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Chemical and Physical Quality, Fermentation Characteristics, Aerobic Stability, and Ruminal Degradability of Sorghum Silage Inoculated with *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum*

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ABSTRACT

This study was carried out to determine the effect of homo (*Lactiplantibacillus plantarum* FNCC 0020) and hetero (*Limosilactobacillus fermentum* BN21) fermentative lactic acid bacteria on chemical compositions, fermentation characteristics, aerobic stability, and ruminal digestibility of sorghum (*Sorghum bicolor* L. Moench) silage. The sorghum forage was harvested at the milk ripening phase with a dry matter content of 25.6% and fermented for 100 days with different inoculants: treatments without inoculant (CON), *L. plantarum* (LP), *L. fermentum* (LF) as well as a mixture of LP and LF at a ratio of 1:1 (MIX). The experiment was conducted using a completely randomized design with 5 replications per treatment, and all inoculants were applied at 10^5 cfu/g of fresh forage. The results showed that LF silage caused a 66.3% reduction in cyanide acid content, the lowest mold count, and longer aerobic stability compared to LP and CON. The lowest pH ($p<0.05$) and highest organic matter digestibility ($p<0.05$) were obtained on LP silage, while the CON silage showed no significant difference. The LP and LF silage showed the highest total volatile fatty acid ($p<0.05$), while there was no significant between CON and others. The LF silage had the highest acetate and the lowest propionate ($p<0.05$). These results showed that *L. fermentum* was more effective in decreasing cyanide acid content and increasing the aerobic stability of sorghum silage, while *L. plantarum* was able to lower pH and reduce ammonia concentration.

Keywords: aerobic stability; lactic acid bacteria; ruminal degradability; sorghum; silage

INTRODUCTION

Sorghum bicolor L. Moench is a tropical plant widely cultivated as a ruminant feed in Indonesia. In comparison with maize, sorghum has several advantages, including being cultivated in soil with limited water and high salinity with a high concentration of soluble carbohydrates (Zhang *et al.*, 2016) and the ability to regrow after cutting (Astuti *et al.*, 2019). However, the high tannin and cyanide contents of sorghum plants poses a significant challenge to its use as animal feed. One solution to the challenge is to use silage, which minimizes the anti-nutrient effects and prolongs the shelf life of the forage. Failures in silage production are often observed in tropical regions when harvesting during the rainy season or high moisture content (Zhao *et al.*, 2022). During ensiling, forage with high moisture content can produce butyric acid (McDonald *et al.*, 2011), as aerobic stability is often limited in tropical regions due to warm climates and high humidity, which promote the rapid growth of pathogenic fungi and bacteria (Bernardes *et al.*, 2018). Aerobic stability has been observed to persist longer in subtropical climates, with an average duration of approximately 149.2 hours (Joo *et al.*, 2018). Therefore, the addition of lactic acid bacteria (LAB) as an inoculant is a promising potential additive for modifying the ensilage process due to the ability to stimulate organic acid production, lower pH, inhibit the growth of harmful microorganism, and reduce nutrient losses (McDonald *et al.*, 2011).

Previous studies have shown that silage quality can be improved using homofermentative LAB by producing a high level of lactic acid, which lowers the pH rapidly (McDonald *et al.*, 2011) and creates an acidic environment. This process hinders the growth of harmful microorganisms and maintains feed nutrition effectively (Liu *et al.*, 2022). According to Danner *et al.* (2003) and Muck *et al.* (2018), heterofermentative LAB produces acetic acid acting as an antifungal agent which improves aerobic stability by reducing yeast and mold growth (Paradhipta *et al.*, 2021). It was reported that heterofermentative LAB produced acetic acid more prevalently compared to homofermentative LAB (Joo *et al.*, 2018). Although LAB inoculation in the ensilage process can increase silage digestibility due to fibrolytic enzyme activity (Paradhipta *et al.*, 2020), the effects of both bacteria as silage additives on ruminal methane emission have not been widely reported. LAB can produce bacteriocins

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which are capable of reducing methane by inhibiting methanogenic growth (Doyle *et al.,* 2019).

As homofermentative LAB, *Lactiplantibacillus plantarum* can improve silage quality by enhancing lactic acid levels, lowering pH, and reducing ammonium content (Zi *et al.,* 2021). *Limosilactobacillus fermentum* has been used as a substitute for preventing aerobic deterioration of silage due to antifungal activity (Chahrour *et al.,* 2013). Based on previous studies, heterofermentative LAB produced acetate, which acted as an antifungal agent with the potential to inhibit the growth of yeast and mold in silage (Danner *et al.,* 2003; Muck *et al.,* 2018; Paradhipta *et al.,* 2019; Paradhipta *et al.,* 2021). The combination of these bacteria has been shown to decrease pH levels, yeast, and mold counts, along with an increase in LAB levels and aerobic stability (Zielińska & Fabiszewska, 2018). There is still a lack of information regarding the effects of homofermentative and heterofermentative LAB on reducing anti-nutrient content and improving aerobic stability in tropical silage. Therefore, this study was conducted to determine the effects of *L. plantarum* and *L. fermentum* on chemical composition, ensilage characteristics, aerobic stability, and ruminal fermentation of sorghum silage.

