

EFFECTS OF ADDITIVES ON THE PHYSICOCHEMICAL CHARACTERISTICS AND POTENTIAL FEEDING VALUE OF CORN AND HYBRID NAPIER GRASS (*Pennisetum purpureum* x *P. glaucum*) SILAGES

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ABSTRACT

Physicochemical characteristics, *in vitro* gas production (IVGP), and *in situ* ruminal degradability of two forage types (FT): whole-plant corn (CS) and hybrid Napier grass silage (NS) with and without additives (ADD) were assessed in this study. The corn and Napier grass were ensiled without additive (control, CON), 2% w/w molasses (MOL), or an inoculant (LAB; *Lactobacillus plantarum* at 5 log CFU/g fresh) for 30 days. Data were analyzed as a completely randomized design (4 replicates) in a 2 FT × 3 ADD factorial arrangement using the PROC GLIMMIX procedure of SAS v.9.4. CS had higher DM content than NS across all ADD. Adding MOL to NS reduced pH and increased the Flieg score ($P<0.05$), but it did not improve the IVGP kinetics and ruminal degradability. The potential gas production (a+b), rapidly soluble (a) DM, and organic matter (OM) fractions were higher ($P<0.05$) in CS than in NS, regardless of ADD. The LAB inoculant did not improve silage quality or potential feeding value of either FT. Overall, CS exhibited superior silage quality and potential feeding value compared with NS, regardless of ADD. However, these findings should be interpreted with caution because microbial populations, organic acids, and alcohol profiles were not evaluated.

Keywords: fermentation kinetics, lactic acid bacteria, dry matter recovery, total soluble sugar, Flieg score

INTRODUCTION

Preservation of forage resources through ensiling can help maintain feed availability and nutritional quality during periods of forage scarcity. Unlike hay, ensiling enables successful forage preservation in tropical regions, including the Philippines, where climatic conditions are characterized by high humidity, frequent rainfall, and highly unpredictable weather. Whole-plant corn has been the predominant forage resource for ensiling worldwide due to its high water-soluble carbohydrate content, optimal standing DM for ensiling, adaptability to a wide range of climatic conditions, and suitability for mechanized production systems (Roth & Heinrich, 2001). In the Philippines, growing corn is often challenging

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due to the high cost of inputs such as seeds, fertilizers, pest control, irrigation, labor, and machinery (Alforque & Pleños, 2026). In contrast, Napier grass (*Pennisetum purpureum*) and its hybrid cultivars are the most widely cultivated forage grasses due to their high biomass yield, drought tolerance, and strong regrowth capacity with minimal input costs (Negawo *et al.*, 2017).

The use of silage additives (e.g., bacterial inoculants, molasses) has been widely investigated for improving silage fermentation. The use of bacterial inoculants in silage is a widely used strategy to improve its nutritional value and shelf life. *Lactobacillus plantarum* is one of the most popular bacterial inoculant species for silage (Fabiszewska *et al.*, 2019). The introduction of exogenous *L. plantarum* encourages the effective conversion of water-soluble carbohydrates from forage materials into organic acids, particularly lactic acid (Muck *et al.*, 2018). Lactic acid is a strong acid that rapidly reduces pH, inhibits spoilage microorganisms, and improves silage quality (Arasu *et al.*, 2014). Another common silage additive, molasses, is a by-product of refining sugarcane into sugar. Molasses is typically added to silage to accelerate acidification by providing readily available, fermentable sugars that support the growth of lactic acid bacteria during ensiling (Qiu *et al.*, 2014). Silage with molasses increases its quality, nutrient content, and palatability to ruminants (Guo *et al.*, 2014).

Jaipolsaen *et al.* (2022) evaluated the effects of microbial inoculants on the fermentation quality of corn and Napier grass silages. However, the inoculant used in their study consisted of *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Pediococcus pentosaceus*, making it difficult to distinguish the specific contributions of individual bacterial species to silage fermentation. Moreover, although molasses is a low-cost, widely accessible additive commonly recommended to improve silage fermentation, studies directly comparing its efficacy with that of a single-species *L. plantarum* inoculant across different forage types remain scarce. To the best of our knowledge, no study has simultaneously evaluated and compared the effects of molasses and a sole *L. plantarum* inoculant on both whole-plant corn and hybrid Napier grass silages under hot, humid tropical conditions. Hence, we investigated the effects of *L. plantarum* inoculant and molasses on the 1) physicochemical characteristics, 2) *in vitro* gas production kinetics, and 3) *in situ* ruminal degradability of corn and hybrid Napier grass silage.

MATERIALS AND METHODS

Ethical Approval

The rumen cannulation, feeding, and management of the animals used in this study were conducted in accordance with the protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños (UPLB), College, Laguna (CAFS-2020-002).

