ketone body levels were reduced ($p < 0.05$) shortly after propylene glycol supplementation. It was concluded that propylene glycol during the early lactation period in Nili-Ravi buffalo effectively increased serum glucose and insulin levels and lowered blood ketone body levels, which reduced the risk of ketosis.

Keywords: propylene glycol, insulin, glucose, beta-hydroxy butyric acid, ketone bodies.

pilenglicol (500 ml/animal/día) comenzó desde el día 7 al día 50 posparto. Estos animales estuvieron bajo observación para mediciones semanales desde 3 semanas antes del parto hasta 10 semanas después del parto. Se realizaron muestras de sangre semanalmente para análisis de glucosa, insulina y ácido beta-hidroxibutírico. La suplementación con propilenglicol no afectó el peso corporal ni la producción de leche ($p > 0,05$). La concentración sérica de glucosa e insulina aumentó significativamente ($p < 0,05$) y los niveles de cuerpos cetónicos se redujeron ($p < 0,05$) poco después de la suplementación con propilenglicol. Se concluyó que el propilenglicol durante el período inicial de lactancia en búfalas Nili-Ravi aumentó efectivamente los niveles séricos de glucosa e insulina y redujo los niveles de cuerpos cetónicos en sangre, lo que redujo el riesgo de cetosis.

Palabras clave: propilenglicol, insulina, glucosa, ácido beta-hidroxibutírico, cuerpos cetónicos.

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Effect of *Arthrospira platensis* (Spirulina) as a dietary supplement during transition period in dairy buffaloes

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ABSTRACT

It is widely recognized that in mammals, the period just before and immediately after calving, until the peak's lactation, is a stressful period impairing animal performance and immune response. Some studies have proposed the use of *Arthrospira platensis* (Spirulina), a natural immunostimulant, as a dietary supplement in cows and sheep to improve the antioxidant response. To date, no similar research has been reported on the buffalo species. Therefore, we investigated the effect of Spirulina on body condition score (BCS), yield and quality of milk, and oxidative stress response through the assessment of reactive oxygen metabolites (d-ROMs test) and the biological antioxidant potential (BAP test) of twenty-four Italian Mediterranean

Efecto de *Arthrospira platensis* (Espirulina) como suplemento dietético durante el período de transición en búfalas lecheras

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RESUMEN

Es ampliamente reconocido que, en los mamíferos, el período justo antes e inmediatamente después del parto, hasta el pico de lactancia, es un período estresante que perjudica el rendimiento animal y la respuesta inmune. Algunos estudios han propuesto el uso de *Arthrospira platensis* (Espirulina), un inmunostimulante natural, como complemento dietético en vacas y ovejas para mejorar la respuesta antioxidante. Hasta la fecha, no se ha informado de ninguna investigación similar sobre la especie bufalina. Por lo tanto, investigamos el efecto de la espirulina en la puntuación de condición corporal (BCS), el rendimiento y la calidad de la leche, así como la respuesta al estrés oxidativo mediante la evaluación de los metabolitos re-

buffaloes. The animals were equally divided into two groups (control=C and Spirulina=S) fed with the same total mixed ration. In top dressing mode, the S group additionally received 50 g/head/d of freeze-dried Spirulina for one month before calving and 100 g/head/d for 90 d after calving. Milk production was recorded daily on the farm's computer system, and milk composition (protein, fat, and lactose) was determined at 30, 60, and 90 d after calving using Milkoscan (Foss, Denmark). BCS was determined at group formation and 30, 60, and 90 days after calving. Colostrum was collected within 6-8 hours post-calving, and its immunoglobulin content was immediately measured by an optical refractometer (Brix degrees). d-ROMs and BAP were measured on serum blood samples taken at group formation, at calving, 15 and 90 d after calving. Data were analyzed by a two-way analysis of variance using treatment, time of sampling, and relative interaction as factors. Where appropriate, the Tukey post hoc test was used to identify significant differences between means. For all variables, the interaction was not significant. Spirulina supplementation did not influence yield, milk quality, BCS, or colostrum quality. Group S showed a significantly higher mean BAP value (U-Carr) than the control (2730.17 vs. 2511.28, $p<0.05$) and a lower level of d-ROMs (54.15 vs. 78.45, $p<0.05$), indicating a better antioxidant response to oxidative stress. As expected, the sampling time significantly affected both BAP and d-ROMs ($p<0.001$), with the highest BAP value at 90 d and the lowest d-ROMs at 30 d before calving. We conclude that Spirulina supplementation during the transition period may benefit the redox status of Italian Mediterranean buffaloes.

Keywords: dairy buffalo, spirulina, oxidative stress, productive performances.

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activos del oxígeno (prueba de d-ROM) y el potencial biológico antioxidante (prueba de BAP). Para este estudio, veinte-cuatro ($n=24$) búfalas mediterráneas italianas se dividieron equitativamente en dos grupos (control=C y Spirulina=S) alimentados con la misma ración mixta total. El grupo S recibió adicionalmente 50 g/cabeza/día de Espirulina liofilizada durante un mes antes del parto y 100 g/cabeza/día durante 90 días después del parto, en modo de aderezo sobre la ración. La producción de leche se registró diariamente en el sistema informático de la finca y la composición de la leche (proteína, grasa y lactosa) se determinó a los 30, 60 y 90 días después del parto utilizando Milkoscan (Foss, Dinamarca). La BCS se determinó en el momento de la formación del grupo y a los 30, 60 y 90 días después del parto. El calostro se recogió entre 6 y 8 horas después del parto y su contenido de inmunoglobulinas se midió inmediatamente con un refractómetro óptico (grados Brix). Los d-ROM y BAP se midieron en muestras de sangre sérica tomadas en la formación del grupo, al parto, 15 y 90 días después del parto. Los datos se analizaron mediante un análisis de varianza bidireccional utilizando el tratamiento, el momento del muestreo y la interacción relativa como factores. Cuando fue apropiado, se utilizó la prueba post hoc de Tukey para identificar diferencias significativas entre las medias. Para todas las variables, la interacción no fue significativa. La suplementación con espirulina no influyó en el rendimiento y la calidad de la leche, la CC y la calidad del calostro. El grupo S mostró un valor medio de BAP (U-Carr) significativamente mayor que el control (2730,17 frente a 2511,28, $p<0,05$) y un nivel más bajo de d-ROM (54,15 frente a 78,45, $p<0,05$), lo que indica una mejor respuesta antioxidante al estrés oxidativo. Como se esperaba, el tiempo de muestreo afectó significativamente tanto a BAP como a d-ROM ($p<0,001$), con el valor más alto de BAP a los 90 días y el d-ROM más bajo a los 30 días antes del parto. Concluimos que la suplementación con espirulina durante el período de transición puede tener un efecto beneficioso sobre el estado redox de búfalos italianos Mediterráneos.

Palabras clave: búfala lechera, espirulina, estrés oxidativo, desempeños productivos.

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Soil-plant-animal relationships and their effect on pasture-based milk production in Hacienda Cuba, Colombia

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ABSTRACT

Milk production from buffaloes has experienced significant growth in Colombia over the past 20 years. These systems are primarily located in the low tropics, where production systems face limitations in intensification. At Hacienda Cuba, situated in Montelíbano, Córdoba, Colombia, various sustainable intensification strategies have been implemented to enhance buffalo milk production. This study describes the soil, plant, and animal components to assess the capacity for high-quality buffalo milk production. We collected soil and forage samples from the grazing area for chemical characterization and quantified the available forage supply. Subsequently, the milk production potential of the animals was determined based on the forage diet provided, as well as the impact and quantification of intensification practices, such as strategic supplementation, on buffalo milk production. Our findings revealed that soil composition was 10% sand, 46% silt, and 44% clay, pH of 5.0, CEC of 15.16, and P of 11. Microbiological analyses indicated that most functional groups were within acceptable ranges. Characterizing the forage component revealed that buffaloes primarily consume a mixture of forages, including *Paspalum fasciculatum*, *Dichanthium annulatum*, *Brachiaria decumbens*, *Dichanthium aristatum*, *Brachiaria arrecta*, and *Panicum virgatum*. On average, these forages exhibited the following nutritional quality characteristics: crude protein of 10.25%, neutral detergent fiber of 65.60%, the *in vitro* digestibility of dry matter was 56.45%, and the metabolizable energy was 2.75 Mcal Kg DM-1. Our results indicate that buffaloes received a substantial forage supply or allocation, ranging between 7 and 8 kg DM/100 kg of live weight. The production potential is based on a pasture yielding 4.04 liters of daily milk with 6% fat. When the intensification practices were adopted (2 kg of balanced feed and 6 kg of sorghum silage), an increase of 78.5% in milk production was observed. These sustainable intensification practices, such as strategic supplementation in dairy buffaloes in Colombia, enhance milk production and may improve milk quality (it needs to be evaluated). Consequently, these prac-

Relaciones suelo-planta-animal y su efecto en la producción de leche basado en pastos en Hacienda Cuba, Colombia

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RESUMEN

La producción de leche de búfala ha experimentado un crecimiento significativo en Colombia durante los últimos 20 años. Estos sistemas están ubicados principalmente en los trópicos bajos, donde los sistemas de producción enfrentan limitaciones en cuanto a intensificación. En Hacienda Cuba, situada en Montelíbano, Córdoba, Colombia, se han implementado varias estrategias de intensificación sostenible para mejorar la producción de leche de búfala. Este estudio describe los componentes del suelo, las plantas y los animales para evaluar la capacidad de producción de leche de búfala de alta calidad. Recolectamos muestras de suelo y forraje del área de pastoreo para su caracterización química y cuantificamos el suministro de forraje disponible. Posteriormente, se determinó el potencial de producción de leche de los animales con base en la dieta forrajera proporcionada, así como el impacto y cuantificación de las prácticas de intensificación, como la suplementación estratégica, en la producción de leche de búfala. Nuestros hallazgos revelaron que la composición del suelo era 10% arena, 46% limo y 44% arcilla, pH de 5,0, CEC de 15,16 y P de 11. Los análisis microbiológicos indicaron que la mayoría de los grupos funcionales estaban dentro de rangos aceptables. La caracterización del componente forrajero reveló que los búfalos consumen principalmente una mezcla de forrajes, incluyendo *Paspalum fasciculatum*, *Dichanthium annulatum*, *Brachiaria decumbens*, *Dichanthium aristatum*, *Brachiaria arrecta* y *Panicum virgatum*. En promedio, estos forrajes presentaron las siguientes características de calidad nutricional: proteína cruda de 10,25%, fibra detergente neutra de 65,60%, la digestibilidad *in vitro* de la materia seca fue de 56,45% y la energía metabolizable fue de 2,75 Mcal Kg DM-1. Nuestros resultados indican que los búfalos recibieron un suministro o asignación sustancial de forraje, oscilando entre 7 y 8 kg de MS/100 kg de peso vivo. El potencial de producción se basa en un pasto que rinde 4,04 litros de leche diarios con un 6% de grasa. Cuando se adoptaron las prácticas de intensificación (2 kg de alimento balanceado y 6 kg de ensilaje de sorgo), se observó un aumento del 78,5% en la producción de leche.

tices can potentially increase profit since payment for milk is linked to these parameters.

Keywords: tropical forages, buffalo milk, soil, supplementation.

Estas prácticas de intensificación sostenible, como la suplementación estratégica en búfalas lecheras en Colombia, mejoran la producción de leche y pueden mejorar la calidad de la leche (es necesario evaluarla). En consecuencia, estas prácticas pueden potencialmente aumentar las ganancias, ya que el pago de la leche está vinculado a estos parámetros.

Palabras clave: forrajes tropicales, leche de búfala, suelo, suplementación.

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Effect of different diets on the fatty acid composition of buffalo bulk milk

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ABSTRACT

Feed can affect the fatty acids (FA) composition of buffalo milk. Therefore, diet could improve the nutritional value of buffalo milk such as increasing polyunsaturated fatty acids n-3 (PUFA n-3), and conjugated linoleic acid (CLA), with high nutritional value for humans. The study aimed to evaluate the effect of diets and seasons on buffalo milk fatty acid composition. During a year, 120 samples of bulk buffalo milk were taken from ten farms. Animals were fed with ten different isoenergetic and isoproteic diets. They were provided as a total mixed ratio where different components (about 3% in dry matter of total ration) were added to the essential diet for each group: basic diet (B) was composed of maize silages, alfalfa hay, corn grain, and soybean as concentrates, the second diet contained linseed as an additional constituent to B (L), another brewer's spent grain (T), additional alfalfa and mixed hay (H), additional corn grain (M), grass (G); other farms included in B diet both grass and cottonseed (CG), grass and linseed (GL) or grass and brewer's spent grain (TG) and at last grass linseed and brewer's spent grain (LTG). Fatty acids in buffalo milk were quantified after extraction and methylation by GC/FID. Data were analyzed by a bi-factorial model with interaction (season x diet) using GLM procedure and the statement "contrast" by SAS Software to highlight the effect of the constituents of diets. Compared to the others, seasonal effects were evident in milk FA, mainly for spring and summer, with an increase in saturated fatty acids (SFA) and a decrease in PUFA of about 10%. Linseed diets (L, LG, LTG) showed an increase ($p < 0.001$) of PUFA n-3 (0.63 vs.

Efecto de diferentes dietas sobre la composición de ácidos grasos de la leche a granel de búfala

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RESUMEN

El alimento puede afectar la composición de ácidos grasos (AG) de la leche de búfala. Por tanto, la dieta podría mejorar el valor nutricional de la leche de búfala, aumentando los ácidos grasos poliinsaturados n-3 (PUFA n-3) y ácido linoleico conjugado (CLA), de alto valor nutricional para el ser humano. El estudio tuvo como objetivo evaluar el efecto de las dietas y las estaciones sobre la composición de ácidos grasos de la leche de búfala. Durante un año se tomaron 120 muestras de leche de búfala a granel de diez granjas. Los animales fueron alimentados con diez dietas isoenergéticas e isoproteicas diferentes. Se proporcionaron como una proporción mixta total donde se agregaron diferentes componentes (alrededor del 3% en materia seca de la ración total) a la dieta esencial para cada grupo: la dieta básica (B) estuvo compuesta por ensilajes de maíz, heno de alfalfa, grano de maíz y soja como concentrados. La segunda dieta contenía linaza como componente adicional de B (L), y el grano gastado de mosto cervecero (T), alfalfa adicional y heno mixto (H), grano de maíz adicional (M), pasto (G). Otras granjas incluyeron en la dieta B tanto pasto como semilla de algodón (CG), pasto y linaza (GL) o pasto y orujo (TG) y por último, pasto, linaza y grano gastado de cervecería (LTG). Los ácidos grasos en la leche de búfala se cuantificaron después de la extracción y metilación mediante GC/FID. Los datos fueron analizados mediante un modelo bifactorial con interacción (estación x dieta) utilizando el procedimiento GLM y la declaración "contraste" del paquete estadístico SAS para resaltar el efecto de los constituyentes de

0.38 g/100g of fat) compared with the others. While milk from the CG and TG diets showed an increase ($p < 0.001$) in PUFA n-6, the differences were also evident in the n6/n3 ratio (3.1 vs. 5.2, $p < 0.001$). Diets T and M showed an increase ($p < 0.002$) in SFA and a decrease $p < 0.001$ in CLA (-20%). Instead, the G diet allowed a higher ($p < 0.0004$) content of CLA (0.90 vs 0.74 g/100g of fat), highlighting the positive contribution of grass on ruminal activity. Instead, the addition of linseed and cottonseed to the G diet caused a decrease ($p < 0.001$) in CLA and branch fatty acids due to the excessive presence of PUFA. The feeds supplied attend all metabolic pathways, and in particular, linseed and grass could enrich the milk with nutraceuticals. However, the right balance must be found between the constituents to maximize their positive contribution.

Keywords: buffalo milk, grass feed, linseed supplementation, season effect, fatty acids, CLA.

las dietas. En comparación con los demás, los efectos estacionales fueron evidentes en los AG de la leche, principalmente en primavera y verano, con un aumento de los ácidos grasos saturados (AGS) y una disminución de los AGPI de aproximadamente el 10%. Las dietas de linaza (L, LG, LTG) mostraron un aumento ($p < 0,001$) de PUFA n-3 (0,63 vs. 0,38 g/100 g de grasa) en comparación con las demás. Mientras que la leche de las dietas CG y TG mostró un aumento ($p < 0,001$) en AGPI n-6, las diferencias también fueron evidentes en la relación n6/n3 (3,1 vs. 5,2, $p < 0,001$). Las dietas T y M mostraron un aumento ($p < 0,002$) en AGS y una disminución $p < 0,001$ en CLA (-20%). En cambio, la dieta G permitió un mayor ($p < 0,0004$) contenido de CLA (0,90 vs 0,74 g/100g de grasa), destacando la contribución positiva del pasto sobre la actividad ruminal. En cambio, la adición de semillas de linaza y algodón a la dieta G provocó una disminución ($p < 0,001$) en CLA y ácidos grasos ramificados debido a la presencia excesiva de PUFA. Los piensos suministrados atienden todas las vías metabólicas y, en particular, la linaza y las hierbas podrían enriquecer la leche con nutraceuticos. Sin embargo, debe encontrarse el equilibrio adecuado entre los componentes para maximizar su contribución positiva.

Palabras clave: leche de búfala, piensos a base de pasto, suplementación con linaza, efecto estacional, ácidos grasos, CLA.

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Comparing corn silage with beet pulp silage fed separate or mixed with concentrate in buffalo calves

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ABSTRACT

Twelve male calves of Nili-Ravi buffalo (aged 14 ± 2 months and weighing 133 ± 5 kg) were randomly assigned one of four experimental diets. Group A was fed only concentrate in the morning and corn silage in the afternoon. Group B was offered a total mixed ratio (TMR) of corn silage. Group C was fed only concentrate in the morning and then beet pulp silage in the afternoon. Group D was given TMR of beet pulp silage. A 2X2 factorial design was applied in this study. Concentrate was formulated with 17% CP and used in a ratio of 50:50 concentrate and silage, respectively. All diets were iso-nitrogenous and

Comparación del ensilaje de maíz con el ensilaje de pulpa de remolacha azucarera alimentado por separado o mezclado con concentrado en terneros búfalos

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RESUMEN

A doce terneros machos de búfalos de la raza Nili-Ravi (de 14 ± 2 meses y con un peso de 133 ± 5 kg) se les asignó aleatoriamente una de cuatro dietas experimentales. El grupo A fue alimentado únicamente con concentrado por la mañana y ensilaje de maíz por la tarde. Al grupo B se le ofreció una proporción mixta total (TMR) de ensilaje de maíz. El grupo C fue alimentado únicamente con concentrado por la mañana

iso-caloric. Data was presented as means and SE. The data was statistically evaluated by Minitab 15 software using analysis of variance in a completely randomized design. The p-value used to compare the significance was set at ≤ 0.05 . Results showed that Group D, fed TMR of sugar beet pulp silage with concentrate, gained significantly more daily weight than other groups. The highest daily weight gain in grams was observed in Group D (852 ± 0.03), followed by Group C (755 ± 0.03), Group B (741 ± 0.03) and Group A (758 ± 0.03). There were no significant differences in feed intake. However, the feed conversion ratio revealed significant improvement in Group D (5.913 ± 0.81) followed by Group C (6.391 ± 0.81), Group B (6.467 ± 0.81) and Group A (6.535 ± 0.81). The digestibility values of dry matter (DM), crude fiber (CF), and crude protein (CP) were significantly better in groups B and D. The digestibility values of DM, CP, and CF were 66.37 ± 2.01 , 69.55 ± 1.25 , 63.15 ± 1.12 in Group B and 65.90 ± 2.01 , 68.55 ± 1.25 , 62.33 ± 1.12 in Group D respectively. The significantly highest value of nutrient digestibility of neutral detergent fiber (66.12 ± 1.67) and acid detergent fiber (60.15 ± 1.78) was observed in Group D. The nitrogen intake and retention by all the treatment groups showed non-significant differences. Regarding serological examination, cholesterol levels showed significantly lower values in Group D, while blood glucose and urea nitrogen exhibited no differences. In the economic analysis, the costs of the different groups were calculated, and it revealed that beet pulp silage and concentrate TMR were economically favorable for the fattening of calves in terms of input-to-output ratio. The better results in group D might be due to the synergic effect of sugar beet pulp silage and concentrate by improving the digestibility of nutrients and their effective utilization. In conclusion, sugar beet ensiled with ground corn cobs is a simple and viable strategy for effective silage making. However, sugar beet pulp silage with concentrate TMR had a pronounced economic impact on the fattening of buffalo calves in terms of cost-to-gain ratio.

Keywords: buffalo calves, corn silage, sugar beet pulp, economic viability.

na y luego con ensilaje de pulpa de remolacha azucarera por la tarde. El grupo D recibió TMR de ensilaje de pulpa de remolacha. En este estudio se aplicó un diseño factorial 2X2. El concentrado se formuló con 17% de proteína cruda (CP) y se utilizó en una proporción de 50:50 de concentrado y ensilaje, respectivamente. Todas las dietas fueron isonitrogenadas e isocalóricas. Los datos se presentaron como medias y error estándar (SE). Los datos fueron evaluados estadísticamente mediante el software Minitab 15 mediante análisis de varianza en un diseño completamente al azar. El valor de p utilizado para comparar la significancia se fijó en $p \leq 0.05$. Los resultados mostraron que el grupo D, alimentado con TMR de ensilaje de pulpa de remolacha azucarera con concentrado, ganó significativamente más peso diario que los otros grupos. El mayor aumento de peso diario en gramos se observó en el Grupo D (852 ± 0.03), seguido del Grupo C (755 ± 0.03), Grupo B (741 ± 0.03) y Grupo A (758 ± 0.03). No hubo diferencias significativas en el consumo de alimento. Sin embargo, el índice de conversión alimenticia reveló una mejora significativa en el Grupo D ($5,913 \pm 0,81$), seguido por el Grupo C ($6,391 \pm 0,81$), el Grupo B ($6,467 \pm 0,81$) y el Grupo A ($6,535 \pm 0,81$). Los valores de digestibilidad de la materia seca (MS), fibra cruda (CF) y proteína cruda (CP) fueron significativamente mejores en los grupos B y D. Los valores de digestibilidad de MS, PB y CF fueron 66.37 ± 2.01 , 69.55 ± 1.25 , $63,15 \pm 1,12$ en el Grupo B y $65,90 \pm 2,01$, $68,55 \pm 1,25$, $62,33 \pm 1,12$ en el Grupo D respectivamente. El valor significativamente más alto de digestibilidad de nutrientes de la fibra detergente neutra ($66,12 \pm 1,67$) y la fibra detergente ácida ($60,15 \pm 1,78$) se observó en el Grupo D. La ingesta y retención de nitrógeno en todos los grupos de tratamiento no mostraron diferencias significativas. En cuanto al examen serológico, los niveles de colesterol mostraron valores significativamente más bajos en el grupo D, mientras que la glucosa en sangre y el nitrógeno ureico no mostraron diferencias. En el análisis económico se calcularon los costos de los diferentes grupos y se reveló que el ensilaje de pulpa de remolacha y el concentrado TMR fueron económicamente favorables para el engorde de terneros en términos de relación insumo-producto. Los mejores resultados en el grupo D podrían deberse al efecto sinérgico del ensilaje y concentrado de pulpa de remolacha azucarera al mejorar la digestibilidad de los nutrientes y su utilización efectiva. En conclusión, el ensilaje de remolacha azucarera con mazorcas de maíz molidas es una estrategia simple y viable para la elaboración de ensilaje eficaz. Sin embargo, el ensilaje de pulpa de remolacha azucarera con concentrado TMR tuvo un impacto económico pronunciado en el engorde de terneros de búfalo en términos de relación costo-beneficio.

Palabras clave: búfalos, ensilaje de maíz, pulpa de remolacha azucarera, viabilidad económica.

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Productive performance in Italian Mediterranean buffaloes after partially replacing dietary starch with simple sugars

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ABSTRACT

This study aimed to partially replace starch with simple sugars as a carbohydrate source in the diet of Italian Mediterranean buffaloes, substituting corn with molasses. Sixty lactating buffaloes located on a commercial farm in Caserta province were divided into two groups according to days in milk (DIM), parity, and milk yield (MY), recorded both in the previous two weeks and the previous lactation: Group C (Control; n=30; 48.7±4.1 DIM; average MY: 13.69±0.5 kg) and group T (Treated; n=30; 49.4±4.3 DIM; average MY: 13.65±0.5 kg). The two groups were maintained in separate paddocks throughout the study, which lasted 120 days. Two diets with the same dry matter (DM) content (16.5 kg) and nutritional characteristics on a DM basis (crude protein: 14.9%; NDF: 38.5%; Ash: 6.3%; NSC: 34.5%; energy density: 0.93 Milk Forage Units) were prepared. In the T diet, 1.7 kg of molasses was inserted, replacing corn meal and maize silage; thus, starch content was 19.8% and 17.2% in Diet C and T, respectively. The diets were administered twice/day in each group, and refusals were weighed and sampled daily to calculate average feed intake. Individual milk yield was recorded daily, and 15 days apart, an individual milk sample was collected for milk quality analysis (fat, protein, casein, lactose, urea, β-hydroxybutyrate [BHB] and fatty acids profile) through mid-infrared spectroscopy (Milkoscan FT6000®, Foss Electric). Data were analyzed through a mixed ANOVA. The average milk yield throughout the experimental period was 11.7±0.1 vs 12.2±0.1 kg, Group C and T, respectively. Buffaloes in Group C showed lower ($p<0.01$) fat content compared to Group T (8.33±0.1 vs. 8.58±0.1 in Group C and T, respectively) and similar protein and casein content (protein 4.41±0.0 vs 4.43±0.0 and casein 3.54±0.0 vs 3.57±0.0 in Group C and T, respectively). Interestingly, the urea concentration was higher in Group C compared to Group T (43.1±0.1 vs. 35.7±0.1, in Group C and T, respectively). In contrast, following simple sugars administration, BHB content was lower (0.22±0.1 vs 0.28±0.1 in Group C and T, respectively), probably because

Comportamiento productivo en búfalos mediterráneos italianos tras la sustitución parcial del almidón dietético por azúcares simples

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RESUMEN

Este estudio tuvo como objetivo sustituir parcialmente el almidón por azúcares simples como fuente de carbohidratos en la dieta de búfalos mediterráneos italianos, sustituyendo el maíz por melaza. Sesenta búfalas lactantes ubicadas en una granja comercial en la provincia de Caserta fueron divididas en dos grupos según los días en lactancia (DIM), el número de partos y la producción de leche (MY), registrados tanto en las dos semanas anteriores como en la lactancia anterior: Grupo C (Control; n=30; 48,7±4,1 DIM; promedio MY: 13,69±0,5 kg) y grupo T (Tratado; n=30; 49,4±4,3 DIM; promedio MY: 13,65±0,5 kg). Los dos grupos se mantuvieron en potreros separados durante todo el estudio, que duró 120 días. Dos dietas con el mismo contenido de materia seca (MS) (16,5 kg) y características nutricionales en base a MS (proteína cruda: 14,9%; FND: 38,5%; cenizas: 6,3%; NSC: 34,5%; densidad energética: 0,93 Unidades de Leche Forrajera) estaban preparadas. En la dieta T se insertó 1,7 kg de melaza, en sustitución de harina de maíz y ensilaje de maíz; así, el contenido de almidón fue del 19,8% y del 17,2% en las dietas C y T, respectivamente. Las dietas se administraron dos veces al día en cada grupo, y los rechazos se pesaron y tomaron muestras diariamente para calcular el consumo promedio de alimento. La producción de leche individual se registró diariamente y, con 15 días de diferencia, se recolectó una muestra de leche individual para el análisis de la calidad de la leche (grasa, proteína, caseína, lactosa, urea, β-hidroxibutirato [BHB] y perfil de ácidos grasos) mediante espectroscopía de infrarrojo medio (Milkoscan FT6000®, Foss Electric). Los datos se analizaron mediante un ANOVA mixto. La producción de leche promedio durante todo el período experimental fue de 11,7±0,1 vs 12,2±0,1 kg Grupo C y T, respectivamente. Los búfalos del Grupo C mostraron un menor contenido de grasa ($p<0,01$) en comparación con el Grupo T (8,33±0,1 vs. 8,58±0,1 en el Grupo C y T, respectivamente) y un contenido similar de proteína y caseína (proteína 4,41±0,0 vs 4,43±0,0 y caseína 3,54±0,0 vs 3,57±0,0 en el grupo C y

of higher butyric acid production. Regarding the acidic profile, only medium-chain fatty acids were lower in Group C vs. Group T (3,135±77 vs 3,379±79 in groups C and T, respectively). In conclusion, simple sugar administration in the diet of lactating buffaloes seems to increase milk yield and fat content, probably through an improvement in rumen efficiency.

Keywords: buffalo, nutrition, starch, simple sugars, milk yield, and quality.

T, respectivamente). Curiosamente, la concentración de urea fue mayor en el Grupo C en comparación con el Grupo T (43,1 ± 0,1 frente a 35,7 ± 0,1, en el Grupo C y T, respectivamente). Por el contrario, después de la administración de azúcares simples, el contenido de BHB fue menor (0,22 ± 0,1 frente a 0,28 ± 0,1 en el grupo C y T, respectivamente), probablemente debido a una mayor producción de ácido butírico. En cuanto al perfil ácido, sólo los ácidos grasos de cadena media fueron inferiores en el grupo C frente al grupo T (3.135 ± 77 frente a 3.379 ± 79 en los grupos C y T, respectivamente). En conclusión, la simple administración de azúcar en la dieta de búfalas lactantes parece aumentar la producción de leche y el contenido de grasa, probablemente a través de una mejora en la eficiencia del rumen.

Palabras clave: búfala, nutrición, almidón, azúcares simples, producción y calidad de leche.

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The potential of buffalo as a meat producer

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ABSTRACT

The buffalo should not only be recognized for its good production of milk and dairy products. They should also be appreciated for converting forage into quality meat and their use as a work animal. To evaluate the potential of the buffalo as a meat-producing animal, 181 Murrah buffaloes weaned between 9 and 9.5 months of age with an average of 188 ± 10 kg (minimum 175, maximum 198) and a coefficient of variation (CV) of 5.32% were evaluated. From birth to weaning, they were fed under the Voisin grazing (Vg) modality with a single milking (from their mothers) receiving the milk produced from the right anterior teat until four months of age, and later, they only received the draining of the udder of the buffalo once the milking was concluded. After weaning, their feeding was at Vg without the supply of concentrated feed and *ad libitum* mineral supplement. They were weighed with 18 hours of fasting every 14 days during the 15.5 months of fattening. The weighing was performed with a digital scale without decimals and calibrated every ten weighings. PROC MEANS was used to calculate descriptive statistics, and a correlation analysis was performed between weaning weight and slaughter weight using the statistical package SAS version 9.1. The average gain was 285 ± 14.3 kg and CV 5.02% (weaning to slaughter). The total fattening and fattening period was 15.5 months (472 days); the average slaughter weight was 473 ± 17.5 kg and CV of 3.69% (minimum 450, maximum 496 kg) at 25 months of age. The average gain/day was 0.604 ± 0.048 g/day, with a CV of

El potencial del búfalo como productor de carne

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RESUMEN

La búfala no sólo debe ser reconocida por su buena producción de leche y productos lácteos. También deben ser apreciados por convertir el forraje en carne de calidad y por su uso como animal de trabajo. Para evaluar el potencial del búfalo como animal productor de carne, se destetaron 181 búfalas Murrah entre los 9 y 9,5 meses de edad con un promedio de 188 ± 10 kg (mínimo 175, máximo 198) y un coeficiente de variación (CV) de 5,32% fueron evaluados. Desde el nacimiento hasta el destete, fueron alimentados bajo la modalidad de pastoreo Voisin (Vg) con un solo ordeño (de sus madres) recibiendo la leche producida del pezón anterior derecho hasta los cuatro meses de edad, y posteriormente, solo recibieron el drenaje de la ubre de la búfala una vez concluido el ordeño. Después del destete, su alimentación fue Vg sin el suministro de alimento concentrado y suplemento mineral *ad libitum*. Fueron pesados con 18 horas de ayuno cada 14 días durante los 15,5 meses de engorde. El pesaje se realizó con balanza digital sin decimales y calibrada cada diez pesajes. Se utilizó PROC MEANS para calcular estadísticas descriptivas y se realizó un análisis de correlación entre el peso al destete y el peso al sacrificio utilizando el paquete estadístico SAS versión 9.1. La ganancia promedio fue de 285 ± 14,3 kg y CV 5,02% (destete al sacrificio). El engorde y el periodo de engorde totales fue de 15,5 meses (472 días); el peso promedio al sacrificio fue de 473 ± 17,5 kg y CV de 3,69% (mínimo 450, máximo 496 kg) a los 25 meses de

7.94%. Weight loss from farm to slaughter was 10.62 ± 0.65 kg with a CV of 6.12%. The average weight of the hot carcass (HC) was 240.86 ± 6.77 kg with a CV of 2.82%. The yield in HC was 50.92% (minimum 49.96 and maximum 52.33%). Out of the 181 buffaloes, 12 were selected with an average slaughter weight of 460 kg, and the body weight distribution was evaluated: head: 17.38 kg; blood: 11.8 kg; skin: 46.68 kg; tail and ears: 3.72 kg; feet: 8.41 kg; meat: 182.15 kg; red and white bone: 73.29; white viscera: 30.26; red viscera: 21.79; fat: 24.30 and gastrointestinal contents: 40.22 kg. This group of buffaloes averaged 236.90 kg in HC with a 51.5% yield. No correlation was observed between weaning weight and slaughter weight. It can be concluded that the buffalo has an excellent meat yield, 39.59% of meat concerning live weight.

Keywords: buffalo, meat yield, carcass.

edad. La ganancia promedio/día fue de $0,604 \pm 0,048$ g/día, con un CV de 7,94%. La pérdida de peso desde la finca hasta el sacrificio fue de $10,62 \pm 0,65$ kg con un CV de 6,12%. El peso promedio de la canal caliente (HC) fue de $240,86 \pm 6,77$ kg con un CV de 2,82%. El rendimiento en HC fue de 50,92% (mínimo 49,96 y máximo 52,33%). De los 181 búfalos, se seleccionaron 12 con un peso promedio al sacrificio de 460 kg, y se evaluó la distribución del peso corporal: cabeza: 17,38 kg; sangre: 11,8 kg; piel: 46,68 kg; cola y orejas: 3,72 kg; pies: 8,41 kg; carne: 182,15 kg; hueso rojo y blanco: 73,29; vísceras blancas: 30,26; vísceras rojas: 21,79; grasa: 24,30 y contenido gastrointestinal: 40,22 kg. Este grupo de búfalos promedió 236,90 kg en canal con un rendimiento del 51,5%. No se observó correlación entre el peso al destete y el peso al sacrificio. Se puede concluir que el búfalo tiene un excelente rendimiento cárnico, 39,59% de carne respecto al peso vivo.

Palabras clave: búfalo, rendimiento de carne, cadáver.

GENETICS & ANIMAL BREEDING

Mejoramiento y Genética

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Classification by production: an alternative criterion to categorization

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ABSTRACT

At the beginning of the Genealogical Control and Registration Program, it was found that the main herds that formed the national buffalo breeding stock did not have the pertinent records for mating, even though the vast majority of this biological material had certificates of registration from their country of origin, initially from Bulgaria and India and later from Brazil and Colombia. In the absence of an institution in charge of the Stud Book, over time, this scarce information was lost to the point where it was impossible to recover it. Applying a genealogical criterion through five generations of crossbreeding by upgrading is impossible, namely, from the LA category to the PO category, with intermediate products LA1, LA2, LA3 and LA4. However, it was well known that the origin of the national inventory was mainly pure and of high genetic value, reasons for which it was considered justifiable to establish an alternative criterion, based on the simultaneous fulfillment of two principles: 1) The phenotypic approximation to the pattern of the corresponding breed; 2) Production adjusted to 244 days and standardized, as explained: LA1 $0.5 \leq z < 1$; LA2 $1 \leq z < 1.5$; LA3 $1.5 \leq z$. From this process, the achievements have been to: a) recognize the conformation and origin of the national buffalo herd; b) incorporate productive female buffaloes into the Program; c) consider the environmental and management conditions of each herd;

Clasificación por producción: un criterio alternativo a la categorización

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RESUMEN

Al inicio del Programa de Control y Registro Genealógico, se encontró que los principales rebaños que conformaban el plantel nacional de cría de búfalos no contaban con los registros pertinentes para el apareamiento, a pesar de que la gran mayoría de este material biológico contaba con certificados de registro de su país de origen, inicialmente de Bulgaria e India y posteriormente de Brasil y Colombia. Al no existir una institución encargada del Libro Genealógico, con el tiempo la escasa información se fue perdiendo hasta el punto de resultar imposible recuperarla. Aplicar un criterio genealógico a través de cinco generaciones de mestizaje mediante mejora es imposible. Es decir, de la categoría LA a la categoría PO, con los productos intermedios LA1, LA2, LA3 y LA4. Sin embargo, era bien sabido que el origen del inventario nacional era principalmente puro y de alto valor genético, razones por las cuales se consideró justificable establecer un criterio alternativo, basado en el cumplimiento simultáneo de dos principios: 1) La aproximación fenotípica a el patrón de la raza correspondiente; 2) Producción ajustada a 244 días y estandarizada, según se explica: LA1 $0,5 \leq z < 1$; LA2 $1 \leq z < 1,5$; LA3 $1,5 \leq z$. De este proceso se ha logrado: a) Reconocer la conformación y origen de la manada bufalina nacional; b) Incorporar al Programa búfalas productivas; c) Considerar las condiciones ambientales y de

d) identify situations to improve both the weighing control report and its management.

Keywords: criterion for categorization, stud book, registered buffalo.

manejo de cada rebaño; d) Identificar situaciones a mejorar tanto en el informe de control de pesaje como en su gestión.

Palabras clave: criterio de categorización, libro genealógico, búfalo registrado.

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Estimating heritabilities and genetic correlations with parent-offspring regression using data from Anatolian water buffalo population raised in Istanbul for weights at different ages

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Estimación de heredabilidades y correlaciones genéticas mediante la regresión entre padres e hijos utilizando datos de la población de búfalos de agua de Anatolia criados en Estambul para pesos a diferentes edades

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ABSTRACT

Determining the breeding potential and direction of the population in terms of traits of interest depends on the genetic variation. Therefore, it is essential to know the genetic parameters of economic traits to improve yields in animal breeding. This study investigated the heritability and genetic correlations among growth traits in Anatolian buffaloes of İstanbul province. Estimating genetic parameters such as heritability of traits and genetic correlation among the traits in farm animal population populations is a major prerequisite to apply in animal genetic improvement programs. The material of this research was composed of the buffaloes included in the sub-project implemented in the Istanbul buffalo breeders' association within the scope of the "community-based water buffalo improvement programs" project under the coordination of the General Directorate of Agricultural Research and Policies. In the study, 1597 buffalo cows were obtained between 2012-2022, and the birth, six- and one-year-old weights recorded parents, and their offspring for the growth characteristics of the calf born from these cows were used. The analysis of variance was performed with the general linear model option to measure the effects of village, year, season, and sex factors on the data of individuals with the same characteristics in two generations. The effect of the factors determined to affect the examined characteristics significantly was eliminated using the standardization programs. The heritability estimates for birth, sixth month, and yearling weights were 0.37, 0.12, and 0.08±0.07, respectively. Genetic correlation among growth traits was generally significant and positive, ranging from 0.068 to 0.673. Consequently, the moderate to high estimates of heritability genetic correlations on growth traits showed some opportunities for genetic improvement in buffaloes of İstanbul.

Keywords: buffaloes, Istanbul, improvement program, genetic.

RESUMEN

La determinación del potencial reproductivo y la dirección de la población, en términos de las características de interés, depende de la variación genética. Por tanto, es fundamental conocer los parámetros genéticos de los rasgos económicos, para mejorar los rendimientos en la cría de animales. El objetivo de este estudio fue investigar la heredabilidad y las correlaciones genéticas entre los rasgos de crecimiento en los búfalos de Anatolia de la provincia de Estambul. La estimación de parámetros genéticos, como la heredabilidad y la correlación genética entre los caracteres en las poblaciones de animales de granja, es un requisito previo importante para aplicar en los programas de mejora genética animal. El material de esta investigación estuvo compuesto por los búfalos incluidos en el subproyecto implementado en la asociación de criadores de búfalos de Estambul dentro del alcance del proyecto "programa comunitario de mejora del búfalo de agua" bajo la coordinación de la Dirección General de Investigación y Políticas Agrícolas. En el estudio se utilizaron 1597 búfalas nacidas entre 2012-2022 y se estudiaron los pesos al nacimiento, seis y un año de edad, se registraron además, los pesos de los padres y sus crías, para determinar las características de crecimiento del ternero nacido de estas vacas. El análisis de varianza se realizó con la opción del modelo lineal general (GLM) para medir los efectos de los factores de finca, año, estación y sexo, sobre los datos de individuos con las mismas características en dos generaciones. Los resultados obtenidos indican que los factores evaluados afectaron significativamente las características examinadas, razón por la cual, se procedió a la estandarización de las mismas. Las estimaciones de heredabilidad para los pesos al nacer, al sexto mes y al año fueron 0,37; 0,12 y 0,08 ± 0,07 respectivamente. La correlación gené-

tica entre los rasgos de crecimiento fue significativa y positiva y osciló entre 0,068 y 0,673. Como consecuencia, los valores de moderados a altos de las heredabilidades y al igual que de las correlaciones genéticas, se concluye que existen en estos rebaños excelentes oportunidades para la mejora genética en los búfalos de Estambul.

Palabras clave: búfalos, Estambul, programa de mejoramiento genético.

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Evaluation of non-linear models for genetic parameters estimation of the growth curve of Nili-Ravi buffalo calves

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ABSTRACT

The study aimed to estimate the growth curve and find the best-fitting non-linear model for the Nili-Ravi buffalo calves. The pedigree and monthly body weight data for 6644 calves born from 2010-2020 in six herds maintained by the Govt. of Punjab were collected. Various non-linear models (Brody, Gompertz, Logistic, & Von Bertalanffy) were used to associate age with weight using the easyreg package in R (version 3.3.0). Model fitting criteria included adjusted coefficient of determination, Akaike's information criterion, Bayesian information criterion, and root-mean-square error. The model with the highest coefficient of determination and the lowest values for the other parameters was considered the best fit for defining the growth curve. The Brody model proved the best-fitted model, with the following values of curve parameters 0.6648 (R2adj), 627871.80 (AIC), 627908.10 (BIC), and 30.793 (RMSE), for the combined analysis. However, the values of R2adj, AIC, BIC, and RMSE for males were 0.6275, 321831.50, 321865.10, and 32.502, while for females, they were 0.7055, 305336.40, 305369.90, and 28.809, respectively. The growth curve parameters for the Brody model were

Evaluación de modelos no lineales para la estimación de parámetros genéticos de la curva de crecimiento de bucerros Nili-Ravi

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RESUMEN

El estudio tuvo como objetivo estimar la curva de crecimiento y encontrar el modelo no lineal que mejor se ajustó para los bucerros Nili-Ravi. Los datos de pedigrí y pesos corporales mensuales de 6644 terneros nacidos entre 2010 y 2020, en seis rebaños, mantenidos por el Gobierno de Punjab, fueron recolectados. Se utilizaron varios modelos no lineales (Brody, Gompertz, Logistic y Von Bertalanffy) para asociar la edad con el peso utilizando el paquete easyreg en R (versión 3.3.0). Los criterios de ajuste del modelo incluyeron el coeficiente de determinación ajustado, el criterio de información de Akaike, el criterio de información bayesiano y el error cuadrático medio. El modelo con el mayor coeficiente de determinación y los valores más bajos para los demás parámetros se consideró como el que mejor se ajustaba para definir la curva de crecimiento. El modelo de Brody demostró ser el modelo mejor ajustado, con los siguientes valores de los parámetros de la curva 0,6648 (R2adj), 627871,80 (AIC), 627908,10 (BIC) y 30,793 (RMSE), para el análisis combinado. Sin embargo, los valores de R2adj, AIC, BIC y RMSE para los machos fue-

943.99±101.38Kg (A), 0.96±0.004Kg (B), and 0.0005±0.00Kg (K) for all animals; 1160.21±251.89Kg, 0.97±0.006Kg, and 0.0004±0.00Kg for males, and 813.56±90.80Kg, 0.96±0.004Kg, and 0.0006±0.00Kg for females respectively. A higher K-value in females indicates early maturity compared with males in this breed, potentially due to management and additional feed supplementation provided to female calves as they were grown as replacement heifers. The heritability estimates for the growth curve traits in the Brody model were low, while the values of genetic correlations were higher than those of phenotypic correlations. The data revealed that Asymptotic weight (A) and birth weight (B) were positively correlated with each other, while the rate of maturity (K) was negatively correlated with initial and final body weights.

Keywords: Growth curve estimation, non-linear models, R-studio, Nili-Ravi buffalo calves.

ron 0.6275, 321831.50, 321865.10 y 32.502, mientras que para las hembras fueron 0.7055, 305336.40, 305369.90 y 28.809, respectivamente. Los parámetros de la curva de crecimiento para el modelo de Brody fueron 943,99±101,38Kg (A), 0,96±0,004Kg (B) y 0,0005±0,00Kg (K) para todos los animales; 1160,21±251,89Kg, 0,97±0,006Kg y 0,0004±0,00Kg para los machos, y 813,56±90,80Kg, 0,96±0,004Kg y 0,0006±0,00Kg para las hembras, respectivamente. Un valor K más alto en las hembras indica una madurez más temprana en comparación con los machos de esta raza, posiblemente debido al manejo y al suplemento alimenticio adicional proporcionado a las terneras a medida que crecían como bubillas de reemplazo. Las estimaciones de heredabilidad para los rasgos de la curva de crecimiento en el modelo de Brody fueron bajas, mientras que los valores de las correlaciones genéticas fueron más altos que los de las correlaciones fenotípicas. Los datos revelaron que el peso asintótico (A) y el peso al nacer (B) se correlacionaban positivamente entre sí, mientras que la tasa de madurez (K) se correlacionaba negativamente con los pesos corporales inicial y final.

Palabras clave: estimación de curvas de crecimiento, modelos no-lineales, R-studio, Bucerros Nili-Ravi.

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Body morphometric measurements of Azakheli buffaloes: A neglected animal genetic resource of Pakistan

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ABSTRACT

The current study aims to morphologically characterize the Azakheli buffalo breed and develop phenotypic standards for this neglected animal genetic resource of Pakistan, which is present in some specific regions of the country's northern areas. The body morphometric measurements of 220 adult (33 males and 187 females) animals were taken, in centimeters, for 26 different biometric parameters, including chest girth (CG), body length (BL), barrel depth (BD), height-at-withers (HW), hip height (HH), face length (FL), face width (FW), ear length (EL), ear width (EW), horn length (HL), horn circumference (HC), neck length (NL), neck width (NW), rump length (RL), rump width (RW), thurl width (TW), tail length (TL), tail circumference (TC), pin-bone distance (PBD), fore-teat length (FtL), fore-teat circumference (FtC), rear-teat length (RtL), rear-teat circumference (RtC), testes length (TesL), testes width (TesW), and

Mediciones morfométricas corporales de búfalos de Azakheli: un recurso genético animal olvidado en Pakistán

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RESUMEN

El presente estudio tuvo como objetivo caracterizar morfológicamente la raza de búfalo Azakheli y desarrollar estándares fenotípicos para este recurso zoogenético, olvidado de Pakistán que está presente en alguna región específica de las zonas del norte del país. Se tomaron medidas morfométricas corporales de 220 animales adultos (33 machos y 187 hembras), en centímetros, para 26 parámetros biométricos diferentes, incluidos la circunferencia del pecho (CG), la longitud del cuerpo (BL), la profundidad del tronco (BD) y la altura a la cruz. (HW), altura de la cadera (HH), longitud de la cara (FL), ancho de la cara (FW), longitud de la oreja (EL), ancho de la oreja (EW), longitud del cuerno (HL), circunferencia del cuerno (HC), longitud del cuello (NL), ancho del cuello (NW), largo de la grupa (RL), ancho de la grupa (RW), ancho de la garganta (TW), largo de la cola (TL), circunferencia de la cola (TC), distancia entre los huesos

scrotal circumference (SC) with the help of a measuring tape and scale. The data were subjected to descriptive and Pearson correlation analysis in R-studio. The values of mean plus-minus standard error for male morphometry were 183.43±1.140 (CG), 147.26±0.556 (BL), 69.85±0.523 (BD), 130.22±0.500 (HW), 125.60±0.558 (HH), 50.06±0.416 (FL), 24.38±0.325 (FW), 23.08±0.919 (EL), 16.10±0.289 (EW), 42.2402±0.957 (HL), 17.34±0.355 (HC), 55.93±0.403 (NL), 44.09±0.568 (NW), 42.59±0.309 (RL), 51.91±0.614 (RW), 46.83±0.708 (TW), 24.05±0.375 (PBD), 67.86±1.551 (TL), 17.19±0.292 (TC), 9.85±0.332 (TesL), 7.46±0.248 (TesW), and 19.32±0.584 (SC). Similarly, in females the values observed were 192.65±0.464 (CG), 145.89±0.424 (BL), 67.05±0.312 (BD), 129.41±0.353 (HW), 123.29±0.325 (HH), 49.63±0.279 (FL), 24.89±0.195 (FW), 23.87±0.266 (EL), 16.45±0.180 (EW), 46.58±0.523 (HL), 19.83±0.332 (HC), 57.25±0.325 (NL), 44.14±0.307 (NW), 43.02±0.261 (RL), 54.25±0.304 (RW), 49.58±0.363 (TW), 26.00±0.261 (PBD), 74.85±0.774 (TL), 17.14±0.254 (TC), 7.72±0.170 (FtL), 10.79±0.154 (RtL), 6.88±0.114 (FtC), 8.20±0.129 (RtC). The highest values of correlation coefficient observed were 0.893 for the FtL/FtC, 0.883 for the TesW/SC, 0.881 for the RtL/RtC, 0.856 for the FtL/RtL, 0.789 for the NL/NW and RW/TW, 0.624 for the NW/RW, 0.612 for the CG/HH, and 0.599 for the HH/BL. It is suggested that these data would help identify this buffalo breed's purebred animals for future breeding and selection programs.

Key words: morphometric measurements, Phenotypic correlation, Azakheli buffaloes, Pakistan.

(PBD), longitud de los pezones anteriores (FtL), circunferencia del pezón anterior (FtC), longitud del pezón posterior (RtL), circunferencia del pezón posterior (RtC), longitud de los testículos (TesL), ancho de los testículos (TesW) y circunferencia escrotal (SC) con la ayuda de una cinta métrica y una báscula. Los datos fueron analizados mediante estadística descriptiva y además se midió la correlación de Pearson, mediante el programa R-studio. Los valores obtenidos ± error estándar correspondieron en los machos a: 183,43 ± 1,140 (CG), 147,26 ± 0,556 (BL), 69,85 ± 0,523 (BD), 130,22 ± 0,500 (HW), 125,60 ± 0,558 (HH), 50,06 ± 0,416 (FL), 24,38±0,325 (FW), 23,08±0,919 (EL), 16,10±0,289 (EW), 42,2402±0,957 (HL), 17,34±0,355 (HC), 55,93±0,403 (NL), 44,09±0,568 (NO), 42,59±0,309 (RL), 51,91±0,614 (RW), 46,83±0,708 (TW), 24,05±0,375 (PBD), 67,86±1,551 (TL), 17,19±0,292 (TC), 9,85±0,332 (TesL), 7,46±0,248 (TesW) y 19,32±0,584 (SC). De igual forma, en las hembras los valores observados fueron 192.65±0.464 (CG), 145.89±0.424 (BL), 67.05±0.312 (BD), 129.41±0.353 (HW), 123.29±0.325 (HH), 49.63±0.279 (FL), 24.89±0.195 (FW), 23.87±0.266 (EL), 16.45±0.180 (EW), 46.58±0.523 (HL), 19.83±0.332 (HC), 57.25±0.325 (NL), 44.14±0.307 (NW), 43.02 ±0.261 (RL), 54.25±0.304 (RW), 49.58±0.363 (TW), 26.00±0.261 (PBD), 74.85±0.774 (TL), 17.14±0.254 (TC), 7.72±0.170 (FtL), 10.79±0.154 (RtL), 6.88 ± 0.114 (PtC), 8.20 ± 0.129 (RtC). Los valores más altos del coeficiente de correlación observados fueron 0,893 para FtL/FtC, 0,883 para TesW/SC, 0,881 para RtL/RtC, 0,856 para FtL/RtL, 0,789 para NL/NW y RW/TW, 0,624 para NW/RW, 0,612 para el CG/HH y 0,599 para el HH/BL. Se sugiere que estos datos ayudarían en la identificación de animales puros de esta raza de búfalo, y utilizarlos para diversos programas de cría y selección en el futuro.

Palabras clave: medidas morfométricas, correlación fenotípica, búfalos Azakheli, Pakistán.

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Genetic parameters of production and reproduction traits of Mediterranean buffaloes under tropical conditions

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ABSTRACT

In the tropics, there needs to be more information on Mediterranean buffaloes' productive and reproductive behavior. For this reason, the zootechnical performance of a herd of Mediterranean buffaloes in Puerto Concha, Zulia state, Ven-

Parámetros genéticos de características de producción y reproducción de búfalos mediterráneos en condiciones tropicales

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RESUMEN

En el trópico existe información limitada sobre el comportamiento productivo y reproductivo de los búfalos mediterráneos. Por tal motivo, se evaluó el desempeño zootécnico de un rebaño de búfalos mediterráneos ubicados en el poblado de

ezuela, was evaluated. The phenotypic behavior and genetic parameters of the characteristics of milk yield (MY), lactation length (LL), calving interval (CI), age at first calving (AFC), and peak lactation (PLAC) were analyzed. The evaluated data comes from the genetic center "Hacienda Caño Negro" and corresponds to the productive and reproductive records from 2004 to 2021, inclusive. Descriptive statistics and analysis of variance were carried out for the mentioned characters. The variance components and genetic parameters of MY, LL, CI, and AFC were estimated using the mixed model methodology with an animal model in the MTDFREML program. After filtering and validating records, the information of 2350 closed lactations in the 18 years under study was available. The unadjusted and adjusted average for MY was 1819.5±10.7 kg and 1864.0±10.1 kg, respectively. The adjusted mean for LL and CI was 290.5±3.9 days and 477.4±3.6 days, respectively. The AFC average was 38.5±1.2 months. The PLAC is reached on average at 36 days with 8 kg of milk. The PLAC is achieved at 48 days with an average of 6.8 kg of milk for the first calving buffaloes. Concerning the genetic analysis, 3142 pedigrees were read, and 3243 individuals made up the relationship matrix. There are 732 inbred animals, with an average consanguinity of 4.2%. The heritability coefficient estimates were 0.37, 0.13, 0.05, and 0.27 for MY, LL, CI, and AFC, respectively. The moderate heredity index obtained for total milk production indicates the possibility of genetic improvement of this trait through selection programs. At the same time, the low heritability estimate for the calving interval reveals that the greatest variation in reproductive traits can be due to non-genetic factors, so the strategies to be followed for the progress of these characters in the herd will obey more to improvements in their management and environmental conditions.

Keywords: genetic, heritability, milk, first calving, buffaloes.

Puerto Concha, estado Zulia, Venezuela. Se analizó el comportamiento fenotípico y los parámetros genéticos de las características de producción de leche (MY), duración de la lactancia (LL), intervalo entre partos (IC), edad al primer parto (AFC) y pico de lactancia (PLAC). Los datos evaluados provienen del centro genético "Hacienda Caño Negro" y corresponden a los registros productivos y reproductivos del 2004 al 2021, inclusive. Se realizaron estadísticas descriptivas y análisis de varianza para los caracteres mencionados. Los componentes de la varianza y los parámetros genéticos de MY, LL, CI, AFC se estimaron utilizando la metodología de modelo mixto con un modelo animal en el programa MTDFREML. Luego del proceso de depuración y validación de registros, se dispuso de la información de 2350 lactancias cerradas en los 18 años de estudio. Para MY, el promedio no ajustado y ajustado fue de 1.819,5±10,7 kg y 1.864,0±10,1 kg, respectivamente. La media ajustada para LL y CI fue 290,5±3,9 días y 477,4±3,6 días, respectivamente. El promedio de la AFC fue de 38,5±1,2 meses. El PLAC se alcanza en promedio a los 36 días, con 8 kg de leche. Para las búfalas de primer parto, el PLAC se logra a los 48 días con un promedio de 6,8 kg de leche. En relación al análisis genético se leyeron 3142 genealogías diferentes, un total de 3243 individuos que conformaron la matriz de parentesco (A). Se encontraron 732 animales consanguíneos (F), con un valor promedio del 4,2%. Las estimaciones del coeficiente de heredabilidad fueron 0,37, 0,13, 0,05, 0,27 para MY, LL, CI y AFC, respectivamente. El moderado índice de herencia obtenido para la producción total de leche indica la posibilidad de mejoramiento genético de este rasgo a través de programas de selección; mientras que el bajo valor de heredabilidad para el intervalo entre partos revela que la mayor variación en los rasgos reproductivos puede deberse a factores no genéticos, por lo que, las estrategias a seguir para el progreso de estas características debe estar dirigida a mejorar el manejo y las condiciones ambientales.

Palabras clave: genética, heredabilidad, leche, primer parto, búfalas.

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Genetic parameters for birth and weaning weight in a herd of Mediterranean buffaloes under tropical conditions

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ABSTRACT

The objectives of the present study were to estimate variance components and genetic parameters for birth weight (BW)

Parámetros genéticos para el peso al nacimiento y al destete en un rebaño de búfalos mediterráneos en condiciones tropicales

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RESUMEN

Los objetivos del presente estudio fueron estimar los componentes de la varianza y los parámetros genéticos para

and weaning weight (WW). The data comes from Mediterranean buffalo calves born during the period from August 2003 to December 2021 on the Livestock Farm "Hacienda Caño Negro", located near the population of Santa Barbara del Zulia, Venezuela. A total of 2225 records collected for BW and WW were included in the analysis. Variance components and genetic parameters were estimated by REML using the MTDFREML program, applying a bivariate animal model that includes direct, maternal, and permanent maternal effects. The population average BW of Buffalo calves was 42.7 ± 0.1 kg, whereas for males, 43.9 ± 0.2 and for females, 41.7 ± 0.2 kg. The population average for WW was 174.5 ± 0.9 kg. According to sex, the WW parameters were 178.5 ± 1.3 kg and 170.5 ± 1.1 kg in males and females, respectively. The direct heritability estimates were 0.03 and 0.23 for BW and WW, respectively, whereas maternal heritability values were 0.78 for BW and 0.24 for WW. The estimated total heritability for both characters was 0.23 (BW) and 0.32 (WW). The genetic correlation between BW and WW was 0.57, while the maternal genetic correlation for BW and WW was 0.01. The results indicated moderate total heritability value for BW and WW; further analysis is needed to estimate the genetic parameters, perhaps including other breed herds.

Keywords: genetic, heritability, birth, weaning, weight.

el peso al nacer (BW) y el peso al destete (WW) de un grupo de bucerros. Los datos provienen de crías de búfalos mediterráneos nacidas durante el período de agosto de 2003 a diciembre de 2021, en la Finca Ganadera "Hacienda Caño Negro", ubicada cerca de la población de Santa Bárbara del Zulia, Venezuela. Se incluyeron en el análisis un total de 2225 registros recopilados para BW y WW. Los componentes de varianza y los parámetros genéticos fueron estimados por REML utilizando el programa MTDFREML, aplicando un modelo animal bivariado que incluye efectos maternos directos and maternos permanentes. El peso corporal promedio de la población de los bucerros fue $42,7 \pm 0,1$ kg; mientras que, el de los machos fue $43,9 \pm 0,2$ y el de las hembras correspondió a $41,7 \pm 0,2$ kg. El promedio poblacional para WW fue $174,5 \pm 0,9$ kg. Según sexo los parámetros de PV fueron $178,5 \pm 1,3$ kg y $170,5 \pm 1,1$ kg en machos y hembras, respectivamente. Las estimaciones de heredabilidad directa fueron 0,03 y 0,23 para BW y WW, respectivamente, mientras que, los valores de heredabilidad materna fueron 0,78 para BW y 0,24 para WW. La heredabilidad total estimada para ambos caracteres fue de 0,23 (BW) y 0,32 (WW). La correlación genética entre BW y WW fue de 0,57, mientras que la correlación genética materna para BW y WW fue de 0,01. Los resultados indicaron un valor de heredabilidad total moderado para BW y WW; se necesitan análisis adicionales para la estimación de los parámetros genéticos, tal vez incluyendo otros rebaños de la raza.

Palabras clave: genética, heredabilidad, nacimiento, destete, peso.

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Phenotypic characteristics of indigenous dairy buffaloes of Bangladesh

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ABSTRACT

The utility of buffalo in Bangladesh has shifted from draught to dairy. People of Bangladesh are now aware of outstanding food value of buffalo milk and meat. However, the market share of buffalo milk and meat is very low compared to cattle milk and meat as evidence from the number of buffalo and cattle. Bangladesh possesses 0.64 million buffaloes and 295 million cattle. Dairy buffalo of the country has no recognized breed and is indigenous river type. In absence of animal

Características fenotípicas de las búfalas lecheras autóctonas de Bangladesh

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RESUMEN

La utilidad del búfalo en Bangladesh ha pasado de ser de trabajo a ser lechero. Los habitantes de Bangladesh son ahora conscientes del extraordinario valor alimentario de la leche y la carne de búfala. Sin embargo, la cuota de mercado de la leche y la carne de búfala es muy baja en comparación con la leche y la carne de ganado, como lo demuestra el número de búfalos y ganado. Bangladesh posee 0,64 millones de búfalos y 295 millones de cabezas de ganado. La búfala lechera del país no

identification and recording system, actual lactation period and yield of dairy buffalo is unknown. This study was, therefore, done to find out actual lactation period and yield as well calving interval of indigenous dairy buffaloes in Bangladesh based on animal recording. 384 indigenous dairy buffaloes having 3rd and 4th parity in 7 herds managed under traditional system in two agro-ecological zones covering 4 seasons was subjected to this study. All the cows and calves were ear tagged, date of calving and milk yield was recorded following International Committee for Animal Recording (ICAR) (2017). The actual lactation period, actual lactation yield and calving interval were found out from the record. 300-day lactation yield was calculated after ICAR (2017). Data was analyzed after R statistics to find out the differences among various effects on production. The actual lactation period and actual lactation yield were 267.28 ± 0.68 days and 749.36 ± 15.08 kg, respectively whereas 300-day lactation yield was 766.92 ± 15.32 kg. The minimum, maximum and average dry period were 74 days, 303 days and 185 ± 2.55 days. The minimum, maximum and average calving interval were 369 days, 547 days and 453 ± 5.64 days. Lactation period, lactation yield and calving interval were affected significantly by genotype, herd, season and agro-ecological zone. The results indicate that there is wide variation in lactation period, lactation yield and calving interval. This property can be utilized in practicing selective breeding for genetic improvement of indigenous dairy buffaloes in Bangladesh.

Keywords: Bangladesh, dairy buffalo, productive phenotypes.

tiene raza reconocida y es de tipo río autóctono. A falta de un sistema de identificación y registro de animales, se desconoce el período de lactancia real y el rendimiento de las búfalas lecheras. Por lo tanto, este estudio se realizó para determinar el período de lactancia real y el rendimiento, así como el intervalo entre partos de las búfalas lecheras autóctonas de Bangladesh, basándose en el registro de los animales. Se estudiaron 384 búfalas lecheras autóctonas de 3^o y 4^o parto en 7 rebaños manejados bajo el sistema tradicional en dos zonas agroecológicas que abarcaron 4 temporadas. A todas las búfalas y terneros se les marcaron las orejas, se registró la fecha del parto y la producción de leche siguiendo el Comité Internacional para el Registro Animal (ICAR) (2017). El período de lactancia real, el rendimiento real de la lactancia y el intervalo entre partos se determinaron a partir del registro. El rendimiento de la lactancia a 300 días se calculó según ICAR (2017). Los datos se analizaron a través del programa estadístico R para descubrir diferentes efectos sobre la producción. El período de lactancia real y el rendimiento de la lactancia real fueron $267,28 \pm 0,68$ días y $749,36 \pm 15,08$ kg, respectivamente, mientras que el rendimiento de la lactancia a 300 días fue de $766,92 \pm 15,32$ kg. El período seco mínimo, máximo y promedio fue de 74 días, 303 días y $185 \pm 2,55$ días. El intervalo entre partos mínimo, máximo y promedio fue de 369 días, 547 días y $453 \pm 5,64$ días. El período de lactancia, el rendimiento de la lactancia y el intervalo entre partos se vieron afectados significativamente por el genotipo, el rebaño, la estación y la zona agroecológica. Los resultados indican que existe una amplia variación en el período de lactancia, el rendimiento de la lactancia y el intervalo entre partos. Esta propiedad se puede utilizar en la práctica de la cría selectiva para el mejoramiento genético de búfalas lecheras autóctonas en Bangladesh.

Palabras clave: Bangladesh, búfala lechera, fenotipos productivos.