MATERIALS AND METHODS

Inoculants Preparation

The bacterial strains *L. plantarum* FNCC 0020 and *L. fermentum* BN21 used in this study were collected from the Centre for Food and Nutrition Studies of Gadjah Mada University and the Nutritional Biochemistry Laboratory, Faculty of Animal Science, Gadjah Mada University, respectively. Subsequently, the samples collected were revived by culturing pure cultures in liquid media using de Mann-Rogosa-Sharpe (MRS) broth. MRS broth and culture tubes were sterilized using an autoclave at 121 ^{o}C and 15 psi pressure for 15 minutes. *L. plantarum* bacteria were grown in 10 mL of liquid medium using an Oxidative Stress Environment (OSE) and *L. fermentum* 1 mL in 9 mL of liquid medium. The inoculant was incubated at 37 °C for 48 hours and both LAB strains showed a colony value of 1 x 10^8 cfu/mL. Following incubation, an inoculant was applied to silage at a concentration of 0.1% of the fresh forage weight or 1 x 10^5 cfu/g fresh forage according to the recommendation of previous studies (Muck *et al.,* 2018; Paradhipta *et al.,* 2020).

Silage Production

Sorghum used in this study was *Sorghum bicolor* L. Moench variety samurai 2 obtained from the Research Center for Food Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN). It was grown in the Srandakan sub-district, Bantul regency, Yogyakarta, Indonesia (7.977S 110.224105E ± 3.10m 151^{o}SE). Sorghum was harvested at the milk ripening phase, with a moisture content of 25.6%. The sample was cut to a length of 3-5 cm and ensiled in 20 L mini silos (5 kg) with 5 replications for 100 days. The inoculant treatment used was as follows: silage without inoculant, added distilled water of 50 μL/g fresh sorghum (CON), and silage with *L. plantarum* FNCC 0020 1x10^5 cfu/g fresh sorghum (LP). Other treatments included silage with *L. fermentum* BN21 1x10^5 cfu/g fresh sorghum (LF) and silage with a mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at a ratio of 1:1 1x10^5 cfu/g fresh sorghum (MIX). A total of 500 g of fresh sorghum was taken for chemical composition analysis before ensiling. After 100 days of ensiling, 300 g of each silage sample was taken for chemical composition analysis and *in vitro* digestibility.

Chemical Composition

The fresh forage and silage sorghum samples were dried in a 55 °C oven for 72 hours and then ground using a Willey mill (Tube Mill Control, IKA, Germany) with a 1 mm diameter for analysis of chemical composition and in vitro digestibility. The fresh forage and silage sorghum samples were also dried in a 105 °C oven for 24 hours to measure dry matter content (DM) analysis (AOAC number 934.01; AOAC, 2005). The ash content was determined in a furnace at 600 °C for 2 hours (AOAC number 942.05; AOAC, 2005). The crude protein (CP) and ether extract (EE) were determined using the Kjeldahl and Soxhlet methods, respectively (AOAC number 984.13; AOAC number 920.39; AOAC, 2005). Neutral detergent fiber (NDF) analysis (AOAC number 2002.04; AOAC, 2005) and acid detergent fiber (ADF) analysis (AOAC number 973.13; AOAC, 2005) were conducted using a fiber analyzer (Ankom A 200, US). The tannin content of sorghum was determined by subtracting the total phenol content from the non-tannin phenol content based on the method described by Makkar (2003). The cyanide acid content was determined using the picrate base spectrophotometry method, based on the method described by Fukushima *et al*. (2016). The data of chemical compositions and tannin were expressed as % of DM while the data for hydrogen cyanide was expressed as ppm.

Fermentation Characteristics

A total of 20 g of silage samples were blended with 200 mL of distilled water, followed by filtering using gauze to obtain silage extract. In this study, silage extract was divided into 3 bottles to determine pH, ammonia, silage microbes, lactic acid, and volatile fatty acid (VFA). Ammonia levels were measured using the colorimetric principal method (Chaney & Marbach, 1962). After centrifuging silage extract at 3,000 rpm for 10 minutes, the filtrate was centrifuged at 15,000 rpm 10 minutes for lactic acid and VFA tests. Subsequently, the concentrations of lactate and VFA were determined using HPLC equipped with a diode array detector (DAD) (LC-2030C, Shimadzu, Japan) and a column (Shim-pack GIST C18, Shimadzu, Japan) according to Vargas *et al*. (2020) and Reuter *et al*. (2015).

Microbiological Counts

The microbial counts of LAB, yeast, and mold were determined from silage extract diluted to 10^{-5} to 10^{-7} and inoculated on selective agar media, with 3 replicates for each dilution series. The De MRS media was used to count the number of LAB, and potato dextrose agar (PDA) media was applied to yeasts and molds. The MRS agar plates were placed in a CO_2 incubator at 38 °C for 48 hours, and PDA agar was incubated in an aerobic incubator at 38 °C for 72 hours. This was followed by counting visible colonies, and the results were shown as log10 cfu per gram of silage (Paradhipta *et al.*, 2021).

Aerobic Stability

Aerobic stability was assessed by placing 3 kg of silage in a 20 L mini silo under aerobic conditions. The temperature was measured every 6 hours until the silage temperature increased by 2 °C above the ambient temperature. Aerobic stability was determined by calculating the time (in hours) taken for silage temperature to increase by 2 °C above the ambient temperature (Fernandes *et al.*, 2020).

