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, September 2024, 47(3):312-320
DOI: https://doi.org/10.5398/tasj.2024.47.3.312
Available online at https://journal.ipb.ac.id/index.php/tasj

Nutritive Value, Digestibility, and Gas Production of *Pennisetum purpureum* Silage Supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

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(Received 17-01-2024; Revised 27-03-2024; Accepted 01-04-2024)

ABSTRACT

The purpose of this study was to evaluate the use of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* as silage additives and their combinations on the physico-chemical and microbiological quality of *Pennisetum purpureum* cv. Mott silage and assess fermentation characteristics, digestibility, and *in vitro* gas production. The experiment used a completely randomized design with 4 treatments and 5 replications. The treatments are: T0= dwarf elephant grass silage + 3% molasses, T1= T0 + *S. cerevisiae*, T2= T0 + *L. plantarum*, and T3= T0 + *S. cerevisiae* + *L. plantarum*. Variables measured were organoleptic, physical, microbial, nutrient composition, *in vitro* fermentation characteristics and nutrient digestibility, as well as total gas and methane productions. The result showed that all silages had good physical quality, low pH (<3.8) and NH_3 content, and high fleigh point. Inoculants decreased the percentage of dry matter, crude protein, ether extract ($p<0.05$), increased crude fiber, and decreased the composition of nitrogen-free extract and non-fiber carbohydrate ($p<0.05$) but it was able to increase Ca and P. Total gas production of T0 was the highest among treatments, while the T3 had the lowest total gas and methane productions ($p<0.05$). The supplementation of *S. cerevisiae* and *L. plantarum* as a silage improved organoleptic, physical, and microbiological qualities. Although the nutrient composition did not increase significantly, the combination of 2 (two) inoculants was able to improve fermentation activity in the rumen, increase total volatile fatty acid (VFA), dry matter and organic matter digestibility, reduce total gas production and the ratio of methane gas production to VFA.

Keywords: inoculants; Lactobacillus plantarum; Pennisetum purpureum; Saccharomyces cerevisiae; silage

INTRODUCTION

Forage as the main feed for ruminants is very important, if it is not fulfilled, farmers usually give field grass or rice straw which has low nutritional value. In tropical areas, especially during the dry season, the productivity of ruminants fed on forages is limited due to the low quality of forage species, low soil fertility, and other factors. Some tropical grasses, such as *Pennisetum purpureum* cv Mott (dwarf elephant grass) stand out among species, with good palatability and nutritional quality so it was very promising as a source of sustainable feed for ruminants (Li *et al.*, 2014). To support the availability of nutritive forage, a preservation technology is needed by making silage. Silage is a product of forage fermentation through an ensilage process carried out anaerobically. The basic principle of making silage is the fermentation of forage by microbes that produce lactic acid, namely lactic acid bacteria (LAB), so there is a decrease in pH, making it durable. One of the efforts to maintain silage quality is the use of inoculants as additives, such as *Saccharomyces cerevisiae* and LAB, in the form of *Lactobacillus plantarum* (Hapsari *et al.*, 2016) but in reality this potential has not been able to meet the requirement of feed both in sustainable quantity and quality. Silage made with the use of liquid fermentation additive (FA).

The addition of inoculants is used to improve silage quality. Some studies reported the benefits of using *S. cerevisiae* as an inoculant for ruminants. *S. cerevisiae* is yeast culture that can be used as silage inoculants. *S. cerevisiae* as an oxygen-user agent can shorten the aerobic phase so that it can support the growth of lactic acid bacteria (Muck *et al.*, 2018). *S. cerevisiae,* besides reducing oxygen to increase the development of lactic acid bacteria, also has the activity of digesting fiber in the high oxygen phase (at the beginning of the ensilage process). The addition of *S. cerevisiae* as yeast treatment produced a faster and greater reduction in pH over 20 days (Savage *et al.*, 2014). *S. cerevisiae* may be beneficial as inoculants in 2 different areas: inhibiting detrimental silage microorganisms and growing yeasts that are currently used as direct-fed microbials (Muck *et al.*, 2018). The *S. cerevisiae* strain can be used as a probiotic yeast, reducing oxygen in the rumen (Riyanti *et al.*, 2016).

