SHEEP FOOD SUPPLEMENT OBTAINED BY SOLID STATE FERMENTATION (SSF) BASED ON SUGAR CANE POST-HARVEST WASTE†

[SUPLEMENTO ALIMENTICIO PARA OVINOS OBTENIDO POR FERMENTACIÓN EN ESTADO SÓLIDO (FES) CON BASE EN RESIDUOS POSCOSECHA DE CAÑA DE AZÚCAR]

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SUMMARY

Background: The growing demand for foods such as corn or soy has favored the study and implementation of biotechnologies that use agroindustrial waste and transform them so that they can be used to obtain alternative diets for animals with a high biological value. **Objective:** To increase the feed value of sugarcane bagasse through fermentation with probiotic microorganisms under solid-state conditions. **Methodology:** A diet based on by-products of sugarcane bagasse harvest treated by solid-state fermentation (SSF) was prepared. The fibrous material was collected, dried, ground, and mixed in a proportion of 1.0 and 2.0 inclusion with alfalfa flour (1.5), molasses (0.25), sodium sulfate (0.05), calcium carbonate (0.05), mineralized salt (0.05), urea (0.15), microbial preparation (0.25), and potato (6.7 or 5.7, respectively). The prepared feed was subjected to FES and evaluated by compositional and microbiological analysis. The effect of the percentage of sugarcane bagasse inclusion on obtaining dry matter, humidity, ash, ethereal extracts, crude protein, neutral and acid detergent fiber, organic matter and in situ digestibility of dry matter was analyzed. **Results:** The inclusion percentage significantly affects the production of ash (7.23 and 6.63%, respectively), crude protein (16.3 and 14.1%, respectively), organic matter (92.7 and 93.3%, respectively), and in situ digestibility (74.6 and 63.8, respectively). The microbiological analysis determined that the count of lactic acid bacteria and aerobic mesophiles increased with fermentation time; no growth of molds, yeasts, or *Salmonella* was observed. **Implications:** Solid-state fermentation proves its value as a sustainable strategy, a cost-effective alternative, and an easily applicable approach for the utilization and valorization of agroindustrial waste by incorporating it into functional diets for sheep**. Conclusion:** Under the conditions of this study, it was demonstrated that the biological value of sugarcane bagasse can be effectively increased through solid- state fermentation when combined with other raw materials, making it a viable component in formulating diets for sheep.
Key words: Agroindustrial waste; bagasse; processing; *Lactobacillus*; animal nutrition.

RESUMEN

Antecedentes: La creciente demanda de alimentos como maíz o soya ha favorecido el estudio e implementación de biotecnologías que utilicen residuos agroindustriales y los transformen de forma que puedan ser aprovechados para obtener dietas alternativas para animales con un alto valor biológico. **Objetivo:** Incrementar el valor alimenticio del bagazo de caña de azúcar mediante fermentación con microorganismos probióticos en condiciones de estado sólido.

† *Submitted May 21, 2024 – Accepted July 2, 2025.* http://doi.org/10.56369/tsaes.5648

ISSN: 1870-0462.
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Metodología: Se preparó una dieta basada en subproductos de la cosecha de bagazo de caña de azúcar tratados mediante fermentación en estado sólido (FES). Se recolectó material fibroso, se secó, molió y mezcló en proporción de 1.0 y 2.0 de inclusión con harina de alfalfa (1.5), melaza (0.25), sulfato de sodio (0.05), carbonato de calcio (0.05), sal mineralizada (0.05), urea (0.15), preparado microbiano (0.25) y papa (6.7 or 5.7, respectivamente). El alimento preparado se sometió a FES y se evaluó por medio de análisis composicional y microbiológico. Se analizó el efecto del porcentaje de inclusión de bagazo de caña en la obtención de materia seca, humedad, cenizas, extractos etéreos, proteína cruda, fibra detergente neutra y ácida, materia orgánica y digestibilidad *in situ* de materia seca. **Resultados:** Se encontró que el porcentaje de inclusión afecta significativamente la producción de cenizas (7.23 and 6.63%, respectivamente), proteína cruda (16.3 and 14.1%, respectivamente), materia orgánica (92.7 and 93.3%, respectivamente) y digestibilidad *in situ* (74.6 and 63.8, respectivamente). El análisis microbiológico determinó que el recuento de bacterias ácido lácticas y de mesófilos aerobios aumentó con el transcurrir del tiempo de la fermentación, no se observó crecimiento de mohos, levaduras ni *Salmonella*. **Implicaciones:** La fermentación en estado sólido demuestra su valor como estrategia sostenible, alternativa rentable y de fácil implementación para el aprovechamiento y valorización de residuos agroindustriales, al integrarlos en dietas funcionales para ovinos. **Conclusión:** Bajo las condiciones de este estudio, se demostró que el valor biológico del bagazo de caña de azúcar puede incrementarse eficazmente mediante fermentación en estado sólido al combinarlo con otras materias primas, lo que lo convierte en un componente viable para la formulación de dietas para ovinos.
Palabras clave: Residuos agroindustriales; bagazo; procesamiento; *Lactobacilos*; nutrición animal.

