p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, May 2025, 48(3):257-266
DOI: https://doi.org/10.5398/tasj.2025.48.3.257
Available online at https://journal.ipb.ac.id/index.php/tasj

Preservation of High-Moisture Sorghum Silage Using Combination of Biological and Chemical Additives in the Tropical Region

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(Received 13-02-2025; Revised 30-03-2025; Accepted 08-04-2025)

ABSTRACT

This study aimed to investigate the effect of the biological and chemical additives on fermentation characteristics, aerobic stability, and ruminal digestibility of high-moisture sorghum silage. A mixture of *Lactiplantibacillus plantarum* FNCC 0020 (LP) and *Limosilactobacillus fermentum* BN21 (LF) was used as a biological additive and potassium sorbate as a chemical additive. At the milk ripening stage (26.8% of DM), sorghum was harvested, chopped to 3-5 cm length, and ensiled into 20 L silo (5 kg) for 100 days. Subsequently, various additives were added, including a control group without additives (CON), LF + LP with a ratio of 1:1 at 1 x 10^5 cfu/g fresh weight (INO), potassium sorbate at 1 g/kg fresh weight (PS), and INO + PS (MIX). Each treatment used 5 silos as replication. The results showed that INO silage had the lowest ($p<0.05$) pH with the highest ($p<0.05$) contents of lactate and acetate, as well as lactic acid bacteria (LAB) count. PS silage produced the minimum contents of lactate and acetate but had lower yeast compared to CON silage. MIX silage had lower ($p<0.05$) lactate and acetate contents than CON silage, with a similar effect on yeast inhibition to PS silage. Furthermore, PS and MIX silages had higher ($p<0.05$) aerobic stability and *in vitro* digestibility of dry matter and organic matter than CON and INO. These results showed that combining biological and chemical additives was more effective in improving fermentation, aerobic stability, and ruminal digestibility of high-moisture sorghum silage.

Keywords: Lactiplantibacillus plantarum; Limosilactobacillus fermentum; potassium sorbate; high-moisture sorghum silage

INTRODUCTION

In the tropical region, the reduction of forage quantity and quality is often affected by the dry season, causing a decrease in ruminant production (Salmoral *et al*., 2020). To address this challenge, the application of silage can help to reduce nutrient loss and increase the number of probiotic bacteria in the feed (Huang *et al*., 2021). However, high temperatures and humidity in tropical regions promote the growth of yeasts and molds, with most forage ensiled in high-moisture silage (HMS). This process is conducted because most forage is harvested during the rainy season, when the wilting process is difficult to conduct due to high rainfall intensity and humidity.

Sorghum bicolor (L.) Moench is the alternative tropical forage that has adaptation in drought conditions (Liu *et al*., 2024), high biomass production, re-growth ability (Terler *et al*., 2021), and elevated content of water-soluble carbohydrates (WSC) (Zhang *et al*., 2024). In the field, sorghum still contains high moisture content despite being harvested with soft or hard dough (Orrico Junior *et al*., 2020; Arriola *et al*., 2021; Dong *et al*., 2022). Preservation of high-moisture sorghum as silage can promote the growth of clostridia bacteria and increase butyric acid production (Wan *et al*., 2021).

The use of homofermentative lactic acid bacteria (LAB) was reported to accelerate the pH reduction in silage because of their capability to produce lactate during fermentation and decrease nutrient losses during ensiling (Kim *et al*., 2021). Moreover, heterofermentative LAB was applied due to the acetate production as an antibacterial compound to prevent yeast and mold when silos were exposed to the air (Ni *et al*., 2016). The combination of homofermentative and heterofermentative LAB evidently improved the fermentation of silage in several studies, such as increased acetate and lactate of mixed grass, high moisture corn, and high-moisture sorghum silage (Paradhipta *et al*., 2019; Auerbach and Nadeau, 2020; Da Silva *et al*., 2024). The application of *Lactiplantibacillus plantarum* (*L. plantarum*) as a homofermentative bacteria can improve silage quality through rapid pH reduction, increase silage digestibility (Li *et al*., 2020), and reduce NH_3-N content (Xie *et al*., 2020). Furthermore, the addition of *Limosilactobacillus fermentum* (*L. fermentum*) as a heterofermentative LAB is considered to be an antibacterial agent due to its ability to prevent

contamination by Clostridia and enterobacteria (Zheng *et al*., 2011). The prior studies had proven that the inoculation of *L. fermentum* could enhance acetate production and perform longer aerobic stability in corn, stylo, and sugarcane tops silage (Puntillo *et al*., 2020; Pitiwittayakul *et al*., 2021; Chauhan *et al*., 2024).

In previous studies, the combination of the homofermentative bacteria *L. plantarum* and the heterofermentative bacteria *L. fermentum* was insufficient to prevent nutrient loss and improve fermentation characteristics in sorghum silage under tropical conditions (Fitriani *et al*., 2024). The addition of potassium sorbate can be used to control fungal activity, thereby preventing aerobic deterioration (Pahlow *et al*., 2015) and increasing silage fiber digestibility by the degradation of lignocellulose (Singh *et al*., 2021). The combination of PS and LAB in silage showed a positive relationship which decreased pH as well as increased lactate, acetate, and aerobic stability (Hafner *et al*., 2015; Wang *et al*., 2023a; Juráček *et al*., 2024). Therefore, this study aimed to investigate the effects of biological and chemical additives on fermentation characteristics, aerobic stability, and ruminal *in vitro* digestibility of high-moisture sorghum silage.