The other inoculants commonly used as inoculants for silage are lactic acid bacteria (LAB). *L. plantarum,* in previous studies, can be used as a silage inoculant to

modify silage fermentation. *L. plantarum* as a homofermentative LAB produces lactic acid rapidly and then the pH will drop quickly, making the silage more durable. Lactic acid produced during the fermentation process will act as a preservative so it prevents the growth of detrimental silage microorganisms (Khota *et al.*, 2016; Okoye *et al.*, 2023). The use of *L. plantarum* inoculant with various variations and concentrations has a good effect on the quality of silage as feed (Amaral *et al.*, 2020). *L. plantarum* inoculant is one type of lactic acid bacteria that produces lactic acid under anaerobic conditions. The lactic acid will reduce the acidity of the silage pile. Mixed silage additive is more effective for improving high-moisture silage quality (Wu *et al.*, 2020). The combination of *S. cerevisiae* and *L. plantarum* inoculant is expected to improve silage quality during the ensilage process. The purpose of this study was to evaluate the use of silage additives in the form of *S. cerevisiae* and *L. plantarum* inoculants and their combinations on the organoleptic, physical, chemical, and microbiological quality of *P. purpureum* cv. Mott silage and evaluate fermentation characteristics, nutrient digestibility, and *in vitro* gas and methane productions.

MATERIALS AND METHODS

Inoculants Preparation

The *S. cerevisiae* strain used is isolate code IPBCC.y.05.0546, a collection culture from IPB Culture Collection (IPBCC) Department of Biology, IPB University. *S. cerevisiae* was cultured on Potato Dextrose Agar (PDA) media in test tubes and incubated at 30 °C for 48 hours. The stock inoculant on solid media was stored in a refrigerator. Liquid inoculant was prepared by inoculating 3 oses of *S. cerevisiae* in 10 mL of Potato Dextrose Broth (PDB) media. Furthermore, for silage inoculant, 5% (v/v) of *S. cerevisiae* was inoculated in 100 mL of PDB media. The population of *S. cerevisiae* was calculated using total plate count (TPC) with a population of 3.9×10^7 cfu/mL.

L. plantarum isolate 1A-2 was obtained from the Indonesian Culture Collection (InaCC), National Research and Innovation Agency, Republic of Indonesia. *L. plantarum* was cultured on de Man Ragosa Sharpe Agar (MRSA) media in petri dishes and incubated at 30 °C for 48 hours (Hapsari *et al.*, 2016). The stock inoculant on solid media was stored in a refrigerator. Liquid inoculant was prepared by inoculating 3 oses of *L. plantarum* in 10 mL of de Man Ragosa Sharpe Broth (MRSB) media. Furthermore, for silage inoculant, 5% (v/v) of *L. plantarum* was inoculated in 100 mL of MRSB media. The population of *L. plantarum* was calculated using total plate count (TPC) with a population of 7.1×10^9 cfu/mL.

Ensilage Process

The dwarf elephant grass used in this study was harvested at the age of 40 days from the pasture of the Department of Animal Husbandry, Bogor Agricultural Development Polytechnic, Indonesia. The grass was wilted for 24 hours at ambient temperature (21-30 °C) and it was chopped by chopper in 3-5 length. A total of 10 kg of chopped grass was spread on a tarpaulin. Molasses was added at a dose of 3% of the fresh weight of the grass. The dose of *S. cerevisiae* and *L. plantarum* was 0.1% of grass weight (v/w) each. Inoculants were suspended in molasses, and then all ingredients for each treatment were mixed homogeneously. The silage was compacted and packed with 2 layers of Low-Density Polyethylene (LDPE) plastic liner with a thickness of 0.7 and a size of 50 x 85 cm. Silage was incubated for 21 days at ambient temperature (21-30 °C). After 21 days, the silage was opened, and physic-chemical and microbiological tests were conducted.

Physical assessment of the silage was evaluated after 21 days of incubation. The silage bag was opened and immediately observed. Five persons of expert panelists asses and evaluate color, flavor, texture, and the presence of mold. The level of color by scoring methods was described as dark (score: 1), brown dark (score: 2), brown (score: 3), and green-browning (score: 4). Level of flavor was described as off-flavor (score: 1), less fragrant (score: 2), medium fragrant (score: 3), and heavy fragrant (score: 4). Level of texture was described high clot and slimy (score: 1), intermediate clot and slimy (score: 2), slightly clot and slimy (score: 3), and no clot and slimy (score: 4). Level of mold contamination (LFC) percentage on the surface area with categories i.e., severe (score 1: >15%), medium (score 2: 5%-15%), mild (score 3: <5%), and no contamination (score 2: 0%) (Sofyan *et al.*, 2017).

Fermentation Profile

Samples used for pH and NH_3 measurements were made by taking a 10 g silage sample and adding 100 mL of distilled water (Hapsari *et al.*, 2016). After that, the sample was blended for 1 minute at a speed of 4000 rpm. The electrode on the pH meter was inserted into the sample and pH readings were taken after stabilization. The concentration of NH_3 in silage was measured by Micro-diffusion Conway Method (General Laboratory Procedure, 1966).