INTRODUCTION

The sugarcane (*Saccharum officinarum*) is significant in over 130 countries, covering approximately 1.9% of the total cultivated area globally (Gómez-Merino *et al*., 2017). In Colombia, it is predominantly cultivated in Santander, Cundinamarca, Antioquia, and Boyacá. In 2019, 200,470 hectares were cultivated, yielding 1,098,207 tons of panela (Rodríguez *et al*., 2019). It occupies the second place in cultivated areas after coffee (Barona *et al*., 2021). Sugarcane is utilized for bioethanol production, sugar for agroindustry, human consumption, and as forage for cattle (Fernández *et al*., 2016; Giacomini *et al*., 2014).

During the sugarcane harvesting process, it is known that to produce 8 million tons of sugar, over 70 million tons of sugarcane must be crushed, resulting in around 20 million tons of bagasse. Typically left in the field, bagasse becomes an agricultural waste, often burned before cutting to maximize harvest productivity. Due to the limited development of mechanized sugarcane-cutting technologies, there is a lack of assistance in the collection, preparation, and handling of agroindustrial waste (Aguilar *et al.,* 2016; Munagala and Yogendra, 2020).

The processing of this crop reveals a substantial amount of underutilized raw material with unknown nutritional value. An economically viable option is to utilize these agricultural residues as low-cost raw materials for animal feed. Through simple biotechnologies like solid-state fermentation (SSF), the nutritional value, including energy and protein, can be enhanced, potentially replacing or minimizing the inclusion of raw materials such as corn and soybeans in animal feed—raw materials also used for human consumption (Álvarez *et al*., 2025).

Considering animal proteins as a global food source, indicators for Colombia show a per capita consumption of 17.1 kg of beef, 36.3 kg of chicken, and 13 kg of pork (Fedegan, 2022). Although relatively low, these figures indicate the large number of animals required to meet national demand, which in turn increases the need for feed derived from raw materials that are often also suitable for human consumption.

The digestibility of raw materials depends on their lignin content. In general, plant biomass contains high levels of lignocellulosic material, comprising cellulose, hemicellulose, and lignin, which form structurally complex matrices that are difficult to degrade, thereby limiting their nutritional availability for animals (Buxton and Redfearn, 1997). Specifically, sugarcane bagasse is rich in lignocellulosic compounds, and its high fiber content and compact structure make it particularly resistant to enzymatic degradation (Alokika *et al*., 2021). This recalcitrance represents a constraint for its direct use in ruminant feed (Paroha *et al*., 2020).

Fiber is a key component in ruminant diets, playing a vital role not only as an energy source but also in maintaining rumen function and overall animal health. Digestible fiber, primarily cellulose and hemicellulose, can be fermented by rumen microbiota to produce volatile fatty acids (VFAs), which serve as the main energy substrates for ruminants. However, the presence of lignin and the complex structure of plant cell walls often reduce fiber digestibility, thus limiting its nutritional value (Alves *et al*., 2016). This challenge is particularly relevant for sugarcane bagasse, which, despite its abundance, exhibits low digestibility due to its high lignin content (Paroha *et al*., 2020).