In Vitro Ruminal Degradability

The rumen fluids were collected in the morning before feeding from one Bali Cattle, which had been fed grass and concentrate with a 6:4 ratio. All animal care and *in vitro* procedures followed the ethical standards of the Ethics Committee of the LPPT, UGM (No. 00007/III/UN1/LPPT/EC/2024). The rumen fluid was filtered through 2 layers of gauze and blended with an anaerobic culture medium at a ratio of 1:4, according to the Tilley & Terry (1963) method. A 0.5 g of silage sample from each silo was placed in a 100 mL glass (fermentor) in duplicates. Rumen fluid of 50 mL was introduced into the fermentor and CO_2 gas was allowed to flow for 5 seconds, creating an anaerobic environment. Subsequently, the fermentor with the sample was incubated at 39 °C for 48 hours, and the *in vitro* rumen digestibility was performed on 3 separate occasions as replication.

***In vitro* gas production**. After 48 hours, total gas production was collected using a syringe and 3 mL of gas was stored in a 3 mL vacuum blood tube for methane gas analysis. The analysis was carried out using gas chromatography (GC) apparatus equipped with a flame ionization detector (FID), designated GC-14B and manufactured by Shimadzu in Japan.

***In vitro* degradability**. After incubating for 48 hours, the fermenter was opened and the content was filtered through crucible filters coated with glass wool to separate the rumen buffer from the remaining sample. Subsequently, the remaining sample was used to calculate the dry matter digestibility (IVDMD) and the organic matter digestibility (IVODMD).

Ruminal fermentation characteristics. The supernatant was applied for the pH test, which was conducted using a pH meter (Mettler Toledo LE438, US) (Paradhipta *et al.*, 2021). Ammonia was analyzed using the colorimetric principle (Chaney & Marbach, 1962), while VFA was quantified GC with a FID (GC-2010 Plus, Shimadzu, Japan) and a column (CP FFAP CB, Shimadzu, Japan).

Organoleptic Appearances

After 100 days, sorghum silage was opened and organoleptic tests were conducted to evaluate color, aroma, and texture using a method described by Trisnadewi & Cakra (2020). A total of 26 non-experts (individuals without training in sensory assessment but with basic knowledge of silage area) completed a questionnaire containing various parameter questions. The organoleptic parameters included color (yellowish green, yellow, brownish yellow, and blackish brown), aroma (very sour, sour, less sour, and rotten), and texture (not lumpy, lumpy, slightly lumpy, and very lumpy).

Statistical Analysis

This study was conducted using a CRD with 4 treatments and 5 replicates. The data were analyzed using one-way ANOVA to determine significant differences between treatments. Duncan's multiple range test (Steel & Torie, 1993) was used for evaluation, and calculations were carried out using SAS® Studio software (Steel *et al.*, 1997). The relationships of chemical characteristics and silage variables were analyzed using Principal Component Analysis (PCA). The variables included in the analysis were organic acid, pH, ammonia, and microbial count. The orientation and extent of the vectors were interpreted to represent the correlation between variables related to silage. The PCA was performed in R software using the FactoMineR package according to the procedure described in (Le *et al.*, 2014).

RESULTS

Chemical Compositions

The results showed that sorghum forage before ensiling had 25.6% DM, 89.5% OM, 7.13% CP, 69.9% NDF, 0.43% tannins, and 187 ppm hydrogen cyanide, as presented in Table 1. The presence of tannins and hydrogen cyanide could be a toxicant with negative effects on animal health in high doses. After 100 days of ensiling, LF silage showed the highest decrease in cyanide acid content, followed by MIX, CON, and LP with 66.3%, 60.7%, 52.0%, and 30.1%, respectively. The LP silage had the highest hydrogen cyanide concentration ($p=0.012$; 131 ppm vs. 89.7, 63.1, and 29.73 ppm) compared to the others (Table 2). However, the application of inoculant treatments had no effects on tannin concentration after ensiling. The addition of inoculants did not affect the concentrations of DM, OM, EE, CP, NDF, ADF, and tannin. The average concentrations of DM, OM, CP, and

NDF from all treatments were 22.6%, 89.8%, 6.71%, and 64.4%, respectively.

Fermentation Characteristics and Microbial Count

The LP silage had the lowest pH (p=0.005; 3.93 vs. 4.06, 4.15, and 4.07) and ammonia concentration (p=0.001; 0.02% vs. 0.05%, 0.06%, and 0.04%) compared to the others, as shown in Table 3. Both LP and LF silage as single inoculant treatment had higher concentrations of major organic acids, such as lactate (p=0.029; 2.19% and 2.03% vs. 0.98%) and acetate (p=0.011; 1.95% and 2.10% vs. 0.95%), compared to the CON silage. However, MIX silage as a mixture inoculant treatment showed no significant difference in lactate and acetate concentration compared to others. Propionate and butyrate concentrations were not detected in all silages. The lowest mold counts were observed in LF silage, followed by LP and MIX, and CON (p<0.001; 4.85 log10 cfu/g vs. 5.81 and 5.75 log10 cfu/g vs. 7.11 log10 cfu/g). The addition of inoculant did not affect LAB and yeast counts.