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Present status of buffalo breeding in Bangladesh

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ABSTRACT

Bangladesh possesses 0.64 million buffaloes. Genetic studies conducted by various scientists during the last 30 years indicate that these buffaloes are indigenous in nature and river type, except the buffaloes of the northeastern part of the country, which are swamp type. The milk yield and body weight of indigenous buffaloes are lower than those of recognized buffalo breeds. The body of adult buffalo ranges from 250 kg to 450 kg. There are approximately 12000 milking buffaloes. The milk yield of dairy buffaloes was 550 kg in 374 days. The outstanding food value of buffalo milk and meat is now well-recognized in the country. The government has come forward with private enterprises for the genetic improvement of indigenous dairy buffaloes of Bangladesh. The present buffalo breeding policy allows the maintenance of yielding buffalo breeds, viz., Murrah/Nili-Ravi/ Italian Mediterranean buffalo under an intensive management system and 50% X Murrah/Nili-Ravi/Italian Mediterranean buffalo under a semi-intensive or extensive management system. *Inter se* mating programs involving outcrossing will be practiced in both systems. Three nucleus herds with pure 281 Murrah buffaloes have been set in three government farms. Four more government buffalo farms with pure Murrah buffalo are being set. These farms will supply pure Murrah buffalo heifers to the dairy farmers who will maintain dairy buffaloes under an intensive management system. Department of Livestock Services, Bangladesh, and three private enterprises have been conducting an artificial system to produce 50% crossbred buffaloes since 2019. So far, four buffalo bull stations have been set to produce frozen semen in the country. Semen of 100% Murrah buffalo/Italian Mediterranean buffalo is being used for artificial insemination. So far, 16500 buffalo cows have been inseminated artificially. The conception rate is 38%, and 1855 crossbred progeny have been produced since June 2023. No crossbred has come to production yet. Animal identification and animal recording systems have been introduced at the farm and farmer levels to implement the *Inter se* mating program for the genetic improvement of dairy buffaloes.

Keywords: Bangladesh, buffalo, breeding practices.

Estado actual de la cría de búfalos en Bangladesh

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RESUMEN

Bangladesh posee 0,64 millones de búfalos. Los estudios genéticos realizados por diversos científicos durante los últimos 30 años indican que estos búfalos son de naturaleza indígena y de tipo de río, excepto los búfalos del noreste del país que son de tipo de pantano. La producción de leche y el peso corporal de las búfalas autóctonas son bajos en comparación con los de las razas de búfalas reconocidas. El peso de un búfalo adulto oscila entre 250 kg y 450 kg. Hay aproximadamente 12.000 búfalas lecheras. La producción de leche de las búfalas lecheras es de 550 kg en 374 días. El excepcional valor alimentario de la leche y la carne de búfala es ahora bien reconocido en el país. El gobierno ha presentado junto con empresas privadas un programa para la mejora genética de las búfalas lecheras autóctonas de Bangladesh. La actual política de cría de búfalos permite mantener una raza de búfalo de alto rendimiento, a saber, Murrah/Nili-Ravi/búfalo Mediterráneo italiano bajo un sistema de gestión intensiva y 50% X Murrah/Nili-Ravi/búfalo Mediterráneo italiano bajo un sistema de gestión semiintensivo o extensivo. En ambos sistemas se practicará un programa de apareamiento entre razas que implica cruces externos. Actualmente, se han establecido tres rebaños centrales con 281 búfalos Murrah puros en tres granjas gubernamentales. Se están estableciendo cuatro granjas de búfalos gubernamentales más con búfalos Murrah puros. Estas granjas suministrarán bubillas Murrah pura a los productores de leche, quienes mantendrán a las búfalas lecheras bajo un sistema de manejo intensivo. El Departamento de Servicios Ganaderos de Bangladesh y tres empresas privadas han estado implementando un sistema de inseminación artificial para producir un 50% de búfalos cruzados desde 2019. Hasta ahora se han instalado cuatro estaciones de toros de búfalo para producir semen congelado en el país. Para la inseminación artificial se utiliza semen 100% de búfalo Murrah/ búfalo Mediterráneo italiano. Hasta el momento se han inseminado artificialmente 16.500 búfalas. La tasa de concepción es del 38% y se han producido 1855 progenies cruzadas desde junio de 2023. Ningún cruce ha llegado a producción todavía. Se ha introducido un sistema de identificación

y registro de animales a nivel de granja y de granjero, para implementar el programa de apareamiento *Inter se* para el mejoramiento genético de búfalas lecheras.

Palabras clave: Bangladesh, búfalo, prácticas de manejo, mejoramiento genético.

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Evaluation of buffalo milk production in several states of Venezuela

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ABSTRACT

The buffalo is used as an efficient fodder converter in bovine production systems into milk and quality meat. To know the potential of milk production (MP) in Venezuela, a database was evaluated from 1992 to February 2023 from 36 farms (F) in 12 states of Venezuela, corresponding to 182,413 buffaloes, 198,056 lactations, and 1,440,448 milk controls. The factors evaluated were: ecological zone (EZ), farm (F), breed (B): Murrah predominance (Mu), Mediterranean predominance (Me) and undefined crossbreeding (UC), year of study (YS), number of calvings (NC), lactation duration (LD), dry days (DD) and maternal milk production (MMP). Analysis of variance (ANOVA) was used, using the statistical package SAS version 9, 1. The linear additive model used was:

$$y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + \beta_1(x_1 - \bar{x}_1) + \beta_2(x_2 - \bar{x}_2) + \beta_3(x_3 - \bar{x}_3) + e_{ijklmn}$$

where y_{ijklmn} = MP, μ = overall average, a_i = effect of ecological zone, b_j = breed effect, c_k = effect of year, d_l = farm effect, f_m = number of calvings, β_1 = regression coefficient for the lactation duration covariate, x_1 = covariate duration of lactation, \bar{x}_1 = mean for the values of the covariate duration of lactation, β_2 = regression coefficient for the dry days covariate, x_2 = covariate dry days, \bar{x}_2 = mean for the values of the covariate dry days, β_3 = regression coefficient for the milk production covariate, x_3 = covariate mother's milk production, \bar{x}_3 = mean for values of the covariate mother's milk production and e_{ijklmn} = residual error. The averages were calculated by least squares. The equation for regression analysis was: $y = 36,08x + 1068$ con un $R^2 = 0,8581$. The overall mean for MP was 1645.24 ± 550.12 kg, with a coefficient of variation of 33.25%. The average LD was 266.69 ± 11.19 days, with the lowest values observed in

Evaluación de la producción láctea bufalina, en algunos estados de Venezuela

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RESUMEN

En los sistemas de producción bovina, la búfala se utiliza como un eficiente convertidor de forraje en leche y carne de calidad. Con el objetivo de conocer el potencial de producción de leche (MP) en Venezuela, se evaluó una base de datos desde 1992 a febrero de 2023 de 36 fincas (F) en 12 estados de Venezuela, correspondientes a 182.413 búfalas, 198.056 lactancias y 1.440.448 controles de leche. Los factores evaluados fueron: zona ecológica (EZ), finca (F), raza (B): predominio Murrah (Mu), predominio mediterráneo (Me) y cruce indefinido (UC), año de estudio (YS), número de partos (NC), duración de la lactancia (LD), días secos (DD) y producción de leche materna (MMP). Se utilizó análisis de varianza (ANOVA), utilizando el paquete estadístico SAS versión 9, 1. El modelo aditivo lineal utilizado fue:

$$y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + \beta_1(x_1 - \bar{x}_1) + \beta_2(x_2 - \bar{x}_2) + \beta_3(x_3 - \bar{x}_3) + e_{ijklmn}$$

donde y_{ijklmn} = MP, μ = promedio general, a_i = efecto de la zona ecológica, b_j = efecto de la raza, c_k = efecto del año, d_l = efecto de la finca, f_m = número de partos, β_1 = coeficiente de regresión para la covariable de duración de la lactancia, x_1 = covariable duración de la lactancia, \bar{x}_1 = media de los valores de la covariable duración de la lactancia, β_2 = coeficiente de regresión para la covariable de días secos, x_2 = covariable de días secos, \bar{x}_2 = media de los valores de la covariable de días secos, β_3 = coeficiente de regresión para la covariable de producción de leche, x_3 = covariable de producción de leche materna, \bar{x}_3 = media de los valores de la covariable de producción de leche materna y e_{ijklmn} = error residual. Los promedios se calcularon por mínimos cuadrados. La ecuación para el análisis de regresión fue: $y = 36,08x + 1068$ con un $R^2 = 0,8581$. La media

first-calving buffaloes between 180 and 195 days; the highest LD was observed in eighth and ninth-calving buffaloes at 310 and 330 days. The average daily milk per lactation was 6.16 ± 1.80 with a CV of 29.22%, with values as low as 3.93 kg/day; the highest average was 7.35 kg/day, but values higher than 10 kg/day/buffalo were also observed in the data analyzed. The factors EZ, F, NC, LD, DD, and MMP were statistically significant ($p < 0.05$). Therefore, it is concluded that these factors cause variability in MP. Factor B was not statistically significant ($p > 0.05$).

Keywords: buffaloes, dairy production, genetic factors, non-genetic factors.

global de MP fue de 1645,24 ± 550,12 kg, con un coeficiente de variación de 33,25%. La LD promedio fue: 266.69 ± 11.19 días, observándose los valores más bajos en búfalas de primer parto entre los 180 y 195 días, la LD más alta se observó en búfalas de octavo y noveno parto entre 310 y 330 días. El promedio de leche diario por lactancia fue de 6,16 ± 1,80 con un C.V. de 29,22%, con valores tan bajos como 3,93 kg/día; el promedio más alto fue de 7,35 kg/día, pero también se observaron valores superiores a 10 kg/día/búfalo en los datos analizados. Los factores EZ, F, NC, LD, DD y MMP fueron estadísticamente significativos ($p < 0.05$), por lo que se concluye que estos factores causan variabilidad en MP. El factor B no fue estadísticamente significativo ($p > 0,05$).

Palabras clave: búfalos, producción de leche, factores genéticos, factores no-genéticos.

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Percentage of consanguinity in Murrah buffaloes in the Zulia state of Venezuela

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ABSTRACT

Consanguinity (F) is the product of mating between animals that have one or more ancestors in common. Increased F can have positive effects but has many adverse effects, such as decreased fertility, increased embryonic losses and fetal death, decreased growth rates, decreased milk and meat production, and decreased milk and meat quality. For a given trait, genetic gain depends on genetic variation within a population. Therefore, since F reduces genetic variation, potential genetic progress may decrease. It is necessary to know the percentage F of individuals and the average F of the herd to detect problems caused by F and to be able to plan non-inbred crosses in the population. The estimation of the percentage of F was carried out with the genealogical records of the Río Lindo and Manantiales farms, belonging to Agropecuaria Mega 21 located in the Baralt municipality (Menegrande) of the state of Zulia-Venezuela. A total of 520 animals were registered in the database from September 2014 to January 2023. The herd composition consisted of 6 father buffaloes, corresponding to 1% of the herd, 108 mother buffaloes, corresponding to 21% of the herd, and 406 animals with known fathers representing 78% of the herd. F coefficients were estimated by the tabular method, which

Porcentaje de consanguinidad en un rebaño Murrah, en el estado Zulia, Venezuela

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RESUMEN

La consanguinidad (F) se define como el producto del apareamiento entre animales que tienen uno o más ancestros en común. El aumento de F puede tener efectos positivos, pero tiene muchos efectos adversos, como disminución de la fertilidad, aumento de las pérdidas embrionarias y muerte fetal, disminución de las tasas de crecimiento, disminución de la producción de leche y carne, y disminución de la calidad de la leche y la carne. Para un rasgo determinado, la ganancia genética depende de la variación genética dentro de una población. Por lo tanto, dado que F reduce la variación genética, el progreso genético potencial puede disminuir en el futuro. Es necesario conocer el porcentaje F de individuos, y el F medio del rebaño, para detectar problemas provocados por F y poder así planificar cruces no endogámicos en la población. La estimación del porcentaje de F se realizó con los registros genealógicos de las fincas Río Lindo y Manantiales, pertenecientes a Agropecuaria Mega21 ubicadas en el municipio Baralt (Mene Grande) del estado Zulia-Venezuela. Un total de 520 animales fueron registrados en la base de datos, desde septiembre de 2014 hasta enero de 2023. La composición del rebaño estuvo compuesta por: 6 búfalos padres correspondientes al 1%

uses the parentage matrix (Matrix A) coefficients to calculate consanguinity. The mathematical formula used to calculate the F coefficients was:

$$F_{ii} = a_{ii} - 1$$

where F_{ii} is the F coefficient for each individual, and a_{ii} is the coefficient of the diagonal of Matrix A for each animal. The database evaluated came from the information in the field notebooks, which are registered in the Gansoft® software, then migrated to Excel, where the information was edited, and finally, the BVOLS genetic evaluation web system (a statistical analysis system applied to animal genetic improvement) was used to calculate the consanguinity. The average percentage of consanguinity of the herd was 0.096%, a value very close to 0, which indicates that in this population of animals, the crossbreeding has been carried out in a directed way to avoid mating between related animals, the tiny percentage of consanguinity found in the herd is the product of the realization of three planned crossbreeding (inbreeding). It can be concluded that the mating program of the studied herd has been effective since the F values are very close to zero, so no adverse effects on productive characteristics are expected.

Keywords: buffaloes, consanguinity, Murrah.

del rebaño; 108 búfalas madres correspondientes al 21% del rebaño y 406 animales de padre conocido que representan el 78% del rebaño. Los coeficientes F se estimaron mediante el método tabular, que utiliza los coeficientes de la matriz de parentesco (Matriz A) para calcular la consanguinidad. La fórmula matemática utilizada para calcular los coeficientes C fue:

$$F_{ii} = a_{ii} - 1$$

donde F_{ii} es el coeficiente F para cada individuo y a_{ii} es el coeficiente de la diagonal de la Matriz A (parentesco) para cada animal. La base de datos evaluada surgió de la información de los cuadernos de campo, los cuales se registran en el software Gansoft®, luego se migró a Excel donde se editó la información y finalmente se utilizó el sistema web de evaluación genética BVOLS (sistema de análisis estadístico aplicado al mejoramiento genético animal). para calcular la consanguinidad. El porcentaje medio de consanguinidad del rebaño fue del 0,096%, valor muy cercano a 0, lo que indica que en esta población de animales el cruzamiento se ha realizado de forma dirigida para evitar el apareamiento entre animales emparentados, siendo muy pequeño el porcentaje de consanguinidad. encontrado en el rebaño es producto de la realización de tres cruces planificados (endogamia). Se puede concluir que el programa de apareamiento del rebaño estudiado ha sido efectivo, ya que los valores de F son muy cercanos a cero, por lo que no se esperan efectos negativos sobre las características productivas.

Palabras clave: búfalos, consanguinidad, Murrah.

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Heritability for IBMI index of Italian buffaloes used in artificial insemination

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ABSTRACT

The Italian National Association of Buffalo Breeders "ANASB", as of February 2018, introduced innovations, and according to these considerations, the indexes for productive and morphological traits have been calculated using a multi-character model that replaces the IQM index. The IBMI index aims to obtain better animals both from the productive point of view and their morphological and functional characteristics. The IBMI is a value that can be heritable. Since it is a product of productive

Heredabilidad del índice IBMI de búfalos italianos utilizados en inseminación artificial

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RESUMEN

La Asociación Nacional Italiana de Criadores de Búfalos cuya sigla "ANASB", implementó a partir de febrero de 2018, nuevas innovaciones en su índice de selección, de la raza mediterránea, según estas consideraciones, los índices de caracteres productivos y morfológicos se calcularon utilizando un modelo multicarácter que vino a reemplazar el anterior índice IMQ. El índice IBMI tiene como objetivo obtener mejores animales tanto desde el punto de vista productivo como de sus

and morphofunctional characteristics evaluations, it is crucial to consider its heritability (h^2) to know how many of the differences observed in the IBMI can be inherited. Eighty-three IBMI data of buffaloes from the semen catalog, which had self-identification and known parents, were analyzed. The number of parents in the analysis was 29, and the number of animals evaluated was 86. The following linear statistical model calculates the heritability index (h^2) and its variance components:

$$y_{ij} = \mu + s_i + e_{ij}$$

where y_{ij} = IBMI, μ = herd average, s_i = effect of the animal (breeding buffalo) and e_{ij} = residual error. The mathematical formula was used to estimate the h^2 of IBMI:

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_e^2}$$

where h^2 = heritability, σ_s^2 = variance between progeny of different fathers (buffalo) and σ_e^2 = variance of progeny of the same buffalo. The restricted maximum likelihood method (REML) was used to estimate the σ_s^2 and σ_e^2 components. The SAS statistical package version 9.1 was used for the statistical calculations, using the MIXED procedure. The h^2 for IBMI was 23.7%. The overall mean for IBMI-Reproducers (catalog) was 119.98 ± 16.24 with a coefficient of variation of 13.53%. The mean for IBMI-Parents was 113.26 ± 19.21 . The mean for IBMI-Mothers was 123.05 ± 14.72 . The mean for IBMI-Paternal Grandparents was 107.80 ± 16.63 . The mean for IBMI-Paternal Grandmothers was 111.54 ± 15.39 . The mean for IBMI-Maternal Grandparents was 122.03 ± 19.21 . The mean for IBMI-Maternal Grandmothers was 113.73 ± 11.60 . Based on the results obtained, an h^2 was estimated for IBMI of 23.7%, which is a low heritability (< 25%), i.e., differences in IBMI among animals are mostly due to non-genetic factors, indicating that by performing selection processes for animals with higher IBMI, a favorable but moderate selection response will be obtained in new generations.

Keywords: IBMI index, Mediterranean buffalo, heritability.

características morfológicas y funcionales. El IBMI es un valor que puede ser heredable y al ser producto de evaluaciones de características productivas y morfo-funcionales, es importante tomar en consideración su heredabilidad (h^2), con el fin de saber cuántas de las diferencias observadas en el IBMI se puede heredar. Se analizaron ochenta y tres datos de IBMI de búfalos del catálogo de semen, que tenían auto-identificación y padres conocidos. El número de progenitores en el análisis fue 29 y el número de animales evaluados fue 86. Para calcular el índice de heredabilidad (h^2) y sus componentes de varianza se utilizó el siguiente modelo estadístico lineal:

$$y_{ij} = \mu + s_i + e_{ij}$$

donde y_{ij} = IBMI, μ = promedio del rebaño, s_i = efecto del animal (búfalo reproductor) y e_{ij} = error residual. Para estimar el h^2 del IBMI se utilizó la fórmula matemática:

$$h^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_e^2}$$

donde h^2 = heredabilidad, σ_s^2 = varianza entre descendencia de diferentes padres (búfalo) y σ_e^2 = varianza de descendencia del mismo búfalo. Se utilizó el método de máxima verosimilitud restringida (REML) para estimar los componentes σ_s^2 y σ_e^2 . Para los cálculos estadísticos se utilizó el paquete estadístico SAS versión 9.1, mediante el procedimiento MIXED. El h^2 del IBMI fue del 23,7%. La media global para IBMI-Reproductores(catálogo) fue de $119,98 \pm 16,24$ con un coeficiente de variación del 13,53%. La media del IBMI-Padres fue $113,26 \pm 19,21$. La media del IBMI-Madres fue $123,05 \pm 14,72$. La media del IBMI-Abuelos Paternos fue de $107,80 \pm 16,63$. La media del IBMI-Abuelas Paternas fue de $111,54 \pm 15,39$. La media del IBMI-Abuelos Maternos fue $122,03 \pm 19,21$. La media del IBMI-Abuelas Maternas fue de $113,73 \pm 11,60$. Con base en los resultados obtenidos, se estimó un h^2 para IBMI de 23.7%, lo cual es una heredabilidad baja (< 25%), es decir, las diferencias en IBMI entre animales se deben en su mayoría a factores no genéticos, lo que indica que al realizar procesos de selección para se deben seleccionar individuos con mayor IBMI se obtendrá una respuesta favorable pero moderada en las nuevas generaciones.

Palabras claves: IBMI, búfalos mediterráneos, heredabilidad.

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Repeatability for weight at birth in Murrah buffaloes in the Zulia state of Venezuela

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ABSTRACT

To improve livestock breeding, aspects such as genetic evaluation must be included, and clear objectives must be set for the selection of animals to be future breeders. In this way, the estimation of repeatability (R) and its use in the most probable production capacity allows the detection of the best females, considering their performance and productive life. Birth weight (BW) is a character that has importance in the future performance of the animal. Therefore, strategies should be applied to improve this character. Thus, the application of the calculation of R is a strategy that should be implemented as a genetic improvement strategy; for this purpose, 257 BW records of the offspring of 108 buffaloes of the Murrah breed of the Mega 21 farm located in the Baralt municipality (Menegrande) of Zulia-Venezuela state were analyzed. The calves (birth weight) were weighed in the first 24 hours of the animal's life. The following linear additive mixed effects model was used to calculate R and its variance components for BW:

$$y_{ijklm} = \mu + d_i + b_j + c_k + d_l + e_{ijklm}$$

where y_{ijklm} = birth weight, μ = herd average, d_i = effect of the buffalo (mother), b_j = effect sex of the animal, c_k = effect of year of birth, d_l = effect of season of birth and e_{ijklm} = error residual. The following mathematical formula was used to estimate the R value:

$$R = \frac{\sigma_d^2}{\sigma_d^2 + \sigma_e^2}$$

where R = repeatability, σ_d^2 = variance of BW of the calves from different dams (inter-calf variance) and σ_e^2 = variance of BW of the calves of each buffalo (within buffalo variance). To estimate the components σ_d^2 and σ_e^2 the restricted maximum likelihood method (REML) was used. Statistical calculations were performed using the statistical package SAS version 9.1 using the MEANS and MIXED procedures. The mean BW for the herd was: 36.05 kg with a coefficient of variation (C.V.) of 12.82%. The R-value for BW in this herd was low: 5.435%; therefore, the correlation between BW records from calves of the same dam

Repetibilidad del peso al nacer en búfalos Murrah del estado Zulia de Venezuela

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RESUMEN

Con el objeto de mejorar la cría de ganado se deben incluir aspectos como la evaluación genética y fijar objetivos claros para la selección de los animales que serán futuros reproductores. De esta manera, la estimación de la repetibilidad (R) y su utilización en la capacidad productiva más probable, el cual permite detectar las mejores hembras, teniendo en cuenta su desempeño y vida productiva. El peso al nacer (BW) es un carácter que tiene importancia en el desempeño futuro del animal, por lo tanto, se deben aplicar estrategias para mejorar este carácter, por lo tanto, la aplicación del cálculo de R, es una estrategia que se debe implementar como mejoramiento genético; para ello se analizaron 257 registros de PC de las crías de 108 búfalos de la raza Murrah del establecimiento Mega21 ubicado en el municipio Baralt (Mene grande) del estado Zulia-Venezuela. El pesaje de los terneros (nacer) se realizó en las primeras 24 horas de vida del animal. Para calcular R y sus componentes de varianza, para BW, se utilizó el siguiente modelo lineal aditivo de efectos mixtos:

$$y_{ijklm} = \mu + d_i + b_j + c_k + d_l + e_{ijklm}$$

donde y_{ijklm} = peso al nacer, μ = promedio del rebaño, d_i = efecto de la búfala (madre), b_j = efecto sexo del animal, c_k = efecto del año de nacimiento, d_l = efecto de la estación de nacimiento y e_{ijklm} = error residual. Se utilizó la siguiente fórmula matemática para estimar el valor R:

$$R = \frac{\sigma_d^2}{\sigma_d^2 + \sigma_e^2}$$

donde R = repetibilidad, σ_d^2 = varianza del BW de los terneros de diferentes madres (varianza entre búfalos) y σ_e^2 = varianza del BW de los terneros de cada búfalo (varianza dentro del búfalo). Para estimar los componentes σ_d^2 y σ_e^2 se utilizó el método de máxima verosimilitud restringida (REML). Los cálculos estadísticos se realizaron utilizando el paquete estadístico SAS versión 9.1 utilizando los procedimientos MEANS y MIXED. El peso corporal medio del rebaño fue: 36,05 kg con un coeficiente de variación (C.V.) de 12,82%. El valor R para

is low, suggesting that a large number of data would be needed to evaluate the performance of a buffalo. The fixed effects (sex, season of birth, and year of birth) had a significant effect on BW ($p < 0.05$). In this population, several records of a buffalo's calves would be needed to correctly predict the BW of a future calf because R is low.

Keywords: buffaloes, repeatability, murrh, birth weight.

BW en este rebaño fue bajo: 5,435%, por lo tanto, la correlación entre los registros de BW de crías de la misma madre es baja, lo que sugiere que se necesitaría una gran cantidad de datos para evaluar el desempeño de un búfalo. Los efectos fijos (sexo, época y año de nacimiento) tuvieron un efecto significativo sobre el peso al nacer ($p < 0,05$). Se puede concluir que en esta población se necesitarían varios registros de crías de una búfala para predecir correctamente el peso corporal de una futura cría, debido a que R es bajo.

Palabras clave: búfalos, repetibilidad, Murrh, peso al nacer.

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Morfometric evaluation of young buffalo males in two Argentinean herds

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ABSTRACT

Bubaline production has increased substantially during the last few years, mainly in the northeastern provinces of Argentina. Exploring biometric parameters on bovine productive traits has driven the development of techniques for greater productivity and reproductive efficiency. This work aimed to evaluate live weight (LW), body score (BS), thoracic perimeter (TP), animal (cross, AH) sacrum height (SH), and scrotal circumference (SC) in 12-month-old buffalo males located in two farms in Corrientes Province (Itatí and Empedrado), Argentina. Linear regression and correlation between variables were evaluated. Forty males of the Mediterranean breed, 20 from each herd, were employed. Descriptive statistics and analysis of variance were performed, and the herd was considered a source of variation in a Randomized Block Design. The degree of linear association was verified by Pearson's test, and linear regression analysis was applied to all the variables explored; Infostat software 2020 was used. The results obtained were LW 323,65±7,01 and 365,5±6,84 k, BS 3,28±0,1 and 3,63±0,1 cm, TP 163,65±1,62 and 169,13±2,56 cm, AH 117,7±0,90 and

Evaluación morfométrica de machos jóvenes de búfalo en dos rebaños argentinos

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RESUMEN

La producción de búfalos ha aumentado sustancialmente durante los últimos años principalmente en las provincias del noreste de Argentina. La exploración de parámetros biométricos sobre rasgos productivos bovinos ha impulsado el desarrollo de técnicas en busca de una mayor eficiencia productiva y reproductiva. El objetivo de este trabajo fue evaluar: peso vivo (PV), puntuación corporal (BS), perímetro torácico (TP), altura del animal (cruz, AH), altura del sacro (SH) y circunferencia escrotal (SC) en búfalos machos de 12 meses de edad, ubicados en dos fincas de la Provincia de Corrientes (Itatí y Empedrado), Argentina. Se evaluó la regresión lineal y la correlación entre variables. Se emplearon 40 machos de raza mediterránea, 20 de cada rebaño. Se realizó estadística descriptiva y análisis de varianza, se consideró el rebaño como fuente de variación en un Diseño de Bloques Al Azar. El grado de asociación lineal se verificó mediante la prueba de Pearson y se aplicó análisis de regresión lineal a todas las variables exploradas, se utilizó el software Infostat 2020. Los resultados obtenidos fueron LW 323,65±7,01 y 365,5±6,84 k, BS 3,28±0,1 y 3,63±0,1 cm, TP

125,15±1,37 cm, SH 123,58±1,35 and 126,98±2,13 cm, SC 19,28±0,44 and 21,15±0,69 cm for Empedrado and Itatí respectively. In the farm located in Itatí, the males showed higher LW, TP, HH, and SC values. A significant correlation between LW and TP and LW with AH, SH, and SC was observed in both herds. There were significant values of Pearson's equation in the correlation between BS and SC in the animals from the Empedrado locality and when considering the animals from both locations, but not in those from the Itatí establishment. The correlation values between BS and SC were 0.37 for the locality of Empedrado, 0.28 for the animals from the locality of Itatí, and 0.33 when evaluating all animals together. It is concluded that exploring morphometric variables from an early age allows the follow-up of the growth and development of the buffalo males selected as future bulls, highlighting the significant correlation of scrotal circumference with the morphometric variables explored. Agroecological characteristics in the Itatí locality were more favorable, possibly translated into a greater body and isometric development of the testicles in the buffaloes of this area.

Keywords: buffaloes, body development, non-genetic factors, variation.

163,65±1,62 y 169,13±2,56 cm, AH 117,7±0,90 y 125,15±1,37 cm, SH 123,58±1,35 y 126,98±2,13 cm, SC 19,28±0,44 y 21,15±0,69 cm para Empedrado e Itatí, respectivamente. En la finca ubicada en Itatí, los machos presentaron mayores valores de LW, TP, HH y SC. En ambos rebaños se observó una correlación significativa entre LW y TP y LW con AH, SH y SC. Hubo valores significativos de la ecuación de Pearson en la correlación entre BS y SC en los animales de la localidad de Empedrado y al considerar los animales de ambas localidades, pero no en los del establecimiento Itatí. Los valores de correlación entre BS y SC fueron de 0,37 para la localidad de Empedrado, 0,28 para los animales de la localidad de Itatí y 0,33 al evaluar a todos los animales en conjunto. Se concluye que la exploración de variables morfométricas desde edades tempranas permite el seguimiento del crecimiento y desarrollo de los búfalos machos seleccionados como futuros toros, destacando la correlación significativa de la circunferencia escrotal con las variables morfométricas exploradas. Las características agroecológicas son más favorables en la localidad de Itatí, lo que posiblemente se tradujo en un mayor desarrollo corporal e isométrico de los testículos en los búfalos de esta zona.

Palabras clave: búfalos, desarrollo corporal, factores no genéticos, variación.

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Correlation of the internal genetic evaluation and the ANASB genomic index of a buffalo herd in Venezuela

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ABSTRACT

In a buffalo herd in Venezuela, absorption crosses to the Mediterranean breed have been carried out for 30 years. The semen used in the artificial insemination programs comes from Italy. Each year, the buffalo used are selected based on the available germplasm supply. A Pearson correlation analysis was conducted to determine the degree of association between the traits evaluated in the ANASB (Associazione Nazionale Allevatori Specie Bufalina) genomic index and the internal genetic evaluation carried out within the herd under study. The estimated genetic values of 10 buffalo with evaluated progeny were used; the number of offspring born (♂ and ♀) for each

Correlación entre la evaluación genética local y el índice genómico ANASB en un rebaño de búfalos en Venezuela

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RESUMEN

En un rebaño de búfalos en Venezuela se llevan realizando cruces de absorción con la raza mediterránea desde hace 30 años. El semen utilizado en los programas de inseminación artificial procede de Italia. Cada año se seleccionan los búfalos que se utilizarán en función del suministro de germoplasma disponible. Razón por la cual, se realizó un análisis de correlación de Pearson para determinar el grado de asociación entre los rasgos evaluados en el índice genómico de la ANASB (Associazione Nazionale Allevatori Specie Bufalina) y la evaluación genética interna realizada dentro del rebaño en estudio. Se utilizaron los valores genéticos estimados de 10

of them ranged from 12 to 154, and the number of completed lactations of their daughters was from 10 to 249 for the buffalo with the lowest and highest number of records of their daughters, respectively. The correlation analysis considered the estimated breeding value for weight at birth (WB), weaning weight adjusted to 270 days (W270), and milk production per lactation (MPL). The 2023 Buffalo and Bull Genomic Index of the ANASB, the RESA, and IgBMI indices were obtained for these same buffalo, in addition to the genetic estimates for milk (M), % protein (%P), % fat (%F), extremities (feet - EF) and mammary gland (MG). The correlation between MPL and M was high (0.80) and ($p \leq 0.01$). Negative correlations were obtained between MPL and %P, %F, and RESA with values of -0.25, -0.13, and -0.22, respectively. None of them present statistical significance. Although positive, the correlation between MPL with IgBMI, EF, and MG was low and insignificant (0.17, 0.03, and 0.16, respectively). The correlations of WB with the IgBMI and EF were 0.59 and 0.65, respectively ($p \leq 0.05$). The WB and W270 correlation were 0.76 ($p \leq 0.05$). The ranking of the ten buffalo by their estimated genetic value for milk in both tests, the first and second place coincided, and the last three. The data suggested that Mediterranean buffalo semen can be selected based on genetic indices for milk generated in Italy.

Keywords: correlation, evaluation, genomic, buffalo.

búfalas con descendencia evaluada, el número de crías nacidas (♂ y ♀) para cada una de ellas osciló entre 12 y 154 y el número de lactancias cerradas de sus hijas fue de 10 a 249, para la búfala con menor y mayor número de registros de sus hijas, respectivamente. El análisis de correlación consideró el valor genético estimado para el peso al nacer (WB), el peso al destete ajustado a 270 días (W270) y la producción de leche por lactancia (MPL). Para estos mismos búfalos se obtuvieron el Índice Genómico de Búfalo y Toro 2023 de la ANASB, los índices RESA e IgBMI, además de las estimaciones genéticas para leche (M), % proteína (%P), % grasa (%F), extremidades (pies - EF) y glándula mamaria (MG). La correlación entre MPL y M fue alta (0,80) y ($p \leq 0,01$). Se obtuvieron correlaciones negativas entre MPL y %P, %F, RESA con valores de -0,25, -0,13, -0,22 respectivamente. Ninguno de ellos presenta significación estadística. La correlación entre MPL con IgBMI, EF, MG, aunque positiva, fue baja y no significativa (0,17, 0,03 y 0,16, respectivamente). Las correlaciones del WB con el IgBMI y la FE fueron de 0,59 y 0,65, respectivamente, ambas ($p \leq 0,05$). La correlación WB y W270 fue de 0,76 ($p \leq 0,05$). En la clasificación de las 10 búfalas por su valor genético estimado para la leche, en ambas pruebas coincidieron el primer y segundo lugar, así como los tres últimos. Los datos sugirieron que el semen de búfala del Mediterráneo puede seleccionarse basándose en índices genéticos de la leche generada en Italia.

Palabras clave: correlación, evaluación, genómica, búfalo.

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Maximizing udder health through a selection index: a focus on udder traits in Italian Mediterranean Buffaloes

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ABSTRACT

The Italian Mediterranean Buffalo is the most important buffalo's breeds for milk production and its derivatives, which has a selection program implemented more than 20 years ago. The selection is based on an aggregate index that includes dairy, mozzarella yield, and functional traits. The improvement of udder conformation (UC) traits is an aspect that plays an essential role in udder health and longevity; it is of great interest due to its direct relationship with economic losses caused by mastitis or poor milkability. The aim of this study was the development of an aggregate selection index (ASI) considering

Maximizar la salud de la ubre a través de un índice de selección: un enfoque en las características de la ubre en los búfalos mediterráneos italianos

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RESUMEN

El Búfalo Mediterráneo Italiano es la raza bufalina más importante para la producción de leche y sus derivados, que cuenta con un programa de selección implementado hace más de 20 años. La selección se basa en un índice agregado que incluye lácteos, rendimiento de mozzarella y rasgos funcionales. La mejora de los rasgos morfológicos de la ubre (CU) es un aspecto que juega un papel esencial para la salud y longevidad de la ubre, es de gran interés por su relación directa con las pérdidas económicas provocadas por mastitis o mala ordeñabilidad. El objetivo de este estudio fue el desarrollo de un índice

MP (milk production), MSCS150 (geometric mean of somatic cell score at 150 days in milk), and UC traits (fore udder attachment– FUA; rear udder width– RUW; rear legs height– RLH; teat direction – TD; teat length – TL and teat position – TP). The first step was to estimate the genetic (co)variances between the UC, MP, and MSCS150 traits and to estimate the relative weight for each UC trait. The phenotypic records of 15,275 females and a pedigree with 43,395 animals were used. A multi-trait animal model that included CG (herd-year-calving season), calving month, and parity as fixed effects, and the animal as a random effect was used. The estimated heritability for MP and MSCS150 were 0.391 and 0.134, respectively, and varied between 0.11 and 0.23 for UC traits. Low negative genetic correlations were observed between MP and RLH (–0.119), the same pattern between MSCS150 and RLH (–0.350) and MSCS150 and FUA (–0.220). A positive correlation was found between FUA and MP (0.4272) and MP and RUW (0.645). Using ASI as selection criteria, an annual reduction of –0.03 is expected for MSCS150 and an increase of 0.26 kg for MP. During a selection period of ten years, the expected cumulative genetic gains would be –0.32 for MSCS150 and an increase of MP in 2.57 kg. Selective breeding programs aim to improve specific traits over time by selecting individuals with desirable genetic characteristics as parents of the next generation. According to the results, by focusing on RUW, RLH, and TD traits, genetic improvements are expected to lead to reduced severity of MSCS150 while maintaining or increasing milk production.

Keywords: udder health, genetic selection, Italian Mediterranean buffalo.

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ce de selección agregado (ASI) considerando MP (producción de leche), MSCS150 (media geométrica del puntaje de células somáticas a los 150 días en leche) y rasgos UC (inserción de la ubre anterior – FUA; ancho de la ubre trasera). – RUW; altura de las patas traseras – RLH; dirección de los pezones – TD; longitud de los pezones – TL y posición de los pezones – TP). El primer paso fue estimar las (co)varianzas genéticas entre los rasgos UC, MP y MSCS150, y estimar el peso relativo de cada rasgo UC. Se utilizaron los registros fenotípicos de 15.275 hembras y un pedigrí con 43.395 animales. Se utilizó un modelo animal multirasgo que incluyó CG (rebaño-año-época de parto), mes de parto y paridad como efectos fijos, y el animal como efecto aleatorio. La heredabilidad estimada para MP y MSCS150 fue 0,391 y 0,134, respectivamente, y varió entre 0,11 y 0,23 para los rasgos UC. Se observaron correlaciones genéticas negativas bajas entre MP y RLH (–0,119), el mismo patrón entre MSCS150 y RLH (–0,350) y MSCS150 y FUA (–0,220). Se encontró una correlación positiva entre FUA y MP (0,4272) y también entre MP y RUW (0,645). Utilizando ASI como criterio de selección, se espera una reducción anual de –0,03 para MSCS150 y un aumento de 0,26 kg para MP. Durante un periodo de selección de diez años, las ganancias genéticas acumuladas esperadas serían –0,32 para MSCS150 y un aumento de MP en 2,57 kg. Los programas de cría selectiva tienen como objetivo mejorar rasgos específicos a lo largo del tiempo seleccionando individuos con características genéticas deseables como padres de la próxima generación. Según los resultados, al centrarse en los rasgos RUW, RLH y TD, se espera que las mejoras genéticas conduzcan a una reducción de la gravedad del MSCS150 y al mismo tiempo mantengan o aumenten la producción de leche.

Palabras clave: Salud de la ubre, selección genética, búfalo mediterráneo italiano.

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On-farm molecular epidemiology and therapeutic insights into multidrug-resistant *Staphylococcus aureus* isolated from bubaline mastitis

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ABSTRACT

Staphylococcus aureus (*S. aureus*) is a pathogen of veterinary and public health concern; majorly responsible for bubaline mastitis and huge farm economic losses. The current study, aimed to investigate the on-farm epidemiology, associated antibiotic resistance and possible resistance modulation strategies against *S. aureus* isolated from bubaline mastitis. *S. aureus* methicillin and vancomycin-resistant (MRSA and VRSA) from buffalo (n = 384) herds of district Bahawalpur and Rahim yar khan, Pakistan were identified based on bacteriological, biochemical, and molecular confirmation of *nuc*, *mecA*, and *vanb* genes, respectively. The antibiotic susceptibility profiling of *S. aureus* isolates was done by Kirby-Bauer's disc diffusion method. The resistant antibiotics were combined with non-antibiotics (NSAIDs and ivermectin) using well diffusion method and checkerboard assay to evaluate resistance modulation. The risk factor analysis was done by chi-square and logistic regression analysis to find out the potential risk factors associated with mastitis in buffalo herds. The results found 50.26% and 38.02% samples positive for subclinical mastitis and *S. aureus*, respectively. Among local isolates, 62.5% and 20.83% isolates were confirmed MRSA and VRSA, respectively. The final logistic regression analysis revealed that host-related risk factors like (udder depth, teat pathology) and farm management risk factors (udder hygiene during milking, hock score, post-milking disinfection, and dry cow therapy) were significantly associated with the occurrence of sub-clinical mastitis in buffaloes. Antibiotic resistance profiling of local isolates showed significantly ($p < 0.05$) higher resistance to gentamicin, oxytetracycline, and cotrimoxazole. The antimicrobial effects

Epidemiología molecular en granja y conocimientos terapéuticos sobre *Staphylococcus aureus* multirresistente aislado de mastitis bubalina

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RESUMEN

Staphylococcus aureus (*S. aureus*) es un patógeno de preocupación veterinaria y de salud pública; principal responsable de la mastitis bubalina y de enormes pérdidas económicas en las granjas. El presente estudio tuvo como objetivo investigar la epidemiología en granja, la resistencia a los antibióticos asociada y las posibles estrategias de modulación de la resistencia contra *S. aureus* aislado de mastitis bubalina. Se identificó *S. aureus*, resistente a metilina y vancomicina (MRSA y VRSA) de manadas de búfalos (n = 384) del distrito de Bahawalpur y Rahim yar khan, Pakistán, basándose en la confirmación bacteriológica, bioquímica y molecular de *nuc*, *mecA* y *vanb*. genes, respectivamente. El perfil de susceptibilidad a los antibióticos de los aislados de *S. aureus* se realizó mediante el método de difusión en disco de Kirby-Bauer. Los antibióticos resistentes se combinaron con no antibióticos (AINE e ivermectina) utilizando el método de difusión en pozo y el ensayo de tablero de damas para evaluar la modulación de la resistencia. El análisis de factores de riesgo se realizó mediante chi-cuadrado y análisis de regresión logística para conocer los posibles factores de riesgo asociados con la mastitis en rebaños de búfalos. Los resultados encontraron que el 50,26% y el 38,02% de las muestras eran positivas para mastitis subclínica y *S. aureus*, respectivamente. Entre los aislamientos locales, el 62,5% y el 20,83% de los aislamientos fueron confirmados MRSA y VRSA, respectivamente. El análisis final de regresión logística reveló que los factores de riesgo relacionados con el huésped, como (profundidad de la ubre, patología de los pezones) y factores de riesgo del manejo de la granja (higiene de la ubre durante el ordeño, puntuación del corvejón,

of non-antibiotics against study isolates showed higher zones of inhibition for meloxicam followed by flunixin, ketoprofen, and ivermectin. The combinations of resistant antibiotics with non-antibiotics showed that the combinations of amoxicillin/meloxicam, cotrimoxazole/flunixin, cotrimoxazole/ketoprofen, and gentamicin/flunixin on well diffusion method and cotrimoxazole/flunixin, amoxicillin/ketoprofen, and gentamicin/flunixin on checkerboard assay revealed synergistic interactions. The study concluded that *S. aureus*, MRSA, and VRSA is an emerging and prevailing causes of bubaline mastitis in dairy farms in Pakistan. The increasing antibiotic resistance in *S. aureus* can be modulated by combining the resistant antibiotics with NSAIDs, especially flunixin and ketoprofen.

Keywords: buffalo, antibiotic resistance, mastitis, resistance modulation, risk factors.

desinfección post-ordeño y terapia de vaca seca) se asocia significativamente con la aparición de mastitis subclínica en búfalos. El perfil de resistencia a los antibióticos de los aislados locales mostró una resistencia significativamente mayor ($p < 0,05$) a la gentamicina, la oxitetraciclina y el cotrimoxazol. Los efectos antimicrobianos de los no antibióticos contra los aislados del estudio mostraron zonas de inhibición más altas para el meloxicam, seguido del flunixin, el ketoprofeno y la ivermectina. Las combinaciones de antibióticos resistentes con no antibióticos mostraron que las combinaciones de amoxicilina/meloxicam, cotrimoxazol/flunixin, cotrimoxazol/ketoprofeno y gentamicina/flunixin en el método de difusión en pozo y cotrimoxazol/flunixin, amoxicilina/ketoprofeno y gentamicina/flunixin en el ensayo de tablero de damas revelaron interacciones sinérgicas. El estudio concluyó que *S. aureus*, MRSA y VRSA son causas emergentes y predominantes de mastitis bubalina en las granjas lecheras de Pakistán. La creciente resistencia a los antibióticos en *S. aureus* se puede modular combinando los antibióticos resistentes con AINE, especialmente flunixin y ketoprofeno.

Palabras clave: búfalo, resistencia a antibióticos, mastitis, modulación de resistencia, factores de riesgo.

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Vaccination with Delta-PGM in the control of buffalo (*Bubalus bubalis*) brucellosis

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ABSTRACT

Brucellosis in buffalos represents an unresolved problem for consolidating the species in Venezuela. In 2020, a control program started in 5 associated farms with an estimated > 20% prevalence. The program focused on: vaccination with Delta-PGM (Agropharma Laboratories, Buenos Aires, Argentina), 2ml subcutaneous injection in 3- to 8- month females, aiming to secure two dosages before the service (2nd dosage seven months after the first), and detection/elimination of the infected through serial diagnosis with buffered plate antigen test (BPA, Agropharma Laboratories, Buenos Aires, Argentina) and fluorescence polarization assay (FPA, Biotehnika Laboratories, Serbia). The objectives included showing the vaccine's pro-

Vacunación con Delta-PGM en el control de la brucelosis del búfalo (*Bubalus bubalis*)

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RESUMEN

La brucelosis en búfalos representa un problema aún no resuelto para la consolidación de la especie en Venezuela. En 2020 se inició un programa de control en 5 fincas asociadas con una prevalencia estimada de > 20%. El programa se centró en: vacunación con Delta-PGM (Laboratorios Agropharma, Buenos Aires, Argentina), inyección subcutánea de 2ml en hembras de 3 a 8 meses, con el objetivo de asegurar dos dosis antes del servicio (segunda dosis 7 meses después de la primera); y detección/eliminación de infectados mediante diagnóstico seriado con prueba de antígeno en placa tamponada (BPA, Laboratorios Agropharma, Buenos Aires, Argentina) y ensayo de fluorescencia polarizada (FPA, Laboratorios Bioteh-

tection capacity against the disease in natural conditions with important naturally occurring challenges (prevalence > 20%) and differentiating infected from vaccinated animals (DIVA) with the previously described diagnostic test. In October 2022, diagnostic evaluation was carried out in 1,340 replacement females from this system, vaccinated or not, and divided into four groups according to body weight (G1: less than 170 kg; G2: between 171 and 220; G3: from 221 to 270; G4: above 270 kg). The data were analyzed through Friedman's non-parametric statistical test, allowing comparisons between the observed characters. Ninety animals (90) without vaccination were detected, belonging to groups G1 and G2, one being positive for brucellosis (1/90; 1.11%; $p>0.05$). Of the total evaluated population, 47 were reactive to BPA (47/1340; 3.5%), and only 16 were confirmed positive for brucellosis with FPA (16/1340; 1.2%; $p<0.05$). Of them, 8 (50%) belonged to G2 and another 8 (50%) to G3, without detection in G1 or G4, showing statistical differences ($p<0.05$). Regarding the vaccination and positivity correlation, it is noteworthy that 14 (14/1160; 1.20%) of the positives for brucellosis had received primary vaccination and a booster; none with two boosters was identified as positive ($p<0.05$). On the other hand, it stands out that three positives had a history, as they came from mothers reported to be reactive to BPA. Concerning the origin of the positives, the majority (68.8%) belonged to two problem farms ($p<0.05$). In conclusion, under the studied conditions, a high efficiency of the established control model was verified, which was associated with the protective capacity of the Delta-PGM strain vaccine. Using two applications prior to service, this model resulted in a significant reduction of infections in relation to their dams ($p<0.05$). It also showed that Delta-PGM allows differentiation between infected, vaccinated, and healthy animals (DIVA); therefore, its use does not lead to any diagnostic confusion when using BPA and/or FPA.

Keywords: buffalo brucellosis, Delta-PGM, control by vaccination.

hnika, Serbia). Los objetivos se basaron en mostrar la capacidad de protección de la vacuna contra la enfermedad en condiciones naturales con importante desafío natural (prevalencia > 20%); y diferenciar animales infectados de vacunados (DIVA) con la prueba diagnóstica descrita anteriormente. En octubre de 2022, se realizó evaluación diagnóstica a 1.340 hembras de reposición de este sistema, vacunadas o no, y divididas en cuatro grupos según el peso corporal (G1: menos de 170 kg; G2: entre 171 y 220; G3: de 221 a 270; G4: por encima de 270 kg). Los datos fueron analizados mediante la prueba estadística no paramétrica de Friedman, permitiendo comparaciones entre los caracteres observados. Se detectaron noventa animales (90) sin vacunación, pertenecientes a los grupos G1 y G2, siendo uno positivo a brucelosis (1/90; 1,11%; $p>0,05$). Del total de la población evaluada, 47 fueron reactivos al BPA (47/1340; 3,5%), y sólo 16 se confirmaron positivos a brucelosis con FPA (16/1340; 1,2%; $p<0,05$). De ellos, 8 (50%) pertenecían al G2 y otros 8 (50%) al G3; sin detección en G1 o G4; mostrando diferencias estadísticas ($p<0,05$). En cuanto a la correlación vacunación y positividad, destaca que 14 (14/1160; 1,20%) de los positivos a brucelosis habían recibido vacunación primaria y un refuerzo. Ninguno con dos refuerzos fue identificado como positivo ($p<0,05$). Por otro lado, se destaca que 3 positivos tenían antecedentes, pues provenían de madres reportadas como reactivas al BPA. En relación al origen de los positivos, la mayoría (68,8%) pertenecía a dos fincas problemáticas ($p<0,05$). En conclusión, bajo las condiciones estudiadas se verificó una alta eficiencia del modelo de control establecido, lo que se asoció a la capacidad protectora de la vacuna cepa Delta-PGM. Este modelo, utilizando dos aplicaciones previas al servicio, resultó en una reducción significativa de contagios en relación a sus madres ($p<0,05$). También, muestra que Delta-PGM permite diferenciar entre animales infectados, vacunados y sanos (DIVA); por lo que su uso no conduce a ningún tipo de confusión diagnóstica al utilizar BPA y/o FPA.

Palabras clave: brucelosis bufalina, Delta-PGM, control mediante vacunación.

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Serum calcium and its relationship with immunoglobulin G (IgG) levels in prepartum buffaloes from the Venezuelan humid tropics

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ABSTRACT

The immune system partially recognizes specific antigens due to the role of calcium (Ca²⁺) as an intracellular second messenger that regulates the humoral immune response. To consider a physiological association between serum Ca²⁺ levels and its modulatory effect on immunoglobulin G (IgG) synthesis before calving in dairy buffaloes (*Bubalus bubalis*) in southern Lake Maracaibo, Venezuela, twenty clinically healthy Murrah x Mediterranean crossbred female buffaloes with excellent body condition were considered for this study. Ten were young females of 33.6±14.15 months, with nine months of gestation and 432±44.27 kg body weight. The other ten were adult buffaloes of 563±53.6 kg body weight at nine months of gestation and reproductive history of four calvings. The sampling consisted of collecting 6 milliliters (mL) of blood by jugular venipuncture in tubes without anticoagulant; then, the serum was separated by centrifugation at 3,300 r.p.m. for 20 minutes at 25 °C using a BOECO®-Germany centrifuge. Next, 1 mL of serum was taken to determine the serological parameters under study. Ca²⁺ was found in the blood through the Arsenazo III method using a Selectra ProsS ELITech®-Netherlands Analyzer and, to quantify the levels of IgG; the immunoturbidimetry technique was used with the Cobas c311 Roche® Analyzer®-USA. The data were subjected to the Kruskal-Wallis nonparametric test and Spearman correlation analysis. According to the results obtained, primiparous *B. bubalis* females close to parturition presented serum Ca²⁺ levels (8.03±0.25 mg/dL) similar to multiparous buffaloes (8.07±0.7 mg/dL) adjusted to the reference range (6.05-11.98 mg/dL) for water buffaloes. In contrast, IgG in young females was higher (761.33±21.81 mg/dL) concerning adult buffaloes (735.83±17.39 mg/dL) with no statistically significant differences ($p>0.05$) between groups for both variables; however, the correlation test indicated that, in first calving buffaloes, the circulating Ca²⁺ level is negatively associated ($r=-0.56$) with IgG concentration, a physiological response that

Calcio sérico y su relación con los niveles de inmunoglobulina G (IgG) en búfalas parto del trópico húmedo venezolano

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RESUMEN

El sistema inmunológico reconoce antígenos específicos en parte debido al papel del calcio (Ca²⁺) como segundo mensajero intracelular que regula la respuesta inmune humoral. Para considerar una asociación fisiológica entre los niveles séricos de Ca²⁺ y su efecto modulador sobre la síntesis de inmunoglobulina G (IgG) antes del parto en búfalas lecheras (*Bubalus bubalis*) en el sur del Lago de Maracaibo, Venezuela, se consideraron veinte búfalas cruzadas Murrah x Mediterráneo clínicamente sanas y con excelente condición corporal. Diez eran hembras jóvenes de 33,6±14,15 meses de edad, con nueve meses de gestación y 432±44,27 kg de peso corporal. Las otras diez fueron búfalas adultas de 563±53,6 kg de peso corporal, con nueve meses de gestación y antecedentes reproductivos de cuatro partos. El muestreo consistió en recolectar 6 mililitros (mL) de sangre mediante venopunción yugular en tubos sin anticoagulante; luego el suero se separó mediante centrifugación a 3.300 r.p.m. durante 20 minutos a 25 °C utilizando una centrifuga BOECO®-Alemania. Luego se tomó 1 mL de suero para determinar los parámetros serológicos en estudio. Ca²⁺ se encontró en sangre mediante el método Arsenazo III utilizando un Analizador Selectra ProsS ELITech®-Netherlands y, para cuantificar los niveles de IgG, se utilizó la técnica de inmunoturbidimetría con el Cobas c311 Roche® Analyzer®-USA. Los datos fueron sometidos a la prueba no paramétrica de Kruskal-Wallis y al análisis de correlación de Spearman. Según los resultados obtenidos, las hembras primíparas cercanas al parto presentaron niveles séricos de Ca²⁺ (8,03±0,25 mg/dL) similares a las búfalas multíparas (8,07±0,7 mg/dL) ajustados al rango de referencia (6,05-11,98 mg/dL) para los búfalos de agua, mientras que la IgG en hembras jóvenes fue mayor (761,33±21,81 mg/dL) respecto a los búfalos adultos (735,83±17,39 mg/dL) sin diferencias estadísticamente significativas ($p>0,05$) entre grupos para ambas variables. Sin embargo, la prueba de correlación indicó que,

was statistically different ($p < 0.05$) concerning multiparous buffaloes ($r = 0.83$). There is evidence of an association between serum calcium concentration and IgG levels that depends on the number of calvings, possibly because different metabolic pathways for Ca^{2+} exchange determine the immune response among the categories of buffaloes included in the study. Thus, the functional significance of Ca^{2+} signaling for the parturition *B. bubalis* immune system is just beginning to emerge.

Key words: *Bubalus bubalis*, IgG, B lymphocyte, immune response, cell signaling.

en búfalas primíparas, el nivel de Ca^{2+} circulante se asocia negativamente ($r = -0,56$) con la concentración de IgG, respuesta fisiológica que fue estadísticamente diferente ($p < 0,05$) con respecto a las búfalas multiparas ($r = 0,83$). Existe evidencia de una asociación entre la concentración de calcio sérico y los niveles de IgG que depende del número de partos, posiblemente porque existen diferentes vías metabólicas para el intercambio de Ca^{2+} que determinan la respuesta inmune entre las categorías de búfalas incluidas en el estudio. Por lo tanto, la importancia funcional de la señalización de Ca^{2+} para el sistema inmunológico parto de *B. bubalis* apenas está comenzando a emerger.

Palabras clave: *Bubalus bubalis*, IgG, linfocitos B, respuesta inmune, señalización celular.

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Molecular characterization and therapeutic insights into biofilm positive *Staphylococcus aureus* isolated from subclinical mastitis in dairy buffaloes

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ABSTRACT

To investigate the prevalence and molecular characterization of biofilm-positive *Staphylococcus aureus* (*S. aureus*) isolates from a total of 384 quarter milk samples of buffaloes from 25 dairy farms in Faisalabad and Rawalpindi, Pakistan, a convenient sampling method was used. The sampling was performed per the National Mastitis Council, USA standard procedure. The study also highlighted the *in vitro* resistance pattern of biofilm-positive *S. aureus* and the role of commonly used NSAIDs and non-antibiotics in modulating the *S. aureus*-associated antibiotic resistance. Based on the California mastitis test, the results depicted the prevalence of subclinical mastitis in buffaloes as 50.26% (193/384). In comparison, the prevalence of *S. aureus* was noted to be 38.02%, out of which 25.79% isolates were biofilm-positive based on the Congo red agar, microtitre plate test, and presence of the *icaA* gene. Phylogenetic analysis of the studied isolates showed a high similarity with Egyptian and Indian *icaA*-positive *S. aureus* isolates. The findings of antibiogram profiling showed that biofilm-positive isolates showed higher resistance against oxacillin (87.5%), followed by trimethoprim/ sulfamethoxazole (79.16%), gentamicin (75%), oxytetracycline (66.66%), and cefoxitin (62.5%), while fusidic acid and linezolid were among the least resistant antibiotics

Caracterización molecular y conocimientos terapéuticos sobre *Staphylococcus aureus* positivo a biopelículas aislado de mastitis subclínica en búfalas lecheras

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RESUMEN

El presente estudio tuvo como objetivo investigar la prevalencia y caracterización molecular de aislamientos de *Staphylococcus aureus* (*S. aureus*) positivos para biopelículas de un total de 384 muestras de cuartos de leche de búfalas de 25 granjas lecheras del distrito de Faisalabad y Rawalpindi, Pakistán, utilizando un método de muestreo conveniente. El muestreo se realizó según el procedimiento estándar del Consejo Nacional de Mastitis, EE. UU. El estudio también destacó el patrón de resistencia *in vitro* mostrado por *S. aureus* con biopelículas positivas y el papel de los AINE y los no antibióticos de uso común para modular la resistencia a los antibióticos asociada a *S. aureus*. Los resultados mostraron que la prevalencia de mastitis subclínica en búfalos, según la prueba de mastitis de California, era del 50,26% (193/384), mientras que la prevalencia de *S. aureus* era del 38,02%, de los cuales el 25,79% de los aislados eran biofilm positivos. basado en agar rojo Congo, prueba en placa de microtitulación y presencia del gen *icaA*. El análisis filogenético de los aislados estudiados mostró una alta similitud con los aislados de *S. aureus* positivos para *icaA* egipcios e indios. Los hallazgos del perfil de antibiograma mostraron que los aislados positivos para biopelículas mostraron mayor resistencia contra oxacilina (87,5%), seguida de trimetoprim/sulfametoxazol (79,16%), gentamicina

against biofilm-positive isolates. The combinations of resistant antibiotics with non-antibiotics were investigated using the well diffusion method and checkerboard assay. Antimicrobial effects of non-antibiotics against study isolates accessed through the well diffusion method showed higher inhibition zones for meloxicam followed by flunixin, ketoprofen, and ivermectin. The combinations of amoxicillin/meloxicam, cotrimoxazole/flunixin, cotrimoxazole/ ketoprofen, and gentamicin/flunixin on well diffusion method, and cotrimoxazole/ flunixin, amoxicillin/ketoprofen and gentamicin/flunixin on checkerboard assay revealed synergistic interactions. The study concluded that biofilm-positive *S. aureus* is an emerging and prevailing cause of bubaline mastitis in dairy farms of the study area. The increasing antibiotic resistance of *S. aureus* can be modulated by combining the resistant antibiotics with NSAIDs, especially flunixin and ketoprofen, in *in vitro* trials. However, further research on *in vivo* trials needs to be conducted to evaluate the actual efficacy of these combinations in animal models.

Keywords: antibiotic resistance biofilm, bovine mastitis, resistance modulation, *Staphylococcus aureus*.

(75%), oxitetraciclina (66,66%) y cefoxitina (62,5%), mientras que el ácido fusídico y el linezolid se encontraban entre los antibióticos menos resistentes contra los aislados positivos para biopelículas. Las combinaciones de antibióticos resistentes con no antibióticos se investigaron utilizando el método de difusión de pozo y el ensayo de tablero de damas. Los efectos antimicrobianos de los no antibióticos contra los aislados del estudio a los que se accedió mediante el método de difusión de pozo mostraron zonas de inhibición más altas para el meloxicam seguido de flunixin, ketoprofeno e ivermectina. Las combinaciones de amoxicilina/meloxicam, cotrimoxazol/flunixin, cotrimoxazol/ketoprofeno y gentamicina/flunixin en el método de difusión de pozo y cotrimoxazol/flunixin, amoxicilina/ketoprofeno y gentamicina/flunixin en el ensayo de tablero de damas revelaron interacciones sinérgicas. El estudio concluyó que *S. aureus* positivo a biopelículas es una causa emergente y predominante de mastitis bubalina en las granjas lecheras del área de estudio. La creciente resistencia a los antibióticos de *S. aureus* puede modularse combinando los antibióticos resistentes con AINE, especialmente flunixin y ketoprofeno, en ensayos *in vitro*. Sin embargo, es necesario realizar más investigaciones en ensayos *in vivo* para evaluar la eficacia real de estas combinaciones en modelos animales.

Palabras clave: biofilm de resistencia a antibióticos, mastitis bovina, modulación de resistencia, *Staphylococcus aureus*.

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Anatomical and pathological findings in long-lived female *Bubalus bubalis*

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ABSTRACT

The objective of this work was to describe the anatomical and pathological findings in female, long-lived *Bubalus bubalis* from Portuguesa state, Venezuela. The age of 10 females was determined using the dental chronometry technique. Rectal palpation was performed to detect pregnancy. The udder integrity was evaluated, and days open between pregnancies were calculated. The animals were sent to slaughter. The ages of buffaloes were between 9 and 19 years old, and none of them were pregnant. In addition, 30% presented anomalies in the nipples and had a mean of 252 days open. At the slaughterhouse, the organs were collected: lung, mediastinal lymph

Hallazgos anatómicos y patológicos en hembras longevas de *Bubalus bubalis*

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ABSTRACT

El objetivo de este trabajo fue describir los hallazgos anatómicos y patológicos en hembras longevas de *Bubalus bubalis* del estado Portuguesa, Venezuela. Se determinó la edad de 10 hembras mediante la técnica de cronometría dental. Se realizó palpación rectal para detectar preñez. Se evaluó la integridad de la ubre y se calcularon los días abiertos entre gestaciones. Los animales fueron enviados al matadero. Las edades de las búfalas oscilaron entre 9 y 19 años y ninguna estaba preñada. Además, el 30% presentó anomalías en los pezones y tenían una media de 252 días abiertos. En el

nodes, heart, liver, kidney, spleen, and uterus. A 10% buffered formaldehyde solution was used for fixing and transporting the tissues. Later on, histological slices and routine HE stains were performed for examination using an optical microscope. The observed lesions, in percentage (%), were the following: chronic lymphoproliferative pneumonia (60), chronic lymphohistiocytic hyperplastic pericarditis (90), concentric cardiac hypertrophy (90), sarcocystosis (20) and lymphoid hyperplasia in the mediastinal lymph node (40). In the spleen: hemosiderosis (40), hemorrhage (40), lymphoid depletion (40), chronic multifocal non-suppurative hepatitis (70). In the kidney: interstitial lymphoplasmacytic membrane-proliferative glomerulonephritis (60), polycystic kidney (40), parenchymal atrophy (40), and non-suppurative chronic interstitial nephritis (90). In the reproductive organs: mild non-suppurative chronic cervicitis (70), mild non-suppurative chronic endometritis with severe glandular atrophy (70), oviduct fibroplasia (50), ovarian atrophy (80) and persistent corpus luteum (20). Severe restrictive pericarditis, which led to cardiac remodeling as a progressive response to acute or chronic damage of an etiology not studied in this investigation, accompanied by hypertrophy of the ventricles, were changes in the organ's shape that could have compromised its functioning. These anatomopathological findings in long-lived female *Bubalus bubalis* propose the need to study long-lived animals that are sacrificed or die naturally to obtain information on organ damage that can contribute to establishing the basis for the possible causes of low productivity and fertility. This species, considered resistant to tropical diseases, is resilient enough to survive up to 19 years, some with severe damage to vital organs. Finally, this research raises the need to keep productive, reproductive, and health records during the animal's life, accompanied by results of routine tests carried out by qualified personnel.

Keywords: anatomical & pathological, buffalo, long-lived buffalo, buffaloes.

matadero se recogieron los órganos: pulmón, ganglios linfáticos mediastínicos, corazón, hígado, riñón, bazo y útero. Se utilizó una solución tamponada de formaldehído al 10% para fijar y transportar los tejidos. Posteriormente, se realizaron cortes histológicos y tinciones HE de rutina para examinarlos mediante un microscopio óptico. Las lesiones observadas, en porcentaje (%) fueron las siguientes: neumonía linfoproliferativa crónica (60), pericarditis hiperplásica linfohistiocítica crónica (90), hipertrofia cardíaca concéntrica (90), sarcocistosis (20) e hiperplasia linfoide en el ganglio linfático mediastino (40). En el bazo: hemosiderosis (40), hemorragia (40) y depleción linfoide (40), hepatitis crónica multifocal no supurativa (70). En el riñón: glomerulonefritis proliferativa de membrana linfoplasmocítica intersticial (60), riñón poliquistico (40), atrofia parenquimatosa (40) y nefritis intersticial crónica no supurativa (90). En los órganos reproductores: cervicitis crónica leve no supurativa (70), endometritis crónica leve no supurativa con atrofia glandular severa (70), fibroplasia de oviductos (50), atrofia ovárica (80) y cuerpo lúteo persistente (20). Pericarditis restrictiva severa, que condujo a un remodelado cardíaco como respuesta progresiva a un daño agudo o crónico de etiología no estudiada en esta investigación, acompañada de hipertrofia de los ventrículos, eran cambios en la forma del órgano que podrían haber comprometido su funcionamiento. Estos hallazgos anatomopatológicos en hembras longevas bufalinas plantean la necesidad de estudiar animales longevos que son sacrificados o mueren naturalmente para obtener información sobre daño orgánico que pueda contribuir a establecer las bases de las posibles causas de la baja productividad y subfertilidad. Esta especie, considerada resistente a enfermedades tropicales, es resiliente para sobrevivir hasta 19 años, algunos con severos daños en órganos vitales y finalmente esta investigación plantea la necesidad de llevar registros productivos, reproductivos y de salud durante la vida del animal, acompañados de resultados de pruebas rutinarias realizadas por personal cualificado.

Palabras clave: anatomo-patológico, búfalos, búfalo longevo.