Preparation of inoculant

Active cultures of *Lactobacillus plantarum* BIOTECH 1223 were obtained from the Philippine National Collection of Microorganisms (PNCM) at the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, Laguna, Philippines. The inoculant was prepared by cultivation of *L. plantarum* in

de Man, Rogosa, and Sharpe (MRS) broth. After incubation at 37 °C for 24 h, cultures were propagated in a sterile 2% (w/v) molasses solution and stored at 5 °C the day before the experiment. Pour-plating revealed a microbial count of 7 log CFU/ml. Subsequently, 10 ml of the inoculant was incorporated into the forage material to achieve an inoculation concentration of 5 log CFU per g of forage (Abdul Rahman *et al.*, 2017).

Silage preparation

Hybrid corn (NK 6414, Syngenta Philippines, Inc., Taguig City, Philippines) was established in the experimental site (~2 ha) located at the Dairy Training and Research Institute (DTRI), University of the Philippines Los Baños, College, Laguna, Philippines (14°09'22" N 121°14'40" E). The site has an average annual rainfall of 160 mm and an annual average temperature of 27.2°C (DOST-PAGASA, 2021). The field was plowed twice, harrowed, and rotavated twice. Dolomitic lime was manually broadcast on September 24, 2020. Afterward, the field was rotavated again to mix the lime and soil. Furrowing was performed on September 25, 2020, with furrows spaced 0.75 m apart and 6-8 cm deep. Broadcast fertilization and planting were performed simultaneously on September 26, 2020. Dolomitic lime and fertilizer (urea and complete fertilizer) were applied to the field following the recommendations of the Bureau of Plant Industry - Department of Agriculture for a genetically-trait hybrid. The entire corn plant was manually harvested on December 23, 2020, from 4 random locations in the field at a cutting height of 15–17 cm. Napier grass, v. Florida, was manually harvested on the same day from 4 randomly selected locations within an established stand, following 45-day standardization at a cutting height of approximately 7.5 cm. Although the two forages differed in chronological age, each crop was harvested at its respective agronomically recommended stage for silage production rather than at equivalent growth stages. The corn was harvested at 88 days after planting, as it had reached the appropriate maturity and the recommended standing DM (30-40%) for ensiling. Napier grass was harvested after a 45-day regrowth period, a standard interval used to optimize yield and nutritive value (Onjai-uea *et al.*, 2022).

The harvested whole corn plants and Napier grass were chopped into 2.54 cm-long particles using a machete. The chopped materials from corn and Napier grass were homogenized and subdivided into 3 independent piles corresponding to the 3 treatments. The treatments were corn or Napier grass silage with no additive (CON, control), with 2% w/w molasses (MOL), and with *L. plantarum* inoculant (LAB). The treatments were applied to 1 pile, and each pile was divided into 4 to represent the 4 replications. The LAB inoculant was applied to achieve a concentration of 5 log CFU/g of forage material. The application rate of the *Lactobacillus plantarum* inoculant was based on the recommendation of Hu *et al.* (2009), who reported that this inoculation level effectively promotes lactic acid fermentation and improves the preservation of corn silage. The 2% (w/w) molasses application rate was selected based on established forage ensiling practices at the Dairy Training and Research Institute. This inclusion rate has been routinely used to provide readily fermentable carbohydrates that support the growth of lactic acid bacteria and facilitate a rapid decline in silage pH, particularly when ensiling tropical forages that may be limited in water-soluble carbohydrates. After treatment application, each replicate pile, including the control, was evenly mixed. The chopped forages (~500 g fresh) were manually packed into 1000 ml transparent glass jar silos to minimize air space. Samples were covered with a polyethylene sheet and tightly sealed with tin lids and packaging tape. The silo jars were individually

weighed and then stored at room temperature and away from direct sunlight.

Analyses of Physicochemical Characteristics of Silage

After 30 days of storage, the silage jars were weighed again to determine DM recovery. Afterward, the physical and chemical properties of the ensiled materials were assessed. The physical characteristics, including odor, texture, and color, were evaluated independently by 3 trained panels. The panels comprised three researchers from the Dairy Training and Research Institute with experience in forage and silage quality assessment using standardized visual and sensory criteria. The odor was evaluated based on the modified recommendation of Van Saun and Heinrich (2016). The texture was evaluated based on the modified procedure of Lyimo *et al.* (2018). The texture index was evaluated as poor=slimy and watery; moderate=firm, less slimy and wet; good=moderately firm, non-slippery and wet; and very good=very firm, non-slippery and slightly wet. The silage colors were evaluated visually using a color chart.

