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ORIGINAL ARTICLE

Effect of the *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* on corn and sorghum silage quality and sheep energy partition under tropical conditions

Frederico Patrus Ananias de Assis Pires[1] | Thierry Ribeiro Tomich[2] | Luiz Gustavo Ribeiro Pereira[2] | Fernanda Samarini Machado[2] | Mariana Magalhães Campos[2] | Alan Figueiredo de Oliveira[1] | Guilherme Lobato Menezes[1] | Rafael Araújo de Menezes[1] | Pamella Grossi de Sousa[1] | Diogo Gonzaga Jayme[1] | Lúcio Carlos Gonçalves[1]

[1]Department of Animal Science, Federal University of Minas Gerais, Belo Horizonte, Brazil

[2]Brazilian Agricultural Research Corporation—Dairy Cattle, Juiz de Fora, Brazil

Correspondence
Frederico Patrus Ananias de Assis Pires, Department of Animal Science, Federal University of Minas Gerais, 31270-901, Belo Horizonte, Minas Gerais, Brazil.
Email: fredericoppires@gmail.com

Abstract

This study aimed to evaluate the silage quality, ingestive behaviour, and sheep energy partition fed corn and sorghum silages, with or without inoculation with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*. Whole plants of one dent corn hybrid (DCS), one flint corn hybrid (FCS), and one forage sorghum hybrid (SS) were ensiled with or without an inoculant containing *L. plantarum* and *L. buchneri* (4×10^5 CFU g^{-1}), totalling six treatments (3×2 factorial scheme). The treatments were ensiled in metal drums with 200 L capacity. The lactic acid concentrations in the inoculated FCS and DCS were higher by 13.4% and 12.8%, respectively, than those in the non-inoculated plants. In contrast, the lactic acid concentration in the inoculated SS was 23.1% lower than that in the non-inoculated SS. Furthermore, there were differences in pH and acetic acid concentrations only in SS, which were 2.3% and 45.2% higher, respectively, in inoculated silage than in non-inoculated silage. In inoculated DCS and SS, propionic acid concentrations were 1.7 times higher (for both silages), and 1-propanol was 3.7 and 1.8 times higher compared than those in non-inoculated silages. There was a main effect of the inoculant on 1,2-propanediol concentrations, which were 37.5% higher in inoculated silages than in non-inoculated silages. However, ingestive behaviour, heat and methane production, and silage net energy concentrations were not affected by inoculant use. Fermentative modifications caused by inoculation with *L. plantarum* and *L. buchneri* in whole plant corn or sorghum silage did not modify sheep energy partition.

KEYWORDS
methane, respirometry, silage additive, *sorghum bicolor*, *Zea mays*

1 | INTRODUCTION

Microbial inoculants based on *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* have been used to improve the silage fermentation process and preservation (Muck et al., 2018). These effects occur due to the acceleration of pH drop, which results in lactate production by *L. plantarum* and the antifungal properties of acetate produced in the lactate degradation process by *L. buchneri*

(Borreani et al., 2018). Although acetate production results in higher dry matter (DM) losses during fermentation, it may also decrease losses during the aerobic phase (Muck et al., 2018).

The lactate degradation process also results in the production of 1,2-propanediol, which can be converted into 1-propanol and propionic acid, compounds with antifungal properties (Oude Elferink et al., 2001). These fermentative changes could improve the nutritional value of inoculated silages, resulting in lower DM losses (Dong et al., 2020; Muck et al., 2018). This improvement is related to increased nutrient intake and silage digestibility (Basso et al., 2018; Santos et al., 2021). In addition, other authors have also reported improvements in animal performance due to the probiotic effects of lactic acid bacteria in the rumen (Rabelo et al., 2017).

Despite the possible improvements in silage nutritional value, some studies did not find any difference on the performance of animals consuming silages inoculated with *L. plantarum* or *L. buchneri* (Arriola et al., 2021; Oliveira et al., 2017). Thus, it is important to emphasize the response to inoculant use depends on factors such as the epiphytic microbial population and inoculated bacterial ability of the plant to grow and survive during the fermentation process (Muck, 2010). Furthermore, specifically in relation to *L. buchneri*, the lactate degradation process depends on the strain (Kleinschmit et al., 2005), dose (Muck et al., 2018), forage (Arriola et al., 2021; Lee et al., 2019), and environmental conditions used (Oude Elferink et al., 2001). In this context, the use of lactic acid bacteria in silages seems to have a great effect on animal performance in tropical climate regions (Rabelo et al., 2016) because of the high microbial activity in warm climate regions (Bernardes et al., 2018; Ferrero et al., 2021). Furthermore, chemical and physical differences between whole-plant corn or sorghum silages can interact with the fermentation process. However, more studies are needed to understand the effects of fermentative modifications of corn and sorghum silages inoculated with *L. plantarum* and *L. buchneri* on animal performance in tropical regions.

In this context, as far as we know, the effects of the fermentative modifications caused by inoculation with *L. plantarum* and *L. buchneri* on the net energy (NE) content of corn and sorghum silages have not yet been studied. Therefore, this study aimed to evaluate the silage quality, ingestive behaviour, and sheep energy partition fed whole plant corn or sorghum silages inoculated or not inoculated with *L. plantarum* and *L. buchneri* in Brazil.

2 | METHODS

2.1 | Planting, harvesting, and ensiling

All animal procedures were approved by the Ethics Committee on the Use of Animals of Embrapa Dairy Cattle (CEUA/EGL) (CEUA Protocol—n° 1,989,120,318). Silages of corn hybrid BRS 3046 with dent grains (DCS) (developed by Embrapa Sete Lagoas, Brazil), corn hybrid RB9308 with flint grains (FCS) (developed by RIBER KWS®, Patos de Minas, Brazil), and forage sorghum hybrid BRS 658 (SS) (developed by Embrapa Sete Lagoas, Brazil) were evaluated. The forages were cultivated at Embrapa Dairy Cattle, Coronel Pacheco, MG, Brazil (21°33′22′ S, 43°06′15′ W, 856 m altitude) in three areas of 8000 m^2 each, randomly distributed in the same location, with similar soil characteristics. A row spacing of 0.7 m was used, and the crops were fertilized at planting (32, 112, and 64 kg/ha of N, P, and K) and by covering with 120 kg/ha of N, 30 days after planting.