Table 1. Chemical compositions of sorghum before ensiling

Items	Amount
Dry matter (% DM)	25.6
Organic matter (% DM)	89.5
Ether extract (% DM)	2.68
Crude protein (% DM)	7.13
Neutral detergent fiber (% DM)	69.9
Acid detergent fiber (% DM)	38.3
Tannins (% DM)	0.43
Hydrogen cyanide (ppm)	187

Aerobic Stability

The increase in silage temperature at 2 °C compared to the ambient temperature showed the end of aerobic stability of the silage after the silo opened. The tropical climate had a warmer temperature than the subtropical climate, which could decrease the aerobic stability of sorghum silage. Therefore, microbial additives were used to improve silage quality at the feed-out phase. At the 50th hour after the silo was opened, the temperatures of CON (31.9 °C) and LP (30.2 °C) silage were 2 °C higher than the ambient temperature (27.9 °C), as shown in Figure 1. At the 52nd hour after the silo was opened, temperatures of LF (30.3 °C) and MIX (30.6 °C) silage were 2 °C higher than ambient temperature (28.7 °C). These results showed that LF and MIX silage had a longer aerobic stability (p<0.05; 52.2 and 52.0 h vs. 49.6 48.8 h) silage, as presented in Figure 2. The inoculation of *L. fermentum* as a single or mixture could increase the aerobic stability of sorghum silage. At the 54th hour after the silo was opened, the highest and lowest temperature was observed in CON at 37.3 °C and LF silage

Table 2. Chemical composition of sorghum silages treated with different inoculants after ensiled for 100 days

Items	Treatments				SEM	p value
	CON	LP	LF	MIX		
Dry matter (% DM)	22.7	22.7	22.9	22.1	0.827	0.494
Organic matter (% DM)	89.8	90,0	89.6	89.8	0.266	0.229
Ether extract (% DM)	3.82	3.8	3.8	3.26	0.559	0.338
Crude protein (% DM)	6.74	6.79	6.6	6.69	0.274	0.733
Neutral detergent fiber (% DM)	63.4	65.0	66.0	63.0	1.850	0.067
Acid detergent fiber (% DM)	35.7	37.3	36.8	36.8	1.084	0.146
Tannins (% DM)	0.72	0.72	0.58	0.59	0.112	0.103
Hydrogen cyanide (ppm)	89.7^{b}	131^{a}	63.1^{b}	73.4^{b}	29.73	0.012

Note: Means in the same row with different superscripts differ significanly (p<0.05). CON= sorghum silage applied without inoculant; LP= sorghum silage with *Lactiplantibacillus plantarum* FNCC 0020; LF= sorghum silage with *Limosilactobacillus fermentum* BN21; MIX= sorghum silage with mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at ratio 1:1. SEM= standard error of the mean.

Table 3. Ensilage characteristics and microbial counts of sorghum silage treated with different inoculants after ensiled for 100 days

Items	Treatments				SEM	p value
	CON	LP	LF	MIX		
Ensilage characteristics						
pH	4.06^{a}	3.93^{b}	4.15^{a}	4.07^{a}	0.081	0.005
Ammonia (% DM)	0.05^{a}	0.02^{b}	0.06^{a}	0.04^{a}	0.012	0.001
Lactate (% DM)	0.98^{b}	2.19^{a}	2.03^{a}	1.66^{ab}	0.612	0.029
Acetate (% DM)	0.95^{c}	1.95^{ab}	2.10^{a}	1.35^{bc}	0.527	0.011
Lactate : acetate	1.06	1.13	0.94	1.36	0.274	0.139
Microbial count						
LAB log10 cfu/g	7.48	7.72	7.51	7.7	0.866	0.955
Yeast log10 cfu/g	7.72	7.4	7.24	7.38	0.470	0.444
Mold log10 cfu/g	7.11^{a}	5.81^{b}	4.85^{c}	5.75^{b}	0.484	<.0001

Note: Means in the same row with different superscripts differ significanly (p<0.05). CON= sorghum silage applied without inoculant; LP= sorghum silage with *Lactiplantibacillus plantarum* FNCC 0020; LF= sorghum silage with *Limosilactobacillus fermentum* BN21; MIX= sorghum silage with mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at ratio 1:1. SEM= standard error of the mean.

at 31.9 °C, respectively, with the ambient temperature of 26.5 °C.

In Vitro Rumen Degradability

The addition of inoculants did not affect *in vitro* total gas production, methane gas concentration, ammonia concentration, and IVDMD, as shown in Table 4. The result of IVOMD was higher in LP silage compared to the LF and MIX silages (p=0.047; 67.4% vs. 65.2% and 65.2%), while the CON showed no significant difference from the other treatments. Ruminal fermentation characteristics showed that the CON silage had the highest pH (p=0.005; 6.97 vs. 6.89, 6.91, and 6.90). The LP and LF silage had higher total VFA values than the MIX silage (p=0.005; 110.0 and 102.0 mM/L vs. 58.0 mM/L), while the CON treatment did not differ significantly from the others. The acetate concentration was the highest in the LF silage (p=0.009; 67.9% vs. 58.5%, 63.3%, and 57.8 %). In comparison, the LF silage had a lower propionate concentration than the CON and MIX silage (p=0.030; 23.8% vs. 29.0% and 29.0%), while the LP showed no significant difference from the other treatments. The MIX silage had a higher butyrate concentration compared to LP and LF silage (p=0.002; 12.9% vs. 10.2% and 8.40%). The LF silage produced the highest acetate to propionate ratio (p=0.007; 2.91% vs. 2.07%, 2.39%, and 2.00%).