To assess the quality of silages after 30 days of ensiling, the Flieg score (FP) was calculated from the pH and DM contents of mixed silages at the end of the fermentation period using Equation 1 (Kilic, 1986). Following the index, values of <20, 21-40, 41-60, 61-80, and 81-100 were classified as bad, poor, medium, good, and very good, respectively.

$$\text{Flieg score} = 220 + (2 \times \% \text{DM} - 15) - 40 \times \text{pH} \quad \text{Equation 1}$$

The temperature of each silage jar was measured by inserting a thermometer 2 inches into the container immediately after opening. The pH was determined by homogenizing 25 g of silage in 100 mL of distilled water (Bolsen *et al.*, 1992). The pH of the resulting solution was measured after 15 minutes using a pH meter electrode (Handylab pH 11 Set, Schott® Instruments, Mainz, Germany). The total soluble sugar (TSS) content of the silage was also measured by extracting sap from different sampling points in the jar using a garlic press. The sap was then placed in the glass prism of the ATAGO® PAL-1 pocket refractometer (Brix range: 0 to 53%; Atago Co., Ltd., Bellevue, Washington, USA) until a stable reading was obtained.

Nutrient Analyses

Approximately 300 g of fresh ensiled samples were weighed and completely dried at 70°C until constant weight. Dried samples were ground using a Wiley Mill and passed through a 1 mm sieve. The ground samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), crude fat (CF), and ash following AOAC (2016) methods. The neutral detergent fiber (NDF) was analyzed using the Ankom® 200 Fiber Analyzer (Ankom Technology Corp., Fairport, NY, USA) using the neutral detergent solution, amylase, and sodium sulfite (Van Soest *et al.*, 1991). Non-fibrous carbohydrates (NFC) were then calculated using Equation 2.

$$\text{NFC} = 100 - (\% \text{CP} + \% \text{NDF} + \% \text{EE} + \% \text{ash}) \quad \text{Equation 2}$$

In vitro gas production determination

Rumen fluid (RF) samples were collected from three cannulated Holstein x Sahiwal dairy bulls. Approximately 200 mL of RF was filtered using three layers of cheesecloth

and placed into a 50-ml Terumo® plastic syringe (Terumo Corporation, Shibuya-ku, Tokyo, Japan) containing 120 mg of ground CS or NS, 5 mL of phosphate buffer (0.05 M, pH 6.9), and 1 mL of magnesium sulfate (0.154 M). The syringes were filled to the 15 mL mark. The air space in the syringe was removed by pushing the plunger. The syringes were placed into zip-loc bags and incubated at 39 °C for 3, 6, 12, 24, and 48 h. The gas produced (GP) was estimated from the air space volume measured using the syringe graduations. Data were fitted into the Orskov and McDonald model except that the parameters *a*, *b*, and *c* were associated with gas production instead of DM degradation (Equation 3):

$$Y = a + b(1 - e^{-ct}) \quad \text{Equation 3}$$

wherein *Y* was GP (ml) at the time of incubation *t*, *a* was GP from the slowly degradable fraction (ml), *a+b* was the potential GP (ml), *c* was the GP rate constant, and *e* was the base of the natural logarithm.

The effective gas production (EP) was calculated using Equation 4:

$$EP = a + \left[\frac{b \cdot c}{(c + k)} \right] \quad \text{Equation 4}$$

where *k* is the rate of particulate outflow from the rumen, *k*. The 0.02 ml/h rumen outflow rate recommended by Ørskov & McDonald (1979) was used. The gas production kinetics were estimated using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA).

***In situ* ruminal degradation**

Samples were dried at 70 °C until constant moisture and ground to pass through a 2 mm sieve. Ground samples (3 g) were placed in a digestion bag (9 x 15 cm) woven using polyester and silk (40-60 microns perforations) and then incubated in three (3) ruminally cannulated Holstein x Sahiwal dairy bulls. The animals were fed a consistent supply of fresh grass, corn silage, and concentrates. Following a randomized complete block design with the bulls as the blocking factor, samples were incubated for 3, 6, 12, 24, and 48 h using a gradual in-all-out method. The sample bags intended for the 48-hour incubation were placed first, followed by the 24-hour bags, and so forth. All the bags were taken out after 48 hours of incubation. The bags were soaked and washed with water multiple times until the water became clear. The bags were clip-hung, air-dried for one day, and dried at 70°C until constant weight was reached. To account for the loss of sample weight during incubation and washing, control samples were soaked, washed, and dried to correct the data. Blanks were also incubated in the rumen to account for weight changes during sample soaking in ruminal liquor. The samples were subjected to DM and OM measurements following the AOAC (2016) methods. The DM and OM degradability were then estimated using Equation 5 by fitting the data to the exponential equation proposed by Ørskov & McDonald (1979):

$$y = a + b(1 - e^{-ct}) \quad \text{Equation 5}$$

where *y* = DM or OM disappearance in the rumen (%) at time *t*, *a* = the rapidly soluble fraction (%), *b* = the potentially degradable fraction (%), *c* = the constant rate of degradation of *b* (%/h), and *t* = incubation time. The model parameters were estimated using the non-

linear procedure of GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA). The effective degradability of DM and OM was calculated following the equation: $ED = a + (bc / (c + k))$, where (a), (b), and (c) are the same as in Equation 1, and (k) is the rumen outflow rate. The outflow rate was based on the NRC (2001) recommendation, with a rumen outflow rate of 2%/h for a dairy animal in maintenance condition.