Chemical and Microbiological Analysis of Silage

After 3 weeks, the silage was opened and analyzed for chemical composition. A total of 2 kg of silage was weighed and oven-dried at 60 °C for 48 hours. Silage samples were ground with a blender (Philips HR 2116) and sieved with a sieve size of 0.5-1.5 mm for further analysis. Dry matter, ash, crude protein, crude fat, and crude fiber were analyzed according to the Association of Official Analytical Chemists method (AOAC, 2005). Analysis of fiber fractions, such as NDF, ADF, cellulose, and lignin, used the Van Soest method (Van Soest *et al.*, 1991).

Microbiological observations were made by measuring the number of total yeast and total lactic acid bacteria (LAB) using the total plate count method. A total of 1 g of silage sample was weighed and mixed with 10 mL of sterile distilled water. The liquid was used in serial

dilutions to count the microbial population. The LAB was counted on deMan Ragosa Sharpe (MRS) medium after 48 hours of incubation at 30 °C. The number of yeasts was enumerated on potato dextrose agar (PDA) after the 48-hour incubation at 30 °C (Modification of Mugabe *et al.,* 2019). Fleigh point is also used to measure silage quality, where the value is obtained from the pH and DM of silage. Fleigh point is calculated by the formula (Moselhy *et al*., 2015): fleigh points = 220 + (2 x %DM – 15) – 40 x pH.

In Vitro Digestibility

In vitro fermentation was conducted according to the method of Tilley and Terry (1963). Into each fermentation tube, 500 mg of sample, 40 mL Mc Dougall buffer, and 10 mL rumen fluid were collected in a fistula. Fermenter tubes were flowed by CO_2 for 30 s (pH 6.5-6.9) and incubated in a shaker water bath at a temperature of 39 °C. Rumen fluid was collected from the rumen of fistulated Friesian Holstein cattle. After 4 hours of incubation, rumen fluid sample was collected for pH, NH_3, and VFA measurements. The rumen pH was measured with a pH meter (Hanna Instrument HI-98108 I pHep with ATC I). The concentration of N-NH_3 in rumen fluid was measured by Micro-diffusion Conway Method (General Laboratory Procedure, 1966). A rumen fluid sample (1 mL) was pipetted into an Eppendorf tube, 0.003 g of sulfa 5 salicylic acid dihydrate was added. The mixture was centrifuged for 10 min at 12.000 rpm at 7 °C and then injected into the Gas Chromatography (GC Bruker S/N BR 1303 M 70 with the type model Scion 436-GC5) equipped with Bruker-1ms column (0.25 mm ID x 15 m x 0.25 μm). Identify volatile fatty acid profiles using standard (Supelco Volatile Free Acid Mix, Sigma-Aldrich). After 48 h incubation, the supernatant was added with 50 mL of 0.2% pepsin HCl solution. This mixture was re-incubated for 48 h. The residue was filtered with Whatman paper and determined the dry matter content by putting the sample in oven 105 °C for 24 h. After that, the sample was burned in a furnace for 6 h to determine the organic matter content.

In Vitro Gas Production

Fermentation digestion was performed *in vitro* using the method of Theodorou *et al*. (1994). *In vitro* digestion was done by putting 0.75 g of silage sample into a 100 mL infusion bottle, then adding 25 mL of rumen fluid and 50 mL of Mc Dougall solution. The infusion bottle was closed using a rubber bottle cover and then sealed using a crimper. The infusion bottle was placed in a water bath at 39 °C and gas was collected at hours 2, 4, 6, 8, 10, 12, and 24. The amount of gas production is measured with a 50 mL syringe. Gas production kinetics are obtained through the exponential equation according to Ørskov & McDonald (1979) as follows: $p = a + b\ (1 - e^{-ct})$, where p is the cumulative gas production at time t hours, while a, b, and c are constants of the exponential equation. The constants a, b, and c can be interpreted as gas production from soluble fractions (a), gas production from insoluble but fermentable fractions (b), and the reaction rate of gas formation (c). Thus, a+b can be interpreted as the maximum gas production that can be formed during the fermentation process at time t approaching infinity (Ørskov & McDonald, 1979). Calculations were performed using nonlinear regression equations using SPSS. The collection of methane gas parameters was carried out after 24 hours of incubation using a syringe equipped with a needle and inserted in a bottle that had been sealed and vacuumed. Methane concentration was measured using Gas Chromatography.

