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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 buffaloe 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 CH_4 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 CH_4. 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 búfalas 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 CH_4. 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 methanogenic 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 (CH_4) produced by the rumen, beef, and dairy production also contributes carbon dioxide (CO_2; feed), nitrous oxide (N2O; feed production, manure), and other CH_4 (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 CO_2-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

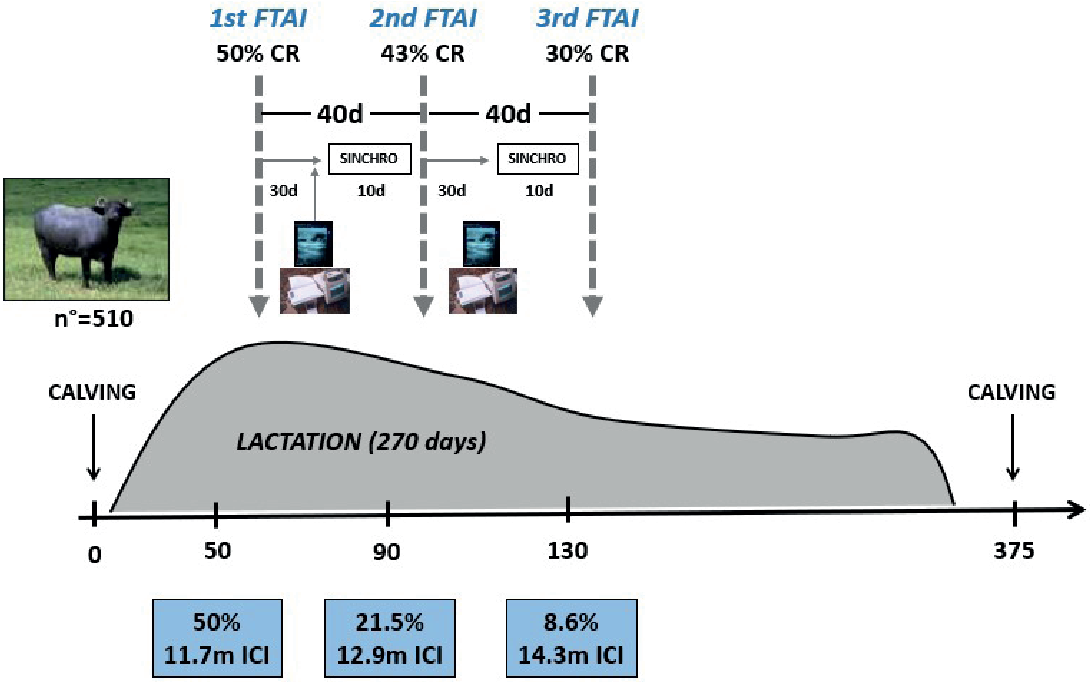

FIGURE 1. Conception rate (P/AI) of lactating buffalo (n=510) submitted to fixed time AI following resynchronization in non-pregnant cows. Ultrasonography evaluation was performed to detect non-pregnant buffalos 30 days after AI for resynchronization. Pregnant buffaloes from the 1st FTAI with 50 days 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 prepuberal 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 point that makes 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 CO_2-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 CO_2 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 CO_2-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 nutrition 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 (N_2O), and CO_2 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 (CO_2, N_2O), and 1.92% for manure (FIG. 2).

As enteric methane has the most significant impact on the production of CO_2 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 CH_4 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 CO_2-eq emitted during the buffalo dairy pro-

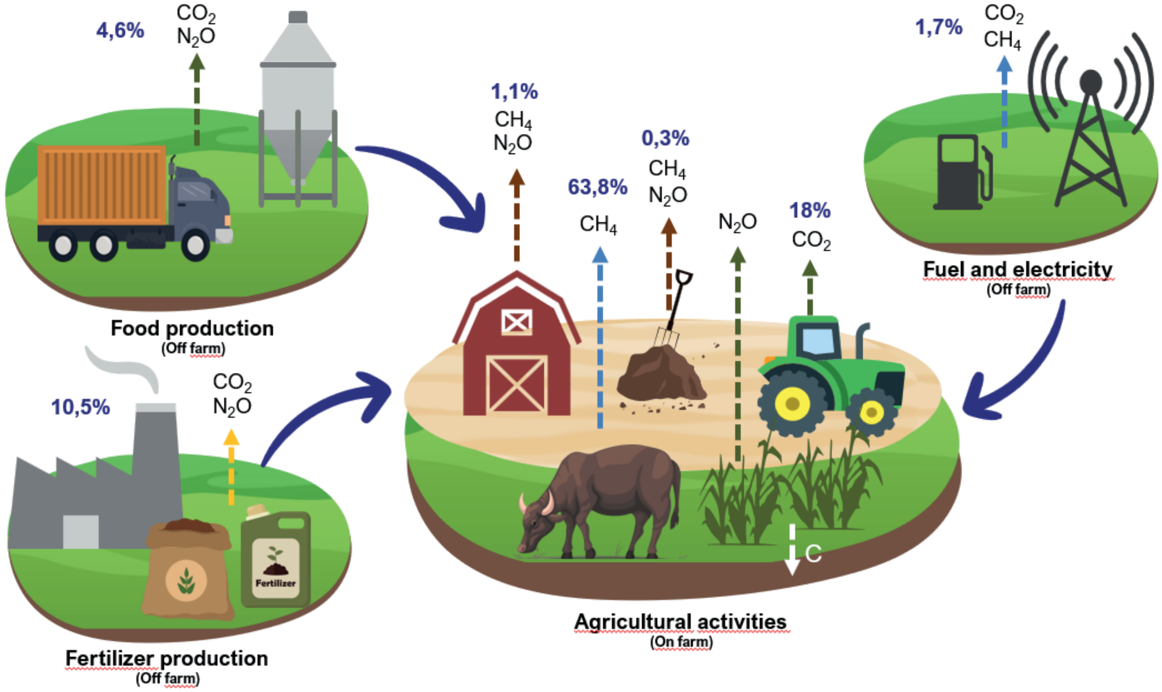

FIGURE 2. Main emission pathways related to a buffalo dairy farm in Brazil, quantified by life cycle assessment (LCA) methodology [Methodology standardized by ISO 14040 (ISO, 2006a) and ISO 14044 (ISO, 2006b); de Vries et al., 2015; Kyttä et al., 2022]. On-farm emissions represent 83.2% (agriculture 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.

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