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Assessment of infectious diseases in commercial frozen semen straws of water buffalo bulls. Preliminary results

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ABSTRACT

Different infectious diseases in water buffaloes may be disseminated through semen, such as *Leptospira* spp. The objective of this investigation was to determine the presence of

Evaluación de enfermedades infecciosas en pajuelas comerciales de semen congelado de toros de búfalo de agua. Resultados preliminares

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RESUMEN

A través del semen se pueden transmitir diferentes enfermedades infecciosas en los búfalos de agua, como *Leptospira* spp. El objetivo de esta investigación fue determinar la presen-

antibodies against the Infectious Bovine Rhinotracheitis (IBR), Bovine Viral Diarrhea Virus (BVDV) and *Neospora caninum*, as well as the contamination with *Leptospira* spp. in frozen semen from water buffalo bulls (*Bubalus bubalis*). For this, 14 straws from seven farms in the states of Barinas (farm C and D), Cojedes (farm A, F and G), Mérida (farm B) and Zulia (farm E), Venezuela, were evaluated. The animals from farms A, B, C, E and F were vaccinated against IBR and BVDV; farms D, and G did not vaccinate against these diseases. The detection of antibodies against the gB glycoprotein of the IBR virus and the p80 protein of the BVDV and against *Neospora caninum* was performed using the ELISA technique according to the detection kit manufacturer IDvet and IDEXX specifications. In addition, semen was cultured in Ellinghausen McCullough, Johnson and Harris medium (EMJH), which is selective to isolate *Leptospira* spp. The presence of antibodies against IBR was detected in 28,6% of the straws evaluated (farm: A 1/2; D 1/1; F 1/3 and G 1/2); however, on farm A and F the animals were vaccinated 180 days before semen collection and the antibodies detected could come from the secretion of the accessory glands (seminal plasma); the antibodies detected in straws from farms D and G are attributed to an active infection of the IBR virus and are the product of a systemic response of the animal. In the case of BVDV and *Neospora caninum*, 100% of the straws were negative. In the culture for the isolation of *Leptospira* spp, 50% of the straws were positive (farm: A 2/2; D 1/1; E 2/2 and G 2/2). *Leptospira* infection in males is often subclinical, and the bacteria can remain in the seminal vesicles, increasing the chances of spreading. In conclusion, the use of straws without due prior disease control represents a significant risk factor in their spread, which is why these results demonstrate the need for screening for IBR, BVDV, *Neospora caninum*, *Leptospira* spp., and other diseases in water buffalo bulls destined for commercial freezing of semen to reduce the risk of spreading venereal diseases. It is recommended to comply with the indications of the Terrestrial Animal Health Code (Chapter 4.7) from the World Organization for Animal Health (WOAH) and include other pathologies established in this code and that were not contemplated in this study.

Keywords: semen, straws, antibodies, rhinotracheitis, culture.

cia de anticuerpos contra la Rinotraqueítis Infecciosa Bovina (IBR), Virus de la Diarrea Viral Bovina (BVDV) y *Neospora caninum*, así como la contaminación con *Leptospira* spp. en semen congelado de toros de búfalo de agua (*Bubalus bubalis*). Para ello se evaluaron 14 pajas provenientes de siete fincas de los estados Barinas (finca C y D), Cojedes (finca A, F y G), Mérida (finca B) y Zulia (finca E), Venezuela. Los animales de las granjas A, B, C, E y F fueron vacunados contra IBR y BVDV; las granjas D y G no vacunaron contra estas enfermedades. La detección de anticuerpos contra la glicoproteína gB del virus IBR y la proteína p80 del BVDV y contra *Neospora caninum* se realizó mediante la técnica ELISA según las especificaciones del fabricante del kit de detección IDvet y IDEXX. Además, el semen se cultivó en medio Ellinghausen McCullough, Johnson y Harris (EMJH), que es selectivo para aislar *Leptospira* spp. La presencia de anticuerpos contra IBR se detectó en el 28,6% de las pajas evaluadas (granja: A 1/2; D 1/1; F 1/3 y G 1/2); sin embargo, en la granja A y F los animales fueron vacunados 180 días antes de la recolección de semen y los anticuerpos detectados pudieron provenir de la secreción de las glándulas accesorias (plasma seminal); los anticuerpos detectados en pajas de las granjas D y G se atribuyen a una infección activa del virus IBR y son producto de una respuesta sistémica del animal. En el caso de BVDV y *Neospora caninum* el 100% de las pajitas resultaron negativas. En el cultivo para el aislamiento de *Leptospira* spp el 50% de las pajitas resultaron positivas (granja: A 2/2; D 1/1; E 2/2 y G 2/2). La infección por *Leptospira* en los hombres suele ser subclínica y la bacteria puede permanecer en las vesículas seminales, lo que aumenta las posibilidades de propagación. En conclusión, el uso de pajitas sin el debido control previo de enfermedades representa un importante factor de riesgo en su propagación, por lo que estos resultados demuestran la necesidad de realizar pesquisas para IBR, BVDV, *Neospora caninum*, *Leptospira* spp., y otras enfermedades en toros búfalo de agua. destinado a la congelación comercial de semen para reducir el riesgo de propagación de enfermedades venéreas. Se recomienda cumplir con las indicaciones del Código Sanitario para los Animales Terrestres (Capítulo 4.7) de la Organización Mundial de Sanidad Animal (WOAH) e incluir otras patologías establecidas en este código y que no fueron contempladas en este estudio.

Palabras clave: semen, pajuelas, anticuerpos, rinotraqueítis, cultivo.

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Characterization of cellular immune system at different ages in water buffalo (*Bubalus bubalis*)

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ABSTRACT

The immune system is part of a regulatory network linking physiology, pathophysiology and behavior and it can be regarded as a source of biomarkers for monitoring health and animal welfare. In recent years, there was an increased interest in understanding the immune system of water buffalo due to the growing economic impact of this species. In Italy, buffalo farming constitutes an important livestock resource to produce typical mozzarella cheese. In this study, to characterize the myeloid and lymphoid cells in peripheral blood of buffalo, we assessed three flow cytometric multicolor panels of antibodies: panel 1, with anti CD3, CD4, CD8 α and TCR- δ mAbs; panel 2, with anti CD79a and CD21 mAbs; panel 3 with anti CD172a, CD14, CD16, CD335 mAbs. Seventy-eight animals from three farms were divided into 3 groups by age: 80-100 day old calves (N=26); 16-18 months old heifers (N=26) and 3-6 year old cows in dry status (N=26). Heparinized blood samples were labelled with each antibody cocktail and collected on a CytoFLEX flow cytometer. Significant differences were found by Mann-Whitney test between adult and heifer groups in the mean value of percentage of granulocytes (19.5 vs 13.2; $p=0.0158$), PBMC (76.3 vs 84.6; $p=0.0015$), total monocytes (6.8 vs 4.3), T helper (55.6 vs 33.3), T cytotoxic (33.1 vs 54.3), $\gamma\delta$ lymphocytes (13.3 vs 32.7) with $p<0.0001$, and NK cells (5.4 vs 3.5; $p=0.0109$) and between adult and calf groups in total monocytes (6.8 vs 4.5; $p=0.0123$), T helper (55.6 vs 27.9), T cytotoxic (33.1 vs 56.1), $\gamma\delta$ lymphocytes (13.3 vs 40.4), ($p<0.0001$), and NK cells (5.4 vs 7.0; $p=0.0112$). Significant differences between heifer and calf groups were found only in $\gamma\delta$ lymphocytes (32.7 vs 40.4), ($p=0.0002$), and NK cells (3.5 vs 7.0; $p<0.0001$). Furthermore, the Kruskal-Wallis test highlighted statistical differences in the adult group between the farms in the mean value of percentage of granulocytes ($p=0.0030$), PBMC ($p=0.0120$), total ($p=0.0152$), helper ($p=0.0047$), cytotoxic ($p=0.0019$) and $\gamma\delta$ T lymphocytes ($p=0.0013$); total monocytes ($p=0.0100$), cM and ncM subsets ($p=0.0320$; $p=0.0252$); CD21⁺ B lymphocytes ($p=0.0007$). The calves showed differences in total monocytes

Caracterización del sistema inmunológico celular a diferentes edades en búfalo de agua (*Bubalus bubalis*)

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RESUMEN

El sistema inmunológico forma parte de una red reguladora que vincula la fisiología, la fisiopatología y el comportamiento y puede considerarse como una fuente de biomarcadores para controlar la salud y el bienestar animal. En los últimos años ha habido un mayor interés en comprender el sistema inmunológico del búfalo de agua debido al creciente impacto económico de esta especie. En Italia, la cría de búfalos constituye un importante recurso ganadero para producir el típico queso mozzarella. En este estudio, para caracterizar las células mieloides y linfoides en sangre periférica de búfalo, evaluamos tres paneles multicolores de anticuerpos por citometría de flujo: panel 1, con mAb anti CD3, CD4, CD8 α y TCR- δ ; panel 2, con mAb anti CD79a y CD21; panel 3 con mAb anti CD172a, CD14, CD16, CD335. Setenta y ocho animales de tres granjas se dividieron en 3 grupos por edad: terneros de 80 a 100 días (N=26); novillas de 16 a 18 meses (N=26) y vacas de 3 a 6 años en estado seco (N=26). Se etiquetaron muestras de sangre heparinizada con cada cóctel de anticuerpos y se recogieron en un citómetro de flujo CytoFLEX. Se encontraron diferencias significativas mediante la prueba de Mann-Whitney entre los grupos de adultos y novillas en el valor medio del porcentaje de granulocitos (19,5 vs 13,2; $p=0,0158$), PBMC (76,3 vs 84,6; $p=0,0015$), monocitos totales (6,8 vs 4,3), células T auxiliares (55,6 frente a 33,3), T citotóxica (33,1 frente a 54,3), linfocitos $\gamma\delta$ (13,3 frente a 32,7) con $p<0,0001$ y células NK (5,4 frente a 3,5; $p=0,0109$) y entre grupos de adultos y terneros en monocitos totales (6,8 frente a 4,5; $p=0,0123$), T auxiliares (55,6 frente a 27,9), T citotóxica (33,1 frente a 56,1), linfocitos $\gamma\delta$ (13,3 frente a 40,4), ($p<0,0001$) y células NK (5,4 frente a 7,0; $p=0,0112$). Se encontraron diferencias significativas entre los grupos de novillas y terneros sólo en los linfocitos $\gamma\delta$ (32,7 frente a 40,4), ($p=0,0002$) y las células NK (3,5 frente a 7,0; $p<0,0001$). Además, la prueba de Kruskal-Wallis destacó diferencias estadísticas en el grupo de adultos entre las granjas en el valor medio del porcentaje de granulocitos ($p=0,0030$),

($p=0.0010$), cM and ncM subsets ($p=0.0335$ and $p=0.0065$), respectively. The heifers showed differences only in the CD21⁺ B lymphocyte subset ($p=0.0439$). In summary, this study provides, for the first time, the composition of myeloid and lymphoid cells in this species highlighting large differences between calves, young and adult buffaloes and between different herds. Our results provide a resource for the further study of the immune system and to monitor health and welfare at animal and farm level.

Keywords: buffalo, immune system, age, flow cytometry.

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PBMC ($p=0.0120$), total ($p=0.0152$), auxiliar ($p=0.0047$), citotóxicos ($p=0.0019$) y linfocitos T $\gamma\delta$ ($p=0.0013$); monocitos totales ($p=0.0100$), subconjuntos cM y ncM ($p=0.0320$; $p=0.0252$); linfocitos B CD21⁺ ($p=0.0007$). Los terneros mostraron diferencias en monocitos totales ($p=0.0010$), subconjuntos de cM y ncM ($p=0.0335$ y $p=0.0065$), respectivamente. Las novillas mostraron diferencias sólo en el subconjunto de linfocitos B CD21⁺ ($p=0.0439$). En resumen, este estudio proporciona, por primera vez, la composición de las células mieloides y linfoides en esta especie, destacando grandes diferencias entre crías, búfalos jóvenes y adultos y entre diferentes rebaños. Nuestros resultados proporcionan un recurso para seguir estudiando el sistema inmunológico y para monitorear la salud y el bienestar a nivel animal y de granja.

Palabras clave: búfalo, sistema inmunológico, edad, citometría de flujo.

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Association of *Neospora caninum* and abortions in water buffaloes in Venezuela

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Asociación de *Neospora caninum* y abortos en búfalos de agua en Venezuela

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ABSTRACT

Buffaloes are an important natural host of *Neospora caninum*; this protozoan causes abortions in bovines. In addition, transplacental transmission is considered the main route of transmission. This research aimed to study the seroprevalence of *N. caninum* of 223 *Bubalus bubalis* females; the herd comes from 10 farms in 4 states of Venezuela; in addition, the association between the presence of antibodies against *N. caninum* with parity and the history of abortions was analyzed. The data was extracted from the SG Software Ganadero version 23, exported to Excel version 2306, and analyzed with IBM SPSS Statistics 20. Antibodies were determined by ELISA using a commercial kit (IDEXX®). The cut-off value to define positive cases was the coefficient sample/positive ≥ 0.500 . A chi-square test was performed to estimate the association between the presence of antibodies against *N. caninum* with parity and abortions. Of the 223 animals analyzed, 59.2% of the samples were positive for *N. caninum* antibodies. The percentages of seropositive animals were: 4.0%, 66.4%, 13.0%, 9.0%, 1.8%, 0.4%,

RESUMEN

Los búfalos son un importante huésped natural de *Neospora caninum*; este protozoo provoca abortos en bovinos. Además, la transmisión transplacentaria se considera la principal vía de transmisión. La presente investigación tuvo como objetivo estudiar la seroprevalencia de *N. caninum* de 223 hembras de *Bubalus bubalis*; la manada proviene de 10 fincas en 4 estados de Venezuela; además, se analizó la asociación entre la presencia de anticuerpos contra *N. caninum* con la paridad y el historial de abortos. Los datos se extrajeron del software SG Ganadero versión 23, se exportaron a Excel versión 2306 y se analizaron con IBM SPSS Statistics 20. Los anticuerpos se determinaron mediante ELISA utilizando un kit comercial (IDEXX®). El valor de corte para definir casos positivos fue el coeficiente muestra/positivo $\geq 0,500$. Se realizó una prueba de chi-cuadrado para estimar la asociación entre la presencia de anticuerpos contra *N. caninum* con la paridad y los abortos. De los 223 animales analizados, el 59,2% de las muestras fueron positivas para anticuerpos contra *N. caninum*. Los porcentajes

1,3%, and 4% for animals with 0 to 7 calving, respectively. The percentages of positives to *N. caninum* for buffaloes of parity 1 and 2 were 54.05% and 62.06%, respectively ($X^2 = 0.630$, $p=0.427$), while the percentage of positives to *N. caninum* in buffaloes without abortion and with one previous abortion were 54.17% and 74.51%, respectively ($X^2 = 6.68$, $p=0.01$). In addition, buffaloes with a history of abortion had more odds of being seropositive to *N. caninum* than those without abortions (2.478, 95%CI:1.2874-4.8834, $p = 0.0087$). Therefore, it is concluded that *N. caninum* serology and parity are independent, while *N. caninum* serology and the occurrence of abortions are associated. These results demonstrate that *N. caninum* is a risk factor for the occurrence of abortions in water buffaloes, so it is suggested to include this pathology in preventive health programs in herds.

Keywords: *Neospora caninum*, ELISA, abortion, serology, buffaloes.

de animales seropositivos fueron: 4,0%; 66,4%; 13,0%; 9,0%; 1,8%; 0,4%; 1,3% y 4% para animales de 0 a 7 partos, respectivamente. Los porcentajes de positivos a *N. caninum* para búfalas de parto 1 y 2 fueron 54,05% y 62,06%, respectivamente ($X^2 = 0,630$, $p=0,427$), mientras que el porcentaje de positivos a *N. caninum* en búfalas sin aborto y con un aborto previo fueron 54,17% y 74,51%, respectivamente ($X^2 = 6,68$, $p=0,01$). Además, las búfalas con antecedentes de aborto tuvieron más probabilidades de ser seropositivas a *N. caninum* que aquellas sin abortos (2,478, IC95%:1,2874-4,8834, $p =0,0087$). Por lo tanto, se concluye que la serología de *N. caninum* y la paridad son independientes, mientras que la serología de *N. caninum* y la ocurrencia de abortos están asociadas. Estos resultados demuestran que *N. caninum* es un factor de riesgo para la ocurrencia de abortos en búfalos de agua, por lo que se sugiere incluir esta patología dentro de los programas de salud preventiva en rebaños.

Palabras clave: *Neospora caninum*, ELISA, aborto, serología, búfalos.

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The use of bacterial culture for Leptospirosis diagnosis in female water buffaloes

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ABSTRACT

The test most used for leptospirosis diagnosis is the Microagglutination Test (MAT). However, the low antibody titers in chronic infections induced by strains adapted to bovines can limit the interpretation of the correct results. The bacteriological culture of *Leptospira* spp. is the gold standard test because it can demonstrate the agent's presence. Therefore, this research aimed to reveal the presence of *Leptospira* spp. in aborted fetuses, genital-urinary secretions of female and male *Bubalus bubalis*, food raw materials, and rodent feces using bacteriological culture. The analyses were performed on a farm located in the state of Cojedes, Venezuela. This farm has an abortion rate of 17,05% and 2,98% mortality at birth. For this, 18 blood samples (16 females and two males) were extracted by puncture of the jugular vein, and these were allowed to coagulate to obtain the serum and urine samples from 2 female buffaloes, one male buffalo, and semen from 2 other animals, were collected. Additionally, two fetuses of 120 and 75 days of gestation were collected. Also, after the necropsy, fluid samples were obtained from the abdomen, umbilical artery, ocular and thoracic cavities, and amniotic liquid, and two samples of liver,

El uso del cultivo bacteriano para el diagnóstico de Leptospirosis en búfalas de agua hembras

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RESUMEN

La prueba más utilizada para el diagnóstico de leptospirosis es la Prueba de Microaglutinación (MAT). Sin embargo, las bajas concentraciones de anticuerpos en infecciones crónicas inducidas por cepas adaptadas a bovinos pueden limitar la interpretación de los resultados correctos. El cultivo bacteriológico de *Leptospira* spp. es la prueba estándar de oro porque puede demostrar la presencia del agente. Por lo tanto, esta investigación tuvo como objetivo revelar la presencia de *Leptospira* spp. en fetos abortados, secreciones genito-urina-rias de *Bubalus bubalis* hembra y macho, materias primas alimentarias y heces de roedores mediante cultivo bacteriológico. Los análisis se realizaron en una finca ubicada en el estado Cojedes, Venezuela. Esta finca tiene una tasa de aborto del 17,05% y una mortalidad al nacer del 2,98%. Para ello se extrajeron 18 muestras de sangre (16 hembras y dos machos) mediante punción de la vena yugular, que se dejaron coagular para obtener el suero, así como muestras de orina de 2 búfalas hembra, un búfalo macho y semen recogido de otros 2 animales. Además, se recolectaron dos fetos de 120 y 75 días de gestación. Tras la necropsia se obtuvieron muestras

spleen, kidney, lung, heart, and brain were collected. Furthermore, samples of food ration and of the four by-products (corn silage, hay, urea, mineral) that compose it were collected, besides the rodent feces found in the storage where the food was preserved. The standard serological MAT was performed with a panel of strains of 7 *Leptospira* serovars. The fluids and tissue samples were inoculated in Ellinghausen–McCullough–Johnson–Harris medium (EMJH), a liquid culture medium selective for *Leptospira*. A portion of the tissue sample was fixed in 10% buffered formalin for histological slices and routine H-E staining. The MAT results indicated titers of 1:50 for the *Icterohaemorrhagiae* serovar at 5.5%, *Pomona* at 5.5%, and *Bratislava* at 5.5%. The serovars *Canicola*, *Grippityphosa*, *Harjo bovis*, and *Harjo pratjino* were negative. There was a growth of *Leptospira* spp. in fetal fluids from the abdomen, umbilicus, thorax, liver, spleen, lung, kidney, and heart tissues; in the same way, *Leptospira* spp. growth was obtained after the culture of urine and semen samples, food, corn silage, hay, urea-mineral and rodent feces. Histopathology analysis showed lesions in the spleen as extra-medullary hematopoiesis and hemorrhage; in the kidney (multifocal hemorrhages, tubular degeneration, and edema) while in the lungs, perivascular hemorrhages and degeneration of the vascular wall were observed. In conclusion, these preliminary results suggest that the MAT cannot diagnose all Leptospirosis cases correctly, especially chronic infections characterized by low antibodies. At the same time, the culture in EMJH seems to solve this weakness, as *Leptospira* spp. growth was obtained from different biological samples. Additionally, these preliminary findings confirm *Leptospira* is an agent involved in abortions, as we obtained *Leptospira* spp. growth from aborted fetus samples. Finally, it is likely that this bacterium growing in the bacteriological cultures belongs to a serovar different from those in the panel of strains used in MAT, so the cultures were preserved for subsequent studies that contemplate molecular identification.

Keywords: *Leptospira*, antibodies, MAT, buffaloes.

de fluidos del abdomen, arteria umbilical, cavidades ocular y torácica y líquido amniótico, y se recogieron dos muestras de hígado, bazo, riñón, pulmón, corazón y cerebro. Además, se recolectaron muestras de la ración alimenticia y de los cuatro subproductos (ensilaje de maíz, heno, urea, mineral) que la componen, además de heces de roedores encontradas en el depósito donde se conservaba el alimento. El MAT serológico estándar se realizó con un panel de cepas de 7 serovares de *Leptospira*. Los fluidos y muestras de tejido se inocularon en medio Ellinghausen-McCullough-Johnson-Harris (EMJH), un medio de cultivo líquido selectivo para *Leptospira*. Una porción de la muestra de tejido se fijó en formalina tamponada al 10% para cortes histológicos y tinción HE de rutina. Los resultados de MAT indicaron concentraciones de 1:50 para el serovar *Icterohaemorrhagiae* al 5,5%, *Pomona* al 5,5% y *Bratislava* al 5,5%. Los serovares *Canicola*, *Grippityphosa*, *Harjo bovis* y *Harjo pratjino* resultaron negativos. Hubo un crecimiento de *Leptospira* spp. en fluidos fetales del abdomen, ombligo, tórax, hígado, bazo, pulmón, riñón y tejidos del corazón; de la misma manera, se obtuvo el crecimiento de *Leptospira* spp. luego del cultivo de muestras de orina y semen, alimentos, ensilaje de maíz, heno, urea-mineral y en heces de roedores. El análisis histopatológico mostró lesiones en el bazo como hematopoyesis extramedular y hemorragia; en el riñón (hemorragias multifocales, degeneración tubular y edema); mientras que en el pulmón, se observaron hemorragias perivasculares y degeneración de la pared vascular. En conclusión, estos resultados preliminares sugieren que el MAT no puede diagnosticar correctamente todos los casos de Leptospirosis, especialmente las infecciones crónicas caracterizadas por niveles bajos de anticuerpos. Al mismo tiempo, el cultivo en EMJH parece solucionar esta debilidad, ya que el crecimiento de *Leptospira* spp. se obtuvo de diferentes muestras biológicas. Además, estos hallazgos preliminares confirman que *Leptospira* es un agente involucrado en abortos, ya que obtuvimos *Leptospira* spp. crecimiento de muestras de fetos abortados. Finalmente, es probable que esta bacteria que crece en los cultivos bacteriológicos pertenezca a un serovar diferente a los del panel de cepas utilizadas en MAT, por lo que los cultivos se conservan para estudios posteriores que contemplen la identificación molecular.

Palabras clave: *Leptospira*, anticuerpos, MAT, búfalos.

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Experimental use of mycobacterial antigens in tuberculin skin test on water buffalo

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ABSTRACT

In Italy, bovine tuberculosis (TB) control in the water buffalo population is mainly carried out using the tuberculin skin test (TST). The test measures the increase in skin thickness 72 hours (h) after the intradermal injection of mycobacterial purified protein derivatives (PPD). The TST can involve the single intradermal test with bovine PPD (PPDB), or the comparative test adding a second injection with avian tuberculin (PPDA), using the couple of PPDs reduces false positives caused by non-tuberculosis mycobacteria. This pilot study aimed to evaluate the potential use of PPDB, PPDA, along with 6 kDa early secretory antigenic target (ESAT-6), 10 kDa culture filtrate protein (CFP-10), and Early Secretory Proteins (ESP) in the TST, in healthy and naturally TB infected buffaloes. Twenty-one buffaloes, gamma-interferon (IFN- γ) test positive, from a TB-outbreak herd and 11 buffaloes from a TB-free herd were selected. All subjects were also submitted to the IFN- γ test using the same antigens (Ag) as the TST to verify the cellular response *in vitro* and to exclude anaerobic animals that did not respond to *in vitro* stimulation with the mitogen. The TST was performed according to the TB Italian National eradication program, and to Reg. EU 2020/689. Eight intradermal injection sites were used on each animal shoulder (4 on the right and 4 on the left side). Three concentrations of the ESAT6-CFP10 protein cocktail (10, 20 and 30 μ g) and two of ESP (50 and 100 μ g) were inoculated. PPDB and PPDA were always included in the TST, along with PBS as negative control. Skin reactions were measured with

Uso experimental de antígenos micobacterianos en la prueba cutánea de tuberculina en búfalos de agua

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RESUMEN

En Italia, el control de la tuberculosis bovina (TB) en la población de búfalos de agua se lleva a cabo principalmente mediante la prueba cutánea de la tuberculina (PT). La prueba mide el aumento del grosor de la piel 72 horas (h) después de la inyección intradérmica de derivados proteicos purificados (PPD) de micobacterias. La PT puede implicar la prueba intradérmica única con PPD bovino (PPDB), o la prueba comparativa añadiendo una segunda inyección con tuberculina aviar (PPDA). El uso de un par de PPD reduce los falsos positivos causados por micobacterias no tuberculosas. Este estudio piloto tuvo como objetivo evaluar el uso potencial de PPDB, PPDA, junto con un objetivo antigénico secretorio temprano de 6 kDa (ESAT-6), una proteína filtrada de cultivo de 10 kDa (CFP-10) y proteínas secretoras tempranas (ESP) en la TST, en búfalos sanos y naturalmente infectados con tuberculosis. Se seleccionaron veintinueve búfalos con resultados positivos para interferón gamma (IFN- γ) de un rebaño con brote de tuberculosis y 11 búfalos de un rebaño libre de tuberculosis. Todos los sujetos también fueron sometidos a la prueba de IFN- γ utilizando los mismos antígenos (Ag) que la TST para verificar la respuesta celular *in vitro* y excluir animales anaérgicos que no respondieron a la estimulación *in vitro* con el mitógeno. La PT se realizó según el programa nacional italiano de erradicación de la tuberculosis y el Reg. UE 2020/689. Se utilizaron ocho sitios de inyección intradérmica en cada hombro de animal (4 en el lado

calipers before the test and every 24 h for 4 days. The TST was considered positive in animals with an increase in skin thickness between post and pre-dermal test readings of > 4 mm, doubtful if between 2 and 4 mm, negative <2 mm. Negative subjects also exhibited a skin response to PPDB at 24 and 48 h, but it became completely negative at 72 h, the prescribed reading time. In the positive buffaloes, the kinetics of skin responses showed that the strongest response was to PPDB at 24h, with some subjects becoming doubtful at 72 and 96 h. A peak response was observed at 48 hours to PPDA and to 20 µg of the ESAT6-CFP10 protein cocktail, followed by a decrease. The response to ESP-100 µg remained high at 24 and 48 h and then decreased, remaining positive at 72 h. ESP showed the best performance with higher reactivity in positive animals and no reactivity in negative animals at 72 h. Therefore, ESP could be an excellent candidate for further extensive studies in the buffalo species to improve TST Sensitivity and Specificity.

Keywords: water buffalo, tuberculosis, diagnosis, tuberculin skin test.

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derecho y 4 en el lado izquierdo). Se inocularon tres concentraciones del cóctel proteico ESAT6-CFP10 (10, 20 y 30 µg) y dos de ESP (50 y 100 µg). PPDB y PPDA siempre se incluyeron en la TST, junto con PBS como control negativo. Las reacciones cutáneas se midieron con calibradores antes de la prueba y cada 24 h durante 4 días. La PT se consideró positiva en animales con un aumento del espesor de la piel entre las lecturas de la prueba post y predérmica de > 4 mm, dudoso si entre 2 y 4 mm, negativo < 2 mm. Los sujetos negativos también mostraron una respuesta cutánea al PPDB a las 24 y 48 h, pero se volvió completamente negativa a las 72 h, el tiempo de lectura prescrito. En los búfalos positivos, la cinética de las respuestas de la piel mostró que la respuesta más fuerte fue al PPDB a las 24 h, y algunos sujetos se volvieron dudosos a las 72 y 96 h. Se observó una respuesta máxima a las 48 horas con PPDA y con 20 µg del cóctel de proteínas ESAT6-CFP10, seguida de una disminución. La respuesta a ESP-100 µg se mantuvo alta a las 24 y 48 h y luego disminuyó, permaneciendo positiva a las 72 h. ESP mostró el mejor desempeño con mayor reactividad en animales positivos y ninguna reactividad en animales negativos a las 72 h. Por lo tanto, ESP podría ser un excelente candidato para estudios más extensos en la especie de búfalo para mejorar la sensibilidad y especificidad de la TST.

Palabras clave: búfalo de agua, tuberculosis, diagnóstico, prueba cutánea de tuberculina.

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Therapeutic effectiveness of three commercial anthelmintics against strongylids in buffalo calves (*Bubalus bubalis*)

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ABSTRACT

Gastrointestinal parasites and their control in buffaloes (*Bubalus bubalis*) represent increasing production costs. To improve sanitary plans in buffalo herds, experimental field re-

Efectividad terapéutica de tres antihelmínticos comerciales contra strongílidos en becerros (*Bubalus bubalis*)

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RESUMEN

Los parásitos gastrointestinales y su control en búfalos (*Bubalus bubalis*) representan costos de producción crecien-

search was carried out to evaluate the efficacy of three commercial anthelmintics against strongylids parasites in buffalo calves. The effectiveness of the anthelmintics was estimated through faecal egg count (FEC) reduction percentage. The study was conducted in a buffalo system dedicated to milk and meat production with crossbreed animals (Murrah and Mediterranean breeds) in the South of Lake Maracaibo, Venezuela. Twenty-eight buffalo calves, naturally infected, with a parasite load ≥ 350 eggs per gram of faeces (EPG), and age between 2 and 3 months, with an average weight of 65.9 kgs, were selected. The animals were grouped into four treatments: T0 control without anthelmintic; T1 fenbendazole 10%, Intervet laboratory, Brazil, at 5mg/kg orally; T2 ivermectin 1%, SIGMA laboratory, Venezuela, at 0.2 mg/kg subcutaneously; and T3 levamisole hydrochloride 22.3%, Valmor laboratory, Venezuela, at 4.5 mg/kg subcutaneously. The anthelmintics were applied in a single dose on day zero (0) of the research. A coprological diagnosis was performed prior to treatment and at 14 (A), 21 (B) and 28 (C) days after treatment. The faecal samples were examined by applying McMaster's technique with saturated saline. Data was analyzed with descriptive statistics and the nonparametric Friedman test. The FEC reduction percentage was calculated for each anthelmintic. Moreover, the lower limit of the 95% confidence interval was calculated for each anthelmintic (low-CI95%). The mean EPG before treatment (BT) was 907.1 EPG, 978.6 EPG, 1057.1 EPG, and 921.4 EPG for T0, T1, T2, and T3, respectively. The mean of EPG during the evaluation of the different treatments showed significant variations ($p < 0.05$): T1 in A (42.9), B (200) and C (178.6). For T2 in A (671.4), B (571.4) and C (964); and for T3 in A (41.7), B (92.9) and C (121.4). The FEC reduction percentage at times A, B and C at T1 was 97%, 81.1% and 84.7% and a low-CI 95% of 67%; for T2 it was 51.5%, 46% and 17.4% and a low-CI 95% of -7.2%; and for T3 it was 97%, 91.2% and 89.6% and a low-CI 95% of 82.9%. Conclusions are that T1 and T3 presented a mean of EPG at low levels (≤ 200) during the evaluated period in comparison to T2, which remained at a high level of infection (> 500). Furthermore, levamisole was most effective against strongylids. In the case of ivermectin, the FEC reduction percentage was less than 90% during the trial, resulting in a low-CI 95% of -7.2%, showing possible anthelmintic resistance.

Keywords: anthelmintic resistance, antiparasites, coprology, deworming, parasites.

tes. Para mejorar los planes sanitarios en rebaños bufalinos, se realizó una investigación experimental de campo para evaluar la eficacia de tres antihelmínticos comerciales contra parásitos estrogilidos en becerros. La eficacia de los antihelmínticos se estimó mediante el porcentaje de reducción del recuento de huevos en heces (FEC). El estudio se realizó en un sistema bufalino dedicado a la producción de leche y carne con animales mestizos (razas Murrah y Mediterránea) en el Sur del Lago de Maracaibo, Venezuela. Se seleccionaron 28 búfalos, naturalmente infectados, con una carga parasitaria ≥ 350 huevos por gramo de heces (EPG), de edad entre 2 y 3 meses, con un peso promedio de 65,9 kg. Los animales se agruparon en cuatro tratamientos: T0 control sin antihelmíntico; T1 fenbendazol 10%, laboratorio Intervet, Brasil, a 5 mg/kg por vía oral; T2 ivermectina 1%, laboratorio SIGMA, Venezuela, a 0,2 mg/kg por vía subcutánea; y T3 clorhidrato de levamisol 22,3%, laboratorio Valmor, Venezuela, a 4,5 mg/kg por vía subcutánea. Los antihelmínticos se aplicaron en dosis única el día cero (0) de la investigación. Se realizó un diagnóstico coprológico previo al tratamiento y a los 14 (A), 21 (B) y 28 (C) días después del tratamiento. Las muestras fecales se examinaron aplicando la técnica de McMaster con solución salina saturada. Los datos fueron analizados con estadística descriptiva y la prueba no paramétrica de Friedman. Se calculó el porcentaje de reducción de FEC para cada antihelmíntico. Además, se calculó el límite inferior del intervalo de confianza del 95% para cada antihelmíntico (bajo-IC95%). La media de EPG antes del tratamiento (BT) fue de 907,1 EPG, 978,6 EPG, 1057,1 EPG y 921,4 EPG para T0, T1, T2 y T3, respectivamente. La media de EPG durante la evaluación de los diferentes tratamientos mostró variaciones significativas ($p < 0,05$): T1 en A (42,9), B (200) y C (178,6). Para T2 en A (671,4), B (571,4) y C (964); y para T3 en A (41,7), B (92,9) y C (121,4). El porcentaje de reducción de FEC en los momentos A, B y C en T1 fue de 97%, 81,1% y 84,7% y un IC bajo 95% de 67%; en T2 fue 51,5%, 46% y 17,4% y un IC bajo 95% de -7,2%; y en T3 fue 97%, 91,2% y 89,6% y un IC bajo 95% de 82,9%. Se concluye que T1 y T3 presentaron la media de EPG en niveles bajos (≤ 200) durante el período evaluado en comparación con T2, que se mantuvo en un nivel alto de infección (> 500). Además, el levamisol resultó ser lo más eficaz contra los estrogilidos. En el caso de la ivermectina, el porcentaje de reducción de FEC fue inferior al 90% durante el ensayo, lo que resultó en un IC 95% bajo de -7,2% y mostró una posible resistencia a los antihelmínticos.

Palabras clave: resistencia a los antihelmínticos, antiparasitarios, coprología, desparasitación, parásitos.

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Assessment tuberculin skin test specificity in water buffalo using five different antigens

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ABSTRACT

Tuberculosis (TB) control involves "test and cull strategies". The primary diagnostic test is the tuberculin skin test (TST) which requires intradermal inoculation with specific *Mycobacterium bovis* antigens, bovine tuberculin (PPDB) in the single intradermal reaction test (SIT) and avian tuberculin (PPDA) in the comparative intradermal reaction test (SICT). Using other antigens such as 6 kDa early secretory antigenic target (ESAT-6), 10 kDa culture filtrate protein (CFP-10) and Early Secretory Proteins (ESP) could increase test specificity. Therefore, this study aims to evaluate the specificity of TST using five different antigens. A total of 100 buffaloes from 3 TB free Italian herds were enrolled. Five intradermal injection sites on the animal's shoulder (three on the right and two on the left side) were used on each animal, with a volume of 0.1 mL for all antigens (ESAT-6 and CFP-10 at 20 µg/mL, ESP at 100 µg/mL, PPDA and PPDB at 100 µg/mL), and PBS as negative control. Skin reactions were measured using calipers. Results were expressed in millimeters as the difference between the two measurements, i.e., before and 72h after the inoculation of tuberculin. The reaction was considered positive if skin thickness increased by ≥ 4mm, doubtful if >2 and <4mm, and negative if ≤ 2 mm. All 100 buffaloes were also submitted to the gamma-interferon (IFN-γ) test to confirm TB negativity. Of the 100 negative subjects, 31 gave doubtful SIT results to PPDB while the number of doubtful subjects decreased to 11 with the SICT. The number of doubtful results with ESP stimulation was 26 and with the ESAT-6 and CFP-10 protein cocktail, it was 21. If we introduce the responses to stimulation with ESP and ESAT-

Evaluación de la especificidad de la prueba cutánea de tuberculina en búfalos de agua utilizando cinco antígenos diferentes

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RESUMEN

El control de la tuberculosis (TB) implica "estrategias de prueba y eliminación". La prueba de diagnóstico primaria es la prueba cutánea de la tuberculina (TST), que requiere la inoculación intradérmica con antígenos específicos de *Mycobacterium bovis*, la tuberculina bovina (PPDB) en la prueba de reacción intradérmica única (SIT) y la tuberculina aviar (PPDA) en la prueba de reacción intradérmica comparativa (SICT). El uso de otros antígenos como prueba antigénica secretora temprana de 6 kDa (ESAT-6), proteína filtrada de cultivo de 10 kDa (CFP-10) y proteínas secretoras tempranas (ESP) podría aumentar la especificidad de la prueba. Por tanto, este estudio tiene como objetivo evaluar la especificidad de la TST utilizando cinco antígenos diferentes. Se inscribieron un total de 100 búfalos de 3 rebaños italianos libres de tuberculosis. En cada animal se utilizaron cinco sitios de inyección intradérmica en el hombro del animal (tres en el lado derecho y dos en el lado izquierdo), con un volumen de 0,1 ml para todos los antígenos (ESAT-6 y CFP-10 a 20 µg/ml, ESP a 100 µg/mL, PPDA y PPDB a 100 µg/mL) y PBS como control negativo. Las reacciones cutáneas se midieron utilizando calibradores. Los resultados se expresaron en milímetros como la diferencia entre las dos mediciones, es decir, antes y 72 h después de la inoculación de tuberculina. La reacción se consideró positiva si el espesor de la piel aumentaba ≥ 4 mm, dudosa si > 2 y < 4 mm, y negativa si ≤ 2 mm. Los 100 búfalos también fueron sometidos a la prueba de interferón gamma (IFN-γ) para confirmar la negatividad de la tuberculosis. De los 100 sujetos negativos, 31 dieron resultados dudosos del SIT al

6 and CFP-10 protein cocktail combined with the interpretation of SICT, the 11 doubtful results would be reduced to 5 and 4, respectively. The doubtful outcomes of SIT and SICT found in negative animals with PPDB and PPDA stimulation, could be due to non-specific responses. In buffalo, this non-specificity could be due to atypical mycobacterial infections as well as to the peculiarities of buffalo skin structure compared to bovine skin (e.g., dermis thickness). Regarding ESAT-6 and CFP-10 protein cocktail and ESP, they induced fewer non-specific reactions, and thus, they could be used in SICT in association with traditional PPDs. Due to the IFN- γ test, we excluded the presence of anergic subjects, since all 100 subjects reacted to stimulus with the mitogen. Furthermore, the IFN- γ test in the same animals gave negative results according to the interpretation criteria of the official EU IFN- γ test, even if with slight non-specific reactions, but excluding false-positive results.

Keywords: water buffalo, tuberculosis diagnosis, tuberculin skin test, specificity.

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PPDB mientras que el número de sujetos dudosos disminuyó a 11 con el SICT. El número de resultados dudosos con estimulación ESP fue de 26 y con cóctel de proteínas ESAT-6 y CFP-10 fue de 21. Si introducimos las respuestas a la estimulación con ESP y cóctel de proteínas ESAT-6 y CFP-10 combinado con la interpretación de SICT, los 11 resultados dudosos se reducirían a 5 y 4, respectivamente. Los resultados dudosos de SIT y SICT encontrados en animales negativos con estimulación PPDB y PPDA, podrían deberse a respuestas no específicas. En búfalo, esta inespecificidad podría deberse a infecciones micobacterianas atípicas, así como a las peculiaridades de la estructura de la piel del búfalo en comparación con la del bovino (por ejemplo, grosor de la dermis). En cuanto al cóctel de proteínas ESAT-6 y CFP-10 y ESP, indujeron menos reacciones no específicas y, por lo tanto, podrían usarse en SICT en asociación con los PPD tradicionales. Debido a la prueba de IFN- γ excluimos la presencia de sujetos anérgicos, ya que los 100 sujetos reaccionaron al estímulo con el mitógeno. Además, la prueba de IFN- γ en los mismos animales arrojó resultados negativos según los criterios de interpretación de la prueba oficial de IFN- γ de la UE, aunque con ligeras reacciones inespecíficas, pero excluyendo los resultados falsos positivos.

Palabras clave: búfalo de agua, diagnóstico de tuberculosis, prueba cutánea de tuberculina, especificidad.

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Buffalo milk quality in southern Italy: trace elements & endocrine disruptors. A One Health analysis

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ABSTRACT

The production of buffalo milk and its derivatives represents an important economic sector in Southern Italy, espe-

Calidad de la leche de búfala en el sur de Italia: oligoelementos y disruptores endocrinos: un análisis de One Health

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RESUMEN

La producción de leche de búfala y sus derivados presenta un sector económico importante en el sur de Italia,

cially in the Campania region. Buffalo milk is mainly used for the production of “mozzarella di bufala”, a soft, un-ripened cheese variety of the *pasta filata* family, highly appreciated worldwide for its good characteristics and particular taste. In the period 2015-2018, buffalo milk production in Italy increased by 27%, from 195,270 to 247,158 tons. The entire buffalo milk production chain can be contaminated with trace elements (TE), bisphenol (BPA) and others endocrine disruptors (EDs). The source of these pollutants can come from feed, forage and food additives. Given the importance of this foodstuff in the Campania region, the presence of BPA and EDs was investigated in all production stages of the buffalo supply chain, from water to feed to milk. The aim of this study was also to evaluate how the geochemical impact of agricultural soils can influence the chemical element profile of buffalo milk. Using a “One Health” approach, the fate of trace elements and endocrine disruptors was considered, starting with the transfer of these elements to buffalo milk after feed ingestion by the animal. Trace element analysis came from different areas of the Campania Region, where buffalo farms were randomly selected for sampling of milk (n:69). At the same way, samples of milk (n:46) and serum (n:190) were collected from 10 buffalo farms in the Campania Region for analysis of endocrine disruptors. For both research lines, forage, feed (n:207) and groundwater (n: 486) samples were also collected. The results of samples collected from buffalo farms distributed throughout the Campania region were statistically analysed using Cluster Analysis (CA) and Factor Analysis (FA). The results indicate that among the endocrine disruptors investigated, BPA is present in all farms studied. Bisphenol contamination has been found in all types of feed and its presence in buffalo milk can be mainly attributed to feed, especially feed additives. In buffalo milk, BPA is present at levels between 0.5 and 5.6 ng/mL. Regarding the presence of metals, of the twenty trace elements analysed, only lead is regulated in the EU and no sample exceeded the maximum limit (LM = 0.02 mg/kg). Potentially toxic elements such as chromium (< 0.141 mg/kg), cobalt (< 0.008 mg/kg), manganese (< 0.035 mg/kg), selenium (< 0.044 mg/kg), vanadium (< 0.016 mg/kg), zinc (< 4.31 mg/kg) and strontium (< 0.947 mg/kg) have been found in milk at levels that do not constitute a health risk; furthermore their presence could be related to the geographical origin. An important presence of strontium and copper was revealed in forages and drinking water. Strontium is naturally present in forage and soil of the Campania region. The presence of copper can be attributed to its extensive use in agronomic practices. The presence of chemical elements in milk could be a tool for geo-referenced traceability down to small-scale, regional soil background levels.

Keywords: buffalo milk, trace elements, endocrine disruptor, BPA, Italy, Campania.

especialmente en la región de Campania. La leche de búfala se utiliza principalmente para la producción de la “mozzarella di búfala”, una variedad de queso blando y sin madurar de la familia de la *pasta filata*, muy apreciado en todo el mundo por sus buenas características y su particular sabor. En el período 2015-2018, la producción de leche de búfala en Italia aumentó un 27%, de 195.270 a 247.158 toneladas. Toda la cadena de producción de leche de búfala puede estar contaminada con oligoelementos (TE), bisfenol (BPA) y otros disruptores endocrinos (DE). La fuente de estos contaminantes puede provenir de piensos, forrajes y aditivos alimentarios. Dada la importancia de este alimento en la región de Campania, se investigó la presencia de BPA y EDs en todas las etapas de producción de la cadena de suministro de búfalas, desde el agua hasta el pienso y la leche. El objetivo de este estudio también fue evaluar cómo el impacto geoquímico de los suelos agrícolas puede influir en el perfil de elementos químicos de la leche de búfala. Utilizando el enfoque “Una sola salud-One Health”, se consideró el destino de los oligoelementos y los disruptores endocrinos, empezando por la transferencia de estos elementos a la leche de búfala después de la ingestión del alimento por parte del animal. El análisis de oligoelementos provino de diferentes áreas de la región de Campania, donde se seleccionaron aleatoriamente granjas de búfalas para el muestreo de leche (n:69). Del mismo modo, se recogieron muestras de leche (n:46) y suero (n:190) de 10 granjas de búfalos en la región de Campania para análisis de disruptores endocrinos. Para ambas líneas de investigación se recolectaron también muestras de forraje, piensos (n:207) y aguas subterráneas (n:486). Los resultados de las muestras recogidas en granjas de búfalos distribuidas por toda la región de Campania se analizaron estadísticamente mediante análisis de conglomerados (CA) y análisis factorial (FA). Los resultados indican que entre los disruptores endocrinos investigados, el BPA está presente en todas las granjas estudiadas. Se ha encontrado contaminación por bisfenol en todos los tipos de piensos y su presencia en la leche de búfala puede atribuirse principalmente a los piensos, especialmente a los aditivos alimentarios. En la leche de búfala, el BPA está presente en niveles entre 0,5 y 5,6 ng/ml. En cuanto a la presencia de metales, de los veinte oligoelementos analizados, sólo el plomo está regulado en la UE y ninguna muestra superó el límite máximo (LM = 0,02 mg/kg). Elementos potencialmente tóxicos como cromo (< 0,141 mg/kg), cobalto (< 0,008 mg/kg), manganeso (< 0,035 mg/kg), selenio (< 0,044 mg/kg), vanadio (< 0,016 mg/kg), zinc (< 4,31 mg/kg) y estroncio (< 0,947 mg/kg) se han encontrado en la leche en niveles que no constituyen un riesgo para la salud; además su presencia podría estar relacionada con el origen geográfico. En forrajes y agua de bebida se reveló una importante presencia de estroncio y cobre. El estroncio está presente de forma natural en el forraje y el suelo de la región de Campania. La presencia de cobre puede atribuirse a su uso extensivo en prácticas agronómicas. La presencia de elementos químicos en la leche podría ser una herramienta para la trazabilidad

georreferenciada hasta niveles regionales de fondo del suelo a pequeña escala.

Palabras clave: leche de búfala, oligoelementos, disruptor endocrino, BPA, Italia, Campania.

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Diagnosis of tuberculosis in buffaloes in Argentina: intradermoreaction, compatible lesions and bacteriological culture

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ABSTRACT

Bovine tuberculosis is a zoonotic disease distributed worldwide and present in Argentine herds. In Argentina, bovine and buffalo cattle are under a tuberculosis control and eradication program, which includes tuberculin purified protein derivative bovine (PPDB) diagnosis and slaughter of positive reagents. The use of this control tool is controversial in buffaloes. The aim of this study is to generate information about buffalo tuberculosis in Corrientes, Argentina, through evaluation of field diagnostic test performance, detection of tuberculosis compatible lesions in previously tuberculinized animals and, confirmation by bacteriological culture. In 8 farms, tuberculin PPDB was inoculated in the anocaudal skin fold of 566 buffaloes of 2 years old. The applied dose was 0.1ml (1mg/ml). Reading was 72 hours after inoculation with a cutimeter. Because of the lack of standardized parameters for this species, two interpretation criteria were applied, a qualitative one (reactive, when any size increase was evident in the inoculation area/non-reactive), and the quantitative one following cattle-regulated criteria (positive reaction of more than 5mm, suspicious from 3 to 5mm and negative up to 3mm). Qualitatively, 13% (73/566) reacted and 87% (493/566) did not. Quantitatively, 5% (30/566) were positive, 2% suspicious (12/566) and 93% (524/566) negative. Fifteen animals (10 reactants and 5 not reactant) were selected and followed until slaughter, where the presence of tuberculo-

Diagnóstico de tuberculosis en búfalos en Argentina: intradermoreacción, lesiones compatibles y cultivo bacteriológico

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RESUMEN

La tuberculosis bovina es una enfermedad zoonótica distribuida mundialmente y presente en rebaños argentinos. En Argentina, el ganado vacuno y bubalino se encuentra bajo un programa de control y erradicación de la tuberculosis, que incluye el diagnóstico del derivado proteico purificado de la tuberculina bovina (PPDB) y el sacrificio de reactivos positivos. El uso de esta herramienta de control es polémico en búfalos. El objetivo de este estudio es generar información sobre la tuberculosis bufalina en Corrientes, Argentina, mediante la evaluación de la realización de pruebas diagnósticas de campo, la detección de lesiones compatibles con tuberculosis en animales previamente tuberculinizados y la confirmación por cultivo bacteriológico. En 8 granjas se inoculó tuberculina PPDB en el pliegue cutáneo anocaudal de 566 búfalos de 2 años de edad. La dosis aplicada fue de 0,1 ml (1 mg/ml). La lectura fue 72 horas después de la inoculación con un cutímetro. Debido a la falta de parámetros estandarizados para esta especie, se aplicaron dos criterios de interpretación, uno cualitativo (reactivo, cuando se evidenció algún aumento de tamaño en el área de inoculación/no reactivo), y el cuantitativo siguiendo criterios regulados en bovinos (reacción positiva de más de 5mm, sospechoso de 3 a 5mm y negativo hasta 3mm). Cualitativamente el 13% (73/566) reaccionó y el 87% (493/566) no. Cuantitati-

sis-compatible lesions was observed, and samples of lymph nodes and organs were taken for subsequent analysis by bacteriological culture and molecular typing. Of the 5 non-reactive animals, 4 of them were negative by bacteriological culture; the other buffalo turned out to be a false negative animal because it resulted positive for bacteriological culture. In 10 reacting buffaloes, 8 were confirmed positive by culture and two of them were negative. Of the 8 buffaloes with positive cultures, only 2 presented macroscopic tuberculosis-compatible lesions, one in the mediastinal lymph node and lung; and the other in the retropharyngeal ganglion. In molecular tests, the *hsp65* gene was amplified to determine the genus *Mycobacteria*, as well as the insertion sequence IS6110, the most widely used marker for the detection of *M. tuberculosis* complex. According to these results, the values of sensitivity, specificity, positive and negative predictive values were 0.89; 0.67; 0.8 and 0.8, respectively. In the scenario of a previous unknown situation, this diagnostic method was used as a screening test to avoid transmission, so a highly sensitive test is needed. If the performance values continue as n increases, it could be inferred that the tuberculin test in buffaloes in the anocaudal fold would meet the premises to determine that it is a useful test for tuberculosis diagnosis.

Keywords: tuberculinization, immune response, buffalo health, zoonoses.

vamente el 5% (30/566) fueron positivos, el 2% sospechosos (12/566) y el 93% (524/566) negativos. Se seleccionaron quince animales (10 reactivos y 5 no) y se siguieron hasta el sacrificio, donde se observó la presencia de lesiones compatibles con tuberculosis y se tomaron muestras de ganglios linfáticos y órganos para su posterior análisis mediante cultivo bacteriológico y tipificación molecular. De los 5 animales no reactivos, 4 de ellos resultaron negativos mediante cultivo bacteriológico; el otro búfalo resulta ser un animal falso negativo porque resulta positivo al cultivo bacteriológico. De 10 búfalos que reaccionaron, 8 fueron confirmados positivos por cultivo y dos de ellos fueron negativos. De los 8 búfalos con cultivos positivos, sólo 2 presentaron lesiones macroscópicas compatibles con tuberculosis, uno en ganglio mediastínico y pulmón; y el otro en ganglio retrofaringeo. En pruebas moleculares se amplificó el gen *hsp65* para determinar el género *Mycobacteria*, así como la secuencia de inserción IS6110, marcador más utilizado para la detección del complejo *M. tuberculosis*. Según estos resultados, los valores de sensibilidad, especificidad, valores predictivos positivos y negativos fueron 0,89; 0,67; 0,8 y 0,8, respectivamente. En el caso de una situación previa desconocida, este método de diagnóstico se utiliza como prueba de detección para evitar la transmisión, por lo que se necesita una prueba altamente sensible. Si los valores de rendimiento continúan a medida que n aumenta, se podría inferir que la prueba de tuberculina en búfalos del pliegue anocaudal cumpliría con las premisas para determinar que es una prueba útil para el diagnóstico de tuberculosis.

Palabras clave: tuberculinización, respuesta inmune, salud del búfalo, zoonosis.

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The use of antimicrobials in Italian buffalo farms

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ABSTRACT

Antimicrobial resistance (AMR) is a major global health emergency; 25,000 people die each year in Europe from infections sustained by resistant bacteria. Inappropriate antimicrobial use (AMU) in humans and animals is reinforcing AMR, threatening public health. The European Medicines Agency (EMA) has classified antimicrobials according to their importance in human medicine. Specifically, the EMA's Category B refers to critical antimicrobials that are registered for use in animals but should only be administered in the absence of viable alternatives. Therefore, there is growing institutional attention to implementing actions for the optimization of AMU. In particular, the World Health Organization's Global Plan of Action on Antimicrobial Resistance recommends a close intersectoral collaboration between public health and animal health (One Health). The purpose of this study was to report the first data on AMU in Italian dairy buffalo farms and the principal causes of treatment. Data from 102 farms were collected over a three-year period (2015-2017); AMU was estimated separately by age group (adults, heifers and calves) using the Defined Daily Dose for Italy (DDDAit). Antimicrobials were used almost exclusively on adults, where overall AMU was relatively low, averaging 1.72 DDDAit/head. The three most common causes of AMU in adult buffaloes were: urogenital (39.2%), dry cow therapy (26.2%), and mastitis (22.8%). The main molecules used are represented by tetracyclines followed by III-IV generation cephalosporins (III-IVGenCep) and I-II generation cephalosporins. With regard to the use of the categories of critical drugs (III-IV-GenCep and Quinolones), most of the use (65.4%) is linked to adult animals, while in relation to the ratio between DDDAit from critical drugs/total DDDAit for each category of animals, the values relating to calves are high (31.29%), proving that, even if fewer drugs are used, the incidence of criticals in this category should be reduced. Although direct comparisons may

El uso de antimicrobianos en granjas de búfalos italianas

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RESUMEN

La resistencia a los antimicrobianos (RAM) es una importante emergencia sanitaria mundial; 25.000 personas mueren cada año en Europa a causa de infecciones provocadas por bacterias resistentes. El uso inadecuado de antimicrobianos (AMU) en humanos y animales está reforzando la resistencia a los antimicrobianos y amenazando la salud pública. La Agencia Europea de Medicamentos (EMA) ha clasificado los antimicrobianos según su importancia en la medicina humana. Específicamente, la Categoría B de la EMA se refiere a antimicrobianos críticos que están registrados para su uso en animales pero que sólo deben administrarse en ausencia de alternativas viables. Por lo tanto, existe una creciente atención institucional a la implementación de acciones para la optimización del AMU. En particular, el Plan de Acción Mundial sobre Resistencia a los Antimicrobianos de la Organización Mundial de la Salud recomienda una estrecha colaboración intersectorial entre la salud pública y la salud animal (Una Salud-One Health). El objetivo de este estudio fue presentar los primeros datos sobre el AMU en las granjas de búfalos lecheras italianas y las principales causas del tratamiento. Se recopiló datos de 102 granjas durante un período de tres años (2015-2017); la AMU se estimó por separado por grupo de edad (adultos, novillas y terneros) utilizando la Dosis Diaria Definida para Italia (DDDAit). Los antimicrobianos se utilizaron casi exclusivamente en adultos, donde la AMU general fue relativamente baja, con un promedio de 1,72 DDDAit/cabeza. Las tres causas más comunes de AMU en búfalos adultos fueron: urogenital (39,2%), terapia con vaca seca (26,2%) y mastitis (22,8%). Las principales moléculas utilizadas están representadas por las tetraciclinas, seguidas de las cefalosporinas de generación III-IV (III-IVGenCep) y las cefalosporinas de generación I-II. En cuanto al uso de las categorías de fármacos críticos (III-IVGenCep y Quinolonas), la mayor parte del

have some limitations, AMU seems to be lower in buffalo than in other species reared in Italy as reported in the bibliography (Mazza et al. 2021). The identification of the principal causes of AMU in buffalo represents an important step towards a more efficient AMU analysis of structural and management critical aspects connected to the buffalo breeding system finalized to reduce to sustainable mode the AMU, in particular critical antimicrobials, and their connected AMR.

Keywords: AMU, DDDAit, buffalo.

uso (65,4%) está vinculado a animales adultos, mientras que en cuanto a la relación entre DDDAit de fármacos críticos/DDDAit total para cada categoría de animales, los valores relativos a los terneros son elevados (31,29%), lo que demuestra que, aunque se utilicen menos fármacos, la incidencia de fármacos críticos en esta categoría debería reducirse. Aunque las comparaciones directas pueden tener algunas limitaciones, la AMU parece ser menor en el búfalo que en otras especies criadas en Italia, como se informa en la bibliografía (Mazza et al. 2021). La identificación de las principales causas de la AMU en el búfalo representa un paso importante hacia un análisis más eficiente de la AMU de los aspectos críticos estructurales y de gestión relacionados con el sistema de cría del búfalo, finalizado para reducir a modo sostenible la AMU, en particular los antimicrobianos críticos, y su RAM asociada.

Palabras clave: AMU, DDDAit, búfalo.

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Diagnosis of bovine viral diarrhoea in *Bubalus bubalis* dairy farms

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ABSTRACT

Bovine viral diarrhoea (BVD) is a disease with worldwide distribution and endemic in most bovine populations, causing a wide range of clinical manifestations, including reproductive disorders, such as embryo mortality and abortions, and low milk production. The purpose of this research was to describe the situation of BVD in *Bubalus bubalis* females from one dairy farm, in Portuguesa state, Venezuela. Fifty-two 52 samples were taken in August 2022. From these, 22 were from a low milk yield group (3.35 ± 0.30 liters/day) and 30 from a high milk yield group (6.91 ± 0.82 liters). The farmer reported fluctuations in milk production, weak calves, the presence of blisters in the oral cavity and dead animals. The ELISA test was performed to detect BVD p80 antibody using the IDvet[®] kit, and the positive cases were defined using a cut-off value sample/negative ≤ 40 . The seropositive results for the non-structural protein p80, in general, were 55.76%. In the group of buffaloes with low milk yield, 54.54% were positive, while in the group with high milk yield, 53.33% were positive, with no significant differences between animal groups ($p > 0.05$). These results show a humoral immune response against potential BVD infections (regardless of the milk yield) since p80 is a non-structural protein that manifests during viral replication. Therefore, it is suggested to implement sanitary plans for the control of BVDV to minimize the economic losses associated with the disease.

Keywords: buffalo, BVD, ELISA, p80.

Diagnóstico de diarrea viral bovina en granjas lecheras de *Bubalus bubalis*

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RESUMEN

La diarrea viral bovina (DVB) es una enfermedad de distribución mundial y endémica en la mayoría de las poblaciones bovinas, que causa una amplia gama de manifestaciones clínicas, que incluyen trastornos reproductivos, como mortalidad embrionaria y abortos, y baja producción de leche. El propósito de esta investigación fue describir la situación de la DVB en hembras *Bubalus bubalis* de una granja lechera, en el estado Portuguesa, Venezuela. Se tomaron 52 muestras en agosto de 2022. De ellas, 22 fueron de un grupo de baja producción de leche ($3,35 \pm 0,30$ litros/día) y 30 de un grupo de alta producción de leche ($6,91 \pm 0,82$ litros). El ganadero informó fluctuaciones en la producción de leche, terneros débiles, presencia de ampollas en la cavidad bucal y animales muertos. Se realizó la prueba ELISA para detectar el anticuerpo BVD p80 mediante el kit IDvet[®], y los casos positivos se definieron utilizando un valor de corte muestra/negativo ≤ 40 . Los resultados seropositivos para la proteína no estructural p80, en general, fueron 55,76%. En el grupo de búfalos con baja producción de leche el 54,54% fueron positivas, mientras que en el grupo de alta producción de leche el 53,33% fueron positivas, no existiendo diferencias significativas entre los grupos de animales ($p > 0.05$). Estos resultados muestran una respuesta inmune humoral contra posibles infecciones por BVD (independientemente de la producción de leche), ya que p80 es una proteína no estructural que se manifiesta durante

la replicación viral. Por lo que se sugiere implementar planes sanitarios para el control del BVDV para minimizar las pérdidas económicas asociadas a la enfermedad.

Palabras clave: búfalo, BVD, ELISA, p80.

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Prevalence of foodborne zoonotic pathogens in milk and milk products along the water buffalo milk value chain in Bangladesh

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ABSTRACT

Buffalo milk and milk products are considered highly valued due to their superior nutritional quality and health benefits. Therefore, assessing the prevalence of zoonotic pathogens in buffalo milk is crucial for ensuring the safety of the consumers. The present study aimed to estimate the prevalence of important foodborne zoonotic pathogens in milk and milk products and identify the associated factors in the buffalo milk chain nodes in Bangladesh. One hundred and forty-three samples

Prevalencia de patógenos zoonóticos transmitidos por los alimentos en la leche y los productos lácteos a lo largo de la cadena de valor de la leche de búfala en Bangladesh

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RESUMEN

La leche y los productos lácteos de búfala se consideran muy valorados debido a su calidad nutricional superior y sus beneficios para la salud. Por lo tanto, evaluar la prevalencia de patógenos zoonóticos en la leche de búfala es crucial para garantizar la seguridad de los consumidores. El presente estudio tuvo como objetivo estimar la prevalencia de importantes patógenos zoonóticos transmitidos por los alimentos en la leche y

were collected from farm bulk milk (n = 34), middleman (n = 37), milk collection center (n = 37), and milk product shops (n = 35) and were analyzed using RT-PCR to estimate the prevalence of seven important zoonotic pathogens: *Staphylococcus aureus*, *Escherichia (E.) coli*, Shiga toxin-producing *Escherichia (E.) coli* O157:H7, *Campylobacter (C.) jejuni*, *Listeria (L.) monocytogenes*, *Salmonella (S.) enterica*, and *Yersinia (Y.) enterocolitica*. *Escherichia coli* was the most prevalent pathogen along the milk chain nodes. The prevalence of the pathogens was high over the buffalo milk value chain. Three classical enterotoxin-encoded genes for *E. coli* O157:H7 were tested e.g., *eae*, *stx1*, and *stx2* of which the *stx2* genotype was most prevalent and was most common in milk products (74%). The prevalence of *L. monocytogenes* and *Y. enterocolitica* were more prevalent on the farms (65 - 79%) than in the later milk value chain nodes. The prevalence of *S. enterica* was rather low (0 - 2.9%) in all the milk chain nodes and all the samples were negative for *C. jejuni*. These results suggest that buffalo milk consumers in Bangladesh are at risk for *L. monocytogenes* and *Y. enterocolitica* on the farms and *E. coli* O157:H7 with *stx2* genotype at the milk product shops. *S. enterica* and *C. jejuni* are not frequent contaminants of the buffalo milk chain in Bangladesh. Avoiding plastic containers and dirty clothes for cleaning milk containers may help reduce contamination in the buffalo milk value chain.

Keywords: water buffalo, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica*.

los productos lácteos e identificar los factores asociados en los nodos de la cadena de la leche de búfala en Bangladesh. Se recolectaron ciento cuarenta y tres muestras de leche a granel de granja (n = 34), intermediarios (n = 37), centros de recolección de leche (n = 37) y tiendas de productos lácteos (n = 35) y se analizaron mediante RT-PCR para estimar la prevalencia de siete patógenos zoonóticos importantes: *Staphylococcus aureus*, *Escherichia (E.) coli*, *Escherichia (E.) coli* O157:H7 productora de toxina Shiga, *Campylobacter (C.) jejuni*, *Listeria (L.) monocytogenes*, *Salmonella (S.) enterica* y *Yersinia (Y.) enterocolitica*. *Escherichia coli* fue el patógeno más prevalente a lo largo de los nodos de la cadena láctea. La prevalencia de los patógenos fue alta en la cadena de valor de la leche de búfala. Se probaron tres genes clásicos codificados por enterotoxinas para *E. coli* O157:H7, por ejemplo, *eae*, *stx1* y *stx2*, de los cuales el genotipo *stx2* fue el más prevalente y común en los productos lácteos (74%). La prevalencia de *L. monocytogenes* e *Y. enterocolitica* fue mayor en las granjas (65 - 79%) que en los nodos posteriores de la cadena de valor de la leche. La prevalencia de *S. enterica* fue bastante baja (0 - 2,9%) en todos los nodos de la cadena láctea y todas las muestras fueron negativas para *C. jejuni*. Estos resultados sugieren que los consumidores de leche de búfala en Bangladesh corren riesgo de contraer *L. monocytogenes* e *Y. enterocolitica* en las granjas y *E. coli* O157:H7 con genotipo *stx2* en las tiendas de productos lácteos. *S. enterica* y *C. jejuni* no son contaminantes frecuentes de la cadena de la leche de búfala en Bangladesh. Evitar recipientes de plástico y ropa sucia para limpiar los recipientes de leche puede ayudar a reducir la contaminación en la cadena de valor de la leche de búfala.