Experimental Design and Statistical Analysis

The experiment used a completely randomized design with 4 treatments and 5 replications. The treatments are: T0= dwarf elephant grass silage with 3% molasses, T1= T0 + *S. cerevisiae*, T2= T0 + *L. plantarum*, and T3= T0 + *S. cerevisiae* + *L. plantarum*. Data on the physical characteristics of silage were tested by using descriptive statistics. Data of chemical composition, *in vitro* fermentability and digestibility, and gas production were tested by using analysis of variance (ANOVA), and the differences among treatment means were examined by Duncan's Multiple Range Test (DMRT) (Steel & Torie, 1980).

RESULTS

Physical and Microbial Quality of Silage

The use of inoculants *S. cerevisiae* and *L. plantarum* or their combination did not show significant differences in the color, texture, and presence of mold, but it made a difference in the flavor of silage ($p<0.05$) (Table 1). T2 and T3 had a heavy fragrant (mean score of 3.6 and 4, respectively), while T0 and T1 had a medium fragrant (mean score of 3). The color of silage in all treatments was tawny (average score 4). Silage texture in all treatments was no clot and slimy (score 4) and there was a little mold in all silages (score 3-3.4).

Table 2 shows the fermentation characteristics and microbial population of silage supplemented with inoculants. The provision of inoculants had a significant effect on the pH of silage ($p<0.05$). pH values showed a

Table 1. Physical characteristics of *Pennisetum purpureum* silage supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

Parameters	Treatments			
	T0	T1	T2	T3
Color	Green-browning	Green-browning	Green-browning	Green-browning
Flavor	Medium fragrant	Medium fragrant	Heavy fragrant	Heavy fragrant
Texture	No clot and slimy	No clot and slimy	No clot and slimy	No clot and slimy
LFC	Mild	Mild	Mild	Mild

Note: T0= Dwarf elephant grass + 3% molasses, T1= T0 + *S. cerevisiae*, T2= T0 + *L. plantarum*, T3= T0 + *S. cerevisiae* + *L. plantarum*. LFC= Level of fungal contamination.

range of 3.52-3.58, where the lowest pH was in T2 with a value of 3.52 and the highest pH was in T0 with a value of 3.58. The provision of inoculants had a significant effect on dry matter, NH_3, fleigh point, yeast population, and LAB population ($p<0.05$). The dry Matter (DM) of silage was the highest in the T0 treatment and the lowest in T3, with DM values of 23.51% and 20.67%, respectively. The provision of inoculants also decreased the NH_3 concentration of silage where the NH_3 concentration in T3 was the lowest with a value of 2.68 mM. The fleigh point of silage in the highest treatment with an average point was 108.5 and the lowest in T3 with an average point was 104.43. All treatments had a fleigh point with a very good category. The use of inoculants increases the population of yeast and LAB. The highest yeast population was found in T3 with an average population of 9.06 log cfu/mL and the lowest in T0 with an average of 6.12 log cfu/g. LAB population was the highest in T2 with an average population of 9.16 log cfu/g and the lowest in the control treatment without adding LAB with a population of 5.33 log cfu/mg.

Nutrient Composition of Silage

The use of *S. cerevisiae* and *L. plantarum* inoculants and their combination on silage significantly affected the variables of dry matter (DM), ash, crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen-free extract (NFE) and non-fiber carbohydrate (NFC) ($p<0.05$). The use of inoculants decreased the percentages of DM, CP, EE ($p<0.05$), increased CF, and decreased the compositions of NFE and NFC ($p<0.05$) but was able to increase Ca and P minerals with the highest Ca and P content in T2 with percentages were 1.96% and 1.04%, respectively (Table 3).

The use of inoculants *S. cerevisiae* and *L. plantarum* and their combinations could also affect fiber fraction variables, such as NDF, ADF, hemicellulose, cellulose, and lignin ($p<0.05$). Combining 2 inoculants in T3 increased NDF ($p<0.05$) with an average NDF of 58.11%. Using *S. cerevisiae* inoculant in T1 and *L. plantarum* in T2 decreased ADF value ($p<0.05$) with percentages of 34.36% and 35.24% compared to T0 and T3, respectively. Hemicellulose increased with the addition of inoculants ($p<0.05$) with the highest percentage in T2 was 19.31%. Cellulose also increased with the addition of inoculants ($p<0.05$) with the highest percentage in the combination of inoculants T3 with a percentage of 30.03% and a decrease in the percentage of lignin in silage with single inoculant with a percentage of lignin of T1 3.81% and T2 3.70%.