Statistical Analyses

All collected data were analyzed as a completely randomized design (4 replicates) in a 2 FT × 3 ADD factorial arrangement using the PROC GLIMMIX procedure of SAS v.9.4 (SAS Institute Inc., 2014). The silage jars were set as the experimental unit for the physicochemical characteristics. In the IVGP and *in situ* degradability procedure, independent syringes and nylon bags, respectively, were used for each incubation time. The individual cannulated bulls were set as a blocking factor in the IVGP and *in situ* procedure to account for animal-to-animal variation. The SLICE option was used to analyze significant interactions between FT and ADD. Data were tested for normality using the Shapiro-Wilk test. Significance was declared at $P \leq 0.05$. Means were separated using Fisher's protected LSD.

RESULTS

The nutrient composition of CS and NS with and without additives is presented in Table 1. CS had higher DM, NFC, and TSS than NS ($P < 0.01$) across all ADD. No differences between ADD and FT were observed for CP, CF, and silage temperature ($P > 0.05$). An interaction effect ($P < 0.01$) between ADD and FT was observed for the ash content. The MOL had the lowest ash content for CS. Meanwhile, LAB had the lowest ash content for NS. NS had higher ash content than CS for all ADD. For NDF, differences were only observed for CS, with LAB having the lowest NDF content (ADD × FT; $P = 0.05$). CS had higher NFC than NS across all ADD ($P < 0.01$). LAB had the highest NFC compared to MOL and CON across all FT ($P = 0.02$). The ADD and FT also had interaction effects in the DM recovery ($P < 0.01$). CS-CON had a lower pH than NS-CON (3.71 vs 4.25; $P < 0.01$). A similar trend was observed between CS-LAB and NS-LAB (3.73 and 4.30, respectively; $P < 0.01$). The pH of NS-MOL did not differ from that of CS-MOL (3.67 vs 3.71; $P < 0.01$). The MOL increased the TSS of both NS and CS ($P < 0.01$). The Flieg score for CS was higher than that for NS in CON (57.1 vs 35.5) and LAB only (56.4 vs 33.6). MOL increased the Flieg score only for NS, and it did not differ from CS-MOL (58.5 vs 57.4).

The physical characteristics of CS and NS with and without ADD are shown in Table 2. The CS-MOL and NS-MOL both had a very firm, non-slippery, and slightly wet texture, indicating a very good silage texture. The CS-CON had a tobacco/caramelized odor, whereas CS-MOL and CS-LAB had a sharp, sweet odor. The NS-MOL was olive-brown, whereas NS-CON and NS-LAB were olive-green. The NS with and without ADD had different odors. The NS-CON had a firm, less slimy, and wet texture.

The *in vitro* gas fermentation kinetics of NS and CS with and without ADD are presented in Table 3. NS had a lower gas production from the rapidly soluble fraction (a) than CS across all ADD. NS had a higher gas production rate (c) than CS ($P < 0.01$) across all ADD (0.06 vs 0.04 mL/h). Across all ADD, the potential gas production (a+b) was higher

($P < 0.04$) in CS than in NS. Tables 4 and 5 present the DM and OM degradation kinetics for CS and NS, with and without ADD. CS had a higher ($P < 0.05$) rapidly soluble DM fraction (a) than NS across all ADD (24.1 vs 12.0 %). A similar trend was observed for the rapidly soluble OM fraction between NS and CS (16.2 vs 2.65%) across all ADD. However, CS had a lower amount of slowly degradable DM and OM fraction (b) ($P < 0.05$) than NS for all ADD. The MOL in CS increased the potentially degradable fraction of DM (a+b) compared to the NS-MOL. Furthermore, the MOL (74.4%) increased the potentially degradable fraction (a+b) of DM more than CON (55.0%) and LAB (57.1%) at CS only. CS-MOL increased ED of DM (60.8%) more than NS-MOL (51.2%). CS-MOL also had a higher ED of OM than NS-MOL (55.9 vs 43.5%). MOL had higher ED of DM and OM than CON and LAB at CS only.

Table 1. Nutrient composition of corn (*Zea mays*) and Napier grass (*Pennisetum purpureum* x *P. glaucum*) silages with and without additives.