Organoleptic Tests and Relationship between Parameters

Organoleptic tests on silage included color, aroma, and texture. Based on the results, all silages were mostly yellowish green in color (Figure 3), with aroma ranging

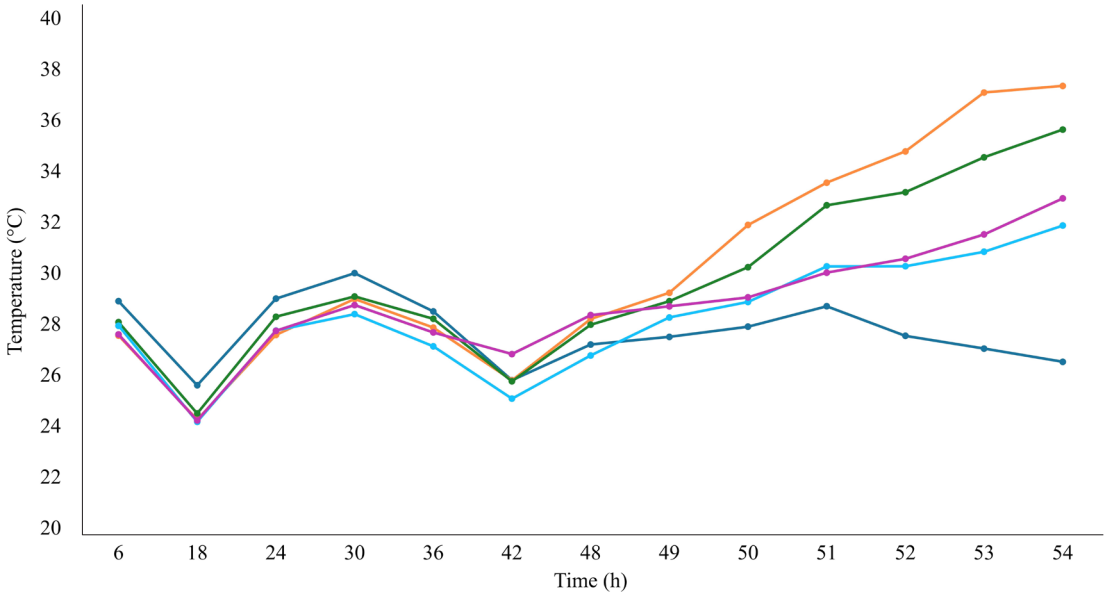

Figure 1. Change of temperature from sorghum silage treated with different inoculants during aerobic exposure (Time). LINK= Ambient; CON= sorghum silage applied without inoculant; LP= sorghum silage with *Lactiplantibacillus plantarum* FNCC 0020; LF= sorghum silage with *Limosilactobacillus fermentum* BN21; MIX= sorghum silage with mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at ratio 1:1.

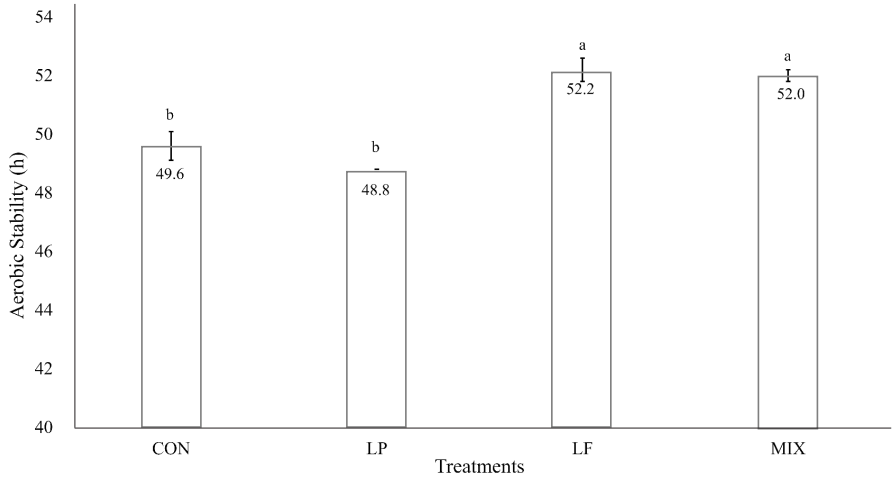

Figure 2. The aerobic stability of sorghum silage treated with different inoculants after being ensiled for 100 days. LINK= Ambient; CON= sorghum silage applied without inoculant; LP= sorghum silage with *Lactiplantibacillus plantarum* FNCC 0020; LF= sorghum silage with *Limosilactobacillus fermentum* BN21; MIX= sorghum silage with mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at ratio 1:1.

from less sour to sour (Figure 4) and the texture was mostly not lumpy (Figure 5). Regarding aroma, LP silage had the highest percentage of being very sour compared to the others (26.9% vs. 15.4%, 11.5%, and 19.2%). The relationship between parameters was analyzed using PCA, as shown in Figure 6. In Dim1, variables with positive loadings were mold (>0.5) and yeast (<0.5). In Dim2, variables with negative loadings were ammonia, pH, LAB (<0.5), lactate, and acetate (<0.5). PCA biplot results for the microbial count and chemical characteristics of silage showed that the Dim1 value was 34.3% and Dim2 value was 65.1% of the cumulative variation value of the data.