MATERIALS AND METHODS

Inoculum Preparation

Isolate *L. plantarum* bacteria strain FNCC 0020 was obtained from the Food and Nutrition Center Laboratory of Universitas Gadjah Mada in solid culture form. Additionally, *L. fermentum* bacteria strain BN21 was obtained from the Nutritional Biochemistry Laboratory, Faculty of Animal Science, Universitas Gadjah Mada, in liquid form. Bacteria were recultured by growing pure cultures in liquid media using De Man-Rogosa-Sharpe (MRS) Broth (Merck KgaA Darmstadt, Germany) and incubated at 30°C until the bacterial colony reached a minimum of 1×10^8 cfu/mL.

Silage Production

Sorghum bicolor L. Moench variety Samurai-2 (National Research and Innovation Agency; BRIN) was planted in Yogyakarta, Indonesia. Subsequently, sorghum forage was harvested at the milk ripening stage (26.8% of DM) and chopped into 3-5 cm. The chopped forage was divided into 5 treatments following: (1) silage without additive, added pure water up to 50 µL/g fresh sorghum (CON), (2) silage with a combination inoculant *L. plantarum* FNCC 0020 and *L. fermentum* BN21 ratio 1:1 ($1x10^5$ cfu/g fresh matter) (INO), (3) silage with addition potassium sorbate 1 g/kg fresh matter (PS), and (4) silage with combination of INO and PS (MIX). Each treatment was sub-sampled (500 g) for chemical composition analysis. Subsequently, 5 kg from each treated forage was put into a 20 L mini silo and ensiled with 5 replication for 100 days. After 100 days of ensiling, 300 g sample was collected for oven drying at 55 °C for chemical composition analysis and *in vitro* digestibility. A total of 20 g sample was blended with 200 mL pure water for fermentation characteristics and microbial analysis (Paradhipta *et al*., 2021). In the end, 3 kg of each silage remained in 20 L mini silo for an aerobic stability test.

Chemical Composition

Fresh forage and silage sorghum content was dried at 55 °C for 48 h. The dried samples were ground using a Willey mill with a 1.0 mm sieve and used for dry matter (DM) analysis (AOAC number 934.01; AOAC, 2005) and organic matter (OM) (AOAC number 942.05; AOAC, 2005). The crude protein (CP) contents were determined using the Kjeldahl (AOAC number 984.13; AOAC, 2005). Ether extract (EE) contents were analyzed using Soxhlet (AOAC number 920.39; AOAC, 2005). Neutral detergent fiber (NDF) (AOAC number 2002.04; AOAC, 2005) and acid detergent fiber (ADF) (AOAC number 973.13; AOAC, 2005) analyses were performed using fiber analyzer (ANKOM A200, US). Subsequently, non-fiber carbohydrate (NFC) contents were determined according to Hall (2003). Tannin contents were analyzed by subtracting the total phenol content from the non-tannin phenol content (Makkar, 2003).

Fermentation Characteristics

Silage extraction was prepared to be used for measuring pH levels alongside lactate, volatile fatty acid (VFA), and ammonia-N (NH3-N). The preparation was conducted according to a previous study (Arriola *et al*., 2012), where 20 g silage samples from each silo were blended with 200 mL distilled water for 30 sec. The mixture was then filtered through a two-layer gauze. The silage pH was measured with a pH meter (Mettler Toledo LE438, US). Ammonia-N contents were analyzed using the colorimetric method (Chaney & Marbach, 1962). The sample of silage extract was centrifuged at 3,000 rpm for 10 min, and the filtrate was used to determine lactate and VFA contents through high-performance liquid chromatography (HPLC; Shimadzu LC-2030C 3D Plus, Japan) with a C18 column (Shimadzu Shim-pack GIST, Japan) according to Vargas *et al*. (2020).

Microbial Counts

A total of 20 g of silage sample from each silo were diluted into 180 mL sterilized distilled water. The mixture was continued to several serial dilutions from 10^{-5} to 10^{-7}. The number of LAB was grown on MRS agar (Merck KgaA Darmstadt, Germany) and incubated at 30°C for 48 h under anaerobic conditions in a CO_2 incubator (Binder GmbH, Germany), whereas yeast and mold were grown on potato dextrose agar (PDA; Merck KgaA Darmstadt, Germany) and incubated at 30°C for 72 h in an aerobic incubator (Binder GmbH, Germany). Visible colonies were determined using a colony counter (Inter-science International, France), and the results were transformed into $\log_{10}$ cfu/g of silage (Paradhipta *et al*., 2021).

Aerobic Stability

A total of 3 kg silage was opened to be exposed aerobically and placed at room temperature, which was recorded using a sensitive temperature for each hour. The aerobic stability of silage was determined as the length of hours before the temperature increased by 2 °C above room temperature (Auerbach *et al*., 2020).