The corn hybrids were harvested on 15 February 2018, when the grains showed a maturation stage between half and two-thirds of the milk line (DM = 306 g/kg for DCS and 288 g/kg for FCS). The sorghum hybrid was ensiled on 01 March 2018, when the grains reached a milky stage (DM = 257 g/kg). Whole forage plants were harvested using a self-propelled forage harvester with a corn grain processor 20 cm from the ground, and adjusted to a theoretical cutting length of 12 mm.

After harvesting, chopped material from each forage was separated into two parts. One half received microbial inoculants and the other half received similar amount of mineral water. A bacterial inoculant composed of two strains of *L. plantarum* (DSM3676 and DSM3677) and one strain of *L. buchneri* (DSM13573) (Feedtech™ F600 DeLaval, Tumba, Sweden) was used. At least 10^{11} colony forming units (CFU) were observed per gram of product for each microorganism species. Two grams of the product per ton of forage was applied to guarantee a total concentration of 4×10^5 CFU g^{-1} (2×10^5 *L. plantarum* and 2×10^5 *L. buchneri*). The product was diluted in mineral water and evenly distributed over forage using a back pump with constant agitation.

The material was compacted to reach a density equivalent to 600 ± 45 kg of fresh matter/m^3 in metal drums with 200 L capacity, internally lined with plastic bags. After filling, the silos were closed with lids and sealed with an adhesive tape. Fourteen silos were prepared for each treatment, for a total of 84 silos. Five silos per treatment were randomly chosen for sample collection to determine silage quality and fermentation profiles. Silage samples were collected during the experiment with animals and, therefore, were not carried out on the same day. On the sampling days (five different sampling days), one sample from each treatment was collected. The forage of all silos was used to evaluate intake, digestibility, and energy partitioning in sheep.

2.2 | Experimental design

The experiment consisted of six treatments arranged in a 3 × 2 factorial scheme (three forages × two inoculations [with or without inoculation]). During storage, the silos were kept in environment protected from sunlight at an average temperature of 22.9 ± 4.9°C an average relative humidity of 75.2% ± 16.9%. The maximum temperature was 36.7°C during summer on 12 March 2018, and the minimum temperature was 9.5°C during winter, on 11 August 2018 (data obtained from the automatic weather station of the Brazilian National Institute of Meteorology, located 5 km from the shed). After 545 days of ensiling,

TABLE 1 Chemical composition (g/kg DM, unless noted) of whole-plant corn dent and flint hybrids and whole-plant sorghum hybrid before fermentation[a]

Variables	Flint corn	SD[j]	Dent corn	SD[j]	Sorghum	SD[j]
DM[b] (g/kg NM[c])	288	5.74	306	3.59	257	4.27
CP[d]	77.1	0.29	75.6	1.50	75.0	0.97
aNDFomp[e]	498	5.87	455	3.32	635	4.57
aADFomp[f]	198	1.68	182	1.42	341	0.42
ADL[g]	28.3	8.31	28.6	5.03	71.8	12.0
EE[h]	25.5	1.86	22.2	1.18	24.8	2.23
Ash	49.0	0.74	50.7	0.66	57.1	1.49
NFComp[i]	351	7.17	397	8.05	208	9.23
Starch	268	5.28	304	4.98	154	4.86

[a]*n* per treatment = 4.
[b]dry matter;
[c]natural matter;
[d]crude protein;
[e]neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash and protein;
[f]acid detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash and protein;
[g]acid detergent lignin;
[h]ether extract;
[i]non-fibrous carbohydrate corrected for ash and residual proteins;
[j]standard deviation.

the silos were opened for the animal experiment and silage quality analysis.

2.3 | Chemical composition and fermentative profile

Four homogeneous samples of each fresh forage were collected at the ensiling time to characterize the chemical composition of the material before fermentation (Table 1). The samples were dried, weighed, and ground to 1 mm in a Wiley type mill (Thomas Wiley Model 4, Thomas Scientific, Swedesboro, NJ, USA). Concentrations of DM (AOAC, 1990; method 934.01), ash (AOAC, 1990; method 942.05), crude protein (CP) (AOAC, 1990; method ID 954.01), and ether extract (EE) (AOAC, 1990; method 920.39) were determined. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) concentrations were determined using the sequential method described by Van Soest et al. (1991). The aNDFomp concentrations were determined through the addition of 2 ml of heat-stable amylase and corrected for residual ash and proteins. The aADFomp concentrations were corrected for residual ash and proteins, and ADL was determined using cellulose solubilization with sulphuric acid. Starch concentrations were determined via the enzymatic method using a commercial Megazyme kit (Total Starch Assay kit—K-TSTA-100A, WGK, Germany) (AOAC, 1990; method 996.11). The non-fibrous carbohydrate concentrations (NFComp) were calculated using the equation proposed by NRC (2001), considering the values of residual ash and proteins corrected in the aNDFomp: NFComp = 100 − (% aNDFomp + % CP + % EE + ash).

After silo opening and exclusion of the superficial layer of losses, two representative samples of the fermented material were collected. One sample was used to determine DM, CP, aNDFomp, aADFomp, EE, ash, and starch using previously described methodologies. The other sample was used to obtain silage juice extracted with a hydraulic press (2.5 kgf/cm^2) to determine the pH and concentrations of ammonia nitrogen as the proportion of total nitrogen (NH_3–N/TN) and volatile compounds.

