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ENHANCEMENT OF NAPIER GRASS SILAGE QUALITY AND AEROBIC STABILITY USING *Lactobacillus* spp.

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Abstract

This study investigated the effects of Lactobacillus spp. on Napier grass silage quality, dry matter loss (DM loss), and aerobic stability. The experiment employed a 3 × 3 factorial design in a completely randomized design (CRD), with three levels (0, 1×10^5, and 1×10^6 cfu/g fresh weight) of Lactobacillus buchneri and Lactobacillus plantarum. The silage was ensiled for 24 days. The following parameters were analyzed: DM loss, fermentation characteristics, nutritive values, fiber fractions and anaerobic microbial profiles. Subsequently, the silage was exposed to air for 2, 4, and 6 days to determine aerobic stability characteristics and microbial profiles. The results showed that the highest inoculation LB level improved silage aerobic stability but increased DM loss and negatively affected fermentation quality. However, co-inoculation with the highest levels of both lactic acid bacteria resolved the DM loss problem and improved overall fermentation quality while maintaining enhanced aerobic stability compared with sole inoculation of LB. In conclusion, co-inoculation with the highest levels of lactic acid bacteria improved dry matter recovery, fermentation quality, and aerobic stability of Napier grass silage.

Key words*: aerobic stability, Lactobacillus buchneri, Lactobacillus plantarum, Napier grass, molasses.*

INTRODUCTION

Optimal silage fermentation is fundamentally dependent on the characteristics of the forage, particularly its moisture levels, buffering capacity, and the availability of water-soluble carbohydrates and buffering capacity (Wilkinson, 2005). While adding molasses boosts energy, the remaining sugars can trigger aerobic deterioration by fostering the growth of yeasts and mold once the silage is exposed to oxygen (Guan et al., 2002; Moon, 1983). Consequently, silage quality deteriorates due to aflatoxin contamination from mold proliferation once the silage is exposed to air (Ferrero et al., 2019).

Lactobacillus buchneri produces acetic acid, which inhibits the proliferation of aerobic microorganisms (Jatkauskas et al., 2025). However, the use of LB has been associated with DM losses in the form of CO_2 (Jin, et al., 2024; Oude Elferink et al., 2001), resulting in economic losses (Goeser et al., 2015) and affecting silage fermentation quality. Previous studies have demonstrated that inoculating LB in combination with LP can address DM loss and improve corn silage quality (Huisden et al., 2009). However, the application of LB combined with LP in molasses-treated Napier grass has not been confirmed, and the appropriate concentration (cfu/g fresh weight) of both bacterial species for this purpose is unclear.

This study hypothesized that combinations of both bacterial species at various levels can increase acetic acid content, decrease DM loss, improve fermentation quality, and enhance the aerobic stability of silage. The objectives were to evaluate the inoculation of LB combined with LP at various concentrations (cfu/g fresh forage weight) in molasses-treated Napier grass silage, specifically examining its effects on DM loss, fermentation characteristics, and aerobic stability.

MATERIALS AND METHODS

Experimental Design

A 3×3 factorial arrangement within a completely randomized design was implemented to evaluate the treatments in this research. Two factors were studied: *Lactobacillus plantarum* (LP) and *Lactobacillus buchneri* (LB). Each factor

consisted of three inoculation rates: 0, 1×10^5, and 1×10^6 cfu/g of fresh grass weight.

Ensiling process

Napier grass, harvested from the Suranaree University of Technology farm in Thailand, has a dry weight of approximately 25% (DM). Forage preparation involved chopping the Napier grass into 1-2 cm segments, followed by the incorporation of molasses at a rate of 5% on a fresh-weight basis. Napier grass was treated with lactic acid bacteria according to the factorial design described above. The total volume of inoculants was adjusted to 2% of fresh forage weight by adding deionized water and was mixed uniformly into the chopped, molasses-treated Napier grass. Each treatment was replicated four times, with 500 g of fresh forage weight per replicate. The silage was sealed in polyethylene bags, stored at 25°C, and ensiled for 24 days.