In Vitro Ruminal Digestibility

The rumen fluid was collected in the morning before feeding from non-pregnant (heifer) Bali Cattle-fed grass and concentrate with a 6:4 ratio. All animal care and *in vitro* procedures were carried out in line with the ethical standards of the Ethics Committee of the LPPT, UGM (No. 00007/III/UN1/LPPT/EC/2024). A total of 0.5 g samples from each treatment were placed into 100 mL *in vitro* bottles. Rumen fluid was placed in a thermos adjusted to a temperature of 39 °C. Furthermore, *in vitro* digestibility analysis was carried out according to Tilley & Terry (1963). During *in vitro* implementation, the rumen fluid collected from healthy cattle was filtered using double-layer gauze and artificial saliva solution (McDougall solution) in a ratio of 1: 4. When filling the solution into the tube, CO_2 gas was circulated, ensuring that anaerobic conditions in the bottle is incubated. The procedure was conducted at three different rumen fluid collection times with duplicates. A total of 40 incubation bottles containing 20 samples with duplicates for each replication were incubated at 39 °C for 48 h **(**Paradhipta *et al*., 2020). After incubation, all bottles were unplugged, and the liquids were filtered through a crucible. Residue from the sample was collected for dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) (Paradhipta *et al*., 2019). Meanwhile, the supernatant was carried out and separated for pH analysis using a pH meter (Mettler Toledo LE438, US), ammonia analysis (Chaney & Marbach, 1962), and VFA through gas chromatography (GC) with flame ionization detector (FID) (GC-2010 Plus, Shimadzu, Japan) and a column (CP FFAP CB, Shimadzu, Japan).

Organoleptic Appearances

A total of 26 non-expert panelists completed the question form based on several indicators. Organoleptic appearance indicators included color (dark, young green, brownish green), aroma (neutral, sourish, sour), and texture (slimy, damp, dampish). The data on organoleptic appearance were used to support Principal Component Analysis (PCA).

Statistical Analysis

Data were analyzed using one-way Analysis of Variance (ANOVA), and significant differences ($p<0.05$) between treatments were determined by conducting Duncan's multiple range test. Subsequently, SAS® Studio software was used to perform calculations (Steel *et al*., 1997). The internal relationships between silage quality variables were examined using PCA. The silage variables examined were organoleptic test, organic acid, pH, ammonia, and microbial count (Lê *et al*., 2008).

RESULTS

Chemical Composition

The mean concentrations of DM, OM, CP, EE, NDF, ADF, NFC, and tannin in fresh forage were 26.8, 88.8, 7.23, 1.42, 69.9, 38.3, and 0.43 %DM, respectively (Table 1). Based on the results, the contents of DM in PS and MIX silages (27.0 and 26.2 %DM) were higher than CON silage (22.7 %DM) ($p=0.031$) (Table 2). Furthermore, the contents of OM in PS and MIX silages (88.7 and 88.5 %DM) were lower than in CON and INO (89.5 and 89.5 %DM) ($p<0.001$). The contents of CP in PS and MIX silages (7.04 and 6.98 %DM) were higher than in INO and CON (6.28 and 6.17 %DM) ($p<0.001$). In line with CP, the contents of NFC in PS and MIX silages (19.6 and 18.8 %DM) were higher than CON and INO silages (16.1 and 14.9 %DM) ($p=0.007$). Compared to the others, NDF contents in PS and MIX silages (58.5 and 58.5 %DM) were lower than in CON and INO silages (63.33 and 64.80 %DM) ($p=0.013$). The content of ADF in PS silage (33.3 %DM) was also lower than in CON and INO silages (35.7 and 36.9 %DM) ($p=0.007$), while EE and tannins were not affected by the treatments.

Fermentation Characteristics

The pH in INO silage (4.01) was lower than in CON silage (4.16), while PS was higher ($p<0.0001$) (Table 3). The concentrations of Ammonia-N in PS and MIX silages (0.09 and 0.19 %DM) were lower than in CON and INO silages (0.45 and 0.42 %DM) ($p<0.001$). In comparison with pH, the concentration of lactate in INO silage (2.56 %DM) was higher than in CON silage (1.78 %DM), while PS silage (0.35 %DM) was lower. ($p<0.001$). The concentration of acetate in INO silage (3.75 %DM) was also higher than in CON silage (1.26 %DM) ($p<0.001$). The ratio of lactate:acetate in INO and MIX silages (0.68 and 0.66) were lower than in CON and PS silages (1.41 and 1.12) ($p<0.001$). No propionate or butyrate was detected in the analysis.