The pH values of the silage juice were measured directly using a digital potentiometer (MS Tecnopon, MPA 210, Piracicaba-SP, Brazil). NH_3-N concentration was determined through distillation using Kjeldahl equipment (AOAC, 1990; method 941.04). Silage ethanol and organic acid concentrations were determined after filtering and centrifuging the silage juice for 15 min at 10,000 rpm. Gas chromatograph with mass detector (GC–MS QP 2010 plus, Shimadzu®, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, USA [60 m × 0.25 mm × 0.25 µm, cross bond carbowax polyethylene glycol]). The lactic acid concentration was determined through gas–liquid chromatography using Waters Alliance HPLC e2695 126 equipment with a PDA 2998 detector (Waters, Milford, MA, USA). The separation was performed on a reverse phase C18 column ODS 80 A (150 mm × 4.6 mm × 5 µm). The analysis conditions consisted of isocratic mobile phase solution prepared of phosphoric acid with a pH of 2.35, flow 1.0 ml/min, oven temperature 40 ± 5°C, sample injection volume of 10 µl, run for 20 min and detection at a reception wavelength of 210 nm.

2.4 | Nutrient intake and digestibility

Six castrated adult male dorper sheep with an average live weight of 90.4 ± 12.8 kg were used to conduct the intake, digestibility, energy partition, methane (CH_4) emission, and energy losses assays. The

animals were vaccinated, dewormed, shortened, and weighed at the beginning and end of the collection phase in each experimental period. The sheep were housed in individual metabolic cages that were suitable for collecting urine and faeces simultaneously. The animals received water and mineral mixture ad libitum.

The experiment began after 20 days of adaptation of the animals to the cages and daily handling. Six experimental periods were conducted, each consisting of 7 days of adaptation to the diet and 5 days of total collection. The silages were fed twice a day (6:00 AM and 15:00 PM) in quantities adjusted to obtain 15% orts. Weighing and individual sampling (10% of the total measured each day) of the offered silage, orts, and faeces were performed. The volume of urine excreted was determined, and individual samples were collected (10% of the total measured volume). Urine collection was performed in 20 L plastic containers, sealed, and refrigerated in polystyrene boxes with ice. After the end of each collection period, the samples were pooled to obtain composite samples. Subsequently, the composite samples of offered silage, orts, and faeces were used to determine DM, CP, aNDFomp, aADFomp, EE, and ash using the same methodology as described previously. Urine samples were analyzed for total content.

Nutrient intake was determined in grams per unit of metabolic size per day (UMS) (g UMS/day), considering the daily DM intake (kg OF–kg OR, where: kg OF = amount of diet offered, in kg of DM; kg OR = amount of orts removed, in kg of DM), and the live weight of the animal was 0.75. Nutrient apparent digestibility was obtained using the Equation (1): $AD = ((OF - SB - CF)/OF - SB) \times 100$ proposed by Maynard et al. (1984), where AD refers to apparent digestibility; OF refers to offered feed ([Offered feed amount in kg DM] × [Offered nutrient content in % of DM]/100); SB refers to orts feed [(Removed orts feed in kg DM) × (Orts nutrient content in % of DM)/100]; CF refers to collected faeces [(Collected faeces in kg DM) × (Collected faeces content in % of DM)/100]. The nitrogen retained (g/day) was obtained using Equation (2): $NR = NI - (NF + NU)$, where NR refers to nitrogen retained, NI refers to ingested nitrogen (g/day), NF refers to faecal nitrogen (g/day), and NU refers to urinary nitrogen (g/day).

2.5 | Methane emission, energy partition, and energy losses

Sheep CH_4 production and silage metabolizable (ME) and digestible energy (DE) contents were determined using respirometry. Three open-flow respirometric chambers were used, made of transparent acrylic plates (6 mm thick) with external dimensions of 1.2 m (width) × 2.0 m (height) × 2.1 m (length). The chambers were placed 1 m apart to avoid animal stress due to isolation and ensure animal welfare. Data were collected with the simultaneous use of the three chambers.

The respirometry test was performed in two stages (fed and fasting animal evaluations). In these two stages, CH_4 and carbon dioxide (CO_2) production and oxygen consumption (O_2) were measured. In addition, animal heat production (indirect calorimetry) was calculated according to Equation (3): $H\ (kj) = (16.2 \times O_2\ (L)) + (5.02 \times CO_2\ (L)) - (5.88 \times NU\ (g)) - (2.17 \times CH_4\ (L))$ proposed by Brouwer (1965), where H refers to heat production and NU refers to urinary nitrogen. Gas exchange measurements were performed in a respirometric chamber for 24 h. After opening the chamber, the urine excreted volume was measured and sampled. In the first phase, the animals were fed silage twice a day and received water and mineral mixtures ad libitum. This process occurred at the end of each period of the Latin square, with the rotation of the three chambers in duplicate (4 days of data collection for each period). In the second phase, the animals were evaluated after 48 h of fasting and remained inside the chamber for 24 h, with only water ad libitum. This second phase took place after the last evaluation period, with the objective of measuring the incremental caloric increase (IC) by the difference between the heat production observed for silage-fed and fasting animals.

The equipment and methodology described by Rodriguez et al. (2007) were used for the indirect calorimetry procedure. Atmospheric air entered each chamber at a constant flow of 1 L of air per kg of animal body weight and was mixed with the animal's exhaled air. The air contained inside each chamber was aspirated with the aid of a pump with constant flow and controlled using a mass flow meter, which automatically corrected the air volume to pressure, temperature, and humidity conditions.

External and internal air samples from the chamber were collected alternately every 5 min to determine the CH_4, CO_2, and O_2 concentrations according to Chwalibog (2004). The CH_4, CO_2, and O_2 analyzers were calibrated daily before the beginning of the animal gas exchange measurement using gases with known concentrations and external air. The gas concentration results and airflow were automatically recorded by specific software, which calculated the volume (L) of CH_4 and CO_2 produced and O_2 consumed by animals. The air temperature and humidity inside the chamber were controlled by air conditioning and were recorded during the first and last readings.

The gross energy (GE) of the material offered, orts, faeces, and urine was determined by combustion in an adiabatic calorimetric bomb model PARR 2081 (PARR Instrument Company, Moline, IL, USA). The DE was obtained from the difference between feed, orts, and faecal GE. The ME was obtained from the difference between DE and energy losses in urine and CH_4 emissions. To calculate the energy lost in CH_4 emissions, an energy value of 13.3 kcal/g and a density of 0.714 g/L were considered. The caloric increment (IC) was calculated as the difference between the heat production observed in the silage-fed and fasting animals. NE was obtained from the difference between the ME and energy losses as IC. In addition, the amount of silage offered and orts were used to calculate the intake of gross, DE, ME, and NE.