Chemical analysis

Dry matter content was assessed using a freeze-drying machine. The percentage of dry matter loss was calculated by subtracting the %DM content after ensiling from the %DM content prior to ensiling. The dried samples were ground using a laboratory blender to achieve an approximate particle size of 1 mm. Water-soluble carbohydrate analysis was conducted through sugar extraction, following the method outlined by Chow and Simon (2004). The sugar content of the extracted solution was evaluated using the procedure described by Dubois et al. (1956). Nutrient compositions, including crude protein, fat, and ash, were analyzed using standard methods according to the AOAC (1995). Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined using detergent fiber analysis according to Van Soest et al., (1991). A 10× dilution was prepared to assess the forage buffering capacity following the method of Playne and McDonald (1966), as well as to determine NH_3-N, pH, lactic acid, and volatile fatty acids (VFAs). A well-homogenized sample of 50 g fresh weight was diluted tenfold by adding 450 mL of deionized water and thoroughly blended using a laboratory blender. The concentrations of lactic acid and volatile fatty acids (VFAs) were analyzed using a gas chromatography instrument (Agilent 7890B GC) with an Agilent CP-Sil 5 CB column (0.32 mm × 25 m fused silica), following the procedure described in the Agilent application note for lactic acid and C2–C7 VFA analyses. NH_3-N concentration was determined using a method adapted from Weatherburn (1967).

Aerobic stability

The silage sample was collected on day 24 of ensiling to determine aerobic stability characteristics, as described by Ashbell et al. (1987; 1991). The silage was exposed to air for 2, 4, and 6 days. A 10× dilution of each sample (as described above) was prepared to measure pH and aerobic bacteria (including yeast, lactate-assimilating yeast, and mold). WSC loss was calculated by subtracting the WSC content at day 24 of ensiling from the WSC content measured after 2, 4, and 6 days of air exposure.

Microbial enumerations

A series of 10-fold serial dilutions ranging from 10^{-1} to 10^{-10} was prepared. From each dilution, 0.1 ml is onto the appropriate culture medium using a pipette. The pour-plate (pour-on) technique was applied to ensure even distribution and thorough mixing of the inoculum with the medium. Enumeration of *Lactobacillus* spp.: Lactobacillus MRS agar was used as the selective culture medium. The medium was prepared by dissolving 67.17 g of MRS agar powder in 1000 ml of distilled water and heating until completely dissolved. The medium was sterilized by autoclaving at 121°C. After cooling to approximately 60°C, cycloheximide was added at a concentration of 0.4 g per 1000 ml of agar and mix thoroughly. Enumeration of *Clostridium* spp.: Reinforced Clostridium Medium (RCM) was prepared by dissolving 30.50 g of RCM powder in 1000 ml of distilled water and heating until a rolling boil was achieved. The medium was sterilized by autoclaving at 121°C. Once cooled to 60°C, the medium was supplemented with D-cycloserine (200 mg dissolved in 20 ml phosphate buffer per 1000 ml of agar) and methyl red (50 mg per 1000 ml of agar) and mixed thoroughly. Enumeration of *Enterobacterium* spp.: Violet Red Bile Glucose Agar (VRBGA) was used as the culture medium. The agar was prepared by

suspending 38.53 g of powder in 1000 ml of distilled water and heating until fully dissolved. This medium should not be sterilized by autoclaving.

Statistical Analysis

Statistical analyses were performed using SPSS version 23. To meet ANOVA assumptions, the normality of data was assessed using the Shapiro-Wilk test ($p > 0.05$). For variables that exhibited non-normal distribution, data transformations (e.g., logarithmic or square root transformations) were applied to achieve skewness and kurtosis values near zero. Specifically, the Box-Cox transformation (1964) was employed for fermentation parameters when standardized residuals failed to meet the assumptions of normality and homogeneity of variance.

Data for all parameters (excluding microbial profiles) were subjected to a two-way Analysis of Variance (ANOVA) using SPSS version 23 (IBM Corp, 2016). The statistical model was designed to evaluate the main effects of LP inoculation, LB inoculation, and their interaction (LP × LB). Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test, with statistical significance declared at $p < 0.05$.