DISCUSSION

The sorghum used in this study had a chemical composition similar to that of previous studies (Mccary & Faciola, 2020; Zhao *et al*., 2022). Inoculant application after ensilage did not affect the chemical composition

Table 4. Gas production, *in vitro* rumen degradability, and fermentation characteristics of sorghum silage treated with different inoculants after incubation for 48 h

Item	Treatments				SEM	p value
	CON	LP	LF	MIX		
In vitro gas production						
Gas production (mL)	47.1	49.3	46.2	48.0	2.925	0.420
CH_4 (%)	4.82	4.44	4.78	4.75	0.284	0.189
In vitro degradability						
IVDMD (% DM)	60.2	60.8	60.4	59.9	1.282	0.679
IVOMD (% DM)	66.6^{ab}	67.4^{a}	65.2^{b}	65.2^{b}	1.323	0.047
Ruminal fermentation characteristics						
pH	6.97^{a}	6.89^{b}	6.91^{b}	6.90^{b}	0.031	0.005
Ammonia (mg/100 mL)	3.99	3.93	4.24	3.75	0.465	0.455
Total VFA (mM/L)	76.3^{ab}	110.0^{a}	102.0^{a}	58.0^{b}	28.57	0.039
Acetate (% of molar)	58.5^{b}	63.3^{b}	67.9^{a}	57.8^{b}	4.494	0.009
Propionate (% of molar)	29.0^{a}	26.5^{ab}	23.8^{b}	29.2^{a}	2.908	0.030
Butyrate (% of molar)	12.5^{ab}	10.2^{bc}	8.40^{c}	12.9^{a}	1.740	0.002
Acetate : Propionate ratio	2.07^{b}	2.39^{b}	2.91^{a}	2.00^{b}	0.382	0.007

Note: Means in the same row with different superscripts differ significanly (p<0.05). CON= sorghum silage applied without inoculant; LP= sorghum silage with *Lactiplantibacillus plantarum* FNCC 0020; LF= sorghum silage with *Limosilactobacillus fermentum* BN21; MIX= sorghum silage with mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at ratio 1:1. SEM= standard error of the mean; IVDMD= *in vitro* dry matter digestibility; IVOMD= *in vitro* organic matter digestibility.

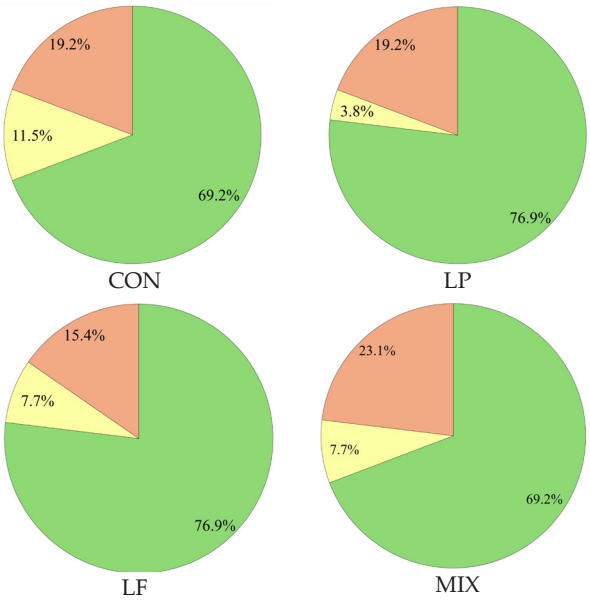

Figure 3. Colour of sorghum silage treated with different inoculants. LINK= Ambient; CON= sorghum silage applied without inoculant; LP= sorghum silage with *Lactiplantibacillus plantarum* FNCC 0020; LF= sorghum silage with *Limosilactobacillus fermentum* BN21; MIX= sorghum silage with mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at ratio 1:1. = Yellowish green; = Yellow; = Brownish yellow; = Blackfish brown.

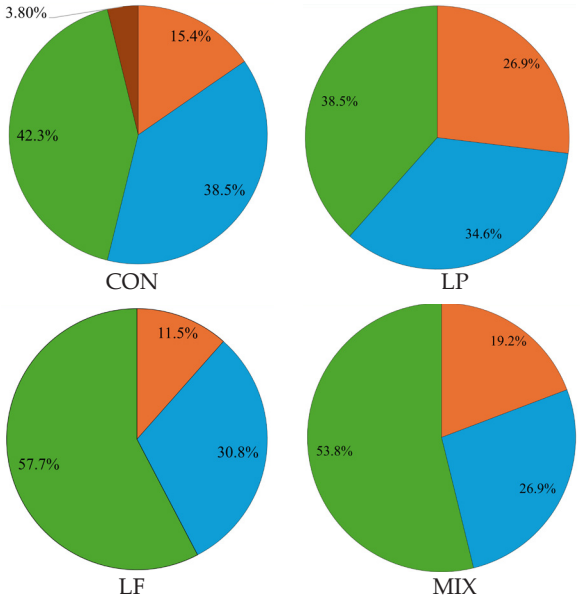

Figure 4. Aroma of sorghum silage treated with different inoculants. LINK= Ambient; CON= sorghum silage applied without inoculant; LP= sorghum silage with *Lactiplantibacillus plantarum* FNCC 0020; LF= sorghum silage with *Limosilactobacillus fermentum* BN21; MIX= sorghum silage with mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at ratio 1:1. = Very sour; = Sour; = Less sour; = Rotten.