Palabras clave: búfalo de agua, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica*.

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Transfer of aflatoxin M₁ and sterigmatocystin from buffalo milk to soft cheeses

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ABSTRACT

Aflatoxin M₁ (AFM₁) may be present in milk as a result of the ingestion of aflatoxin B₁ (AFB₁), which could potentially be contained in dairy feed due to contamination by molds such as *Aspergillus flavus* and *A. parasiticus*. AFM₁ is a toxic molecule classified by the IARC as "potentially carcinogenic to humans" in Group 1. In addition to the four aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂), these fungi also produce other toxins such as Sterigmatocystin (STC), while Aflatoxicol (AFL) is a metabolite of AFB₁. The aim of this study was to investigate the transfer of AFM₁ and STC from naturally contaminated buffalo milk to soft cheeses. During routine activities carried out in the Lazio region (Italy) between July 2022 and May 2023, eight bulk buffalo milk samples, with AFM₁ incidence, were found on eight different farms. Bulk milk samples were immediately collected to conduct eight experimental mini-cheese-making procedures, following the traditional production method of mozzarella and ricotta. These soft cheeses were manufactured, and samples of whey, and spinning water were collected. All samples were analysed for AFM₁, AFL, and STC was determined with the LC-MS/MS system. The concentrations in milk were: AFM₁ range of 6-87 ng/kg; STC range of 0.3-0.8 ng/kg (detected in only five lots). AFL was never detected (LoQ>4 ng/kg). Mycotoxins detected in cheese were, for mozzarella, AFM₁ concentration range of 15-233 ng/kg; STC concentration range of 0.9-10 ng/

Transferencia de aflatoxina M₁ y esterigmatocistina de la leche de búfala a los quesos blandos

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RESUMEN

La aflatoxina M₁ (AFM₁) puede estar presente en la leche como resultado de la ingestión de aflatoxina B₁ (AFB₁), que potencialmente podría estar contenida en los alimentos lácteos debido a la contaminación por mohos como *Aspergillus flavus* y *A. parasiticus*. AFM₁ es una molécula tóxica clasificada por la IARC como "potencialmente cancerígena para los humanos" en el Grupo 1. Además de las cuatro aflatoxinas (AFB₁, AFB₂, AFG₁ y AFG₂), estos hongos también producen otras toxinas como la esterigmatocistina (STC), mientras que el aflatoxicol (AFL) es un metabolito de AFB₁. El objetivo de este estudio fue investigar la transferencia de AFM₁ y STC de la leche de búfala contaminada naturalmente a los quesos blandos. Durante las actividades de rutina, realizadas en la región del Lazio (Italia) entre julio de 2022 y mayo de 2023, se encontraron ocho muestras de leche a granel de búfala con incidencia AFM₁, en ocho granjas diferentes. Inmediatamente, se recolectaron muestras de leche a granel para realizar ocho elaboraciones experimentales de miniquesos, siguiendo el método tradicional de producción de mozzarella y ricotta. Se fabricaron estos quesos blandos y se recogieron muestras de suero y agua de hulado. Todas las muestras se analizaron para determinar AFM₁, AFL y STC con el sistema LC-MS/MS. Las concentraciones en la leche fueron: rango de AFM₁ de 6 a 87 ng/kg; rango de STC de 0,3-0,8 ng/kg (detectado solo en cinco lotes). AFL nunca se

kg; while for ricotta, AFM₁ concentration range 4.2-92 ng/kg and STC concentration range 0.5-4 ng/kg. AFL was never detected (LoQ>10 ng/kg). Interestingly, the range concentration of AFM₁ in the spinning water was 1.2 – 21 ng/kg. Even though it is to a small extent, the processing technology, which uses hot water to spin the mozzarella, can act as a detoxifier. The assessment of the AFM₁ enrichment factor (EF) in cheeses made with contaminated milk, revealed an EF of 2.4 for mozzarella and 1.2 for ricotta; EFs were calculated as the ratio (%) between AFM₁ in the respective cheese and AFM₁ in milk. In conclusion, the EFs found in our study are similar to those reported in the literature for these cheeses, and below the suggested value proposed by the Italian Ministry of Health of 3.0 for soft cheese like mozzarella and ricotta. Among the other toxins analysed, only STC was found, although in low concentrations, while AFL was never detected.

Keywords: Aflatoxin M₁, sterigmatocystin, Buffalo cheeses, Enrichment Factor.

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detectó (LoQ>4 ng/kg). Las micotoxinas AFM₁ detectadas en el queso mozzarella tenían un rango de concentración de 15 a 233 ng/kg y el rango de concentración de STC de 0,9 a 10 ng/kg; para ricotta el rango de concentración de AFM₁ es de 4,2 a 92 ng/kg y el rango de concentración de STC de 0,5 a 4 ng/kg. Además, en el caso de los quesos, nunca se detectó AFL (LoQ>10 ng/kg). Curiosamente, el rango de concentración de AFM₁ en el agua de hilado fue de 1,2 a 21 ng/kg. Aunque sea en pequeña medida, la tecnología de procesamiento, que utiliza agua caliente para hacer girar la mozzarella, puede actuar como desintoxicante. La evaluación del factor de enriquecimiento (FE) AFM₁ en quesos elaborados con leche contaminada reveló un FE de 2,4 para mozzarella y 1,2 para ricotta; Los FE se calcularon como la relación (%) entre AFM₁ en el queso respectivo y AFM₁ en la leche. En conclusión, los FE encontrados en nuestro estudio son similares a los reportados en la literatura para estos quesos, y por debajo del valor sugerido por el Ministerio de Salud italiano de 3,0 para quesos blandos como mozzarella y ricotta. Entre el resto de las toxinas analizadas, sólo se encontró STC, aunque en bajas concentraciones, mientras que nunca se detectó AFL.

Palabras clave: Aflatoxina M₁, esterigmatocistina, quesos de búfalo, factor de enriquecimiento.

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Molecular identification of *Leptospira* spp. in urine samples from female water buffaloes. Preliminary results

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ABSTRACT

Animal leptospirosis is a zoonotic disease that affects multiple domestic and wild species. It causes generalized vasculitis, which in pregnant females results in placentitis and triggers abortion. Furthermore, it causes hemoglobinuria, icterus (jaundice), and congestion in different mucosa. The objective of this study was to establish whether the occurrence of hemoglobinuria and changes in the pigmentation of the vaginal mucosa was associated with the presence of *Leptospira* spp. DNA in the urine. Therefore, 38 urine samples from lactating female buffaloes showing hemoglobinuria, alterations in the pigmentation of the vaginal mucosa, history of abortion, and low-weight calves were evaluated. Additionally, hematocrit

Identificación molecular de *Leptospira* spp. en muestras de orina de búfalas de agua hembras. Resultados preliminares

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RESUMEN

La leptospirosis animal es una enfermedad zoonótica que afecta a múltiples especies domésticas y salvajes. Provoca vasculitis generalizada, que en mujeres embarazadas provoca placentitis y desencadena el aborto. Además, provoca hemoglobinuria, ictericia y congestión en diferentes mucosas. El objetivo de este estudio fue establecer si la aparición de hemoglobinuria y cambios en la pigmentation de la mucosa vaginal se asociaban con la presencia de *Leptospira* spp. ADN en la orina. Por lo tanto, se evaluaron 38 muestras de orina de búfalas lactantes que mostraron hemoglobinuria, alteraciones en la pigmentation de la mucosa vaginal, antecedentes de aborto y terneros de bajo peso. Además, el hematocrito se determinó

was determined by blood centrifugation in a capillary tube, and the presence of hemoparasites was determined by staining of blood smears. The DNA of *Leptospira* spp. was detected by PCR using two pairs of primers for the markers G1-G2, derived from a sequence from the genomic library of *L. interrogans* serovar *icterohaemorrhagiae*, strain RGA, which was described by Gravekamp et al. (1993). Additionally, Internal 1 and Internal 2 were derived from the sequence of the gene encoding the LipL32 protein and specific to identify pathogenic leptospira, described by Haake et al. (2000). Data analysis was performed with R (Fisher's exact test). The hematocrit mean was 21%, and all females were negative for hemoparasites (*Anaplasma marginale*, *Babesia* spp., and *Trypanosoma* spp.). The percentage of *Leptospira* spp. DNA-positive samples with at least one of the markers used was 63%. The marker G1-G2 detected more positive samples in comparison with the marker Internal 1-Internal 2 (60.5% vs 7.9%, $p < 0.0001$ respectively). A significant association was observed between the mucosa's appearance and the presence of *Leptospira* spp. DNA in urine when the G1-G2 was used ($p < 0.0001$). In the case of females with icteric vaginal mucosa, the percentage of positive samples (85%, 17/20, $p = 0.001227$) was higher than that in buffaloes with normal mucosa (26.66%, 4/15). In addition, in females with icteric vaginal mucosa, the odds of a positive sample for *Leptospira* spp. DNA (using the G1-G2 marker) were 14 (95% CI: 2.3427-119.1974) times more than in females with normal vaginal mucosa. There was no significant difference in the percentage of positive samples between buffaloes grouped as icteric mucosa, and congestive mucosa ($p = 0.4529$), nor between the females grouped as congestive and normal mucosa ($p = 0.2451$). When the Internal 1-2 marker was used, there was no association between the appearance of the mucosa and the percentage of buffaloes with *Leptospira* spp. DNA in the urine ($p = 0.1714$). The presence of hemoglobinuria was not associated with *Leptospira* spp. DNA in the urine, regardless of the marker used for its detection ($p > 0.05$). In conclusion, the data from this study indicates that the G1-G2 marker was more efficient in determining the presence of *Leptospira* spp. DNA in urine, and using this marker, the icteric vaginal mucosa is associated with *Leptospira* spp. DNA in urine, thus suggesting that this clinical sign should be considered suggestive of the disease.

Keywords: *Leptospira*, PCR, icterus, water buffaloes.

mediante centrifugación de la sangre en un tubo capilar y la presencia de hemoparásitos se determinó mediante tinción de frotis de sangre. El ADN de *Leptospira* spp. se detectó mediante PCR utilizando dos pares de cebadores para los marcadores G1-G2, derivados de una secuencia de la biblioteca genómica de *L. interrogans* serovar *icterohaemorrhagiae*, cepa RGA, que fue descrita por Gravekamp et al. (1993). Además, Internal 1 e Internal 2 se derivaron de la secuencia del gen que codifica la proteína LipL32 y son específicos para identificar leptospiras patógenas, descrito por Haake et al. (2000). El análisis de los datos se realizó con R (prueba exacta de Fisher). La media del hematocrito fue del 21% y todas las hembras resultaron negativas para hemoparásitos (*Anaplasma marginale*, *Babesia* spp. y *Trypanosoma* spp.). El porcentaje de muestras de ADN *Leptospira* spp. positivas con al menos uno de los marcadores utilizados fue del 63%. El marcador G1-G2 detectó más muestras positivas en comparación con el marcador Interno 1-Interno 2 (60,5% vs 7,9%, $p < 0,0001$ respectivamente). Se observó una asociación significativa entre la apariencia de la mucosa y la presencia de ADN de *Leptospira* spp. en la orina cuando se utilizó el G1-G2 ($p < 0,0001$). En el caso de hembras con mucosa vaginal icterica, el porcentaje de muestras positivas (85%, 17/20, $p = 0,001227$) fue mayor que en búfalas con mucosa normal (26,66%, 4/15). Además, en hembras con mucosa vaginal icterica, las probabilidades de una muestra positiva para ADN de *Leptospira* spp. (utilizando el marcador G1-G2) fue 14 (IC 95%: 2,3427-119,1974) veces más que en hembras con mucosa vaginal normal. No hubo diferencia significativa en el porcentaje de muestras positivas entre las búfalas agrupadas como mucosa icterica y mucosa congestiva ($p = 0,4529$), ni entre las hembras agrupadas como mucosa congestiva y normal ($p = 0,2451$). Cuando se utilizó el marcador Interno 1-2 no hubo asociación entre la apariencia de la mucosa y el porcentaje de búfalos con *Leptospira* spp. ADN en orina ($p = 0,1714$). La presencia de hemoglobinuria no se asoció con *Leptospira* spp. ADN en orina, independientemente del marcador utilizado para su detección ($p > 0,05$). En conclusión, los datos de este estudio indican que el marcador G1-G2 fue más eficiente para determinar la presencia de *Leptospira* spp. ADN en orina, y utilizando este marcador, se asocia la mucosa vaginal icterica con *Leptospira* spp. ADN en orina, lo que sugiere que este signo clínico debe considerarse sugestivo de la enfermedad.

Palabras clave: *Leptospira*, PCR, ictericia, búfalos de agua.

REPRODUCTION

Reproducción

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Anogenital distance in water buffalo females and relationship with reproductive performance. Preliminary results

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ABSTRACT

Recently, anogenital distance (AGD) has been studied in cattle, and cows with shorter AGD have a better reproductive performance. There needs to be more information about AGD and its influence on reproductive performance in the case of water buffaloes. Therefore, the objectives of this preliminary study were to characterize the anogenital distance in water buffalo females and evaluate if this trait is related to reproductive performance. Data for this study was registered in a commercial farm in El Vigía, Merida, Venezuela, with crosses of Mediterranean x Murrah water buffalo breeds (*Bubalus bubalis*). The collected data from 171 females (26 nulliparous and 145 with at least one calving) included anogenital distance (millimeters), age (days), weight (kilograms), height at the withers (millimeters), and hip height (millimeters). The calving-to-conception interval (days) was registered from 88 females. Descriptive statistics and correlation analysis (Pearson's correlation coefficient) were carried out between AGD, age, weight, wither height, and hip height. Water buffalo cows with at least one previous calving were categorized as short or long AGD based on the median AGD. The calving-to-conception interval was modeled against the AGD category using a

Distancia anogenital en hembras de búfalo de agua y relación con el desempeño reproductivo. Resultados preliminares

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RESUMEN

Recientemente, se ha estudiado la distancia anogenital (AGD) en bovinos, y las vacas con AGD más corta tienen un mejor desempeño reproductivo. Es necesario disponer de más información sobre la AGD y su influencia en el rendimiento reproductivo en el caso de los búfalos de agua. Por lo tanto, los objetivos de este estudio preliminar fueron caracterizar la distancia anogenital en hembras bufalinas y evaluar si este rasgo está relacionado con el desempeño reproductivo. Los datos para este estudio se registraron en una finca comercial en El Vigía, Mérida, Venezuela, con cruces de razas de búfalo de agua Mediterráneo x Murrah (*Bubalus bubalis*). Los datos recopilados de 171 hembras (26 nulíparas y 145 con al menos un parto) incluyeron distancia anogenital (milímetros), edad (días), peso (kilogramos), altura a la cruz (milímetros) y altura de la cadera (milímetros). Se registró el intervalo parto-concepción (días) de 88 hembras. Se realizaron estadísticas descriptivas y análisis de correlación (coeficiente de correlación de Pearson) entre AGD, edad, peso, altura a la cruz y altura de la cadera. Las búfalas con al menos un parto previo se clasificaron como AGD corta o larga según la mediana AGD. El intervalo parto-concepción se modeló contra la categoría AGD utilizando

variance analysis adjusted by parity and season of calving. All analyses were carried out with R studio version R4.2.2. The anogenital distance averaged 152 ± 20.60 mm, and it was correlated positively with age (0.5516; $p < 0.05$), weight (0.4574; $p < 0.05$), height at the withers (0.4056, $p < 0.05$), and hip height (0.3703; $p < 0.05$). Water buffalo cows with at least one previous calving were categorized as short-AGD or long-AGD (short < 157.0 mm $>$ long), and as expected, the mean of AGD differed between groups (145.61 vs. 180.50 mm, respectively, $p < 0.05$). It was observed that the calving-to-conception interval was shorter in water buffalo cows with short AGD (70.82 vs. 96.60 days, $p < 0.05$). In conclusion, anogenital distance in water buffalo females positively correlates with age, weight, wither height, and hip height. Additionally, a long anogenital distance was associated with lower reproductive performance (longer calving-to-conception interval). This fact could be attributed to the adverse effects of prenatal exposure to androgens on reproductive organs and endocrine pathways; however, more research is needed to understand this better. This could be the first study establishing the relationship between anogenital distance and reproductive performance in *Bubalus bubalis*. However, considering the reduced number of animals and observations, testing, and validating these findings with a bigger population is necessary. In the same direction, there is a need to carry out more research to clarify the mechanism involved in improving reproductive performance and the heritability of AGD.

Keywords: water buffalo cows, anogenital distance, reproductive performance, calving-to-conception interval.

un análisis de varianza ajustado por paridad y temporada de parto. Todos los análisis se realizaron con R Studio versión R4.2.2. La distancia anogenital promedió $152 \pm 20,60$ mm y se correlacionó positivamente con la edad (0,5516; $p < 0,05$), peso (0,4574; $p < 0,05$), altura a la cruz (0,4056, $p < 0,05$) y altura de la cadera (0.3703; $p < 0.05$). Las búfalas con al menos un parto previo se clasificaron como AGD corta o AGD larga (corta $< 157,0$ mm $>$ de largo) y, como era de esperar, la media de AGD difirió entre grupos (145,61 vs. 180,50 mm, respectivamente, $p < 0,05$). Se observó que el intervalo parto-concepción fue más corto en búfalas con AGD corto (70,82 vs. 96,60 días, $p < 0,05$). En conclusión, la distancia anogenital en hembras búfalas se correlaciona positivamente con la edad, el peso, la altura a la cruz y la altura de la cadera. Además, una distancia anogenital larga se asoció con un menor rendimiento reproductivo (intervalo parto-concepción más largo). Este hecho podría atribuirse a los efectos adversos de la exposición prenatal a los andrógenos sobre los órganos reproductivos y las vías endocrinas; sin embargo, se necesita más investigación para comprender esto mejor. Este podría ser el primer estudio que establece la relación entre la distancia anogenital y el rendimiento reproductivo en *Bubalus bubalis*. Sin embargo, considerando el número reducido de animales y observaciones, es necesario probar y validar estos hallazgos con una población más grande. En la misma dirección, es necesario realizar más investigaciones para aclarar el mecanismo implicado en la mejora del rendimiento reproductivo y la heredabilidad de la AGD.

Palabras clave: búfalos de agua, distancia anogenital, desempeño reproductivo, intervalo parto-concepción.

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Relationship between the postpartum moment of pregnancy and productivity in water buffalo cows. Preliminary results

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ABSTRACT

One of the most important facts in the livestock industry is the need to increase reproductive performance to improve profitability. In the case of water buffalo cows, a calving-to-con-

Relación entre el momento posparto de la gestación y la productividad en vacas búfala de agua. Resultados preliminares

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RESUMEN

Uno de los hechos más importantes en la industria ganadera es la necesidad de incrementar el rendimiento reproductivo para mejorar la rentabilidad. En el caso de las búfalas

ception interval maximum of 90 days is recommended; however, achieving this reproductive target could be challenging. This study evaluated how the moment water buffalo cows get pregnant (before or after 90 days postpartum) affects the lactation length, total milk yield, calving interval, milk yield per day of the calving interval, and dry period. In this study, day 90 is considered the maximum postpartum moment in which the buffaloes must become pregnant to reach a calving interval near 400 days, regarded as the maximum calving interval without a negative economic impact on farm profitability. This retrospective cohort study analyzed 3060 observations from 1459 water buffalo cows from three farms in a similar agroecological environment (Catatumbo and Jesus Maria Semprun counties, Zulia State, Venezuela). A linear mixed model of the mean of parity, lactation length, total milk yield, calving interval, milk yield per day of calving interval, milk yield per year, dry period, and calving interval was built with the postpartum moment of pregnancy (before or after 90 days) as a fixed effect. All analyses were performed in Stata 15.1, and comparisons were adjusted by Sidak's method. Water buffalo cows getting pregnant after 90 days postpartum had lower parity (1.85 vs. 2.25, $p<0.05$), a more extended lactation period (321.77 days vs. 254.01 days, $p<0.05$), a total milk yield of 18.09% higher (1452.91 kg vs. 1230.31 kg, $p<0.05$) and the calving interval was 149.43 days longer than those getting pregnant in the first 90 days postpartum (512.93 vs. 363 days, $p<0.05$). Both the milk yield per day of calving interval and the milk yield per year were higher in water buffaloes getting pregnant during the first 90 days postpartum (3.39 kg and 1237.5 kg vs. 2.9 kg and 1072.11 kg, respectively, $p<0.05$) and the dry period was shorter (108.98 vs. 191.16 days, $p<0.05$). In conclusion, water buffalo cows getting pregnant in the first 90 days postpartum had more milk yield per day of calving interval, more milk per year, and fewer non-productive days than those getting pregnant after 90 days postpartum. Adopt measurements to increase the reproductive performance of water buffalo cows and, in consequence, to maximize profitability must be a rule and not an exception.

Keywords: water buffalo cows, milk yield, productivity, calving interval, non-productive days

de agua, se recomienda un intervalo parto-concepción máximo de 90 días; sin embargo, lograr este objetivo reproductivo podría ser un desafío. Este estudio evaluó, cómo el momento en que las búfalas de agua quedan preñadas (antes o después de los 90 días posparto) afecta la duración de la lactancia, la producción total de leche, el intervalo entre partos, la producción de leche por día del intervalo entre partos y el período seco. En este estudio, se consideró el día 90 como el momento máximo posparto en el que las búfalas deben quedar preñadas para alcanzar un intervalo entre partos cercano a los 400 días, considerado como el máximo intervalo entre partos sin un impacto económico negativo en la rentabilidad de la explotación. Este estudio de cohorte retrospectivo analizó 3060 observaciones de 1459 búfalas de agua de tres fincas en un ambiente agroecológico similar (condados de Catatumbo y Jesús María Semprún, estado Zulia, Venezuela). Se construyó un modelo lineal mixto de la media de paridad, duración de la lactancia, producción total de leche, intervalo entre partos, producción de leche por día de intervalo entre partos, producción de leche por año, período seco e intervalo entre partos con el momento posparto de la preñez (antes o después de 90 días) como efecto fijo. Todos los análisis se realizaron en Stata 15.1 y las comparaciones se ajustaron mediante el método de Sidak. Las búfalas que quedaron preñadas después de 90 días posparto tuvieron una menor paridad (1,85 vs. 2,25, $p<0,05$), un período de lactancia más prolongado (321,77 días vs. 254,01 días, $p<0,05$), una producción total de leche de 18,09% mayor (1452,91 kg vs. 1230,31 kg, $p<0,05$) y un intervalo entre partos 149,43 días más largo que las que quedaron preñadas en los primeros 90 días posparto (512,93 vs. 363 días, $p<0,05$). Tanto la producción de leche por día de intervalo entre partos como la producción de leche por año fue mayor en las búfalas de agua que preñaron durante los primeros 90 días posparto (3,39 kg y 1237,5 kg vs. 2,9 kg y 1072,11 kg, respectivamente, $p<0,05$) y el período seco fue menor (108,98 vs. 191,16 días, $p<0,05$). En conclusión, las búfalas que quedaron preñadas en los primeros 90 días posparto tuvieron mayor producción de leche por día de intervalo entre partos, más leche por año y menos días no productivos que las que quedaron preñadas después de los 90 días posparto. Adoptar medidas para incrementar el rendimiento reproductivo de las búfalas de agua y, en consecuencia, maximizar la rentabilidad debe ser una regla y no una excepción.

Palabras clave: vacas búfala de agua, producción de leche, productividad, intervalo entre partos, días no productivos.

R-105 Rev. Cientif. FCV-LUZ, XXXIII, SE, 243-244, 2023, <https://doi.org/10.52973/rcfcv-wbc096>**Percentage of primiparous water buffalo cows becoming pregnant in the first 90 days postpartum****Héctor Nava-Trujillo¹, José Colmenares-Duque², Frank Díaz-Huerta³, Adriana Morgado-Osorio⁴, Armando Quintero-Moreno⁵**¹ Division of Animal Sciences, University of Missouri, Columbia, Missouri, USA.² Ejercicio libre. La Fría, Tachita Venezuela³ Mejoramiento Agroproductivo Técnico e Integral C.A (MATICA). La Fría, Tachita Venezuela.⁴ Producción Animal, Instituto de Investigaciones Agropecuarias, Universidad de los Andes, Mérida, Mérida, Venezuela⁵ Facultad de Ciencias Veterinarias, Universidad del Zulia, Maracaibo, Zulia, Venezuela*Corresponding author: Hector Nava-Trujillo (hectornava00@gmail.com).**ABSTRACT**

Reproductive performance is a critical factor affecting the profitability of buffalo systems, and parity is a factor affecting reproductive performance. This study aimed to evaluate the effect of parity on the percentage of pregnant buffaloes for day 90 postpartum and determine if the postpartum treatment with GnRH increases the reproductive efficiency of primiparous water buffalo cows. In the first experiment, water buffalo cows from one commercial farm located in Coloncito, Tachira, Venezuela, were grouped into primiparous (n=77) or multiparous (n=341), and the percentage of pregnant buffaloes at day 90 postpartum was compared with Chi-square. In a second experiment, the effect of the treatment with GnRH intramuscular (21 µg of Buserelin acetate, Gestar®, OVER, Argentina) on the rate of pregnant primiparous buffaloes for day 90 postpartum was evaluated. For this, 47 primiparous buffaloes from a commercial farm (La Fría, Tachira, Venezuela) calving between October-December, with a BCS of at least 3 (scale of 1 to 5) and without follicular or luteal structures at day 30 postpartum, were divided into three groups: Control (n=11), no treatment; G30 (n=18), treated with 21µg of GnRH at day 30 postpartum; and G45 (n=18), treated with 21 µg of GnRH at day 45 postpartum. The association between the treatment and the proportion of water buffaloes pregnant at day 90 postpartum was determined with the Chi-square test. In both experiments, animals grazed in pastures consisting of creeping river grass (*Echinochloa polystachya*), tanner (*Bachiaria arrecta*), and humidicola (*Brachiaria humidicola*) in one-day paddock rotations. The bull was present continuously with the herd of buffaloes, and the pregnancy diagnosis was carried out by rectal palpation at day 90 postpartum. In experiment 1, the percentage of buffaloes be-

Porcentaje de vacas búfalas de agua primíparas que quedan preñadas en los primeros 90 días postparto

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El desempeño reproductivo es un factor crítico que afecta la rentabilidad de los sistemas bufalinos, y la paridad es un factor que afecta el desempeño reproductivo. Este estudio tuvo como objetivo evaluar el efecto de la paridad en el porcentaje de búfalas preñadas para el día 90 posparto y determinar si el tratamiento posparto con GnRH aumenta la eficiencia reproductiva de vacas búfalas de agua primíparas. En el primer experimento, se agruparon búfalas de agua de una finca comercial ubicada en Coloncito, Táchira, Venezuela, en primíparas (n=77) o multíparas (n=341), y se comparó el porcentaje de búfalas preñadas al día 90 posparto con Chi-cuadrado. En un segundo experimento se evaluó el efecto del tratamiento con GnRH intramuscular (21 µg de acetato de buserelina, Gestar®, OVER, Argentina) sobre la tasa de búfalas primíparas preñadas al día 90 posparto. Para ello, se estudiaron 47 búfalas primíparas de una finca comercial (La Fría, Táchira, Venezuela) paridas entre octubre-diciembre, con una condición corporal (BCS) de al menos 3 (escala de 1 a 5) y sin estructuras foliculares ni lúteas al día 30 posparto. dividido en tres grupos: Control (n=11), sin tratamiento; G30 (n=18), tratado con 21 µg de GnRH el día 30 posparto; y G45 (n=18), tratados con 21 µg de GnRH el día 45 posparto. La asociación entre el tratamiento y la proporción de búfalas de agua preñadas al día 90 posparto se determinó mediante la prueba de Chi-cuadrado. En ambos experimentos, los animales pastaron en pastos que consistían en pasto alemán (*Echinochloa polystachya*), pasto Tanner (*Bachiaria arrecta*) y humidicola (*Brachiaria humidicola*) en rotaciones de potreros de un día. El toro estuvo presente continuamente con el rebaño de búfalas, y el diagnóstico de preñez se realizó mediante palpación rectal al día 90 pospar-

coming pregnant in the first 90 days postpartum was lower in primiparous (25.97% vs. 43.98%, Chi-square = 6.573, $p < 0.05$). The odds of pregnancy for day 90 in primiparous buffaloes was only 0.4468 (95% CI: 0.2571-0.7763) and was statistically significant ($p = 0.0043$). In experiment 2, the overall rate of water buffaloes becoming pregnant during the first 90 days postpartum was 59.57% (28/47). In the control group, it was 45.45%, while in the groups treated with GnRH, it was 77.77% for G30 and 50% for G45. The numerical difference in the percentage of pregnant buffaloes between treatment at day 30 versus treatment at day 45 or the control group did not reach statistical significance ($p > 0.05$). Similarly, no significant differences were observed between the control group and the buffaloes treated at day 45 (Chi-square = 0.055, $p = 0.8152$). In a separate experience, on a farm located in Tres Islas, Tachira, Venezuela, 31/34 of primiparous buffaloes treated with 21 μg of GnRH at day 30 postpartum became pregnant (a control group was not included, and therefore no statistical analysis was made). In conclusion, given the lower percentage of primiparous buffaloes becoming pregnant during the first 90 days postpartum, this category must be considered a group with a high risk of reproductive failure, and differential management must be implemented to avoid this situation. Using 21 μg of GnRH at day 30 postpartum resulted only in a numerical increase in pregnancy rate at day 90 postpartum in primiparous buffaloes. Therefore, this treatment could be an economical alternative to improve the reproductive efficiency of primiparous buffaloes calving in the short photoperiod season, managed with natural service, and having a good body condition score. Still, the results of this treatment must be validated with a larger population.

Keywords: water buffaloes, primiparous, pregnancy, GnRH, reproductive failure.

to. En el experimento 1, el porcentaje de búfalas que quedaron preñadas en los primeros 90 días posparto fue menor en primíparas que en múltiparas (25,97% vs. 43,98%, respectivamente; Chi-cuadrado = 6,573, $p < 0,05$). La probabilidad de preñez para el día 90 en búfalas primíparas fue de sólo 0,4468 (IC 95%: 0,2571-0,7763) y fue estadísticamente significativa ($p = 0,0043$). En el experimento 2, la tasa general de búfalas de agua que quedaron preñadas durante los primeros 90 días posparto fue del 59,57% (28/47). En el grupo control fue del 45,45%, mientras que en los grupos tratados con GnRH fue del 77,77% para G30 y del 50% para G45. La diferencia numérica en el porcentaje de búfalas preñadas entre el tratamiento del día 30 versus el tratamiento del día 45 o el grupo control no alcanzó significación estadística ($p > 0,05$). De manera similar, no se observaron diferencias significativas entre el grupo control y las búfalas tratadas el día 45 (Chi-cuadrado = 0,055, $p = 0,8152$). En una experiencia separada, en una finca ubicada en Tres Islas, Táchira, Venezuela, 31/34 de búfalas primíparas tratadas con 21 μg de GnRH el día 30 posparto quedaron preñadas (no se incluyó grupo control, por lo que no se realizó análisis estadístico). En conclusión, dado el menor porcentaje de búfalas primíparas que quedan preñadas durante los primeros 90 días posparto, esta categoría debe considerarse un grupo con alto riesgo de fracaso reproductivo, debiendo implementarse manejos diferenciales para evitar esta situación. El uso de 21 μg de GnRH el día 30 posparto dio como resultado solo un aumento numérico en la tasa de preñez el día 90 posparto en búfalas primíparas. Por lo tanto, este tratamiento podría ser una alternativa económica para mejorar la eficiencia reproductiva de búfalas primíparas que paren en la temporada de fotoperiodo corto, manejadas con servicio natural y con un buen puntaje de condición corporal. Aún así, los resultados de este tratamiento deben validarse con una población más grande.

Palabras clave: búfalas de agua, primíparas, gestación, GnRH, fracaso reproductivo.

R-111 Rev. Cientif. FCV-LUZ, XXXIII, SE, 245-246, 2023, <https://doi.org/10.52973/rfcv-wbc097>**Ovarian stimulation alternatives for *in vitro* production of embryos in water buffaloes****Bandeo, Alexis^{1,2}; Konrad, José Luis^{1,2}; Vallejos, Natalia¹; Ponce, Pablo^{1,2}; Sansinena, Marina J.^{2,4,5}; Crudeli, Gustavo⁶; Maldonado-Vargas, Pablo¹**¹Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina.²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.³Facultad de Ingeniería y Ciencias Agrarias, Universidad Católica Argentina⁴Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador⁵Universidad Nacional del Chaco Austral (UNCAUS).*Corresponding author: Bandeo, Alexis (bandeoalexissebastian@gmail.com).**ABSTRACT**

Currently, one of the most promising tools to increase the number of transferable embryos in buffaloes is *in vitro* production (IVP). However, IVP still has certain limitations that prevent its optimal efficiency for commercial application. One of these limitations is the reduced number and low competence of oocytes obtained from transvaginal follicular aspiration in this species. It has been found that ovarian stimulation with follicle-stimulating hormone (FSH) before aspiration improves the technique's efficiency, primarily due to the increased competence of the obtained oocytes. The objective of this study was to evaluate the response to different hormonal stimulation treatments in buffalo donors, their effect on oocyte quality, and subsequent embryo production. This field study was conducted at the Pedro Antonio Silva buffalo farm, located in the General Paz department, Province of Corrientes, in 2021. A total of 60 OPU sessions were performed on Murrah and Mediterranean breed donors, and four ovarian stimulation treatments were applied. Treatment (TRT) 1, which was used as control (n=20), consisted of a day 0 insertion of an intravaginal progesterone device (IP) + 2 mg estradiol benzoate (EB) i.m., with OPU performed on day 7. Treatment (TRT) 2 (n=10) consisted of day 0 insertion of IP + 2 mg EB, followed by four applications of FSH (Folltropin-V®) i.m. with a total of 160 mg distributed in decreasing doses every 12 hours for two days (50mg, 50mg, 30mg, 30mg), OPU was then performed 36 hours after the last application. Treatment (TRT) 3 (n=20) consisted of day 0 insertion of IP + EB, followed by a day 4 application of 1050 IU of recombinant eCG (FoliRec®) i.m., OPU was performed on day 7; Treatment (TRT) 4 (n=10) consisted of day 0 insertion of IP + EB, followed by day 4 application of 2500 IU of serum eCG (Ecegon®) i.m., OPU was performed 72 hours later. Prior

Alternativas de estimulación ovárica para la producción *in vitro* de embriones en búfalas de agua**Bandeo, Alexis^{1,2}; Konrad, José Luis^{1,2}; Vallejos, Natalia¹; Ponce, Pablo^{1,2}; Sansinena, Marina J.^{2,4,5}; Crudeli Gustavo⁶; Maldonado-Vargas, Pablo¹**¹Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina.²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.³Facultad de Ingeniería y Ciencias Agrarias, Universidad Católica Argentina⁴Facultad de Ciencias Agrarias y Veterinarias, Universidad del Salvador⁵Universidad Nacional del Chaco Austral (UNCAUS).*Autor de correspondencia: Bandeo, Alexis (bandeoalexissebastian@gmail.com).**RESUMEN**

Actualmente, una de las herramientas más prometedoras para aumentar el número de embriones transferibles en búfalas es la producción *in vitro* (PIVE). Sin embargo, la PIVE todavía tiene ciertas limitaciones que limitan su eficiencia para aplicaciones comerciales. Una de estas limitaciones es el número reducido y la baja competencia de los ovocitos obtenidos de la aspiración folicular transvaginal en esta especie. Se ha descubierto que la estimulación ovárica con hormona folículo estimulante (FSH) antes de la aspiración mejora la eficacia de la técnica, principalmente debido a la mayor competencia de los ovocitos obtenidos. El objetivo de este estudio fue evaluar la respuesta a diferentes tratamientos de estimulación hormonal en donantes bufalinas, su efecto sobre la calidad de los ovocitos y la posterior producción de embriones. Este estudio de campo se realizó en el criadero de búfalos Pedro Antonio Silva, ubicado en el departamento General Paz, Provincia de Corrientes, durante el año 2021. Se realizaron un total de 60 sesiones de OPU a donantes de raza Murrah y Mediterránea, y cuatro tratamientos de estimulación ovárica se aplicaron. El tratamiento (TRT) 1, que se utilizó como control (n=20), consistió en la inserción el día 0 de un dispositivo de progesterona intravaginal (IP) + 2 mg de benzoato de estradiol (EB) por vía intramuscular, y la OPU se realizó el día 7. Tratamiento (TRT) 2 (n=10) consistió en la inserción del día 0 de IP + 2 mg de EB, seguida de cuatro aplicaciones de FSH (Folltropin-V®) i.m. con un total de 160 mg distribuidos en dosis decrecientes cada 12 horas durante dos días (50 mg, 50 mg, 30 mg, 30 mg), luego se realizó OPU 36 horas después de la última aplicación. El tratamiento (TRT) 3 (n=20) consistió en la inserción de IP + EB el día 0, seguida de una aplicación el día 4 de 1050 UI de eCG recombinante (FoliRec®) i.m., la OPU se realizó el día 7; El tra-

to each follicular aspiration, antral follicles were counted using a portable ultrasound with a linear probe (Mindray, DP30-Vet) and classified into small (≥ 3 mm \emptyset), medium (4-8 mm \emptyset), and large (> 8 mm \emptyset) follicles. The aspirated oocytes or COCs (cumulus-oocyte complexes) were classified into grades (1 to 4) based on the number of cumulus layers present, according to the International Embryo Transfer Society method (IETS Manual). *In vitro* fertilization (IVF) was performed after 24 hours of oocyte *in vitro* maturation using cryopreserved semen from buffalo bulls of proven fertility. *In vitro* culture (IVC) was carried out for 6.5 days, and all embryos that reached the blastocyst stage were graded and vitrified. Descriptive statistics and analysis of variance were conducted on the obtained data, considering population and follicular size, quantity and quality of oocytes, and embryo production, with a significance level (α) of 5%. The total observed follicular population did not differ among treatments ($p > 0.05$). However, stimulation in TRT2 (FSH) and TRT4 (serum eCG) increased the proportion of medium-sized follicles (4-8 mm) available for OPU ($p < 0.05$), which correlates with better quality. In comparison, the control group had the highest number of small follicles (< 3 mm). The number of collected oocytes per OPU did not vary between treatments ($p > 0.05$). Regarding oocyte quality, TRT2 (FSH) and TRT4 (serum eCG) resulted in a higher quantity of grade 1 oocytes ($p < 0.05$). *In vitro* embryo production was higher in TRT2 (1.5 embryos/buffalo/OPU), followed by TRT3 (1.05 embryos/buffalo/OPU), and lower in TRT4 and TRT1 (0.8 and 0.6 embryos/buffalo/OPU, respectively) ($p < 0.05$). No significant difference was found in the number of zygotes (CIV) between treatments ($p < 0.05$), and cleavage was higher in TRT3 ($p < 0.05$). In conclusion, this study has shown that ovarian super stimulation before OPU increases the proportion of medium-sized follicles available for the aspiration procedure, resulting in a higher proportion of viable oocytes. This effect is mainly achieved using FSH and serum eCG. Stimulation with FSH also resulted in higher rates of blastocysts and embryos produced per OPU session. These are promising for the advancement of the OPU/IVP technique in water buffaloes. Further work is needed to optimize the outcomes, especially with these new ovarian stimulation alternatives.

Keywords: buffalo, stimulation, follicular aspiration, *in vitro*, embryo.

tamiento (TRT) 4 (n=10) consistió en la inserción del día 0 de IP + EB, seguida de la aplicación del día 4 de 2500 UI de eCG sérico (Ecegon®) por vía intramuscular; la OPU se realizó 72 horas después. Antes de cada aspiración folicular, los folículos antrales se contaron mediante un ultrasonido portátil con sonda lineal (Mindray, DP30-Vet) y se clasificaron en pequeños (≥ 3 mm \emptyset), medianos (4-8 mm \emptyset) y grandes (> 8 mm \emptyset) folículos. Los ovocitos aspirados o COCs (complejos cúmulo-ovocito) se clasificaron en grados (1 a 4) según el número de capas de cúmulo presentes, según el método de la Sociedad Internacional de Transferencia de Embriones (Manual IETS). La fertilización *in vitro* (FIV) se realizó después de 24 horas de maduración *in vitro* de ovocitos utilizando semen criopreservado de toros bufalinos de fertilidad comprobada. Se realizó cultivo *in vitro* (CIV) durante 6,5 días y todos los embriones que alcanzaron el estadio de blastocisto fueron clasificados y vitrificados. A los datos obtenidos se les realizó estadística descriptiva y análisis de varianza, considerando tamaño poblacional y folicular, cantidad y calidad de ovocitos y producción de embriones, con un nivel de significancia (α) del 5%. La población folicular total observada no difirió entre tratamientos ($p > 0.05$). Sin embargo, la estimulación en TRT2 (FSH) y TRT4 (eCG sérica) aumentó la proporción de folículos de tamaño mediano (4-8 mm) disponibles para OPU ($p < 0,05$), lo que se correlaciona con una mejor calidad. En comparación, el grupo de control tuvo la mayor cantidad de folículos pequeños (< 3 mm). El número de ovocitos recolectados por OPU no varió entre tratamientos ($p > 0.05$). En cuanto a la calidad de los ovocitos, TRT2 (FSH) y TRT4 (eCG sérica) resultaron en una mayor cantidad de ovocitos grado 1 ($p < 0,05$). La producción de embriones *in vitro* fue mayor en TRT2 (1,5 embriones/búfalo/OPU), seguida de TRT3 (1,05 embriones/búfalo/OPU), y menor en TRT4 y TRT1 (0,8 y 0,6 embriones/búfalo/OPU, respectivamente) ($p < 0,05$). No se encontró diferencia significativa en el número de cigotos (CIV) entre tratamientos ($p < 0,05$), y la escisión fue mayor en TRT3 ($p < 0,05$). En conclusión, este estudio ha demostrado que la superestimulación ovárica antes de la OPU aumenta la proporción de folículos de tamaño mediano disponibles para el procedimiento de aspiración, lo que resulta en una mayor proporción de ovocitos viables. Este efecto se logra principalmente utilizando FSH y eCG sérico. La estimulación con FSH también resultó en tasas más altas de blastocistos y embriones producidos por sesión de OPU. Estos son prometedores para el avance de la técnica OPU/IVP en búfalos de agua. Se necesita más trabajo para optimizar los resultados, especialmente con estas nuevas alternativas de estimulación ovárica.

Palabras clave: búfalo, estimulación, aspiración folicular, *in vitro*, embrión.

R-120 Rev. Cientif. FCV-LUZ, XXXIII, SE, 247-248, 2023, <https://doi.org/10.52973/rcfcv-wbc098>**Body weight gain and development of reproductive tract in crossbred Murrah × Mediterranean water buffalo heifers****González Oswaldo J.^{1*}, Acuña Amílcar², Bradley Solaine², Cañizalez Ysis², Espinoza Jorge², Paniagua Cindy², Suárez María Virginia³, Fuentes Mónica³**¹ Médico Veterinario en ejercicio libre² Estudiantes del Decanato de Ciencias Veterinarias de la Universidad Centroccidental "Lisandro Alvarado", Barquisimeto, Venezuela³ Decanato de Ciencias Veterinarias, Universidad Centroccidental Lisandro Alvarado, Barquisimeto, Venezuela*Corresponding author: González Oswaldo J (ojosegg@gmail.com).**ABSTRACT**

The objective of this study was to evaluate the effects of supplementation that include a *Saccharomyces cerevisiae* commercial yeast culture (Nutrit Yeast®, Barquisimeto, Venezuela) on average daily gain (ADG) and development of the reproductive tract in crossbred Murrah x Mediterranean water buffalo heifers with poor weight gain in the central-western region of Venezuela, Lara state. The buffalo heifers (middle age: 710 days old and middleweight: 237 Kg) were randomly assigned to one of two treatments for 174 days. A treated group (n=35) was fed 1kg of a concentrate animal/day plus 5g of *Saccharomyces cerevisiae* yeast/animal/day, and a Control group (n=36) did not receive the supplementation. The heifers grazed in the same pastures and received the same fresh-cut grass at the herd and the same mineral supplementation. All animals were weighed and evaluated, and the reproductive tract score (RTS) was assessed by rectal palpation every 45 days. During the test, the animals gained 94.5 ± 25.5 Kg and 59.6 ± 38.5 Kg, treated and non-treated heifers, respectively. At the end of the study, the qualifiers of the reproductive tract, 26.5%, and 3.6%, were observed in a cyclical state by treated and non-treated, respectively. The weights of the animals were analyzed by linear regression, and the qualifier of the reproductive tract by non-parametric statistics. When comparing the results between supplemented and non-supplemented heifers, highly significant differences ($p < 0.01$) were observed between both groups in ADG since supplementation began and was kept throughout the study. In addition, for the gynecological examination, a median of Wilcoxon comparison test was performed, showing highly significant differences between supplemented and non-supplemented from the beginning of supplementation, observing a lower percentage of heifers in gynecological infantile uterus condition in supplemented ones, even increasing the percentage of heifers in the development and active stage. In conclusion, the supple-

Aumento de peso corporal y desarrollo del tracto reproductivo en bubillas mestizas Murrah × Mediterránea

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El objetivo de este estudio fue evaluar los efectos de la suplementación que incluye un cultivo comercial de levadura *Saccharomyces cerevisiae* (Nutrit Yeast®, Barquisimeto, Venezuela) sobre la ganancia diaria promedio (ADG) y el desarrollo del tracto reproductivo en bubillas mestizas Murrah x Mediterránea con pobre ganancia de peso en la zona centro-occidental de Venezuela, estado Lara. Las bubillas (edad media: 710 días de edad y peso medio: 237 kg) fueron asignadas aleatoriamente a uno de dos tratamientos durante 174 días. Un grupo Tratado (n=35) fue alimentado con 1kg de concentrado animal/día más 5g de levadura *Saccharomyces cerevisiae*/animal/día, y un grupo Control (n=36) no recibió la suplementación. Los animales pastorearon en los mismos potreros y recibieron adicionalmente pasto recién cortado y el mismo suplemento mineral. Todos los animales fueron pesados y evaluados, y la puntuación del tracto reproductivo (RTS) se evaluó mediante palpación rectal cada 45 días. Durante la prueba, los animales ganaron 94,5 ± 25,5 Kg y 59,6 ± 38,5 Kg por parte de las novillas tratadas y no tratadas, respectivamente. Al final del estudio, los calificadores del tracto reproductivo, 26,5% y 3,6%, se observaron en estado cíclico por tratados y no tratados, respectivamente. Los pesos de los animales fueron analizados mediante regresión lineal, y el calificador del tracto reproductivo mediante estadística no paramétrica. Al comparar los resultados entre bubillas suplementadas y no suplementadas, se observaron diferencias significativas ($p < 0.01$) entre ambos grupos en relación a la ADG desde que comenzó la suplementación y se mantuvo durante todo el estudio. Además, para el examen ginecológico se realizó una prueba de comparación de media de Wilcoxon, mostrando diferencias significativas ($p < 0.01$) entre suplementadas y no suplementadas desde el inicio de la suplementación, encontrándose, un menor porcentaje de bubillas en condición de útero infantil y una mayor proporción de hembras con estado reproductivo en desarrollo y etapa

mentation, including the yeast culture increase, significantly enhanced the body weight gain and reproductive tract development in water buffalo heifers.

Keywords: buffalo, supplementation, Yeast, RTS, average daily gain, *Saccharomyces cerevisiae*.

activa al examen ginecológico en las suplementadas. En conclusión, la suplementación, incluyendo el aumento del cultivo de levadura, mejoró significativamente el aumento de peso corporal y el desarrollo del tracto reproductivo en bubillas mestizas Murrah x Mediterránea.

Palabras clave: búfalos, suplementación, Levadura, RTS, ganancia diaria promedio, *Saccharomyces cerevisiae*.

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Impact of post-breeding administration of hCG and flunixin meglumine on luteal function and pregnancy rates in anestrus Nili-Ravi buffaloes

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ABSTRACT

This study was conducted to determine the impact of post-breeding administration of human chorionic gonadotropin hormone (hCG) and flunixin meglumine on luteal function and pregnancy rates in postpartum anoestrous Nili-Ravi buffaloes synchronized through a CIDR-GnRH based protocol during low breeding season. It was hypothesized that administering flunixin meglumine, an inhibitor of PGF2 α synthesis, alone or in combination with hCG before implantation will increase the pregnancy rate by improving luteal functions in buffaloes. The sixty buffaloes (having a postpartum period of 90-120 days) were synchronized through the CIDR-GnRH protocol. Briefly, CIDR was placed intravaginally in all buffaloes for 7 days, then an injection of PGF2 α was given on day 06 and GnRH on day 09. Timed artificial insemination (TAI) was done at 14-16 hrs following GnRH. After TAI, animals were randomly divided into four groups for different treatments. The T1 (n=15) buffaloes were given IM injection of hCG (3000 IU/animal) on day 07 after TAI. The T2 (n=15) buffaloes received an IM injection of flunixin meglumine (1.1mg/kg; b.i.d.) on days 14-15 post-TAI. The T3 (n=15) buffaloes received IM injections of hCG (3000 IU/animal) on day 07 and IM injection of flunixin meglumine (1.1mg/kg; b.i.d.) on day 14-15 post-TAI. In the T4 group (n=15), the control group received IM injection of normal saline on day 7 and day 14-15 after TAI. The size of the corpus luteum was measured by scanning ovaries through trans-rectal ultrasonog-

Impacto de la administración post-reproducción de hCG y flunixin meglumina sobre la función lútea y las tasas de preñez en búfalos Nili-Ravi en anestro

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RESUMEN

Este estudio se realizó para determinar el impacto de la administración post-reproducción de la hormona gonadotropina coriónica humana (hCG) y flunixin meglumina sobre la función lútea y las tasas de preñez en búfalos Nili-Ravi en anestro posparto sincronizadas a través de un protocolo basado en CIDR-GnRH durante la temporada baja de reproducción. Se planteó la hipótesis de que la administración de flunixin meglumina, un inhibidor de la síntesis de PGF2 α , sola o en combinación con hCG antes de la implantación aumentaría la tasa de preñez al mejorar las funciones lúteas en las búfalas. Las sesenta búfalas (que tenían un período posparto de 90 a 120 días) se sincronizaron mediante el protocolo CIDR-GnRH. Brevemente, se colocó CIDR por vía intravaginal en todas las búfalas durante 7 días, luego se administró una inyección de PGF2 α el día 6 y GnRH el día 9. La inseminación artificial a tiempo fijo (IATF) se realizó entre 14 y 16 horas después de la GnRH. Después de la TAI, los animales se dividieron aleatoriamente en cuatro grupos para diferentes tratamientos. Las búfalas T1 (n=15) recibieron una inyección IM de hCG (3000 UI/animal) el día 7 después de la IATF. Las búfalas T2 (n=15) recibieron una inyección IM de flunixin meglumina (1,1 mg/kg; dos veces al día) los días 14-15 después de la IATF. Los búfalos T3 (n=15) recibieron inyecciones IM de hCG (3000 UI/animal) el día 07 y una inyección IM de flunixin meglumina (1,1 mg/kg; dos veces al día) el día 14-15 después de la IATF. En el grupo T4 (n=15),

raphy, and blood samples for serum progesterone assessment were collected on day 14 and day 20 post-TAI. Progesterone level was measured through ELISA. The pregnancy was diagnosed through ultrasonography on day 35 post-TAI. The results showed that the mean progesterone concentration was higher ($p < 0.05$) in pregnant T1 and T3 buffaloes on day 14 post-TAI. However, progesterone concentration was similar in all groups on day 20 post-TAI. The corpus luteum size was higher ($p < 0.05$) in pregnant buffaloes of group T1 and T3 at days 14 and 20. The pregnancy rates were higher ($p < 0.05$) in T1 and T3 compared to T2 and Control Group. It is concluded that post-breeding administration of hCG improved luteal functions and pregnancy rate. However, flunixin meglumine alone or combined with hCG did not impact these parameters in Nili-Ravi buffaloes during the low breeding season.

Keywords: hCG, flunixin meglumine, anestrous, Nili-Ravi buffalo.

el grupo de control recibió una inyección IM de solución salina normal el día 7 y el día 14-15 después de la IATF. El tamaño del cuerpo lúteo se midió escaneando los ovarios mediante ecografía transrectal y se recogieron muestras de sangre para evaluar la progesterona sérica los días 14 y 20 después de la IATF. El nivel de progesterona se midió mediante ELISA. La preñez fue diagnosticada mediante ecografía el día 35 post-IATF. Los resultados mostraron que la concentración media de progesterona fue mayor ($p < 0,05$) en búfalas preñadas de los grupos T1 y T3 el día 14 post-IATF. Sin embargo, la concentración de progesterona fue similar en todos los grupos el día 20 después de la IATF. El tamaño del cuerpo lúteo fue mayor ($p < 0,05$) en las búfalas preñadas del grupo T1 y T3 en los días 14 y 20. Las tasas de preñez fueron mayores ($p < 0,05$) en T1 y T3 en comparación con T2 y el grupo Control. Se concluye que la administración de hCG después del servicio mejoró las funciones lúteas y la tasa de preñez. Sin embargo, el flunixin meglumina solo o combinado con hCG no afectó estos parámetros en los búfalos Nili-Ravi durante la temporada baja de reproducción.

Palabras clave: hCG, flunixin meglumina, anestro, búfalos Nili-Ravi.

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Seasonal variation of different seminal parameters of breeding buffalo bulls

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ABSTRACT

This study evaluated seasonal variation in seminal parameters and post-thaw motility after cryopreservation in buffalo bulls. To conduct this study, five Nili Ravi buffalo bulls were placed at a private semen production unit, Al-Haiwan Sires, Sahiwal, under uniform nutritional and management conditions. Semen samples were taken twice a week from May 2019 to April 2020. A total of 389 ejaculates were collected with the help of a pre-warmed artificial vagina (42°C) and then transferred to the semen evaluation room. Then, semen ejaculates were evaluated for physical characteristics, including ejaculatory volume, mass motility, individual sperm motility, sperm concentration, number of ejaculates, motility after dilution, and post-thaw motility. Post-thaw semen evaluation was carried out by Computer Assisted Sperm Analyzer (CASA) at the Theriogenology laboratory, UVAS Lahore. Climatic data was also collected from the Regional Meteorological Center, Sahiwal, during the study period. The seminal parameter data of Nili Ravi buffalo bull was analyzed using the analysis of variance in the SPSS 13.0 program. The results showed that season significantly ($p < 0.05$)

Variación estacional de diferentes parámetros seminales de toros búfalos reproductores

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RESUMEN

Este presente estudio se realizó para evaluar la variación estacional en los parámetros seminales y la motilidad post-descongelación después de la criopreservación en búfalos reproductores. Para realizar este estudio, se colocaron cinco búfalos Nili Ravi en una unidad privada de producción de semen, Al-Haiwan Sires, Sahiwal, bajo condiciones nutricionales y de manejo uniformes. Se tomaron muestras de semen dos veces por semana desde mayo de 2019 hasta abril de 2020. Se recogieron un total de 389 eyaculados con ayuda de una vagina artificial precalentada (42°C) y luego se transfirieron a la sala de evaluación de semen. Luego, se evaluaron las características físicas de los eyaculados de semen, incluido el volumen eyaculatorio, la motilidad masal, la motilidad de los espermatozoides individuales, la concentración de espermatozoides, el número de eyaculados, la motilidad después de la dilución y la motilidad post-descongelación. La evaluación del semen posterior a la descongelación se llevó a cabo mediante un analizador de esperma asistido por computadora (CASA) en el laboratorio de teriogenología de UVAS Lahore. También

affected different seminal parameters, as no. of ejaculates/bull, motility after dilution, and number of doses were significantly higher in autumn. In contrast, semen volume and individual motility were highest in spring. Interestingly, sperm concentration was highest in the dry summer compared to other seasons. The sperm motility parameters and motion characteristics of buffalo bulls after post-thaw revealed that total motility, progressive motility, and rapid motility were not affected by season. In contrast, VSL, VCL, and ALH were significantly higher in the spring season than others. In conclusion, it was found that breeding bulls exhibited a seasonal pattern as their seminal parameters were found best in the spring and autumn seasons; however, most post-thaw variables have no seasonal effect.

Keywords: buffalo, Ejaculates, CASA, post thaw, cryopreservation.

se recopilaron datos climáticos del Centro Meteorológico Regional, Sahiwal, durante el período de estudio. Los datos de parámetros seminales de los búfalos Nili Ravi se analizaron mediante el análisis de varianza en el programa SPSS 13.0. Los resultados mostraron que la temporada afectó significativamente diferentes parámetros seminales, ya que el número de eyaculados/toro, la motilidad después de la dilución y el número de dosis fueron significativamente mayores en otoño. Por el contrario, el volumen de semen y la motilidad individual fueron mayores en primavera. Curiosamente, la concentración de esperma fue mayor en el verano seco en comparación con otras estaciones. Los parámetros de motilidad de los espermatozoides y las características de movimiento de los búfalos después de la descongelación revelaron que la motilidad total, la motilidad progresiva y la motilidad rápida no se vieron afectadas por la estación. Por el contrario, VSL, VCL y ALH fueron significativamente más altos en la temporada de primavera que otras. En conclusión, se encontró que los búfalos reproductores exhibieron un patrón estacional ya que sus parámetros seminales se encontraron mejor en las estaciones de primavera y otoño; sin embargo, la mayoría de las variables posteriores al deshielo no tienen efectos estacionales.

Palabras clave: búfalo, eyaculados, CASA, post descongelación, criopreservación.

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Exploring the effect of cold stress on the semen quality parameters of buffalo bulls

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ABSTRACT

The objective of the current study was to evaluate how buffalo bulls' semen quality is affected by cold stress. A total of 26 adult Murrah buffaloes were taken at the Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, India. Semen collection of these bulls was done twice during the peak of winter and spring seasons with overall average ambient temperature (°C) of 12.55±0.22 and 20.21±0.68,

Explorando el efecto del estrés por frío en los parámetros de calidad del semen de búfalos

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RESUMEN

El objetivo del presente estudio fue evaluar cómo la calidad del semen de los búfalos se ve afectada por el estrés por frío. Se escogieron un total de 26 búfalos Murrah adultos en el Centro de Investigación de Mejora Artificial del Instituto Nacional de Investigación Láctea ICAR, Karnal, India. La recolección de semen de estos toros se realizó dos veces du-

respectively, and blood was collected on the same day for biochemical analysis. Routine seminal attributes, viz., semen volume, sperm concentration, mass activity, individual motility, viability, acrosome integrity, and hypo-osmotic swelling test (HOST), were evaluated immediately after semen collection, and diluted semen was cryopreserved for further studies. Additionally, fresh and frozen sperm were subjected to flow cytometric analysis utilizing the Luminex MUSE Cell Analyzer and CytoFLEX, Beckman Coulter-Life Sciences, respectively. Sperm motility characteristics were assessed using a CASA (Hamilton Thorne IVOS II) with the help of eight chambered Leja slides. Further, serum hormones were assayed through the CMA technique, and the antioxidant status of both serum and seminal plasma was done using standard protocol. Statistical analyses were performed using IBM SPSS Statistics software (v.20.0). The THI during the winter and spring seasons was 54.85 ± 0.38 (THI range; 40.93 – 60.38) and 66.89 ± 1.03 (THI range; 60.04 – 77.99), respectively, while the cold stress index was 857.62 ± 8.89 and 793.08 ± 14.10 , respectively, demonstrating that the values varied significantly between the seasons ($p=0.001$). Fresh seminal attributes such as volume (2.86 ± 0.20 vs. 2.73 ± 0.15 ml, $p=0.616$), concentration (1181.94 ± 71.32 vs. $1115.10 \pm 78.34 \times 10^6$ /ml, $p=0.529$), mass activity (2.28 ± 0.09 vs. 2.47 ± 0.08 , $p=0.094$), viability (81.95 ± 1.07 vs. $82.13 \pm 0.89\%$, $p=0.896$), and acrosome integrity (86.17 ± 0.86 vs. $87.53 \pm 0.75\%$, $p=0.239$), did not vary between the extreme winter and comfortable spring seasons. However, individual motility, HOST (66.75 ± 1.41 vs. $71.13 \pm 1.25\%$, $p=0.022$), and discard rate (48.36 ± 1.92 vs. $36.44 \pm 5.51\%$, $p=0.024$) of semen were affected by cold stress. ROS (+) cells and apoptotic cells in fresh semen were not affected by cold stress, despite their higher values in the winter season. A similar trend was observed with MitoSOX (+) cells and live acrosome intact cells in frozen semen. Further, cold stress had a significant influence on the sperm kinematic characteristics of both fresh and frozen-thawed semen. Serum hormones such as Triiodothyronine (5.23 ± 0.16 vs. 4.41 ± 0.14 ng/mL, $p=0.000$), testosterone (0.99 ± 0.12 vs. 1.68 ± 0.17 ng/mL, $p=0.002$), cortisol (3.66 ± 0.43 vs. 2.29 ± 0.12 ng/mL, $p=0.004$), and LDH (1078.54 ± 12.32 vs. 988.23 ± 13.54 U/L, $p=0.000$) were affected by the cold weather. In addition, GSH and MDA concentrations in both seminal plasma and serum were affected by cold stress. In conclusion, frigid temperatures also contribute as a stress factor, further leading to a significant reduction in the semen quality of buffalo bulls.

Keywords: buffalo bulls, cold stress, CASA, flow cytometry, semen quality.

rante el pico de las temporadas de invierno y primavera con una temperatura ambiente promedio general (°C) de $12,55 \pm 0,22$ y $20,21 \pm 0,68$, respectivamente, y la sangre se recogió el mismo día para análisis bioquímicos. Los atributos seminales de rutina, a saber, volumen de semen, concentración de espermatozoides, motilidad masal, motilidad individual, viabilidad, integridad acrosómica y test de resistencia hiposmótica, se evaluaron inmediatamente después de la recolección de semen y el semen diluido se crioconservó para estudios adicionales. Además, los espermatozoides frescos y congelados se sometieron a análisis de citometría de flujo utilizando el analizador de células Luminex MUSE y CytoFLEX, Beckman Coulter-Life Sciences, respectivamente. Las características de la motilidad de los espermatozoides se evaluaron utilizando un CASA (Hamilton Thorne IVOS II) con la ayuda de ocho diapositivos en cámara Leja. Además, se analizaron las hormonas séricas mediante la técnica CMA y el estado antioxidante tanto del suero como del plasma seminal se realizó mediante un protocolo estándar. Los análisis estadísticos se realizaron utilizando el software IBM SPSS Statistics (v.20.0). El THI durante las temporadas de invierno y primavera fue de $54,85 \pm 0,38$ (rango del THI; 40,93 – 60,38) y $66,89 \pm 1,03$ (rango del THI; 60,04 – 77,99), respectivamente, mientras que el índice de estrés por frío fue de $857,62 \pm 8,89$ y $793,08 \pm 14,10$, respectivamente, demostrando que los valores variaron significativamente entre las estaciones ($p=0.001$). Los atributos del semen fresco como volumen (2.86 ± 0.20 vs. 2.73 ± 0.15 ml, $p=0.616$), concentración (1181.94 ± 71.32 vs. $1115.10 \pm 78.34 \times 10^6$ /ml, $p=0.529$), motilidad masal (2.28 ± 0.09 vs. 2.47 ± 0.08 , $p=0.094$), viabilidad (81.95 ± 1.07 vs. $82.13 \pm 0.89\%$, $p=0.896$) e integridad acrosómica (86.17 ± 0.86 vs. $87.53 \pm 0.75\%$, $p=0.239$), no variaron entre los inviernos extremos y cómodas estaciones de primavera. Sin embargo, la motilidad individual, HOST ($66,75 \pm 1,41$ vs. $71,13 \pm 1,25\%$, $p=0,022$) y la tasa de descarte ($48,36 \pm 1,92$ vs. $36,44 \pm 5,51\%$, $p=0,024$) del semen se vieron afectados por el estrés por frío. Las células ROS (+) y las células apoptóticas en el semen fresco no se vieron afectadas por el estrés por frío, a pesar de sus valores más altos en la temporada de invierno. Se observó una tendencia similar con células MitoSOX (+) y células vivas intactas del acrosoma en semen congelado. Además, el estrés por frío tuvo una influencia significativa en las características cinemáticas del esperma tanto del semen fresco como del congelado-descongelado. Hormonas séricas como Triyodotironina (5.23 ± 0.16 vs. 4.41 ± 0.14 ng/mL, $p=0.000$), testosterona (0.99 ± 0.12 vs. 1.68 ± 0.17 ng/mL, $p=0.002$), cortisol (3.66 ± 0.43 vs. 2.29 ± 0.12 ng/mL, $p=0.004$) y LDH ($1078,54 \pm 12,32$ vs. $988,23 \pm 13,54$ U/L, $p=0,000$) se vieron afectados por el clima frío. Además, las concentraciones de GSH y MDA tanto en plasma seminal como en suero se vieron afectadas por el estrés por frío. En conclusión, las temperaturas gélidas también contribuyen como factor de estrés, lo que conduce aún más a una reducción significativa en la calidad del semen de los búfalos.

Palabras clave: toros bufalinos, estrés por frío, CASA, citometría de flujo, calidad del semen.