Regarding microbial profiles, due to the lack of experimental replication, these data were not subjected to statistical analysis. Instead, the results are presented as **descriptive data** to provide a general overview of the microbial community, though we recognize this limits the ability to draw definitive statistical conclusions.

RESULTS AND DISCUSSIONS

Silage Fermentation

This study examined the impact of inoculating two species of lactic acid bacteria at varying levels on the fermentation quality of Napier grass silage with molasses addition. The findings indicated that the combination of both bacterial species, especially when LB was inoculated at the highest level, adversely affected silage fermentation quality compared with the combination using the highest levels of both bacterial species. Notably, there was a significant accumulation of acetic acid due to the high-level of LB inoculation (Table 2). As previously reported, LB can anaerobically convert lactic acid to acetic acid (Oude Elferink et al., 2001). Therefore, a high-level inoculation of LB led to an increased conversion of lactic acid to acetic acid under anaerobic conditions. These results align those of Kung et al. (2018), who noted that LB inoculation resulted in higher acetic acid levels and lower lactic acid levels.

LP is classified as a homofermentative lactic acid bacterium (Salvetti et al., 2012) and is recognized for its rapid and substantial production of lactic acid (Kung Jr., 1998; Muck & Kung, 1997; Oliveira et al., 2017). Higher inoculation levels of LP enable a more rapid pH decline in silage, which inhibits the metabolism of epiphytic lactic acid bacteria, including LP, and suppresses other anaerobic microorganisms through acidic conditions more quickly than without LP inoculation. Therefore, the addition of LP was expected to solve the problem of reduced lactic acid levels caused by the anaerobic degradation of lactate to acetic acid by LB. However, the study found that LP did not solve the problem as expected in this case. The study showed that increasing the level of LP along with different levels of LB did not increase the lactic acid levels. Higher levels of LP inoculation resulted in lower lactic acid content than treatments without LP inoculation (Oliveira et al., 2017).

Table 1. Nutritional composition and microbiological characteristics of Napier grass with/without 5% molasses added by fresh weight

Items	Napier grass added molasses	Napier grass
DM (% FW)	22.2	26.2
CP (% DM)	8.3	8.4
Fat (% DM)	3.5	2.8
Ash (% DM)	10.8	9.5
NDF (% DM)	55.2	70.0
ADF (% DM)	30.1	39.3
ADL (% DM)	8.5	10.3
WSC (g WSC/kg DM)	164.7	62.9
BC (meq NaOH/kg DM)	86.5	90.0
LAB (log_{10} cfu/kg FW)	9.4	9.9
ENT (log_{10} cfu/kg FW)	9.4	9.8
CLOS (log_{10} cfu/kg FW)	7.5	8.0

Note: DM = Dry Matter, FW = Fresh weight, BC = Buffering capacity, LAB = Lactic acid bacteria, ENT = *Enterobacteria* spp., CLOS = *Clostridium* spp.

Inoculation with the highest level of LB, combined with varying amounts of LP, did not lead to a significant reduction in lactic acid

concentration as LP levels increased. This resulted in a higher pH, which was influenced by the highest level of LB (LB6) in this study. Consequently, there was a notable increase in acetic acid content whereas lactic acid levels remained low (Table 2).

Thus, the highest LB inoculation level triggered secondary anaerobic fermentation. Elevated pH facilitates the fermentation processes of both LB and epiphytic lactic acid bacteria also promoting the metabolism of undesirable anaerobic microorganisms (Oliveira et al., 2017; Wilkinson, 2005). The residual WSC results of this study were consistent with the findings of Huisden et al. (2009), who studied inoculants containing LB for corn silage. They found that forage treated with the LB inoculant had lower residual WSC than the control group, which resulted from more complete utilization of forage WSC for lactic acid fermentation by lactic acid bacteria in the inoculant-treated forage.