2.6 | Ingestive behaviour

Behavioural evaluations were carried out in each experimental period, after the end of the 7 days of adaptation to the diet and before starting the collections. The animals were visually evaluated every 5 min

for 24 h, for a total of 288 observations. The observations were based on verifying whether the animal was ingesting feed, ruminating, idling, or performing another activity. In addition, over the 24 h of observation, three evaluations were carried out per animal to obtain the number of mericic chews (chewing during rumination) per ruminal bolus and the average chewing time for each ruminal bolus (seconds/bolus) using a digital stopwatch. Data were collected in triplicate, and the evaluation periods ranged from 1000 to 1200, 1700 to 1900, and 2100 to 2300, totalling nine evaluations per animal. Animals were kept under artificial lighting conditions.

The results related to the factors of ingestive behaviour were chosen according to Burger et al. (2000), by relations: Chewing time (min/day), = Time spent feeding (min/day) + Time spent ruminating (min/day), Efficiency in feeding (g/DM/h) = DM intake (g/day)/Time spent feeding (h), Efficiency in rumination (g/DM/h) = DM intake (g/day)/Time spent ruminating (h/day), Number of ruminal boluses = Time spent ruminating (h/day)/Time spent on mericic chews per bolus (Polli et al., 1995), Mericic chews (day) = Number of chews per bolus * Number of ruminal boluses.

2.7 | Statistical analyses

The whole-plant chemical composition data from each hybrid prior to fermentation were descriptive only. Only the averages and standard deviations were calculated. Silage chemical composition and fermentation profile data were evaluated in a completely randomized design, and sheep energy intake and partition in a 6 × 6 Latin square. Data were analyzed in a 3 × 2 factorial arrangement (three forages and two inoculations) using Two-way ANOVA. A mixed model was used, considering the fixed effects of the addition of the inoculant, effect of forage, and interaction between these factors. The means were compared using Tukey's test, and statistical significance was considered when $p \leq .05$ and marginal significance when $p \leq .1$.

3 | RESULTS

3.1 | Chemical composition and fermentative profile

The chemical composition of fresh forages is shown in Table 1. There was no interaction effect between the inoculant and forage or inoculant fixed effects on silage chemical composition ($p > .05$) (Table 2). When considering the fixed forage effect, only EE and CP did not change. Furthermore, there was no interaction between forage and inoculant for ethanol, 1,2-propanediol, and isobutyric acid concentrations (Table 3). However, this interaction was significant ($p \leq .05$) or showed a trend ($p \leq .1$) in all other evaluated fermentation profile variables.

The pH of inoculated SS was 2.3% higher than that of non-inoculated SS, with no differences observed in other silages. In the inoculated FCS and DCS, the total acid concentrations were 13.0% and 12.4% higher, respectively, than in the non-inoculated silages. Similarly, lactic acid concentrations in inoculated FCS and DCS were 13.4% and 12.8% higher than those in non-inoculated FCS and DCS, respectively. On the other hand, in inoculated SS, total acid and lactic acid concentrations were 20.7% and 23.14% lower, respectively, than in non-inoculated silages. There were differences in acetic acid concentrations only in SS, which were 45.2% higher in inoculated silage. Butyric acid and NH_3–N/TN concentrations were altered only in the FCS, with values 11.6% and 18.3% lower in the inoculated silage than in the non-inoculated silage.

Propionic acid concentrations were approximately 1.7 times higher in inoculated DCS and SS than in non-inoculated. Inoculant use also increased 1-propanol concentrations by 3.7 times in DCS and 1.8 times in SS. Isopropyl alcohol concentrations in inoculated DCS and SS were 2.7 and 1.9 times higher compared to non-inoculated. Furthermore, there was a fixed effect of inoculant on 1,2-propanediol concentrations, with an average increase of 37.5% in the inoculated silages.

Valeric acid concentrations were 34.7% lower in the inoculated DCS and 47.3% lower in the inoculated FCS than in the non-inoculated DCS. In contrast, inoculant use increased isovaleric acid concentrations by 2.1 times in SS and reduced by 2.4 times in FCS. Ethyl acetate concentrations were 1.9 times higher in inoculated DCS and 1.3 times higher in inoculated SS than in non-inoculated. Ethyl lactate concentrations were 26% higher in non-inoculated SS than in inoculated SS. There was no interaction effect between forage and inoculant or inoculant fixed effect on propyl acetate concentrations. When considering the forage fixed effect, among all fermentation profile variables, only ethyl lactate concentrations were similar.

3.2 | Nutrient intake and digestibility, methane emission, energy partition and energy losses

There was no interaction effect between inoculant and forage or inoculant fixed effects on silage intake and digestibility ($p > .05$) (Table 4). When considering the forage fixed effect, only CP intake and aNDFomp digestibility did not change. In general, corn silage intake and digestibility were superior to those of SS. Regarding nitrogen balance, energy partition, methane emissions, and energy losses, there were also no interactions between the factors or inoculant fixed effects in any of the evaluated variables. When considering the forage fixed effect, the values of NR, DE, ME, NE, CH_4 (L/animal/day), and energy lost in faeces were similar among the corn silages and were 68.7%, 17.0%, 20.5%, 53.0%, and 25.4% lower and 14.8% higher, respectively, in SS (Table 5). The other evaluated variables were not affected by forage ($p > .1$).