R-129 Rev. Cientif. FCV-LUZ, XXXIII, SE, 252-253, 2023, <https://doi.org/10.52973/rcfcv-wbc102>**Effect of Dalmavital and Ovulik Plus on conception rate in Ovsynch synchronized Nili-Ravi buffaloes****Muhammad Tayyab Khan¹, Muhammad Muzammal Niaz¹, Ghulam Shabbir Khan²**¹Department of Theriogenology, University of Veterinary and Animal Sciences, outfall Road, Lahore (54000), Pakistan²PMAS Arid Agriculture University Rawalpindi, Sub Campus Khushab. Pakistan*Corresponding author: Muhammad Tayyab Khan
(tayyabkhan686@gmail.com)**ABSTRACT**

Nili-Ravi buffalo are known as the “Black Gold of Pakistan”. It is the most prized dairy animal in Pakistan. Silent estrus and shy behavior are among the main causes of poor reproductive efficiency in buffalo, leading to prolonged calving intervals. Ovulik Plus® is a supplementary mixture for stimulating fertility. It provides energy, eliminates the deficiency of β-carotene, as well as giving a rich source of vitamins and microelements to elevate reproductive efficiency. Dalmavital® is another product that contains β-carotene and acts as an antioxidant source. Our objective was to compare the impact of these supplements in enhancing conception rates of Ovsynch synchronized Nili-Ravi buffaloes. For this purpose, buffaloes (n=45) with healthy reproductive tract, BCS (2.5-3.5), and mixed parity (2-3) were enrolled into three groups: G1: Ovsynch + Dalmavital (IM), G2: Ovsynch + Ovulik (PO) and G3: Ovsynch without supplementation as control. For the Ovsynch protocol, buffaloes were administered with 2ml (IM) injection of GnRH (Lecirelin acetate 25µg/ml) at day 0, followed by 2ml (IM) injection of PGF2α (D-cloprostenol 75µg/ml) at day 7. Finally, 2ml GnRH was injected (IM) at day 9. Dalmavital was injected (IM) at the rate of 1ml/40kg body weight at day 5 in G1, and Ovulik Plus was offered by drenching gun just after 1st AI as a single dose of 1000 g/ buffalo. Double inseminations were performed at standing estrus, morning and evening, at intervals of 12 and 24 h after the last GnRH injection. Pregnancies were confirmed through ultrasonography at day 40. Data were analyzed through the Chi-Square test and Binary Logistic Regression model using SPSS. Results revealed no difference ($p>0.05$) in estrus response among all groups. Conception rates were higher ($p<0.05$) in G2 compared to G3 (60% vs. 46.66%, respectively). However, there was no difference ($p>0.05$) in conception rates of G1 and G2 (53.33% vs. 60%, respectively). In conclusion, Ovulik Plus per oral was superior to intramuscular administration of Dalmavital in improving conception rates of Ovsynch synchronized Nili-Ravi buffaloes.

Keywords: Ovulik Plus, Dalmavital, β-carotene, Ovsynch, Nili-Ravi Buffalo.

Efecto de Dalmavital y Ovulik Plus sobre la tasa de concepción en búfalas Nili-Ravi sincronizadas con Ovsynch

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(tayyabkhan686@gmail.com)**RESUMEN**

Los búfalos Nili-Ravi son conocidos como el “oro negro de Pakistán”. Es el animal lechero máspreciado de Pakistán. El estro silencioso y el comportamiento tímido se encuentran entre las principales causas de la mala eficiencia reproductiva en las búfalas, lo que lleva a intervalos entre partos prolongados. Ovulik Plus® es una mezcla complementaria para estimular la fertilidad. Aporta energía, elimina la deficiencia de β-caroteno, además de ser una rica fuente de vitaminas y microelementos para elevar la eficiencia reproductiva. Dalmavital® es otro producto que contiene β-caroteno y actúa como fuente antioxidante. Nuestro objetivo fue comparar el impacto de estos suplementos en la mejora de las tasas de concepción de búfalas Nili-Ravi sincronizadas con Ovsynch. Para este propósito, se utilizaron búfalas (n=45) con tracto reproductivo sano, condición corporal (BCS: 2,5-3,5) y paridad mixta (2-3) en tres grupos: G1: Ovsynch + Dalmavital (IM), G2: Ovsynch + Ovulik (PO) y G3: Ovsynch sin suplementación como control. Para el protocolo Ovsynch, a los búfalos se les administró una inyección de 2 ml (IM) de GnRH (acetato de lecirelina 25 µg/ml) el día 0, seguida de una inyección de 2 ml (IM) de PGF2α (D-cloprostenol 75 µg/ml) el día 7. Finalmente, Se inyectaron 2 ml de GnRH (IM) el día 9. Se inyectó Dalmavital (IM) a razón de 1 ml/40 kg de peso corporal el día 5 en G1, y Ovulik Plus se ofreció mediante pistola de inyección oral justo después de la primera IA como una dosis única de 1000 g/ búfalo. Dos inseminaciones se realizaron después del estro estático, por la mañana y por la tarde, a intervalos de 12 y 24 h después de la última inyección de GnRH. Las preñeces se confirmaron mediante ecografía el día 40. Los datos se analizaron mediante la prueba de Chi-Cuadrado y el modelo de Regresión Logística Binaria utilizando SPSS. Los resultados no revelaron diferencias ($p>0.05$) en la respuesta al estro entre todos los grupos. Las tasas de concepción fueron mayores ($p<0,05$) en el G2 en comparación con el G3 (60% vs. 46,66%, respectivamente). Sin embargo, no hubo diferencias ($p>0,05$) en las tasas de concepción de G1 y G2 (53,33% vs. 60%, respectivamente). En conclusión, Ovulik Plus por vía oral fue superior a la admi-

nistración intramuscular de Dalmavital para mejorar las tasas de concepción de búfalos Nili-Ravi sincronizadas con Ovsynch.

Palabras clave: Ovulik Plus, Dalmavital, β -caroteno, Ovsynch, Nili-Ravi Buffalo.

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Cloned buffalo bulls and their somatic cell donor bulls exhibit similar *in-vitro* embryo production efficiency

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ABSTRACT

Somatic cell nuclear transfer (SCNT) has emerged as a valuable assisted reproductive technology for faster multiplication of animals. This helps to preserve the valuable superior germplasm. The cloning of superior sires and their use in breeding programs helps rapidly disseminate superior genetics in animal populations. However, questions remain regarding cloned bulls' fertility status compared to their somatic cell donor bulls, especially in buffaloes. We studied the semen kinetic parameters and *in vitro* fertilization (IVF) rate of frozen-thawed cloned bulls' semen (n=2) compared to those of somatic cell donor bulls' semen (n=2). The kinematics were studied using a Computer Assisted Semen Analyzer (CASA; IVOS II, IMV, France). Semen from cloned bulls vs. donor bulls showed progressive motility with distance average path (17.70 ± 0.835 vs. $18.72 \pm 0.53 \mu\text{m}$), distance curvilinear (28.30 ± 2.85 vs. $31.61 \pm 1.71 \mu\text{m}$), distance straight line (15.89 ± 0.56 vs. $16.36 \pm 0.20 \mu\text{m}$), velocity average path (74.84 ± 7.23 vs. $69.59 \pm 9.96 \mu\text{m/s}$), velocity curvilinear (113.77 ± 15.28 vs. $113.49 \pm 16.94 \mu\text{m/s}$), and velocity straight line (66.71 ± 6.01 vs. $61.94 \pm 9.13 \mu\text{m/s}$). This suggested that the kinematic parameters of cloned bull semen were not significantly different ($p < 0.05$) from those of the somatic cell donor bull semen. Furthermore, cloned and donor bull semen was used for IVF to assess the blastocyst production rate. Immature oocytes were matured *in vitro* for 24 h in a humidified CO₂ incubator (5% CO₂ in air; RH>95%) at 38.5°C. After 24 h, mature oocytes were co-incubated with processed semen in the fertilization Bracket and Oliphant (BO) medium for 18 h. The presumptive zygotes were cultured in Research Vitro Cleavage medium (RVCL; Cook, Australia) for up to 8 days. The cleavage and blastocyst rates for cloned bulls, $68.72 \pm 0.72\%$ and $14.11 \pm 1.89\%$, respectively, did not differ significantly ($p < 0.05$) from those of the donor bulls, i.e., $67.44 \pm 0.16\%$ and $13.96 \pm 1.24\%$, respectively. Furthermore,

Los toros bufalinos clonados y sus donantes de células somáticas exhiben una eficiencia de producción de embriones *in vitro* similar

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RESUMEN

La transferencia nuclear de células somáticas (SCNT) se ha convertido en una valiosa tecnología de reproducción asistida para una multiplicación más rápida de los animales. Esto ayuda a preservar el valioso germoplasma superior. La clonación de toros superiores y su uso en programas de reproducción ayuda a difundir rápidamente una genética superior en las poblaciones animales. Sin embargo, quedan dudas sobre el estado de fertilidad de los toros clonados en comparación con sus toros donantes de células somáticas, especialmente en los búfalos. Estudiamos los parámetros cinéticos del semen y la tasa de fertilización *in vitro* (FIV) del semen de búfalos reproductores clonados congelados-descongelados (n=2) en comparación con los del semen de búfalos donantes de células somáticas (n=2). La cinemática espermática se estudió utilizando un analizador de semen asistido por computadora (CASA; IVOS II, IMV, Francia). El semen de toros clonados vs. toros donantes mostró motilidad progresiva con distancia trayectoria promedio (17.70 ± 0.835 vs. $18.72 \pm 0.53 \mu\text{m}$), distancia curvilínea (28.30 ± 2.85 vs. $31.61 \pm 1.71 \mu\text{m}$), distancia línea recta (15.89 ± 0.56 vs. $16.36 \pm 0.20 \mu\text{m}$), velocidad de trayectoria promedio (74.84 ± 7.23 frente a $69.59 \pm 9.96 \mu\text{m/s}$), velocidad curvilínea (113.77 ± 15.28 frente a $113.49 \pm 16.94 \mu\text{m/s}$) y velocidad en línea recta (66.71 ± 6.01 frente a $61.94 \pm 9.13 \mu\text{m/s}$). Esto sugirió que los parámetros cinemáticos del semen de toro clonado no eran significativamente diferentes ($p < 0.05$) de los del semen de toro donado de células somáticas. Además, se utilizó semen de toro clonado y donado para la FIV para evaluar la tasa de producción de blastocistos. Los ovocitos inmaduros se maduraron *in vitro* durante 24 h en una incubadora de CO₂ humidificada (5% de CO₂ en aire; RH>95%) a 38,5°C. Después de 24 h, los ovocitos maduros se incubaron conjuntamente con el semen procesado en el

the expression of pluripotency-related genes (*OCT4*, *SOX2*, and *NANOG*) was similar in blastocysts produced in both experimental groups. Thus, the semen characteristics and *in vitro* embryo production rate of cloned bulls are like those of their somatic cell donor bulls. This preliminary study suggests that farmers can use semen from cloned bulls for Artificial Insemination programs and other ART procedures.

Keywords: buffalo, semen, IVF, blastocyst.

Acknowledgement: This work was financially supported by Buffalo Cloning Project (NASF/BGAM(SM)-9001/2022-23) ICAR-NASF, New Delhi.

medio de fertilización Bracket y Oliphant (BO) durante 18 h. Los presuntos cigotos se cultivaron en medio Research Vitro Cleavage (RVCL; Cook, Australia) durante 8 días. Las tasas de escisión (clivaje) y blastocisto de los toros clonados, $68,72 \pm 0,72\%$ y $14,11 \pm 1,89\%$, respectivamente, no difirieron significativamente ($p < 0,05$) de las de los toros donantes, es decir, $67,44 \pm 0,16\%$ y $13,96 \pm 1,24\%$, respectivamente. Además, la expresión de genes relacionados con la pluripotencia (*OCT4*, *SOX2* y *NANOG*) fue similar en los blastocistos producidos en ambos grupos experimentales. Por lo tanto, las características del semen y la tasa de producción de embriones *in vitro* de los toros bufalinos clonados son similares a las de sus toros donantes de células somáticas. Este estudio preliminar sugiere que los ganaderos pueden utilizar semen de toros clonados para programas de inseminación artificial y otros procedimientos de reproducción asistida.

Palabras clave: búfalo, Semen, FIV, blastocisto.

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Relationship between biometric characteristics with semen quality of buffalos (*Bubalus bubalis*)

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ABSTRACT

Several factors, including environmental and nutritional management, as well as individual-associated intrinsic factors, influence dramatically the semen quality. This study aimed to relate biometric characteristics with the semen quality of buffalos (*Bubalus bubalis*). For this purpose, 11 Murrah buffaloes between 2 and 7 years old, located at the Centro de Investigaciones de Biotecnología Bufalina (CIBB) in Antioquia, Colombia, were used under the same feeding and management conditions. Each buffalo underwent a complete breeding soundness examination; scrotal circumference was assessed by a scrotometer, accessory sex glands were assessed by rectal palpation and ultrasonography, and libido was assessed on a 1-10 scale by observing sexual stimulation signs. For four months, a semen sample was collected weekly from each buffalo by the artificial vaginal method; the date and time of collection and the weight of each male were recorded. For each ejaculate, volume (VOL) and concentration (CON) were evaluated, and a computerized

Relación entre características biométricas con la calidad del semen de búfalos (*Bubalus bubalis*)

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RESUMEN

Varios factores, incluido el manejo ambiental y nutricional, así como factores intrínsecos asociados al individuo, influyen dramáticamente en la calidad del semen. Este estudio tuvo como objetivo relacionar las características biométricas con la calidad del semen de búfalos (*Bubalus bubalis*). Para ello se utilizaron 11 búfalos Murrah de entre 2 y 7 años de edad, ubicados en el Centro de Investigaciones de Biotecnología Bufalina (CIBB) en Antioquia, Colombia, bajo las mismas condiciones de alimentación y manejo. Cada búfalo se sometió a un examen completo de estimación del potencial reproductivo. La circunferencia escrotal se evaluó mediante un escrotómetro, las glándulas sexuales accesorias se evaluaron mediante palpación rectal y ecografía, y la libido se evaluó en una escala del 1 al 10 mediante la observación de signos de estimulación sexual. Durante cuatro meses, se recolectó semanalmente una muestra de semen de cada búfalo por el método de vaginal artificial; se registró la fecha y hora de recolección y el peso de

semen analysis system assessed sperm motility and kinetics, vitality, and abnormal sperm morphology (AM) by eosin-negrosin staining and the integrity of the plasma membrane by the hypoosmotic test. Descriptive statistics and a Pearson correlation analysis were performed. Means for prostate diameter, scrotal circumference, and libido were 18.7 ± 3.7 mm, 28.8 ± 3.6 cm, and 8.6 ± 0.1 , respectively. Regarding seminal quality, means for VOL of 2.3 ± 1.1 ml, CON of $1470.7 \pm 33.4 \times 10^6$ cells/ml, sperm vitality of $77.9 \pm 10\%$, AM of $20.8 \pm 12.5\%$, total motility of $88.9 \pm 6.4\%$ and progressive motility of $73.7 \pm 10.4\%$, were found. Positive correlations ($p < 0.05$) between buffalo weight and sperm concentration (0.32), plasma membrane integrity (0.68), and sperm kinetics were found. Also, a positive correlation ($p < 0.05$) was observed between scrotal circumference and sperm mass motility (0.36). On the contrary, a negative correlation ($p < 0.05$) was found between libido and slow sperm (-0.22) and non-progressive sperm (-0.34) and between the prostate diameter and these same two parameters (-0.31 and -0.33, respectively). It is concluded that biometric parameters such as buffalo weight, scrotal circumference, libido, and prostate diameter are related to the semen quality of Murrah buffalos.

Keywords: buffalo, freezing, libido, semen quality, scrotal circumference.

cada macho. Para cada eyaculado, se evaluaron el volumen (VOL) y la concentración (CON), y un sistema computarizado de análisis de semen evaluó la motilidad y cinética de los espermatozoides, mientras que la vitalidad y la morfología anormal de los espermatozoides (AM) mediante tinción con eosina-negrosina y la integridad de la membrana plasmática mediante la prueba hipoosmótica. Se realizó estadística descriptiva y análisis de correlación de Pearson. Las medias para el diámetro de la próstata, la circunferencia escrotal y libido fueron $18,7 \pm 3,7$ mm, $28,8 \pm 3,6$ cm y $8,6 \pm 0,1$, respectivamente. En cuanto a la calidad seminal, se encontraron medias de VOL de $2,3 \pm 1,1$ ml, CON de $1470,7 \pm 33,4 \times 10^6$ células/ml, vitalidad espermática de $77,9 \pm 10\%$, AM de $20,8 \pm 12,5\%$, motilidad total de $88,9 \pm 6,4\%$ y motilidad progresiva de $73,7 \pm 10,4\%$. Se encontraron correlaciones positivas ($p < 0,05$) entre el peso del búfalo y la concentración de esperma (0,32), la integridad de la membrana plasmática (0,68) y la cinética del esperma. Además, se observó una correlación positiva ($p < 0,05$) entre la circunferencia escrotal y la motilidad de la masa espermática (0,36). Por el contrario, se encontró una correlación negativa ($p < 0,05$) entre la libido y los espermatozoides lentos (-0,22) y no progresivos (-0,34) y entre el diámetro prostático y estos mismos dos parámetros (-0,31 y -0,33, respectivamente). Se concluye que parámetros biométricos como el peso del búfalo, la circunferencia escrotal, la libido y el diámetro de la próstata están relacionados con la calidad del semen de los búfalos Murrah.

Palabras clave: búfalo, congelación, libido, calidad del semen, circunferencia escrotal.

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Birth and weaning weights in buffalo calves resulting from artificial insemination or natural mating in Venezuela

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ABSTRACT

A retrospective study (2016-2018) was conducted at the commercial farm "La Fortuna", located south of Lake Ma-

Pesos al nacer y al destete en terneros bufalinos resultantes de inseminación artificial o monta natural en Venezuela

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RESUMEN

Se realizó un estudio retrospectivo (2016-2018) en la finca comercial "La Fortuna", ubicada al sur del Lago de Ma-

racaiabo, Zulia state, Venezuela, to analyze the effect of fixed-time artificial insemination (TAI) and natural mating (NM) on birth weight (BW), weaning weight (WW), age at weaning and post-weaning weights in dairy buffalo calves. The effect of buffalo calf sex on these variables was also assessed. After birth, calves were kept with their mother for 15 days. After that, they were suckled twice daily until weaning and went daily to the pasture from 30 days postpartum. During the study period, 15 AI and ten natural mating bulls were used. Data were analyzed by the general linear model of SAS. BW was greater in calves born from TAI ($n=141$; 36.2 ± 0.1 kg) than from NM ($n=131$; 35.7 ± 0.2) ($p=0.0320$). The age at weaning was similar between breeding methods (TAI: 285.1 ± 3.7 d; NM: 290.9 ± 3.8 d; $p=0.2731$). Weaning weight was ~ 10 kg greater in calves born from TAI (178.8 ± 3.8 kg) than from NM (168.6 ± 4.0 kg) ($p=0.0450$). Adjusted weight at 205 (202.1 ± 4.6 vs. 179.2 ± 4.7 kg respectively; $p=0.0006$) and 365 days (248.2 ± 6.1 vs 230.8 ± 6.3 respectively; $p=0.0466$) was significantly greater in buffaloes resulting from TAI than from NM. Female buffaloes ($n=157$) were born lighter than males (35.7 ± 0.1 vs 36.2 ± 0.2 kg; $p=0.0500$). The former (295.8 ± 3.5) reached an older age at weaning than the latter (280.2 ± 4.1 d) ($p=0.0041$). The weight at weaning was similar between sexes (174.9 ± 3.6 and 172.6 ± 4.2 kg respectively; $p=0.6838$). Adjusted weight at 205 days was greater in females (198.6 ± 4.3 kg) than in males (182.7 ± 4.3) ($p=0.0178$); however, the weight at 365 days did not vary between the sexes (females: 243.2 ± 5.7 kg; males: 235.8 ± 6.3 kg; $p=0.3663$). This study demonstrated that artificial insemination is a valuable tool for improving weaning and yearling weights in buffalo herds. Although female buffalo calves were born at lower birth weights and weaned at older ages, they achieved better post-weaning weights.

Keywords: *Bubalus bubalis*, birth and weaning weights, breeding methods.

racaiabo, estado Zulia, Venezuela, para analizar el efecto de la inseminación artificial a tiempo fijo (IATF) y monta natural (MN) sobre el peso al nacer (PN), peso al destete (PD), edad al destete y peso post-destete en terneras bufalinas lecheras. También se evaluó el efecto del sexo de las crías sobre estas variables. Después del nacimiento, las crías se mantuvieron con su madre durante 15 días. Posteriormente, fueron amamantadas dos veces al día hasta el destete y fueron diariamente al pasto a partir de los 30 días posparto. Durante el periodo de estudio se utilizaron 15 toros IA y diez toros de MN. Los datos fueron analizados mediante el modelo lineal general de SAS. El PN fue mayor en los terneros nacidos de IATF ($n=141$; $36,2 \pm 0,1$ kg) que de MN ($n=131$; $35,7 \pm 0,2$) ($p=0,0320$). La edad al destete fue similar entre métodos de servicio (IATF: $285,1 \pm 3,7$ d; MN: $290,9 \pm 3,8$ d; $p=0,2731$). El peso al destete fue ~ 10 kg mayor en los terneros nacidos de IATF ($178,8 \pm 3,8$ kg) que de MN ($168,6 \pm 4,0$ kg) ($p=0,0450$). El peso ajustado a los 205 ($202,1 \pm 4,6$ vs. $179,2 \pm 4,7$ kg respectivamente; $p=0,0006$) y 365 días ($248,2 \pm 6,1$ vs $230,8 \pm 6,3$ respectivamente; $p=0,0466$) fue significativamente mayor en los búfalos resultantes de IATF que de MN. Las crías hembras ($n=157$) nacieron más ligeras que los machos ($35,7 \pm 0,1$ vs $36,2 \pm 0,2$ kg; $p=0,0500$). Las bucerras ($295,8 \pm 3,5$) alcanzaron una edad mayor al destete que los bucerros ($280,2 \pm 4,1$ d) ($p=0,0041$). El peso al destete fue similar entre sexos ($174,9 \pm 3,6$ y $172,6 \pm 4,2$ kg respectivamente; $p=0,6838$). El peso ajustado a los 205 días fue mayor en las hembras ($198,6 \pm 4,3$ kg) que en los machos ($182,7 \pm 4,3$) ($p=0,0178$); sin embargo, el peso a los 365 días no varió entre sexos (hembras: $243,2 \pm 5,7$ kg; machos: $235,8 \pm 6,3$ kg; $p=0,3663$). Este estudio demostró que la inseminación artificial es una herramienta valiosa para mejorar el peso al destete y a los 365 días en rebaños bufalinos. Aunque las terneras bufalinas nacieron con pesos más bajos al nacer y fueron destetadas a edades más avanzadas, alcanzaron mejores pesos después del destete.

Palabras clave: *Bubalus bubalis*, pesos al nacer y al destete, métodos de reproducción.

R-141 Rev. Cientif. FCV-LUZ, XXXIII, SE, 257, 2023, <https://doi.org/10.52973/rcfcv-wbc106>**Pregnancy rate after estrus synchronization and timed artificial insemination in Mediterranean buffaloes in two seasons in Venezuela****Miguel Chacón¹, Néstor Morán¹, Iván Cárdenas², Mariana S. Perea³, Fernando P. Perea⁴, Aitor De Ondiz⁵⁻⁶**¹Agropecuaria Caño Negro, Zulia, Venezuela.²Departamento de Ing. Producción Animal-Unet,³Ejercicio privado.⁴Facultad de Ciencias Agropecuarias, Universidad de Cuenca. Cuenca, Ecuador.⁵Facultad de Ciencias Veterinarias, Maracaibo, Venezuela.⁶Eville & Jones, Leeds, United Kingdom.*Corresponding author: Miguel Chacón
miguelarturochacon@gmail.com**ABSTRACT**

A retrospective study (2016-2018) was conducted in a commercial farm from Zulia state, Venezuela, to analyze the effectiveness of ovulation synchronization followed by fixed-time artificial insemination (TAI) at two seasons of the year: 1) May-September [unfavorable (U), n=386] 2) October-December [favorable (F), n=308]. A total of 694 ovulation synchronization protocols were applied in 190 heifers- and 504 cow-buffaloes. In season U, an intravaginal device (IVD) was used for 9 days (day 0: IVD + 2 mg estradiol benzoate; day 9: 200-230 IU eCG; day 11: 1 mg GnRH) and TAI at 64-66 hours post IVD removal. Ovsynch (IATF 16 to 18 hours after the second GnRH injection) or IVD protocols were used in season F. Calving to synchronization interval was 102.2 ± 51.8 and 126.0 ± 74.8 days for seasons U and F, respectively. Data were analyzed by the Chi-square test of SAS. Pregnancy rate (pregnant buffaloes divided by inseminated buffaloes after ovulation synchronization $\times 100$) did not vary between seasons: 43% (166/386) and 45.8% (141/308) for U and F, respectively ($p>0.05$). In season U, buffalo heifers had a pregnancy rate of 50.8% (62/122) and buffalo cows 39.9% (104/264) ($p<0.05$). In season F, the pregnancy rate was similar between groups: 44.1% (30/68) and 46.2% (111/240) for heifers and buffalo cows, respectively ($p>0.05$). In this tropical buffalo herd, the pregnancy rate after IATF was comparable between seasons; however, in the unfavorable season, buffalo heifers were more fertile than buffalo cows.

Keywords: *Bubalus bubalis*, hormonal protocol, TAI, season.

Tasa de preñez tras sincronización del estro e inseminación artificial a tiempo fijo en búfalas mediterráneas en dos temporadas en Venezuela

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miguelarturochacon@gmail.com**RESUMEN**

Se realizó un estudio retrospectivo (2016-2018) en una finca comercial del estado Zulia, Venezuela, para analizar la efectividad de la sincronización de la ovulación seguida de inseminación artificial a tiempo fijo (IATF) en dos estaciones del año: 1) mayo-septiembre [desfavorable (U), n=386] 2) Octubre-diciembre [favorable (F), n=308]. Se aplicaron un total de 694 protocolos de sincronización de la ovulación en 190 bubillas y 504 búfalas. En la temporada U, se utilizó un dispositivo intravaginal (DIV) durante 9 días (día 0: DIV + 2 mg de benzoato de estradiol; día 9: 200-230 UI de eCG; día 11: 1 mg de GnRH) e IATF a las 64-66 horas posteriores. Extracción del DIV. En la temporada F se utilizaron protocolos Ovsynch (IATF de 16 a 18 horas después de la segunda inyección de GnRH) o IVD. El intervalo entre parto y sincronización fue de $102,2 \pm 51,8$ y $126,0 \pm 74,8$ días para las temporadas U y F, respectivamente. Los datos fueron analizados mediante la prueba de Chi-cuadrado de SAS. La tasa de preñez (hembras preñadas divididas por hembras inseminadas después de la sincronización de la ovulación $\times 100$) no varió entre estaciones: 43% (166/386) y 45,8% (141/308) para U y F, respectivamente ($p>0,05$). En la temporada U, las bubillas tuvieron una tasa de preñez del 50,8% (62/122) y las búfalas del 39,9% (104/264) ($p<0,05$). En la temporada F, la tasa de preñez fue similar entre grupos: 44,1% (30/68) y 46,2% (111/240) para bubillas y búfalas, respectivamente ($p>0,05$). En este rebaño de búfalos tropicales, la tasa de preñez después de la IATF fue comparable entre temporadas; sin embargo, en la estación desfavorable, las bubillas fueron más fértiles que las búfalas.

Palabras clave: *Bubalus bubalis*, protocolo hormonal, TAI, estación.

R-147 Rev. Cientif. FCV-LUZ, XXXIII, SE, 258-259, 2023, <https://doi.org/10.52973/rcfcv-wbc107>**Validating the reproductive tract score (RTS) technique in pre-service buffalo heifers****Freddys Escalona-Ramírez^{1,2}, Jessenia Mercado-Carrillo¹, Balbino A. Jaimes-Vazques¹, Antonio Landaeta-Hernández²**¹Servicios Veterinarios S&S del Sur, Barinas, Venezuela.²Universidad del Zulia, Facultad de Ciencias Veterinarias, Unidad de Investigaciones Zootécnicas. Maracaibo, Venezuela*Corresponding author: Freddys Escalona-Ramirez (freddys.escalonara@gmail.com)**ABSTRACT**

The fast growth of buffalo (*Bubalus bubalis*) herds has placed this livestock species as a suitable alternative for animal protein production in the Venezuelan tropics. As with any domestic species with food production interest, the buffalo's reproductive performance has a paramount relevance, which may be improved by applying the technique of reproductive tract scores (RTS). Using rectal palpation, the RTS (scale 1-5) assign the following scores: 1=flabby uterus and ovaries both <10 mm of diameter with no detectable follicles; 2=flabby uterus and ovaries both 10-15mm of diameter with <8 mm follicles; 3=soft tone uterus and ovaries both <15-20 mm of diameter with 8-10 mm follicles; 4=good tone uterus with 20-25 mm of diameter, ovaries >20mm with >10 mm follicles; 5= good tone uterus >25 mm of diameter, ovaries >20mm with evident luteal tissue. Therefore, the objective of this study was to validate the technique of RTS in pre-service buffalo heifers as a management strategy to improve the pregnancy rate. One-hundred and ten buffalo heifers with ages of 27 + 3 months and body weight of 354,32 + 24,14 kg were assessed for RTS before starting a breeding season of 120 days (Sep-Dec 2020) with four buffalo bulls in a proportion male to female 1:30. 67% of buffalo heifers were found with RTS among 3-5 (mature tract), while 33% were found with RTS among 1-2 (immature tract). Pregnancy was diagnosed 60 days after the end of the breeding season. Data was analyzed by Logistic Regression. A linear and positive increase association ($p<0.02$) was observed between RTS and pregnancy rate (RTS=5:85%, RTS=4:80%, RTS=3:74%, RTS=2:62%, and RTS=1:50%) indicating a beneficial effect of reproductive tract maturity on pregnancy rate. Likewise, body weight at service and pregnancy rate were also associated ($p<0.0001$). It was concluded that RTS is a good predictor of pregnancy rate in buffalo heifers. Thus, applying RTS at a field level should be a beneficial management tool.

Keywords: buffaloes, fertility, heifer, reproduction, management.**Validación de la técnica del puntaje del tracto reproductivo (RTS) en novillas búfalas en preservicio****Freddys Escalona-Ramírez^{1,2}, Jessenia Mercado-Carrillo¹, Balbino A. Jaimes-Vazques¹, Antonio Landaeta-Hernández²**¹Servicios Veterinarios S&S del Sur, Barinas, Venezuela.²Universidad del Zulia, Facultad de Ciencias Veterinarias, Unidad de Investigaciones Zootécnicas. Maracaibo, Venezuela*Autor de correspondencia: Freddys Escalona-Ramirez (freddys.escalonara@gmail.com)**RESUMEN**

El rápido crecimiento del rebaño de búfalos (*Bubalus bubalis*) ha colocado a esta especie ganadera como una alternativa adecuada para la producción de proteína animal en el trópico venezolano. Como ocurre con cualquier especie doméstica con interés en la producción de alimentos, el desempeño reproductivo del búfalo tiene una relevancia primordial, que puede mejorarse aplicando la técnica de puntuación del tracto reproductivo (RTS). Mediante palpación rectal, la RTS (escala 1-5) asigna las siguientes puntuaciones: 1 = útero y ovarios flácidos, ambos <10 mm de diámetro sin folículos detectables; 2 = útero y ovarios flácidos, ambos de 10 a 15 mm de diámetro con folículos <8 mm; 3=útero y ovarios de tono suave, ambos <15-20 mm de diámetro con folículos de 8-10 mm; 4=útero de buen tono con 20-25 mm de diámetro, ovarios >20mm con folículos >10 mm; 5= útero de buen tono >25 mm de diámetro, ovarios >20mm con cuerpo lúteo evidente. Por lo tanto, el objetivo de este estudio fue validar la técnica de RTS en bubillas en preservicio como estrategia de manejo para mejorar la tasa de preñez. Ciento diez novillas bufalinas con edades de 27 ± 3 meses y peso corporal de 354,32 ± 24,14 kg fueron evaluadas para RTS antes de iniciar una temporada de reproducción de 120 días (septiembre-diciembre 2020) con cuatro búfalos reproductores en una proporción macho:hembra 1:30. El 67% de las bubillas se encontraron con RTS entre 3-5 (tracto maduro), mientras que el 33% se encontró con RTS entre 1-2 (tracto inmaduro). La gestación se diagnosticó 60 días después del final de la temporada reproductiva mediante palpación rectal. Los datos fueron analizados mediante regresión logística. Se observó una asociación de aumento lineal y positivo ($p<0,02$) entre RTS y tasa de embarazo (RTS=5:85%, RTS=4:80%, RTS=3:74%, RTS=2:62% y RTS= 1:50%), lo que indica un efecto beneficioso de la madurez del tracto reproductivo sobre la tasa de preñez. Asimismo, el peso corporal al servicio y la tasa de preñez también se asociaron ($p<0,0001$). Se concluyó que la RTS es un buen predictor de la tasa de preñez en novillas

bufalinas. Por lo tanto, la aplicación de RTS a nivel de campo debería ser una herramienta de gestión beneficiosa.

Palabras clave: búfalos, fertilidad, bubilla, reproducción, manejo.

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Fixed timed artificial insemination in buffalo heifers with fresh and frozen-thawed semen in Argentina

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ABSTRACT

Buffalo meat and milk production is a constantly growing activity, especially in America. Artificial insemination (AI) is a better tool with a low application in this species due to the particularities of its physiology; an alternative is the application of fixed-time insemination (FTAI). The aim of the present work was to evaluate the effect of sperm source on pregnancy rates using an IATF protocol in heifers. It was performed in Argentina in four buffalo farms, during the reproductive season of 2021. A total of 627 Murrah and Mediterranean heifers were included, with age, body condition and genital development of 2.61±0.52 years, 3.58±0.58 (1-5 score), 2.49±0.50 respectively, the permeability of the cervix was also evaluated. They were synchronized using the Ovsynch protocol supplemented with 400 IU of equine chorionic gonadotrophin (eCG) on day 7. Fresh semen (30±6.5x10⁶ spermatozoa) and frozen semen (40±4.4x10⁶ spermatozoa) from two breeders were used. 35 days after insemination, pregnancy diagnosis was performed by ultrasonography. The data obtained were recorded in Excel spreadsheets. A comparison of gestation results was performed using the Kruskal-Wallis nonparametric test. A pregnancy rate of 50.3% (149/296) was obtained with frozen-thawed semen and 55.9% (185/331) with refrigerated semen, these differences were not statistically significant ($p=0.1918$). No significant differences were observed between farms ($p>0.05$), nor between the bulls used with the two variables of straws, with 50% and 58% of pregnancies for bull 1 and 58% and 52% and 54% of pregnancies for frozen-thawed and refrigerated semen for bull

Inseminación artificial a tiempo fijo en búfalas con semen fresco y congelado-descongelado en Argentina

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RESUMEN

La producción de carne y leche de búfalo es una actividad en constante crecimiento, especialmente en América. La inseminación artificial (IA) es una buena herramienta con baja aplicación en esta especie debido a las particularidades de su fisiología; una alternativa es la aplicación de la inseminación a tiempo fijo (IAFT). El objetivo del presente trabajo fue evaluar el efecto de la fuente de esperma sobre las tasas de preñez utilizando un protocolo IATF en novillas. Se realizó en Argentina en cuatro granjas bufalinas, durante la temporada reproductiva de 2021. Se incluyeron un total de 627 novillas Murrah y Mediterránea, con edad, condición corporal y desarrollo genital de 2,61±0,52 años, 3,58±0,58 (puntuación 1-5) y 2,49±0,50 respectivamente, también se evaluó la permeabilidad del cuello uterino. Se sincronizaron mediante el protocolo Ovsynch suplementado con 400 UI de gonadotropina coriónica equina (eCG) el día 7. Se utilizó semen fresco (30±6,5x10⁶ espermatozoides) y semen congelado (40±4,4x10⁶ espermatozoides) de dos reproductoras. 35 días después de la inseminación se realizó el diagnóstico de preñez mediante ecografía. Los datos obtenidos se registraron en hojas de cálculo Excel. Se realizó una comparación de los resultados de la gestación mediante la prueba no paramétrica de Kruskal-Wallis. Se obtuvo una tasa de preñez del 50,3% (149/296) con semen congelado-descongelado y del 55,9% (185/331) con semen refrigerado, estas diferencias no fueron estadísticamente significativas ($p=0,1918$). No se observaron diferencias significativas entre granjas ($p>0,05$), ni entre los toros utilizados con las dos va-

2, respectively ($p>0.05$). The fertility of frozen-thawed semen was lower than that of frozen-thawed semen, without being significant, which should motivate veterinarians and breeders to cryopreserve semen from breeding stock. It is shown how acceptable pregnancy rates are obtained independently of the semen condition, which allows visualizing the application of artificial insemination in this species to achieve the necessary improvement to satisfy the needs of the industry.

Keywords: IATF, frozen semen, fresh semen, improvement.

riables de pajuela, con 50% y 58% de preñeces para el toro 1 y 58% y 52% y 54% de preñeces para el toro congelado. semen descongelado y refrigerado para el toro 2, respectivamente ($p>0.05$). La fertilidad del semen congelado-descongelado fue menor que la del semen congelado-descongelado, sin ser significativa, lo que debería motivar a veterinarios y criadores a criopreservar el semen de reproductores. Se muestra cómo se obtienen tasas de preñez aceptables independientemente del estado del semen, lo que permite visualizar la aplicación de la inseminación artificial en esta especie para lograr la mejora necesaria para satisfacer las necesidades de la industria.

Palabras clave: IAFT, semen congelado, semen fresco, mejoramiento.

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Month and lunar cycle at calving influence and reproductive performance of a tropical buffalo herd

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ABSTRACT

A retrospective study (2000-2022) was conducted in a commercial buffalo farm in Mérida State, Venezuela, to assess the effect of month and lunar cycle at calving on some reproductive variables. According to the percentage of moonlight, the lunar cycle (29.52 days) was divided into 30 periods (0.98 days each). The intervals calving-estrus and calving-conception and the number of services/conception were analyzed by the general linear model. The conception rate at first service and calving frequency were analyzed by the Chi-square test ($n=3510$). The month of parturition did not affect the calving-estrus interval ($p=0.1438$), which varied between 93.3 (January) and 112.5 days (February). An effect of month of parturition was observed in the calving-conception interval ($p=0.0006$), with the greatest value in February (170.4 ± 11.3 days) and lowest values in January (137.7 ± 6.3 days), July (138.9 ± 7.3 days) and August (136.1 ± 5.4); however, no differences were found in the services/conception ($p=0.4692$) (1.41 in July and August, and 1.49 in June) and first service conception rate ($p=0.8504$) (~63% in

Mes y ciclo lunar en la influencia del parto y el desempeño reproductivo de una manada de búfalos tropicales.

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RESUMEN

Se realizó un estudio retrospectivo (2000-2022) en un rebaño comercial de búfalos en el estado Mérida, Venezuela, para evaluar el efecto del mes y ciclo lunar al parto sobre algunas variables reproductivas. Según el porcentaje de luz lunar, el ciclo lunar (29,52 días) se dividió en 30 periodos (0,98 días cada uno). Los intervalos parto-estro y parto-concepción y el número de servicios/concepción fueron analizados mediante el modelo lineal general. La tasa de concepción al primer servicio y la frecuencia de partos se analizaron mediante la prueba de Chi-cuadrado ($n=3510$). El mes de parto no afectó el intervalo parto-estro ($p=0,1438$), el cual varió entre 93,3 (enero) y 112,5 días (febrero). Se observó un efecto del mes de parto en el intervalo parto-concepción ($p=0.0006$), con el mayor valor en febrero (170.4 ± 11.3 días) y los valores más bajos en enero (137.7 ± 6.3 días), julio (138.9 ± 7.3 días) y agosto ($136,1 \pm 5,4$); sin embargo, no se encontraron diferencias en el número de servicios/concepción ($p=0,4692$) (1,41 en julio y agosto, y 1,49 en junio) y tasa de concepción del primer servicio

May and June, and 68% in January). The distribution of calving varied significantly along the year ($p < 0.0001$); the lowest frequency of calving occurred between February and July (range 6.7 to 7.2%), and the greatest took place between August and January (9.6 to 10.9%). There was no effect of the lunar cycle at parturition on the calving-estrus interval ($p = 0.3837$). However, there was an effect on the calving-conception interval ($p = 0.0021$), which was more significant around the new moon (154.9 ± 4.5 days) than around the full moon (141.9 ± 4.0 days) and most of the waning period (138.0 ± 4.3 days). No effect of the lunar cycle was observed on services/conception ($p = 0.8196$) or first service conception rate ($p = 0.8539$). As has been observed in other species (cattle, swine, and guinea pigs), the frequency of calving was significantly greater ($p = 0.0026$) ~2 days around the new moon (17.5%) and full moon (14.6%) than in the remaining 26 periods of the moon cycle (67.9%). This study showed a significant effect of month and lunar cycle at calving on calving-conception interval and calving frequency in a tropical buffalo population.

Keywords: *Bubalus bubalis*, reproduction, calving month, moon cycle.

($p = 0,8504$) (~63% en mayo y junio, y 68% en Enero). La distribución de partos varió significativamente a lo largo del año ($p < 0,0001$); la menor frecuencia de partos ocurrió entre febrero y julio (rango 6,7 a 7,2%), y la mayor entre agosto y enero (9,6 a 10,9%). No hubo efecto del ciclo lunar al parto sobre el intervalo parto-estro ($p = 0,3837$). Sin embargo, hubo un efecto en el intervalo parto-concepción ($p = 0,0021$), que fue más significativo alrededor de la luna nueva ($154,9 \pm 4,5$ días) que alrededor de la luna llena ($141,9 \pm 4,0$ días) y la mayor parte del período menguante ($138,0 \pm 4,3$ días). No se observó ningún efecto del ciclo lunar sobre los servicios/concepción ($p = 0,8196$) o la tasa de concepción del primer servicio ($p = 0,8539$). Como se ha observado en otras especies (vacunos, porcinos y cuyes), la frecuencia de partos fue significativamente mayor ($p = 0,0026$) ~2 días alrededor de la luna nueva (17,5%) y la luna llena (14,6%) que en los 26 períodos restantes del ciclo lunar (67,9%). Este estudio mostró un efecto significativo del mes y el ciclo lunar en el momento del parto sobre el intervalo parto-concepción y la frecuencia de partos en una población de búfalas tropicales.

Palabras clave: *Bubalus bubalis*, reproducción, mes del parto, ciclo lunar.

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Reproductive management and parity influence reproductive activity in a tropical buffalo herd

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ABSTRACT

A retrospective study (2000-2022) was conducted on a commercial buffalo farm in Merida State, Venezuela, to evaluate the effect of reproductive management and parity on reproductive performance. Reproductive management was split into two periods: 1) 2000-2013 (n=1382) and 2) 2014-2022 (n=2128). In the former, estrus synchronization protocols were applied mainly between November and February, while in the latter, the hormonal protocols were applied during the whole year. The number of calving was divided into 1 (1P) and ≥ 2 (2P). The general linear model analyzed the intervals calving-estrus and calving-conception and the number of services

El manejo reproductivo y la paridad influyen en la actividad reproductiva en una manada de búfalos tropicales

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RESUMEN

Se realizó un estudio retrospectivo (2000-2022) en una rebaño comercial de búfalos en el estado Mérida, Venezuela, para evaluar el efecto del manejo reproductivo y la paridad en el desempeño reproductivo. El manejo reproductivo se dividió en dos períodos: 1) 2000-2013 (n=1382) y 2) 2014-2022 (n=2128). En los primeros los protocolos de sincronización de estrus se aplicaron principalmente entre noviembre y febrero, mientras que en los segundos los protocolos hormonales se aplicaron durante todo el año. El número de partos se dividió en 1 (1P) y ≥ 2 (2P). El modelo lineal general analizó los in-

per conception. The conception rate at first service and calving frequency were analyzed by the Chi-square test. Reproductive management affected calving-estrus ($p < 0.0001$) (109.4 ± 2.0 and 99.2 ± 1.3 days for periods 1 and 2) and calving-conception ($p = 0.0476$) (135.9 ± 3.7 and 149.6 ± 2.7 days for periods 1 and 2) intervals, and services/conception ($p < 0.0001$) (1.52 ± 0.01 and 1.39 ± 0.01 for periods 1 and 2). Conception rate at first service was lower in period 1 (60.1%) than in period 2 (69.1%) ($p < 0.0001$). Calving distribution varied significantly throughout the year ($p < 0.0001$). In period 1, the most significant proportion of calving (~60%) occurred in the last five months of the year (August-December), with the highest peaks in September (13%) and October (12.9%), and the lowest in March (5.4%), April (4.6%) and May (3.1%). In period 2, however, parturitions were evenly distributed across the year, with the most significant proportion occurring in December (9.3%) and January (10.3%) and the lowest in June (6.4%) and July (6.9%). Parity influenced calving-1st service ($p < 0.0001$) (117.2 ± 2.7 and 99.6 ± 1.4 days for P1 and P2) and calving-conception ($p = 0.0026$) (161.5 ± 5.0 and 140.9 ± 2.7 days for P1 and P2) intervals, but not services/conception ($p = 0.8467$). The conception rate at first service was similar between buffalo cows with 1 and ≥ 2 parturitions (66.8 and 66.3%, respectively). Collectively, these results showed a significant influence of reproductive management and parity on the reproductive performance of buffalo cows under tropical rearing conditions. According to these findings, deseasonalization of reproduction under tropical conditions can be applied without impairing the herd's reproductive efficiency.

Keywords: *Bubalus bubalis*, management, reproductive activity, parity.

intervalos parto-estro y parto-concepción y el número de servicios por concepción. La tasa de concepción al primer servicio y la frecuencia de partos se analizaron mediante la prueba de Chi-cuadrado. El manejo reproductivo afectó los intervalos parto-estro ($p < 0.0001$) (109.4 ± 2.0 y 99.2 ± 1.3 días para los períodos 1 y 2) y parto-concepción ($p = 0.0476$) (135.9 ± 3.7 y 149.6 ± 2.7 días para los períodos 1 y 2) y servicios/concepción ($p < 0.0001$) ($1,52 \pm 0,01$ y $1,39 \pm 0,01$ para los períodos 1 y 2), respectivamente. La tasa de concepción en el primer servicio fue menor en el período 1 (60,1%) que en el período 2 (69,1%) ($p < 0,0001$). La distribución de partos varió significativamente a lo largo del año ($p < 0,0001$). En el periodo 1, la proporción más significativa de partos (~60%) se produjo en los últimos cinco meses del año (agosto-diciembre), con los picos más altos en septiembre (13%) y octubre (12,9%), y los más bajos en marzo (5,4%), abril (4,6%) y mayo (3,1%). Sin embargo, en el periodo 2, los partos se distribuyeron uniformemente a lo largo del año, con la proporción más significativa en diciembre (9,3%) y enero (10,3%) y la más baja en junio (6,4%) y julio (6,9%). La paridad influyó en los intervalos parto-1^{er} servicio ($p < 0.0001$) (117.2 ± 2.7 y 99.6 ± 1.4 días para P1 y P2) y parto-concepción ($p = 0.0026$) (161.5 ± 5.0 y 140.9 ± 2.7 días para P1 y P2), pero no los servicios/concepción ($p = 0,8467$). La tasa de concepción al primer servicio fue similar entre búfalas con 1 y ≥ 2 partos (66,8 y 66,3%, respectivamente). En conjunto, estos resultados mostraron una influencia significativa del manejo reproductivo y la paridad en el desempeño reproductivo de búfalas en condiciones de crianza tropicales. Según estos hallazgos, la desestacionalización de la reproducción en condiciones tropicales se puede aplicar sin perjudicar la eficiencia reproductiva del rebaño.

Palabras clave: *Bubalus bubalis*, manejo, actividad reproductiva, paridad.

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Extracellular vesicles (EVs) in seminal plasma of buffalo bulls possess spermatozoa specific fertility associated proteins and enzymes

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ABSTRACT

Buffaloes are the backbone of the Indian dairy and livestock industry. They contribute nearly 53% of milk and 30% of meat production in India. Bull fertility is vital to productivity, sustainability, and efficient buffalo farming. The current study aims

Las vesículas extracelulares (EV) en el plasma seminal de los toros búfalo poseen proteínas y enzimas asociadas a la fertilidad específicas de los espermatozoides

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RESUMEN

Los búfalos son la columna vertebral de la industria ganadera y láctea de la India. Contribuyen con casi el 53% de la producción de leche y el 30% de la producción de carne en

to assess the extracellular vesicles (EVs) based fertility marker in the seminal plasma of buffalo bulls. The seminal plasma of buffalo bulls of contrasting fertility was utilized to purify EVs by size exclusion chromatography (SEC). Diffraction light scattering (DLS), nano-tracking assay (NTA), and Western blotting were used to confirm seminal plasma EVs. A pure population of EVs was obtained in 7-12 SEC fractions with an average EV size of 161nm. The NTA analysis provides a quantitative abundance of EVs, and the EVs population was 1.5×10^9 to 2.9×10^{11} EVs/ml in seminal plasma. Utilizing the Western blotting approach, the abundance of SPAM-1 protein in EVs was significantly high in high fertile (HF) bulls as compared to low fertile (LF) group ($p < 0.05$). However, disintegrin and metalloproteinase domain-containing protein 7 (ADAM-7) and acrosomal protein SP-10 were not significantly different in seminal EVs of HF and LF bulls. Similarly, the abundance of Gelectin-3 and aldose reductase (AR) was comparatively higher in HF groups, although it was insignificant. The fertility factor, CD9 protein, was significantly abundant in the seminal EVs of HF vis a vis LF buffalo bulls, suggesting that CD9 of seminal EVs origin could help assess the fertility status of buffalo bulls. Enzymatic phospholipase A2 (PLA2) activity in HF was higher than in LF seminal EVs, indicating superior PLA2 activity in highly fertile bull semen. Uptake of EV-associated fertility proteins by spermatozoa was assessed. We observed that protein cargo was successfully assimilated with sperm. Finally, LC-MS/MS-based high throughput proteome was performed to identify the differential abundance of protein cargo in seminal EVs of contrasting fertility bulls. We identified the top twenty differently abundant proteins in seminal EVs of HF bulls *vis-à-vis* LF bulls. The identified proteins were strongly related to sperm functions. Thus, the present study supports that buffalo seminal EVs possess vital fertility-associated proteins and enzymes, which could be important markers for assessing buffalo bull fertility.

Keywords: extracellular vesicles, seminal plasma, fertility, protein.

la India. La fertilidad de los toros es vital para la productividad, la sostenibilidad y la cría eficiente de búfalos. El presente estudio tuvo como objetivo evaluar el marcador de fertilidad basado en vesículas extracelulares (EV) en el plasma seminal de toros búfalinos. El plasma seminal de búfalos de fertilidad contrastante se utilizó para purificar vesículas extracelulares mediante cromatografía de exclusión por tamaño (SEC). Se utilizaron dispersión de luz por difracción (DLS), ensayo de nanoseguimiento (NTA) y Western Blot para confirmar las EV del plasma seminal. Se obtuvo una población pura de vesículas extracelulares en fracciones de 7 a 12 SEC con un tamaño de vesículas extracelulares promedio de 161 nm. El análisis NTA proporciona una abundancia cuantitativa de EV, y la población de EV fue de $1,5 \times 10^9$ a $2,9 \times 10^{11}$ EV/ml en plasma seminal. Utilizando el método de transferencia Western, la abundancia de la proteína SPAM-1 en las vesículas extracelulares fue significativamente alta en los toros de alta fertilidad (HF) en comparación con los del grupo de baja fertilidad (LF) ($p < 0,05$). Sin embargo, la proteína 7 que contiene el dominio de desintegrina y metaloproteinasas (ADAM-7) y la proteína acrosómica SP-10 no fueron significativamente diferentes en las EV seminales de toros HF y LF. De manera similar, la abundancia de Gelectina-3 y aldosa reductasa (AR) fue comparativamente mayor en los grupos con HF, aunque fue insignificante. El factor de fertilidad, la proteína CD9, fue significativamente abundante en las vesículas extracelulares seminales de los toros búfalo HF en comparación con los búfalos LF, lo que sugiere que el CD9 de origen de las vesículas extracelulares seminales podría ayudar a evaluar el estado de fertilidad de los búfalos. La actividad enzimática de la fosfolipasa A2 (PLA2) en HF fue mayor que en las EV seminales de LF, lo que indica una actividad de PLA2 superior en semen de toro altamente fértil. Se evaluó la absorción de proteínas de fertilidad asociadas a EV por parte de los espermatozoides. Observamos que la carga proteica fue asimilada exitosamente por los espermatozoides. Finalmente, se realizó un proteoma de alto rendimiento basado en LC-MS/MS para identificar la abundancia diferencial de la carga de proteínas en vesículas extracelulares seminales de toros de fertilidad contrastante. Identificamos las veinte proteínas con abundancia diferente en las vesículas extracelulares seminales de toros HF frente a toros LF. Las proteínas identificadas estaban fuertemente relacionadas con las funciones de los espermatozoides. Por lo tanto, el presente estudio respalda que las vesículas extracelulares seminales de búfalos poseen proteínas y enzimas vitales asociadas a la fertilidad, que podrían ser marcadores importantes para evaluar la fertilidad de los búfalos.

Palabras clave: vesículas extracelulares, plasma seminal, fertilidad, proteínas.

R-186 Rev. Cientif. FCV-LUZ, XXXIII, SE, 264-265, 2023, <https://doi.org/10.52973/rcfcv-wbc112>**Association between sperm motility in thawed semen and pregnancy rate in Mediterranean buffaloes****Armando Quintero-Moreno^{1,2,4,5*}, Carla Osorio Meléndez⁴, Héctor Nava-Trujillo^{3,4}, Jorge Rubio Guillén⁴, Juan Carlos Gutiérrez-Añez⁴, Adriana Camacho de Gutiérrez⁵**¹*Department of Physiology, Faculty of Veterinary Medicine, University of Murcia, International Excellence Campus for Higher Education and Research (Campus Mare Nostrum), Murcia, Spain*²*Maria Zambrano Program of the European Union*³*Division of Animal Sciences, University of Missouri, Columbia MO, USA*⁴*Andrology Laboratory. Faculty of Veterinary Sciences (FCV). University of Zulia (LUZ). Venezuela*⁵*BIOTERVET (Biotecnología Reproductiva Veterinaria, C.A), Maracaibo, Venezuela**Corresponding author: quintearma@gmail.com**ABSTRACT**

Progressive individual motility is the main parameter used to determine the reproductive potential of a buffalo bull. This information contributes to deciding to use and process or not the ejaculate obtained and therefore assume that the spermatozoa from the seminal sample could achieve pregnancy in inseminated buffaloes. The association of sperm motility to fertility and/or pregnancy rate (PR) has yielded controversial results. To contribute to knowledge in buffalo reproduction, an experiment was carried out where four sperm motility descriptors (VCL: curvilinear velocity, VSL: straight-line velocity, VAP: average path velocity, and LIN: linearity) were evaluated using a Computerized Semen Analysis System (Sperm Class Analyzer®, CASA System, Barcelona, Spain) in samples obtained from 8 frozen-thawed straws of 8 Mediterranean buffaloes. The PR data for each buffalo bull were obtained from 2 farms where the management and FTAI protocol employed was handled by the same professionals and straws of similar provenance and proper nitrogen thermos management were used. The PR data for each buffalo bull was confirmed by ultrasonography, organized in Excel® and analyzed with SAS®, associating VAP to PR, using a Chi-square analysis. To achieve the objective, the PR obtained was categorized from highest to the lowest value (A: PR>70%, B: PR>60≤70%, C: PR>50≤60%, D: PR>40≤50%), in addition to creating sperm subpopulations (Sp) based on the quality of sperm movement, for which it was decided to use the VAP, since it is a variable that integrates the VCL and the VSL and indirectly assumes the LIN, creating the following categories: Z= Sp with VAP value ≥ 80 μm/s, Y: Sp with a VAP value

Asociación entre la motilidad de los espermatozoides en semen descongelado y la tasa de preñez en búfalos Mediterráneo

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La motilidad individual progresiva es el principal parámetro utilizado para determinar el potencial reproductivo de un búfalo. Esta información contribuye a decidir el uso y procesamiento o no del eyaculado obtenido y por tanto suponer que los espermatozoides de la muestra seminal podrían lograr la preñez en búfalos inseminados. La asociación entre la motilidad de los espermatozoides con la fertilidad y/o la tasa de preñez (TP) ha arrojado resultados controvertidos. Para contribuir al conocimiento en la reproducción del búfalo, se llevó a cabo un experimento donde se evaluaron cuatro parámetros de la motilidad de los espermatozoides (VCL: velocidad curvilínea, VSL: velocidad en línea recta, VAP: velocidad de la trayectoria promedio y LIN: linealidad) utilizando un Sistema Computarizado de Análisis de Semen. (Sperm Class Analyzer®, CASA System, Barcelona, España) en muestras obtenidas de 8 pajuelas congeladas-descongeladas de 8 búfalos mediterráneos. Los datos de TP de cada macho reproductor se obtuvieron de 2 granjas donde el manejo y protocolo IATF empleado fue manejado por los mismos profesionales y se utilizaron pajuelas de similar procedencia y adecuado manejo de termos de nitrógeno. Los datos de TP de cada búfalo fueron confirmados mediante ecografía, organizados en Excel® y analizados con SAS®, asociando VAP a TP, mediante análisis de Chi-cuadrado. Para lograr el objetivo se categorizó la TP obtenida de mayor a menor valor (A: TP>70%, B: TP>60≤70%, C: TP>50≤60%, D: TP>40≤50%), además de crear subpoblaciones de espermatozoides (Sp) en función de la calidad del movimiento espermático, para lo cual se decidió utilizar la VAP, ya que es una variable que integra el VCL y el

≥ 50 and < 80 $\mu\text{m/s}$, X= Sp with a VAP ≥ 30 and < 50 $\mu\text{m/s}$, W= Sp with a VAP ≥ 10 < 30 $\mu\text{m/s}$, V= VAP < 10 $\mu\text{m/s}$. The SAS® GLM procedure was used to assess the VAP velocity according to the fertility rate obtained. The results show that the buffalo bulls with high fertility (A and B) presented in their ejaculates sperm with the best VAP (Z: 14.6% and Y: 15.84%) and less quantity of immobile sperm (W: 7.14% and V: 1.63%) ($P < 0.01$). In contrast, the buffalo bulls with lower fertility (C and D) had a considerable decrease in sperm with high velocity (Z: 4.84%) or immobile (6.24%) ($p > 0.01$). There is an evident association between the average speed (VAP) provided by the CASA system and the PR in this species. This type of analysis provides the opportunity to quantify sperm movements and group them to generate more accurate sperm motility values, which allows the prediction of the fertility and/or PR in this species.

Keywords: sperm motility, CASA system, pregnancy rate.

VSL e indirectamente asume el LIN, generando lo siguiente categorías: Z= Sp con valor de VAP ≥ 80 $\mu\text{m/s}$, Y: Sp con valor de VAP ≥ 50 y < 80 $\mu\text{m/s}$, X= Sp con valor de VAP ≥ 30 y < 50 $\mu\text{m/s}$, W= Sp con VAP ≥ 10 < 30 $\mu\text{m/s}$, V= VAP < 10 $\mu\text{m/s}$. Se utilizó el procedimiento SAS® GLM para evaluar la velocidad de VAP según la tasa de fertilidad obtenida. Los resultados muestran que los machos reproductores con alta fertilidad (A y B) presentaron en sus eyaculados espermatozoides con mejor VAP (Z: 14,6% e Y: 15,84%) y menor cantidad de espermatozoides inmóviles (W: 7,14% y V: 1,63. %) ($p < 0,01$). En contraste, los búfalos con menor fertilidad (C y D) tuvieron una disminución considerable de espermatozoides con alta velocidad (Z: 4,84%) o inmóviles (6,24%) ($p > 0,01$). Existe una evidente asociación entre la velocidad promedio (VAP) proporcionada por el sistema CASA y la TP en esta especie. Este tipo de análisis brinda la oportunidad de cuantificar los movimientos de los espermatozoides y agruparlos para generar valores de motilidad espermática más precisos, lo que permite predecir la fertilidad y/o TP en esta especie.

Palabras clave: motilidad espermática, sistema CASA, tasa de preñez.

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Testicular biometrics of Murrah buffaloes in different age groups

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ABSTRACT

Knowledge of the testicles' biometric parameters is fundamental to performing an andrological evaluation of a breeder. The present study was conducted to determine Murrah buffaloes' testicular biometry. Twenty-four buffaloes were classified into three age groups: G1 (15 to 19 months), G2 (20 to 23 months), and G3 (>24 months), located in Montelíbano, Córdoba, Colombia, from March to November 2022. The measurements analyzed were scrotal circumference (SC), spermatic cord circumference (CC), Testicular volume (TV), Testicular length (TL), and width (TW). To measure the TL, testes were measured, excluding the epididymal tail in the dorsal-ventral direction. In contrast, TW was measured in the middle portion of each testis in the lateral-medial direction. The TV was determined using the equation of the spheroid prolate and represented the average of both testes. Descriptive statistics were performed for each variable; for the SC and TV, a linear regression model as a function of age and weight was analyzed,

Biometría testicular de búfalos Murrah en diferentes grupos de edad

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RESUMEN

El conocimiento de los parámetros biométricos del testículo es fundamental para realizar una evaluación andrológica de un reproductor. El presente estudio se realizó para determinar la biometría testicular de los búfalos Murrah. Se clasificaron veinticuatro búfalos en tres grupos de edades: G1 (15 a 19 meses), G2 (20 a 23 meses) y G3 (>24 meses), ubicados en Montelíbano, Córdoba, Colombia, de marzo a noviembre de 2022. Las medidas analizadas fueron circunferencia escrotal (SC), circunferencia del cordón espermático (CC), volumen testicular (TV), longitud testicular (TL) y ancho testicular (TW). Para medir la TL se midieron los testículos excluyendo la cola del epidídimo en dirección dorsal-ventral. Por el contrario, la TW se midió en la porción media de cada testículo en dirección lateral-medial. El TV se determinó mediante la ecuación de aproximación del esferoide alargado y representó el promedio de ambos testículos. Se realizaron estadísticas descriptivas para cada variable; para SC y TV se analizó un modelo de

and simple Pearson correlations were performed. The mean values observed for G1, G2, and G3 were SC (21.7 ± 2.2 cm; 24.2 ± 1.2 cm and 27.4 ± 1.2 cm), CC (18.3 ± 2.7 cm; 20.0 ± 2.7 cm and 25.7 ± 2.1 cm), TV (79.7 ± 41.6 cm³; 142.2 ± 32.8 cm³ and 220.7 ± 39.5 cm³), right TL (6.8 ± 1.7 cm; 8.2 ± 0.8 cm and 9.0 ± 0.4 cm), left TL (6.7 ± 1.7 cm; 8.2 ± 0.8 cm and 9.0 ± 0.5 cm), Right TW (3.5 ± 0.5 cm; 4.2 ± 0.3 cm and 5.0 ± 0.4 cm) and left TW (3.4 ± 0.5 cm; 4.2 ± 0.2 cm and 5.0 ± 0.3 cm). The average weights of the groups were: G1 (350.0 ± 27.6 kg), G2 (387.9 ± 45.6 kg), and G3 (469.3 ± 40.1 kg). Positive and significant correlations of weight and age were found with SC (R: 0.86 and 0.90, respectively), as well as with TV (R: 0.85 and 0.87, respectively). From the linear regression models, the estimated monthly variations were for 1.16 ± 0.01 cm ($p < 0.01$), and the TV grows by 8.2 ± 0.3 cm³ ($p < 0.01$). In addition, it was estimated that, for each kilogram of live weight that the animal gains, the SC grows 0.05 ± 0.00 cm ($p < 0.01$) and TV 0.42 ± 0.1 cm³ ($p < 0.01$). Some testicular biometry parameters typical of the Murrah breed were known, and the variations concerning age were identified. In addition, it was possible to estimate the relationship with body weight, which will allow us to establish precision and comparison of testicular development models. More studies are necessary to standardize the morphofunctional changes of buffaloes chronologically.

Keywords: andrology, testicular biometrics, Murrah buffalo, scrotal circumference, testicular volumen.

regresión lineal en función de la edad y el peso y se realizaron correlaciones simples de Pearson. Los valores medios observados para G1, G2 y G3 fueron SC ($21,7 \pm 2,2$ cm; $24,2 \pm 1,2$ cm y $27,4 \pm 1,2$ cm), CC ($18,3 \pm 2,7$ cm; $20,0 \pm 2,7$ cm y $25,7 \pm 2,1$ cm), TV ($79,7 \pm 41,6$ cm³; $142,2 \pm 32,8$ cm³ y $220,7 \pm 39,5$ cm³), LT derecha ($6,8 \pm 1,7$ cm; $8,2 \pm 0,8$ cm y $9,0 \pm 0,4$ cm), LT izquierda ($6,7 \pm 1,7$ cm; $8,2 \pm 0,8$ cm y $9,0 \pm 0,5$ cm), DT derecha ($3,5 \pm 0,5$ cm; $4,2 \pm 0,3$ cm y $5,0 \pm 0,4$ cm) y DT izquierda ($3,4 \pm 0,5$ cm; $4,2 \pm 0,2$ cm y $5,0 \pm 0,3$ cm). Los pesos promedio de los grupos fueron: G1 ($350,0 \pm 27,6$ kg), G2 ($387,9 \pm 45,6$ kg) y G3 ($469,3 \pm 40,1$ kg). Se encontraron correlaciones positivas y significativas de peso y edad con SC (R: 0,86 y 0,90, respectivamente), así como con TV (R: 0,85 y 0,87, respectivamente). A partir de los modelos de regresión lineal, las variaciones mensuales estimadas fueron de $1,16 \pm 0,01$ cm ($p < 0,01$), y la TV crece de $8,2 \pm 0,3$ cm³ ($p < 0,01$). Además, se estimó que, por cada kilogramo de peso vivo que gana el animal, el SC crece $0,05 \pm 0,00$ cm ($p < 0,01$) y el TV $0,42 \pm 0,1$ cm³ ($p < 0,01$). Se conocieron algunos parámetros de biometría testicular propios de la raza Murrah y se identificaron las variaciones respecto a la edad. Además, se logró estimar la relación con el peso corporal, lo que permitirá establecer precisión y comparación de modelos de desarrollo testicular. Son necesarios más estudios para estandarizar cronológicamente los cambios morfofuncionales de los búfalos.