Item	Additives			Mean	SEM	P-values		
	CON	MOL	LAB			ADD	FT	ADD × FT
Dry matter, %					1.13	0.11	<0.01	0.34
Corn silage	25.0	29.2	27.1	27.1 ^A				
Napier silage	12.9	13.7	12.8	13.2 ^B				
Mean	19.0	21.5	20.0					
Crude protein					1.36	0.59	0.28	0.71
Corn silage	8.28	6.57	7.49	7.45				
Napier silage	6.96	6.58	5.86	6.47				
Mean	7.62	6.58	6.68					
Ash					0.10	<0.01	<0.01	<0.01
Corn silage	7.04 ^{B, a}	4.63 ^{B, c}	5.38 ^{B, b}	5.68				
Napier silage	14.3 ^{A, a}	14.2 ^{A, ab}	14.1 ^{A, b}	14.2				
Mean	10.7	9.42	9.74					
Crude fat					1.44	0.92	0.12	0.90
Corn silage	2.32	2.55	2.41	2.42				
Napier silage	4.23	3.37	3.92	3.84				
Mean	3.28	2.96	3.17					
Neutral detergent fiber					1.09	0.06	0.78	0.05
Corn silage	63.4 ^a	61.9 ^a	57.6 ^b	61.0				
Napier silage	60.8	60.5	60.9	60.7				
Mean	62.1	61.2	59.3					
Non-fibrous carbohydrate					2.20	0.02	<0.01	0.13
Corn silage	16.9	24.4	27.1	22.8 ^A				
Napier silage	13.8	14.8	15.8	14.8 ^B				
Mean	15.4 ^b	19.6 ^{ab}	21.5 ^a					

Table 1 continued . . .

Item	Additives			Mean	SEM	P-values		
	CON	MOL	LAB			ADD	FT	ADD × FT
Dry matter recovery					0.03	0.09	0.08	<0.01
Corn silage	99.7	99.6 ^B	99.7 ^A	99.6				
Napier silage	99.6 ^b	99.8 ^{A, a}	99.6 ^{B, b}	99.7				
Mean	99.7	99.7	99.7					
Temperature					0.50	0.95	1.00	0.95
Corn silage	29.5	29.7	29.8	29.7				
Napier silage	29.7	29.7	29.7	29.7				
Mean	29.6	29.7	29.8					
pH					0.05	<0.01	<0.01	<0.01
Corn silage	3.71 ^B	3.71	3.73 ^B	3.71				
Napier silage	4.25 ^{A, a}	3.67 ^b	4.30 ^{A, a}	4.07				
Mean	3.98	3.69	4.02					
Total soluble sugars					0.54	0.06	<0.01	0.66
Corn silage	5.97	7.47	5.73	6.39 ^A				
Napier silage	3.60	4.57	3.83	4.00 ^B				
Mean	4.78 ^b	6.01 ^a	4.78 ^b					
Flieg score					1.92	<0.01	<0.01	<0.01
Corn silage	57.1 ^A	57.4	56.4 ^A	57.0				
Napier silage	35.5 ^{B, b}	58.5 ^a	33.6 ^{B, b}	42.5				

^{A,B} Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

^{a-c} Means with different lowercase superscripts within a row are significantly different ($P \leq 0.05$).

SEM=standard error of the mean. CON=untreated silage (i.e., no additive), MOL=silage with 2% w/w molasses, LAB=silage with *Lactobacillus plantarum* inoculant at 5 log CFU/g forage.

ADD=Additive effects; F=forage effects

Flieg score: <20, very bad silage quality; 21-40, bad; 41-60, medium quality; 61-80, good quality; 81-100, very good quality.

Table 2. Physical characteristics of corn (*Zea mays*) and Napier grass (*Pennisetum purpureum* x *P. glaucum*) silage with and without additives.

Item	Additives		
	CON	MOL	LAB
Color			
Corn silage	yellowish brown	yellowish brown	yellowish brown
Napier silage	olive green	olive brown	olive green
Odor ¹			
Corn silage	tobacco/caramel	sharp sweet	sharp sweet
Napier silage	rancid butter	sharp sweet	alcohol
Texture ²			
Corn silage	moderate firm, non-slippery, and wet	very firm, non-slippery, and slightly wet	moderate firm, non-slippery, and wet

Table 2 continued . . .

Item	Additives		
	CON	MOL	LAB
Texture ²			
Napier silage	firm, less slimy, and wet	very firm, non-slippery, and slightly wet	moderate firm, non-slippery, and wet

CON=untreated silage (i.e., no additive), MOL=silage with 2% w/w molasses, LAB=silage with *Lactobacillus plantarum* inoculant at 5 log CFU/g forage.

¹odor was evaluated based on the modified recommendation of Van Saun and Heinrich (2016).

²based on the method described by Lyimo *et al.* (2018).

Table 3. *In vitro* gas fermentation kinetics of corn (*Zea mays*) and napier grass (*Pennisetum purpureum* x *P. glaucum*) silage with and without additives.