3.3 | Ingestive behaviour

There was no interaction effect between the inoculant and forage or inoculant fixed effects in any of the ingestive behaviour variables

TABLE 2 Chemical composition (g/kg DM, unless noted) of whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

	FCS		DCS		SS			*p*-value[b]		
Variables	LP + LB[c]	CON[d]	LP + LB	CON	LP + LB	CON	SEM[a]	*F*	*I*	*F* * *I*
DM[l] (g/kg NM[e])	272	283	285	290	227	229	12.87	<.001	.559	.477
CP[f]	75.4	77.2	77.8	75.1	80.0	76.0	1.890	.583	.277	.270
aNDFomp[g]	466	452	415	434	653	641	44.45	<.001	.867	.452
aADFomp[h]	244	237	211	218	399	383	34.16	<.001	.413	.297
DL[i]	38.6	32.3	31.5	33.5	87.1	76.3	10.71	<.001	.250	.475
EE[j]	35.5	32.6	33.8	35.9	35.2	32.8	4.040	.970	.753	.807
Ash	51.7	51.3	51.7	52.2	70.9	66.9	3.830	<.001	.398	.439
NFComp[k]	371	387	422	403	173	183	43.76	<.001	.779	.431
Starch	270	278	306	312	153	150	28.25	<.001	.297	.314

[a]standard error of mean;
[b]F = forage effect, I = inoculant effect, F * I = interaction effect between forage and inoculant;
[c]*Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1});
[d]control *n* per treatment = 5;
[e]natural matter;
[f]crude protein;
[g]neutral detergent fibre assayed with a heat-stable amylase and expressed exclusively of residual ash and protein;
[h]acid detergent fibre assayed with a heat-stable amylase and expressed exclusively of residual ash and protein;
[i]acid detergent lignin;
[j]ether extract;
[k]non-fibrous carbohydrate corrected for ash and residual proteins;
[l]dry matter.

TABLE 3 Fermentative parameters (g/kg DM, unless noted) of whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

	FCS		DCS		SS			*p*-value[b]		
Variables	LP + LB[c]	CON[d]	LP + LB	CON	LP + LB	CON	SEM[a]	*F*	*I*	*F* * *I*
pH	3.50	3.52	3.54	3.56	4.01	3.92	0.087	<.001	.367	.005
NH_3–N/TN[e] (g/kg N)	3.90	4.60	4.84	5.19	3.57	3.12	0.367	<.001	.164	.007
Total acids	61.0	54.0	59.0	52.5	27.6	34.8	5.536	<.001	.045	<.001
Lactic acid	60.3	53.2	58.3	51.7	25.9	33.7	5.666	<.001	.069	<.001
Acetic acid	0.69	0.64	0.70	0.72	1.67	1.15	0.167	<.001	.003	<.001
Propionic acid	0.10	0.10	0.10	0.06	0.18	0.11	16.73	<.001	<.001	<.001
Butyric acid	0.29	0.33	0.31	0.29	0.17	0.20	28.48	<.001	.131	.065
Ethanol (g/kg DM)	0.06	0.05	0.06	0.06	0.10	0.10	0.011	<.001	.187	.279
1,2-propanediol (mg/kg DM)	27.1	21.5	32.0	21.3	24.7	18.2	2.774	.078	<.001	.496
1-propanol (mg/kg DM)	3.97	4.60	6.63	1.77	8.07	4.45	1.028	.005	<.001	<.001
Isopropyl alcohol (mg/kg DM)	3.10	3.70	2.18	0.82	8.73	4.69	1.298	<.001	.022	.027
Isobutyric acid (mg/kg DM)	4.06	4.51	2.92	2.48	5.48	3.13	0.718	.030	.150	.105
Isovaleric acid (mg/kg DM)	3.15	7.58	6.97	6.18	5.35	2.49	1.023	.005	.670	<.001
Valeric acid (mg/kg DM)	6.22	11.8	9.53	14.6	4.53	4.08	1.760	<.001	<.001	<.001
Ethyl acetate (mg/kg DM)	5.98	6.66	4.63	2.42	19.5	15.1	2.632	<.001	<.001	.002
Propyl acetate (mg/kg DM)	0.90	0.72	0.37	0.71	1.08	1.00	0.144	.001	.786	.104
Ethyl lactate (mg/kg DM)	241	217	202	233	202	267	15.06	.330	.012	.001

[a]standard error of mean;
[b]*F* = forage effect, *I* = inoculant effect, *F* * I = interaction effect between forage and inoculant;
[c]*Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1});
[d]control *n* per treatment = 5;
[e]ammonia nitrogen as a proportion of total nitrogen.

TABLE 4 Intake and nutrient digestibility of sheep fed whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

	FCS		DCS		SS			*p*-value[b]		
Variables	LP + LB[c]	CON[d]	LP + LB	CON	LP + LB	CON	SEM[a]	*F*	*I*	*F* * *I*
Nutrient intake (g/UMS[e]/day)										
Dry matter	39.1	39.8	40.7	42.6	29.9	36.0	3.141	.015	.188	.567
Organic matter	37.0	37.8	38.6	40.4	27.8	33.6	3.046	.009	.193	.563
NFComp[f]	14.7	15.8	17.8	17.3	4.70	6.70	2.556	<.001	.633	.463
Crude protein	2.95	3.06	3.14	3.20	2.56	2.74	0.226	.109	.499	.760
aNDFomp[g]	18.4	17.8	16.7	18.4	19.6	23.1	1.618	.009	.157	.416
aADFomp[h]	10.1	9.93	9.01	9.85	12.7	14.6	1.110	<.001	.567	.218
Nutrient digestibility (g/kg DM)										
Dry matter	653	677	689	667	557	575	25.0	<.001	.631	.639
Organic matter	671	695	709	686	574	586	25.8	<.001	.514	.696
Crude protein	556	602	594	576	485	481	26.6	<.001	.547	.460
aNDFomp	474	508	470	461	511	506	29.2	.478	.782	.937
aADFomp	407	488	394	381	489	471	42.5	.100	.565	.607

[a]standard error of mean;
[b]*F* = forage effect, *I* = inoculant effect, *F* * *I* = interaction effect between forage and inoculant;
[c]*Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1});
[d]control *n* per treatment = 6;
[e]unit of metabolic size (live weight$^{0.75}$);
[f]non-fibrous carbohydrate corrected for ash and residual proteins;
[g]neutral detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash and protein;
[h]acid detergent fibre assayed with a heat-stable amylase and expressed exclusive of residual ash and protein.

