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In vitro evaluation of agro-industrial by-products as alternative feed sources for guinea pigs (*Cavia porcellus*)

Evaluación *in vitro* de subproductos agroindustriales como ingredientes alternativos en la alimentación de cobayos (*Cavia porcellus*)

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ABSTRACT

The guinea pig (*Cavia porcellus*) is a fiber-adapted herbivorous rodent with a particular digestive system based on cecal fermentation to maximize nutrient utilization. However, Knowledge regarding the nutritional requirements of guinea pigs and the evaluation of feedstuffs remains limited. This study assessed the *in vitro* digestibility of agro-industrial by-products as alternative feed sources for guinea pigs, using a three-phase digestion model simulating gastric, intestinal, and cecal fermentation. Agro-industrial by-products from horticultural sources (broccoli and cabbage) and agricultural sources (corn husk and sugarcane bagasse), as well as a conventional guinea pigs diet, were evaluated. Chemical composition, *in vitro* dry matter digestibility, and gas production throughout cecal fermentation were determined. Significant differences in chemical composition were observed among the tested by-products. Horticultural wastes showed lower fiber and higher crude protein content (19.7 – 22.3 %) compared to agricultural by-products (1.76 – 5.08 %, P < 0.05). *In vitro* dry matter digestibility was higher (67 – 73 %, P < 0.05) in horticultural by-products, whereas gas production was lower in corn husk and sugarcane bagasse (253 – 290 mL/g of DM), exhibiting lower fermentative activity. In conclusion, agro-industrial by-products represent a feasible nutritional alternative for guinea pigs, and horticultural residues demonstrating superior digestibility and fermentative potential (574 – 587 mL/g of DM). These findings support the development of more sustainable and cost-effective feeding strategies for guinea pig production, while also providing a non-invasive alternative that eliminates the need for animal slaughter.

Key words: guinea pigs; *in vitro* digestibility; *in vitro* cecal fermentation; agro-industry by-products.

RESUMEN

El cuy (*Cavia porcellus*) es un roedor herbívoro que posee un sistema digestivo adaptado al consume fibra, basado en la fermentación cecal como mecanismo clave para maximizar la utilización de nutrientes. Sin embargo, la información sobre sus requerimientos nutricionales y la evaluación de los alimentos sigue siendo limitada. Este estudio evaluó la digestibilidad in vitro de subproductos agroindustriales como fuentes alternativas de alimento para cobayos, utilizando un modelo de digestión trifásico que simula la fermentación gástrica, intestinal y cecal. Se evaluaron subproductos agroindustriales de origen hortícola (brócoli y col) y agrícola (hojas envolventes de maíz y bagazo de caña de azúcar), y alimentos convencionales. Se determinó la composición química, la digestibilidad *in vitro* de la materia seca y la producción de gas durante la fermentación cecal. Se observaron diferencias significativas en la composición química entre los subproductos probados. Los residuos de origen hortícolas mostraron un menor contenido de fibra y un mayor contenido de proteína bruta (19.7 – 22.3 %) en comparación con los subproductos agrícolas (1.76 – 5.08 %, P < 0,05). La digestibilidad *in vitro* de la materia seca fue mayor (67 – 73 %, P < 0,05) para los subproductos hortícolas, mientras que la producción de gas fue menor para las hojas envolventes de maíz y el bagazo de caña de azúcar (253 – 290 mL/g de MS), que mostraron una menor actividad fermentativa. En conclusión, los subproductos agroindustriales representan una alternativa nutricional viable para los cobayos y los residuos de origen hortícola demuestran una digestibilidad y un potencial fermentativo superiores (574 – 587 mL/g de MS). Estos valores respaldan el desarrollo de estrategias de alimentación más sostenibles y rentables para la producción de cobayos, además, que proporcionan una alternativa no invasiva que elimina la necesidad de sacrificar animales.

Palabras clave: cobayos; digestibilidad *in vitro*; fermentación *in vitro*; subproductos agroindustriales.