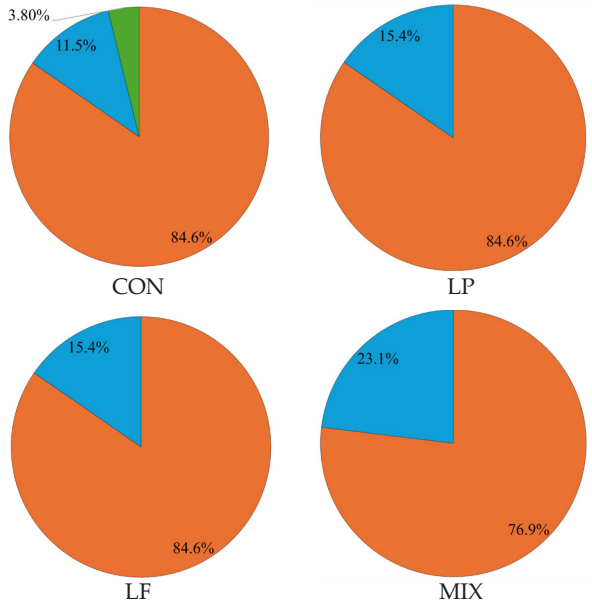

Figure 5. Texture of sorghum silage treated with different inoculants. LINK= Ambient; CON= sorghum silage applied without inoculant; LP= sorghum silage with *Lactiplantibacillus plantarum* FNCC 0020; LF= sorghum silage with *Limosilactobacillus fermentum* BN21; MIX= sorghum silage with mixture of *L. plantarum* FNCC 0020 and *L. fermentum* BN21 at ratio 1:1. = Not lumpy; = Slightly lumpy; = Lumpy; = Very lumpy.

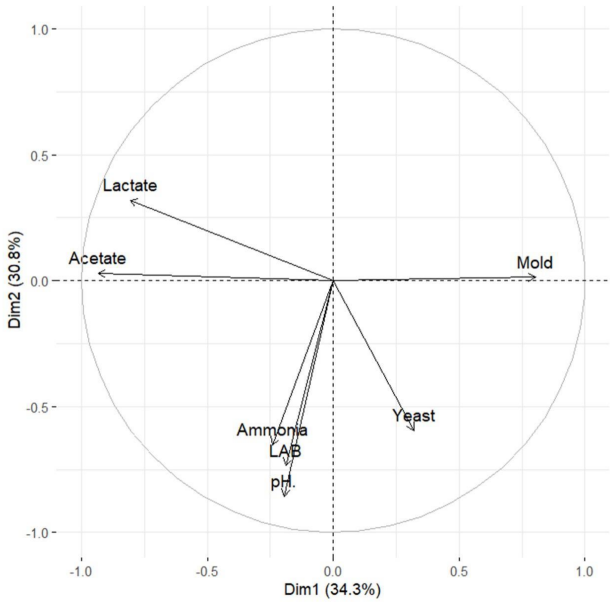

Figure 6. Principal component biplots of silage variable. LAB= Lactic acid bacteria. Dim1= first principal component; Dim2= second principal component.

of sorghum due to favorable acidification of silage. As shown in Table 3, pH value lower than 4.2 stabilized the fermentation process by inhibiting microbe growth (Kung *et al.,* 2018). These results were consistent with previous reports by Joo *et al.* (2018) and Paradhipta *et al.* (2021).

LP silage had the highest cyanogenic acid content compared to the other treatments. This was due to the rapid pH reduction during the initial fermentation phase, leading to less efficient linamarase enzyme production by LP silage bacteria and the lowest pH value. The optimal conditions for linamarase enzyme activity were pH of 7 and a temperature of 45 °C (Adeleke *et al.,* 2017). The linamarase enzyme hydrolyzed cyanogenic glycosides to cyanohydrin, which decomposed to acetone and hydrogen cyanide, is readily soluble in water or released into the air (Murugan *et al.,* 2012). Meanwhile, the inoculation of *L. fermented* caused the highest reduction in hydrogen cyanide concentration after ensiling. Previous studies reported that *L. fermentum* showed the highest linamarase enzyme activity compared to the other LAB (Nwokoro, 2016). According to Khota *et al.* (2023), the fermentation process could reduce cyanide acid content by 47%–51%.

The tannin content of sorghum forage was lower compared to sorghum. The variation in results was due to a change in water content before and after silage making, affecting the tannin content as a percentage of DM. The application of *L. fermentum* has shown a 19.4% reduction in tannin levels compared to the control sample. Although *L. plantarum* alone did not significantly reduce tannin content, its combination with *L. fermentum* resulted in a notable 18.05% reduction. According to Nasyatul-Ekma *et al.* (2018), there was an observed 20.8% decrease in tannin levels in *Acacia mangium* silage when inoculants were applied, attributing the effect of LAB producing tannase (Chávez-González *et al.,* 2018).

All treatments showed favorable fermentation characteristics based on pH levels, including the absence of butyrate (Paradhipta *et al.,* 2020) and appropriate ammonia concentrations (Wang *et al.,* 2018). The silage treated with *L. plantarum* showed the lowest pH due to the increased lactic acid production and reduced ammonia (Chotimah *et al.,* 2023). As a homofermentative LAB, *L. plantarum* effectively converts glucose and fructose into lactic acid (McDonald *et al.,* 2011), leading to a lower pH (Kung *et al.,* 2018). This was in line with Liu *et al.* (2022), where *L. plantarum* significantly reduced the pH of tropical forage silage compared to the control group. Additionally, LF showed the lowest ammonia concentration because of its ability to inhibit protein degradation (Kung *et al.,* 2018) and amino acid deamination (Liu *et al.,* 2022). Specifically, *L. plantarum* silage showed a protein loss of 4.77%, lower than the control (5.46%), MIX (6.17%), and *L. fermentum* (7.43%). A lower pH inhibited protease activity, which reduced ammonia formation (Zi *et al.,* 2021). Wang *et al.* (2018), Gang *et al.* (2020), and Liu *et al.* (2022) also observed lower ammonia levels in silages treated with *L. plantarum* compared to untreated controls.

