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Effects of *Lactobacillus brevis* and *Lactobacillus plantarum* on Fermentation, Aerobic Stability, and Ruminal Digestibility of Sorghum Silage under Tropical Conditions

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ABSTRACT

This study aimed to evaluate the effects of *Lactobacillus brevis*, a heterofermentative bacterium, and *Lactobacillus plantarum,* a homofermentative bacterium, on reducing nutrient loss, extending aerobic stability, and improving the digestibility of sorghum silage under tropical conditions. Sorghum forage was harvested at 110 days and ensiled for 60 days with the following treatments: without inoculant (CON); *Lactobacillus brevis* FNCC0265 as a heterofermentative inoculant (LB); *Lactobacillus plantarum* FNCC0020 as a homofermentative inoculant (LP). Both LB and LP inoculants were applied at a concentration of 1×10^5 cfu/g of fresh forage. Each treatment used five mini silos (5 kg each) as replicates. The mini silo consisted of a plastic bag placed inside a 20-L container. After opening the silos, both LB and LP silages resulted in lower DM (dry matter) loss than the CON silage ($p<0.05$). The concentration of acid detergent fibre, organic matter, crude protein and neutral detergent fibre was not impacted by inoculant application. According to the fermentation characteristics, both LB and LP silages showed a lower pH, along with increased lactate and LAB (lactic acid bacteria) count compared to CON silage ($p<0.05$). In addition, mould was not detected in LB and LP silages. Acetate concentration and aerobic stability were higher in LB silage than in LP and CON silages. In the rumen, LP and LB silage showed greater *in vitro* organic matter digestibility than CON silage ($p<0.05$). This study concluded that both *Lactobacillus brevis* and *Lactobacillus*

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plantarum improved silage quality and *in vitro* ruminal digestibility, while Lactobacillus *brevis* additionally enhanced aerobic stability of sorghum silage under tropical conditions.

Keywords: Aerobic stability, *in vitro* digestibility, *Lactobacillus brevis, Lactobacillus plantarum,* organic acid, sorghum silage, tropical climate

INTRODUCTION

Sorghum (*Sorghum bicolour* L. Moench) has emerged as a promising alternative forage crop for ruminant production due to its adaptability to drought-prone environments, high biomass yield, and competitive nutritive value compared with conventional forages such as corn (Reddy & Blümmel, 2020). Sorghum can replace corn as a forage crop for dairy and beef cattle in tropical regions, and its heat tolerance enables sorghum to thrive in such environments (Singh et al., 2014). Sorghum is widely recognised as a potential feed resource in tropical areas facing seasonal forage shortages (Dahlya et al., 2025; Pazla et al., 2025). However, its use in ruminants is limited by the presence of antinutritional factors. Antinutritional aspects in sorghum, including tannins and cyanogenic glycosides (HCN), reduce nutrient use and palatability in animals (Etuk et al., 2012). Previous studies have reported that ensiling can reduce tannin and HCN levels while preserving nutrients (Ardiansyah et al., 2025; Etuk et al., 2012; Fitriani et al., 2024). Thus, ensiling sorghum is an effective strategy to enhance its utilisation in ruminant feeding systems.

Forage is abundant during the rainy season but becomes scarce and declines in quality during the dry season (Cooke et al., 2025). Silage technology helps stabilise supply, reduce anti-nutritional factors in sorghum, and preserve roughage quality (Fitriani et al., 2024; Selim et al., 2024). However, tropical silage production is hindered by humidity, forage moisture, and high temperature (Ardiansyah et al., 2025; Bernardes et al., 2018). These conditions cause silages to undergo rapid spoilage and aerobic deterioration, which decreases their nutritional value (Bernardes et al., 2018; Borreani et al., 2018; Kung et al., 2018).