Table 2. Fermentation characteristic and microbial population of *Pennisetum purpureum* silage supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

Variables	Treatments			
	T0	T1	T2	T3
pH	3.58±0.02^{a}	3.53±0.03^{b}	3.52±0.03^{b}	3.54±0.02^{b}
DM (%)	23.51±1.51^{a}	22.43±0.91^{ab}	21.40±0.72^{bc}	20.67±0.43^{c}
NH_3 (mM)	2.98±0.27^{bc}	3.85±0.31^{a}	3.15±0.25^{b}	2.68±0.24^{c}
Fleigh point	108.50±2.70^{a}	108.51±2.83^{a}	106.76±2.24^{ab}	104.43±1.66^{b}
Yeast population (log cfu/g)	6.12±0.37^{c}	7.29±0.13^{b}	7.42±0.40^{b}	9.06±0.25^{a}
LAB population (log cfu/g)	5.33±1.18^{c}	7.02±0.85^{b}	9.16±0.91^{a}	9.11±0.24^{a}

Note: T0= Dwarf elephant grass + 3% molasses, T1= T0 + *S. cerevisiae*, T2= T0 + *L. plantarum*, T3= T0 + *S. cerevisiae* + *L. plantarum*. Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 3. The chemical composition of *Pennisetum purpureum* silage supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

Variables	Treatments			
	T0	T1	T2	T3
Dry matter (%)	23.51±1.01^{a}	22.43±0.91^{ab}	21.40±0.72^{bc}	20.67±0.43^{c}
Ash (% DM)	13.16±0.33^{b}	14.05±0.33^{a}	14.08±0.70^{a}	13.31±0.43^{b}
Crude protein (% DM)	16.92±0.48^{a}	16.29±0.84^{ab}	15.91±0.73^{b}	15.46±0.46^{b}
Ether extract (% DM)	2.53±0.80^{ab}	2.81±0.34^{a}	2.56±0.28^{ab}	2.33±0.16^{b}
Crude fiber (% DM)	31.11±0.64^{c}	30.65±0.81^{c}	32.73±1.75^{b}	34.24±0.95^{a}
Nitrogen-free extract (% DM)	36.27±0.62^{a}	36.20±1.21^{a}	34.71±0.94^{b}	34.65±0.36^{b}
Non-fiber carbohydrate (%)	12.37±0.87^{ab}	13.83±1.30^{a}	12.88±1.34^{a}	10.77±1.53^{b}
Ca (%)	0.69±0.07^{d}	0.84±0.09^{c}	1.30±0.07^{b}	1.96±0.09^{a}
P (%)	0.33±0.07^{c}	0.41±0.06^{c}	0.80±0.07^{b}	1.04±0.08^{a}
Neutral detergent fiber (%)	55.00±0.68^{b}	53.02±0.85^{c}	54.55±1.95^{bc}	58.11±1.42^{a}
Acid detergent fiber (%)	39.14±0.57^{a}	34.36±0.85^{b}	35.24±0.53^{b}	38.96±2.01^{a}
Hemicellulose (%)	15.86±0.95^{b}	18.66±1.44^{a}	19.31±2.04^{a}	19.15±1.31^{a}
Cellulose (%)	28.13±0.59^{b}	26.42±0.69^{b}	27.5±1.49^{b}	30.03±1.75^{a}
Lignin (%)	5.43±0.73^{a}	3.81±0.51^{b}	3.70±0.31^{b}	4.60±0.87^{ab}

Note: T0= Dwarf elephant grass + 3% molasses, T1= T0 + *S. cerevisiae*, T2= T0 + *L. plantarum*, T3= T0 + *S. cerevisiae* + *L. plantarum*. Means in the same row with different superscripts differ significantly ($p<0.05$). NFC (%) = OM (%) - CP (%) - NDF (%) - EE (%) (Kurniawan *et al*., 2022).

In Vitro Fermentation and Digestibility

The use of inoculant had a significant effect on pH value, NH3 concentration, total VFA concentration, DMD, and OMD ($p<0.05$) (Table 4). The use of inoculants in silage was able to reduce rumen pH ($p<0.05$) in T1, T2, and T3 compared to control/T0 with pH values of 6.81, 6.75, and 6.77, respectively, vs. 6.94 in control. The pH value ranges of all treatments were 6.77-6.94. Supplementation of inoculants was also able to increase NH3 concentration ($p<0.05$) with the highest value in T1 and T3 with concentrations of 8.93 mM and 9.02 mM, respectively, compared to T0 and T2 with concentrations of 7.79 mM and 7.96 mM, respectively. There was an increase in total VFA concentration in the combination treatment of 2 inoculants with the highest VFA concentration of 62.42 mM compared to the other treatments T0, T1, and T2, with 48.54, 42.58, and 43.18 mM, respectively. Inoculants increased DMD with the highest percentage in T3, which was 67.87%, followed by T1 with a percentage of 65.46%, and T0 and T2 had the same digestibility of 61.38% and 62.66% (Table 4). The highest percentage of OMD was also in T3 addition of a combination of 2 inoculants with a percentage was 67.34%, followed by T1= 64.25%, T2= 62.26%, and T0= 60.41%. Inoculant supplementation not significantly affected the molar proportion of acetate and propionate butyrate, isobutyrate, valerate, isovalerate, and A/P ratio.