Microbial Counts

LAB in INO silage (7.90 log 10 cfu/g) had the highest, followed by CON, and MIX silage (7.40 and 6.76

Table 1. Chemical compositions of pre-ensiled sorghum forage (% DM)

Variables	% DM
Dry matter	26.8
Organic matter	88.8
Ether extract	1.42
Crude protein	7.23
Neutral detergent fiber	69.9
Acid detergent fiber	38.3
Tannins	0.43

Table 2. Chemical compositions of sorghum silages after 100 days of ensiling with different additives (% DM)

Variables	Treatments				SEM[5]	p-Value
	CON[1]	INO[2]	PS[3]	MIX[4]		
Dry matter	22.7[c]	23.7[bc]	27.0[a]	26.2[ab]	0.616	0.031
Organic matter	89.5[a]	89.5[a]	88.7[b]	88.5[b]	0.122	<0.001
Ether extract	3.89	3.57	3.52	4.19	0.133	0.255
Crude protein	6.17[b]	6.28[b]	7.04[a]	6.98[a]	0.101	<0.001
Neutral detergent fiber	63.3[a]	64.8[a]	58.5[b]	58.5[b]	0.776	0.013
Acid detergent fiber	35.7[ab]	36.9[a]	33.3[c]	33.6[bc]	0.475	0.007
Non-fiber carbohydrate	16.1[b]	14.9[b]	19.6[a]	18.8[a]	0.612	0.007
Tannins	0.82	0.80	0.70	0.74	0.081	0.050

Note: [a-c] Means in the same row with different superscripts differ significantly (p<0.05); [1]CON: sorghum silage without additive (Control); [2]INO: sorghum silage inoculated with the mixture of *L. plantarum* and *L. fermentum* at 1:1 ratio (1x10[5] cfu/g fresh matter); [3]PS: sorghum silage with potassium sorbate addition (1 g/kg fresh matter); [4]MIX: INO + PS; [5]SEM: standard error of the mean.

Table 3. Fermentation characteristics of sorghum silages ensiled for 100 days with different additives (% DM)

Variables	Treatments				SEM[5]	p-Value
	CON[1]	INO[2]	PS[3]	MIX[4]		
pH	4.13[b]	4.01[c]	4.31[a]	4.16[b]	0.026	<0.001
Ammonia-N (% DM)	0.45[a]	0.42[a]	0.09[b]	0.19[b]	0.037	<0.001
Lactate (% DM)	1.78[b]	2.56[a]	0.35[c]	1.36[b]	1.999	<0.001
Acetate (% DM)	1.26[c]	3.75[a]	0.31[d]	2.31[b]	3.205	<0.001
Propionate (% DM)	ND	ND	ND	ND	ND	NA
Butyrate (% DM)	ND	ND	ND	ND	ND	NA
Lactate:acetate	1.41[a]	0.68[c]	1.12[b]	0.66[c]	0.081	<0.001

Note: [a-c] Means in the same row with different superscripts differ significantly (p<0.05); [1]CON: sorghum silage without additive (Control); [2]INO: sorghum silage inoculated with the mixture of *L. plantarum* and *L. fermentum* at 1:1 ratio (1x10[5] cfu/g fresh matter); [3]PS: sorghum silage with potassium sorbate addition (1 g/kg fresh matter); [4]MIX: INO + PS; [5]SEM: standard error of the mean. ND: not detected; NA: not applicable.

Table 4. Microbial counts of sorghum silages ensiled for 100 days with different additives (log 10 cfu/g)

Variables	Treatments				SEM[5]	p-Value
	CON[1]	INO[2]	PS[3]	MIX[4]		
Lactic acid bacteria	7.40[b]	7.90[a]	6.05[c]	6.76[b]	0.210	<0.001
Yeast	8.01[a]	7.43[ab]	6.76[c]	7.21[bc]	0.136	0.003
Mold	7.43[a]	6.55[b]	6.22[b]	6.31[b]	0.143	0.002

Note: [a-c] Means in the same row with different superscripts differ significantly (p<0.05); [1]CON: sorghum silage without additive (Control); [2]INO: sorghum silage inoculated with the mixture of *L. plantarum* and *L. fermentum* at 1:1 ratio (1x10[5] cfu/g fresh matter); [3]PS: sorghum silage with potassium sorbate addition (1 g/kg fresh matter); [4]MIX: INO + PS; [5]SEM: standard error of the mean.

log 10 cfu/g, respectively). Meanwhile, PS silage (6.05 log 10 cfu/g) had the lowest (p<0.001) (Table 4). Yeast count was the lowest in PS (6.76 log 10 cfu/g) followed by MIX and INO silages (7.21 and 7.43 log 10 cfu/g, respectively) and the highest in CON silage (8.01 log 10 cfu/g, respectively) (p<0.003). Mold population in PS, MIX, and INO silages with values of 6.22, 6.31, and 6.55 log 10 cfu/g, respectively, were lower than CON silage at 7.43 log 10 cfu/g (p=0.002).

Aerobic Stability

Based on Figure 1, the aerobic stability of MIX, PS, and INO silages (57.8, 57.2, and 52 h) were higher than CON silage (48.2 h) (p<0.001). This was further supported by the changes presented in Figure 2, which showed the temperature fluctuation of HMS when exposed to air. Specifically, CON silage was the fastest to reach 2 °C above room temperature.

In Vitro Rumen Digestibility

The percentages of IVDMD in MIX and PS silages (65.7 and 64.96 %DM) were higher than in INO and CON silages (60.25 and 59.7 %DM) (p<0.001). In the same line, IVOMD in MIX and PS silages (61.5 and 60.5 %DM) were also higher than CON and INO silages (56.9 and 56.86 %DM) (p<0.001) (Figure 3). *In vitro* rumen digestibility showed that ammonia in PS and MIX silages (4.36 and 4.31 mg/100 mL) were higher than CON and INO silages (3.84 and 3.94 mg/100 mL) (p=0.006) (Table 5). In comparison, the total production of ruminal VFA in MIX silage (69.4 mmol) was the highest, followed by PS (64.2 mmol), CON silages (58.9 mmol), and INO (54.3 mmol) (p=0.004). The percentage of butyrate in MIX silage (11.5% of total VFA) was the lowest, followed by PS, CON, and INO silages (12.1, 13.5, and 13.7% of total VFA) (p=0.021).