The residual WSC results of this study were significantly influenced by the main effects of LB and LP, as indicated in Table 2. The lowest residual WSC was observed in silage inoculated with the highest level of LB, where WSC was largely used for fermentation and lactic acid synthesis. During secondary anaerobic fermentation triggered by high LB levels, anaerobic microorganisms consume WSC.

The NH_3-N results were similarly affected by LB and LP. As the levels of lactic acid bacteria increased, NH_3-N levels decreased. The addition of molasses resulted in a high abundance of WSC, and inoculation at greater levels of both species facilitated more extensive WSC fermentation than at lower levels. This led to rapid lactic acid production and a swift drop in silage pH, which inhibits plant proteolytic enzymes during the initial ensiling phase and suppresses proteolytic bacteria during both the initial ensiling and secondary anaerobic fermentation phases (Oliveira et al., 2017; Rooke & Hatfield, 2003). As a result, the reduction of NH_3-N in silage increased with higher levels of LP, since the pH decreased more quickly than with lower inoculation levels of either species.

The highest silage pH was observed in silage inoculated with the greatest amount of LB, which correlated with the significant presence of acetic acid. This finding aligns with the meta-analysis by Kleinschmit and Kung (2006). Acetic acid, produced through LB fermentation, is a weaker acid than lactic acid, which is predominantly generated by LP fermentation (Kleinschmit & Kung, 2006; Muck et al., 2018). Inoculation with the highest level of LB did not effectively suppress secondary anaerobic fermentation by undesirable microorganisms, such as *Clostridium* spp. and *Enterobacteria*, compared to the untreated control (LP0LB0). This was evidenced by the butyric acid results, which revealed notable levels of butyric acid on day 24 of ensiling in silage inoculated with high levels of both lactic acid bacterial species. This situation arose because LP has a slow growth rate at pH levels above 5 during the early stages of silage (Kung, 1998). Generally, the two main groups found in silage are *Saccharolytic* and *Proteolytic clostridia*.

Clostridia grow optimally at a pH of 7.0-7.4, and wet forage conditions before ensiling are conducive to Clostridia growth (McDonald et al., 2010). Therefore, the initial ensiling conditions and high moisture content of the forage promoted Clostridia proliferation. An increase in silage pH promotes secondary anaerobic fermentation, which activates undesirable anaerobic microorganisms. *Saccharolytic clostridia* can degrade lactic acid into acetic and butyric acid (McDonald et al., 2010).

Theoretically, inoculation with the highest level of LP should produce more lactic acid than inoculation at lower levels. Consequently, *Saccharolytic clostridia* would have more substrate (lactic acid) available for conversion into butyric acid than treatments with lower LP levels.

Microbial enumeration of Napier grass silage supplemented with molasses on day 24 of ensiling (Table 1) was used to support the fermentation results observed at this stage. The silage was inoculated with various treatment combinations involving two lactic acid bacterial species at different levels. As previously described, LP grows slowly at higher pH; therefore, clostridia were not immediately suppressed by acidic conditions during the early stage of ensiling. In addition,

inoculation with the highest LB levels resulted in higher silage pH values than other treatment combinations. Moreover, the highest level of LP produced greater amounts of lactic acid, which served as a substrate for *Saccharolytic clostridia*. Consequently, higher *Clostridium* spp. counts were observed in treatments combining LP with the highest LB levels.

Although the silage pH values in these treatment combinations were insufficient to inhibit undesirable anaerobic bacterial fermentation compared with the untreated silage or other inoculation treatments, the butyric acid concentrations at day 24 of ensiling remained within the recommended range established by the Agriculture and Food Development Authority (Teagasc) (Kung et al., 2018).

The standard recommendation for optimum silage butyric acid concentration specifies that levels should not exceed the range of approximately 10-40 g of butyric acid/kg DM silage. Despite the silage pH values of both treatment combinations (LP5LB6, LP6LB6) falling within the recommended standard range (not exceeding pH 4.5 and remaining below pH 3.7, which is necessary to inhibit fermentation by undesirable anaerobic microorganisms), the treatment combinations of both lactic acid bacterial species did not significantly affect the overall silage fermentation quality. This can be attributed to the fact that all silage analytical parameters remained within recommended standards. However, the combined treatment with the highest level of LB resulted in a positive effect on silage fermentation quality compared to the untreated control group for both lactic acid bacteria species.