Table 5 shows the gas production (mL/750 mg DM) of silage in the rumen at 2, 4, 6, 8, 10, 12, and 24 hours. The provision of inoculants had a significant effect on reducing rumen gas production ($p<0.05$). The highest gas production at T0 and T2 every hour, with gas production at 24 hours was 126.97 and 124.91 mL/750 mg DM, and the lowest gas production at T3 with a value of 121.26 mL/750 mg DM. Table 6 shows the kinetics of total gas production and methane gas production. Inoculant supplementation had a significant effect on gas production potential (a+b) ($p<0.05$) but no significant effect on gas production rate (c). Inoculant supplementation in silage had a significant effect on gas production/DM ($p<0.05$) and methane gas production ($p<0.05$). The highest gas production was in the T2 treatment, with a total gas production of 151.17 mL/g DM and the lowest in the control treatment, with a value of 131.02 mL/g. The highest methane production in T2= 12.22%, same with the T1= 11.61%, T3= 12.10%, and the lowest in T0= 10.36%. The ratio of methane production to VFA showed a significant difference ($p<0.05$), with the highest ratio value in T2= 3.84 mL/mM and the lowest in T3= 2.30 mL/mM.

DISCUSSION

Nutritive Value of Silage

In general, the silage produced has good quality, as seen from physical observations with very good fleigh

Table 4. *In vitro* fermentability and digestibility of *Pennisetum purpureum* silage supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

Variables	Treatments			
	T0	T1	T2	T3
pH	6.94±0.07[a]	6.81±0.04[b]	6.75±0.03[b]	6.77±0.05[b]
NH_3 (mM)	7.79±0.33[b]	8.93±0.61[a]	7.96±0.84[b]	9.02±0.45[a]
Total VFA (mM)	48.54±8.77[b]	42.58±10.39[b]	43.18±13.34[b]	62.42±6.23[a]
Acetate (%mM)	48.16±11.48	55.05±16.78	35.45±14.48	45.91±12.74
Propionate (%mM)	23.37±2.49	20.93±4.92	23.62±4.80	26.15±9.48
Butyrate (%mM)	13.35±3.42	11.13±5.74	15.25±2.47	10.77±4.60
Isobutyrate %(mM)	4.88±3.3.32	4.96±2.19	8.91±4.66	5.76±2.74
Valerate (%mM)	4.79±1.92	3.53±2.43	8.05±5.68	5.12±2.93
Isovalerate (%mM)	5.45±2.10	4.40±2.63	8.72±5.31	6.29±2.81
A/P Ratio	2.11±0.72	2.95±1.76	1.50±0.59	1.96±0.94
DMD (%)	61.38±1.40[c]	65.46±0.91[b]	62.66±0.81[c]	67.87±0.55[a]
OMD (%)	60.41±1.52[d]	64.25±0.75[b]	62.26±0.67[c]	67.34±0.65[a]

Note: T0= Dwarf elephant grass + 3% molasses, T1= T0 + *S. cerevisiae*, T2= T0 + *L. plantarum*, T3= T0 + *S. cerevisiae* + *L. plantarum*. Means in the same row with different superscripts differ significantly ($p<0.05$). VFA= volatile fatty acid, DMD= dry matter digestibility, OMD= organic matter digestibility.

Table 5. Gas production (mL/750 mg DM) of *Pennisetum purpureum* silage supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

Treatments	Incubation time (h)						
	2	4	6	8	10	12	24
T0	27.24±0.94[a]	40.70±0.96[a]	51.76±0.99[a]	69.46±1.05[a]	80.52±1.09[a]	87.15±1.11[a]	126.97±1.28[a]
T1	26.48±1.64[a]	39.45±1.69[a]	50.26±1.74[a]	67.55±1.81[a]	78.36±1.86[a]	84.84±1.89[b]	123.75±2.07[b]
T2	26.73±1.29[a]	39.82±1.35[a]	50.73±1.39[a]	68.18±1.48[a]	79.09±1.53[a]	85.64±1.57[ab]	124.91±1.79[ab]
T3	23.29±1.44[b]	36.36±1.49[b]	47.24±1.53[b]	67.66±1.60[b]	75.54±1.65[b]	82.08±1.67[c]	121.26±1.84[c]

Note: T0= Dwarf elephant grass + 3% molasses, T1= T0 + *S. cerevisiae*, T2= T0 + *L. plantarum*, T3= T0 + *S. cerevisiae* + *L. plantarum*. Means in the same column with different superscripts differ significantly ($p<0.05$).