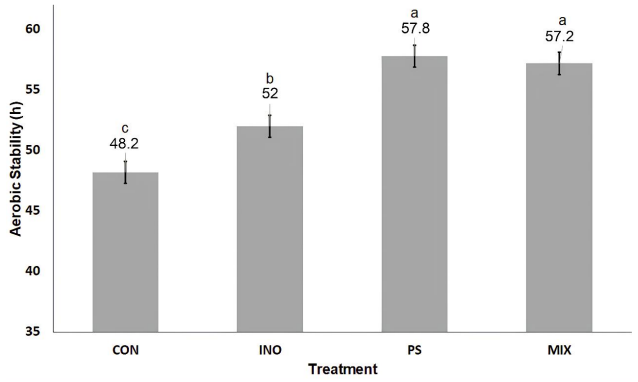

Figure 1. Aerobic stability of sorghum silages with different additives. [a-c] Means in the same row with different superscripts differ significantly (p<0.05); CON: sorghum silage without additive (Control); INO: sorghum silage inoculated with the mixture of *L. plantarum* and *L. fermentum* at 1:1 ratio (1x10^5 cfu/g fresh matter); PS: sorghum silage with potassium sorbate addition (1 g/kg fresh matter); MIX: INO + PS.

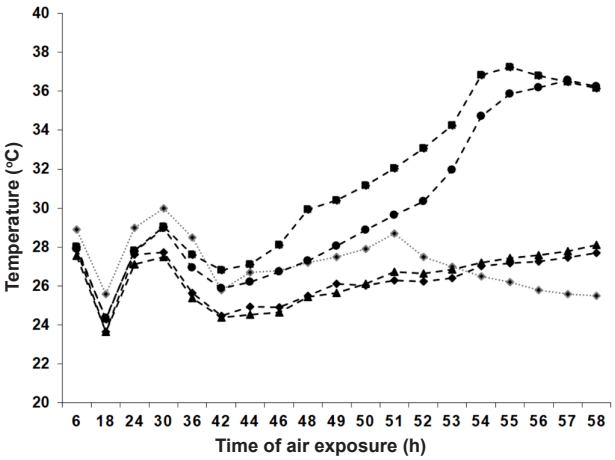

Figure 2. Dynamics of temperature change when sorghum silage with different additives exposed to the air. AMBIENT (♦): ambient temperature change; CON (■): sorghum silage without additive (Control); INO (●): sorghum silage inoculated with the mixture of *L. plantarum* and *L. fermentum* at 1:1 ratio (1x10^5 cfu/g fresh matter); PS (♦): sorghum silage with potassium sorbate addition (1 g/kg fresh matter); MIX (▲): INO + PS.

Organoleptic Appearance

Organoleptic tests of silage included color, aroma, and texture. As shown in Figure 4, the silage color for all treatments was yellowish green. The aroma of INO silage was sour, while the others were less sour. Furthermore, the texture for all treated silage was not lumpy.

Relationship between Variables

The relationship between variables was analyzed using principal component analysis (PCA), as shown in Figure 5. Lactate was strongly negatively correlated with pH value (p<0.01) while having a positive correlation with LAB (p<0.05). In line with the results, acetate tended to be positively correlated with aroma and negatively related to pH value (p<0.1). The tendency of a positive correlation was also performed in yeast and mold (p<0.1). According to Dim1 (58.1%

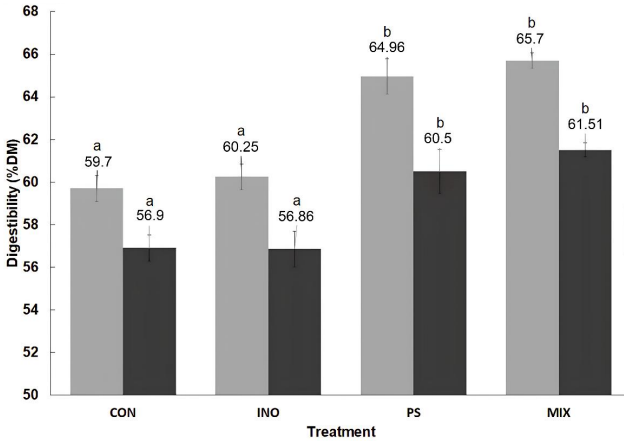

Figure 3. The *in vitro* rumen digestibility of sorghum silage with different additives after 48 h of ruminal incubation. [a-c] Means with different superscripts differ significantly (p<0.05); CON: sorghum silage without additive (Control); INO: sorghum silage inoculated with the mixture of *L. plantarum* and *L. fermentum* at 1:1 ratio (1x10^5 cfu/g fresh matter); PS: sorghum silage with potassium sorbate addition (1 g/kg fresh matter); MIX: INO + PS. IVDMD (■): *in vitro* dry matter digestibility; IVOMD (■): *in vitro* organic matter digestibility.