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INTRODUCTION

The guinea pig (*Cavia porcellus*) is a herbivorous rodent with a digestive system specialized for the efficient utilization of high-fiber diets, which are essential to meet its nutritional requirements [1]. However, information regarding its specific nutrient requirements and the nutritional value of the feedstuffs included its diet remains limited [2]. Additionally, most of the available studies consider the guinea pigs as a companion or laboratory animal, leaving aside its importance as a species of zootechnical interest [3, 4].

In the Andean countries of South America, guinea pigs represent a species of significant cultural and economic value and serve as a crucial source of high-quality animal protein in household diets [5]. The feeding and nutritional management of this species have been extensively studied through in vivo digestibility trials, relying on the interactions between the digestive system, intestinal microbiota, and diet composition [1, 2].

Nevertheless, *in vitro* digestibility methodologies, which enable the simulation of digestive processes and yield highly accurate digestibility coefficients, remain poorly established for this species [6, 7, 8].

The three-stage *in vitro* digestibility method comprising acid digestion, intestinal digestion, and cecal fermentation aims to simulate the digestive processes of the guinea pig's gastrointestinal tract by incubating feed samples of known chemical composition with digestive and cecal fluids [7, 9]. Rabbits are often considered a comparable physiological model due to similarities in hindgut fermentation, cecotrophy behavior, and fiber digestion dynamics, all of which are essential for the efficient utilization of fibrous diets [6, 7]. However, unlike the well-established methodologies developed for rabbits, the in vitro determination of digestibility and cecal fermentation in guinea pigs remains insufficiently characterized.

In this study, the *in vitro* methodology proposed by Haro *et al.* [10] was adapted by replacing sheep-derived ruminal fluid with cecal fluid from guinea pigs and utilizing food waste digestion phases as the fermentation substrate. Additionally, the in vitro cecal fermentation of both conventional and non-conventional feedstuffs was assessed, focusing on agro-industrial by-products that are currently discarded but may serve as viable nutritional alternatives for guinea pigs feeding.

The aim of this study was to evaluate the potential agro-industrial by-products as feed ingredients for guinea pigs by analyzing their chemical composition, dry matter digestibility, and cecal fermentation using *in vitro* methods.

MATERIALS AND METHODS

Ethical statement

The trial was conducted at the University of Cuenca, including the Department of Veterinary Medicine and the Bromatology Laboratory, under the supervision of the Animal Production Group. All experimental procedures involving animals were reviewed and approved by the Human Research Ethics Committee of the University of Cuenca (CEISH-UC), Cuenca, Ecuador under the protocol code 2023-016EO-VIUC.

Animals and feeding

The donors of the soft faeces used for dry matter digestion and cecal fermentation in vitro were 10 guinea pigs (558 g ± 7.0 g body weight), fed a mixed diet composed of a forage mix containing 60 % Lolium multiflorum, 13 % *Cenchrus clandestinus*, 25 % *Trifolium repens*, and 2 % miscellaneous weed species, together with commercial concentrate containing 17 % crude protein, 4 % ether extract, 8 % crude fiber, 94 % organic matter, and 87 % dry matter, offered in a 9:1 ratio and adjusted to maintain energy levels [3]. Animals also received a premix of vitamins and minerals during the trial.

Agro-industrial by-products

The feeds analyzed included agro-industrial by-products from horticultural sources (HBP): broccoli (*Brassica oleracea var. italica*) and cabbage (*Brassica oleracea* var. capitata) and agro-industrial by-products from agricultural sources (ABP: corn husk and sugarcane bagasse), sourced from vegetable marketing centers that discarded non-commercial vegetable fractions during their selection processes. In addition, commonly used guinea pig feed ingredients such as ground corn (*Zea mays*) and forage mix were included for comparative purposes. All samples were ground using a CM 290 Cemotec™ mill (FOSS, CM 290, Denmark), equipped with a1 mm sieve, to obtain a uniform particle size for the respective analyses.

Trial design and samplings

In vitro dry matter digestibility and cecal fermentation were assessed using a multi enzymatic procedure to simulate the digestive processes of the guinea pig gastrointestinal tract, based on the modified method described by Ocasio-Vega *et al.* [7] and Haro *et al*. [10]. The process involves three sequential incubation phases simulating gastric digestion, intestinal digestion, and microbial fermentation under anaerobic conditions.