LAB (lactic acid bacteria) inoculants are widely utilised to enhance the quality of nasilage in tropical climates (Bernardes et al., 2018; Fitriani et al., 2024). Without them, heat and moisture can promote butyrate production by *Clostridium* (Bernardes et al., 2018; Kung et al., 2018; Muck et al., 2018; Paradhipta et al., 2019). LAB inoculants modify the fermentation characteristics of silage during ensiling. Homofermentative LAB, such as *Lactobacillus plantarum*, primarily ferments soluble carbohydrates into lactic acid, which results in decreasing pH and suppressing harmful microbes (Arriola et al., 2011; Queiroz et al., 2013). Heterofermentative LAB, like *Lactobacillus brevis*, produce acetic acid, which has antifungal properties that improve the silage aerobic stability during the feed-out phase

(Holzer et al., 2003; Paradhipta et al., 2021). Therefore, the current study was designed to evaluate the effects of hetero and homofermentative LAB as inoculants on nutrient loss, aerobic stability, fermentation characteristics, and ruminal digestibility of sorghum silage under tropical situations.

MATERIAL AND METHODS

Silage Production

Sorghum forage (*Sorghum bicolour* L. Moench) with variety Samurai 2 was planted at Bantul Region, Province of Daerah Istimewa Yogyakarta (-7.866458, 110.408891). A total of 100 kg of sorghum forage was harvested at day 110, containing 27.7% of dry matter (DM). Forage was chopped to a length of 3-5 centimetres and ensiled in 20-litre mini silos (5 kg) following a previously published method (Paradhipta et al., 2024). A plastic bag placed inside a container was used as a mini silo with 60 cm of diameter. The fermentation was conducted for 60 days with different inoculant treatments. The treatments consisted of without inoculant (CON); *Lactobacillus brevis* FNCC0265, as a heterofermentative employed at 1×10^5 cfu/g of forage (LB); *Lactobacillus plantarum* FNCC0020 (LP), as a homofermentative employed at 1×10^5 cfu/g of forage. Each treatment used five mini silos as replicates. All silos were placed at the feed warehouse under semi-field conditions with temperatures approximately 27.4°C to 33.8°C. Both *L. brevis* and *L. plantarum* were obtained from the Centre for Food and Nutrition Studies of Universitas Gadjah Mada. After the ensiling period for 60 days, 200 g of each sample was taken for *in vitro* ruminal digestibility tests and chemical composition (Ardiansyah et al., 2025; Fitriani et al., 2024; Paradhipta et al., 2024). For fermentation characteristics, approximately 20 g of silage was extracted, while aerobic stability was evaluated using 1 kg samples incubated under aerobic conditions in 20 L mini silos (Ardiansyah et al., 2025; Fitriani et al., 2024; Paradhipta et al., 2019).

Chemical Compositions

Samples of fresh sorghum and silage were dried at 55°C for 48 hours and then ground using a Willey mill to pass through a 1 mm sieve. These were analysed for chemical composition and *in vitro* digestibility. DM (dry matter) content was measured after drying at 105°C for 24 h (Association of Official Analytical Chemists [AOAC], 2005; method 934.01). Organic matter (OM) was quantified by ashing at 600°C for 2 hours (AOAC, 2005; method 942.05). CP (crude protein) was measured using the Kjeldahl method (AOAC, 2005; method 954.01), while EE (ether extract) was measured using the Soxhlet method (AOAC, 2005; method 920.39). Fibre fractions, including NDF (neutral detergent fibre) and ADF (acid detergent fibre), were analysed with a fibre analyser (Ankom A200, USA) based on the AOAC method (AOAC, 2005; method 973.18).

Fermentation Characteristics

Silage fermentation parameters were determined by blending 180 g of the sample with 200 mL of distilled water for 30 s, followed by filtration through double-layered gauze. The extract was used for ammonia, pH, VFA (volatile fatty acid), and lactate measurements. A pH-meter was utilised to analyse the value of pH (Ohaus AB23PH-F, China). Ammonia concentration was assessed colourimetrically according to a previous study (Chaney & Marbach, 1962).