Table 6. Gas production kinetics and methane gas production of *Pennisetum purpureum* silage supplemented with *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

Variables	Treatments			
	T0	T1	T2	T3
a+b (mL)	130.12±0.89[a]	127.16±0.71[c]	128.34±1.00[b]	128.05±0.64[bc]
c (mL/hour)	0.67±0.00	0.67±0.00	0.67±0.00	0.67±0.00
Gas production/DM (mL/g)	131.02±1.43[c]	144.46±1.96[b]	151.17±5.51[a]	142.74±2.04[b]
Methane (%)	10.36±0.37[b]	11.61±0.88[a]	12.22±0.38[a]	12.10±0.45[a]
Methane (mL)	13.58±0.58[c]	16.77±1.37[b]	18.49±1.19[a]	17.28±0.82[ab]
Methane/VFA (mL/mM)	2.76±0.45[ab]	3.61±1.11[ab]	3.84±1.41[a]	2.30±0.24[b]

Note: T0= Dwarf elephant grass + 3% molasses, T1= T0 + *S. cerevisiae*, T2= T0 + *L. plantarum*, T3= T0 + *S. cerevisiae* + *L. plantarum*. Means in the same row with different superscripts differ significantly ($p<0.05$). a+b= potential gas production, c= gas production rate.

points (>85) (Moselhy *et al.,* 2015). The addition of inoculant *L. plantarum* and the combination of *S. cerevisiae* and *L. plantarum* were able to give a heavy fragrant flavor due to the high population of yeast and LAB in T2 and T3, which allows the production of lactic acid. This is also conveyed by Chen *et al*. (2016), who states that adding *L. plantarum* can enhance homolactic fermentation, thus increasing the lactic acid content in silage. Yeast and LAB populations in this study were higher than the results of research by Silva *et al.* (2021) on a 30-day ensiling silage with LAB population of 7.72 log cfu/g and yeast population of 1.75 log cfu/g. The yeast strain is beneficial in preventing and inhibiting the development of other detrimental microorganisms in silage (Muck *et al.,* 2018). The figure of the silage can be seen in Figure 1.

The silage fermentation has good fermentation characteristics with a low pH value (<3.80). The decrease in pH value in inoculant silage can also be influenced by the high population of LAB in T2 and T3, while in T0 the decrease in pH can occur due to the use of additives in the form of molasses, which allows the ensilage process. The supplementation of inoculants has not been able to maintain the percentage of silage dry matter (DM). The factors affecting DM losses include pre-ensiling condition, respiration, and temperature at ensiling (Borreani *et al*., 2018). The results of this study are different from the results of research by Silva *et al.* (2022), which states that the supplementation of inoculants *L. plantarum* can maintain DM value and different results are also reported by Amaral *et al.* (2020), which states an increase in DM value with the supplementation of *L. plantarum* inoculants. *L. plantarum* reduced the NH_3 concentration of the silage. This is in accordance with the research of Muck *et al*. (2018), which found that the use of *L. plantarum* inoculant decreases NH_3 concentration.

Low dry matter (DM) content in inoculant treatment is associated with loss of DM during ensiling. The range of DM content silage was 20.67%-23.51%. DM content of the study is higher than the result of Guan *et al.* (2020). The nutritive value of the forage is determined by nutrient composition, particularly NFC, NDF, ADF, and CP, as reported by Lyons *et al.* (2019). NFC includes rapidly fermentable carbohydrates such as sugar and soluble sugar calculated through a formula that uses ash, EE, CP, and NDF (Table 3).

NDF content represents structural carbohydrates in plants. Structural carbohydrates also function as an energy source for ruminants but have a slow rate value of degradation (Kondo *et al.,* 2014). The NDF content of elephant grass silage at 30 days of fermentation was

Figure 1. The color of the *Pennisetum purpureum* silage after 21 days. Note: (a) T0= *P. purpureum* + 3% molasses, (b) T1= T0 + *Saccharomyces cerevisiae*, (c) T2= T0 + *Lactobacillus plantarum*, (d) T3= T0 + *S. cerevisiae* + *L. plantarum*.

73.87% and the ADF content was 47.02% (Khota *et al.*, 2016). The highest content of ADF was associated with lignin content that was shown in T1 and T3, but the control silage had the highest lignin content. There was an increase in Ca and P content in the inoculant supplementation treatment.