Processing fibrous post-harvest residues through solid-state fermentation (SSF) using probiotic microorganisms presents a promising approach for producing nutrient-enriched, cost-effective feed for small ruminants, such as sheep and goats. Therefore, this study aimed to improve the nutritional value of sugarcane bagasse through solid-state fermentation in the presence of probiotic microorganisms.

MATERIALS AND METHODS

Preparation of food supplement with sugarcane bagasse

The food supplement was prepared using finely milled sugarcane bagasse from Socotá-Boyacá, Colombia. Ingredients, energy sources, and the lactic acid solution from the microbial preparation reported by Borrás-Sandoval *et al*. (2017) were added and mixed in two clean containers, as detailed in Table 1. The content was mixed until a homogeneous paste was obtained. Aliquots of 120 g were taken, placed in loosely sealed plastic bags, and incubated at 20 °C for 48 h. Each bag represented an experimental unit, and two treatments with three replicates were performed (Table 2).

Table 1. Components and inclusion percentage evaluated in the solid-state fermentation of bagasse.

Component	Inclusion percentage T1	T2
Cane bagasse	10	20
Alfalfa flour	15	15
Urea	1.5	1.5
Sodium chloride (NaCl)	0.5	0.5
Molasses	2.5	2.5
Microbial preparation	2.5	2.5
Sodium sulfate (Na_2SO_4)	0.5	0.5
Calcium carbonate ($CaCO_3$)	0.5	0.5
Potato	67	57

T: Treatments; T1: 10% sugarcane bagasse, T2: 20% sugarcane bagasse

Physicochemical Analysis of the Food Supplement

Physicochemical analyses included pH measurement, humidity (%H), dry matter (%DM), ash, crude protein (%CP), ether extracts (%EE), neutral detergent fiber (%NDF), acid detergent fiber (%ADF) (Van Soest *et al*., 1991), organic matter, in situ dry matter digestibility, and microbiological analysis. The latter determined the presence or absence of mesophilic aerobes (CFU/g) (AOAC, 2005), total and fecal coliforms (NMP) (Navarro, 2017), *Clostridium* sulfite reducer spores (CFU/g) (ISO 15213:2003), fungi and yeasts (CFU/g) (Bird *et al*., 2015), *Salmonella* (A-P/25g) (Mooijman *et al*., 2019), and mesophilic lactic acid bacteria (CFU/g) (Blagoeva *et al*., 2014).

To measure pH at each fermentation time (0 h, 24 and 48 h after incubation at 20°C), 5 g of the sample were taken, placed in Erlenmeyer flasks (100 ml) with the addition of 45 ml of sterile distilled water, and agitated at 120 rpm for 30 minutes on a stirring plate. pH was measured using an Okaton® automatic potentiometer. The remaining 115 g of each sample was used for bromatological and microbiological analyses.

Table 2. Treatments based on inclusion percentage of sugarcane bagasse, temperature, and fermentation time.

Treatment	Inclusion percentage	Conditions Time (H)	Temperature (°C)
1	10	0	20
2	20		
3	10	24	
4	20		
5	10	48	
6	20		

Statistical analysis

A randomized complete block design was employed, and analysis of variance (ANOVA) was conducted. Tukey's test was conducted for treatments showing statistically significant differences to understand the effect of inclusion percentage on obtaining H, DM, ash, EE, CP, NDF, ADF, organic matter, and *in situ* digestibility. The data were analyzed using the statistical program R for Windows.

RESULTS AND DISCUSSION

During the fermentation of the dietary supplement, it was observed that the inclusion percentage of sugarcane bagasse did not significantly affect the pH. In the performed assays, fermentation started at a pH of 6.3, which steadily decreased to 5.4 and 5.3 at 24 h and further to 5.1 and 5.2 at 48 hours, respectively, for each percentage inclusion of sugarcane bagasse. This gradual decrease in pH may be attributed to the metabolic activity of microorganisms, as reported by Carrasco *et al*. (2003). Lactic acid bacteria (LAB) are associated with a progressive decline in pH during fermentation, resulting from the production of organic acids, including lactic, acetic, and butyric acids. These metabolic byproducts are formed when LAB