Cecal inoculum preparation

Soft faeces inoculum was obtained from adult guinea pigs through manual collection of soft faeces directly from the rectum at 06:00 h. A maximum of 10 g of soft faeces per animal was collected to preserve animal welfare and sample integrity. Soft faeces from each guinea pig were mixed with Goering and Van Soest [11] culture medium in a proportion of 10 g of soft faeces per 100 mL of medium. The mixture was homogenized for 2 min and filtered through a 46-µm nylon mesh following the procedure described by Ocasio-Vera *et al*. [7]. The inoculum preparation was used for both *in vitro* digestibility and gas production assays.

Trial 1: Dry matter digestibility

Dry matter digestibility was determined using the *in vitro* methodology described by Ocasio-Vera *et al*. [7], adapted by replacing rabbit soft feces inoculum with soft feces inoculum obtained from guinea pigs. For the gastric digestion phase, 250 mg of dry matter (DM) from each feed sample were weighed into 100-mL glass vials. A total of 24 vials containing substrate (four feeds per four inoculum) and vials without substrate (two blanks per inoculum) to correct for endogenous losses.

Each vial received 25 mL of a bicarbonate-buffered solution containing NaHCO3 and KCl (0.1 M, pH 6.0) and 10 mL of 0.2 M HCl, adjusting the final pH to 1.5. Subsequently, 1 mL of a pepsin solution containing 25 mg of porcine pepsin (2000 FIP-U/g, Merck No. 7190) was added to each vial. To prevent bacterial growth during the enzymatic digestion phases, 0.5 mL of a gentamicin solution (0.5 g / 100 mL of ethanol) was included. The vials were sealed with rubber stoppers and incubated at 39 ºC for 1.5 h in a laboratory incubator (Memmert, IN55, Germany). Intestinal digestion (pH 6.8) was simulated by incubating the residues obtained from the gastric phase in a buffer solution containing porcine pancreatin (0.1 g/100 mL; P-7545, Sigma-Aldrich, St. Louis, MO, USA) at 39 ºC for 3.5 h under the same incubation conditions.

Samples predigested with pepsin and pancreatin were filtered and subjected to cecal fermentation to determine DM digestibility. Fermentation vials were filled with 25 mL of inoculum mixture, sealed with rubber stoppers, and incubated at 39 ºC for 19 h under anaerobic conditions maintained by continuous CO2 flushing. After incubation, the residues were filtered and washed with cold water for DM determination.

Trial 2: Gas production kinetics

Gas production kinetics were evaluated following the methodological framework described by Haro *et al*. [10], with modifications consisting of the replacement of sheep ruminal fluid by guinea pig cecal inoculum obtained from soft faeces. Fermentation vials containing the respective substrates were filled with 25 mL of inoculum mixture, sealed with rubber stoppers, and continuously flushed with CO2 to maintain anaerobic conditions throughout incubation.

Gas production was measured using a pressure transducer (Delta Ohm HD2304.0 TP705-2BGI, Herter Instruments SL, Barcelona, Spain) at different time intervals of 2, 4, 8, 16, 24, 48, 72, 96, and 120 h, using four vials per substrate. The potential degradability of DM was estimated using two vials per substrate, following the same incubation protocol, the vials were uncovered at 120 h, filtered, and washed with cold water for DM analysis.

Chemical analyses

The chemical composition of the samples used in the trial was analyzed in triplicate. Dry matter (934.01), ash (942.05), nitrogen (984.13), ether extract (920.39), and gross energy (920.87) were determined according to the procedures outlined by the AOAC [12]. Cell wall components were analyzed following the methods described by Van Soest *et al*. [13].

Calculation, chemical and statistical analyses

Gas production values measured at each time were corrected for the amount of gas produced in the corresponding blanks to correct for endogenous production. Gas production was fitted with time using the exponential model:

$$\mathrm{GAS} = V\,(1 - e^{(-k\,(t-lag))})$$

Where: V is the asymptotic gas production, *k* is the fractional rate of gas production, *lag* is the time before starting gas production, and t is the time of gas measurement. Data fitting was performed using the nonlinear regression procedure (PROC NLIN) of the SAS statistical software (Version 9.4; SAS Institute Inc., Cary, NC, USA) [14].