Item	Additives			Mean	SEM	P-values		
	CON	MOL	LAB			ADD	FT	ADD × FT
a, mL					0.65	0.95	0.05	0.73
Corn silage	-0.06	-0.55	-0.14	-0.25 ^A				
Napier silage	-1.69	-1.17	-1.27	-1.38 ^B				
Mean	-0.88	-0.86	-0.71					
b, mL					2.21	0.45	0.09	0.16
Corn silage	11.1	15.4	18.6	15.0				
Napier silage	12.5	11.7	10.8	11.7				
Mean	11.8	13.6	14.7					
c, mL/h					0.01	0.54	<0.01	0.18
Corn silage	0.05	0.04	0.03	0.04 ^B				
Napier silage	0.06	0.06	0.07	0.06 ^A				
Mean	0.06	0.05	0.05					
a+b, mL					2.35	0.45	0.04	0.22
Corn silage	11.0	14.8	18.4	14.8 ^A				
Napier silage	10.9	10.6	9.55	10.3 ^B				
Mean	10.9	12.7	14.0					
EP, mL					0.64	0.18	0.06	0.39
Corn silage	7.85	9.72	8.17	8.58 ^A				
Napier silage	7.69	7.01	7.77	7.49 ^B				
Mean	7.77	8.37	7.97					

^{A,B} Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

^{a-c} Means with different lowercase superscripts within a row are significantly different ($P \leq 0.05$).

SEM=standard error of the mean. CON=untreated silage (i.e., no additive), MOL=silage with 2% w/w molasses, LAB=silage with *Lactobacillus plantarum* inoculant at 5 log CFU/g forage.

A=Additive effects; F=forage effects

a= gas production from the immediately soluble fraction (mL); b= gas production from the slowly degradable fraction (mL);

c= gas production rate constant (mL/h); a+b= potential gas production (mL); EP=effective gas production (mL).

Table 4. Dry matter degradation kinetics of corn (*Zea mays*) and napier grass (*Pennisetum purpureum* x *P. glaucum*) silages with and without additives.

Item	Additives			Mean	SEM	P-values		
	CON	MOL	LAB			ADD	FT	ADD × FT
a, %					5.65	0.99	0.02	0.61
Corn silage	21.8	26.9	23.6	24.1 ^A				
Napier silage	14.4	8.39	13.3	12.0 ^B				
Mean	18.1	17.6	18.5					
b, %					4.40	0.18	<0.01	0.26
Corn silage	33.2	47.5	33.5	38.1 ^B				
Napier silage	48.5	49.9	49.4	49.2 ^A				
Mean	40.9	48.7	41.5					
c, %/h					0.05	0.70	0.14	0.29
Corn silage	0.09	0.06	0.08	0.08				
Napier silage	0.09	0.17	0.09	0.12				
Mean	0.09	0.12	0.09					
a+b, %					4.72	0.27	0.82	0.05
Corn silage	55.0 ^b	74.4 ^{A, a}	57.1 ^b	62.2				
Napier silage	62.9	58.3 ^B	62.7	61.3				
Mean	59.0	66.4	59.9					
ED, %					3.61	0.09	0.51	0.05
Corn silage	48.5 ^b	60.8 ^{A, a}	50.3 ^b	53.2				
Napier silage	51.5	51.2 ^B	52.5	51.7				
Mean	50.0	56.0	51.4					

^{A, B} Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).

^{a, b} Means with different lowercase superscripts within a row are significantly different ($P \leq 0.05$).

SEM=standard error of the mean. CON=untreated silage (i.e., no additive), MOL=silage with 2% w/w molasses, LAB=silage with *Lactobacillus plantarum* inoculant at 5 log CFU/g forage.

A=Additive effects; F=forage effects

a=rapidly soluble fraction; b=slowly degradable fraction; c=degradation rate; a+b = potentially degradable fraction;

ED=effective degradability.

Table 5. Organic matter degradation kinetics of corn (*Zea mays*) and napier grass (*Pennisetum purpureum* x *P. glaucum*) silages with and without additives.

Item	Additives			Mean	SEM	P-values		
	CON	MOL	LAB			ADD	FT	ADD × FT
a, %					5.29	0.95	<0.01	0.52
Corn silage	12.4	19.8	16.3	16.2 ^A				
Napier silage	4.55	-0.29	3.70	2.65 ^B				
Mean	8.48	9.76	10.0					

Table 5 continued . . .

Item	Additives			Mean	SEM	P-values		
	CON	MOL	LAB			ADD	FT	ADD × FT
b, %					5.29	0.95	<0.01	0.52
Corn silage	37.8	46.7	37.8	40.8 ^B				
Napier silage	50.8	51.2	51.9	51.3 ^A				
Mean	44.3	49.0	44.9					
c, %/h					0.03	0.72	0.39	0.22
Corn silage	0.11	0.07	0.09	0.09				
Napier silage	0.09	0.16	0.09	0.11				
Mean	0.10	0.12	0.09					
a+b, %					4.11	0.37	0.39	0.06
Corn silage	50.2	66.5	54.1	56.9				
Napier silage	55.3	50.9	55.6	53.9				
Mean	52.8	58.7	54.9					
ED, %					2.27	0.07	0.02	0.04
Corn silage	43.9 ^b	55.9 ^{A, a}	47.5 ^b	49.1				
Napier silage	44.1	43.5 ^B	44.9	44.2				
Mean	44.0	49.7	46.2					

^{A,B} Means with different uppercase superscripts within a column are significantly different ($P \leq 0.05$).
^{a,c} Means with different lowercase superscripts within a row are significantly different ($P \leq 0.05$).
SEM=standard error of the mean. CON=untreated silage (i.e., no additive), MOL=silage with 2% w/w molasses, LAB=silage with *Lactobacillus plantarum* inoculant at 5 log CFU/g forage.
A=Additive effects; F=forage effects.
a=rapidly soluble fraction (%); b=slowly degradable fraction (%); c=degradation rate (ml/h); a+b = potentially degradable fraction; ED=effective degradability (%).