Volatile fatty acid and lactate concentrations were analysed with HPLC (high-performance liquid chromatography). Prior to HPLC analysis, aqueous samples were centrifuged at 12,000 × *g* for 15 min to remove suspended solids and proteins. The supernatant was diluted with Milli-Q water, recentrifuged, and filtered through a 0.22 μm syringe filter into HPLC vials. The mobile phase comprised an acidified phosphate buffer (pH 2.0, adjusted with orthophosphoric acid) and a mixture of acetonitrile and methanol. The acidic buffer minimised fatty acid ionisation, thereby improving retention and separation on a reversed-phase C18 column. The method allowed accurate determination of the target fatty acids. The analysis was performed using a diode array detector (LC-2030C, Shimadzu, Japan) and a Shim-pack GIST C18 column, following methods outlined by Vargas et al. (2020) and Reuter et al. (2015). The column temperature was maintained at 30°C, the injection volume was applied at 20 μL, and the working wavelength was 210 nm with a flow rate of 0.750 mL/min.

Microbial Count

Silage extracts were serially diluted up to 10^{-7}, and appropriate dilutions (10^{-5} to 10^{-7}) were used to enumerate LAB, yeast, and mould populations. LAB were cultured on MRS agar, and yeast and mould on PDA, with all assays conducted in triplicate. Plates of MRS were incubated using low oxygen in a CO_2 incubator at 30°C for 48 hours, whereas plates of PDA were incubated aerobically at 37°C for 72 hours. The difference between yeast and mould was based on morphological characteristics such as appearance and filamentous growth. Microbial populations were expressed as $\log_{10}$ cfu/g (Paradhipta et al., 2021).

Aerobic Stability

Aerobic stability was evaluated by exposing 1 kilogram of silage to aerobic conditions in 20 L mini silos. Temperatures of both the ambient environment and the silage were measured every hour. Aerobic deterioration was considered to occur when the silage temperature increased by more than 2°C above the ambient temperature (Paradhipta et al., 2021).

In vitro Ruminal Digestibility

Rumen fluid was collected from two fistulated Balinese cattle with an average body weight of 300 kg. During maintenance, all animals were fed with the standard diet to ensure an isonitrogenous and isoenergic condition. The standard diet consisted of a 3:7 concentrate-to-forage diet (DM basis), containing 12% of CP and 10 kcal/kg of metabolizable energy. All animal care procedures and *in vitro* analyses were approved by the Ethics Committee of LPPT, Universitas Gadjah Mada (No. 00007/III/UN1/LPPT/EC/2024). Approximately 500 mL of rumen fluid was obtained before feeding in the morning, placed into anaerobic bottles, and transported to the laboratory for analysis. The buffer for the rumen was created by combining rumen fluid and McDougall solution at a ratio of 1:4. Approximately 0.5 g of dried silage sample was incubated with 40 millilitres of rumen buffer in CO_2-flushed bottles, which were then sealed and maintained at 39°C for 48 hours, with agitation performed every 8 hours. All of the procedures followed the method of Tilley & Terry (1963). Each treatment was applied in triplicate, including two blanks and two standard samples (Pangola grass). After incubation, the contents of the incubation bottle were filtered through Gooch crucibles, and the residues were used to determine *in vitro* DM digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). The rumen buffer pH was determined using a pH meter (Ohaus AB23PH-F, China).

The results were expressed as IVDMD and IVOMD. The rumen buffer after incubation was collected to measure the pH value using a pH meter (Ohaus AB23PH-F, China).

Statistical Analysis

A completely randomised design (CRD) with the PROC GLM (General Linear Model) procedure in SAS version 9.4 was used to analyse data. The model of statistics was $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} represents the observed response, μ denotes the overall mean, T_i the treatment impact of inoculant, and e_{ij} the random error. Each silo was considered the experimental unit (n = 5 per treatment). Analytical measurements, including fermentation characteristics, microbial counts, and *in vitro* ruminal digestibility, were conducted in triplicate, and the resulting values were averaged within each silo prior to statistical analysis. Post hoc comparisons were carried out using Tukey's test, with significance declared at $p \leq 0.05$.