Nutritional value of Napier grass silage after 24 days

The nutritional value of silage was influenced by inoculation with both lactic acid bacterial species, primarily as a consequence of silage DM loss. This loss of DM directly lead to a reduction in the nutritive value. As previously noted, the lowest DM loss at the highest LB inclusion level was achieved when it was combined with LP (LB6LP5 and LB6LP6). This improvement was attributed to the acidic conditions established by LP inoculation, which inhibited LB metabolic activity. Consequently, the combination of the highest LB level with varying LP levels resulted in a higher silage DM content after 24 days of ensiling than treatments without LP. These effects were observed when both lactic acid bacterial species were inoculated into Napier grass supplemented with molasses and ensiled for 24 days, the treatment combinations did not significantly affect the fiber fractions NDF and ADF but did significantly increase ADL content. This is expected because lactic acid bacteria cannot ferment forage fibers due to their lack of fibrinolytic enzymes (Dewar et al., 1963; Pahlow et al., 2003). Therefore, lactic acid bacteria inoculation did not reduce the NDF or ADF content of the silage. However, inoculation with both bacterial species affected silage DM loss, as the lost dry matter consisted of substrates that could be fermented by anaerobic silage microorganisms.

As a result, NDF and ADF tended to increase but without statistical significance, whereas ADL showed statistically significant differences due to the decrease in fermentable fractions. Additionally, the crude protein content of silage was affected by the inoculation of the two bacterial species, demonstrating a significant LB x LP interaction. The crude protein content of the silage decreased as the LP level increased when mixed with uninoculated LB. This was likely due to the slower pH decline during initial fermentation compared to other treatment groups (as explained above), which may have allowed proteolytic clostridia to ferment forage crude protein into other products during early fermentation (Oliveira et al., 2017). The crude protein content of the silage also decreased with increasing DM loss. These results were confirmed by DM loss measurements, where the highest losses occurred with sole LP inoculation, resulting in a lower crude protein content. Furthermore, at the highest level of LB inoculation, the silage crude protein content increased with increasing LP levels, consistent with improved dry matter recovery.

Aerobic stability characteristics of Napier grass silage after exposure to air for 2, 4, and 6 days

Silage from each treatment combination was exposed to air for 2, 4, and 6 days after opening

the sealed plastic bags at 24 days of ensiling to evaluate aerobic deterioration characteristics, including silage pH, DM loss, residual water-soluble carbohydrates (WSC), and microbial populations. Overall, the treatment combinations improved the aerobic stability of the silage following air exposure.

Sole inoculation with LP resulted in the highest silage pH after 2 days of air exposure compared with the other treatment combinations. This result agrees with previous studies indicating that LP inoculation leads to lower acetic acid production and higher residual WSC concentrations, thereby increasing susceptibility to aerobic deterioration relative to treatments combined with LB (Tabacco et al., 2011; Weinberg et al., 2002).

After 4 days of air exposure, silage inoculated with 1×10^5 cfu LB/g fresh forage weight (LB5) in combination with various levels of LP exhibited only minor increases in pH; however, the treatment combined with 1×10^5 cfu LP/g fresh forage weight (LP5) showed a higher silage pH than the other combinations. In addition, treatments inoculated with the highest level of LB combined with different levels of LP rarely exhibited increases in silage pH after 4 days of air exposure.

Furthermore, inoculation with the highest level of LB combined with various levels of LP (LP0LB6, LP5LB6, and LP6LB6) markedly improved the aerobic stability of the silage. In contrast, combinations containing lower LB levels failed to enhance aerobic stability after 6 days of air exposure. Consequently, the highest LB level combined with different LP levels resulted in the lowest silage DM losses and the lowest silage pH values on day 6 of air exposure compared with treatments containing lower LB levels.