Palabras clave: andrología, biometría testicular, búfalo Murrah, circunferencia escrotal, volumen testicular.

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Are the acute phase proteins related to anestrus in primiparous water buffaloes? Preliminary results

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ABSTRACT

Primiparous water buffaloes are a group with more risk for low reproductive performance, mainly due to a long period of postpartum anestrus, which could have a multifactorial origin. It is unknown if inflammatory diseases may promote the long anestrus period in primiparous buffaloes. Therefore, this

¿Están las proteínas de fase aguda relacionadas con el anestro en búfalas de agua primíparas? Resultados preliminares

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RESUMEN

Las búfalas de agua primíparas son un grupo con mayor riesgo de bajo rendimiento reproductivo, debido principalmente a un largo período de anestro posparto, lo que podría tener un origen multifactorial. Se desconoce si las enfermedades inflamatorias pueden promover el largo período de anestro en búfa-

preliminary analysis aimed to compare C reactive protein and fibrinogen levels, two acute phase proteins, and body condition score and cholesterol levels in primiparous water buffaloes during the early postpartum and after a long anestrus period. To this, ten primiparous water buffaloes from a commercial farm in La Fria, Tachira, Venezuela, were submitted to a reproductive ultrasonographic evaluation; no signs of uterine disease or *corporal lutea* were observed in any buffalo; and therefore, these were considered in anestrus and grouped as early postpartum ($n=4$, mean of postpartum days 15 ± 2.70) or prolonged anestrus ($n=6$, mean of postpartum days 124.5 ± 36.55 , $p < 0.05$). Moreover, the body condition score was evaluated using a 1 to 5 scale with 0.25 increments (1 = thin, 5 = fat), and blood samples were collected to measure the levels of C-reactive protein (by ELISA), fibrinogen (by Clauss assay), and cholesterol (by Colorimetric method). All the means were compared by T-test using R studio; additionally, a correlation analysis was carried out. No differences were observed for any of the studied variables. Body condition score was lower than 3 points in both groups (2.31 ± 0.51 and 2.33 ± 0.37 for early and late postpartum, respectively, $p=0.947$). The cholesterol level was 142.75 ± 9.74 mg/dL and 156 ± 12.26 mg/dL, respectively ($p=0.133$). C-reactive protein averaged 60 ± 24 mg/dL and 33 ± 18 mg/dL for early and late postpartum buffaloes, respectively ($p=0.137$). At the same time, fibrinogen levels were 318.5 ± 161.77 mg/dL for early postpartum and 318.83 ± 69.78 mg/dL, respectively ($p = 0.914$). C-reactive protein and cholesterol levels were negatively correlated ($r = -0.71$, $p=0.048$). Because no differences were observed in the variables studied in both postpartum moments, it is possible to suggest that a body condition score lower than 3.0 and persistent levels of C-reactive protein, fibrinogen, and cholesterol could be related to a prolonged period of anestrus in primiparous water buffaloes. However, given the reduced number of animals included and the absence of cyclic females, the results reported should be taken cautiously and verified in a larger population.

Keywords: water buffalo cows, primiparous, anestrus, C-reactive protein, fibrinogen, cholesterol.

las primíparas. Por lo tanto, este análisis preliminar tuvo como objetivo comparar los niveles de proteína C reactiva y fibrinógeno, dos proteínas de fase aguda, y la puntuación de condición corporal y los niveles de colesterol en búfalas de agua primíparas durante el posparto temprano y después de un largo período de anestro. Para ello, se sometió a una evaluación ultrasonográfica reproductiva a diez búfalas de agua primíparas de una finca comercial en La Fría, Táchira, Venezuela. No se observaron signos de enfermedad uterina o cuerpos lúteos en ninguna búfala y, por lo tanto, se consideraron en anestro y se agruparon como posparto temprano ($n=4$, media de días posparto $15 \pm 2,70$) o anestro prolongado ($n=6$, media de días posparto $124,5 \pm 36,55$, $p < 0,05$). Además, se evaluó la puntuación de condición corporal utilizando una escala de 1 a 5 con incrementos de 0,25 (1 = muy flaca, 5 = muy gorda), y se recogieron muestras de sangre para medir los niveles de proteína C reactiva (mediante ELISA), fibrinógeno (mediante Clauss ensayo), y colesterol (por método colorimétrico). Todas las medias se compararon mediante prueba T utilizando R Studio; además, se realizó un análisis de correlación. No se observaron diferencias para ninguna de las variables estudiadas. La puntuación de condición corporal fue inferior a 3 puntos en ambos grupos ($2,31 \pm 0,51$ y $2,33 \pm 0,37$ para el posparto temprano y tardío, respectivamente, $p=0,947$). El nivel de colesterol fue de $142,75 \pm 9,74$ mg/dL y $156 \pm 12,26$ mg/dL, respectivamente ($p=0,133$). La proteína C reactiva promedió 60 ± 24 mg/dL y 33 ± 18 mg/dL para las búfalas en el posparto temprano y tardío, respectivamente ($p= 0,137$). Al mismo tiempo, los niveles de fibrinógeno fueron $318,5 \pm 161,77$ mg/dL para el posparto temprano y $318,83 \pm 69,78$ mg/dL, respectivamente ($p=0,914$). Los niveles de proteína C reactiva y colesterol se correlacionaron negativamente ($r = -0,71$, $p=0,048$). Debido a que no se observaron diferencias en las variables estudiadas en ambos momentos posparto, es posible sugerir que un puntaje de condición corporal inferior a 3,0 y niveles persistentes de proteína C reactiva, fibrinógeno y colesterol podrían estar relacionados con un período prolongado de anestro en búfalas primíparas. Sin embargo, dado el reducido número de animales incluidos y la ausencia de hembras cíclicas, los resultados informados deben tomarse con cautela y verificarse en una población más grande.

Palabras clave: vacas búfala de agua, primíparas, anestro, proteína C reactiva, fibrinógeno, colesterol.

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Factors influencing pregnancy rate following estradiol-free fixed TAI protocols in water buffaloes

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ABSTRACT

Fixed-Time Artificial Insemination (TAI) protocols efficiency is affected by numerous factors. This study evaluated biological- and technical-inherent factors associated with TAI programs. Two hundred forty-seven buffalo cows (1-14 calvings), with a body condition score (BCS) between 2.5 and 4.5, were used. The TAI protocol consisted of: Day 0, an injection of GnRH analog (10.5µg of buserelin benzoate acetate, Gestar®) intramuscularly (im) and one intravaginal device (IVD) for 9 days [TRIUB®: 1.0gr P4, (n=139); CIDR®: 1.38gr P4 (n=77); or PREGNAHEAT-E®: 250mg of medroxyprogesterone acetate (MPA), (n= 18)]. On day 9, IVD was withdrawn, and 0.5mg PGF2α (Estrumate®, Intervet, MSD Animal Health) plus eCG (500 IU, Folligon®, Intervet, MSD Animal Health) was given im. On day 11, the ovulation was induced using GnRH (10.5µg) im. TAI was performed 8-12 h later using semen from Murrah and Mediterranean bulls. Pregnancy diagnosis was performed 30-32 days after by ultrasound. Biological-inherent factors such breed (Murrah, Mediterranean, undefined), postpartum days (PPD; 30–45, 46–60, 61–90 or >90), calving's number, BCS, milk yield (< 4, 4-6 and > 6 Kg/day), ovarian status at the beginning of the protocol [follicle size (<8 mm, 8-10 mm, >12mm), corpus luteum and cysts], uterine status were studied. In addition, physiological responses at the TAI timepoint, such as tail bending, cervix lubrication, and dilation, were included. Moreover, technical-inherent variables such as AI technician, straw size (0.25 and 0.5), time performing AI technique, kind, IVD expulsion of IVDs, and the entry order to AI were evaluated. Data was analyzed by logistic regression using Proc Logistic from SAS®. The protocol showed a similar pregnancy rate (PR; $p>0.05$) regardless of the season, calving number, BCS, breed, milk yield, follicle size, and PPD period at the time point of a protocol start. Similarly, tail bending, cervix dilation, and lubrication did not influence fertility ($p>0.05$). However, cows with ovarian cysts at the start of the protocol showed a lower PR than those with CL 22.2 vs. 62.8% ($p<0.05$) respectively. Furthermore, animals treated with PGF2α and antibiotics to correct abnormal uterine status showed a higher PR 86% vs 50% than animals with apparently normal uterine status. Tech-

Factores que influyen la tasa de preñez seguidas a un protocolo de sincronización del celo e inseminación a tiempo fijo libre de estradiol en el búfalo de agua

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RESUMEN

La eficiencia de los protocolos de sincronización del celo e inseminación a tiempo fijo (IATF) se ve afectada por múltiples factores. El objetivo de este estudio fue estudiar factores biológicos y técnicos asociados a la IATF. Un total 247 búfalas (1-14 partos), con una condición corporal (CC) entre 2,4 y 4,5 fueron usadas para el experimento. El protocolo de IATF consistió: Día 0, una inyección intramuscular (*im*) de un análogo de GnRH (10,5 µg de acetato de buserelina, Gestar® y la inserción de un dispositivo intravaginal (DIV) mantenido durante 9 días [TRIUB®: 1.0gr P4, (n=139); CIDR®: 1.38gr P4 (n=77); o PREGNAHEAT-E®: 250mg of medroxyacetato de progesterona (MAP), (n= 18)]. El día 9, se retiró el DIV y se inyectó 0.5mg de PGF2α (Estrumate®, Intervet, MSD Animal Health) más eCG (500 IU, Folligon®, Intervet, MSD Animal Health) *im*. El día 11, GnRH (10.5µg) *im*. La IATF se realizó 8-12 hrs más tarde usando semen de toros Murrah o Mediterráneo. El diagnóstico de preñez fue realizado a los 30-32 días después de la IATF a través de ultrasonografía. Fueron estudiados factores biológicos como raza (Murrah, Mediterráneo o indefinido), días postparto (DPP; 30-45, 46-60, 61-90 o >90), número de partos, CC, producción de leche (< 4, 4-6 and > 6 Kg/día), estatus ovárico [tamaño de foliculo (<8 mm, 8-10 mm, >12mm), presencia de cuerpo lúteo o quistes] y estatus uterino al comienzo del protocolo. Adicionalmente se incluyeron factores asociados a la respuesta fisiológica al tratamiento al momento de la IATF como reflejo de enrollamiento de la cola, lubricación y dilatación del cérvix. Además, fueron evaluadas variables técnicas como técnico inseminador, tamaño de la pajueta (0.25 and 0.5), tiempo realizando la inseminación, tipo de DIV, expulsión del DIV y orden de entrada al brete de inseminación. El análisis estadístico fue llevado a cabo mediante un Proc Logistic de SAS®. Los resultados arrojaron que la tasa de preñez (TP) fue similar ($p>0.05$) independientemente de la temporada, número de parto, CC, raza, producción de leche, DPP o tamaño folicular presente al momento de comenzar el protocolo de IATF. De manera similar, variables como reflejo de enrollamiento de la cola, dilatación y lubricación del cérvix no influyen-

nical-inherent factors such as IVD type, expulsion of IVDs before the end of treatment, and straw size did not affect the PR ($p>0.05$). However, when the AI technique was performed in ≤ 2 minutes, the PR was higher, in comparison to ≥ 2 minutes (73.1% vs. 42.9%, respectively) ($p<0.01$). Additionally, docile animals accessing first to the AI showed higher ($p<0.05$) PR compared to those that struggled and entered last (68.8% vs 48.3%, respectively). In conclusion, this protocol showed adequate PR independent of the season, cows' breed, and productive status. Moreover, correcting the abnormal uterine status improved the PR and might increase the number of animals to be included in the TAI program. Additionally, technical factors such as time employed to perform the AI and animal behaviour when applying TAI successfully affected the PR and warrant further investigation.

Keywords: water buffaloes, AI, synchronization, TAI, pregnancy.

ció la fertilidad ($p>0.05$). Sin embargo, búfalas con presencia de quistes ováricos al comienzo del protocolo mostraron una TP más baja ($p<0.05$) que aquellas que tenían cuerpo lúteo (22,2 vs. 62,8%, respectivamente). Además, animales que fueron tratados con PGF2 α y antibióticos para corregir su estatus uterino anormal mostraron una TP mayor (86%) que las búfalas con aparente estatus uterino normal (50%). Factores técnicos como tipo de DIV, expulsión del DIV antes de culminar el tratamiento y tamaño de la pajuela no afectaron la TP ($p>0.05$). Sin embargo, cuanto la técnica de IA se realizó en ≤ 2 minutos la TP fue mayor ($p<0.01$) comparado con aquellas que requirieron ≥ 2 minutos para ejecutar la técnica (73,1% vs 42,9%, respectivamente). Adicionalmente, los animales dóciles que entraron de primero a la IA mostraron una mayor TP comparados con aquellos que se reusaban y entraron de último (68,8% vs 48,3%, respectivamente). En conclusión, este protocolo mostró una adecuada tasa de preñez independientemente de la temporada, la raza de los animales y el estado productivo. Adicionalmente, corregir el estado uterino anormal mejoró la tasa de preñez y podría aumentar el número de animales candidatos a ser incluidos en programas de IATF. Además, factores técnicos como el tiempo empleado para realizar la IA y el comportamiento animal al aplicar la IATF afectaron la preñez y justifican una mayor investigación.

Palabras clave: búfalo de agua, IA, sincronización, IATF, preñez.

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A first study on sperm sexing in water buffalo through magnetic nanoparticles

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ABSTRACT

Gender pre-selection is a genetic tool of economic significance, as it permits animal breeders to produce sex-specific progeny, thereby increasing profitability and genetic gain in the livestock industry. A higher percentage of female calves is preferable for dairy production. In Pakistan, water buffaloes are primarily kept for dairy purposes. Therefore, this study was designed to optimize the magnetic nanoparticles (MNPs) based sperm sexing technique in water buffalo. Qualified semen samples from five buffalo bulls were pooled and divided into four aliquots. Each aliquot of 50 million sperm was diluted in 1 mL of modified human tubal fluid (mHTF). Three ali-

Un primer estudio sobre sexado de espermatozoides en búfalos de agua mediante nanopartículas magnéticas

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RESUMEN

La preselección de sexo en ganadería es una herramienta genética de importancia económica, ya que permite a los criadores de animales producir progenie de sexo específico, aumentando así la rentabilidad y la ganancia genética en la industria ganadera. Es preferible un mayor porcentaje de terneras para la producción lechera. En Pakistán, los búfalos de agua se crían principalmente con fines lácteos. Por lo tanto, este estudio fue diseñado para optimizar la técnica de sexado de semen basada en nanopartículas magnéticas (MNP) en búfalos de agua. Se reunieron muestras de semen calificadas de

quots (sexed groups) were incubated with 0.67 ml of negatively charged MNPs suspension. The fourth aliquot was incubated without MNPs (called "control group"). Each tube containing sperm-MNPs suspension was slowly shaken for 5 minutes for uniform mixing. The MNPs were negatively charged and (provided by Clemente Associates Inc, Prescott, Arizona, USA) had a diameter of 50 nanometers. The interaction between the negative charge of MNPs and the Z electrical potential of spermatozoa was different for those spermatozoa carrying an X chromosome (-20 mV) and those carrying a Y chromosome (-16 mV). Therefore, the Y chromosome-bearing spermatozoa remained closer to MNPs. Three sexed groups were exposed to a magnet for 10, 20, and 30 minutes. The control group was exposed to the magnet for 20 minutes. Consequently, in the sexed groups, the Y-bearing sperm-MNP complexes remained attached to the tube's inner wall due to the magnetic force. In contrast, the X-chromosome spermatozoa remained suspended in the medium. Then, suspended spermatozoa carrying the X chromosome were slowly aspirated. The control group was also aspirated and transferred to a new tube. After aspiration, each group's progressive motility (PM, %) of spermatozoa was assessed through a computer-assisted sperm analyzer (CASA). After that, spermatozoa were centrifuged at $226 \times g$ for 5 minutes to remove the mHTF, and DNA was extracted. Validation of the sexing technique was done through SYBR® Green Real-Time (RT) PCR using two sets of primers for gender-specific genes, i.e., X-linked proteolipid protein (PLP) and sex-determining region Y protein (SRY). Results revealed that PM was similar ($p > 0.05$) in all groups (ranging from 62 to 65%). The mean fold expression of the PLP gene (X chromosome bearing sperm) was higher ($p < 0.05$) in all sexed groups (average: 15.34-fold = 91.09% X chromosome bearing sperm) as compared to control (1.60-fold). In conclusion, the MNPs-based technique appeared to be an effective method for water buffalo sperm sexing as validated by RT PCR.

Keywords: buffalo sperm, sperm sexing, magnetic nanoparticles, Real-Time PCR.

cinco búfalos reproductores y se dividieron en cuatro alícuotas. Cada alícuota de 50 millones de espermatozoides se diluyó en 1 ml de líquido tubárico humano modificado (mHTF). Se incubaron tres alícuotas (grupos sexados) con 0,67 ml de suspensión de MNP cargada negativamente. La cuarta alícuota se incubó sin MNP (llamada "grupo de control"). Cada tubo que contenía suspensión de espermatozoa-MNP se agitó lentamente durante 5 minutos para una mezcla uniforme. Los MNP estaban cargados negativamente y (proporcionados por Clemente Associates Inc, Prescott, Arizona, EE. UU.) y tenían un diámetro de 50 nanómetros. La interacción entre la carga negativa de las MNP y el potencial eléctrico Z de los espermatozoides fue diferente para aquellos espermatozoides que portan un cromosoma X (-20 mV) y aquellos que portan un cromosoma Y (-16 mV). Por lo tanto, los espermatozoides portadores del cromosoma Y permanecieron más cerca de las MNP. Se expusieron tres grupos sexados a un imán durante 10, 20 y 30 minutos. El grupo de control estuvo expuesto al imán durante 20 minutos. En consecuencia, en los grupos sexados, los complejos espermatozoide-MNP con carga cromosómica Y permanecieron adheridos a la pared interna del tubo debido a la fuerza magnética. Por el contrario, los espermatozoides del cromosoma X permanecieron suspendidos en el medio. Luego, se aspiraron lentamente los espermatozoides suspendidos que portaban el cromosoma X. El grupo de control también fue aspirado y transferido a un tubo nuevo. Después de la aspiración, se evaluó la motilidad progresiva (PM, %) de los espermatozoides de cada grupo mediante un analizador de esperma asistido por computadora (CASA). Después de eso, los espermatozoides se centrifugaron a $226 \times g$ durante 5 minutos para eliminar el mHTF y se extrajo el ADN. La validación de la técnica de sexado se realizó mediante PCR en tiempo real (RT-PCR) SYBR® Green utilizando dos conjuntos de cebadores para genes específicos de género, es decir, proteína proteolípida ligada al cromosoma X (PLP) y proteína de la región Y determinante del sexo (SRY). Los resultados revelaron que la MP fue similar ($p > 0,05$) en todos los grupos (entre 62 y 65%). La expresión media del gen PLP (esperma con cromosoma X) fue mayor ($p < 0,05$) en todos los grupos sexados (promedio: 15,34 veces = 91,09% de esperma con cromosoma X) en comparación con el control (1,60 veces). En conclusión, la técnica basada en MNP mostró ser un método eficaz para el sexado de semen de búfalo de agua, validado por RT PCR.

Palabras clave: semen de búfalo, sexado de espermatozoides, nanopartículas magnéticas, RT PCR.

R-231 Rev. Cientif. FCV-LUZ, XXXIII, SE, 271-272, 2023, <https://doi.org/10.52973/rcfcv-wbc117>**Forty-two-year frequencies of river buffaloes calving in the Terecay ranch, Venezuela****Renny Jesús Becerra¹, Inés Valentina Reggeti-Gómez²,
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Néstor Simón Montiel-Urdaneta⁴**¹INSERGAN; C.A^{2,3}Hato Terecay; C.A. Guárico, Venezuela⁴Facultad de Ciencias Veterinarias, Universidad del Zulia,
Maracaibo, Venezuela*Corresponding author: Renny Jesús Becerra (rennybecerra@gmail.com)**ABSTRACT**

Although buffaloes are polyestrous, they exhibit a distinct seasonal variation in displaying estrus, conception rate, and calving, greatly influenced by physiological, sanitary, nutritional, management, and particularly bioenvironmental factors, such as temperature, humidity, precipitation, evaporation, and daylight duration. Venezuela, a tropical country, is located in the intertropical zone of the northern hemisphere, between the Equator and the Tropic of Cancer, between coordinates 00° 38' 53" and 12° 11' 22" LN and 59° 48' 10" and 73° 25' 00" LW. The area (Terecay ranch) under study has two well-defined seasons concerning precipitation, with a bimodal behavior, a rainy season (From May to October with an average of 1,441.3 mm/year) and a season with very little or no precipitation (From November to April with an average of 107.4 mm/year). In order to calculate the calving distribution, the records of 13,720 calvings of buffaloes between 1980-2022 corresponding to the Terecay farm located in the Esteros de Camaguan of Guárico State were used. For calculating calving frequency, the PROC FREQ procedure of the statistical package SAS version 9.1 was used, and a polynomial regression analysis was performed to evaluate the prediction of future behavior of the calving distribution. The percentage of calving according to the months of the year were: January: 6.96%; February: 4.34%; March: 2.3%; April: 0.95%; May: 0.98%; June: 1.34%; July: 4.39%; August: 11.44%; September: 16.38%; October: 22.32%; November: 15.69% and December 12.91%. The months with the highest average of calving percentages were September (16.38%), October (22.32%), and November (15.69%), and the months with the lowest average percentages were April (0.95%) and May (0.98%). The equation of the polynomial regression analysis was $y = -3.0656x^4 + 64.363x^3 - 360.05x^2 + 417.41x + 810.37$ with an $R^2 = 0.9571$. With this R^2 value, it can be inferred that the calving behavior will be maintained during these 42 years. Conclusions are that the frequency of calving showed a very marked seasonality between July and January, with 90.09% and 9.91% between February and June. Calving seasonality should be preserved if environmental, nutritional, sanitary, and

Frecuencias de cuarenta y dos años de partos de búfalos de río en la estancia Terecay, Venezuela

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(rennybecerra@gmail.com)**RESUMEN**

Aunque los búfalos son poliestros, exhiben una clara variación estacional en la presentación del estro, la tasa de concepción y el parto, muy influenciados por factores fisiológicos, sanitarios, nutricionales, de manejo y particularmente bioambientales, como la temperatura, la humedad, las precipitaciones, la evaporación y la duración de la luz del día. Venezuela, un país tropical, está ubicado en la zona intertropical del hemisferio norte, entre el Ecuador y el Trópico de Cáncer, entre las coordenadas 00° 38' 53" y 12° 11' 22" LN y 59° 48' 10" y 73° 25' 00" LO. La zona de estudio (Rancho Terecay) tiene dos estaciones bien definidas en cuanto a precipitación, con un comportamiento bimodal, una estación lluviosa (de mayo a octubre con un promedio de 1.441,3 mm/año) y una estación con muy poca o nula precipitación (de noviembre a abril con un promedio de 107,4 mm/año). Para calcular la distribución de partos se utilizaron los registros de 13.720 partos de búfalos entre 1980-2022 correspondientes a la finca Terecay ubicada en los Esteros de Camaguán del Estado Guárico. Para el cálculo de la frecuencia de partos se utilizó el procedimiento PROC FREQ del paquete estadístico SAS versión 9.1 y se realizó un análisis de regresión polinomial para evaluar la predicción del comportamiento futuro de la distribución de partos. Los porcentajes de parto según los meses del año fueron: enero: 6,96%; febrero: 4,34%; marzo: 2,3%; abril: 0,95%; mayo: 0,98%; junio: 1,34%; julio: 4,39%; agosto: 11,44%; septiembre: 16,38%; octubre: 22,32%; noviembre: 15,69% y diciembre 12,91%. Los meses con mayor porcentaje promedio de partos fueron septiembre (16,38%), octubre (22,32%) y noviembre (15,69%), y los meses con menor porcentaje promedio fueron abril (0,95%) y mayo (0,98%). La ecuación del análisis de regresión polinómica fue $y = -3.0656x^4 + 64.363x^3 - 360.05x^2 + 417.41x + 810.37$ con un $R^2 = 0.9571$. Con este valor de R^2 se puede inferir que el comportamiento de parto se mantendrá durante estos 42 años. Se concluye que la frecuencia de partos presentó una estacionalidad muy marcada entre julio y enero, con 90,09% y 9,91% entre febrero y junio. La estacionalidad de los partos debe preservarse si se mantienen las condiciones ambienta-

management conditions are maintained, especially without hormonal interventions.

Keywords: buffaloes, calving, seasonality.

les, nutricionales, sanitarias y de manejo, especialmente sin intervenciones hormonales.

Palabras clave: búfalas, parto, estacionalidad.

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Effect of different hormonal protocols with timed AI on clinical signs of estrus and conception rates in Bulgarian Murrah buffaloes

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Efecto de diferentes protocolos hormonales con inseminación artificial a tiempo fijo sobre los signos clínicos de estro y las tasas de concepción en búfalas Murrah búlgaras

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ABSTRACT

The objective of the field experiment was to study the effect of different protocols for estrus synchronization with timed artificial insemination (TAI) on the manifestation of clinical signs of estrus (CSE) and conception rates in the Bulgarian Murrah breed. For treatment 75 heifers and 133 buffalo cows were assigned with follicles >8 mm and no corpus luteum and subjected to the following protocols: Presynch/Ovsynch (D1 - Synchrostim + Enzaprost; D4 - Ovarelin; D11 - Enzaprost; D13 - Ovarelin), Ovsynch (D1 - Ovarelin; D8 - Enzaprost; D10 - Ovarelin), Ovsynch+PRID (D1-8 PRID DELTA; D8 - Enzaprost; D10 - Ovarelin). For the buffalo cows, each protocol was applied in the breeding and non-breeding season, while in the heifers, out of season only. Gestation was diagnosed sonographically on day 45 post-TAI. The data were processed via dispersion analysis of a non-orthogonal set of qualitative traits, including the following factors with respective classes: protocol, three classes – as described above; CSE, three classes – without CSE; patency of the cervical canal (excluding mucus); presence of clear mucous discharge (including patency); age, two classes – heifers, cows. Another 3-factor analysis was conducted on buffalo cows only, instead of age including season – with two classes: in season and out of season. The results show that the factor protocol has the most pronounced effect on TAI success ($p < 0.01$), significantly lowest being the pregnancy rate (pi values) under the Presynch/Ovsynch protocol – only 23.9%, compared to 40.7 and 47.4% under Ovsynch and Ovsynch+PRID, respectively. Although the effect of age is non-significant, in the heifers, Ovsynch+PRID and Ovsynch show markedly higher results (50.0 and 52.9%, respectively). At the same time, in the buffalo cows, the differences are lower, the Ovsynch protocol having a relatively low pregnancy rate (38.3%), Ovsynch+PRID – highest (45.0%), and Presynch/Ovsynch – lowest

RESUMEN

El objetivo del experimento de campo fue estudiar el efecto de diferentes protocolos de sincronización del estro con inseminación artificial a tiempo fijo (IATF) sobre la manifestación de los signos clínicos del estro (CSE) y las tasas de concepción en la raza Murrah búlgara. Para el tratamiento se asignaron 75 novillas y 133 búfalas con folículos >8 mm y sin cuerpo lúteo y sometidas a los siguientes protocolos: Presynch/Ovsynch (D1 - Synchrostim + Enzaprost; D4 - Ovarelin; D11 - Enzaprost; D13 - Ovarelin), Ovsynch (D1 - Ovarelin; D8 - Enzaprost; D10 - Ovarelin), Ovsynch+PRID (D1-8 PRID DELTA; D8 - Enzaprost; D10 - Ovarelin). En las búfalas cada protocolo se aplicó en época reproductiva y no reproductiva, mientras que en las bubillas solo fuera de temporada. La gestación se diagnosticó ecográficamente el día 45 después de la IATF. Los datos se procesaron mediante análisis de dispersión de un conjunto no ortogonal de rasgos cualitativos, incluidos los siguientes factores con sus respectivas clases: protocolo, tres clases, como se describió anteriormente; CSE, tres clases – sin CSE; permeabilidad del canal cervical (excluyendo moco); presencia de secreción mucosa clara (incluida la permeabilidad); edad, dos clases: bubillas y búfalas. Se realizó otro análisis de tres factores únicamente en búfalas, en lugar de la edad, incluida la temporada, con dos clases: en temporada y fuera de temporada. Los resultados muestran que dentro de los factores, el protocolo tiene el efecto más pronunciado en el éxito de la IATF ($p < 0,01$), siendo significativamente más bajo la tasa de preñez (valores pi) bajo el protocolo Presynch/Ovsynch: solo 23,9%, en comparación con 40,7 y 47,4% bajo Ovsynch y Ovsynch+PRID, respectivamente. Aunque el efecto de la edad no fue significativo, en las bubillas Ovsynch+PRID y Ovsynch mostraron resultados marcadamente superiores (50,0 y 52,9%,

(30.4%). The season had no significant effect, but it is noteworthy that there are even higher conception rates after out-of-season TAI than in-season – a very well-expressed difference under Ovsynch+PRID (50.0% vs. 37.5%) and smaller under Presynch/Ovsynch (37.5% vs 28.9%) and Ovsynch (40.9% vs 36.0%). CSE is a significant source of variance of conception rates ($p < 0.05$), predictably the highest p_i value belonging to the cases with mucus. The superiority of the Ovsynch+PRID protocol finds expression in the highest incidence of full estrus (with both CSE) in the lactating buffaloes (70%) and even more in the heifers (82%), as compared to Presynch/Ovsynch (56.5 and 52.2% respectively) and Ovsynch (51.1 and 50.0%) protocols. This is a reason to conclude that the Ovsynch+PRID protocol is recommendable for application in both age groups of Bulgarian Murrah buffaloes, as the observed highest manifestation of full estrus is associated with the highest conception rates, for which mucous discharge can be used as an indicator; this to greater extent regards the heifers in which, on the other hand, the Ovsynch protocol also has high success of TAI. The tested protocols show the capacity to mitigate the impact of season on reproduction and can be used in practice for overcoming the species-problematic seasonal anestrus, especially Ovsynch+PRID.

Keywords: buffaloes, gonadotropin, prostaglandin, PRID, conception rate, clinical signs of estrus.

respectivamente). Al mismo tiempo, en las búfalas adultas, las diferencias son menores: el protocolo Ovsynch tuvo una tasa de preñez relativamente baja (38,3%), Ovsynch+PRID – la más alta (45,0%) y Presynch/Ovsynch – la más baja (30,4%). La temporada no tuvo un efecto significativo, pero es digno de mención que hay tasas de concepción aún más altas después de la IATF fuera de temporada que durante la temporada: una diferencia muy bien expresada bajo Ovsynch+PRID (50,0% vs. 37,5%) y menores bajo Presynch/Ovsynch (37,5% vs 28,9%) y Ovsynch (40,9% vs 36,0%). El CSE fue una fuente importante de variación de las tasas de concepción ($p < 0,05$), previsiblemente el valor p_i más alto perteneciente a los casos con moco. La superioridad del protocolo Ovsynch+PRID se expresó en la mayor incidencia de estro completo (con ambos CSE) en las búfalas lactantes (70%) y aún más en las bubillas (82%), en comparación con Presynch/Ovsynch (56,5 y 52,2% respectivamente) y protocolos Ovsynch (51,1 y 50,0%). Esta es una razón para concluir que el protocolo Ovsynch+PRID es recomendable para su aplicación en ambos grupos de edad de búfalas murrah búlgaras, ya que la manifestación más alta observada de estro completo se asocia con las tasas de concepción más altas, para las cuales la secreción mucosa puede usarse como un indicador; esto se aplica en mayor medida a las bubillas en las que, por otro lado, el protocolo Ovsynch también tiene un gran éxito de IATF. Los protocolos probados muestran la capacidad de mitigar el impacto de la estación en la reproducción y pueden usarse en la práctica para superar el anestro estacional problemático para la especie, especialmente Ovsynch+PRID.

Palabras clave: búfalas, gonadotropina, prostaglandina, PRID, tasa de concepción, signos clínicos de estro.

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Effect of supplementing epinephrine in maturation media on the developmental competence of buffalo oocytes

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ABSTRACT

Epinephrine is a catecholamine that plays a vital role during cellular stress by scavenging free radicals. During *in*

Efecto de la suplementación con epinefrina en los medios de maduración sobre la competencia de desarrollo de los ovocitos de búfala

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RESUMEN

La epinefrina es una catecolamina que desempeña un papel vital durante el estrés celular al eliminar los radicales li-

in vitro maturation, oocytes experience stressful conditions that likely compromise their development. The objective of the experiment was to investigate the effect of the addition of epinephrine in IVM media on nuclear maturation and *in vitro* developmental competence of oocytes. Buffalo oocytes were harvested from slaughterhouse ovaries. Oocytes of A and B grade were matured for 24 hours in IVM media supplemented with increasing concentrations of epinephrine 0, 0.01, 1.0, and 100 μ M. The maturation of oocytes was examined based on the nuclear maturation after staining with Hoechst 33342 (Sigma Aldrich, St. Louis, MO, USA). After maturing with epinephrine, oocytes were fertilized for 6 hours with post-thawed semen from a single bull. Presumptive zygotes were cultured in commercial media (IVF Bioscience, Falmouth, UK), and cleavage was observed 48 hours after fertilization. The blastocyst rate was assessed on day 7 of the culture. Maturation and fertilization media were prepared in-house. Blastomere was counted from the hatched blastocyst after staining with Hoechst 33342 using an inverted microscope equipped with Cellsens software. The data was analyzed using the GLIMMIX procedures of SAS. The statistical model included the fixed effect of treatment and random replication. Orthogonal polynomial contrasts were built to assess the dose-response of epinephrine. Results represented as least square means and respective standard error of means (LSM \pm SEM). Adding epinephrine to the maturation media did not affect the nuclear maturation and cleavage. Media supplemented with epinephrine at 0, 0.01, 1.0, and 100 μ M concentrations had a proportion of meiotic I and II oocytes of 83.1 ± 6.0 , 85.5 ± 6.3 , 75.3 ± 5.9 , and $70.9 \pm 6.4\%$ and cleavage rate of 58.2 ± 4.6 , 60.5 ± 4.6 , 54.7 ± 4.5 , and $47.8 \pm 4.4\%$ respectively. However, the proportion of blastocyst out of matured oocytes was increased ($p=0.01$; 35.9 ± 4.5 , 43.2 ± 4.8 , 36.0 ± 4.3 , and $25.8 \pm 3.8\%$) with epinephrine supplementation and the response was maximized (Quadratic effect; $p=0.01$) at 0.01 μ M concentration. Consistently, the proportion of blastocyst out of cleaved embryos tended to increase ($p=0.13$; 62.1 ± 5.0 , 72.4 ± 4.6 , 66.7 ± 4.7 , and $55.3 \pm 5.3\%$) with epinephrine and the response was maximized (Quadratic effect; $p=0.04$) at 0.01 μ M concentration. Supplementation of epinephrine in maturation media increased ($p<0.01$) the blastomere number, and response was maximized (Quadratic effect; $p<0.01$) at 1.0 μ M concentration. It is concluded that supplementing epinephrine in maturation media had a favorable effect on the blastocyst formation, and significant benefits were observed at 0.01 μ M concentration.

Keywords: epinephrine, oocyte, maturation, cleavage rate, blastocyst.

Durante la maduración *in vitro*, los ovocitos experimentan condiciones estresantes que probablemente comprometan su desarrollo. El objetivo del experimento fue investigar el efecto de la adición de epinefrina en medios de maduración *in vitro* (IVM) sobre la maduración nuclear y la competencia de desarrollo *in vitro* de los ovocitos. Los ovocitos de búfalos se recolectaron de ovarios de matadero. Se maduraron ovocitos de grado A y B durante 24 horas en medio IVM suplementado con concentraciones crecientes de epinefrina 0, 0,01, 1,0 y 100 μ M. La maduración de los ovocitos se examinó en función de la maduración nuclear después de la tinción con Hoechst 33342 (Sigma Aldrich, St. Louis, MO, EE. UU.). Después de madurar con epinefrina, los ovocitos fueron fertilizados durante 6 horas con semen postdescongelado de un solo toro. Se cultivaron presuntos cigotos en medios comerciales (IVF Bioscience, Falmouth, Reino Unido) y se observó la escisión 48 horas después de la fertilización. La tasa de blastocistos se evaluó el día 7 del cultivo. Los medios de maduración y fertilización se prepararon internamente. Se contó el número de blastómeras a partir del blastocisto eclosionado después de tefir con Hoechst 33342 utilizando un microscopio invertido equipado con el software Cellsens. Los datos se analizaron utilizando los procedimientos GLIMMIX de SAS. El modelo estadístico incluyó el efecto fijo del tratamiento y la replicación aleatoria. Se construyeron contrastes polinomiales ortogonales para evaluar la dosis-respuesta de epinefrina. Los resultados se representan como medias de mínimos cuadrados y el respectivo error estándar de medias (LSM \pm SEM). La adición de epinefrina a los medios de maduración no afectó la maduración y escisión nuclear. Los medios suplementados con epinefrina a concentraciones de 0, 0,01, 1,0 y 100 μ M tuvieron una proporción de ovocitos meióticos I y II de $83,1\pm 6,0$, $85,5\pm 6,3$, $75,3\pm 5,9$ y $70,9\pm 6,4\%$ y una tasa de escisión de $58,2\pm 4,6$, $60,5\pm 4,6$, $54,7\pm 4,5$ y $47,8\pm 4,4\%$, respectivamente. Sin embargo, la proporción de blastocistos a partir de los ovocitos maduros aumentó ($p=0,01$; $35,9\pm 4,5$, $43,2\pm 4,8$, $36,0\pm 4,3$ y $25,8\pm 3,8\%$) con la suplementación con epinefrina y la respuesta se maximizó (efecto cuadrático; $p=0,01$) a una concentración de 0,01 μ M. Consistentemente, la proporción de blastocistos de los embriones escindidos tendió a aumentar ($p=0,13$; $62,1\pm 5,0$, $72,4\pm 4,6$, $66,7\pm 4,7$ y $55,3\pm 5,3\%$) con epinefrina y la respuesta se maximizó (efecto cuadrático; $p=0,04$) a una concentración de 0,01 μ M. La suplementación con epinefrina en los medios de maduración aumentó ($p<0,01$) el número de blastómeros y la respuesta se maximizó (efecto cuadrático; $p<0,01$) a una concentración de 1,0 μ M. Se concluye que la suplementación con epinefrina en los medios de maduración tuvo un efecto favorable sobre la formación de blastocistos y se observaron beneficios significativos a una concentración de 0,01 μ M.

Palabras clave: epinefrina, ovocito, maduración, tasa de escisión, blastocisto.

R-237 Rev. Cientif. FCV-LUZ, XXXIII, SE, 275-276, 2023, <https://doi.org/10.52973/rcfcv-wbc120>**Effect of partial oocyte maturation before ovum pick up on developmental competence of buffalo oocytes****Amjad Riaz¹, Muhammad K. Ashraf¹, Talha Ashraf¹,
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Compared with *in vivo* matured oocytes, *in vitro* maturation produces half the number of blastocysts in cattle. We hypothesized that partial maturation of oocytes *in vivo* improves blastocyst formation in buffalo. Therefore, we aimed to investigate the effect of partial *in vivo* maturation on *in vitro* embryo production (IVEP). The estrous cycle of buffalo was synchronized by double shot prostaglandin (PGF2 α , Fatro, Ozzano Emilia, Italy) with 11-day intervals. On the last PGF2 α , dominant follicle ablation and supplementation of progesterone by inserting intra-vaginal controlled internal drug release was performed to synchronize the follicle wave emergence and considered as Day 0 of the experiment. Starting on Day 2 of the experiment, the ovaries of buffalo were super-stimulated with six equally divided doses of 33.3 mg per dose of porcine follicle stimulating hormone (Vetoquinol, Magny-Vernois, France) i.m., for three days with 12 h interval. The experiment was randomized, with a complete block design. On Day 5 of the experiment, buffaloes were blocked by parity, and within the block, they were assigned randomly to receive 1500 IU of human chorionic gonadotropin i.m., (hCG, LG Chem, Seoul, South Korea; n = 6) or to remain untreated control (CON; n = 4). Oocytes were aspirated six hours after treatment implementations using ultrasound-guided Ovum Pick Up (OPU). The data was analyzed by using mixed effect models in SAS statistical software. Treatment with hCG did not affect the number of small, medium, and large follicles at the time of OPU. However, hCG treatment increased ($p < 0.01$) cleaved oocytes compared with CON (84.0 vs. 40.5 \pm 7.2%). Similarly, oocytes treated with hCG had greater ($p = 0.04$) development from cultured oocytes to Day 7 embryo (52.5 vs. 18.1 \pm 10.3%). Treatment with hCG did not affect the development of cleaved oocytes to Day 7 embryo. In conclusion, our results indicated that partial oocyte maturation improved the *in vitro* embryo production. However, the impact was pronounced at

Efecto de la maduración parcial de los ovocitos antes de coleccionar los óvulos sobre la competencia del desarrollo de los ovocitos de búfala

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En comparación con los ovocitos madurados *in vivo*, la maduración *in vitro* produce la mitad de los blastocistos en el ganado. Nuestra hipótesis es que la maduración parcial de los ovocitos *in vivo* mejora la formación de blastocistos en búfalas. Por lo tanto, nuestro objetivo fue investigar el efecto de la maduración parcial *in vivo* en la producción de embriones *in vitro* (PIVE). El ciclo estral de las búfalas se sincronizó mediante doble inyección de prostaglandinas (PGF2 α , Fatro, Ozzano Emilia, Italia) con intervalos de 11 días. En la última dosis de PGF2 α , se realizó la ablación del folículo dominante y la suplementación de progesterona mediante la inserción intra-vaginal de un dispositivo de liberación interna controlada del fármaco para sincronizar la aparición de la onda del folículo y se consideró como el día 0 del experimento. A partir del día 2 del experimento, los ovarios de las búfalas fueron superestimulados con seis dosis divididas equitativamente de 33,3 mg por dosis de hormona folículo estimulante (FSH) porcina (Vetoquinol, Magny-Vernois, Francia) por vía intramuscular, durante tres días con un intervalo de 12 h. El experimento fue aleatorio, con un diseño de bloques completos. El día 5 del experimento, las búfalas fueron bloqueadas por paridad y, dentro del bloque, fueron asignadas aleatoriamente para recibir 1500 UI de gonadotropina coriónica humana por vía intramuscular (hCG, LG Chem, Seúl, Corea del Sur; n=6), o permanecer control no tratado (CON; n=4). Los ovocitos se aspiraron seis horas después de la implementación del tratamiento mediante Ovum Pick Up (OPU) guiada por ultrasonido. Los datos se analizaron utilizando modelos de efectos mixtos en el software estadístico SAS. El tratamiento con hCG no afectó la cantidad de folículos pequeños, medianos y grandes en el momento de la OPU. Sin embargo, el tratamiento con hCG aumentó ($p < 0,01$) los ovocitos escindidos en comparación con CON (84,0 frente a 40,5 \pm 7,2%). De manera similar, los ovocitos tratados con hCG tuvieron un mayor desarrollo ($p = 0,04$) desde los ovocitos cultivados

the cleavage rate rather than in the later stages of embryo development.

Keywords: buffalo, ovum pick up, *in vivo* maturation, hCG, cleavage.

hasta el embrión del día 7 (52,5 frente a $18,1 \pm 10,3\%$). El tratamiento con hCG no afectó el desarrollo de los ovocitos escindidos hasta el embrión del día 7. En conclusión, nuestros resultados indicaron que la maduración parcial de los ovocitos mejoró la producción de embriones *in vitro*. Sin embargo, el impacto fue pronunciado en la tasa de escisión más que en las últimas etapas del desarrollo embrionario.

Palabras clave: búfala, recogida de óvulos, maduración *in vivo*, hCG, escisión.

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Crocina improves post-thaw quality, fertility-associated gene expression and fertilization potential of buffalo bull sperm

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ABSTRACT

Buffalo sperm suffer more cryoinjuries due to lipid peroxidation of their high structural polyunsaturated fatty acid contents than cattle. Consequently, the post-thaw fertilization potential of buffalo sperm is compromised. Crocin is a carotenoid known for its antioxidant potential through scavenging reactive oxygen species. The current study investigated the effect of crocin addition in the semen extender on the post-thaw quality, fertility-associated gene expression, and fertilization potential of buffalo bull sperm. Semen samples (n=32) from four Nili-Ravi buffalo bulls were extended with tris-citric acid extender containing different concentrations of crocin (0mM; control, 0.5, 1, 1.5, and 2mM). The extended semen was packed in 0.5 mL French straws (25 x 10⁶ sperm/straw) and cryopreserved using liquid nitrogen vapor freezing protocol. Computer-assisted semen analysis, Normal Apical Ridge assay, Rhodamine 123, Acridine orange, and Propidium iodide staining were used to assess sperm motility parameters, acrosome integrity, mitochondrial membrane potential, DNA integrity, and viability, respectively. Expression levels of sperm acrosome-associated *SPACA3*, DNA condensation-related *PRM1*, anti-apoptotic *BCL2*, pro-apoptotic *BAX*, and oxidative stress-associated *ROMO1* genes were evaluated through qPCR. The *in vivo* fertility of semen doses containing 1mM crocin was compared with control. Buffaloes were inseminated 24 hours after the onset of natural estrus and transrectally palpated for pregnancy at least

La crocina mejora la calidad post-descongelación, la expresión genética asociada a la fertilidad y el potencial de fertilización del semen de búfalo

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RESUMEN

Los espermatozoides de búfalo sufren más criolesiones debido a la peroxidación lipídica de su alto contenido de ácidos grasos poliinsaturados estructurales que el ganado vacuno. En consecuencia, el potencial de fertilización del espermatozoide de búfalo después de la descongelación se ve comprometido. La crocina es un carotenoide conocido por su potencial antioxidante al eliminar especies reactivas de oxígeno. El estudio actual investigó el efecto de la adición de crocina en el diluyente de semen sobre la calidad post-descongelación, la expresión genética asociada a la fertilidad y el potencial de fertilización del espermatozoide de búfalos. Las muestras de semen (n=32) de cuatro toros búfalo Nili-Ravi se extendieron con un diluyente de ácido tris-cítrico que contenía diferentes concentraciones de crocina (0 mM; control, 0,5, 1, 1,5 y 2 mM). El semen extendido se envasó en pajillas (pajuelas) francesas de 0,5 ml (25 x 10⁶ espermatozoides/pajita) y se criopreservó utilizando un protocolo de congelación con vapor de nitrógeno líquido. Se utilizaron análisis de semen asistido por computadora, evaluación de la zona de cresta apical normal, rodamina 123, naranja de acridina y tinción con yoduro de propidio para evaluar los parámetros de motilidad de los espermatozoides, integridad acrosómica, potencial de membrana mitocondrial, integridad del ADN y viabilidad, respectivamente. Los niveles de expresión de los genes *SPACA3* asociado al acrosoma del esperma, *PRM1* relacionado con la condensación de ADN,

60 days post-insemination. Data were analyzed through PROC MIXED or PROC GLIMMIX using SAS. Tukey test was applied for multiple comparisons among treatments. Post-thaw evaluation revealed that 0.5 and 1mM crocin improved total motility, plasma membrane integrity, acrosome integrity, mitochondrial membrane potential, and viability, and one and 1.5mM crocin enhanced catalase activity and reduced lipid peroxidation compared to control ($p < 0.05$). Moreover, 1mM crocin improved progressive motility, kinematics, and DNA integrity than control ($p < 0.05$). Expression levels of *SPACA3*, *PRM1*, and *BCL2* genes were higher ($p < 0.05$) with 1mM crocin, whereas no difference ($p > 0.05$) was observed in the expression of *BAX* gene among all groups. Moreover, *ROMO1* gene expression was higher ($p < 0.05$) with 2mM compared to 1mM and 1.5mM crocin. Semen doses containing 1mM crocin showed a higher fertility rate compared to control [$56 \pm 0.03\%$ (112/200) vs. $46 \pm 0.04\%$ (92/200), respectively; $p = 0.0465$]. In conclusion, 1mM crocin addition in the semen extender improves post-thaw quality, fertility-associated gene expression, and fertilization potential of buffalo bull sperm.

Keywords: crocin, antioxidant, cryopreservation, lipid peroxidation, Protamine 1, fertility.

BCL2 antiapoptótico, *BAX* proapoptótico y *ROMO1* asociado al estrés oxidativo se evaluaron mediante qPCR. La fertilidad *in vivo* de dosis de semen que contenían crocina 1 mM se comparó con la del control. Las búfalas fueron inseminadas 24 horas después del inicio del estro natural y palpadas transrectalmente para detectar preñez al menos 60 días después de la inseminación. Los datos se analizaron mediante PROC MIXED o PROC GLIMMIX utilizando SAS. Se aplicó la prueba de Tukey para comparaciones múltiples entre tratamientos. La evaluación posterior a la descongelación reveló que 0,5 y 1 mM de crocina mejoraron la motilidad total, la integridad de la membrana plasmática, la integridad del acrosoma, el potencial de la membrana mitocondrial y la viabilidad, y una y 1,5 mM de crocina mejoraron la actividad catalasa y redujeron la peroxidación lipídica en comparación con el control ($p < 0,05$). Además, la crocina 1 mM mejoró la motilidad progresiva, la cinemática y la integridad del ADN que el control ($p < 0,05$). Los niveles de expresión de los genes *SPACA3*, *PRM1* y *BCL2* fueron mayores ($p < 0,05$) con crocina 1 mM, mientras que no se observaron diferencias ($p > 0,05$) en la expresión del gen *BAX* entre todos los grupos. Además, la expresión del gen *ROMO1* fue mayor ($p < 0,05$) con crocina 2 mM en comparación con crocina 1 mM y 1,5 mM. Las dosis de semen que contenían crocina 1 mM mostraron una tasa de fertilidad más alta en comparación con el control [$56 \pm 0,03\%$ (112/200) frente a $46 \pm 0,04\%$ (92/200), respectivamente; $p = 0,0465$]. En conclusión, la adición de 1 mM de crocina en el diluyente de semen mejora la calidad post-descongelación, la expresión genética asociada a la fertilidad y el potencial de fertilización del esperma de búfalo.

Palabras clave: crocina, antioxidante, criopreservación, peroxidación lipídica, Protamina 1, fertilidad.

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Preliminary results about the use of injected Lysozyme (LIZOVET) to improve the response to synchronization protocol for insemination in buffalo heifers

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ABSTRACT

Lysozyme is a natural enzyme present in many body fluids in animals and humans, where it fulfills immunostimulant, bactericidal, virucidal, and anti-inflammatory functions. A commercial product based on Lysozyme (LIZOVET, Laboratory

Resultados preliminares sobre el uso de Lisozima inyectada (LIZOVET) para mejorar la respuesta al protocolo de sincronización para inseminación en novillas bufalinas

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RESUMEN

La lisozima es una enzima natural presente en muchos fluidos corporales de animales y humanos, donde cumple funciones inmunoestimulantes, bactericidas, virucidas y antiinflamatorias. Un producto comercial a base de Lisozima (LIZOVET,

Agriquimvet, Aragua State, Venezuela) for use in different animal species has effectively increased production and health in cattle, poultry, and pigs. However, the effect of Lysozyme on buffalo reproduction is little known. The objective of this work was to determine the effect of injectable Lysozyme (Lizovet) over the response to synchronization protocol for insemination in buffalo heifers. We conducted small field trials in buffalo farms in Barinas state, Venezuela. A group of 20 heifers was prepared 21 days before to apply a synchronization protocol for insemination at the beginning of the reproductive season with a complete mineral mix. Additionally, a treatment group (n=10) received treatment with injectable Lysozyme at a dose of 1 cc/60 Kg of intramuscular weight weekly for three weeks. The other heifers were not treated and were left as a control group (n=10). Preliminary results in this study showed that in the case of heifers, 100% of the group treated with LIZOVET were classified as suitable for admission to the insemination program by presenting ovarian follicles with an average size greater than 11 mm and the presence of corpus luteum, compared to the control group where only 30% of them classified for insemination by showing an average size of ovarian follicles of 7.5 mm. In conclusion, Lysozyme (LIZOVET) effectively improved the response to synchronization protocol for insemination in buffalo heifers. Future expectations are probable by favoring follicular growth at the ovarian level. The latest deserves further studies.

Keywords: lysozyme, buffalo heifers, follicular growth.

^A Conflict-of-interest statement: Johnny Ronnel Bracho Ylaraza is an employee in Laboratorio AGRIQUIMVET.

Laboratorio Agriquimvet, Estado Aragua, Venezuela) para uso en diferentes especies animales ha aumentado efectivamente la producción y la salud en vacunos, aves y cerdos. Sin embargo, el efecto de la lisozima sobre la reproducción del búfalo es poco conocido. El objetivo de este trabajo fue determinar el efecto de la lisozima inyectable (Lizovet) sobre la respuesta al protocolo de sincronización para inseminación en novillas bufalinas. Realizamos pequeñas pruebas de campo en granjas de búfalos en el estado Barinas, Venezuela. Se preparó un grupo de 20 bubillas 21 días antes para aplicar un protocolo de sincronización para la inseminación al inicio de la temporada reproductiva con mezcla mineral completa. Además, un grupo de tratamiento (n=10) recibió tratamiento con Lisozima inyectable a una dosis de 1 cc/60 Kg de peso intramuscular semanalmente durante tres semanas. Las otras bubillas no fueron tratadas y quedaron como grupo control (n=10). Los resultados preliminares de este estudio mostraron que, en el caso de las bubillas, el 100% del grupo tratado con LIZOVET fueron clasificadas como aptas para el ingreso al programa de inseminación al presentar folículos ováricos con un tamaño promedio mayor a 11 mm y la presencia de cuerpo lúteo, en comparación con el grupo control donde sólo el 30% de ellas clasificaron para inseminación al presentar un tamaño promedio de folículos ováricos de 7,5 mm. En conclusión, la lisozima (LIZOVET) mejoró efectivamente la respuesta al protocolo de sincronización para la inseminación en bubillas bufalinas. Las expectativas del futuro probablemente sean favorecer el crecimiento follicular a nivel ovárico. Lo último merece más estudios.

Palabras clave: lisozima, bubillas bufalinas, crecimiento follicular.

^A Declaración de conflicto de intereses: Johnny Ronnel Bracho Ylaraza es empleado del Laboratorio AGRIQUIMVET.

BIOTECHNOLOGY & OMICS TECHNOLOGIES

Biotecnología y Tecnologías Ómicas

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Effect of custom-designed transfection buffer on delivery of genome modification components into primary cells of buffalo, cattle, goats, and sheep

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Efecto del tampón de transfección diseñado a medida en la administración de componentes de modificación del genoma en células primarias de búfalos, ganado vacuno, cabras y ovejas

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ABSTRACT

The transfer of genome-modification components into farm animal cells is indispensable for the production of genome-modified and transgenic farm animals. Electroporation is a physical transfection method when appropriately used; this technique is safe, simple to use, affordable, and efficient in transfecting cells from several lineages. Electroporation efficiency depends on various physical parameters, of which cell type is considered a major factor for transfection efficiency. Primary cells are generally less susceptible to transfection than other cell types due to their finite lifespan and limited expansion capacity. Previously, we custom-designed a transfection buffer to deliver exogenous genetic components into mammalian cells. In the present study, we examined the effect of the developed buffer on transfection rates and cell viability of primary somatic cells from buffalo, cattle, goats, and sheep. To achieve the aims of this study, primary somatic cells from skin biopsies were established and were transfected with a Venus-expression vector (pAcGFPs-Venus). We noticed that transfection rates of pAcGFPs-Venus were 22.51%, 17.56%, 22.81%, and 16.16% for buffalo, cattle, goats, and sheep cells, respectively. We also noticed that cell viability and proliferation rates were better in the case of goats, sheep, and cattle cells; also, these cells have less vacuolation than buffalo cells. In addition, we also generated MSTN (myostatin) KO (Knockout) cell clones from these cell populations, in which the efficiency of single-cell clone generation was high for goats and sheep cells. In conclusion, our lab-made transfection buffer can be efficiently used to

RESUMEN

La transferencia de componentes de modificación del genoma a células de animales de granja es indispensable para la producción de animales de granja transgénicos y con el genoma modificado. La electroporación es un método de transfección física cuando se utiliza adecuadamente. Esta técnica es segura, sencilla de utilizar, asequible y eficaz para transfectar células de varios linajes. La eficiencia de la electroporación depende de varios parámetros físicos, de los cuales el tipo de célula se considera un factor importante para la eficiencia de la transfección. Las células primarias son generalmente menos susceptibles a la transfección que otros tipos de células debido a su vida útil finita y su capacidad de expansión limitada. Anteriormente, diseñamos un tampón de transfección personalizado para administrar componentes genéticos exógenos a células de mamíferos. En el presente estudio, examinamos el efecto del tampón desarrollado sobre las tasas de transfección y la viabilidad celular de células somáticas primarias de búfalos, bovinos, caprinos y ovinos. Para lograr los objetivos de este estudio, se establecieron células somáticas primarias de biopsias de piel y se transfecaron con un vector de expresión de Venus (pAcGFPs-Venus). Notamos que las tasas de transfección de pAcGFPs-Venus fueron 22,51%, 17,56%, 22,81% y 16,16% para células de búfalo, ganado vacuno, cabras y ovejas, respectivamente. También notamos que la viabilidad celular y las tasas de proliferación eran mejores en el caso de células de cabras, ovejas y vacunos; además, estas células tienen menos vacuolización que las células de búfalo. También

generate genome-edited or transgenic farm animals for agriculture, biomedical, and veterinary applications.

Keywords: transfection buffer, genome modification, CRISPR.

generamos clones de células MSTN (miostatina) KO (Knockout) a partir de estas poblaciones de células, en las que la eficiencia de la generación de clones unicelulares fue alta para células de cabras y ovejas. En conclusión, nuestro tampón de transfección fabricado en laboratorio se puede utilizar de manera eficiente para generar animales de granja transgénicos o editados con genoma para aplicaciones agrícolas, biomédicas y veterinarias.

Palabras clave: tampón de transfección, modificación del genoma y CRISPR.

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Production of β -lactoglobulin (BLG) gene knock-out blastocyst stage embryos of Indian water buffalo using CRISPR and SCNT technology

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ABSTRACT

In several tropical countries, buffalo milk has a higher-value demand than cow milk due to its nutritional and economic value. In India, the buffalo is the main dairy animal and contributes 45% of the total milk produced in the country. Besides the nutritional value of milk, several allergen proteins such as casein, α -lactalbumin, β -lactoglobulin (BLG), and immunoglobulins have been reported. Breeding strategies, nutritional management, and quantitative genetics have improved milk yield, but these approaches could not lead to significant changes in milk composition. With the development of biotechnology, especially genome editing tools (CRISPRs), it is possible to generate new value-added products such as designer hypoallergenic milk for human health benefits. Keeping this in mind, we planned to utilize the CRISPR tools to disrupt the buffalo β -lactoglobulin (BLG) gene to produce hypoallergenic milk in the long run. In pursuit of our objectives, we designed three single guide RNAs (sgRNAs) targeting the BLG locus in buffalo. Subsequently, we assessed their editing efficiency through a combination of Sanger sequencing, followed by TIDE and ICE analysis. Among three sgRNAs, the most efficient sgRNA was used to generate the clonal population of edited cells. Several single-cell clones were established and screened using the TA cloning (also known as rapid cloning or T cloning) and Sanger sequencing methods. Of 14 single-cell clones screened, eight were found to have BLG gene disruption events (57% editing rates). Using SCNT, we successfully produced cloned blastocyst stage embryos from 4 BLG-gene disrupted clonal cells.

Producción de embriones en etapa de blastocisto con desactivación del gen β -lactoglobulina (BLG) de búfalo de agua indio utilizando tecnología CRISPR y SCNT

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RESUMEN

En varios países tropicales, la leche de búfala tiene una demanda de mayor valor que la leche de vaca debido a su valor nutricional y económico. En la India, la búfala es el principal animal lechero y aporta el 45% del total de leche producida en el país. Además del valor nutricional de la leche, se han informado varias proteínas alergénicas como la caseína, el α -lactoalbúmina, el β -lactoglobulina (BLG) y las inmunoglobulinas. Las estrategias de reproducción, el manejo nutricional y la genética cuantitativa han mejorado la producción de leche, pero estos enfoques no han podido conducir a cambios significativos en la composición de la leche. Con el desarrollo de la biotecnología, especialmente las herramientas de edición del genoma (CRISPR), es posible generar nuevos productos con valor agregado, como el diseño de leche hipoalérgica para beneficiar la salud humana. Teniendo esto en cuenta, planeamos utilizar las herramientas CRISPR para alterar el gen de la β -lactoglobulina de búfala (BLG) y así producir leche hipoalérgica a largo plazo. Para lograr nuestros objetivos, diseñamos tres guías de ARN de guía única (sgRNA) dirigidos al locus BLG en búfalos. Posteriormente, evaluamos su eficiencia de edición mediante una combinación de secuenciación Sanger, seguida de análisis TIDE e ICE. Entre los tres sgRNA, se utilizó el sgRNA más eficiente para generar la población clonal de células editadas. Se establecieron y seleccionaron varios clones unicelulares utilizando los métodos de clonación TA (también

The cloned blastocyst production rates (25 to 30%) were similar to non-edited control cells. Efforts are ongoing to establish pregnancies from BLG-KO cloned embryos. This work can lead to the generation of designer buffaloes to produce hypoallergenic milk for human benefit.

Keywords: buffalo, milk allergy, β -lactoglobulin, CRISPR, SCNT, hypoallergenic milk.

conocida como clonación rápida o clonación T) y secuenciación de Sanger. De los 14 clones unicelulares analizados, se encontró que ocho tenían eventos de alteración del gen BLG (tasas de edición del 57%). Utilizando transferencia nuclear de células somáticas (SCNT), producimos con éxito embriones clonados en etapa de blastocisto a partir de 4 células clonales alteradas por el gen BLG. Las tasas de producción de blastocistos clonados (25 a 30%) fueron similares a las de las células de control no editadas. Se están realizando esfuerzos para establecer preñeces a partir de embriones clonados BLG-KO. Este trabajo puede conducir a la generación de búfalos diseñados para producir leche hipoalérgica para beneficio humano.

Palabras clave: búfala, alergia a la leche, β -lactoglobulina, CRISPR, SCNT, leche hipoalérgica.

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Successful establishment of CRISPR-based genome-edited clonal cell populations from primary cells of buffalo, goats, and sheep

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ABSTRACT

Genome editing technology has great potential for precise DNA modification in mammalian cells. The ability to precisely generate the clonal population of CRISPR-edited genotype is of great importance in gene function/pathway analysis, drug discovery, and production of genome-edited animals. In the present study, we demonstrated an efficient method to generate CRISPR-edited single-cell clonal populations of farm animals, including buffalo, goats, and sheep. To generate clonal cell populations, the primary fibroblasts were established through explant culture and then electroporated with CRISPR/Cas RNPs targeted for the disrupted MSTN gene. We used a single-cell pickup method in which one cell was picked up using an ultra-fined glass capillary and transferred into each well of a 96-well plate. For promoting the growth of single cells, we used growth factor-supplemented media. After seeding a single cell to each well, the plate was kept undisturbed for 5-7 days, and then cell attachment rates were noted. We reported that the cell attachment rates for buffalo, goat, and sheep cells were 40%, 77.08%, and 83.67%, respectively. The proliferation rates were 70.83%, 75.67%, and 78.05% for buffalo, goat, and sheep cells, respectively. We noticed that cell attachment and proliferation rates were better in the case of goat and sheep cells; also, these cells exhibited less vacuolation compared to

Establecimiento exitoso de poblaciones de células clonales editadas con genoma basado en CRISPR a partir de células primarias de búfalo, cabra y oveja

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RESUMEN

La tecnología de edición del genoma tiene un gran potencial para la modificación precisa del ADN en células de mamíferos. La capacidad de generar con precisión la población clonal del genotipo editado con CRISPR es de gran importancia en el análisis de la función/vía genética, el descubrimiento de fármacos y la producción de animales con genoma editados. En el presente estudio, demostramos un método eficiente para generar poblaciones clonales unicelulares de animales de granja editadas con CRISPR, incluidos búfalos, cabras y ovejas. Para generar poblaciones de células clonales, los fibroblastos primarios se establecieron mediante cultivo de explante y luego se sometieron a electroporación con CRISPR/Cas RNP dirigidas al gen MSTN alterado. Utilizamos un método de recogida de una sola célula en el que se recogió una célula utilizando un capilar de vidrio ultrafino y se transfirió a cada pocillo de una placa de 96 pocillos. Para promover el crecimiento de células individuales, utilizamos medios suplementados con factor de crecimiento. Después de sembrar una única célula en cada pocillo, la placa se mantuvo en reposo durante 5 a 7 días y luego se anotaron las tasas de unión de las células. Informamos que las tasas de unión celular para las células de búfalo, cabra y oveja fueron del 40%, 77,08% y 83,67%, respectivamente. Las ta-

buffalo cells. In the present study, we generated 11, 20, and 20 single-cell clones of MSTN-gene-edited buffalo, goat, and sheep cells. In conclusion, our method can be efficiently used to generate genome-edited single-cell clones to harness the potential of CRISPR technologies in farm animals.

Keywords: CRISPR, single cell, clonal population, genome-editing, farm animals.

Las tasas de proliferación fueron del 70,83%, 75,67% y 78,05% para las células de búfalo, cabra y oveja, respectivamente. Notamos que las tasas de proliferación y unión celular eran mejores en el caso de las células de cabra y oveja; además, estas células exhibieron menos vacuolización en comparación con las células de búfalo. En el presente estudio, generamos 11, 20 y 20 clones unicelulares de células de búfalo, cabra y oveja editadas con el gen MSTN (miostatina). En conclusión, nuestro método se puede utilizar de manera eficiente para generar clones unicelulares con genoma editados para aprovechar el potencial de las tecnologías CRISPR en animales de granja.