TABLE 5 Nitrogen balance, energy partition, methane emission and energy losses in sheep fed whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

	FCS		DCS		SS			*p*-value[b]		
Variables	LP + LB[c]	CON[d]	LP + LB	CON	LP + LB	CON	SEM[a]	*F*	*I*	*F* * *I*
Nitrogen balance (g/UMS/day)										
N ingested	0.47	0.49	0.50	0.51	0.41	0.44	0.036	.102	.495	.754
N urinary	0.11	0.10	0.09	0.11	0.10	0.09	0.008	.803	.940	.187
N faecal	0.21	0.20	0.21	0.22	0.21	0.23	0.018	.424	.793	.813
N retained	0.15	0.19	0.20	0.18	0.09	0.12	0.024	.001	.354	.225
Energy partition (Mcal/kg DM)										
Gross energy	4.09	4.04	4.05	4.06	4.11	4.07	0.021	.260	.109	.337
Digestible energy	2.75	2.71	2.77	2.69	2.33	2.32	0.097	<.001	.508	.958
Metabolizable energy	2.26	2.18	2.29	2.20	1.86	1.85	0.091	<.001	.374	.706
Net energy	1.28	1.14	1.32	1.22	0.78	0.89	0.111	<.001	.569	.140
Methane production										
Methane (L/animal/day)	55.5	57.6	51.7	54.9	41.3	46.1	4.129	.006	.242	.951
Methane (g/kg DM)	31.9	34.3	31.8	31.6	31.5	30.6	2.254	.603	.925	.826
Energy losses (kcal/UMS/day)										
Faeces	54.8	52.4	52.6	58.4	57.3	63.0	5.075	.002	.297	.744
Urine	2.54	2.81	2.27	2.86	2.02	2.34	0.386	.432	.169	.956
Heat production	102	106	105	108	102	101	4.332	.527	.507	.701

[a]standard error of the mean;
[b]*F* = forage effect, *I* = inoculant effect, *F* * *I* = interaction effect between forage and inoculant;
[c]*Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1});
[d]control *n* per treatment = 6;

TABLE 6 Ingestive behaviour of sheep fed whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

	FCS		DCS		SS			*p*-value[b]		
Variables	LP + LB[c]	CON[d]	LP + LB	CON	LP + LB	CON	SEM[a]	*F*	*I*	*F* * *I*
Time spent in rumination (min/day)	465	492	438	494	533	540	36.28	.074	.290	.850
Time spent in feeding (min/day)	147	153	164	138	168	173	22.88	.579	.795	.647
Time spent in idle (min/day)	757	715	752	722	647	665	48.24	.103	.620	.731
Time in other activities (min/day)	71.7	80.8	85.8	86.7	93.3	61.7	16.91	.156	.257	.536
Chewing time (min/day)	612	644	603	632	700	713	44.93	.045	.451	.870
Efficiency in feeding (g DM/h)	509	507	570	563	472	474	99.70	.528	.976	.899
Efficiency in rumination (g DM/h)	149	147	166	155	104	123	11.69	<.001	.725	.210
Number of chews per bolus	64.7	64.1	61.6	63.1	64.6	69.2	62.31	.925	.752	.725
Chewing time for bolus (min/day)	48.4	50.6	45.9	47.8	50.4	51.0	4.558	.801	.694	.914
Mericic chews (chews/day)	37,166	37,081	35,084	38,532	41,313	43,795	3038	.077	.400	.873

[a]standard error of the mean;
[b]*F* = forage effect, *I* = inoculant effect, *F* * *I* = interaction effect between forage and inoculant;
[c]*Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1});
[d]control *n* per treatment = 6.

(Table 6). When considering the forage fixed effect, the time spent feeding increased, and there was a trend of increase in the time spent ruminating and the number of mericic chews for the SS. In addition, the rumination efficiency was 35.9% lower in SS than in corn silage.

4 | DISCUSSION

4.1 | Chemical composition and fermentative profile

The evaluation of silage quality and animal performance, especially the determination of NE content, represents an advance in the use of *L. plantarum* and *L. buchneri* in corn and sorghum silages. This advance is due to the possibility of indicating whether fermentative changes caused by the action of these microorganisms can modify the nutritional use of silage.

The chemical compositions of DCS and FCS are similar to those generally observed for corn silages (Ferraretto & Shaver, 2015; Saylor et al., 2020). The SS also had a composition similar to that shown in studies of sorghum silages (Diepersloot et al., 2021; dos Anjos et al., 2018). The lack of an inoculant effect on chemical composition was also observed in other studies that evaluated the use of *L. buchneri* and *L. plantarum* (Lee et al., 2019; Rabelo et al., 2016). Some studies have found lower DM concentrations (Kleinschmit & Kung, 2006) and higher NDF and ADF concentrations in silages inoculated with *L. buchneri* (Basso et al., 2014). These modifications are related to the type of heterofermentative fermentation performed by *L. buchneri*, which results in greater DM losses (McDonald et al., 1991). However, the concomitant use of *L. plantarum* can minimize DM losses and avoid changes in the silage chemical composition (Arriola et al., 2021; Muck et al., 2018), which probably occurred in the present study.

Regarding fermentation parameters, the concentrations of organic acids, NH_3–N/TN, and ethanol indicated that the fermentation process was efficient in preserving the silages, with low development of spoilage microorganisms in all treatments (Kung et al., 2018). The differences found in the silages using *L. plantarum* and *L. buchneri* indicated that the inoculated bacteria probably survived and grew during the fermentation process (Muck, 2010). The highest pH and acetic acid values associated with the lowest lactic acid and total acid concentrations found in inoculated SS have also been previously reported for sorghum silages inoculated with *L. buchneri* (Diepersloot et al., 2021; Fernandes et al., 2020). These modifications were in accordance with the lactate degradation mechanism of *L. buchneri*. This mechanism consists of anaerobic conversion of moderate amounts of lactic acid into acetic acid, ethanol, and 1,2-propanediol (Oude Elferink et al., 2001). Because acetic acid has a lower dissociation constant than lactic acid (McDonald et al., 1991), the pH of the medium increases. However, acetic acid concentrations in all inoculated silages were below the reference values for silages inoculated with *L. buchneri*, which is 3%–4% DM (Kung et al., 2018).