The average gas production rate (AV) was defined as the rate between the incubation start and the time at which half *V* is reached, and it was calculated as:

$$AV = \frac{V\ K}{2\,(ln\,2 + k\ lag)}$$

Data were analyzed using a linear mixed model using PROC MIXED in SAS. Fixed effects included by-product (By-product), feed type (Feeds), and their interaction (Sub x Feeds), while the random effect was animal. A difference with P < 0.05 was considered significant, and values between 0.05 and 0.10 were interpreted as trends.

RESULTS AND DISCUSSION

The proportion and chemical composition of agro-industrial by-products (broccoli, cabbage, corn husk, and sugarcane bagasse) classified as HBP and ABP, as well as the reference feeds, is shown in TABLE I. By-products had low DM content, which ranged from 6.20 to 33.3 % and was lower (P = 0.011) in HBP than in ABP. Compared with ABP, HBP samples had lower (P < 0.001) OM content but higher (P < 0.001) CP and NSC contents. In general, broccoli and cabbage showed lower EE, NDF, ADF, hemicellulose and cellulose contents.

The gross energy and metabolizable energy contents were similar for all samples (P > 0.05). However, among the by-products the ME values were lower (P = 0.035, ranging from 2.31 to 2.62 Kcal/g of DM). Significant differences were observed among the fractions in both groups. Nevertheless, no differences (P > 0.05) were found in OM, NSC, GE, and ME among feedstuffs. Additionally, the interaction between by-products and feeds showed no significant effects (P > 0.05). However, the effect of by-products varied significantly across different feeds (P < 0.001).

The results of chemical composition align well with the high CP content (ranging 19.7 to 22.3 %) and GE values (ranging 3.60 to 4.23 Kcal/g of DM), as well as the low NDF, ADF y lignin levels previously reported for HBP [2, 15]; and the higher fiber content observed in ABP [2, 16]. However, the cell wall content observed in cabbage was higher than the value reported by De Evan *et al*. [17] for brussels sprouts, white cabbage, savoy cabbage, and red cabbage, which showed average NDF, ADF and lignin contents of 23.8, 15.3, and 1.68 %, respectively. It is well established that factors such as growth stage, seasonality, species, variety, soil type, and fertilization significantly influence the chemical composition of plants [8, 18].

TABLE I

Chemical composition of agro-industrial by-products samples (n = 3) and reference feeds (g/100 g of DM unless otherwise stated)

Item[1]	HBP		ABP		SEM[4]	P value			Referent feeds	
	Broccoli	Cabbage	Corn husk	Sugarcane bagasse		By-product	Feeds	Sub x Feeds	Corn	Forage mix
DM (%)	10.1	6.20	17.9	33.3	1.65	< 0.001	0.011	< 0.001	98.6	
OM	88.8	90.2	96.7	97.5	0.84	< 0.001	0.231	0.748	98.3	14.3
CP	22.3	19.7	5.08	1.76	0.77	< 0.001	0.037	0.100	8.10	87.7
EE,	1.26	2.08	3.21	1.10	0.09	< 0.001	< 0.001	< 0.001	3.45	17.2
NSC2	42.9	33.2	10.2	22.9	1.06	< 0.001	0.208	< 0.001	77.2	2.1
NDF	22.3	33.0	78.7	71.8	0.82	< 0.001	0.036	< 0.001	9.54	13.9
ADF	18.8	21.7	21.7	40.2	0.75	< 0.001	< 0.001	< 0.001	3.20	54.5
ADL	1.97	4.22	10.4	5.38	0.33	< 0.001	0.004	< 0.001	1.10	36.2
HEM	3.56	11.3	56.5	31.6	0.24	< 0.001	< 0.001	< 0.001	6.34	5.12
CEL	16.8	17.5	11.2	34.8	0.58	< 0.001	< 0.001	< 0.001	2.10	18.3
GE (Kcal/g of DM)	3.75	3.60	3.73	4.23	256	0.266	0.510	0.242	4.15	31.1
(ME, Kcal/g of DM)[3]	3.08	2.95	2.31	2.62	208	0.035	0.662	0.331	3.41	4.82
										2.99