Table 5. The *in vitro* rumen digestibility and fermentation characteristic of sorghum silage with different additives after 48 h of ruminal incubation

Variables	Treatments				SEM[5]	p-Value
	CON[1]	INO[2]	PS[3]	MIX[4]		
pH	6.89	6.91	6.93	6.93	0.009	0.152
Ammonia (mg/100 mL)	3.84^b	3.94^b	4.36^a	4.31^a	0.096	0.006
Total VFA (mmol)	58.9^{bc}	54.3^c	64.2^{ab}	69.4^a	1.744	0.004
Acetate (% of total VFA)	57.1	58.0	59.3	62.0	0.897	0.232
Propionate ((% of total VFA)	29.4	28.3	28.6	26.5	0.628	0.439
Butyrate ((% of total VFA)	13.5^a	13.7^a	12.1^{ab}	11.5^b	0.322	0.021
Acetate : Propionate ratio	1.96	2.08	2.36	2.11	0.794	0.364

Note: [a-c] Means in the same row with different superscripts differ significantly (p<0.05); [1]CON: sorghum silage without additive (Control); [2]INO: sorghum silage inoculated with the mixture of *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum* at 1:1 ratio (1x105 cfu/g fresh matter); [3]PS: sorghum silage with potassium sorbate addition (1 g/kg fresh matter); [4]MIX: INO + PS; [5]SEM: standard error of the mean; [6]IVDMD, *in vitro dry* matter digestibility; [7]IVOMD, *in vitro* organic matter digestibility.

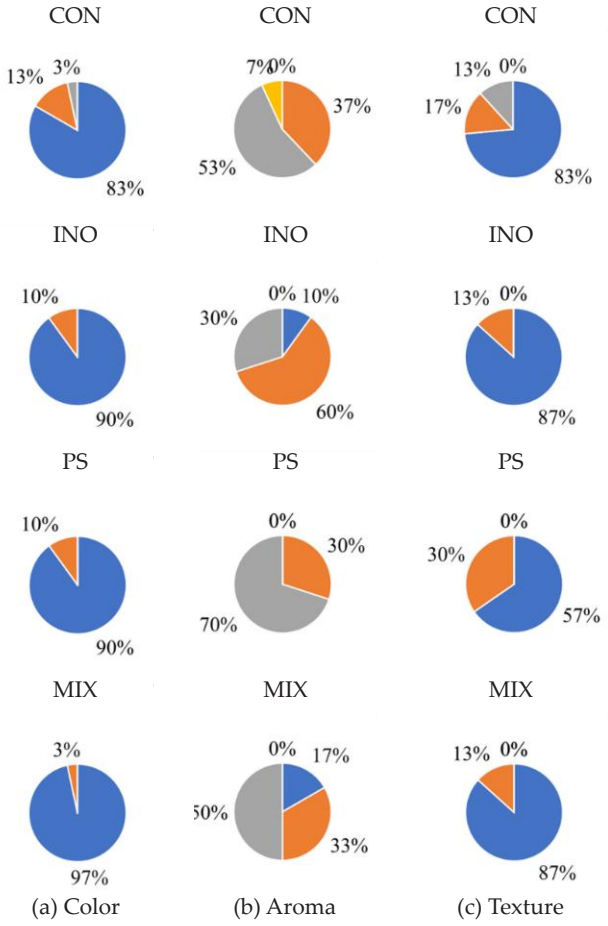

Figure 4. Color, aroma, and texture of sorghum silage ensiled for 100 days with different additives. CON: sorghum silage without additive (Control); INO: sorghum silage inoculated with the mixture of *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum* at 1:1 ratio (1x10^5 cfu/g fresh matter); PS: sorghum silage with potassium sorbate addition (1 g/kg fresh matter); MIX: INO + PS. (a) Color: Yellowish green (■), Yellowish (■), Brownish yellow (■), Blackish brown (■); (b) Aroma: Very sour (■), Sour (■), Less sour (■), Rotten (■); (c) Texture: Not lumpy (■), Slightly lumpy (■), Lumpy (■), Very lumpy (■).

of total variance), LAB, acetate, and lactate generated pH levels in silage. In terms of Dim2 (33.9% of total variance), both yeast and mold were considerable to generate low texture and color appearance.