and certain filamentous fungi consume simple sugars and polysaccharides, coverting them into acids under low-moisture conditions (Wang *et al*., 2021, Álvarez *et al*., 2025). Key advantages of this pH reduction include the inhibition of undesirable or pathogenic microorganisms, generally not resistant to acidic environments (Astashkina *et al*., 2014, Newaj *et al*., 2014, Tripathi and Giri, 2014), an increase of LAB populations (Velázquez-López *et al*., 2018) and substrate transformation through the action of microbial enzymes with differential activities (Barrios-Gonzalez, 2012). Furthermore, the increasingly acidic environment contributes to the preservation of dietary supplements, which is essential for maintaining the functional efficiency of the fermentation system (Caplice and Fitzgerald, 1999, Caicedo *et al*., 2019, Cubillos *et al*., 2024).

Ash, ether extracts, crude protein, and organic matter.

In the formulation of a dietary supplement, it is essential to understand the purity of the ingredients (ash), fat content (EE), enhancing energy utilization efficiency, improving nutrient absorption, and feed quality (CP) related to the growth of microbiota in the supplement. Analyzing each percentage inclusion of bagasse (10% and 20%) separately in the dietary supplement, it is observed that ash, EE, and organic matter remain stable during the fermentation process (Figure 1). This stability may be attributed to the metabolic limitations of LAB because these components are not directly involved in their metabolic pathways. No studies have been found that specifically use LAB to monitor, over time, the contents of ash, ether extract (EE), and total organic matter, while simultaneously reporting their stability. Most research involving LAB focuses on parameters such as pH, lactic acid production, protein content, and fiber degradation, which are more directly affected by their metabolic activity. This gap in the literature may be due to the biochemical nature of LAB, which are not known to produce cellulolytic, ligninolytic, or lipolytic enzymes (Tarraran and Mazzoli, 2018). Accordingly, no significant changes in ash, EE, or organic matter are observed over time in systems inoculated solely with LAB, regardless of the bagasse concentration used.

However, when comparing treatments with 10% and 20% bagasse inclusion, analysis of variance indicates statistically significant effects based on the percentage inclusion of bagasse in the dietary supplement for ash (P=0.033, R^2=71.68), CP (P=0.006, R^2=87.50), organic matter (P=0.033, R^2=71.68), and in situ digestibility (P=0.007, R^2=86.45). A statistical comparison of means reveals that using 10% sugarcane bagasse yields the highest values for ash and CP, suggesting that microbial synthesis of CP from carbohydrates and urea nitrogen occurs at this inclusion percentage (Table 3, Table S1). Supporting evidence emerges from studies involving treatments of bagasse with urea and molasses, which demonstrate significant increases in CP and ash. For instance, Ratchataporn *et al*. (2018) found that treatments with 5 % urea significantly augmented bagasse CP, attributed to microbial proliferation harnessing added nitrogen and readily fermentable carbohydrates. Similarly, Ahmed *et al*. (2013) reported that inclusion of urea increased CP and improved fiber degradability, suggesting microbial conversion of carbohydrates into protein rather than direct assimilation of feed nitrogen alone.