[1]HBP: high-value by-products (broccoli and cabbage); ABP: alternative by-products (corn husk and sugarcane bagasse); DM: Dry Matter; OM: Organic Matter; CP: Crude Protein; EE: Extract Ether; NDF: Neutral Detergent Fiber; ADF: Acid detergent Fiber; ADL: Acid Detergent Lignin; HEM: Hemicellulose; CEL: Cellulose; GE: Gross Energy, Kcal/g of DM; [2]Non-structural carbohydrates calculate as 100 – [(100 – OM) + CP + EE + NDF)]. [3]ME: Metabolic energy, Kcal/g of DM, calculated based on an 82% efficiency of GE utilization. SEM[4]: standard error of mean

The gas production parameters, DM intestinal digestibility, and DM digestibility of agro-industrial by-products and reference feeds are shown in TABLE II. Sub x Feeds interaction was only detected for the DM intestinal digestibility and DM digestibility (P < 0.001), whereas a sub x Feeds interaction tended to be detected for the average gas production rate (P = 0.073). No significant differences (P > 0.05) were observed in asymptotic gas production, fractional rate of gas production, or time before starting gas production among the analyzed feeds and Sub x Feeds interaction. Similarly, no differences were detected in the fractional rate of gas production for the by-products (P = 0.660). However, the average gas production rate was higher (P < 0.001) for both by-products and feeds, and a trend towards a greater effect of by-products among different feeds was observed (P = 0.073).

Agro-industrial by-products from horticultural exhibited higher gas production and time before starting gas production (581 mL/g DM and 7.7 h, respectively), indicating greater fermentability compared to ABP (272 mL/g DM and 3.8 h). Although ABP fermented more rapidly than HBP, the overall fermentation effect was greater in ABP, possibly due to a higher content of readily fermentable carbohydrates and more accessible components for microbial degradation [7, 19, 20].

The DM intestinal digestibility and total DM digestibility were significantly higher (P < 0.001) among by-products, feeds, and their interaction. Among by-products, broccoli showed greater intestinal digestibility and total DM digestibility compared to cabbage, corn husk, and sugarcane bagasse (ranging from 35 to 66% for DMID and 43 to 73 % for DMD). These results indicate that HBP are more efficiently digested and utilized in guinea pigs, which is in agreement with the findings reported by Castro-Bedriñana and Chirinos-Peinado [2], who evaluated the *in vivo* digestibility of conventional and non-conventional feeds used in guinea pigs nutrition, with total DMD values ranging from 50 to 97% for forages, 55 to 96 % for protein feeds, and 51 to 89 % for agro-industrial by-products.

The greater digestibility and gas production observed in horticultural by-products may be associated with their lower structural carbohydrate and lignin contents, as well as their higher concentrations of non-structural carbohydrates, which likely improved microbial accessibility and substrate degradation during cecal fermentation. The elevated NDF, ADF, and ADL

TABLE II

Gas production parameters, dry matter intestinal digestibility, and total dry matter digestibility of agro-industrial by-products and reference feeds

Item[1]	HBP		ABP		SEM[2]	P value			Referent feeds	
	Broccoli	Cabbage	Corn husk	Sugarcane bagasse		By-product	Feeds	Sub x Feeds	Corn	Forage mix
V (mL/g DM)	587	574	290	253	21.92	< 0.001	0.277	0.587	536	297
k (%/h)	0.019	0.015	0.020	0.017	0.002	0.660	0.175	0.566	0.034	0.016
Lag (h)	7.32	8.12	3.14	4.41	0.924	< 0.001	0.288	0.803	10.8	6.31
AV (mL/h)	7.13	5.60	3.76	3.10	0.214	< 0.001	< 0.001	0.073	8.33	3.77
DMID (%)	61	66	52	35	0.763	< 0.001	< 0.001	< 0.001	63	53
DMD (%)	67	73	59	43	0.987	< 0.001	< 0.001	< 0.001	75	58

[1]HBP: high-value by-products (broccoli and cabbage); ABP: alternative by-products (corn husk and sugarcane bagasse); *V*: asymptotic gas production; *k*: fractional rate of gas production; *Lag*: time before starting gas production; *AV*: average gas production rate; DMID: dry matter intestinal digestibility; DMD: dry matter digestibility; [2]SEM: standard error of the mean

contents observed in corn husk and sugarcane bagasse may have reduced microbial attachment and limited enzymatic hydrolysis of cell wall polysaccharides, thereby decreasing fermentability and nutrient utilization. In guinea pigs, cecal fermentation constitutes a key digestive process for the utilization of fibrous feeds and the production of short-chain fatty acids [2, 7, 19, 20]. Therefore, the differences observed in fermentation kinetics among substrates may reflect variations in the efficiency of microbial fermentation within the cecal environment.