RESULTS

Across all treatments, sorghum forage in the current analysis contained 27.7% DM, 90.3% OM, 7.74% CP, 1.45% EE, 48.3% NDF, and 27.8% ADF (Table 1). After ensiling for 60 days, the application of inoculant affected DM loss after ensiling (Figure 1). Both LB and LP silages had lower DM loss than CON silage ($p = 0.005$; 1.17% and 1.27% vs. 1.81%).

In addition, both LB and LP silages also had higher concentrations of DM after ensiling ($p = 0.049$; 27.0% and 27.2% vs. 26.3%) (Table 2). The concentration of EE was greater in LB silage than CON ($p = 0.036$; 3.11% vs. 2.01%), while LP silage did not differ compared to others. The concentration of OM, CP, NDF, and ADF from sorghum silage was not impacted by the application of inoculant. The means of OM, CP, NDF, and ADF from all treatments were 90.5%, 6.27%, 47.4%, and 37.6%, respectively.

Applications of LB and LP resulted in lower silage pH ($p = 0.045$; 3.58 and 3.57 vs. 3.69) than application of CON (Table 3). Ammonia was not impacted by the application of the inoculant. The mean of ammonia-N in all silages was 0.03%. The highest lactate concentration was reported in LP silage, followed by LB silage, and the lowest was in CON silage ($p = 0.002$; 3.03% vs. 2.68% vs. 2.26%). The concentration of acetate was greater in LB silage compared to in LP and CON silage ($p = 0.047$; 3.46% vs. 3.05% and 3.07%). Butyrate was not detected in all silages.

Table 1
Chemical compositions of sorghum forage before ensiling (%, DM)

Item	
Dry matter	27.7 ± 0.32
Organic matter	90.3 ± 0.51
Crude protein	7.74 ± 0.12
Ether extract	1.45 ± 0.02
Neutral detergent fibre	48.3 ± 0.23
Acid detergent fibre	37.8 ± 0.19

Note. DM = dry matter

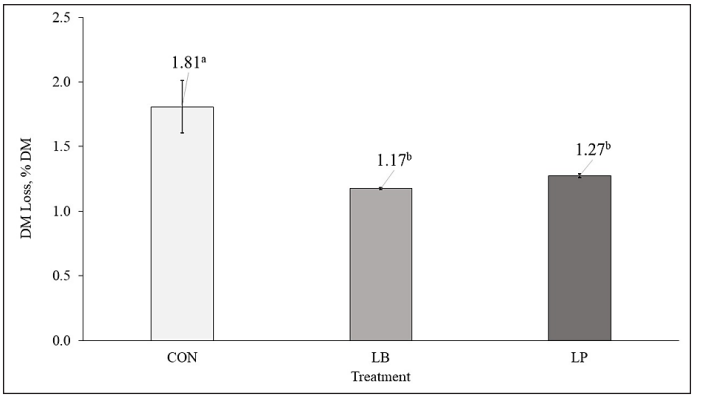

Figure 1. Effects of hetero and homofermentative inoculants on dry matter loss of sorghum silage ensiled for 60 days
Note. CON = silage without inoculant; LB= silage with *Lactobacillus brevis* FNCC0265 at 1 × 105 cfu/g of forage; LP = silage with *Lactobacillus plantarum* FNCC0020 at 1 × 105 cfu/g of forage; [a,b]Means with different superscript letters are significantly different ($p < 0.05$)

Table 2
Effects of hetero and homofermentative inoculants on chemical compositions of sorghum silage ensiled for 60 days (%, DM)

Item	Treatment[1]			SEM	p-value
	CON	LB	LP		
Dry matter	26.3[b]	27.0[a]	27.2[a]	0.518	0.049
Organic matter	90.2	91.0	90.4	0.733	0.212
Crude protein	6.24	6.29	6.30	0.483	0.985
Ether extract	2.01[b]	3.11[a]	2.64[ab]	0.584	0.036
Neutral detergent fibre	48.0	47.1	47.2	3.253	0.920
Acid detergent fibre	38.7	37.6	36.4	2.980	0.511