DISCUSSION

The higher DM content in CS than in NS may be attributed to factors such as plant maturity, environmental conditions, forage species, and harvest management (Ball, 2001). Forage corn is typically harvested for silage at the reproductive stage when its standing DM content reaches 30-40%, which is optimal for successful packing (Kung *et al.*, 2018). Meanwhile, it is recommended to harvest Napier grass for ensiling during its vegetative stage. Harvesting Napier grass after the vegetative stage increases NDF accumulation, thereby hampering digestibility (Chen *et al.*, 2006). Furthermore, wilting of the harvested Napier grass was not performed in this study; thus, it would have higher moisture content. The DM recovery was generally high (>99%) across all treatments, indicating minimal losses during the 30-day ensiling period. The glass jar silo used in our experiment was not fitted with gas traps and an effluent valve, which are used in other silage experiments (Pratama *et al.*, 2025). The high DM recovery observed in the jar silos can be attributed to the strict hermetic nature of the glass jar silos. As demonstrated by the mass-conservation models of Abalone *et al.* (2011) for airtight systems, volatile by-products and gases generated are completely trapped within the sealed jars. In this experiment, because the fermentation by-products

(such as volatile fatty acids and carbon dioxide) were prevented from escaping the system, they remained accounted for during the total mass measurements of the jar silos. This might explain why the difference in DM recovery between treatments ranges only from 0.1 to 0.2%, which is highly unlikely to be biologically significant.

NS had higher ash content than CS because it had lower stubble height at harvest (7.5 cm vs 15-17 cm). It is recommended to harvest Napier grass for silage at a stubble height of approximately 15 cm to maximize DM yield while leaving enough nodes for healthy regrowth (Geren *et al.*, 2020). However, cutting closer to the ground increases the chance of dirt, dust, and mud sticking to the stems and leaves (Lounglawan *et al.*, 2014). Moreover, corn is ideally cut at a length of at least 25 cm, as nitrates tend to accumulate in the lower stems during periods of drought and stress (Block *et al.*, 2021). Nitrates in corn are associated with nitrate toxicity in livestock and with the risk of silo gas exposure in humans (Skjolaas *et al.*, 2012). Non-fibrous carbohydrates refer to the highly digestible fraction of the plant cell, which consists of starch, sugars, organic acids, and pectins (Villalba *et al.*, 2021). The CS had higher NFC content than NS due to the sugar and starch in corn grains. Corn grains are estimated to contain an average of 71.0% starch and 3.7% sugar (Heuze *et al.*, 2017). Meanwhile, the addition of LAB led to increased NFC in both CS and NS. De Mendonça *et al.* (2020) also reported that inoculants made up of LAB led to higher NFC in whole-plant corn silage.

As expected, MOL increased the TSS content in both NS and CS due to the additional fermentable carbohydrates it provided. NS-MOL had a pH and Flieg score similar to that of the CS-MOL. Meanwhile, the addition of LAB failed to lower the pH or increase the Flieg score in NS compared with CS. This showed that, despite the high moisture content of the NS, molasses encouraged more effective fermentation after 30 days. Bureenok *et al.* (2012) reported that molasses lowered the pH value and increased the water-soluble carbohydrates of NS. Silage without molasses often exhibits poor fermentation characteristics, such as high pH, an elevated ammonia-nitrogen-to-total-nitrogen ratio, and butyric acid (Wang *et al.*, 2017). Similarly, molasses decreased the pH value and increased the Flieg score of mixed silage (Wang *et al.*, 2017), fox millet silage (Arbabi & Goorch, 2008), and sorghum-sudangrass hybrid silage (Ramzan *et al.*, 2022).