Kinetics gas production of agro-industrial by-products over 120 h of incubation is shown in FIG. 1. Different fermentation patterns were observed among the evaluated substrates. The broccoli and cabbage showed highest cumulative gas production, reaching approximately 533 and 483 mL/g of DM, respectively. This fermentation performance was similar to that of corn source, which showed the highest value at 523 mL/ g of DM. Conversely, corn husk, sugarcane bagasse and the forage mix exhibited the lowest gas production values, remaining below 300 g of DM throughout the incubation period.

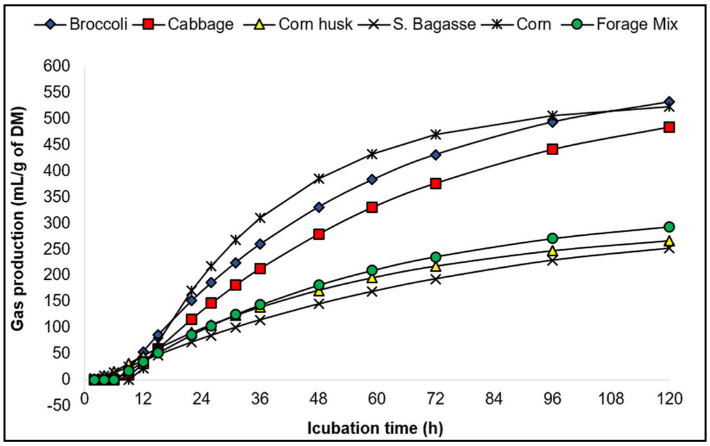

FIGURE 1. *In vitro* gas production kinetics (mL/g DM) of agro-industrial by-products and reference feeds over 120 h of incubation. Broccoli: broccoli by-product; Cabbage: cabbage by-product; Corn husk: corn husk; S. bagasse: sugarcane bagasse; Corn: ground corn; Forage mix: mixed forage diet

In vitro fermentation and gas production measurements are widely used in ruminants to assess feed digestibility and fermentability [10]. Nevertheless, their application in guinea pigs remains limited. To the best of current knowledge, the kinetics feeds fermentation in guinea pigs have not been previously studied. However, similar assays have been conducted in rabbits, a species with a comparable fiber digestion and fermentation process. Studies by Abad-Guzmán *et al*. [6] and Ocasio-Vega *et al.* [7] demonstrated that using soft faeces inoculum yielded fermentation and digestibility parameters comparable to those observed for feed used in this trial.

The rapid gas accumulation observed within the first 24 h in agro-industrial by-products and reference feed expressed a high fermentative potential, likely due to the presence of readily fermentable carbohydrates and accessible fiber fraction [8, 21]. The slow fermentation rates observed in corn husk, sugarcane bagasse, and the forage mix shown a lower availability of fermentable substrates, likely due to higher fiber crystallinity and reduced microbial accessibility [22, 23]. Furthermore, the structural carbohydrate composition and degree of lignification may have played a crucial role in shaping these fermentation patterns [8].

CONCLUSION

Horticultural agro-industrial by-products, particularly broccoli and cabbage, showed higher crude protein concentrations, dry matter digestibility (67 – 73 %), and asymptotic gas production values (574 – 587 mL/g of DM) than corn husk and sugarcane bagasse. Corn husk and sugarcane bagasse showed lower digestibility and fermentative activity, which may limit their nutritional utilization. Additionally, the three-stage *in vitro* methodology proved to be an effective non-invasive approach for evaluating digestibility and cecal fermentation of feedstuffs in guinea pigs without requiring animal slaughter.

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Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

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