Note. [1]CON = silage without inoculant; LB= silage with *Lactobacillus brevis* FNCC0265 at 1×10^5 cfu/g of forage; LP = silage with *Lactobacillus plantarum* FNCC0020 at 1×10^5 cfu/g of forage
*DM = dry matter; SEM = standard error of mean; [a,b] Means with different superscript letters in a row are significantly different ($p < 0.05$)

Table 3
Effects of hetero and homofermentative inoculants on fermentation characteristics of sorghum silage ensiled for 60 days

Item	Treatment[1]			SEM	p-value
	CON	LB	LP		
pH	3.69[a]	3.58[b]	3.57[b]	0.007	0.045
Ammonia-N, % DM	0.03	0.03	0.04	0.008	0.355
Lactate, % DM	2.26[c]	2.68[b]	3.03[a]	0.118	0.002
Acetate, % DM	3.05[b]	3.46[a]	3.07[b]	0.136	0.047
Propionate, % DM	ND	ND	ND	NA	NA
Butyrate, % DM	ND	ND	ND	NA	NA

Note. [1]CON = silage without inoculant; LB= silage with *Lactobacillus brevis* FNCC0265 at 1×10^5 cfu/g of forage; LP = silage with *Lactobacillus plantarum* FNCC0020 at 1×10^5 cfu/g of forage
*DM = dry matter; SEM = standard error of mean; ND = not detected; NA = not applicable; [a,b] Means with different superscript letters in a row are significantly different ($p < 0.05$)

In microbial characteristics, LB and LP silages had greater LAB count ($p = 0.003$; 7.42 $\log_{10}$ cfu/g and 7.45 vs 7.23 $\log_{10}$ cfu/g), but lower mould count ($p = 0.003$; ND and ND vs 3.08 $\log_{10}$ cfu/g) than CON silage (Table 4). Mould was not detected in LB and LP silages. The yeast count was not impacted by the application of inoculant. The mean count of yeast from all silages was 5.24 $\log_{10}$ cfu/g. After the silo was opened, LB silage had a higher aerobic stability than CON and LP silages (Figure 2).

In the rumen, application of inoculant did not affect ruminal pH and IVDMD (Table 5). The means of rumen pH and DMD from all silages were 6.52 and 54.2%, respectively. However, LB and LP silages had a greater IVOMD compared to CON silage ($p = 0.007$; 64.7% and 63.8% vs. 61.8%).

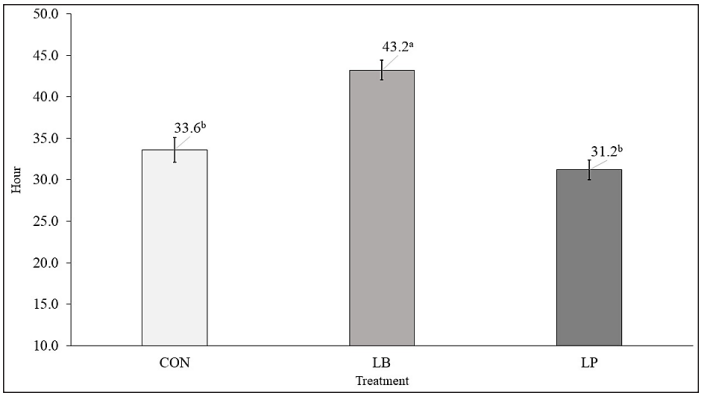

Figure 2. Effects of hetero and homofermentative inoculants on aerobic stability of sorghum silage ensiled for 60 days
Note. CON = silage without inoculant; LB= silage with Lactobacillus brevis FNCC0265 at 1 × 105 cfu/g of forage; LP = silage with Lactobacillus plantarum FNCC0020 at 1 × 105 cfu/g of forage; a,bMeans with different superscript letters are significantly different ($p < 0.05$)

Table 4
Effects of hetero and homofermentative inoculants on microbial counts of sorghum silage ensiled for 60 days (log_{10} cfu/g)

Item	Treatment[1]			SEM	p-value
	CON	LB	LP		
Lactic acid bacteria	7.23[b]	7.42[a]	7.45[a]	0.070	0.003
Yeast	5.30	5.20	5.22	0.010	0.506
Mold	3.08[a]	ND[b]	ND[b]	1,073	0.002