This was due to the highest concentration of acetic acid at the bag opening in the highest LB treatment combinations compared to the other treatments. As is well established, acetic acid inhibiting the proliferation of yeast and mold during air exposure (Muck et al., 2018).

Therefore, inoculation at the highest LB level in molasses-treated Napier grass improved silage aerobic stability after 6 days of air exposure. After 6 days of air exposure, residual WSC in the highest LB inoculation level remained more stable than that in lower LB levels due to the inhibition of yeast and mold growth by acetic acid (Muck et al., 2018). This was confirmed by aerobic microbial profiles 6 days after air exposure (Table 3), which demonstrated lower counts of yeast, lactate-assimilating yeast, and mold at the highest LB inoculation level.

CONCLUSIONS

The experimental results indicate that sole inoculation with *Lactobacillus buchneri* (LB) at its highest level negatively impacts silage quality. This decline is primarily due to the conversion of lactic acid into acetic acid, which raises the silage pH beyond ideal levels. While inoculating with both LB and *Lactobacillus plantarum* (LP) at varying levels initially resulted in lower fermentation quality compared to non-inoculated silage, the overall fermentation products remained within recommended standard ranges, suggesting that combined inoculation does not significantly impair the final product.

The study highlights a critical trade-off regarding dry matter (DM) loss and stability. While the highest level of LB inoculation alone caused the most significant DM loss, the combination of high-level LB with LP effectively mitigated this depletion. Furthermore, this dual-species treatment at maximum levels enhanced aerobic stability, maintaining silage integrity for up to 6 days after air exposure, a performance comparable to the high-level LB treatment alone.

In conclusion, for Napier grass silage supplemented with 5% molasses, the simultaneous inoculation of both bacterial species at their highest levels is the most effective strategy. This approach optimizes the balance between fermentation quality and dry matter preservation while ensuring robust aerobic stability during the feed-out phase.

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Table 2. Effects of treatment combination on the nutritional value of Napier grass silage on day 24

Items	LB0			LB5			LB6			*P - value*		
	LP0	LP5	LP6	LP0	LP5	LP6	LP0	LP5	LP6	LB	LP	LBxLP
DM (%)	23.42^{ab}	21.61^{b}	22.40^{ab}	24.07^{a}	22.78^{ab}	22.97^{ab}	22.26^{b}	23.71^{ab}	23.41^{ab}	NS	NS	< 0.01
CP (%)	9.01^{ab}	8.54^{cd}	8.20^{d}	9.05^{ab}	8.66^{de}	9.13^{a}	8.72^{abc}	9.14^{bc}	9.12^{a}	< 0.01	NS	< 0.01
EE (%)	3.49	2.11	2.02	3.46	3.91	1.58	3.51	2.56	1.50	NS	NS	NS
Ash (%)	12.24	12.09	11.49	12.02	11.3	11.28	11.82	12.05	12.10	NS	NS	NS
NDF (%)	60.04	60.45	59.65	59.88	60.56	60.94	59.40	60.8	61.31	NS	NS	NS
ADF (%)	34.62	34.41	35.79	34.36	34.73	35.26	33.46	35.75	35.54	NS	NS	NS
ADL (%)	9.40^{a}	9.52^{a}	9.40^{a}	7.24^{ab}	9.10^{a}	9.25^{a}	6.09^{b}	9.41^{a}	8.13^{ab}	< 0.01	< 0.01	< 0.05
DM loss (%)	10.77^{ab}	17.66^{a}	13.08^{a}	8.81^{b}	13.21^{a}	12.48^{a}	15.18^{a}	10.72^{ab}	10.81^{ab}	< 0.05	< 0.05	< 0.01
pH	3.80	3.78	3.78	3.83	3.83	3.80	3.88	3.96	3.87	< 0.01	NS	NS
NH_3-N (% of total N)	13.03	9.62	8.12	12.08	8.39	8.88	9.01	6.60	6.55	< 0.05	< 0.01	NS
Residue WSC (g /kg DM)	23.74	25.78	28.23	24.58	24.83	29.49	21.91	19.81	21.63	< 0.01	< 0.01	NS
Lactic acid (g/kg DM)	70.87^{b}	62.78^{b}	55.94^{b}	73.74^{a}	60.17^{b}	56.36^{b}	67.07^{b}	53.40^{b}	63.83^{b}	NS	< 0.01	< 0.05
Acetic acid (g/kg DM)	21.06	24.66	22.58	24.29	21.40	21.51	32.94	46.43	41.05	< 0.01	NS	NS
Propionic acid (g/kg DM)	5.57	6.82	8.49	4.95	6.99	12.33	5.86	9.91	13.85	NS	< 0.01	NS
Butyric acid (g/kg DM)	10.36	10.36	10.65	10.13	11.08	10.60	10.11	13.39	13.23	< 0.01	< 0.01	NS