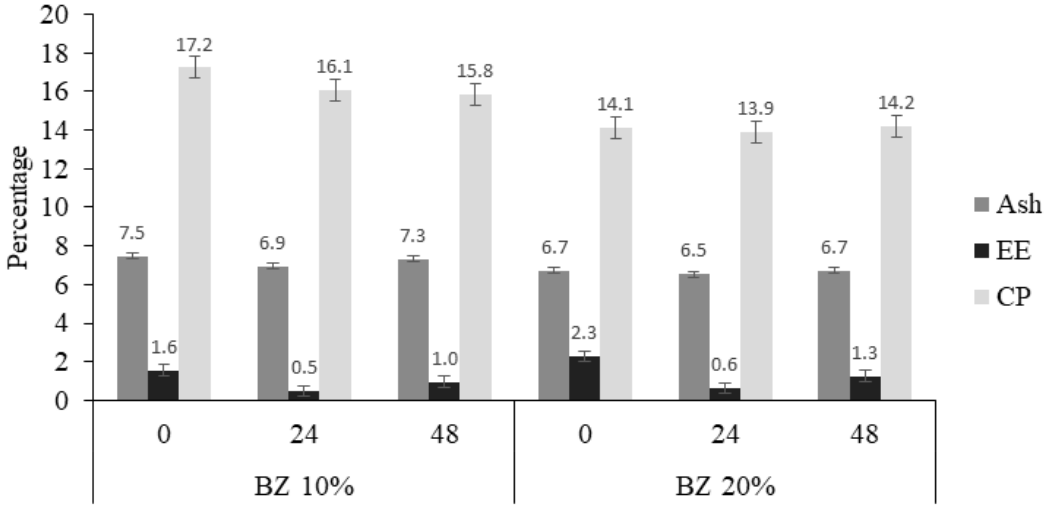

Figure 1. Content of ash, ether extracts (EE), and crude protein (CP) in each percentage inclusion of sugarcane bagasse at the beginning (0 h), 24 h, and 48 h of solid-state fermentation of the dietary supplement.

Importantly, since the bagasse was not sterilized before fermentation, native microbial populations may have remained viable. Among them, cellulolytic fungi, *such as Aspergillus* and *Trichoderma*, could potentially have been present, although their presence was not specifically confirmed in this study (Valiño *et al*., 2002). These microorganisms are known to secrete extracellular enzymes, such as cellulases and laccases, which can contribute to the partial degradation of lignocellulosic components and influence the composition of the extractable fraction. This may explain why treatments with lower bagasse loading (e.g., 10%) show increased values of ash and organic matter. These differences are not the result of LAB activity, but rather reflect the improved aeration, moisture distribution, and microbial accessibility associated with lower substrate loads—factors that are crucial for efficient microbial colonization and enzymatic action in SSF systems (Oiza *et al*., 2022). In contrast, a higher solid loading (20%) results in a denser, more compact matrix that limits microbial colonisation and enzymatic diffusion, reducing extractability.

Table 3. Effect of the inclusion percentage of bagasse on evaluated bromatological indicators.

Indicators (%)	Sugarcane bagasse (Inclusion)		EE + Sign.
	10%	20%	
Ash	7.23[a]	6.63[b]	
Crude protein	16.3[a]	14.1[b]	±0.05; p<0.05
Organic matter	92.7[b]	93.3[a]	
In situ digestibility	74.6[a]	63.8[b]	

Other indicators show no statistically significant differences (±0.05; p>0.05).

Humidity, dry matter, neutral detergent fiber, and acid detergent fiber.

In fermentation processes, humidity (H) is crucial for dissolving nutrients uniformly in the medium, making them available to microorganisms, and maintaining homogeneous conditions such as pH, temperature, and culture concentration (Carrasco *et al*., 2003). When using 10% bagasse, a 14.6 percentage point decrease in H was observed at 48 h compared to hour 0, while including 20% bagasse maintains constant H during fermentation (Figure 2). Similar results regarding H reduction at the same fermentation time were reported by Borrás e*t al*. (2021) using residual harvest raw materials in solid-state fermentation. The decrease can be attributed to the action of microorganisms in the microbial preparation, as well as the addition of urea diluted in distilled water to the microbial preparation and the dietary supplement.

Proportionally, there is a 14.6% increase in dry matter at 48 h compared to hour 0 when adding 10% bagasse, while 20% sugarcane bagasse keeps dry matter stable (Figure 2). This can be explained by the addition of water to the preparation, which dilutes urea (Ramos et al., 2007), and the release of additional water due to metabolic processes developed by microorganisms over time. Rodríguez (2005) reported that microbial action reduces dry matter in solid-state fermentation over time, producing water, CO_2, and volatile fatty acids by utilizing sugars (sucrose, glucose, fructose) and starch in their metabolic processes.

Other reports using sugarcane in solid-state fermentation processes show a 2.0 percentage point decrease in dry matter compared to the initial dry matter for Sacchasorgo and Sacchapulido (Ramos *et al*., 2006). Dry matter reduction has also been reported in Sacchaboniato (Rodríguez, 2005) and Saccharina (Elías *et al*., 1990) as fermentation time increases.

ADF and NDF values are primarily used to calculate the amount of forage that animals can digest, including total digestible nutrients and other energy values. Analyzing neutral detergent fiber determines the composition and quantity of hemicellulose, cellulose, and lignin—less digestible compounds—and digestible cell contents formed by starch and sugars. NDF indicates volume and, consequently, food intake, while ADF indicates digestibility and energy intake.