Rumen Fermentation Characteristic and Digestibility

Supplementation of inoculant in silage maintains normal rumen pH (5.5-7.0). The ability of a single inoculant to produce rumen NH_3 was similar to the inoculant combination treatment. The increase in NH_3 concentration at T1 and T3 was related to the solubility of silage protein. NH_3 is used as a nitrogen source for microbial protein synthesis. However, the increase in NH_3 in treatments T1 and T3 was unrelated to CP content. This indicates that the CP contents in T1 and T3 are easily degraded.

Total VFA concentrations are representations of fermentation rate, digestibility, and gas production (Sugoro *et al.*, 2015; Wahyono *et al.*, 2018). The combination of *S. cerevisiae* and *L. plantarum* inoculant increased the VFA total concentration. The increase in total VFA concentration in the rumen was caused by the stability of rumen pH that eventually stimulated the growth of the bacterial population. Yeast supplementation increased rumen pH and volatile fatty acid concentration (Riyanti *et al.*, 2016). This value is also in line with the high concentrations of acetate and propionate in the inoculant combination treatment but does not affect the A/P ratio, where the lower A/P ratio indicates a balanced proportion of acetate and propionate. All treatments had low A:P ratios, indicating that propionic activity was more dominant in feed degradation. This contradicts the results of Vyas *et al.* (2014) which state inoculant supplementation is more effective for rumen fermentation, resulting in a higher A:P ratio. Supplementation of inoculant does not change the concentration of short chain fatty acid. The growths of specific strains of fiber-digesting bacteria, which have major roles in the digestion of fiber to produce higher short chain fatty acid, were stimulated by yeast supplementation. Isobutyrate and isovalerate enhanced proteolytic bacteria could utilize branch-chain amino acid as the energy source to produce branch-chain fatty acid as the end product (Vyas *et al.*, 2014). A combination of *S. cerevisiae* and *L. plantarum* inoculant increased DMD and OMD. Increased DMD and OMD are influenced by high VFA concentrations in the rumen. Ruminal VFA productions indicate the degradation pattern of carbohydrates (Zhong *et al.*, 2016). OMD and DMD of the silage in this study were higher than in the previous study (Gul, 2023).

Regarding Table 5, the control and single inoculant treatments produced the highest gas production until 10 h, while the inoculant combination had the lowest gas production. The control and single inoculant treatments produced the highest gas production until 10 h, while the inoculant combination had the lowest gas production. The difference in ADF contents will affect the difference in *in vitro* total gas production. The low gas production can also be influenced by the higher VFA production in T3 compared to the other treatments because it is in line with carbohydrate fermentation. Kondo *et al.* (2015) stated that total VFA concentration in tropical grass highly correlates with *in vitro* gas production (r=0.96) and NFC content (r=0.84).

The sum of a and b fractions (a+b) can be interpreted as the optimum gas production, while the c fraction is the degradation rate (Ørskov & McDonald, 1970; Kisworo *et al.*, 2017). Regarding Table 6, the total gas production of T0 was the highest among treatments, while the supplementation of *S. cerevisiae* and *L. plantarum* had the lowest total gas production. However, the rate of gas production in all treatments was the same. Feed components in the form of fiber and protein can affect the production of gas produced during the fermentation process. Optimal gas production in this study was lower than the results of Elghandour *et al.* (2014), with 128-162 mL in 24 h. The lower gas production indicates that the gas production rate decreases with increasing incubation time because the fermented substrate also decreases. A high c value indicates that the feed can be degraded rapidly in a certain unit of time. There are little data in the literature regarding the effect of additional inoculants on gas production. Elghandour *et al.* (2014) showed that the gas production rate (c value) produced in various fibrous feeds was 0.029-0.09 mL/h lower than the c value in our study, and the *S. cerevisiae* addition could improve gas production in Elghandour *et al.* (2014), in contrast to our study.

Methane gas production was the highest in the inoculant treatment. This is in line with the high hemicellulose compositions also in T1, T2, and T3. However, compared to the ratio of methane gas production to VFA production, the T3 treatment has a lower ratio. Methane production will increase, as it is associated with high NDF and hemicellulose content (Wahyono *et al.*, 2019).

CONCLUSION

The supplementation of *S. cerevisiae* and *L. plantarum* as a silage inoculant improved physical and microbiological quality of *P. purpureum* silage. Although the nutrient composition did not increase significantly, the combination of *S. cerevisiae* and *L. plantarum* inoculants was able to improve fermentation activity in the rumen, increase total VFA, dry matter digestibility, organic matter digestibility, reduce total gas production, and reduce the ratio of methane gas production to VFA.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

ACKNOWLEDGEMENT

The research was funded by The Centre of Agricultural Education, the Indonesian Agency

for Agricultural Extension and Human Resources Development, Ministry of Agriculture through Strategic Research Grant 2023.

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