Based on the panel evaluation, the color of the corn silage (CS) was generally retained across additive treatments. Properly fermented corn silage is typically yellowish-green, whereas a more yellow appearance has been associated with elevated acetic acid concentrations, although color alone cannot reliably indicate fermentation characteristics (Muck *et al.*, 2018). The olive-brown color observed in NS-MOL was likely influenced more by the naturally dark color of molasses than by changes in the fermentation process. Olive-green to olive-brown colors are generally considered normal for grass and corn silages (FAO, 2020). Differences in odor were observed among treatments. CS-CON exhibited a tobacco/caramel odor, whereas CS-MOL, CS-LAB, and NS-MOL were characterized by a sweeter aroma. NS-CON had a rancid butter odor, while NS-LAB exhibited an alcoholic odor. These odor descriptors have previously been associated with different fermentation patterns. For example, tobacco/caramel odors have been reported in heat-damaged silages (Van Saun & Heinrich, 2016), rancid butter odors are commonly associated with butyric fermentation, and alcoholic odors have been linked to ethanol production during fermentation (FAO, 2020; Jones, 2019). The texture of the CS treatments ranged from moderately firm, non-slippery, and wet to very firm, non-slippery, and slightly wet, corresponding to good or very good

silage quality. In contrast, NS-CON exhibited a softer, moderately firm texture, which may be consistent with poorer fermentation quality reported in previous studies (Bagg, 2016). Nevertheless, texture alone cannot confirm the extent of proteolysis or microbial activity. Ultimately, we did not perform measurements on the microbial populations (i.e., molds, yeast, lactic acid bacteria), organic acids (i.e., lactic acid, acetic acid, propionic acid, butyric acid), and alcohol contents (i.e., ethanol and 1,2-propanediol) in our silage samples; thus, the result of the organoleptic properties remains speculative and should be interpreted with caution.

As mentioned above, CS contains more NFC, such as starch and sugars, which are highly digestible in the rumen. Moreover, NS contains more lignin than CS (Feedipedia, 2015), thereby inhibiting rumen microbial enzymatic digestion (Atuhaire *et al.*, 2016). Correspondingly, the GP from the immediately soluble fraction (a) was lower for the NS than for the CS, regardless of ADD. The negative rapidly soluble fraction (a) values do not correspond to negative gas production, as the Ørskov and McDonald model does not include a lag parameter; the regression may generate negative intercepts to achieve the best fit to the gas production curve (Cabral *et al.*, 2019). Furthermore, negative estimates commonly occur when little or no gas is produced during the initial stages of incubation and when microbial colonization of the substrate requires a lag period (Guadayo *et al.*, 2019; Poblete *et al.*, 2020). Despite the faster GP rate for NS, the potential GP ($a+b$) of CS was still higher. The availability of nitrogen and fermentable carbohydrates strongly affects the rate of *in vitro* gas production (Kleden *et al.*, 2021). As mentioned, corn grains contain more starch and sugar, which are converted by plant enzymes and microbes into more soluble compounds such as organic acids and soluble proteins (Kung *et al.*, 2018). When rumen microbes ferment readily available carbohydrates into volatile fatty acids, additional gases are produced as by-products (Moran, 2005).

Corn silage contains about 50% and 84% more rapidly soluble DM and OM fractions than NS, regardless of ADD. Moreover, CS contains about 29% and 26% less slowly degradable DM and OM, respectively, than NS. The CS-MOL also had a higher effective OM degradability than all ADD and NS-MOL. As mentioned, CS contains more starch and sugars that are easily degradable in the rumen. Sugars and starch are degraded by amylolytic bacteria, fungi, and protozoans in the rumen, providing sufficient energy for high-yielding ruminants (Huntington, 1997). For CS-MOL, the readily available sugars in molasses increased solubility and the degradation rate in silage. It also promotes increased bacterial attachment to forage material, thereby increasing rumen degradability (Ni *et al.*, 2017).

The low availability of water-soluble carbohydrates compromises the efficacy of silage inoculants. This phenomenon could explain why LAB did not improve the physicochemical characteristics, IVGP, and *in situ* degradability of the CS and NS. Davies *et al.* (1998) explained that when an inoculant was used in low WSC conditions, pH decline tended to be slower. Thus, the slower decline in pH affects the overall efficiency of fermentation. Despite the improved fermentation quality of NS-MOL, as reflected by its pH, Flieg score, and favorable physical characteristics, no corresponding increase in IVGP, DM degradability, or OM degradability was observed. This suggests that the improved silage fermentation achieved by adding molasses was insufficient to overcome the inherent limitations imposed by Napier grass's high structural carbohydrate content. This is also speculative, as we did not measure the ADF (i.e., cellulose and lignin) content in this study. Furthermore, the lower

standing DM of the 45-day-old Napier grass used in this study would imply that a greater quantity is required to provide an equivalent amount of DM and nutrients to ruminants.

CONCLUSION

The addition of a LAB inoculant did not improve the silage quality and potential feeding value of CS and NS. CS exhibited superior fermentation characteristics, IVGP, and ruminal degradability compared to NS. Adding MOL to NS improved its pH and Flieg score. However, these improvements did not translate into enhanced ruminal IVGP kinetics, DM, and OM degradability. CS has better overall silage quality and greater potential feeding value than NS, regardless of whether MOL or LAB are added. However, the results of this study should be interpreted with caution, as measurements of microbial populations, organic acids, and the alcohol profile were not performed.

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