Note. [1]CON = silage without inoculant; LB= silage with *Lactobacillus brevis* FNCC0265 at 1 × 10^5 cfu/g of forage; LP = silage with *Lactobacillus plantarum* FNCC0020 at 1 × 10^5 cfu/g of forage
*DM = dry matter; SEM = standard error of mean; ND = not detected; NA = not applicable; [a,b] Means with different superscript letters in a row are significantly different ($p < 0.05$)

Table 5
Effects of hetero and homofermentative inoculants on ruminal digestibility of sorghum silage ensiled for 60 days

Item	Treatment[1]			SEM	p-value
	CON	LB	LP		
Rumen pH	6.47	6.54	6.55	0.970	0.416
IVDMD, % of DM	53.2	54.4	55.0	2.125	0.567
IVOMD, % of DM	61.8[b]	64.7[a]	63.8[a]	0.660	0.007

Note. [1]CON = silage without inoculant; LB= silage with *Lactobacillus brevis* FNCC0265 at 1 × 10^5 cfu/g of forage; LP = silage with *Lactobacillus plantarum* FNCC0020 at 1 × 10^5 cfu/g of forage
*IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; DM = dry matter; SEM = standard error of mean; [a,b] Means with different superscript letters in a row are significantly different ($p < 0.05$)

DISCUSSION

The chemical compositions of sorghum forage harvested at 110 days in the current analysis were within the normal range, consistent with other studies (Ardiansyah et al., 2025; Fitriani et al., 2024). After ensiling, the use of microbial inoculant in this study improved nutritional quality. According to findings, it was shown that the application of both homo and hetero LAB could decrease the DM loss of sorghum silage. This result was in line with several previous studies (Chotimah et al., 2024; Lee et al., 2021; Paradhipta et al., 2019, 2020). In addition, the level of EE was greater in LB silage than in CON silage, which indicated less lipolysis during ensiling due to the low pH condition (Table 3). The higher acetate concentration and lower pH observed in LB silage (Table 3) might have suppressed undesirable microbial activity, including lipolytic microbes and reduced nutrient degradation during fermentation (Liu et al., 2018; Kung et al., 2018; Lee et al., 2021). Furthermore, the lower DM loss observed in LB silage could have contributed to the relatively greater EE concentration after ensiling. In general, the decrease in nutrient loss by inoculant application was supported by the result of organic acid production (Table 3). Acetate generated by LB had a role as an antimicrobial agent (Holzer et al., 2003), while lactate generated by LP could establish an acidic condition that is unfavourable for the growth of unwanted microbes (Ardiansyah et al., 2025; Lee et al., 2019). The application of LB and LP had no effects on fibre fractions such as NDF and ADF because neither inoculant exhibited fibrolytic activity.

In fermentation characteristics, all inoculated silages resulted in lower pH than the CON silage. This result of pH was caused by higher production of organic acids (lactate and acetate) in LB and LP silages (Table 3). According to previous studies, the application of homofermentative generally increases lactate concentration, while the application of heterofermentative can improve acetate concentration (Arriola et al., 2011; Fitriani et al., 2024; Paradhipta et al., 2019). This is consistent with the results of lactate and acetate in this current study. The level of ammonia-N was not affected in the present study. This occurred because the pH was acidic enough to inhibit proteolytic activity (pH less than 4) (Tao et al., 2012). Moreover, the concentration of CP after ensiling did not differ among silages, which corroborated the findings of this study.

In this examination, the production of acetate was higher than the production of lactate in all silages. Generally, lactate is considered the main fermentation product. However, several previous studies also reported that a higher concentration of acetate than lactate in sorghum silage production conducted in tropical conditions (Ardiansyah et al., 2025; Fitriani et al., 2024). Although Lactobacillus plantarum is classified as a homofermentative LAB, the relatively high acetate concentration observed in LP silage suggests that fermentation may not have been exclusively dominated by the inoculated strain. Epiphytic heterofermentative LAB, enterobacteria, or yeasts naturally present on the forage may also

have contributed to acetate production during ensiling (Li et al., 2019; Muck et al., 2018). In addition, the inoculation level used in this study (1×10^5 cfu/g forage) might not have been sufficient to completely outcompete the native microflora under tropical conditions (Arriola et al., 2011; Kleinschmit et al., 2005).