Palabras clave: CRISPR, células unicelulares, población clonal, edición del genoma, animales de granja.

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Mir-449b improves the epigenetic status of buffalo transgenic embryos produced by handmade cloning

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Mir-449b mejora el estado epigenético de embriones transgénicos de búfalo producidos mediante clonación artesanal

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ABSTRACT

The current study aimed to explore the effects of miR-449b on the epigenetic status of transgenic embryos (containing human insulin gene) produced through handmade cloning. For this, *in vitro* matured oocytes were enucleated by microblade, and two demi-oocytes were fused with a somatic cell (transgenic cells) by electrofusion and after 1 h of electrofusion, the fused embryos (reconstructs) were incubated in 40 nM miR-449b mimic/inhibitor by lipofection method for 1 h at 38.5°C in a CO₂ incubator. After incubation, transfected zygotes were chemically activated and subjected to *in vitro* culture in RVCL (Cook®) medium for 8 days at 38.5°C in 5% CO₂ in air, 90-95% relative humidity condition. Transfection of mi-449b was confirmed through the red fluorescence produced by miR-449b [tagged with 5 carboxytetramethylrhodamine (TAMARA) fluorescence dye] and the effect of miR-449b on epigenetic status was studied by immunofluorescence and qPCR. The transgenic embryos showed a similar development pattern as control embryos. The global level of 5-methyl cytosine was compared among three groups of buffalo transgenic cloned embryos produced by treatment of reconstructed embryos with miR-449b control, mimic, and inhibitor. It was found that the mean pixel intensity was significantly reduced ($p < 0.05$) when reconstructed embryos were cultured for 2 h with miR-449b mimic as compared to control (mimic 7.92 ± 0.34 vs control 16.15 ± 0.50).

RESUMEN

El presente estudio tuvo como objetivo explorar los efectos de miR-449b sobre el estado epigenético de embriones transgénicos (que contienen el gen de la insulina humana) producidos mediante clonación hecha a mano. Para esto, se enuclearon ovocitos madurados *in vitro* mediante microcuchilla, se fusionaron dos demi-ooocitos con una célula somática (células transgénicas) mediante electrofusión y después de 1 h de electrofusión, los embriones fusionados (reconstrucciones) se incubaron en 40 nM de miR-449b imitador/inhibidor por método de lipofección durante 1 h a 38,5°C en una incubadora de CO₂. Después de la incubación, los cigotos transfectedos se activaron químicamente y se sometieron a cultivo *in vitro* en medio RVCL (Cook®) durante 8 días a 38,5 °C en 5% de CO₂ en aire y condiciones de humedad relativa de 90-95%. La transfección de mi-449b se confirmó a través de la fluorescencia roja producida por miR-449b [etiquetada con tinte fluorescente de 5 carboxitetrametilrodamina (TAMARA)] y el efecto de miR-449b sobre el estado epigenético se estudió mediante inmunofluorescencia y qPCR. Los embriones transgénicos mostraron un patrón de desarrollo similar al de los embriones del grupo control. Se comparó el nivel global de 5-metilcitosina entre tres grupos de embriones clonados transgénicos de búfalo producidos mediante el tratamiento de embriones reconstruidos con

At the same time, it was not significantly higher ($p>0.05$) when reconstructed embryos were exposed to miR-449b inhibitor as compared to control (Inhibitor 17.18 ± 0.88 vs control 16.15 ± 0.50). The relative expression level of *HDAC1* and *DNMT1* were significantly reduced ($p<0.05$), up to 0.33 ± 0.39 fold and 0.16 ± 0.74 fold, respectively, compared to control when treated with miR-449b mimic. At the same time, in the case of miR-449b inhibitor, the expression level of *HDAC 1* gene was significantly higher ($p<0.05$) up to 4.89 ± 0.39 fold compared to the control. However, in the case of *DNMT1*, there was no significant difference ($p<0.05$) 1.17 ± 0.66 fold compared to control embryos. This study revealed that miR-449b mimic reduces the global methylation level of buffalo transgenic cloned embryos, hence improving developmental competence and quality.

Keywords: buffalo, miR-449b, handmade cloning, embryo, epigenetics.

control, imitador e inhibidor de miR-449b. Se encontró que la intensidad media de los píxeles se redujo significativamente ($p<0,05$) cuando los embriones reconstruidos se cultivaron durante 2 h con un imitador de miR-449b en comparación con el control (imitado $7,92 \pm 0,34$ frente a control $16,15 \pm 0,50$). Al mismo tiempo, no fue significativamente mayor ($p>0,05$) cuando los embriones reconstruidos se expusieron al inhibidor de miR-449b en comparación con el control (inhibidor $17,18 \pm 0,88$ frente al control $16,15 \pm 0,50$). El nivel de expresión relativo de *HDAC1* y *DNMT1* se redujo significativamente ($p<0,05$), hasta $0,33 \pm 0,39$ veces y $0,16 \pm 0,74$ veces, respectivamente, en comparación con el control cuando se trató con el imitador de miR-449b. Al mismo tiempo, en el caso del inhibidor de miR-449b, el nivel de expresión del gen *HDAC1* fue significativamente mayor ($p<0,05$) hasta $4,89\pm 0,39$ veces en comparación con el control. Sin embargo, en el caso de *DNMT1*, no hubo diferencia significativa ($p<0,05$) arrojando $1,17\pm 0,66$ veces en comparación con los embriones del grupo control. Este estudio reveló que el imitador de miR-449b reduce el nivel de metilación global de los embriones clonados transgénicos de búfalo, mejorando así la competencia y la calidad del desarrollo.

Palabras clave: búfalo, miR-449b, clonación artesanal, embrión, epigenética.

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Semen quality and fertility effectiveness of cloned buffalo (*Bubalus bubalis*) bulls

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ABSTRACT

In India, due to a severe shortage of elite bulls, semen available from progeny-tested bulls covers a limited breedable population of buffalo. Animal cloning has proved beneficial in making identical genetic copies in the shortest possible time to meet the target of providing elite breeding bulls. ICAR-National Dairy Research Institute, Karnal, has produced several cloned breeding bulls, of which two breeding bulls (named Swarn and Rajat) were used to demonstrate the fertility of cloned bulls. The semen parameters such as volume (2.83 ± 0.33 vs. 2.44 ± 0.23 ml), sperm concentration (1607.56 ± 161.90 vs. 1589.44 ± 211.76 million/ml), mass motility (3.17 ± 0.08 vs. 3.11 ± 0.20), progressive motility ($87.78\pm 0.88\%$ vs $85.00\pm 1.44\%$) and acrosome integrity ($85.39\pm 1.66\%$ vs $87.61\pm 1.80\%$), respectively in cloned and non-cloned bull's semen and had no significant ($p<0.05$) difference. Post-thaw motility was also similar to non-cloned

Calidad del semen y efectividad en la fertilidad de toros clonados de búfalo (*Bubalus bubalis*)

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RESUMEN

En la India, debido a una grave escasez de toros élite, el semen disponible de toros probados en la progenie cubre una población reproducible limitada de búfalos. La clonación de animales ha demostrado ser beneficiosa al hacer copias genéticas idénticas en el menor tiempo posible para cumplir el objetivo de proporcionar toros reproductores élite. ICAR-Instituto Nacional de Investigación Láctea, Karnal, ha producido varios toros reproductores clonados, de los cuales dos toros reproductores (llamados Swarn y Rajat) se utilizaron para demostrar la fertilidad de los toros clonados. Los parámetros del semen como volumen ($2,83\pm 0,33$ vs. $2,44\pm 0,23$ ml), concentración de espermatozoides ($1607,56\pm 161,90$ vs. $1589,44\pm 211,76$ millones/ml), motilidad de masa ($3,17\pm 0,08$ vs. $3,11\pm 0,20$), motilidad progresiva ($87,78\pm 0,88\%$ vs $85,00\pm 1,44\%$) y la integridad acrosómica

bulls or their parents. When these spermatozoa were subjected to a transcriptomics study, 27,481 transcripts were identified, out of which 18,703 transcripts were expressed commonly in both cloned and somatic cell donor bull's spermatozoa. Only 566 transcripts were up-regulated, and 410 transcripts were down-regulated significantly ($FC \geq 2$; $P < 0.05$) in clone bulls spermatozoa relative to somatic cell donor bulls spermatozoa. KEGG analysis revealed that the Up- and down-regulated transcripts affect excision repair, autophagy, lipolysis, AMPK, and insulin signaling pathways. A total of 278 miRNAs were found in both cloned and parent bull spermatozoa, of which 239 were common and 28 were unique to cloned bulls spermatozoa. Out of 62 miRNAs (at $FC \geq 2$; $p < 0.05$) found by differential expression analysis, 31 were up-regulated, and 31 were down-regulated in cloned bull spermatozoa compared to donor bulls. The expression pattern of some necessary transcripts and miRNAs associated with spermatogenesis, sperm motility, sperm capacitation, bull fertility, and early embryonic development was almost similar in cloned bulls spermatozoa and parent bulls spermatozoa. To determine the fertility of cloned bulls, 20 female buffaloes were inseminated with the semen of two above-mentioned cloned bulls. A 65% conception rate was achieved following insemination, which is typical in buffaloes. Thirteen healthy calves (seven males and six females) have been produced, and further attempts are ongoing to produce more calves. These calves are physiologically normal, growing well and healthy. In conclusion, with this limited study, we can say that the semen produced by cloned bulls is similar to that of non-cloned bulls and parent bulls, which can be further used in various assisted reproductive technologies and sustainable milk production.

Keywords: cloned buffalo, semen, transcriptome, miRNA, fertility.

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($85,39 \pm 1,66\%$ vs $87,61 \pm 1,80\%$), respectivamente en semen de toro clonado y no clonado y no tuvieron diferencia significativa ($p < 0,05$). La motilidad post-descongelación también fue similar a la de los toros no clonados o a sus padres. Cuando estos espermatozoides se sometieron a un estudio transcriptómico, se identificaron 27.481 transcripciones, de las cuales 18.703 se expresaron comúnmente en espermatozoides de toro de donantes de células somáticas y clonados. Solo 566 transcripciones estaban reguladas positivamente y 410 transcripciones estaban reguladas negativamente significativamente ($FC \geq 2$; $p < 0,05$) en espermatozoides de toros clonados en relación con los espermatozoides de toros donantes de células somáticas. El análisis de KEGG reveló que las transcripciones reguladas hacia arriba y hacia abajo afectan las vías de reparación por escisión, autofagia, lipólisis, AMPK y señalización de insulina. Se encontró un total de 278 miARN en espermatozoides de toro clonados y parentales, de los cuales 239 eran comunes y 28 exclusivos de los espermatozoides de toro clonados. De 62 miARN (en $FC \geq 2$; $p < 0,05$) encontrados mediante análisis de expresión diferencial, 31 estaban regulados positivamente y 31 estaban regulados negativamente en espermatozoides de toro clonados en comparación con toros donantes. El patrón de expresión de algunos transcritos y miARN necesarios asociados con la espermatogénesis, la motilidad de los espermatozoides, la capacitación de los espermatozoides, la fertilidad del toro y el desarrollo embrionario temprano fue casi similar en los espermatozoides de toros clonados y en los espermatozoides de toros parentales. Para determinar la fertilidad de los toros clonados, se inseminaron 20 búfalas con el semen de dos toros clonados antes mencionados. Después de la inseminación se logró una tasa de concepción del 65%, lo cual es típico en las búfalas. Se han producido trece terneros sanos (siete machos y seis hembras) y se están realizando nuevos intentos para producir más terneros. Estos terneros son fisiológicamente normales, crecen bien y están sanos. En conclusión, con este estudio podemos decir que el semen producido por toros clonados es similar al de toros no clonados y al de toros parentales, el cual puede usarse aún más en diversas tecnologías de reproducción asistida y producción sostenible de leche.

Palabras clave: búfalo clonado, semen, transcriptoma, miARN, Fertilidad.

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Oxamflatin and ascorbic acid improves developmental competence and quality of buffalo (*Bubalis bubalis*) cloned embryos

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ABSTRACT

Buffalo cloning is a powerful assisted reproductive tool for multiplying elite buffalo germplasm. However, the live offspring production efficiency is low due to aberrant epigenetic reprogramming. Aberrant epigenetic marks can be modified by culturing donor cells and/or one cell stage fused embryo or both with epigenetic modifiers alone or in combination. In the present study, we examined the effect of oxamflatin (OxF), ascorbic acid (AA), and their combined (OxF+AA) effect on *in vitro* developmental competence, quality, and pregnancy establishment rate of buffalo cloned embryos. Oxamflatin is a histone deacetylase inhibitor, whereas ascorbic acid is a hypomethylating agent. To achieve this aim of the study, reconstructs (fused two enucleated ooplasm + donor cell) were cultured for 8 h, i.e., 4 h post-fusion and 4 h post-activation with 1 μM oxamflatin (OxF), 50 μM ascorbic acid (AA), and there combined (OxF+AA) treatment. There was no significant ($p < 0.05$) difference in cleavage rates when reconstructs were treated with oxamflatin (81.34 \pm 0.81%), ascorbic acid (82.76 \pm 0.51%), combined treatment (82.17 \pm 0.54%) compared with control (82.87 \pm 0.63%). The blastocyst production rate was significantly higher ($p < 0.05$) in combined treatment OxF+AA (41.64 \pm 0.95%) as compared to OxF (34.88 \pm 1.22%), AA (38.99 \pm 0.69%) and control (30.29 \pm 0.77%). The TUNEL assay showed a significantly lower ($p < 0.05$) apoptotic index in combined (OxF+AA) treatment (1.43 \pm 0.43) as compared to oxamflatin (3.54 \pm 0.46), ascorbic acid (3.24 \pm 0.49) and control (5.06 \pm 0.48). The cloned embryos were transferred to the synchronized recipient (n=15 to 18 buffaloes in each group), and the conception rate was observed better in combined treatment (OxF+AA) (46.66%) than oxamflatin (16.66%) and ascorbic acid (12.50%). At the same time, no pregnancy was reported in the control group. In conclusion, the combined treatment with oxamflatin and ascorbic acid improves the *in vitro* and *in vivo* developmental potential in buffalo-cloned embryos, which could probably be due to decreased methylation and increased acetylation of the embryos.

Keywords: oxamflatin, ascorbic acid, buffalo cloning, epigenetic reprogramming, pregnancy.

La oxamflatina y el ácido ascórbico mejoran la capacidad de desarrollo y la calidad de los embriones clonados de búfalo (*Bubalis bubalis*)

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RESUMEN

La clonación de búfalos es una poderosa herramienta de reproducción asistida para multiplicar el germoplasma de búfalos élite. Sin embargo, la eficiencia de la producción de crías vivas es baja debido a una reprogramación epigenética aberrante. Las marcas epigenéticas aberrantes se pueden modificar cultivando células de un donante y/o un embrión fusionado en etapa celular o ambos con modificadores epigenéticos solos o en combinación. En el presente estudio, examinamos el efecto de la oxamflatina (OxF), el ácido ascórbico (AA) y su efecto combinado (OxF+AA) sobre la competencia del desarrollo *in vitro*, la calidad embrionaria y la tasa de establecimiento de la preñez de embriones clonados de búfalo. La oxamflatina es un inhibidor de la histona desacetilasa, mientras que el ácido ascórbico es un agente hipometilante. Para lograr este objetivo del estudio, se cultivaron reconstrucciones (dos ooplasmas enucleados fusionados + células del donante) durante 8 h, es decir, 4 h después de la fusión y 4 h después de la activación con 1 μM oxamflatina (OxF), 50 μM de ácido ascórbico (AA), y allí tratamiento combinado (OxF+AA). No hubo diferencias significativas ($p < 0,05$) en las tasas de escisión cuando las reconstrucciones se trataron con oxamflatina (81,34 \pm 0,81%), ácido ascórbico (82,76 \pm 0,51%), tratamiento combinado (82,17 \pm 0,54%) en comparación con el control (82,87 \pm 0,63). %. La tasa de producción de blastocistos fue significativamente mayor ($p < 0,05$) en el tratamiento combinado OxF+AA (41,64 \pm 0,95%) en comparación con OxF (34,88 \pm 1,22%), AA (38,99 \pm 0,69%) y control (30,29 \pm 0,77%). El ensayo TUNEL mostró un índice apoptótico significativamente menor ($p < 0,05$) en el tratamiento combinado (OxF+AA) (1,43 \pm 0,43) en comparación con oxamflatina (3,54 \pm 0,46), ácido ascórbico (3,24 \pm 0,49) y control (5,06 \pm 0,48). Los embriones clonados fueron transferidos a hembras receptoras sincronizadas (n=15 a 18 búfalas en cada grupo), y la tasa de concepción se observó mejor en el tratamiento combinado (OxF+AA) (46,66%) que con oxamflatina (16,66%) y ácido ascórbico (12,50%). Al mismo tiempo, no se informó ninguna preñez en el grupo de control. En conclusión, el tratamiento combinado con oxamflatina y ácido ascórbico mejora el potencial de desa-

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rollo *in vitro* z la competencia *in vivo* en embriones clonados de búfalo, lo que probablemente podría deberse a una disminución de la metilación y un aumento de la acetilación de los embriones.

Palabras clave: oxamflatina, ácido ascórbico, clonación de búfalos, reprogramación epigenética, embarazo.

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A leap forward: exploring the advantages of single-step genome evaluation in Italian Mediterranean buffalo

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ABSTRACT

Single-step genomic best linear unbiased predictor (ssGBLUP) is a method for jointly estimating breeding values (BV) for genotyped and non-genotyped animals. Genomic information in the Italian Mediterranean Buffalo (IMB) is now available and its inclusion in the genetic evaluation system could increase both accuracy and genetic progress of the traits of interest of the breed. The aim of this study was to test the feasibility of ssGBLUP and to present the first results of the implementation of a genomic evaluation for production and type traits in the IMB. Phenotypic information on production (270-day milk, mozzarella yield (MY), protein and fat kg and %, respectively) and morphology: feet and legs (FL) and mammary system (MS) were used for this study. Production records included 743,904 lactations from 276,451 buffalo cows born from 1984 to 2019. Morphological traits were from 91,966 buffalo cows from 2004 to 2022. Regarding the genotypes, a total of 2,017 buffalo cows and 133 bulls were used. Data were analysed fitting two multi-trait animal models, a 6-trait model for production data and a 2-trait model for morphology data. According to the relationship matrix used, two models were fitted: (i) the BLUP with the

Un salto adelante: explorar las ventajas de la evaluación del genoma en un solo paso en el búfalo mediterráneo italiano

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RESUMEN

El mejor predictor lineal genómico insesgado de un solo paso (ssGBLUP) es un método para estimar conjuntamente los valores genéticos (BV) para animales genotipados y no genotipados. La información genómica del búfalo mediterráneo italiano (IMB) ya está disponible y su inclusión en el sistema de evaluación genética podría aumentar tanto la precisión como el progreso genético de los rasgos de interés de la raza. El objetivo de este estudio fue probar la viabilidad de ssGBLUP y presentar los primeros resultados de la implementación de una evaluación genómica para rasgos de producción y tipo en el IMB. Para este estudio se utilizó información fenotípica sobre producción (leche a 270 días, rendimiento de mozzarella (MY), kg y % de proteína y grasa, respectivamente) y morfología: pies y piernas (FL) y sistema mamario (MS). Los registros de producción incluyeron 743.904 lactancias de 276.451 vacas búfalas nacidas de 1984 a 2019. Los rasgos morfológicos fueron de 91.966 vacas búfalas de 2004 a 2022. Respecto a los genotipos, se utilizaron un total de 2.017 vacas búfalas y 133 toros. Los datos se analizaron ajustando dos modelos animales con múltiples rasgos, un modelo de 6 rasgos para datos de

numerator relationship matrix (A); (ii) the ssGBLUP where A and the genomic relationship matrix (G) are blended into H. BV were estimated with BLUP and ssGBLUP models. Three different scenarios were used, according to the cut-off year used to create the partial datasets, namely 2012, 2015 and 2017. In each scenario, correlation, accuracy, dispersion, and bias statistics were calculated (LR method). Both bulls (N=49) and cows (N=1288) were used for validations. On average, correlation between EBVs from partial and whole datasets estimated with BLUP and ssGBLUP increased from 6 to 49% and from 14 to 17% for production and type traits, respectively. Among the traits analysed, the ones most affected by the change were protein/fat content, MY, and AM. The accuracy increase for these traits was above 20% when using the ssGBLUP. All LR statistics improved also for non-genotyped females. These results showed that implementing ssGBLUP in the breeding program can generate more accurate predictions for important traits in dairy IMB than traditional BLUP.

Keywords: genomics, Italian Mediterranean buffalo, selection.

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producción y un modelo de 2 rasgos para datos de morfología. De acuerdo a la matriz de relaciones utilizada, se ajustaron dos modelos: (i) el BLUP con la matriz de relaciones del numerador (A); (ii) el ssGBLUP donde A y la matriz de relaciones genómicas (G) se combinan en H. BV se estimaron con modelos BLUP y ssGBLUP. Se utilizaron tres escenarios diferentes, según el año de corte utilizado para crear los conjuntos de datos parciales, a saber, 2012, 2015 y 2017. En cada escenario se calcularon estadísticas de correlación, precisión, dispersión y sesgo (método LR). Para las validaciones se utilizaron toros (N=49) y vacas (N=1288). En promedio, la correlación entre los EBV del conjunto de datos parcial y completo estimado con BLUP y ssGBLUP aumentó del 6 al 49% y del 14 al 17% para los rasgos de producción y tipo, respectivamente. Entre los rasgos analizados, los más afectados por el cambio fueron el contenido de proteína/grasa, MY y AM. El aumento de precisión para estos rasgos fue superior al 20% cuando se utilizó ssGBLUP. Todas las estadísticas de LR mejoraron también para las hembras no genotipadas. Estos resultados mostraron que la implementación de ssGBLUP en el programa de mejoramiento puede generar predicciones más precisas para rasgos importantes en el IMB lechero que el BLUP tradicional.

Palabras clave: genómica, búfalo mediterráneo italiano, selección.

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Approaches to modulate buffalo gut microbiome for efficient feed utilization and reduced environmental pollution

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ABSTRACT

Buffaloes are excellent converters of highly fibrous feeds into edible products like milk and meat due to the rumen microbiome structure and their functionality. About 37–44% of global methane emissions, mainly due to enteric fermentation of feeds by fibrolytic bacteria and formation of methane by methanogenic archaea, present in the rumen, is contributed by ruminants and a major source of methane production in the agriculture sector. Though the hyper ammonia-producing bacteria (HAB) are present inside the rumen (*Clostridium aminophilum*, *Peptostreptococcus anaerobius*, and *Clostridium sticklandii*) in low numbers, the concentration of ammonia (>300 nmol NH₃/ mg cell protein/ min) they can produce makes them quantitatively

Enfoques para modular el microbioma intestinal de búfalo para una utilización eficiente del alimento y una reducción de la contaminación ambiental

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RESUMEN

Los búfalos son excelentes convertidores de alimentos altamente fibrosos en productos comestibles como leche y carne debido a la estructura del microbioma del rumen y su funcionalidad. Alrededor del 37% al 44% de las emisiones globales de metano, debido principalmente a la fermentación entérica de los alimentos por bacterias fibrolíticas y la formación de metano por arqueas metanogénicas, presentes en el rumen, son aportadas por los rumiantes y son una fuente importante de producción de metano en el sector agrícola. Aunque las bacterias productoras de hiperamoníaco (HAB) están presentes dentro del rumen (*Clostridium aminophilum*, *Peptostreptococcus anaerobius* y *Clostridium sticklandii*) en cantida-

important. Methane is the second major greenhouse gas contributing to climate change. At the same time, ammonia is a potent environmental pollutant and is liable for the depletion of oxygen in the body water, reduction of soil pH, and generation of harmful aerosol fine particles accompanied by the augmented menace of pulmonary diseases. Therefore, the roles played by the microbes in the rumen microbial ecosystem are fundamental to developing innovative methods to modulate the rumen microbiome to improve buffalo production while reducing environmental impact. The major fiber-degrading bacteria in buffalo rumen are *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *R. albus*, *Prevotella ruminicola*, and *Butyrivibrio fibrisolvens*. However, our recent studies with metagenomic analysis of rumen methanogens showed that *Methanobrevibacter* was the predominant genus for cattle and buffalo. At the same time, *Methanomicrobium* is much higher in buffaloes than cattle (18% vs. 4.5%). *Methanomassiliicoccaceae* methanogens, a major methylophilic group of archaea, was isolated in the rumen of buffaloes for the first time. Hyper ammonia-producing bacteria (HAB) are responsible for a faster rate of ammonia production from dietary protein degradation, resulting in wastage of feed protein and environmental pollution. 16S rRNA sequences of enrichment culture of buffalo rumen content revealed Proteobacteria (61.1 - 68.2%) as most predominant bacteria followed by Unclassified bacteria (24-29%), *Firmicutes* (2.8-6.9%), *Bacteroidetes* (0.9-2%), *Actinobacteria* (0.1-1.1%), *Fusobacteria* (0.04-0.7%) and *Synergistetes* (0.1-0.4%) at phylum level. At the family and genus level, most of the sequences remained unclassified. Among sequences with valid family names, *Acidaminococcaceae* was most predominant, constituting 1.5 to 4.7% of sequences. Similarly, *Acidaminococcus* was most predominant at the genus level among sequences with valid genus names, constituting 1.4 to 4.1% of total bacterial sequences. Supplementation of bioactive compounds viz. essential oils (eugenol, organum oil, thymol), saponins or tannins in pure form or through natural compounds (garlic oil, eucalyptus oil, clove oils, *Sapindus mukorossi* fruit, *Glycyrrhiza glabra* root, *Ficus bengalensis* leaves, *Ficus glomerata* leaves), which are rich in these bioactive were shown to reduce methane (30%) and ammonia production (50%) in buffaloes with improvement in body weight (14%), milk production (10%) and feed efficiency (15%) in Murrah buffaloes. A composite feed additive formulated to reduce the methanogenic archaeal population without affecting fibrolytic rumen microbes has been demonstrated to increase milk production, antioxidant status, and immunity with abatement of enteric methane production in Murrah buffaloes. Strategic supplementation of Moringa leaves or feeding of low lignin sorghum cultivars were demonstrated to enhance feed utilization and production performances of Murrah buffalo with reduction of enteric methane production. Therefore, insight study of buffalo rumen microbiome and dietary interventions through strategic feeding and development of new products for rumen stimulants, anti-methanogenic compounds, or alternate hydrogen sinks in the rumen could be the promising strategies for modulating gut health for efficient conversion of human

des bajas, la concentración de amoníaco (>300 nmol NH₃/ mg de proteína celular/min) que pueden producir hace que ellos sean cuantitativamente importantes. El metano es el segundo gas de efecto invernadero que más contribuye al cambio climático, mientras que el amoníaco es un potente contaminante ambiental y responsable del agotamiento del oxígeno en los cuerpos de agua, la reducción del pH del suelo y la generación de finas partículas de aerosol nocivas, acompañadas de una mayor amenaza de enfermedades pulmonares. Por lo tanto, las funciones que desempeñan los microbios en el ecosistema microbiano del rumen son fundamentales para el desarrollo de métodos innovadores para modular el microbioma del rumen de una manera que mejore la producción de búfalos y, al mismo tiempo, reduzca el impacto ambiental. Las principales bacterias degradantes de la fibra en el rumen de búfalo son *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *R. albus*, *Prevotella ruminicola* y *Butyrivibrio fibrisolvens*. Sin embargo, nuestros estudios recientes con análisis metagenómicos de los metanógenos del rumen mostraron que *Methanobrevibacter* era el género predominante tanto para el ganado vacuno como para los búfalos, mientras que *Methanomicrobium* es mucho mayor en los búfalos que en el ganado vacuno (18% frente a 4,5%). *Methanomassiliicoccaceae* methanogens, un importante grupo metilotrófico de arqueas, fue aislado por primera vez en el rumen de búfalos. Las bacterias hiperproductoras de amoníaco (HAB) son responsables de una tasa más rápida de producción de amoníaco a partir de la degradación de las proteínas de la dieta, lo que resulta en un desperdicio de proteínas en los piensos y en la contaminación ambiental. Las secuencias de ARNr 16S del cultivo de enriquecimiento del contenido del rumen de búfalo revelaron que las *Proteobacterias* (61,1 - 68,2%) eran las bacterias más predominantes, seguidas de las bacterias no clasificadas (24-29%), *Firmicutes* (2,8-6,9%), *Bacteroidetes* (0,9-2%), *Actinobacteria*, (0,1-1,1%), *Fusobacteria* (0,04-0,7%) y *Synergistetes* (0,1-0,4%) a nivel de phylum. A nivel de familia y género, la mayoría de las secuencias permanecieron sin clasificar. Entre las secuencias con nombres de familia válidos, *Acidaminococcaceae* fue la más predominante y constituyó del 1,5 al 4,7% de las secuencias. De manera similar, a nivel de género, entre las secuencias con nombre de género válido, *Acidaminococcus* fue la más predominante y constituyó del 1,4 al 4,1% del total de secuencias bacterianas. Suplementación de compuestos bioactivos, a saber, aceites esenciales (eugenol, aceite de orégano, timol, etc.), saponinas o taninos en forma pura o mediante compuestos naturales (aceite de ajo, aceite de eucalipto, aceites de clavo, fruto de *Sapindus mukorossi*, raíz de *Glycyrrhiza glabra*, hojas de *Ficus bengalensis*, hojas de *Ficus glomerata*) ricos en estos bioactivos, demostró que reduce la producción de metano (30%) y amoníaco (50%) en búfalos con una mejora en el peso corporal (14%), la producción de leche (10%) y la eficiencia alimenticia (15%) en búfalos Murrah. Se ha demostrado que un aditivo alimentario compuesto formulado para reducir la población de arqueas metanogénicas sin afectar los microbios fibrolíticos del rumen aumenta la producción de leche, el

inedible feeds to edible animal products so reducing the impact of buffalo production on environmental pollution.

Keywords: Gut microbiome, environmental pollution, buffalo.

estado antioxidante y la inmunidad con la reducción de la producción de metano entérico en búfalas Murrah. Se demostró que la suplementación estratégica con hojas de Moringa o la alimentación con cultivares de sorgo bajos en lignina mejoran la utilización del alimento y el rendimiento de producción del búfalo Murrah con una reducción de la producción de metano entérico. Por lo tanto, el estudio profundo del microbioma del rumen de búfalo y las intervenciones dietéticas a través de la alimentación estratégica y el desarrollo de nuevos productos para estimulantes del rumen, compuestos antimetanogénicos o sumideros de hidrógeno alternativos en el rumen podrían ser estrategias prometedoras para modular la salud intestinal para una conversión eficiente del alimentos no comestibles para el humano, desde piensos animales comestibles, reduciendo así el impacto de la producción ganadera en la contaminación ambiental.

Palabras clave: microbioma intestinal, contaminación ambiental, búfalo.

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A green forage diet enhances microbial diversity in buffalo rumen

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ABSTRACT

Diet alterations affect the composition of the rumen microbiota and, consequently, the rumen metabolism, animal productivity, and the quality of its products. Aim of the work was to analyze the effects of an experimental feed based on the inclusion of ryegrass green forage (16.1 kg dry matter (DM) and, in kg on DM, 14.4 kg crude protein, 5.0 kg fat, 38.5 kg NDF, 23.4 kg ADF, 33.1 NSC, 15.5 kg and 0.91 Milk forage units; Green group) on the microbial composition of the rumen of buffaloes compared to a traditional total mixed ratio (TMR) diet (16.0 kg DM and, in kg on DM, 14.5 kg crude protein, 4.8 kg fat, 38.0 kg NDF, 24.0 kg ADF, 34.0 NSC, 21.0 kg and 0.93 Milk forage units; Dry group). The forage was just ryegrass at the re-blossoming stage, cut twice a day to avoid any fermentation, and immediately put in the mixing wagon, with no storage, and administered to animals. The forage to concentrates ratio of control buffaloes was 56:44, and that of treated buffaloes was 69:31. The two diets were isonitrogenous and isoenergetic and differed only in the inclusion of green feed in treated buffaloes. The study was carried out over 60 days in a commercial buf-

Una dieta de forraje verde mejora la diversidad microbiana en el rumen de búfalo

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RESUMEN

Las alteraciones de la dieta afectan la composición de la microbiota ruminal y, en consecuencia, el metabolismo ruminal, la productividad animal y la calidad de sus productos. El objetivo del trabajo fue analizar los efectos de un alimento experimental basado en la inclusión de forraje verde Raigrás (16,1 kg de materia seca (MS) y, en kg sobre MS, 14,4 kg de proteína bruta, 5,0 kg de grasa, 38,5 kg de FND, 23,4 kg FDA, 33,1 NSC, 15,5 kg y 0,91 unidades de forraje lácteo; Grupo Verde) sobre la composición microbiana del rumen de búfalos en comparación con una dieta tradicional de proporción mixta total (TMR) (16,0 kg MS y, en kg sobre MS, 14,5 kg proteína cruda, 4,8 kg grasa, 38,0 kg FND, 24,0 kg FDA, 34,0 NSC, 21,0 kg y 0,93 unidades de forraje lácteo; Grupo Seco). El forraje fue forraje verde Raigrás en la etapa de re-floración cortado dos veces al día para evitar cualquier fermentación e inmediatamente puesto en el carro mezclador, sin almacenamiento, y administrado a los animales. La proporción de forraje a concentrados de los búfalos de control fue de 56:44 y la de los búfalos tratados fue de 69:31. Las dos dietas fueron isonitrogénicas e

falo dairy farm in southern Italy using lactating Italian Mediterranean dairy buffaloes (n 8/group). Rumen fluid samples were immediately collected at the slaughterhouse, and bacterial genomic DNA was extracted to perform metagenomic analyses. Firmicutes and Bacteroidota were the most abundant phyla in both Dry and Green groups (over 92% of the total bacteria). Although the abundant microbial taxa of the rumen are not affected by the experimental diet, we observed a significant increase in rare species and an overall increase in total biodiversity in the Green group. Indeed, at the order level, the Peptostreptococcales-Tissierellales, Veillonellales-Selenomonadales, and Bradymonadales groups showed statistically significant differences ($p < 0.05$), with the former bacteria being more abundant in the Dry group and the other two in the Green group. Moreover, at the genus level, Prevotellaceae_YAB2003_group and Colidextribacter were more abundant ($p < 0.05$) in the Dry group, while Selenomonas, Prevotellaceae-UCG-007, Quinella, Oscillospira, and Tyzzerella, were more abundant ($p < 0.05$) in the Green group. We conclude that including green forage in the diet can stimulate a more taxonomically and functionally diverse rumen microbiome with positive effects on microbiota total biodiversity, which might improve the health and productivity of the buffaloes.

Keywords: rumen, microbiome, green feed.

isoenergéticas y diferían sólo en la inclusión de alimento verde en los búfalos tratados. El estudio se llevó a cabo durante 60 días en una granja lechera de búfalos comercial ubicada en el sur de Italia utilizando búfalos lecheras italianas lactantes del Mediterráneo (n 8/grupo). En el matadero se recogieron inmediatamente muestras de líquido ruminal y se extrajo ADN genómico bacteriano para realizar análisis metagenómicos. Firmicutes y Bacteroidota fueron los Filos más abundantes en ambos los grupos seco y verde (más del 92% del total de bacterias). Aunque los abundantes grupos (Taxones) microbianos del rumen no se ven afectados por la dieta experimental, observamos un aumento significativo en especies raras y un aumento general en la biodiversidad total en el Grupo Verde. De hecho, a nivel de Orden, los grupos Peptostreptococcales-Tissierellales, Veillonellales-Selenomonadales y Bradymonadales mostraron diferencias estadísticamente significativas ($p < 0,05$), siendo las primeras bacterias más abundantes en el Grupo Seco y las otras dos en el Grupo Verde. Además, a nivel de Género, Prevotellaceae_YAB2003_group y Colidextribacter fueron más abundantes ($p < 0,05$) en el Grupo Seco, mientras que Selenomonas, Prevotellaceae-UCG-007, Quinella, Oscillospira y Tyzzerella, fueron más abundantes ($p < 0,05$) en el Grupo Verde. Concluimos que la inclusión de forraje verde en la dieta puede estimular un microbioma ruminal más diverso desde el punto de vista taxonómico y funcional, con efectos positivos sobre la biodiversidad total del microbiota que podría mejorar la salud y la productividad de los búfalos.

Palabras clave: rumen, microbioma, alimento verde.

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Exploring the functional role of POU5F1 using CRISPR RNP electroporation of zygote (CRISPR-EZ) in buffaloes

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ABSTRACT

POU5F1, a key transcription factor, plays a pivotal role in maintaining pluripotency and cellular differentiation, making it of immense interest in developmental biology and animal husbandry. In the present study, we employ state-of-the-art CRISPR ribonucleoprotein (RNP) electroporation of zygotes (CRISPR-EZ) methodology to precisely manipulate the *POU5F1* gene in buffalo zygotes for exploring the phenotypic and functional outcomes of embryonic development. Through a combination

Explorando el papel funcional de POU5F1 utilizando la electroporación CRISPR RNP de cigoto (CRISPR-EZ) en búfalos

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RESUMEN

POU5F1, un factor de transcripción clave, desempeña un papel fundamental en el mantenimiento de la pluripotencia y la diferenciación celular, lo que lo hace de inmenso interés en la biología del desarrollo y la cría de animales. En el presente estudio, empleamos la metodología de electroporación de cigotos (CRISPR-EZ) de ribonucleoproteína CRISPR (RNP) de última generación para manipular con precisión el gen *POU5F1* en cigotos de búfalo para explorar los resultados fenotípicos y

of strategies, we found that electroporation of buffalo zygote at 20V/mm, five pulses, 3 msec at 10 h post-insemination (hpi) resulted in greater membrane permeability and higher editing efficiency (88.71%) without affecting embryonic developmental potential. We targeted the buffalo *POU5F1* gene using the abovementioned parameters, which caused nonsense-mediated mRNA decay and led to complete knockout (KO) of the *POU5F1* gene. We analyzed the mutation rates and mosaic mutations using tracking of Indels by decomposition (TIDE) software. We observed no difference in the embryonic developmental competence at cleavage or blastocyst rate between control, *POU5F1*-KO, and electroporated control embryos. We determined the expression of *SOX2*, *Nanog*, and *GATA2* in *POU5F1* intact (Control) and *POU5F1*-KO confirmed blastocyst to better understand the impact of *POU5F1*-KO on other pluripotent genes. *POU5F1*-KO significantly ($p<0.05$) altered *SOX2*, *Nanog*, and *GATA2* expression in blastocyst-stage embryos. In conclusion, direct electroporation of the CRISPR RNP component to the earlier stage of the zygote efficiently created mutations in the *POU5F1* gene. We developed an easy and straightforward protocol for gene editing, which could serve as a valuable method for studying the functional genomics of the buffalo embryos.

Keywords: RNP, electroporation, zygotes, buffalo, *POU5F1*.

funcionales del desarrollo embrionario. A través de una combinación de estrategias, encontramos que la electroporación del cigoto de búfalo a 20 V/mm, 5 pulsos, 3 ms a las 10 h post-inseminación (hpi) resultó en una mayor permeabilidad de la membrana y una mayor eficiencia de edición (88,71%) sin afectar el potencial de desarrollo embrionario. Nos dirigimos al gen *POU5F1* de búfalo utilizando los parámetros mencionados anteriormente, lo que provocó una descomposición del ARNm mediada sin sentido y condujo a una desactivación completa (KO) del gen *POU5F1*. Analizamos las tasas de mutación y las mutaciones en mosaico utilizando el software de seguimiento de Indels por descomposición (TIDE). No observamos diferencias en la competencia de desarrollo embrionario en la tasa de escisión o blastocisto entre los embriones del grupo control, *POU5F1*-KO y los de control electroporados. Determinamos la expresión de *SOX2*, *Nanog* y *GATA2* en *POU5F1* intacto (Control) y en blastocisto confirmado con *POU5F1*-KO para comprender mejor el impacto de *POU5F1*-KO en otros genes pluripotentes. En embriones en etapa de blastocisto, *POU5F1*-KO alteró significativamente ($p<0,05$) la expresión de *SOX2*, *Nanog* y *GATA2*. En conclusión, la electroporación directa del componente CRISPR RNP en la etapa anterior del cigoto fue eficaz para crear mutaciones en el gen *POU5F1*. Desarrollamos un protocolo sencillo y directo para la edición de genes, que podría servir como un método valioso para estudiar la genómica funcional de los embriones de búfalo.

Palabras clave: RNP, electroporación, cigotos, búfalo, *POU5F1*.

BUFFALO'S PRODUCTS & INDUSTRY

Productos e Industria de Búfalos

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Chemical analysis of buffalo meat in a self-consumption system in northeastern of Argentina

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ABSTRACT

The Argentine Northeast region is characterized by its subtropical climate, with low and floodable land with significant environmental variability. The exploitation of buffaloes is presented as an alternative for meat production, as it adapts to special agro-ecological conditions due to waterlogging of the land, the presence of low-digestibility pastures and ectoparasites in which beef cattle do not always express their maximum potential. This research was carried out as part of a larger work at the El Carmen farm, 20 km from Corrientes, which has approximately 30 hectares, where beef cattle and buffaloes coexist. The sample included 15 post-weaning Murrah (n: 5), Mediterranean (n: 5) buffaloes breeds and their crosses (n: 5), with ages ranging from 12 to 15 months and average weights of 380 kg. Animals were raised on natural fields, with access to self-consumption hoppers to supply food composed of alfalfa bales, broken and whole corn, and soybean pellets with a consumption of 1.5% of live weight. The composition of the food supplied as strategic supplementation to achieve the slaughter weight was studied, as well as the chemical composition of the meat, taking samples of the *Longissimus dorsi* muscle between the 11th and 13th ribs, obtaining steaks with a thickness of 2.5 cm., being conditioned in bags of first use and refrigerated at 4 °C until its processing in the laboratory of the Food Technology Service of the Faculty of Veterinary Sciences of Corrientes. With the steak samples (n: 15), we determined the chemical composition of the meat and the food supplied to the buffaloes, referencing the standard methods of the Official Association of Analytical Chemists (AOAC). The fatty acid profile was also determined, with extraction according to the Folch technique and gas chromatography (GC), identifying saturated (SFA), unsat-

Análisis químico de carne de búfalo en un sistema de autoconsumo en el noreste argentino

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RESUMEN

La región del Nordeste argentino se caracteriza por su clima subtropical, con terrenos bajos e inundables con importante variabilidad ambiental. La explotación de búfalos se presenta como una alternativa para la producción de carne, ya que se adapta a condiciones agroecológicas especiales debido al encharcamiento del terreno, la presencia de pastos de baja digestibilidad y ectoparásitos en los que el ganado vacuno no siempre expresa su máximo potencial. Esta investigación se realizó como parte de un trabajo mayor en la finca El Carmen, a 20 km de Corrientes, que cuenta con aproximadamente 30 hectáreas, donde conviven ganado vacuno y búfalos. La muestra incluyó 15 búfalos post-destete de razas Murrah (n:5), Mediterráneo (n:5) y sus cruces (n:5), con edades comprendidas entre 12 y 15 meses y pesos promedio de 380 kg. Los animales fueron criados en campos naturales, con acceso a tolvas de autoconsumo para abastecerse de alimento compuesto por fardos de alfalfa, maíz quebrado y entero, y pellets de soja con un consumo de 1,5% del peso vivo. Se estudió la composición de los alimentos suministrados como suplementación estratégica para lograr el peso de sacrificio, así como la composición química de la carne, tomando muestras del músculo *Longissimus dorsi* entre las costillas 11 y 13, obteniendo filetes con un espesor de 2,5 cm, siendo acondicionado en bolsas de primer uso y refrigerado a 4 °C hasta su procesamiento en el laboratorio del Servicio de Tecnología de Alimentos de la Facultad de Ciencias Veterinarias de Corrientes. Con las muestras de filetes (n:15), determinamos la composición química de la carne y del alimento suministrado a los búfalos, tomando como referencia los métodos estándar del Colegio Oficial de Químicos Analíticos (AOAC). También se determinó el perfil de ácidos

urated (UFA): monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids present in the intramuscular fat. The average values in meat were 28.09% db, 71.91% moisture, 23.69% crude protein, 1.94% ethereal extract (chemical fat), and 1.23% ash. Regarding fatty acids, values of 18.59% SFA and 81.4% UFA were found, standing out within the latter 77.4% MUFA and 4% PUFA. Ratio n-3/PUFA/n-6 PUFA was 1:2. No significant differences in breeds and crosses were detected, concluding that buffalo meat is nutritionally healthy and productively profitable for the region.

Keywords: buffaloes, meat composition, fatty acids.

grasos, con extracción según la técnica de Folch y cromatografía de gases (GC), identificando ácidos grasos saturados (SFA), insaturados (UFA): monoinsaturados (MUFA) y poliinsaturados (PUFA) presentes en la grasa intramuscular. Los valores promedio en carne fueron 28.09% db, 71,91% humedad, 23,69% proteína cruda, 1,94% extracto etéreo (grasa química) y 1,23% ceniza. En cuanto a los ácidos grasos se encontraron valores de 18,59% SFA y 81,4% UFA, destacándose dentro de estos últimos 77,4% MUFA y 4% PUFA. La relación n-3/PUFA/n-6 PUFA fue de 1:2. No se detectaron diferencias significativas entre razas y cruces, concluyendo que la carne de búfalo es nutricionalmente saludable y productivamente rentable para la región.

Palabras clave: búfalos, composición de la carne, ácidos grasos.

BPI-160

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Physicochemical and sensory analysis of fruit juices based on buffalo yogurt whey

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ABSTRACT

Whey is a by-product derived from dairy production and is often discarded, generating environmental contamination. However, it still contains proteins, carbohydrates, and minerals, among others, that could be used in by-products. Among them, fermented drinks might be obtained from the whey mixed with juices and fruit pulp. The objective of this work was to carry out physical-chemical and sensory analysis of juices made with buffalo yogurt whey. The mixtures used 90 mL of whey, 300 mL of fruit juice, and 120 g of sugar in 1 L of filtered water. Mixtures were fermented for 48 h at room temperature, filtered, bottled, and refrigerated. Nineteen samples were grouped into G1: citrus fruits (grapefruit, orange, tangerine) and G2: tropical fruits (passion fruit, pineapple, and mango). Measurements of pH, sugar concentration (°Brix), and density (ρ) were made at the beginning and after fermentation. Sensory analysis was conducted with a non-expert tasting panel of 30 adults between 20 and 35 years old (13 men and 17 women). The juice was evaluated by a 9-point hedonic test, recording the degree of acceptability considering smell, color, and taste, from 1 to 4: I dislike it ("extremely", "a lot", "moderately", "slightly"); 5 "I don't like it, nor do I dislike it"; and from 6 to 9: I like it ("slightly", "moderately", "a lot" and "extremely"). The results were statistically compared by Tuckey with a significance level of 5%. The values on day 0 for G1 were: pH 3.97; ρ 1050.8; °Brix 12.85 and G2: pH 3.69; ρ 1039.44; °Brix 10.26. At 48 h for G1,

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Análisis fisicoquímico y sensorial de jugos de frutas a base de suero de yogur de búfalo

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RESUMEN

El suero es un subproducto derivado de la producción láctea y muchas veces se desecha generando contaminación ambiental. Sin embargo, todavía contiene proteínas, carbohidratos y minerales, entre otros, que podrían utilizarse en subproductos. Entre ellas, se pueden obtener bebidas fermentadas a partir del suero mezclado con jugos y pulpa de frutas. El objetivo de este trabajo fue realizar los análisis físico-químicos y sensoriales de jugos elaborados con suero de yogur de búfalo. Las mezclas utilizaron 90 ml de suero, 300 ml de jugo de frutas y 120 g de azúcar en 1 litro de agua filtrada. Las mezclas se fermentaron durante 48 h a temperatura ambiente, se filtraron, embotellaron y refrigeraron. Diecinueve muestras se agruparon en G1: frutas cítricas (pomelo, naranja, mandarina) y G2: frutas tropicales (maracuyá, piña y mango). Se realizaron mediciones de pH, concentración de azúcar (°Brix) y densidad (ρ) al inicio y después de la fermentación. El análisis sensorial se realizó con un panel de cata no experto formado por 30 adultos de entre 20 y 35 años (13 hombres y 17 mujeres). El jugo fue evaluado mediante una prueba hedónica de 9 puntos, registrando el grado de aceptabilidad considerando olor, color y sabor, de 1 a 4: No me gusta ("extremadamente", "mucho", "moderadamente", "poco"); 5 "No me gusta ni me disgusta"; y del 6 al 9: Me gusta ("poco", "moderadamente", "mucho" y "extremadamente"). Los resultados fueron comparados estadísticamente a través de la prueba Tukey con un nivel de significación del 5%. Los valores

they were: pH 3.68; ρ 1056.7; °Brix 14.52 and G2: pH 3.54; ρ 1042.55; °Brix 10.92. An increase in density and °Brix was observed at 48 h in both groups with significant differences. The sensory evaluation of the juices was good, resulting in G1: 7.23 in men and 7.15 in women. Regarding G2, it was 6.56 for men and 7.49 for women, with a significant difference. 56.6 and 60% of consumers rated 7 to 9 for both groups. The drinks had good levels of acceptance. Among the opinions expressed as positive, the "fine gasified" and "soft color" stand out. Although further studies are needed, whey would be a good raw material for producing naturally fermented juices.

Keywords: by-product, sensory analysis, fruit juice.

del día 0 para G1 fueron: pH 3,97; ρ 1050,8; °Brix 12,85 y G2: pH 3,69; ρ 1039,44; °Brix 10,26. A las 48 h para G1 fueron: pH 3,68; ρ 1056,7; °Brix 14,52 y G2: pH 3,54; ρ 1042,55; °Brix 10,92. Se observó un aumento en la densidad y °Brix a las 48 h en ambos grupos con diferencias significativas. La evaluación sensorial de los jugos fue buena, resultando G1: 7,23 en hombres y 7,15 en mujeres. Respecto al G2 fue de 6,56 para los hombres y de 7,49 para las mujeres, con diferencia significativa. El 56,6 y el 60% de los consumidores calificaron de 7 a 9 para ambos grupos. Las bebidas tuvieron buenos niveles de aceptación. Entre las opiniones expresadas como positivas destacan el "fino gasificado" y el "color suave". Aunque se necesitan más estudios, el suero sería una buena materia prima para producir zumos fermentados de forma natural.

Palabras clave: subproducto, análisis sensorial, jugo de fruta.

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Comparison of meat tenderness parameters and free amino acids content in different species: Buffalo, Yak and Maremmana beef

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ABSTRACT

Meat tenderness and flavor are essential characteristics for consumer satisfaction. These parameters are influenced by several aspects, such as animal species and proteolytic enzyme activity that occurs during post-mortem aging. This work aimed to evaluate the evolution of the proteolytic degradation on Longissimus dorsi muscle in male buffalo (B), yak (Y), and Maremmana beef breed (M) of about 16 months old during the pre-rigor and post-rigor period. Muscle samples were stored at 3 °C and sampled at 3, 6, 24, 72 h, 7, and 14 days after slaughtering five animals for each species. All the samples were stored at -80 °C and then analyzed for free amino acids (FAA) by GC, collagen insoluble content by quantifying hydroxyproline after acidic hydrolysis of meat (AOAC method), myofibrillar degradation (MFI) by spectrometric methods and sarcomere length by optical microscope measurements. On the 14th day, the shear force of cooked meat was determined by the Warner-Bratzler apparatus (WBS). Data were analyzed by ANOVA using a bifactorial model (species and aging times). Furthermore, correlation among data was performed. Among the studied species, M showed less FAA than Y ($p < 0.015$), particularly Val, Leu, and Met. B meat showed an intermediate position. These amino acids are linked to a bitter flavor. B reported a higher value of Asp (0.55 $\mu\text{g/g}$ vs 0.24 $\mu\text{g/g}$) and His (0.85

Comparación de los parámetros de ternura de la carne y contenido de aminoácidos libres en diferentes especies: ternera de búfalo, yak y Maremmana

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RESUMEN

La ternura y el sabor de la carne son características esenciales para la satisfacción del consumidor. Estos parámetros están influenciados por varios aspectos, como la especie animal y la actividad de la enzima proteolítica que se produce durante el envejecimiento post-mortem. Este trabajo tuvo como objetivo evaluar la evolución de la degradación proteolítica en el músculo *Longissimus dorsi* en machos de búfalo (B), yak (Y) y carne de vacuno Maremmana (M) de aproximadamente 16 meses de edad durante el período pre-rigor y post-rigor. Las muestras de músculo se almacenaron a 3 °C y se tomaron muestras a los 3, 6, 24, 72 h, 7 y 14 días después del sacrificio de cinco animales de cada especie. Todas las muestras se almacenaron a -80 °C y luego se analizaron en busca de aminoácidos libres (FAA) mediante GC, contenido de colágeno insoluble mediante la cuantificación de hidroxiprolina después de la hidrólisis ácida de la carne (método AOAC), degradación miofibrilar (MFI) mediante métodos espectrométricos y longitud del sarcómero mediante mediciones con microscopio óptico. El día 14, se determinó la fuerza de corte de la carne cocida mediante el aparato Warner-Bratzler (WBS). Los datos fueron analizados mediante ANOVA utilizando un modelo bifactorial (especies y tiempos de envejecimiento). Además, se realizó

µg/g vs 0.23 µg/g) compared to M. As we expected, the aging time influenced almost all FAA. The increase in FAA generally contributes to the improvement of meat taste. During aging, FAA increased until 24 hours but decreased between 72 hours and seven days to speed up subsequently. Buffalo showed the lowest insoluble collagen percentage (65.59% vs. 69.95% average for Y and M) and the lowest MFI (44.63 vs 52.03, respectively $p < 0.005$). On the contrary, B showed a higher sarcomere length than Y (1.52 vs 1.36 µm). All these parameters contributed to explaining the intermediate shear force value of B (41.4 N) with respect to Y (30.9 N) and M (58.6 N) assessed at 14 days. The shear force resulted inversely related to Leu ($r = -0.74$), Met ($r = -0.63$), and MFI ($r = -0.69$) with significant Pearson coefficients. In conclusion, we can highlight several differences among the species, and buffalo meat reported some indicators of proteolysis favorable for quality.

Keywords: tenderness, free amino acids, ageing time, buffalo, yak, beef.

la correlación entre los datos. Entre las especies estudiadas, M mostró una menor cantidad de FAA en comparación con Y ($p < 0.015$), particularmente Val, Leu y Met. La carne B mostró una posición intermedia. Estos aminoácidos están ligados a un sabor amargo. B informó un valor más alto de Asp (0,55 µg/g frente a 0,24 µg/g) y His (0,85 µg/g frente a 0,23 µg/g) en comparación con M. Como esperábamos, el tiempo de envejecimiento influyó en casi todos los FAA. El aumento de FAA generalmente contribuye a mejorar el sabor de la carne. Durante el envejecimiento, la FAA aumentó hasta las 24 horas, pero disminuyó entre las 72 horas y los siete días para acelerarse posteriormente. La carne de búfalo mostró el porcentaje más bajo de colágeno insoluble (65,59% vs. 69,95% promedio para Y y M) y el MFI más bajo (44,63 vs 52,03, respectivamente $p < 0,005$). Por el contrario, B mostró una longitud de sarcómero mayor que Y (1,52 frente a 1,36 µm). Todos estos parámetros contribuyeron a explicar el valor de fuerza de corte intermedio de B (41,4 N) con respecto a Y (30,9 N) y M (58,6 N) evaluados a los 14 días. La fuerza de corte resultó inversamente relacionada con Leu ($r = -0,74$), Met ($r = -0,63$) y MFI ($r = -0,69$) con coeficientes de Pearson significativos. En conclusión, podemos resaltar varias diferencias entre las especies, y la carne de búfalo reportó algunos indicadores de proteólisis favorables para la calidad.

Palabras clave: ternura, aminoácidos libres, tiempo de envejecimiento, búfalo, yak, ternera.

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Effect of different diets on the fatty acid composition of buffalo bulk milk

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ABSTRACT

What animals feed can affect buffalo milk fatty acids (FA) composition. Therefore, the diet could increase the content of polyunsaturated fatty acids (PUFA), omega 3 (n-3), and conjugated linoleic acid (CLA), improving the nutritional value of buffalo milk. The objective of this work was to evaluate the effect of diet and season on the composition of buffalo milk fatty acids. Over a year, 120 samples of bulk buffalo milk were taken from ten farms. Animals were fed with 10 different isoenergetic and isoproteic diets. The basic diet of each group was supplemented with different components representing about 3% in

Efecto de diferentes dietas sobre la composición de ácidos grasos de la leche a granel de búfala

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RESUMEN

La alimentación de los animales puede afectar la composición de ácidos grasos (AG) de la leche de búfala. Por tanto, la dieta podría aumentar el contenido de ácidos grasos poliinsaturados (PUFA), omega 3 (n-3) y ácido linoleico conjugado (CLA), mejorando el valor nutricional de la leche de búfala. El objetivo de este trabajo fue evaluar el efecto de la dieta y la estación sobre la composición de ácidos grasos de la leche de búfala. A lo largo de un año, se tomaron 120 muestras de leche de búfala a granel de diez granjas. Los animales fue-

dry matter of total ration. The basic diet (B) comprised maize silages, alfalfa hay, corn grain, and soybean as concentrates. The second diet was supplemented with linseed (L), brewer's spent grain (T), alfalfa and mixed hay (H), corn grain (M), and grass (G) as additional constituents. Other farms included both grass and cottonseed (CG), grass and linseed (GL), or grass and brewer's spent grain (TG) and grass, linseed and brewer's spent grain (LTG). Fatty acids in buffalo milk were quantified after extraction and methylation by GC/FID. Data was analyzed through a bifactorial model with interaction (season x diet), using GLM procedure and the statement "contrast" by SAS Software to highlight the effect of the diet constituents. Seasonal effects were evident in milk FA. Spring and summer significantly increase the saturated fatty acids (SFA) and decrease the PUFA by about 10%. Diets containing linseed (L, LG, LTG) showed an increase ($p < 0.001$) in PUFA n-3 (0.63 vs. 0.38 g/100g of fat) compared to the others. While milk from the CG and TG diets showed an increase ($p < 0.001$) in PUFA n-6, the differences were also evident in the n6/n3 ratio (3.1 vs. 5.2, $p < 0.001$). Diets with T and M showed an increase ($p < 0.002$) in SFA and a decrease ($p < 0.001$) in CLA (-20%). Instead, the G diet allowed a higher ($p < 0.0004$) content of CLA (0.90 vs 0.74 g/100g of fat), highlighting the positive contribution of grass on ruminal activity. Contrarily, the addition of linseed and cottonseed to the G diet caused a decrease ($p < 0.001$) in CLA and branch fatty acids due to the excessive presence of PUFA. The feeds supplied attended all metabolic pathways, and in particular, linseed and grass could enrich the milk with functional compounds. However, the right balance must be found between the constituents to maximize their positive contribution.

Keywords: buffalo milk, grass feed, linseed supplementation, season effect, fatty acids, CLA.

ron alimentados con 10 dietas isoenergéticas e isoproteicas diferentes. A la dieta básica de cada grupo se le agregaron diferentes componentes que representaban alrededor del 3% en materia seca de la ración total. La dieta básica (B) comprendía ensilajes de maíz, heno de alfalfa, grano de maíz y soja en forma concentrada. A la segunda dieta se le agregaron semillas de lino (L), bagazo de cerveza (T), alfalfa y heno mixto (H), grano de maíz (M) y pasto (G) como componentes adicionales. Otras explotaciones incluían pasto y semilla de algodón (CG), pasto y linaza (GL), o pasto y bagazo de cerveza (TG) y pasto, linaza y bagazo de cerveza (LTG). Los ácidos grasos en la leche de búfala se cuantificaron después de la extracción y metilación mediante GC/FID. Los datos se analizaron mediante un modelo bifactorial con interacción (estación x dieta), utilizando el procedimiento GLM y la declaración "contraste" del Software SAS para resaltar el efecto de los constituyentes de las dietas. Los efectos estacionales fueron evidentes en los AG de la leche. La primavera y el verano aumentan significativamente los ácidos grasos saturados (AGS) y disminuyen los AGPI en aproximadamente un 10%. Las dietas que contenían linaza (L, LG, LTG) mostraron un aumento ($p < 0,001$) en PUFA n-3 (0,63 vs. 0,38 g/100g de grasa) en comparación con las demás. Mientras que la leche de las dietas CG y TG mostró un aumento ($p < 0,001$) en AGPI n-6, las diferencias también fueron evidentes en la relación n6/n3 (3,1 vs. 5,2, $p < 0,001$). Las dietas con T y M mostraron un aumento ($p < 0,002$) en AGS y una disminución ($p < 0,001$) en CLA (-20%). En cambio, la dieta G permitió un mayor ($p < 0,0004$) contenido de CLA (0,90 vs 0,74 g/100g de grasa), destacando la contribución positiva del pasto sobre la actividad ruminal. Por el contrario, la adición de semillas de linaza y algodón a la dieta G provocó una disminución ($p < 0,001$) en CLA y ácidos grasos ramificados debido a la presencia excesiva de AGPI. Los piensos suministrados atendieron todas las vías metabólicas y, en particular, la linaza y el pasto pudieron enriquecer la leche con compuestos funcionales. Sin embargo, debe encontrarse el equilibrio adecuado entre los componentes para maximizar su contribución positiva.

Palabras clave: leche de búfala, piensos a base de pasto, suplementación con linaza, efecto estacional, ácidos grasos, CLA.

Sensory acceptability of buffalo meat compared to beef**Luis A. de la Cruz-Cruz^{1,2*}, Cristian Larrondo-Cornejo^{3,4}, Patricia Roldán-Santiago⁵, René Rodríguez-Florentino⁶**¹ Escuela de Ciencias de la Salud. Medicina Veterinaria y Zootecnia. Universidad del Valle de México-Coyoacán, Ciudad de México.² Preservación del Bienestar Animal/Manejo de la Fauna Silvestre. Departamento de Producción Agrícola y Animal. Universidad Autónoma Metropolitana-Xochimilco, Ciudad de México, México.³ Núcleo de Investigaciones Aplicadas en Ciencias Veterinarias y Agronómicas, Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Viña del Mar, Chile.⁴ AWEC Advisors S.L. Parc de Recerca Universitat Autònoma de Barcelona, Cerdanyola del Vallès, España.⁵ Departamento de Reproducción. Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, México.⁶ Posgrado en Ganadería, Recursos Genéticos y Productividad, Colegio de Postgraduados, Campus Montecillo, Texcoco, Edo. De México.*Corresponding author: Luis A. de la Cruz-Cruz (ladelacruzcc@gmail.com).**ABSTRACT**

This study aimed to compare the sensory acceptability of buffalo meat regarding beef. The study was conducted at the Dietary Techniques Laboratory of the Universidad del Valle de México (UVM), Mexico City. Three *Longissimus thoracis et lumborum* samples 2.5 cm thick were compared: 1) select beef (slight marbling); 2) select buffalo meat (slight marbling); and 3) prime beef (abundant marbling). The samples were evaluated by 76 students (non-trained panelists) enrolled in the food quality and safety course of the Veterinary Medicine and Zootecnia career, UVM. A seven-point hedonic scale assessed the appearance, odor, flavor, tenderness, juiciness, and overall acceptability. Analysis of variance was conducted (PROC GLM; SAS®), considering the sensory characteristics as dependent variables and the types of meat as independent variables. When statistical differences were noticed, a multiple comparison Tukey test was utilized ($p < 0.05$). Panelists were mainly women (72.36%/n=55), followed by men (22.36%/n=17) and another gender (5.2%/n=4). They were between 19-24 years old (84%/n= 64), followed by 25-30 (10.52%/n=8) and >31 years (5.26%/n=4). The results indicated that the prime beef presented better appearance (5.32±0.18; 4.65±0.16; 4.57±0.16 points, $p=0.0042$) and tenderness (5.77±0.17; 4.38±0.20; 4.09±0.18 points, $p < 0.0001$) compared to select buffalo and select beef,

Aceptabilidad sensorial de la carne de búfalo en comparación con la carne de vacuno**Luis A. de la Cruz-Cruz^{1,2*}, Cristian Larrondo-Cornejo^{3,4}, Patricia Roldán-Santiago⁵, René Rodríguez-Florentino⁶**¹ Escuela de Ciencias de la Salud. Medicina Veterinaria y Zootecnia. Universidad del Valle de México-Coyoacán, Ciudad de México.² Preservación del Bienestar Animal/Manejo de la Fauna Silvestre. Departamento de Producción Agrícola y Animal. Universidad Autónoma Metropolitana-Xochimilco, Ciudad de México, México.³ Núcleo de Investigaciones Aplicadas en Ciencias Veterinarias y Agronómicas, Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Viña del Mar, Chile.⁴ AWEC Asesores S.L. Parc de Recerca Universitat Autònoma de Barcelona, Cerdanyola del Vallès, España.⁵ Departamento de Reproducción. Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, México.⁶ Posgrado en Ganadería, Recursos Genéticos y Productividad, Colegio de Postgraduados, Campus Montecillo, Texcoco, Edo. De México.*Autor de correspondencia: Luis A. de la Cruz-Cruz (ladelacruzcc@gmail.com).**RESUMEN**

Este estudio tuvo como objetivo comparar la aceptabilidad sensorial de la carne de búfalo con respecto a la de carne vacuna. El estudio se realizó en el Laboratorio de Técnicas Dietéticas de la Universidad del Valle de México (UVM), Ciudad de México. Se compararon tres muestras de *Longissimus thoracis et lumborum* de 2,5 cm de espesor: 1) carne selecta de res (ligeramente veteada); 2) carne selecta de búfalo (ligeramente veteada); y 3) carne de res de primera (abundante veteado). Las muestras fueron evaluadas por 76 estudiantes (panelistas no capacitados) matriculados en la carrera de calidad e inocuidad de alimentos de la carrera de Medicina Veterinaria y Zootecnia, UVM. Una escala hedónica de siete puntos evaluó la apariencia, el olor, el sabor, la ternura, la jugosidad y la aceptabilidad general. Se realizó análisis de varianza (PROC GLM; SAS®), considerando las características sensoriales como variables dependientes y los tipos de carne como variables independientes. Cuando se observaron diferencias estadísticas, se utilizó la prueba de Tukey de comparación múltiple ($p < 0,05$). Los panelistas fueron principalmente mujeres (72,36%/n=55), seguidas de hombres (22,36%/n=17) y otro género (5,2%/n=4). Tenían entre 19 y 24 años (84%/n= 64), seguidos de 25-30 (10,52%/n=8) y >31 años (5,26%/n=4). Los resultados indicaron que la carne de primera presen-

respectively. Similarly, a higher score was observed in juiciness for prime beef (5.52±0.19 points), but a better score for buffalo meat (4.52±0.18 points) compared to select beef was identified (3.86±0.19 points) ($p<0.001$). No significant differences were observed in odor ($p=0.67$) and flavor ($p=0.88$), and the overall acceptability showed a positive trend for prime beef ($p=0.06$). Most of the panelists indicated that before the study, they had not consumed buffalo meat (89%/n=68). However, they noted that buffalo meat was like select beef (71%/n=54). Additionally, 47% (n=36) indicated that “maybe” they could include buffalo meat in their diets, followed by 37% (n=28) who indicated that “yes” they would include buffalo meat at least once or twice a week. The panelist highlighted various reasons why buffalo meat is not commonly consumed, such as there is no information on buffalo meat (93.42%/n=71), there are not many products made with buffalo meat (60.52%/n=46), and that it is not available at supermarkets (73.69%/n=56). We conclude that buffalo meat can be a good option for consumers. However, it is vital to increase the information about buffalo meat characteristics (chemical, nutritional, sensory properties, and technological quality) and improve marketing channels that ensure the availability of buffalo products.