The absence of an inoculant effect on the pH and acetate concentrations in FCS and DCS confirms that the magnitude of the lactate degradation process depends on the substrate used (Arriola et al., 2021). Furthermore, the increases in lactic acid and total acid concentrations indicated that the inoculant used in these silages favoured homolactic fermentation (McDonald et al., 1991) differently from SS.

Arriola et al. (2011) found higher lactate concentrations and lower pH in corn silages inoculated with *L. buchneri* than in the control group. The authors justified these differences to the lower consumption of lactate by yeasts, which was inhibited in the inoculated silages. However, the absence of differences between treatments and low ethanol content indicated that this process probably did not occur in the present study. It is noteworthy that the application of *L. plantarum*

generally favours lactate production, with few changes in other organic acids (Lara et al., 2018; Oliveira et al., 2017).

According to Borreani et al. (2018), in association with microorganisms, strains with homofermentative actions must ensure high lactate production and rapid pH reduction. Subsequently, *L. buchneri* slowly converts lactic acid into acetic acid. Our results suggested that inoculant use intensified the fermentation process; however, the action of *L. buchneri* was less pronounced in DCS and FCS than in SS. In addition to *L. plantarum* action, there was a more intense fermentation process in corn silages, considering the pH values of the control silages, which were lower than the values generally observed in literatures (Costa et al., 2021; Kung et al., 2018; Saylor et al., 2020). Therefore, in these silages, there may have been greater competition between epiphytic microorganisms and *L. buchneri*, which may have reduced their growth and performance. This can also explain the difference in SS, which presented higher mean pH values in all treatments and greater evidence of *L. buchneri* development in the inoculated silages.

This may be related to the lower levels of DM at the time of cutting in the SS than in the DCS and FCS. It is known that materials with higher humidity favour heterolactic fermentation (McDonald et al., 1991).

The highest 1,2-propanediol content in the inoculated silages suggests that there was slight activity of *L. buchneri* in all treatments. It is important to highlight that the 1,2-propanediol concentrations found were much lower than the reference values for silages inoculated with *L. buchneri* (0.25 to 1.5% in DM) (Kung et al., 2018). However, 1,2-propanediol can be converted during the storage phase by *Lactobacillus* (*Lentilactobacilli*) *diolivorans*, which are often naturally present in silages. This conversion results in approximately equimolar amounts of 1-propanol and propionic acid (Krooneman et al., 2002), which explains the higher concentrations of these components in the inoculated DCS and SS. Furthermore, the isomer of 1-propanol was isopropyl alcohol, which also explains the increase in this component in the inoculated DCS and SS.

In FCS, the absence of differences in the concentrations of 1-propanol, isopropyl alcohol, and propionic acid indicates that *L. buchneri* and/or *Lactobacillus (Lentilactobacilus) diolivorans* probably acted even more discreetly. Furthermore, the lower butyric acid and NH_3–N/TN concentrations in FCS were probably related to the greater growth inhibition of *Clostridium*. The growth of these microorganisms occurs through the catabolism of amino acids and consumption of glucose and lactate, leading to the production of butyric acid and NH_3-N (McDonald et al., 1991). Notably, in all treatments evaluated, the butyric acid and NH_3–N/TN concentrations were within the reference values for good-quality silages (Kung et al., 2018).

It is noteworthy that ethyl esters are formed in silages by abiotic esterification of carboxylic acids and alcohols at low pH (Weiss, 2017). Thus, ethyl esters, particularly ethyl acetate and ethyl lactate, are positively correlated with this component (da Silva et al., 2018; Weiss, 2017). Ethanol formation occurred during lactate degradation. However, the metabolism of *L. buchneri* is marked by the preferential production of acetic acid and only small amounts of ethanol (Oude Elferink et al., 2001), which explains the absence of differences in this component in inoculated silages. Despite the absence of differences in ethanol concentrations, the higher ethyl acetate concentrations in SS and DCS were likely related to the higher activity of *L. buchneri* in these treatments.

Another ester associated with *L. buchneri* metabolism is propyl acetate, which is conditioned by acetic acid and 1-propanol precursors (da Silva et al., 2018). The absence of modification of this ester indicates that, despite the modifications in the ethyl acetate concentrations, the esterification process occurred discreetly, together with the moderate development of *L. buchneri*. Ethyl lactate, like ethanol, also contains lactic acid as a precursor (Weiss, 2017), which explains the higher concentrations of this ester in non-inoculated SS.

4.2 | Ingestive behaviour, nutrient intake and digestibility, methane emission, energy partition and energy losses in sheep

The performance of animals consuming inoculated silages has rarely been investigated in tropical countries (Rabelo et al., 2016). Furthermore, to the best of our knowledge, our study is the first to evaluate the NE content, heat production, methane emission, and ingestive behaviour of sheep fed silages inoculated or not inoculated with *L. plantarum* and *L. buchneri*. Although fermentative modifications indicated that the inoculated microorganisms developed, none of the animal response variables were affected by microbial inoculant use. Our results corroborate those of the meta-analysis conducted by Arriola et al. (2021), who demonstrated that the intake of silages inoculated with *L. buchneri* associated with homofermentative microorganisms does not interfere with animal performance. However, some studies suggest that bacterial inoculant use can positively affect DM intake and animal performance (Basso et al., 2014; Basso et al., 2018; Rabelo et al., 2016), especially in tropical countries because of the more favourable conditions for the development of inoculated microorganisms (Bernardes et al., 2018; Ferrero et al., 2021).

The main factors identified as responsible for the improvement in animal performance are the improvement in silage preservation (Muck et al., 2018; Rabelo et al., 2017) and the increase in the resistance of silages to deterioration by aerobic microorganisms (Kleinschmit & Kung, 2006). Furthermore, some authors have justified that improvements in DM digestibility and nutrient intake may occur because of the possible probiotic effects of inoculated lactic acid bacteria (Basso et al., 2014). These changes could alter the ingestive behaviour and enteric methane production in animals.