Note: LB0, LB5 & LB6 – *LB* (0, 1×10^{5}, and 1×10^{6} cfu/g fresh forage weight), LP0, LP5 & LP6 = *LP* (0, 1×10^{5}, and 1×10^{6} cfu/g fresh forage weight), NS = non-significant

^{a,b} within the same row, different letters represent significant differences.

Table 3. Effects of treatment combination on residual WSC and pH in air-exposed Napier grass silage

Items	LB0			LB5			LB6			*P - value*		
	LP0	LP5	LP6	LP0	LP5	LP6	LP0	LP5	LP6	LB	LP	LBxLP
pH at 2 days	3.94^{c}	8.25^{a}	8.04^{b}	3.86^{cd}	3.82^{d}	3.82^{d}	3.87^{cd}	3.92^{c}	3.89^{cd}	< 0.01	< 0.01	< 0.01
residual WSC (g/kg DM)	25.86^{a}	19.05^{bc}	25.97^{a}	18.06^{bc}	17.11^{c}	22.84^{ab}	20.95^{abc}	20.47^{bc}	17.80^{bc}	< 0.05	NS	< 0.05
pH at 4 days	7.20^{a}	8.80^{a}	8.67^{a}	4.53^{c}	7.58^{a}	4.85^{b}	3.89^{e}	3.97^{d}	3.93^{ed}	< 0.01	< 0.01	< 0.01
residual WSC (g/kg DM)	26.58^{a}	17.71^{b}	11.35^{b}	21.31^{ab}	20.35^{ab}	20.13^{ab}	16.70^{b}	17.87^{b}	18.21^{b}	< 0.05	NS	< 0.05
pH at 6 days	8.28^{a}	8.87^{a}	8.84^{a}	8.00^{a}	9.10^{a}	8.00^{a}	4.20^{b}	3.70^{b}	4.24^{b}	NS	NS	< 0.05
residual WSC (g/kg DM)	26.97^{a}	19.60^{ab}	18.30^{ab}	16.90^{ab}	16.41^{ab}	19.10^{ab}	13.41^{b}	19.40^{ab}	19.76^{a}	< 0.01	NS	< 0.05

Note: LB0, LB5 & LB6 – *LB* (0, 1×10^{5}, and 1×10^{6} cfu/g fresh forage weight), LP0, LP5 & LP6 = *LP* (0, 1×10^{5}, and 1×10^{6} cfu/g fresh forage weight), NS = non-significant

$^{a-d}$ within the same row, different letters represent significant differences

REFERENCES

AOAC (1995). *Official Methods of Anlysis* (16 ed.). Arlington, VA: Association of Official Analytical Chemists.

Ashbell, G., Pahlow, G., Dinter, B., & Weinberg, Z. G. (1987). Dynamics of orange peel fermentation during ensilage. *Journal of Applied Microbiology, 63*, 275-279.

Ashbell, G., Weinberg, Z. G., Azrieli, A., Hen, Y., & Horev, B. (1991). A simple system to study the aerobic deterioration of silages. *Canadian Agricultural Engineering, 33*, 171-175.

Box, G. E. P., & Cox, D. R. (1964). An analysis of transformation. *JSTOR, 26*(2).