In this study, fiber remained within permissible ranges for the supplement to be digestible and utilized by sheep. However, considering that as ADF increases, digestibility decreases, it is evident that using 20% bagasse increases ADF by 13.3 percentage points at 48 h of fermentation, while with 10% bagasse, it decreases by 5.4 percentage points (Figure 2). This suggests that animals digest the dietary supplement more easily when including 10% sugarcane bagasse. The increase in fiber values is due to a significant increase in the content of cell walls over fermentation time (Hernández, 2010), relative to the starch content of the used product, thus varying the percentages.

Statistical analysis for H, DM, NDF, and ADF factors indicates no statistically significant differences based on the percentage inclusion of sugarcane bagasse. In other words, values for each analyzed factor are equal or show minimal variation with both inclusion percentages.

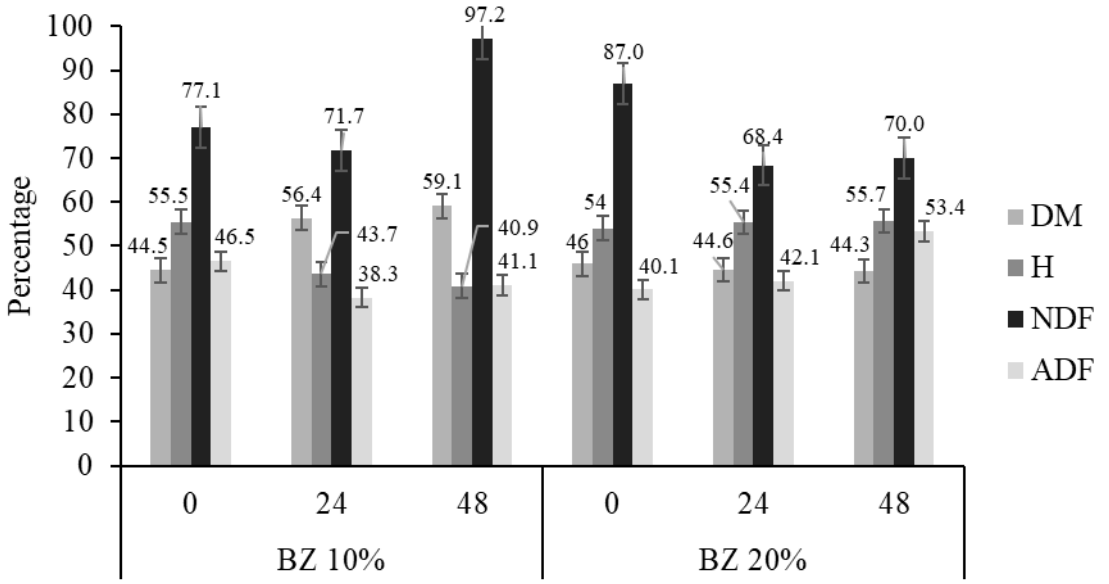

Figure 2. Content of dry matter (DM), humidity (H), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in each percentage inclusion of sugarcane bagasse at the beginning (0 h), 24 h, and 48 h of solid-state fermentation of the dietary supplement.

Finally, results on the in-situ digestibility of DM indicate that a 10% inclusion of sugarcane bagasse increases digestibility by 2.3 percentage points at 48 h of fermentation. In comparison, a 20% inclusion decreases digestibility by 6.9% at the end of the fermentation process. Studies, such as those by Attaelmnan and Ismeal (2019), evaluated sugarcane bagasse treated with 5% urea as a partial replacement for sorghum grain in goat diets. They found that replacing 10 % of sorghum grain with bagasse (approximately 2.6 % of the total ration) maintained moderate to high DM digestibility, around 84 % at 48 h, which was only slightly lower than the control diet's 88.5 %, representing a modest decrease of 4.5 percentage points. These results suggest that at higher inclusion levels, the increased lignocellulosic content dilutes the concentration of fermentable substrates and restricts microbial access, thereby reducing overall DM degradability. This pattern aligns with the results of the present study, which followed a 2.3-point rise in digestibility at a 10 % inclusion rate, indicating that moderate levels of sugarcane bagasse can maintain fermentability without significantly compromising digestibility.