The use of inoculants significantly increased both acetate and lactate concentrations in sorghum silage. Silages treated with *L. plantarum* and *L. fermentum* produced nearly identical levels of lactic and acetic acids, as indicated in Table 3. This is consistent with Andrada *et al.* (2023), who observed similar acid concentrations in silages inoculated with either *L. plantarum* or *L. fermentum*. Previous studies have shown that homofermentative LAB produces more lactic acid, while heterofermentative LAB generates higher acetic acid

levels (Si *et al.,* 2018). In the mixed treatment, the lactic acid concentration was comparable across treatments, while acetate levels were similar to those in both *L. plantarum* and control silages. This could be due to bacterial competition, which may have influenced organic acid production. Additionally, high ambient temperatures in tropical environments created suboptimal conditions for both *L. plantarum* and *L. fermentum* to produce organic acids. These results reflect the influence of multiple factors, such as the specific *Lactobacillus* strain, the composition of the growth medium, and environmental conditions (Bangar *et al.,* 2022). Inoculated silages also exhibited fewer mold colonies compared to the control, with *L. fermentum* silage showing the lowest count, likely due to its higher acetic acid production. Acetic acid is known to inhibit undesirable microorganisms (Zi *et al.,* 2021), which prevents yeast proliferation (Paradhipta *et al.,* 2020). These results align with previous studies that found a lower mold colony count in silage treated with heterofermentative bacteria (Joo *et al.,* 2018).

The data also showed that the addition of *L. fermentum* BN21 led to an increase in temperature compared to the control, indicating that the aerobic stability of both LF and MIX silage was two hours longer than the control and LP silage. The highest temperature 37.3 °C was recorded in the control silage, while the lowest 31.9 °C occurred in silage treated with LF. The growth of yeast, mold, and spoilage organisms was suppressed by acetate, which acted as an antimicrobial (Paradhipta *et al.,* 2019; Li *et al.,* 2016; Zi *et al.,* 2021). Control silage showed lower aerobic stability and higher temperatures, which can be attributed to lower acetate content and a higher mold count, as shown in Table 3. Overall, the aerobic stability of silages with LAB additives in tropical conditions lasted approximately 52 hours, as Pinto *et al.* (2020) reported. This could be due to the high temperature and humidity, which triggered the growth of fungi and bacteria (Bernardes *et al.,* 2018), making silage exposed to oxygen easily damaged. According to Joo *et al.* (2018), the use of heterofermentative LAB could enhance aerobic stability compared to the control in silage.

A lower pH value was observed in the *in vitro* rumen fermentation media due to the addition of inoculants in sorghum silage compared to the control. Despite the differences, the pH values across all treatments remained within the normal range for rumen fermentation (McDonald *et al.,* 2011). A low pH in the *in vitro* rumen fermentation media showed high substrate degradation (Chotimah *et al.,* 2023). The high digestibility of organic matter in LP silage was attributed to the simpler fiber components, which facilitated digestion. Additionally, there was a positive correlation between rumen pH values, total VFA, and IVOMD, resulting in LP silage. The high digestibility of organic matter in LP silage led to the increased total VFA and could lower the pH value of LP silage (Table 4). Gang *et al.* (2020) discovered a positive correlation between nutrient digestibility and VFA production. Enhanced nutrient digestibility caused higher total VFA production. The LF silage produced the highest acetate and acetate:propionate ratio and a lower propionate concentration than the control due to its relatively high acetate content (Table 3). High acetate production is associated with high fiber digestibility, while elevated propionate production is related to high water-soluble carbohydrate (WSC) content (Gang *et al.* 2020). Ammonia concentration, gas production, methane, and dry matter digestibility values were significantly different across all treatments, possibly due to similar nutrient content (Table 2) and LAB count (Table 3) observed in all silage. This was consistent with the results of Gang *et al.* (2020), suggesting that the addition of inoculants does not affect ammonia content *in vitro*.

The results presented in Table 3 showed that silage with high lactic and acetic acid production caused a yellowish-green color, a slightly sour aroma, and no clumping. There was a positive correlation between ammonia concentration, pH, and LAB values, as shown in Figure 6. Furthermore, a significant decrease in pH was accompanied by a decrease in ammonia levels. Zi *et al.* (2021) suggested that a low pH value reduced protease activity, reducing ammonia production. The acetate concentration was positively related to the concentration of lactate (Figure 6). The data showed a positive correlation between lactate and acetate levels in all silages. Higher lactate levels corresponded to higher acetate levels and vice versa. Lactate concentration has a negative relationship with yeast, while mold concentration has a negative relationship with acetate (Figure 6). LF silage produced high acetate concentrations, which were negatively correlated with low mold counts (Table 3). It was also reported that acetate inhibited yeast and mold growth when silage is exposed to oxygen (Li *et al.,* 2016; Paradhipta *et al.,* 2019).

CONCLUSION

In conclusion, this study showed that the inoculation of *L. plantarum* FNCC 0020 as homofermentative LAB had no significant effect on the reduction of anti-nutrient content and improvement of aerobic stability of sorghum silage ensiled in a tropical climate. However, *L. plantarum* FNCC 0020 improved fermentation quality by decreasing pH value and ammonia concentration. The inoculation of *L. fermentum* BN21 as heterofermentative effectively decreased the concentration of hydrogen cyanide, inhibited mold growth, and increased aerobic stability of sorghum silage. The combination of these inoculants showed significant potential to increase aerobic stability and decrease mold growth but was not optimal compared to individual applications. The use of all inoculants in sorghum silage production did not significantly impact the *in vitro* rumen fermentation process.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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