DISCUSSION

In this study, the chemical compositions of sorghum harvested at the hard dough stage in the tropical region were similar to previously published ranges (Jardim *et al*., 2021; Araújo *et al*., 2023). After ensiling, this study showed that the addition of potassium sorbate in PS and MIX silages had a higher DM content than the other additives. The results showed the effectiveness of using potassium sorbate in reducing DM loss of high-moisture sorghum silage. According to Dai *et al*. (2022), potassium sorbate as a silage additive could inhibit the growth of undesirable microbes, which could result in higher DM content after ensiling. This opinion was supported by the results of PS and MIX silages that performed a lower population of yeast and mold in the present study (Table 4). Wang *et al*. (2022) stated that yeast and mold utilize soluble carbohydrates to heat, carbon dioxide, and H2O during ensiling. Besides, low DM loss might be associated with lower carbohydrate use by microbes, which is represented by higher NFC content (Table 2). In addition, MIX and PS silages in the present study also resulted in higher CP content, which was in agreement with Dai *et al*. (2022). The application of potassium sorbate as a silage additive might inhibit proteolytic bacteria during ensiling, as shown by reduced ammonia-N production in both MIX and PS silages (Table 3). The high CP content with low ammonia production after ensiling shows a low rate of proteolysis. Potassium sorbate successfully eliminated ammonia production, leading to the effects of inhibiting proteolytic microbes, such as clostridia and enterobacteria (Auerbach & Nadeau, 2020). Conversely, both MIX and PS silages also succeeded in degrading fiber fraction in silage. In the same line, Wang *et al*.

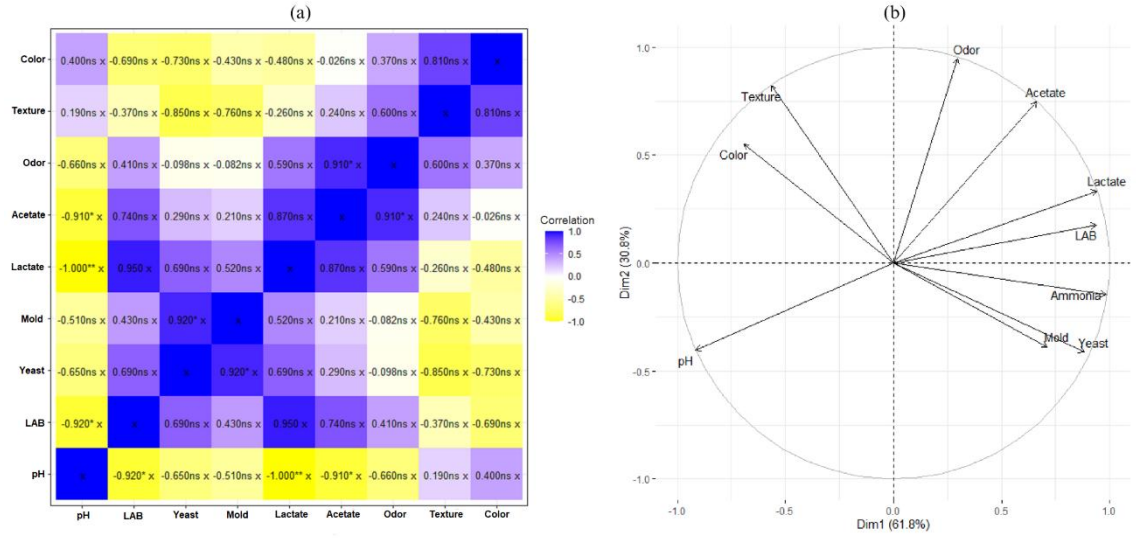

Figure 5. Principal component analysis (PCA) of factors: (a) correlation matrix of variables; (b) loadings of variables in the PCA. LAB: Lactic acid bacteria; Dim1: first principal component; Dim2: second principal component.

(2023b) reported that the addition of potassium sorbate reduced the content of NDF and ADF in the total mixed ration. The most likely explanation for this result was shown by Richa *et al*. (2023), that potassium had the ability to break down the cellulose and hemicellulose bond with lignin through the pyrolysis mechanism. The decrease in NDF and ADF contents observed in this study was associated with a high NFC content, which served as a substrate for microbial activity. An increase in NFC levels in silage indicates optimal lignocellulose degradation (Li *et al*., 2021). Additionally, the high NFC content in PS and MIX silages might be obtained from limited NFC degradation due to a smaller population of bacteria (Auerbach & Nadeau, 2020).

The HMS produced in the present study resulted in a good quality, which had a low pH value (<4.2), undetected butyrate and propionate, and also produced sufficient lactate and acetate, according to Kung *et al*. (2018). This result showed the role of homofermentative bacteria in lowering the pH of silage despite the high moisture content of the forage and the high temperatures, which prevented buffering capacity. These findings were supported by the prior observations in stylo (Pitiwittayakul *et al*., 2021) and alfalfa silage (Li *et al*., 2023). Fawzi *et al*. (2022) explained that the acceleration of acidification and decrement in pH value in the fermentation process was generated by the multiplication of lactate and organic acid as metabolic products from LAB.

Silage production with the addition of INO as a biological additive appeared to influence lactate concentration positively. This increase can be attributed to the conversion of carbohydrates into lactate by LAB (Muck *et al*., 2018). Similarly, studies by Wu *et al*. (2022) and Pitiwittayakul *et al*. (2021) reported high lactate levels in Chinensis and Stylo silages inoculated with *L. plantarum* and *L. fermentum.* This increase was also represented by the lower pH in INO silage, as shown in Table 3. The results showed a strong negative correlation between lactate production and pH decrease (Figure 4b). In line with the analysis, Chen *et al*. (2020) stated that *L. plantarum* was important in boosting lactate production. However, the addition of PS significantly decreased lactate content. The decrease in lactate content was probably related to the antibacterial effects generated by PS silage, which inhibited LAB growth (Xie *et al*., 2020).