Mainly, forage in tropical regions contains high moisture content, which could affect the ensiling process and organic acid production during incubation. Moreover, the temperature in a tropical climate was high, approximately 30°C - 33°C, during the incubation of silage. Similar to the present study, ensiling high-moisture forage at high temperature was reported to increase the potential of acetate production (Kim & Adesogan, 2006; Li et al., 2019). The microbial community has a pivotal role in acetic acid production. High-moisture silages favour *Lactobacillus* and *Enterobacter*, which can increase acetic acid production. In addition, *Clostridium* can produce butyric acid, thereby disrupting lactic fermentation (Li et al., 2019; Kung et al., 2018; Paradhipta et al., 2019). Despite high moisture, butyrate was not found in the silages. Their pH remained less than 4, sufficiently acidic to inhibit *Clostridium* growth (Borreani et al., 2018; Kung et al., 2018; Ogunade et al., 2018). However, parameters such as water-soluble carbohydrates, buffering capacity, and epiphytic microbial populations were not evaluated in the present study, which may limit further interpretation of the fermentation pathways observed in the silages.

This study found that inoculating sorghum silage increased LAB populations, aligning with previous reports (Chotimah et al., 2024; Lee et al., 2021; Paradhipta et al., 2020, 2021). Higher LAB counts in LB and LP silages led to more lactate, creating a low pH that suppressed mould growth (Kung et al., 2018; Muck et al., 2018; Queiroz et al., 2018).

In subtropical regions, silages such as corn, rye, and sorghum remain stable for more than two days (Lee et al., 2019; Paradhipta et al., 2019; Weinberg et al., 2011). In tropical areas, higher temperatures and humidity shorten stability, encouraging spoilage microbes after air exposure (Ardiansyah et al., 2025; Koc et al., 2009; Lee et al., 2019). Temperatures of 30-37°C accelerate deterioration by reducing LAB and promoting yeast, moulds, and CO_2 production (Koc et al., 2009). During this study, ambient temperature averaged 32.3°C in the afternoon and 26.7°C in the evening. LB silage extended shelf-life, linked to higher acetate levels. Heterofermentative LAB in LB silage generate acetate, which inhibits spoilage microbes (Holzer et al., 2003; Danner et al., 2003; Paradhipta et al., 2021). However, excess lactate can reduce stability, as it fuels yeast growth that occurs under aerobic conditions (Danner et al., 2003). Moreover, Danner et al. (2003) reported that yeast and mould use lactate as an energy source for growth during aerobic conditions, which could reduce the aerobic stability of silage.

LAB inoculation increased ruminal digestibility, as LB and LP silages showed higher IVOMD than CON, in line with other studies (Chotimah et al., 2024; Lee, Paradhipta et al., 2021; Paradhipta et al., 2020). LAB may function as probiotics that stimulate cellulolytic

microbes, improving fibre breakdown (Ellis et al., 2016; Oskoueian et al., 2021). However, the present study was based on an *in vitro* ruminal fermentation system and did not directly assess cellulolytic microbial populations or enzyme activities. Therefore, the proposed mechanism should be interpreted with caution, and further *in vivo* studies are needed to confirm whether similar responses occur under practical feeding conditions.

CONCLUSION

In conclusion, the application of both *Lactobacillus brevis* and *Lactobacillus plantarum*, representing hetero- and homofermentative LAB, respectively, improved fermentation quality, reduced nutrient losses and mould growth, and enhanced *in vitro* ruminal digestibility of sorghum silage. However, the use of *Lactobacillus brevis* is more effective, as it provides additional benefits in enhancing the stability of aerobic sorghum silage under tropical situations. In addition, further *in vivo* studies are required to confirm the beneficial effects of hetero- and homofermentative LAB on the nutrient utilisation of animals under practical feeding conditions.

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