In the present study, modifications in the fermentation process were not able to improve the nutritional value, probably because of the relatively small differences between the silages. Thus, considering that all silages were well-fermented, the magnitude of the effect of silage inoculation was less pronounced. In addition, it is noteworthy that the silage removal process after silo opening was highly controlled, with the daily removal layer always greater than 15 cm. These factors limit oxygen exposure and penetration after silo opening (Bolsen, 2018). Thus, the potential of *L. buchneri* to reduce losses due to the development of aerobic microorganisms is reduced, which could occur differently under field conditions (Weng et al., 2021).

Volatile compounds in silage may modify the ingestive behaviour of animals. These changes are related to reduced palatability of the diet and increased rumen osmotic pressure, with impacts mainly on feeding time and DM intake (Daniel et al., 2013; Grant & Ferraretto, 2018). However, it is important to highlight that the acetic acid content generally found in studies that reported behavioural changes and intake reduction was 4% in DM. These concentrations were much higher than those found for inoculated SS in the present study, although previous studies have reported similar acetic acid concentrations (4% DM) in silages inoculated with *L. buchneri* (Grant & Ferraretto, 2018). Therefore, the increase in acetic acid content in inoculated SS was probably not sufficient to interfere with ruminal osmolarity and diet palatability, which justifies the absence of differences in ingestive behaviour and nutrient intake.

Similarly, higher propionic acid concentrations in the inoculated DCS and SS could also interfere with feeding time and DM intake (Maldini & Allen, 2019). This interference could occur because of the ability of propionic acid to stimulate satiety in ruminants (Allen, 2020). However, the propionic acid concentrations observed in the inoculated DCS and SS were far below the concentrations proven to interfere with ingestive behaviour and animal nutrient intake (Grant & Ferraretto, 2018; Maldini & Allen, 2019).

Furthermore, propionate, 1,2-propanediol, and 1-propanol produced directly or indirectly by the action of *L. buchneri* could interfere with energy use efficiency. Propionic acid is the main gluconeogenic precursor used by ruminants (Owens & Goetsch, 1988). The 1,2-propanediol is a compound analogous to propylene glycol and can be converted to propionic acid in the rumen or directly absorbed and converted to glucose in the liver (Kung et al., 2018). The 1-propanol also represents a gluconeogenic substrate, with a metabolism similar to that of 1,2-propanediol (Raun & Kristensen, 2012). It is important to consider that lactate can also be converted to propionate by microorganisms in the rumen. However, this involves energy expenditure and heat production (Owens & Goetsch, 1988). Therefore, the lactate degradation process carried out in the silo by *L. buchneri* could reduce heat production in the ruminal fermentation process and increase the efficiency of ME energy use and silage NE content.

In the present study, despite the increase in 1,2-propanediol concentrations in all inoculated silages and propionate and 1-propanol in SS and DCS, the absolute amounts consumed were low in relation to the total DM intake by the animals, which justifies the equality in the concentrations of NE between the inoculated and non-inoculated treatments. Studies carried out with the direct supply of 1-propanol and 1,2-propanediol that found glycogenic effects used amounts greater than 1% DM of the diet (Maurer et al., 2017; Raun & Kristensen, 2012; da Silva et al., 2017). However, in contrast to our findings on 1,2-propanediol after inoculation, there are reports of 1,2-propanediol concentrations above 3% in DM in silages inoculated with *L. buchneri* in the literature (Kung et al., 2018).

In this context, future studies should evaluate the NE content in silages produced under farm conditions, which may present more challenging conditions for the development of epiphytic microbiota and greater performance of the inoculated microorganisms. In addition, lower control during silage removal under farm conditions could favour silage preservation by *L. buchneri* inoculation and cause differences in the quality of silage consumed by the animals. It is also recommended that in the future, along with the determination of the NE content, the ruminal fermentation parameters and metabolic parameters of animals fed silage inoculated with *L. plantarum* and *L. buchneri* should be evaluated.

Another aspect that can be pointed out by the lack of identification of differences in the variables of feeding efficiency and ingestive behaviour is the type of animal used in the experiment. Adult sheep at the maintenance level of feeding have low energy requirements and low intake proportional to their live weight (NRC, 1985). Therefore, discrete modifications in the fermentation process of inoculated silages could produce different results in animals with high nutritional demand, such as lactating cows (NRC, 2001). Furthermore, considering the economic importance of these animals and the physiological and metabolic differences compared to sheep, studies on the effect of using *L. plantarum* and *L. buchneri* on feed efficiency in cattle should be conducted in the future.

5 | CONCLUSIONS

The use of *L. plantarum* and *L. buchneri* altered the quality of corn and sorghum silages. These changes occurred as a consequence of the intensification of a more heterolactic fermentation pattern in sorghum silages and homolactic pattern in inoculated corn silages. There were slight changes in the content of volatile compounds previously related to changes in behaviour, intake, and energy use efficiency. These compounds were modified to a greater extent in sorghum silage. However, inoculation with *L. plantarum* and *L. buchneri* did not change the ingestive behaviour or the energy use efficiency of sheep fed any of the whole plant corn or sorghum silages.

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None.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

We confirm that we have full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. The data supporting the findings of this study

are available from the corresponding author upon reasonable request.

ORCID

Frederico Patrus Ananias de Assis Pires https://orcid.org/0000-0002-7960-1612
Thierry Ribeiro Tomich https://orcid.org/0000-0002-8788-5715
Mariana Magalhães Campos https://orcid.org/0000-0001-5332-6010
Alan Figueiredo de Oliveira https://orcid.org/0000-0002-1928-6753
Guilherme Lobato Menezes https://orcid.org/0000-0002-9317-3239
Pamella Grossi de Sousa https://orcid.org/0000-0002-7073-3141
Diogo Gonzaga Jayme https://orcid.org/0000-0003-1838-2732
Lúcio Carlos Gonçalves https://orcid.org/0000-0002-1716-4744

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