However, raising the inclusion rate to 20 % or higher appears to have a detrimental effect on digestibility. Attaelmnan and Ismeal (2019) documented that higher bagasse substitution levels (20 % and 30 %) progressively decreased in situ digestibility, and *in vitro* values ranged from 78 % down to 74.5 %, indicating a drop of up to ~6–8 percentage points by the end of the fermentation/incubation period. This agrees qualitatively with our results of 6.9 % decline at 20 % inclusion, reinforcing the concept of a threshold beyond which bagasse dilutes the available fermentable substrate and impairs microbial access.

In a statistically significant effect on the inclusion percentage, with higher digestibility values obtained using 10% sugarcane bagasse inclusion in the dietary supplement is optimal, given that the raw material is of very low nutritional content, economical, and easy to prepare.

Microbiology

The microbiological analysis shows the quality and innocuity of the dietary supplement, aligning with established fermentation dynamics observed in ensiled forages, where LAB dominate and inhibit pathogenic organisms. LAB rapidly acidifies the environment by producing organic acids and bacteriocins, which inhibit the growth of spoilage moulds, yeasts, *Clostridium* spores, and coliforms (Kim *et al*., 2021; Mokoena *et al*., 2021). This mechanistic understanding supports our results, which indicate freedom from bacteria such as *Salmonella* and a decrease in the presence of moulds, yeasts, *Clostridium* spores, faecal, and total coliforms as fermentation time increases. This is important because those microorganisms could cause diseases in animals consuming the feed. Additionally, regarding the count of aerobic mesophiles, a higher quantity is

observed in the treatment with 10% bagasse at 24 h (5.2×10^3 CFU/g) compared to the treatment with 20% inclusion at 48 h (3.4×10^3 CFU/g). These results are consistent with expected fermentation dynamics: initial increases in mesophilic populations reflect active microbial proliferation, particularly of LAB, followed by stabilization or reduction as acid accumulation restricts aerobic microbial growth (Pérez *et al*., 2005). In fact, well-established anaerobic conditions driven by LAB activity tend to suppress aerobic populations after the early stages of fermentation.

For the lactic acid bacteria count, the highest quantity is evident at 24 h of fermentation (8.6×10^3 CFU/g and 3.1×10^4 CFU/g, Table S2) for the 10% and 20% inclusion treatments, respectively. This result indicates the safety of the feed and its suitability as a diet for sheep; a pH decreases and an increase in beneficial probiotic bacteria were observed. High LAB populations are crucial for rapid acidification, pathogen suppression, and the development of a stable, probiotic-rich final product (Mokoena *et al*., 2021).

CONCLUSION

Under the conditions of this study, it was demonstrated that the dietary supplement, which includes 10% sugarcane bagasse and is subject to solid-state fermentation, is optimal for utilizing post-harvest residue of low commercial value, as the nutritional value of the bagasse is increased. Additionally, the dietary supplement was obtained at an economical cost compared to commercial balanced diets.

Funding. We thank the Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación Francisco José de Caldas and the Gobernación de Boyacá for financial support for the project development “Desarrollo de un alimento altamente digestible para la alimentación ovina desde residuos post-cosecha”, contingent recovery financing contract No. 80740-658-2020 and SGI code 2993. A Rodriguez is doctorate student of chemical sciences of the science faculty of the Universidad Pedagógica y Tecnológica de Colombia-Tunja and acknowledge to the program.

Conflicts of interest and authorship contributions. The manuscript was prepared and reviewed with the participation of the authors who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

Compliance with ethical standards. This work does not require approval by a bioethical committee.

Data availability. Data is available upon reasonable request to the corresponding author

Author contribution statement (CRediT). L.M. Gómez-Martínez – Methodology, Investigation, Writing – original draft. **A. Rodríguez-Montaña** – Methodology, Investigation**. J.J. Martínez** – Conceptualization, Funding acquisition, Resources, Writing – review & editing. **L.Y. Rache-Cardenal** – Data curation, Formal analysis, Writing – review & editing. **L.M. Borrás-Sandoval** – Conceptualization, Funding acquisition, Resources, Writing – review & editing.

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