Compared with the other treatments, INO silage performed the highest acetate production, which was in line with lactate production and pH level. This result was coherent with previous reports (Paradhipta *et al*., 2020), which used the combination of homo-heterofermentative bacteria to enhance acetate production in the corn silage due to longer aerobic stability. According to Zhang *et al*. (2023), high acetate restricts dangerous microorganism development, which was correlated with this study on inhibiting mold growth, as shown in Table 4.

This study showed that INO silage produced a higher population of LAB in silage. The improvement was consistent with the other studies (Andrada *et al*., 2023), which reported the increase of LAB population on corn silage due to the inoculation of *L. fermentum* and *L. plantarum*. Su *et al*. (2019) stated that LAB played a role in fermentation by transforming WSC into organic acids to restrict the activity of undesirable bacteria. However, PS decreased the LAB population due to the inhibition effects on LAB growth (Xie *et al*., 2020). Although PS had an effect on reducing the population of LAB, it could restrict the growth of yeast and mold effectively. This restrain was further confirmed by a prior study in Napier grass silage (Dai *et al*., 2022) and total mixed ratio (TMR) (Wang *et al*., 2023c). The results might be determined from the acidic characteristics and effectiveness against bacteria (Dai *et al*., 2022)

Aerobic stability is one of the crucial parameters that shows the defense ability of the silage in aerobic exposure. Aerobic damage is more likely to occur under tropical conditions because high temperatures and humidity promote the development of undesirable microbes. Shan *et al*. (2021) stated that when silage was exposed to air, the aerobic microbes consumed the lactate and the glucose residue to produce heat, CO_2, and H_2O. In this study, the aerobic stability of the silage was determined by the increase of 2 ^{o}C of silage temperature above the ambient, as suggested by Auerbach and Nadeau (2020). Overall, the present study showed that all silage treatment produced higher silage stability during aerobic exposure. The elevated level of stability on INO silage might be related to the effects of antibacterial from *L. fermentum*, as heterofermentative bacteria. According to Puntillo *et al*. (2020), heterofermentative bacteria elevated the production of acetate in corn silage, which might contribute to preserving the nutrient of the silage after the silo opening. Danner *et al*. (2003) explained that higher acetate content was correlated with enhancing antibacterial activity and inhibiting the growth of undesirable microbes. Moreover, potassium sorbate addition as a single or combination with biological additive also performed higher silage stability during aerobic conditions. It was more likely that the stability of PS silage was probably related to antibacterial effects, which related to the depression of yeast and mold population. This result was consistent with previous reports that PS performed higher aerobic stability on alfalfa silage (Xie *et al*., 2020). Dai *et al*. (2022) showed that during aerobic exposure, potassium sorbate depresses the growth of yeast and mold, thereby preserving residual glucose and decreasing ammonia production.

The present study found a significant increase of IVDMD and IVOMD in PS and MIX silages. This was further confirmed by previous studies (Wang *et al*., 2023c; Dai *et al*., 2022) that additional PS performed a higher rate of digestibility on TMR silage. A probable explanation was that the higher availability of carbohydrates and protein in feed was commonly accompanied by the growth improvement of ruminal microbes and fiber degradation in the rumen (Suharti *et al*., 2021). This result was confirmed by the higher CP and NFC content in PS and MIX silages (Table 2.). Moreover, the better ruminal fermentation on PS and MIX silages was also represented by the enrichment concentration of ammonia and total VFA. First, the

elevated production of ammonia rumen might be explained by the fact that the content of CP was higher in PS and MIX silages (Table 2.). In agreement with these findings, Shen *et al*. (2023) verified that enhanced ammonia concentration was correlated to the increase of protein availability in the feed before digestion. Meanwhile, total VFA was increased, probably due to the high concentration of NFC and the enriched ruminal bacteria caused by the increased substrate for growth. According to Li *et al*. (2023), well-preserved silage provided more fermentation substrate for fibrolytic bacteria, enhanced rumen fermentation and produced high VFA concentration. Guo *et al*. (2020) explained that carbohydrates in the rumen were degraded into pentose or hexose and transformed into pyruvate, acetate, propionate, and butyrate.

The present study showed a positive correlation between LAB, lactate, and acetate. In agreement with this result, Andrada *et al*. (2023) showed that the enhanced population of LAB was positively correlated to higher productions of lactate and acetate. Furthermore, Kung *et al*. (2018) explained that the sweet odor from well-fermented silage was triggered by acetate as a secondary product from lactate use. The weak correlation between yeast and mold with physical appearance (texture and color) was consistent with a previous study (Kung *et al*., 2018), where spoilage-causing microbes contributed to protein degradation. This process was associated with a binding reaction between proteins and sugars, leading to a brownish color. Yeast deteriorates silage quality by consuming nutrients and causing a lumpy texture, leading to potential issues in ruminants.

CONCLUSION

In conclusion, the combination of biological and chemical additives in HMS improved chemical composition, digestibility, and aerobic stability while reducing yeast and mold. Therefore, this study recommended combining biological and chemical additives for optimal silage quality.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in this study.

ACKNOWLEDGMENT

The authors are grateful to the Research Center for Food Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency for providing sorghum seeds.

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