Keywords: beef, water buffalo meat, sensory properties.

tó mejor apariencia (5,32±0,18; 4,65±0,16; 4,57±0,16 puntos, $p=0,0042$) y ternura (5,77±0,17; 4,38±0,20; 4,09±0,18 puntos, $p<0,0001$) en comparación con búfalo selecto y carne de res selecto, respectivamente. De manera similar, se observó un mayor puntaje en jugosidad para la carne de res de primera (5,52±0,19 puntos), pero se identificó un mejor puntaje para la carne de búfalo selecta (4,52±0,18 puntos) en comparación con la carne de res selecta (3,86±0,19 puntos) ($p<0,001$). No se observaron diferencias significativas en olor ($p=0,67$) y sabor ($p=0,88$), y la aceptabilidad general mostró una tendencia positiva para la carne de primera calidad ($p=0,06$). La mayoría de los panelistas indicaron que antes del estudio no habían consumido carne de búfalo (89%/n=68). Sin embargo, observaron que la carne de búfalo era como la carne de res selecta (71%/n=54). Además, el 47% (n=36) indicó que “tal vez” podrían incluir carne de búfalo en su dieta, seguido por el 37% (n=28) que indicó que “sí” incluirían carne de búfalo al menos una o dos veces por semana. El panelista destacó diversas razones por las cuales la carne de búfalo no es comúnmente consumida, como no hay información sobre la carne de búfalo (93,42%/n=71), no existen muchos productos elaborados con carne de búfalo (60,52%/n=46), y que no está disponible en los supermercados (73,69%/n=56). Se concluye que la carne de búfalo puede ser una buena opción para los consumidores. Sin embargo, es vital aumentar la información sobre las características de la carne de búfalo (propiedades químicas, nutricionales, sensoriales y calidad tecnológica), así como mejorar los canales de comercialización que aseguren la disponibilidad de los productos de búfalo.

Palabras clave: carne vacuna, carne de búfalo de agua, propiedades sensoriales.

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Frequency and perception of buffalo meat consumption by the population of Belem do Pará, Brazil

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ABSTRACT

Belem City is characterized by its diversity in products of animal and vegetable origin. Currently, it is the second most populous city in the northern region. This large population increases the demand for foodstuffs. The objective of this study was to evaluate the profile, preference, habit, and frequency of

Frecuencia y percepción del consumo de carne de búfalo por la población de Belem do Pará, Brasil

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RESUMEN

La ciudad de Belem se caracteriza por su diversidad de productos de origen animal y vegetal. Actualmente, es la segunda ciudad más poblada de la región norte de Brasil. Esta gran población aumenta la demanda de productos alimenticios. El objetivo de este estudio fue evaluar el perfil, preferencia, hábito y frecuencia de consumo de carne de res, búfalo, pollo,

consumption of beef, buffalo, chicken, fish, and swine meat in Belem - PA. Data collection was carried out in 2019. The sample was composed of 400 interviewees chosen randomly from various points of sale in the city, such as fairs, butchers, and supermarkets, among others. It was found that 51.9% of the consumers were women and 48.1% were men aged between 15 and 29 years; the family environment was composed of three to four people, and 41.6% had income between a minimum wage; 51.9% had completed high school. It was observed that the consumers of Belem preferred beef (51%), followed by these meats: chicken (33%), fish (10%), buffalo (5%) and swine (1%). 64.2% of the frequency of consumption of beef and buffalo was between one and two kilograms per week, while for chicken, fish, and pork, this frequency was 72.4% for the same volume of meat. 70.2% of the respondents had the habit of consuming beef two to three times per week, while 62.7% consumed chicken, fish, and swine at least two to three times a week. Only 7.3% said that they consume buffalo meat once a week. Knowledge about the benefits of buffalo meat was considered low. 70.7% of the sample did not know how to comment about it. 22.8% of the interviewed bought primary beef and buffalo cuts and 53.1% chicken drumsticks. These meats were bought mainly from butchers (48.1%). According to 80.2% of interviewees, chicken is the product that replaces beef and buffalo.

Keywords: comparison of consumer behavior, knowledge of buffalo meat, market.

pescado y cerdo en Belem - Pará. La recolección de datos se realizó en el año 2019. La muestra estuvo compuesta por 400 entrevistados elegidos aleatoriamente de diversos puntos de venta de la ciudad, como ferias, carnicerías y supermercados, entre otros. Se encontró que el 51,9% de los consumidores eran mujeres y el 48,1% eran hombres con edades entre 15 y 29 años; el entorno familiar estaba compuesto por tres a cuatro personas, y el 41,6% tenía ingresos entre un salario mínimo; el 51,9% tenía educación secundaria completa. Se observó que los consumidores de Belem prefirieron la carne vacuna (51%), seguida de las carnes: pollo (33%), pescado (10%), búfalo (5%) y cerdo (1%). El 64,2% de la frecuencia de consumo de carne vacuna y búfala fue entre uno y dos kilogramos semanales, mientras que para pollo, pescado y cerdo esta frecuencia fue del 72,4% para el mismo volumen de carne. El 70,2% de los encuestados tenía el hábito de consumir carne vacuna dos o tres veces por semana, mientras que el 62,7% consumía pollo, pescado y cerdo al menos dos o tres veces por semana. Sólo el 7,3% afirmó consumir carne de búfalo una vez por semana. El conocimiento sobre los beneficios de la carne de búfalo se consideró bajo. El 70,7% de la muestra no supo opinar al respecto. El 22,8% de los entrevistados compró cortes primarios de carne vacuna y búfala y el 53,1% muslo de pollo. Estas carnes fueron compradas principalmente a carniceros (48,1%). Según el 80,2% de los entrevistados, el pollo es el producto que reemplaza a la carne vacuna y al búfalo.

Palabras clave: comparación del comportamiento del consumidor, conocimiento de la carne de búfalo, mercado.

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Buffalo whey proteins as innovative raw material for nanoparticles building

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ABSTRACT

Buffalo whey is the most important byproduct of the cheese industry. Whey proteins are fascinating from a technological and economic point of view. In the presence of polysaccharides (PS), proteins (PR) can form complexes in the nanoscale. The study of PS and PR interactions constitutes a key aspect in building nanomaterials, particularly when the use of

Proteínas de suero de búfalo como materia prima innovadora para la construcción de nanopartículas

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RESUMEN

El suero de búfalo es el subproducto más importante de la industria quesera. Las proteínas del suero son fascinantes desde el punto de vista tecnológico y económico. En presencia de polisacáridos (PS), las proteínas (PR) pueden formar complejos a nanoescala. El estudio de las interacciones PS y PR constituye un aspecto clave en la construcción de na-

scarcely studied protein sources such as Buffalo (*Bubalus bubalis*) whey is intended. In this sense, the objective of this work was to study the formation of biopolymeric structures by electrostatic interactions from buffalo whey proteins (BWPC) and Arabic gum (GA). An aqueous suspension containing BWPC (0.1% w/v) and GA (0.05% w/v) was prepared for this purpose. For comparative purposes, the behavior of mixtures containing cow whey (WPC) and GA, and the single components were also studied. The pH of biopolymeric dispersions was adjusted to 5.5 to 2.5 with HCl. The interactions were monitored through turbidimetric analysis (absorbance by spectrometry at 600 nm), zeta-potential and particle size distribution (by dynamic light scattering), and relative viscosity (in a Cannon-Fenske capillary viscometer). Analytical trials were performed at least in triplicate. Analysis of variance (ANOVA) was performed to find statistical differences among samples. The significance level was defined at $p > 0.05$ (confidence interval of 95%). While GA suspensions did not show changes in the absorbance, both BWPC and WPC displayed a maximum between pH 4 and 4.5, attributable to their isoelectric points. Comparatively, mixtures of BWPC-GA and WPC-GA showed higher turbidity values than single-component suspensions ($p < 0.05$), which could indicate the occurrence of attractive interactions. The gradual increase in the absorbance, intended as the formation of soluble complexes, was verified for BWPC-GA from pH 5, reaching a maximum of 4. In contrast, for WPC-GA, it occurred between 5.5 and 4.5. In the range of formation of conjugates, PS-PR showed intermediate zeta-potential values to the single components, being -27 and -50 mV for BWPC-GA and WPC-GA, respectively. In these conditions, the particles showed a monomodal size distribution centered at 250 nm for both systems. The interaction between biopolymers at the different pH values did not evidence appreciable changes in the rheological properties ($p > 0.05$). Obtained results allow us to consider that BWPC can undergo associative interactions with GA, resulting in soluble nano complexes with good colloidal valuable stability for the nutraceutical industry. The use of BWPC as nanostructured components constitutes a promising alternative for adding value to this byproduct, contributing to the economic benefit of dairy industries, and minimizing the polluting effect of this effluent.

Keywords: nanoparticles, buffalo whey, electrostatic interactions.

nomateriales, particularmente cuando se pretende el uso de fuentes proteicas escasamente estudiadas como el suero de búfalo (*Bubalus bubalis*). En este sentido, el objetivo de este trabajo fue estudiar la formación de estructuras biopoliméricas mediante interacciones electrostáticas de proteínas de suero de búfala (BWPC) y goma arábiga (GA). Con este fin, se preparó una suspensión acuosa que contenía BWPC (0,1% p/v) y GA (0,05% p/v). Con fines comparativos, también se estudió el comportamiento de mezclas que contienen suero de vaca (WPC) y GA, así como de los componentes individuales. El pH de las dispersiones biopoliméricas se ajustó de 5,5 a 2,5 con HCl. Las interacciones se monitorearon mediante análisis turbidimétrico (absorbancia por espectrometría a 600 nm), potencial zeta y distribución del tamaño de partículas (por dispersión dinámica de la luz) y viscosidad relativa (en un viscosímetro capilar Cannon-Fenske). Los ensayos analíticos se realizaron al menos por triplicado. Se realizó un análisis de varianza (ANOVA) para encontrar diferencias estadísticas entre muestras. El nivel de significación se definió en $p > 0,05$ (intervalo de confianza del 95%). Mientras que las suspensiones de GA no mostraron cambios en la absorbancia, tanto BWPC como WPC mostraron un máximo entre pH 4 y 4,5, atribuible a sus puntos isoeléctricos. Comparativamente, las mezclas de BWPC-GA y WPC-GA mostraron valores de turbidez más altos que las suspensiones de un solo componente ($p < 0,05$), lo que podría indicar la ocurrencia de interacciones atractivas. El aumento gradual de la absorbancia, entendido como la formación de complejos solubles, se verificó para BWPC-GA a partir de pH 5, alcanzando un máximo de 4. En cambio, para WPC-GA ocurrió entre 5,5 y 4,5. En el rango de formación de conjugados, PS-PR mostró valores de potencial zeta intermedios para los componentes individuales, siendo -27 y -50 mV para BWPC-GA y WPC-GA, respectivamente. En estas condiciones, las partículas mostraron una distribución de tamaño monomodal centrada en 250 nm para ambos sistemas. La interacción entre biopolímeros a los diferentes valores de pH no registró cambios apreciables en las propiedades reológicas ($p > 0,05$). Los resultados obtenidos nos permiten considerar que BWPC puede sufrir interacciones asociativas con GA, dando como resultado nanocomplejos solubles con buena estabilidad coloidal valiosa para la industria nutraceutica. El uso de BWPC como componentes nanoestructurados constituye una alternativa prometedora para agregar valor a este subproducto, contribuyendo no sólo al beneficio económico de las industrias lácteas sino también a minimizar el efecto contaminante de este efluente.

Palabras clave: nanopartículas, suero de búfala, interacciones electrostáticas.

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Nutritional properties of buffalo meat finished with wrapped alfalfa hay compared with dried hay and maize silage-based diets

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ABSTRACT

Several nutritional compounds characterize ruminant meat. Among these, conjugated linoleic acids (CLA) show relevant functional properties as an intermediary product in ruminal biohydrogenation. Diets rich in green forage increase the CLA content and the level of polyunsaturated fatty acid (PUFA) as linolenic acid, the precursor of the n-3 long-chain fatty acids. This effect is partially lost when the forage is hayed, particularly to the loss of leaves. Hence, hay wrapping could limit these losses and increase the forage quality. This work aimed to evaluate the effect of hay wrapping technology on buffalo meat fatty acid profile. Young beef male buffaloes of about 12 months old were divided into three groups (7 for the group) and fed for five months with three different diets based on maize silage (MS), alfa-alfa dried hay (DH), and wrapped alfalfa hay (WH). The concentrates supply varied to obtain isoenergetic (0.9 UFC/day) and isoproteic (15% of crude protein) diets. After slaughtering, proximate composition and fatty acids (FA) analysis was performed on a piece of the *Longissimus thoracic* muscle. After the extraction and methylation of fat, fatty acids, using the GC/FID instrument, were quantified, and expressed as mg/g of fat. Data were analyzed by ANOVA, considering only diet as a factor. The animals had a similar slaughter weight of 436±20.4 kg with an average yield of 52.4%. The proximate composition of meat did not differ significantly between groups (on average, 21.4%, 1.5%, and 1.1%, respectively for protein, fat, and ash). Monounsaturated fatty acids were higher ($p<0.0041$) in the MS group than in the WH. The wrapping effect was evident in PUFA (186.6, 152.5, and 134.4 mg/g of fat in WH, MS, and DH groups, respectively; $p<0.001$). This was explained in terms of the high content of n-6 and n-3 fatty acids, with the last double in WH concerning MS (13.9 vs. 31.4 mg/g of fat), which significantly affected the n6/n3 ratio (8.77 vs 4.86 in WH and MS groups respectively; $p<0.005$). Furthermore, it is important to underline how the wrapped hay induced a more significant accumulation of CLA ($p<0.001$) when compared to dried hay and maize silage (3.36 vs. 2.93 vs. 2.37 mg/g of fat) due to its ability to retain leaves. In conclusion, the positive effect on the meat quality of a wrapped hay-based diet appears evident thanks to the richness in leaves, which positively stimulate the microbial

Propiedades nutricionales de la carne de búfalo terminada con heno de alfalfa envuelto en comparación con dietas basadas en heno seco y ensilaje de maíz

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RESUMEN

Varios compuestos nutricionales caracterizan la carne de rumiantes. Entre estos, los ácidos linoleicos conjugados (CLA), como producto intermediario en la biohidrogenación ruminal, muestran propiedades funcionales relevantes. Las dietas ricas en forraje verde aumentan el contenido de CLA, así como el nivel de ácidos grasos poliinsaturados (PUFA) como el ácido linolénico, precursor de los ácidos grasos de cadena larga n-3. Este efecto se pierde parcialmente cuando se hena el forraje, particularmente por la pérdida de hojas. Por tanto, el envoltorio de heno podría limitar estas pérdidas y aumentar la calidad del forraje. Este trabajo tuvo como objetivo evaluar el efecto de la tecnología de envoltura de heno sobre el perfil de ácidos grasos de la carne de búfalo. Se dividieron búfalos machos jóvenes de aproximadamente 12 meses de edad en tres grupos (7 para el grupo) y se alimentaron durante cinco meses con tres dietas diferentes basadas en ensilaje de maíz (MS), heno seco de alfalfa (DH) y heno de alfalfa envuelto (WH). El suministro de concentrados varió para obtener dietas isoenergéticas (0,9 UFC/día) e isoproteicas (15% de proteína cruda). Después del sacrificio, de un trozo del músculo torácico *Longissimus* de la región torácica se realizó un análisis aproximado de la composición y de los ácidos grasos (AG). Después de la extracción y metilación de la grasa, los ácidos grasos, utilizando el instrumento GC/FID, se cuantificaron y expresaron como mg/g de grasa. Los datos fueron analizados mediante ANOVA, considerando únicamente la dieta como factor. Los animales tuvieron un peso al sacrificio similar de 436±20,4 kg con un rendimiento promedio del 52,4%. La composición aproximada de la carne no difirió significativamente entre los grupos (en promedio, 21,4%, 1,5% y 1,1%, respectivamente, para proteínas, grasas y cenizas). Los ácidos grasos monoinsaturados fueron mayores ($p<0,0041$) en el grupo MS que en el WH. El efecto envolvente fue evidente en los AGPI (186,6, 152,5 y 134,4 mg/g de grasa en los grupos WH, MS y DH, respectivamente; $p<0,001$). Esto se explicó en términos del alto contenido de ácidos grasos n-6 y n-3, siendo el último doble en WH respecto al MS (13,9 vs. 31,4 mg/g de grasa), lo que afectó significativamente

activity in the rumen and the accumulation in the meat of fatty acids with high nutritional value.

Keywords: buffalo meat, diet, CLA, PUFA, wrapped hay.

la relación n6/n3 (8,77 vs 4,86 en los grupos WH y MS respectivamente; $p < 0,005$). Además, es importante destacar cómo el heno envuelto indujo una acumulación más significativa de CLA ($p < 0,001$) en comparación con el heno seco y ensilado de maíz (3,36 vs. 2,93 vs. 2,37 mg/g de grasa) debido a su capacidad para conservar las hojas. En conclusión, el efecto positivo sobre la calidad de la carne de una dieta a base de heno envuelto parece evidente gracias a la riqueza en hojas, que estimulan positivamente la actividad microbiana en el rumen y la acumulación en la carne de ácidos grasos con alto valor nutricional.

Palabras clave: carne de búfalo, dieta, CLA, PUFA, heno envuelto.

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How can the buffalo carcass be used rationally?

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ABSTRACT

The objective of this study was evaluate the yields of meat cuts and discards obtained from the carcass of buffalo steers. The research was carried out in the Bubalinoculture Sector of the Animal Science Department of the Federal Rural University of Pernambuco, Brazil. 62 carcasses of pasture-finished cross-bred buffalo steers were evaluated. The animals weighed an average of 507.0 kg and were slaughtered before the first molt of the deciduous dentition, with a carcass yield of 49.0%. The carcass of each animal was divided into two half-carcasses, which were identified and weighed. The carcasses were taken to a cold chamber at 4 °C during 24h. To obtain the yield of each integral primary cut, the carcasses were sectioned into front and rear, which were weighed and boned. Boning was carried out by the same employees to maintain the standard cuts and dressing. The cuts with and without bones, the edible flap (meat and fat shavings), the discarded flap (tallow, nerves and skins) and the discarded bones were obtained. The deboning standard used was typical of the Brazilian market with dressing between moderate and severe. The percentages observed for the cuts (in each half carcass) were: soft drumstick, 5.3%; hard

¿Cómo utilizar racionalmente el cadáver de búfalo?

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RESUMEN

El objetivo de este estudio fue evaluar los rendimientos de cortes y descartes de carne obtenidos de la canal de novillos bufalinos. La investigación se llevó a cabo en el Sector de Bubalinocultura del Departamento de Ciencia Animal de la Universidad Federal Rural de Pernambuco, Brasil. Se evaluaron 62 canales de novillos búfalos mestizos en pastoreo. Los animales pesaron en promedio 507,0 kg y fueron sacrificados antes de la primera muda de la dentición temporal, con un rendimiento en canal de 49,0%. La canal de cada animal se dividió en dos medias canales, las cuales fueron identificadas y pesadas. Las canales fueron llevadas a cámara de frío a 4 °C durante 24h. Para obtener el rendimiento de cada corte primario integral, las canales se seccionaron en delantera y trasera, las cuales fueron pesadas y deshuesadas. El deshuesado fue realizado por los mismos empleados para mantener los cortes y faenados estándar. Se obtuvieron los cortes con y sin huesos, el colgajo comestible (carne y virutas de grasa), el colgajo de descarte (sebo, nervios y pieles) y los huesos de descarte. El estándar de deshuesado utilizado fue el típico del mercado brasileño

cushion, 3.7%; duckling, 3.8%; lizard, 1.8%; sirloin steak, 1.1%; grill flank steak, 0.75%; red flank steak, 0.61%; breast, 0.96%; rump baby beef, 1.3%; rump bonbon, 0.8%; ossobuco, 4.2%; muscle, 2.0%; turkey bone, 1.1%; duckling bone, 0.5%; oxtail, 0.38%; ancho steak, 1.3%; chorizo steak, 1.9%; filet mignon, 1.2%; fillet shell, 0.35%; chest, 3.9%; neck, 4.2%; acm, 4.3%; window rib, 8.7%; strip roast, 2.5%; banana, 0.23%; goldfish, 0.87%; palette heart, 0.77%; flat iron, 0.95%; Denver stake, 0.6%; beef steak, 0.40%; edible shavings, 19.43%; fat, 1.8%; scraps of disposal, 0.4%; discard bones, 17.9%. The proportion observed for the front and rear were 45.0 and 55.0%, respectively. There is a high volume of waste bones, which could be used in food, such as bone broth, for example. The volume of edible trimmings should be better used for making meat in cubes or as bait. The ground trimmings can be sold as ground meat or used in the preparation of hamburgers, sausages, meatballs, kafta, among others. Cuts that can be labeled premium make up approximately 25.0% of the carcass. Therefore, it is necessary to stimulate the daily consumption of buffalo meat in order to have a market for the other cuts that make up most of the carcass.

Keywords: *Bubalus bubalis*, meat cuts, yield.

con un procedimiento entre moderado y severo. Los porcentajes observados para los cortes (en cada media canal) fueron: muslo blando, 5,3%; colchón duro, 3,7%; patito, 3,8%; lagarto, 1,8%; solomillo, 1,1%; arrachera a la parrilla, 0,75%; arrachera roja, 0,61%; mama, 0,96%; ternera tierna, 1,3%; bombón de grupa, 0,8%; osobuco, 4,2%; músculo, 2,0%; hueso de pavo, 1,1%; hueso de patito, 0,5%; rabo de toro, 0,38%; filete ancho, 1,3%; filete de chorizo, 1,9%; filet mignon, 1,2%; cáscara de filete, 0,35%; pecho, 3,9%; cuello, 4,2%; acm, 4,3%; nervadura de ventana, 8,7%; asado en tiras, 2,5%; plátano, 0,23%; peces de colores, 0,87%; corazón de paleta, 0,77%; plancha, 0,95%; participación de Denver, 0,6%; filete de ternera, 0,40%; virutas comestibles, 19,43%; grasa, 1,8%; desechos de eliminación, 0,4%; descartar huesos, 17,9%. La proporción observada para la parte delantera y trasera fue del 45,0 y 55,0%, respectivamente. Existe un gran volumen de huesos de desecho, que podrían aprovecharse en alimentos, como caldo de huesos, por ejemplo. El volumen de recortes comestibles debería aprovecharse mejor para hacer carne en cubos o como cebo. Los recortes molidos pueden venderse como carne molida o utilizarse en la preparación de hamburguesas, salchichas, albón-digas, kafta, entre otros. Los cortes que pueden etiquetarse como premium constituyen aproximadamente el 25,0% de la canal. Por lo tanto, es necesario estimular el consumo diario de carne de búfalo para tener mercado para los demás cortes que componen la mayor parte de la canal.

Palabras clave: *Bubalus bubalis*, cortes de carne, rendimiento.

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Smart packaging tools for selected Indian traditional buffalo milk products: a case for on-package freshness indicators

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ABSTRACT

Among the intelligent packaging systems, freshness indicators have been proven to provide real-time quality status of packaged food. They supplement or complement the printed “best before” or “expiry date” and give consumers an informed choice. Freshness indicators work on the principle of colorimetric changes with changes in the headspace volatile components of the packaged food product. With the growing consumer demand for real-time quality status, there is a need to develop colorimetric on-package freshness indicators for

Herramientas de envasado inteligentes para productos seleccionados de leche de búfala tradicional en India: un caso de indicadores de frescura en el envase

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RESUMEN

Entre los sistemas de envasado inteligentes, se ha demostrado que los indicadores de frescura proporcionan el estado de calidad de los alimentos envasados en tiempo real. Suplementan o complementan el “consumir preferentemente antes” o la “fecha de caducidad” impresos y ofrecen a los consumidores una elección informada. Los indicadores de frescura funcionan según el principio de cambios colorimétricos con los cambios en

dairy products. The current study aimed to develop on-package freshness indicators for selected traditional Indian buffalo milk products, namely *khoa*, *burfi*, *dahi*, and *misti dahi*, and correlate their colorimetric responses with quality attributes. The prerequisites for developing freshness indicators were to identify the critical volatile headspace markers using solid phase microextraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) for the respective dairy products, selection of suitable innovative ink components, namely dyes (methyl red, and methyl red sodium salt), plasticizer (glycerol), choose appropriate polymer support (methylcellulose, polyvinyl alcohol or paper), and adopt a suitable production technique such as casting, dipping, or electrospinning. SPME coupled with GC-MS revealed that the key volatile markers for all the products belonged to ketones, aldehydes, acids, and alcohols. The smart ink components were selected based on the key volatile markers, and a suitable fabrication technique was adopted for developing the respective freshness indicators. Later, the developed indicators were exposed to different concentrations of key volatile markers, such as hexanal, heptanal, 2-pentanone, 2-hexanone, 2-heptanone, acetic acid, butanoic acid, among others, in the range of 0.1 to 40 μL and their dose-dependent color responses were noted. The selected freshness indicators were characterized and suitably integrated with the closures to effectively detect and interact with the dynamic headspace volatile compounds while storing the packaged product. Finally, the real-time performance of the developed freshness indicators was evaluated. It was found that the initial yellow color of freshness indicators was changed to reddish-dark brown in the case of *khoa* and *burfi* while to pale pink in the case of *dahi* and *misti dahi* at the end of the storage periods, indicating loss of freshness. The total color difference (TCD) in the freshness indicators was correlated with the quality attributes of the products. Among all the freshness indicators, the TCD was found to be very highly positively correlated with titratable acidity, thiobarbituric acid value, free fatty acids value, and total viable bacterial count while significantly negatively correlated with pH, sensory flavor, and overall acceptability scores. The developed freshness indicators could successfully depict the freshness status of the stored products through visual color changes distinguishing between the fresh and spoiled products.

Keywords: buffalo milk, Indian traditional dairy products, smart packaging, freshness indicator.

el espacio de encabezado de los componentes volátiles del producto alimenticio envasado. Con la creciente demanda de los consumidores de un estado de calidad en tiempo real, existe la necesidad de desarrollar indicadores colorimétricos de frescura en los envases de los productos lácteos. El objetivo del presente estudio fue desarrollar indicadores de frescura en el paquete de productos seleccionados de leche de búfala tradicional de la India, a saber, *khoa*, *burfi*, *dahi* y *misti dahi*, y correlacionar sus respuestas colorimétricas con atributos de calidad. Los requisitos previos para desarrollar indicadores de frescura fueron identificar los marcadores clave de espacio de cabeza volátil mediante microextracción en fase sólida (SPME) junto con cromatografía de gases-espectrometría de masas (GC-MS) para los respectivos productos lácteos, selección de componentes de tinta innovadores adecuados, a saber, colorantes (metil rojo y sal sódica de rojo de metilo), plastificante (glicerol), elegir el soporte polimérico apropiado (metilcelulosa, alcohol polivinílico o papel) y adoptar una técnica de producción adecuada, como fundición, inmersión o electrohilado. SPME junto con GC-MS revelaron que los marcadores volátiles clave de todos los productos pertenecían a cetonas, aldehídos, ácidos y alcoholes. Con base en los marcadores volátiles clave, se seleccionaron los componentes de tinta inteligente y se adoptó una técnica de fabricación adecuada para desarrollar los respectivos indicadores de frescura. Posteriormente, los indicadores desarrollados fueron expuestos a diferentes concentraciones de marcadores volátiles clave como hexanal, heptanal, 2-pentanona, 2-hexanona, 2-heptanona, ácido acético, ácido butanoico, entre otros, en el rango de 0,1 a 40 μL y se observaron sus respuestas de color dependientes de la dosis. Los indicadores de frescura seleccionados se caracterizaron y se integraron adecuadamente con los cierres para detectar e interactuar eficazmente con los compuestos volátiles dinámicos del espacio de cabeza mientras se almacena el producto envasado. Finalmente, se evaluó el desempeño en tiempo real de los indicadores de frescura desarrollados. Se encontró que el color amarillo inicial de los indicadores de frescura cambió a marrón rojizo oscuro en el caso de *khoa* y *burfi*, mientras que a rosa pálido en el caso de *dahi* y *misti dahi* al final de los períodos de almacenamiento, lo que indica pérdida de frescura. La diferencia total de color (TCD) en los indicadores de frescura se correlacionó con los atributos de calidad de los productos. Entre todos los indicadores de frescura, se encontró que el TCD tenía una correlación muy positiva con la acidez titulable, el valor del ácido tiobarbitúrico, el valor de los ácidos grasos libres y el recuento total de bacterias viables, mientras que se correlacionaba significativamente negativamente con el pH, el sabor sensorial y las puntuaciones de aceptabilidad general. Los indicadores de frescura desarrollados podrían representar con éxito el estado de frescura de los productos almacenados mediante cambios de color visuales que distinguen entre los productos frescos y en mal estado.

Palabras clave: leche de búfala, productos lácteos tradicionales de la India, envases inteligentes, indicador de frescura.

BPI-232 Rev. Cientif. FCV-LUZ, XXXIII, SE, 305, 2023, <https://doi.org/10.52973/rcfcv-wbc143>**Effect of rennet on buffalo cheese microbiota, taste and flavor**

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ABSTRACT

Rennet is a critical element that allows the coagulation of milk caseins, the basic process of producing cheese. Traditional cheeses (Traditional Agri-food Products - PAT) are generally produced using artisanal animal rennet. The type of rennet used for cheese making, jointly with several other factors, influences the flavor and aroma of dairy products. In this study, we focused exclusively on rennet's role in developing aromas and flavors, highlighting different tastes depending on the type of rennet used in cheesemaking. Buffalo milk was used to produce cheeses through a single cheesemaking session, where the only difference was the rennet used: commercial calf or artisanal lamb. Cheeses were stored and analyzed monthly at T0, T30, T60, and T90. Microbiota characterization (NGS technology) was performed on all samples, jointly with microbiological and sensory analysis (ISO 11035:1994). The microbiological analysis highlighted differences mainly in quantifying total bacteria count and acid lactic bacteria at T0, significantly higher in cheeses from calf rennet. Furthermore, at T60, a general reduction of all microbiological parameters in cheese based on lamb rennet was observed. The Microbiota analysis showed significant microbiological changes during the seasoning in the same product (α diversity) and between the two products obtained from industrial and artisanal rennet (β diversity). Regarding the sensory analysis, all the judges evaluated the cheeses based on lamb rennet as more appreciable due to their intense flavors and aroma. The study results confirmed the role of lamb rennet in developing flavor in buffalo cheese and, consequently, suggests a larger use of this rennet to obtain cheeses with a particular and typical taste based on buffalo milk.

Keywords: buffalo cheese, rennet, microbiota, sensory assay.

Efecto del cuajo sobre el microbiota, el gusto y el sabor del queso de búfala

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RESUMEN

El cuajo es un elemento crítico que permite la coagulación de las caseínas de la leche, proceso básico en la elaboración del queso. Los quesos tradicionales (Productos Agroalimentarios Tradicionales - PAT) se elaboran generalmente con cuajo animal artesanal. El tipo de cuajo utilizado para la elaboración del queso, junto con varios otros factores, influye en el sabor y aroma de los productos lácteos. En este estudio, nos centramos exclusivamente en el papel del cuajo en el desarrollo de aromas y sabores, destacando diferentes sabores según el tipo de cuajo utilizado en la elaboración del queso. Para la elaboración de quesos se utilizó leche de búfala mediante una única quesería, donde la única diferencia era el cuajo utilizado: ternera comercial o cordero artesanal. Los quesos se almacenaron y analizaron mensualmente en T0, T30, T60 y T90. En todas las muestras se realizó la caracterización de la microbiota (tecnología NGS), junto con análisis microbiológicos y sensoriales (ISO 11035:1994). El análisis microbiológico destacó diferencias principalmente en la cuantificación del recuento de bacterias totales y ácido láctico en T0, significativamente mayor en los quesos a partir de cuajo de ternera. Además, en T60 se observó una reducción general de todos los parámetros microbiológicos en el queso a base de cuajo de cordero. El análisis de Microbiota mostró cambios microbiológicos significativos durante el curado en un mismo producto (diversidad α) y entre los dos productos obtenidos a partir de cuajo industrial y artesanal (diversidad β). En cuanto al análisis sensorial, todos los jueces valoraron el queso a base de cuajo de cordero como más apreciable por sus sabores y aromas más intensos. Los resultados del estudio confirmaron el papel del cuajo de cordero en el desarrollo del sabor del queso de búfala y, en consecuencia, sugieren un mayor uso de este cuajo para obtener quesos con un sabor particular y típico a base de leche de búfala.

Palabras clave: queso de búfala, cuajo, microbiota, ensayo sensorial.

Determination of the physicochemical and microbiological quality of buffalo milk in the south of lake Maracaibo**Álvarez Luis^{1,2}, Zambrano Alexis¹,
Rosales-Zambrano Datty³**¹ *University of the Andes. Chemical, Industrial and Agricultural Analysis Research Laboratory (LIAQIA). Mérida-Venezuela*² *CONLAC, El Vigía. Mérida. Venezuela*³ *Veterinary Advance Technologies C.A. (IVACA). Ejido. Merida. Venezuela**Corresponding author: dattyrsl@gmail.com**ABSTRACT**

The determination of quality parameters in the food industry is a critical point in the transformation process in aspects such as safety and quality attributes required by the market for a product, the cheese making industry does not escape this. In Venezuela, the standards for raw materials (raw milk) and dairy derivatives are established by COVENIN (Venezuelan Commission of Industrial Standards), which determine variables of great importance in milk such as: lipids, proteins, lactose, mineral salts, load microbial, among others. These components are of great importance because they influence the entire production chain. The purpose of this work is to evaluate the physicochemical and microbiological quality of buffalo milk used in a lactuary in western Venezuela, according to the official regulations in force in the country. It was developed at the Chemical, Industrial and Agricultural Analysis Research Laboratory (LIAQIA), in conjunction with the Venezuelan dairy company CONLAC and the VATCA Laboratory. C.A., (Biodiagnostic Unit), in order to provide the company with quality controls on cheeses made from buffalo milk. Sampling of raw pure milk (production units described as A, B, C and D) and milk mixtures (composite samples described as E) and pasteurized milk F was carried out consecutively for three months in 2019. Samples of Milk was taken in triplicate from the tanks according to the COVENIN 938-1983 standard, from the four buffalo production units in the southern area of Lake Maracaibo, collecting 400 mL of milk for each sample, in taking the milk sample. it was homogenized for 5 minutes by stirring. A completely randomized experimental design was carried out, where the parameters evaluated were the mean (\bar{x}), the standard deviation (S), coefficient of variation (CV), Tukey's post hoc tests using the statistical program Minitab 15 V. 2021. The analyses physicochemical tests were performed on buffalo milk, where the parameters of acidity, fat, solid non-fat (SNG), lactose, protein, and cryoscopy were evaluated in the milk. Some of the mentioned tests were performed directly using a Lactoscan. The amount of somatic cells was determined for each milk sample by direct microscopic somatic cell count (CCS) and bacterial

Determinación de la calidad fisicoquímica y microbiológica de leche de búfala en el sur del lago de Maracaibo

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La determinación de parámetros de calidad en la industria de alimento, se constituye en un punto crítico del proceso de transformación en aspectos como la inocuidad y los atributos de calidad que exige el mercado de un producto, la industria de elaboración de quesos no escapa de ello, en Venezuela las normas de materia prima (leche cruda) y derivados lácteos están establecidas por COVENIN (Comisión Venezolana de Normas Industriales), las cuales determinan variables de gran importancia en la leche como son: lípidos, proteínas, lactosa, sales minerales, carga microbiana, entre otros. Estos componentes son de gran importancia debido a que influyen en toda la cadena de producción. El presente trabajo tiene como finalidad evaluar la calidad fisicoquímica y microbiológica de leche de búfala empleada en un lactuario del occidente venezolano, de acuerdo a la normativa oficial vigente en el país. Se desarrolló en el Laboratorio de Investigación en Análisis Químico, Industrial y Agropecuario (LIAQIA), en conjunto con la empresa láctea venezolana CONLAC y el Laboratorio VATCA. C.A., (Unidad de Biodiagnóstico), con el fin de aportar a la empresa controles de calidad en los quesos elaborados con leche de búfala. El muestreo de leche pura cruda (descritas las unidades de producción como A, B, C y D) y mezclas de leche (muestras compuestas descritas como E) y leche pasteurizada F se efectuó consecutivamente durante tres meses en el año 2019. Las muestras de leche se tomaron por triplicado de los tanques según la norma COVENIN 938-1983, de las cuatro unidades de producción de búfalos de la zona Sur del Lago de Maracaibo, se recolectando 400 mL de leche por cada muestra, en la toma de muestra la leche se homogeneizó durante 5 minutos mediante agitación. Se realizó un diseño experimental completamente aleatorizado, donde los parámetros evaluados fueron la media (\bar{x}), la desviación estándar (S), coeficiente de variación (CV), pruebas a posteriori de Tukey utilizando el programa estadístico Minitab 15 V. 2021. Los análisis fisicoquímicos se realizaron a la leche de búfala, donde se evaluaron en la leche los parámetros de acidez, grasa, sólidos no grasos

colony count in a Petri dish (COVENIN 1126-1989, COVENIN 902-1987 and García, 2011). The average physicochemical values of the three raw milk samples from farms evaluated were for A, B, C and D respectively: Acidity: $17.1 \pm 0.8^{**}$; $17.67 \pm 0.5^*$; $16.8 \pm 0.7^*$; $17.6 \pm 0.5^*$; % Fat: $5.2 \pm 0.4^{**}$; $6.5 \pm 0.4^{**}$; $6.4 \pm 0.5^{**}$; $6.6 \pm 0.1^{**}$; Solid non-fat (SNG): $9.3 \pm 0.1^*$; $9.7 \pm 0.2^*$; $9.9 \pm 0.2^{**}$; $9.7 \pm 0.2^*$; Lactose: $5.1 \pm 0.1^*$; $5.3 \pm 0.1^*$; $5.4 \pm 0.1^{**}$; $5.3 \pm 0.1^*$; % Protein: $4.14 \pm 0.03^*$; $4.21 \pm 0.04^*$; $4.25 \pm 0.04^*$; $4.27 \pm 0.06^*$; and Cryoscopy: $0.61 \pm 0.01^*$; $0.64 \pm 0.01^*$; $0.66 \pm 0.02^{**}$; $0.65 \pm 0.02^*$. The averages of the samplings of the milk mixtures E (milk silo) and F (pasteurized milk) obtained: Acidity: $17.6 \pm 0.5^*$; $18.3 \pm 0.7^*$; % Fat: $6.0 \pm 0.2^{**}$; $3.9 \pm 0.6^{**}$; SNG: $9.7 \pm 0.2^{**}$; $8.1 \pm 0.6^{**}$; Lactose: $5.3 \pm 0.1^{**}$; $4.4 \pm 0.3^{**}$; % Protein: $4.16 \pm 0.03^*$; $3.7 \pm 0.3^{**}$ and Cryoscopy: $0.64 \pm 0.02^{**}$; $0.51 \pm 0.04^{**}$. (**). Presents a significant difference between the results obtained during the three months of sampling (Tukey, $p < 0.05$). The somatic cell count (CCS) of farms A, B, C and D were: 103,937, 162,271, 101,842 and 306,816 (cells/mL) respectively. The colony forming units for farms A, B, C, D and E were: 1.2×10^6 ; 2×10^5 ; 6.6×10^5 ; 8.9×10^6 ; 1.3×10^7 (CFU/mL) respectively. It is concluded that buffalo milk in the southern area of Lake Maracaibo is affected by the month in which the sample is taken, especially the percentage of milk fat. The most outstanding properties of buffalo milk is its high fat content, which presents an average value of $6.0 \pm 0.2\%$ fat; Another outstanding value is the amount of protein around $4.16 \pm 0.03\%$. The microbiological quality indicators indicate that the processes for obtaining milk must be improved and framed in practice that guarantee the safety of raw milk.

Keywords: milk, buffalo, CFU/mL, CCS, quality control.

(SNG), lactosa, proteína, y crioscopia. Algunas de las pruebas mencionadas fueron realizadas de forma directa mediante un Lactoscan. A cada muestra de leche se le determinó la cantidad de células somáticas mediante los métodos de conteo microscópico directo de células somáticas (CCS) y recuento de colonias de bacterias en placa de Petri (COVENIN 1126-1989, COVENIN 902-1987 y García, 2011). Los valores físicoquímicos promedio de los tres muestreos de leche cruda de fincas evaluadas fueron para A, B, C y D respectivamente: Acidez: $17,1 \pm 0,8^{**}$; $17,67 \pm 0,5^*$; $16,8 \pm 0,7^*$; $17,6 \pm 0,5^*$; % Grasa: $5,2 \pm 0,4^{**}$; $6,5 \pm 0,4^{**}$; $6,4 \pm 0,5^{**}$; $6,6 \pm 0,1^{**}$; Sólidos no grasos (SNG): $9,3 \pm 0,1^*$; $9,7 \pm 0,2^*$; $9,9 \pm 0,2^{**}$; $9,7 \pm 0,2^*$; Lactosa: $5,1 \pm 0,1^*$; $5,3 \pm 0,1^*$; $5,4 \pm 0,1^{**}$; $5,3 \pm 0,1^*$; % Proteína: $4,14 \pm 0,03^*$; $4,21 \pm 0,04^*$; $4,25 \pm 0,04^*$; $4,27 \pm 0,06^*$; y Crioscopia: $0,61 \pm 0,01^*$; $0,64 \pm 0,01^*$; $0,66 \pm 0,02^{**}$; $0,65 \pm 0,02^*$. Los promedios de los muestreos de las mezclas de leche E (silo de leche) y F (leche pasteurizada) obtuvieron: Acidez: $17,6 \pm 0,5^*$; $18,3 \pm 0,7^*$; % Grasa: $6,0 \pm 0,2^{**}$; $3,9 \pm 0,6^{**}$; SNG: $9,7 \pm 0,2^{**}$; $8,1 \pm 0,6^{**}$; Lactosa: $5,3 \pm 0,1^{**}$; $4,4 \pm 0,3^{**}$; % Proteína: $4,16 \pm 0,03^*$; $3,7 \pm 0,3^{**}$ y Crioscopia: $0,64 \pm 0,02^{**}$; $0,51 \pm 0,04^{**}$. (**). Presenta diferencia significativa entre los resultados obtenidos durante los tres meses de muestreo (Tukey, $p < 0,05$). El conteo de células somáticas (CCS) de las fincas A, B, C y D fueron: 103.937, 162271, 101.842 y 306.816 (células/mL) respectivamente. Las unidades formadoras de colonias para las fincas A, B, C, D y E fueron: $1,2 \times 10^6$; 2×10^5 ; $6,6 \times 10^5$; $8,9 \times 10^6$; $1,3 \times 10^7$ (UFC/mL) respectivamente. Se concluye que la leche de búfala en la zona del Sur del Lago de Maracaibo se ve afectada por el mes en que se toma la muestra, sobre todo el porcentaje de grasa de la leche. Las propiedades más resaltantes de la leche de búfala es su alto contenido graso, el cual presenta un valor promedio de $6,0 \pm 0,2\%$ de grasa; otro valor resaltante es la cantidad de proteína alrededor de $4,16 \pm 0,03\%$. Los indicadores de calidad microbiológicos indican que los procesos de obtención de leche deben ser mejorados y enmarcados en práctica que garanticen la inocuidad de la leche cruda.

Palabras clave: leche, búfala, UFC/mL, CCS, control de calidad.

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The buffalo supply chain in northeast of Brazil

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ABSTRACT

Studies and analyses of supply chains make it possible to assess the different approaches such as technologies, public and private policies, business strategies, and new arrangements and to identify the issues such as improved performance and competitiveness. The objective of this work was to analyze the buffalo (*Bubalus bubalis*) production chain and the contribution of Porter's Five competitive forces to the buffalo production chain in Northeast Brazil. For that, research was carried out through information obtained in the literature. Interviews and questionnaires were applied to the companies involved in the activity. It was observed that the main limiting factor on the buffalo chain in Northeast Brazil is the need for more information about the species, which makes it difficult for a more accurate analysis. Concerning daily activity, the production chain is well organized. Five small and medium-sized dairies process buffalo milk in the region. Other small-scale enterprises are also found. Regarding buffalo breeding for meat, the numbers associated with the activity in Northeast Brazil need to be clarified since buffalo meat is sold as beef, confusing the buffalo herd with the bovine herd. The commercialization of the buffalo carcass as bovine is the main factor responsible for this condition. Buffalo meat needs to be marketed as buffalo meat for the activity to be recognized. The use of Porter's Five Forces efficiently describes the interconnected processes associated with the buffalo production chain in Northeast Brazil and contributes to improving the chain.

Keywords: *Bubalus bubalis*, buffalo farming, competitive forces, economy.

La cadena de suministro del búfalo en el noreste de Brasil

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RESUMEN

Los estudios y análisis de las cadenas de suministro permiten evaluar los diferentes enfoques, como tecnologías, políticas públicas y privadas, estrategias comerciales y nuevos acuerdos, e identificar cuestiones como la mejora del desempeño y la competitividad. El objetivo de este trabajo fue analizar la cadena productiva del búfalo (*Bubalus bubalis*) y la contribución de las cinco fuerzas competitivas de Porter a la cadena productiva del búfalo en el Nordeste de Brasil. Para ello se realizó la investigación a través de información obtenida en la literatura. Se aplicaron entrevistas y cuestionarios a las empresas involucradas en la actividad. Se observó que el principal factor limitante de la cadena del búfalo en el Nordeste de Brasil es la necesidad de mayor información sobre la especie, lo que dificulta un análisis más preciso. En cuanto a la actividad diaria, la cadena de producción está bien organizada. Cinco pequeñas y medianas lecherías procesan leche de búfala en la región. También se encuentran otras empresas de pequeña escala. En cuanto a la cría de búfalos para carne, es necesario aclarar las cifras asociadas a la actividad en el Nordeste de Brasil, ya que la carne de búfalo se vende como carne vacuna, confundiendo el rebaño de búfalos con el rebaño de bovinos. La comercialización de la canal de búfalo como vacuno es el principal factor responsable de esta condición. La carne de búfalo debe comercializarse como carne de búfalo para que se reconozca la actividad. El uso de las Cinco Fuerzas de Porter es eficiente para describir los procesos interconectados asociados a la cadena de producción de búfalo en el Nordeste de Brasil y contribuir a mejorar la cadena.

Palabras clave: *Bubalus bubalis*, cría de búfalos, fuerzas competitivas, economía.

SUSTAINABILITY & SOCIOECONOMICS

Sustentabilidad y Socioeconomía

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Economic-productive analysis of a mixed cattle-bubaline model mixed model in the Northern region of Corrientes Province in Argentina

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ABSTRACT

The production of buffaloes has been consistently growing in Argentina, with an increase in the number of animals and spread in 19 of the 23 provinces of the country, with an evident regional grouping. The Northeastern region of Argentina contains the provinces of Corrientes, Formosa, Chaco, Misiones, and Entre Ríos, where 88.89% of the buffaloes, with Corrientes having the most significant numbers of animals and farmers. The study aimed to analyze a mixed cattle-bubaline model's economic and productive results. Data from farms in the region was used as the analysis unit, gathering data from infrastructure, management practices, and market situations. Quantitative data (target stocking rate, female replacements) and qualitative data (descriptions) were obtained using a survey within an agreement between the provincial government and the University. A simulation model was run with the gathered data to describe and analyze the system. A method developed by INTA (National Institute of Agriculture and Technology) was used, with an Excel worksheet devel-

Análisis económico-productivo de un modelo mixto bovino-bubalino en la región Norte de la provincia de Corrientes en Argentina

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RESUMEN

La producción de búfalos viene creciendo constantemente en Argentina, con un aumento en el número de animales y distribuida en 19 de las 23 provincias del país, con una evidente agrupación regional. La región Noreste de Argentina contiene las provincias de Corrientes, Formosa, Chaco, Misiones y Entre Ríos, donde se encuentran el 88,89% de los búfalos, siendo Corrientes la que concentra el mayor número de animales y ganaderos. El objetivo del estudio fue analizar los resultados económicos y productivos de un modelo mixto ganado-bubalino. Se utilizaron como unidad de análisis datos de fincas de la región, recopilando datos de infraestructura, prácticas de manejo y situaciones de mercado. Los datos cuantitativos (tasa de ocupación objetivo, hembras de reemplazo) y cualitativos (descripciones) se obtuvieron mediante una encuesta dentro de un acuerdo entre el gobierno provincial y la Universidad. Se ejecutó un modelo de simulación con los datos recopilados para describir y analizar el sistema. Se utilizó un método desarrollado por el INTA (Instituto Nacional de Agricultura y Tecnología), con

oped by the technical team of the Chaco-Formosa Regional Centre and modified afterward at the Economic Department of the Experimental Agriculture Station of Colonia Benítez. The main economic and productive characteristics were obtained, which generated helpful information for designing the productive systems with buffaloes in subproductive areas and allowed for decision-making in the diverse business systems. Highlighting the main differences within the simulation model in terms of key performance indicators for buffaloes: weaning percentage (75 to 80%), female productive life (20 years), replacement percentage (10%), and age at first service (24 months). The cattle subsystem is consistent with the traditional breeding system of the region, whose main objective is the sale of calves and culled cows, while the buffalo subsystem is a complete cycle, which results in the sale of steers surplus of replacement females and culled animals. The mixed cattle-bubaline model is utilized within an area of 3,600 ha and 35% of buffaloes of the whole herd of animals. The meat production per ha was 53 kg, and the stock efficiency was 33%. A gross income of \$55,565/ha, net income of \$3,802/ha, and profitability was 5.1%. In contrast, the cattle activity yielded 48 kg/ha of meat and 23% stock efficiency, \$5,473 gross income, and 3% profitability. Simulations and models were formulated to objectively compare and evaluate the economic and productive performance contributing to the different farming systems' mixed revenue sources. This resulted in the emerging subtropical buffaloes' pasture-based systems from the wetlands, such as those from the region, being improved. In the context of climate change, the global increase in droughts and floods and price volatility constitute a real challenge. To achieve and offer the market a critical mass of cattle and buffalo meat that can reliably demonstrate the production processes carried out, the origin and quality of the product should be achieved by selling meat with more information on current demands. In this way, it will be possible to participate more actively in the formation of prices, partially reversing the power of the current market, focusing on adding value and receiving a price premium for what has been done, generating extra income in buffalo production systems as a livestock activity.

Keywords: model, mixed, cattle, *Bubalus bubalis*, Corrientes, Argentina.

una hoja de cálculo en Excel desarrollada por el equipo técnico del Centro Regional Chaco-Formosa y modificada posteriormente en el Departamento Económico de la Estación Agrícola Experimental de Colonia Benítez. Se obtuvieron las principales características económicas y productivas, las cuales generaron información útil para el diseño de los sistemas productivos con búfalos en áreas subproductivas y permitieron la toma de decisiones en los diversos sistemas empresariales. Resaltando las principales diferencias dentro del modelo de simulación en términos de indicadores clave de desempeño para las búfalas: porcentaje de destete (75 a 80%), vida productiva femenina (20 años), porcentaje de reemplazo (10%) y edad al primer servicio (24 meses). El subsistema bovino es consistente con el sistema de crianza tradicional de la región, cuyo objetivo principal es la venta de terneros y vacas de descarte, mientras que el subsistema bufalino es de ciclo completo, lo que resulta en la venta de novillos bufalinos, excedentes de hembras de reposición y animales de descarte. El modelo mixto vacuno-bubalino se utiliza en un área de 3.600 ha y el 35% de búfalos de todo el rebaño de animales. La producción de carne por hectárea fue de 53 kg y la eficiencia ganadera fue del 33%. Un ingreso bruto de \$55.565/ha, un ingreso neto de \$3.802/ha y una rentabilidad del 5,1%. En contraste, la actividad ganadera rindió 48 kg/ha de carne y 23% de eficiencia ganadera, \$5.473 de ingreso bruto y 3% de rentabilidad. La formulación de simulaciones y modelos se utilizó para comparar y evaluar objetivamente el desempeño económico y productivo que contribuye a las fuentes mixtas de ingresos de los diferentes sistemas agrícolas. Esto resultó en la mejora de los sistemas de pastos de búfalos subtropicales emergentes de los humedales, como los de la región. En el contexto del cambio climático, el aumento global de las sequías e inundaciones y la volatilidad de los precios constituyen un verdadero desafío. Para lograr y ofrecer al mercado una masa crítica de carne de vacuno y búfalo que pueda demostrar fehacientemente los procesos productivos realizados, se debe lograr informar el origen y la calidad del producto vendiendo la carne con mayor información sobre las demandas actuales. De esta manera, se podrá participar más activamente en la formación de precios, revirtiendo parcialmente el poder del mercado actual, enfocándose en agregar valor y recibir un sobreprecio por lo hecho, generando ingresos extras en los sistemas de producción bufalina como una actividad ganadera.

Palabras clave: modelo, mixto, bovinos, *Bubalus bubalis*, Corrientes, Argentina.

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Reasons for decline and measures to promote Nili Ravi buffalo in its breeding tract

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ABSTRACT

The Food and Agricultural Organization (2000) termed buffalo as an important asset that is undervalued. Currently, there are 208 million buffaloes spread across 77 countries; the majority of the buffaloes are found in India (56.67%), followed by Pakistan (19.28%) and China (13.46%). India is endowed with a rich biodiversity of buffalo germplasm in the form of 20 recognized breeds and several distinct local types. Buffalo husbandry plays a crucial role in rural livelihood, food security, and the agricultural economy in India. Despite significant contribution to milk production, a decline in buffalo population was observed in dairy progressive states like Haryana and Punjab from 2012 to 2019, with a 28% and 22% decline, respectively. The breed-wise data revealed the highest decline in the Nili Ravi buffalo of 65%. The breeding area for Nili-Ravi buffaloes is located in the districts of Amritsar, Ferozpur, Tarn Taran, and Gurdaspur in the state of Punjab, India. This decline in the buffalo population is alarming and calls researchers to find reasons for the decline in the Nili Ravi buffalo population and its breeding area, despite the breed performance being at par with any other milk-producing buffalo. In this regard, a study about the reason and extent of the buffalo population decline in the Punjab was carried out. For the study, 180 dairy farmers from Hoshiarpur (31.99%), Tarn Tarn (30.76%), and Ferozpur (29.62%) districts were selected purposively, where the highest decline in buffalo population was reported. Milk production performance, family pride and docile nature were the main reasons for most (~70%) of the selected farmers to keep Nili Ravi buffalo. However, frequent reproductive problems, less resale value, and off-breed characteristics (white patches) were the primary reasons that forced dairy farmers to shift from Nili Ravi to Murrah buffalo. Although both the Murrah and Nili Ravi breeds have similar productive and reproductive parameters, the Murrah buffalo breed is gaining popularity due to its higher resale value and a lack of associated cultural constraints. Thus, it is suggested to incentivize the Nili Ravi buffalo owners by introducing milk recording programs, improving the supply of superior germplasm for this vital breed, and addressing reproductive problems through awareness campaigns and training programs in convergence with research institutes and state animal husbandry departments, to propagate and conserve the Nili Ravi buffalo breed in the state.

Keywords: Nili Ravi, buffalo, Punjab, population, decline, India.

Razones de la disminución y medidas para promover el búfalo Nili Ravi en su zona de reproducción

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RESUMEN

La Organización para la Agricultura y la Alimentación (2000) calificó al búfalo como un activo importante que está infravalorado. Actualmente, hay 208 millones de búfalos repartidos en 77 países; la mayoría de los búfalos se encuentran en la India (56,67%), seguida de Pakistán (19,28%) y China (13,46%). La India está dotada de una rica biodiversidad de germoplasma de búfalo en forma de 20 razas reconocidas y varios tipos locales distintos. La cría de búfalos desempeña un papel crucial en los medios de vida rurales, la seguridad alimentaria y la economía agrícola en la India. A pesar de la importante contribución a la producción de leche, se observó una disminución de la población de búfalos en estados lecheros progresistas como Haryana y Punjab de 2012 a 2019, con una disminución del 28% y el 22%, respectivamente. Los datos raciales revelaron la mayor disminución en el búfalo Nili Ravi del 65%. La zona de reproducción de los búfalos Nili-Ravi se encuentra en los distritos de Amritsar, Ferozpur, Tarn Taran y Gurdaspur en el estado de Punjab, India. Esta disminución en la población de búfalos es alarmante y llama a los investigadores a encontrar razones para la disminución de la población de búfalos Nili Ravi en su zona de reproducción a pesar de que el rendimiento de la raza está a la par con el de cualquier otro búfalo productor de leche. En este sentido, se llevó a cabo un estudio sobre las causas y el alcance de la disminución de la población de búfalos en Punjab. Para el estudio, se seleccionaron intencionalmente 180 productores lecheros de los distritos de Hoshiarpur (31,99%), Tarn Tarn (30,76%) y Ferozpur (29,62%), donde se informó la mayor disminución en la población de búfalos. El rendimiento de la producción de leche, el orgullo familiar y la naturaleza dócil fueron las principales razones por las que la mayoría (~70%) de los agricultores seleccionados mantuvieron el búfalo Nili Ravi. Sin embargo, los frecuentes problemas reproductivos, el menor valor de reventa y las características fuera de raza (manchas blancas) fueron las razones principales que obligaron a los productores de leche a cambiar del búfalo Nili Ravi al búfalo Murrah. A pesar de los parámetros productivos y reproductivos similares de las razas Murrah y Nili Ravi, la raza de búfalo Murrah está ganando más popularidad debido a su mayor valor de reventa y a la ausencia de restricciones culturales asociadas. Por lo tanto, se sugiere incentivar a los propietarios de búfalos Nili Ravi introduciendo programas de registro de leche, mejorando el suministro de germoplasma superior de esta raza vital y abordando los problemas reproductivos.

vos a través de campañas de concientización y programas de capacitación en convergencia con institutos de investigación y departamentos estatales de cría de animales, para propagar y conservar la raza de búfalo Nili Ravi en el estado.

Palabras clave: Nili Ravi, búfalo, Punjab, población, disminución, India.

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Mixed pastures and biological efficiency in buffalo production systems: determinants for a sustainable livestock

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ABSTRACT

Livestock production faces important challenges in addressing the integral concept of sustainability. The buffalo (*Bubalus bubalis*) is recognized for its high productivity standards and its advancement urgently demands the promotion of efficient, environmentally friendly, economically viable and socially acceptable pasture-based systems. The analysis of biological systems is characterized by the use of basic calculations during the production process that define the complex dynamics of natural capital, which is directly managed by human activities with a simplification and selection of biodiversity to support ecosystem function while generating products of agri-food interest. In order to consolidate a conceptual approach to sustainable grazing of *B. bubalis*, 674 specialized texts derived from refereed and indexed scientific journals (83.23%), books or book chapters (14.84%), proceedings of scientific events (0.74%) and other dissemination instruments such as official bulletins and brief notes from institutional pages (1.19%), written in English (86.05%), Spanish (12.02%), Portuguese (1.34%) or other languages (0.59%), were analyzed. By bringing together the conclusive comments of each scientific instrument consulted, the current philosophy of sustainable development to establish sustainable livestock systems requires in particular: a) designing agroecosystems that favor the increase of biodiversity; b) monitoring key indicators of biological efficiency and including the evaluation of feasibility to implement corrective measures; and c) taking maximum advantage of highly biodiverse or mixed pastures under technical control of grazing to minimize the emission of pollutant gases. Well-managed pastures have

Pastos mixtos y eficiencia biológica en sistemas de producción bufalina: determinantes para una ganadería sustentable

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RESUMEN

La producción ganadera enfrenta importantes desafíos para abordar el concepto integral de sostenibilidad. El búfalo (*Bubalus bubalis*) es reconocido por sus altos estándares de productividad y su avance exige urgentemente la promoción de sistemas basados en pastos eficientes, amigables con el medio ambiente, económicamente viables y socialmente aceptables. El análisis de los sistemas biológicos se caracteriza por el uso de cálculos básicos durante el proceso de producción que definen la dinámica compleja del capital natural, que es gestionado directamente por las actividades humanas con una simplificación y selección de la biodiversidad para apoyar la función ecosistémica mientras se generan productos de interés agri-alimentario. Con el fin de consolidar un enfoque conceptual sobre el pastoreo sustentable de *B. bubalis*, se presentaron 674 textos especializados derivados de revistas científicas arbitradas e indexadas (83,23%), libros o capítulos de libros (14,84%), actas de eventos científicos (0,74%) y otros instrumentos de divulgación como boletines oficiales y notas breves de páginas institucionales (1,19%), escritas en inglés (86,05%), español (12,02%), portugués (1,34%) u otros idiomas (0,59%). Al reunir los comentarios concluyentes de cada instrumento científico consultado, la filosofía actual del desarrollo sostenible para establecer sistemas ganaderos sostenibles requiere en particular: a) diseñar agroecosistemas que favorezcan el aumento de la biodiversidad; b) monitorear indicadores clave de eficiencia biológica e incluir la evaluación de la viabilidad de implementar medidas correctivas; y c) aprovechar al máximo los pastos de alta biodiversidad o mixtos bajo control técnico

been promoted as a key strategy to recover in a profitable way the sustainable balance of the agroecosystem, taking into account that grazing has a strong influence on grass composition and, therefore, pasture quality; many researchers consider *B. bubalis* herds as living tools for the remediation of pastures degraded by weed infestation thanks to their resistance and low selectivity that allows modulating grazing areas for remediation. If these recommendations are met, production with *B. bubalis* would offer beneficial ecosystem services that would make the buffalo system a safer, more sustainable and resilient supplier of animal protein, since farms that operate under biological diversification criteria are more resistant to climate change, improve soil health, increase animal productivity and make efficient use of nutrients; however, each system is unique and its dynamism among components varies according to the yield goal sought by the producer according to local conditions.

Keywords: agroecosystem, biodiversity, rotational grazing, sustainability.

del pastoreo para minimizar la emisión de gases contaminantes. Los pastos bien manejados han sido promovidos como una estrategia clave para recuperar de manera rentable el equilibrio sostenible del agroecosistema, teniendo en cuenta que el pastoreo tiene una fuerte influencia en la composición de los pastos y, por tanto, en la calidad de los pastos; muchos investigadores consideran a los rebaños de *B. bubalis* como herramientas vivas para la remediación de pastos degradados por infestación de malezas gracias a su resistencia y baja selectividad que permite modular las áreas de pastoreo para la remediación. De cumplirse estas recomendaciones, la producción con *B. bubalis* ofrecería servicios ecosistémicos beneficiosos que harían del sistema búfalo un proveedor de proteína animal más seguro, sostenible y resiliente, ya que las granjas que operan bajo criterios de diversificación biológica son más resistentes al cambio climático, mejoran la salud del suelo, aumentan la productividad animal y hacen un uso eficiente de los nutrientes; sin embargo, cada sistema es único y su dinamismo entre componentes varía según la meta de rendimiento que busca el productor según las condiciones locales.

Palabras clave: agroecosistema, biodiversidad, pastoreo rotacional, sostenibilidad.

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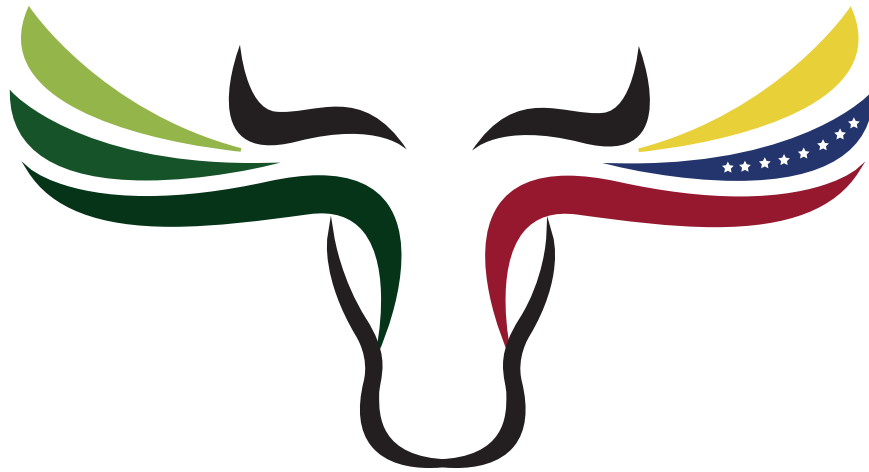
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