Chow, P. S., & Simon, M. L. U. (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology, 24*, 1129-1136.

Dewar, W. A., McDonald, P., & Whittenbury, R. (1963). The hydrolysis of grass hemicelluloses during ensiling. *Journal of the Science of Food and Agriculture, 14*, 411-417.

Dubosis, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, & Fred. Smith. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical chemistry, 28*(350-356).

Ferrero, F., Prencipe, S., Spadaro, D., Gullino, M. L., Cavallarin, L., Piano, S., & Borreani, G. (2019). Increase in aflatoxins due to Aspergillus section Flavi multiplication during the aerobic deterioration of corn silage treated with different bacteria inocula. *Journal of Dairy Science, 102*(2), 1176-1193.

Goeser, J. P., Heuer, C. R., & Crump, P. M. (2015). Forage fermentation product measures are related to dry matter loss through meta-analysis. *The Professional Animal Scientist, 31*(2), 137-145.

Guan, W.-T., Driehuis., F., & Wikselaar., P. V. (2002). The influences of addition of sugar with or without L. buchneri on fermentation and aerobic stability of whole crop maize silage ensiled under anaerobic silos. *Asian-Australasian Journal of Animal Sciences, 15*, 1128-1133.

Jatkauskas, J., R.C.D., Amaral, K.L., Witt, J.N., Joergensen, I., Eisner, & V., Vrotniakiene. (2025). Early Fermentation Dynamics and Aerobic Stability of Maize Silage Improved by Dual-Strain Lactic Acid Bacteria Inoculation. *Fermentation, 11*, 293.

Jin, Y. P., Wang, F., Li, M., Yu, J., Du, Y., Zhao, Q., Yi, H., Tang, & B., Yuan, (2024). The Effects of *Lactobacillus plantarum* and *Lactobacillus buchneri* on the Fermentation Quality, In Vitro Digestibility, and Aerobic Stability of *Silphium perfoliatum L.* Silage. *Animals. 14*, 2279.

Huisden, C. M., Adesogan, A. T., Kim, S. C., & Ososanya, T. (2009). Effect of applying molasses or inoculants containing homofermentative or heterofermentative bacteria at two rates on the fermentation and aerobic stability of corn silage. *Journal of Dairy Science, 92*, 690–697.

IBM Corp. (2016). *IBM SPSS Statistics for Macintosh* (Version 24). Armonk, NY: IBM Corp.

Kleinschmit, D. H., & Kung, J. (2006). A Meta-Analysis of the Effects of *Lactobacillus buchneri* on the Fermentation and Aerobic Stability of Corn and Grass and Small-Grain Silages. *Journal of Dairy Science, 89*, 4005-4013.

Kung, J. L., Shaver, R. D., Grant, R. J., & Schmidt, R. J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science, 101*(5), 4020-4033. doi:https://doi.org/10.3168/jds.2017-13909

Kung, L., Jr. (1998). *A review on silage additives and enzymes.* Paper presented at the 59th Minneapolis Nutrition Conference, Minneapolis, MN. Department of Animal Science, University of Minnesota, St. Paul.

McDonald, P., Greenhalgh, J. F. D., Morgan, C. A., Edwards, R., Sinclair, L. A., & Wilkinson, R. G. (2010). *Animal Nutrition*: Trans-Atlantic Publications, Incorporated.

Moon, N. J. (1983). Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *Journal of applied bacteriology, 55*, 454-460.

Muck, R. E., & Kung, J., L. (1997). *Effects of silage additives on ensiling*. Paper presented at the Silage: Field to Feedbunk North American Conference., Northeast Regional Agricul- tural Engineering Service, Ithaca, NY.

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., & Kung Jr, L. (2018). A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. *Journal of Dairy science, 89*, 4005-4013.

Oliveira, A. S., Weinberg, Z. G., Ogunade, I. M., Cervantes, A. A. P., Arriola, K. G., Jiang, Y., & Adesogan, A. T. (2017). Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *Journal of Dairy Science, 100*, 1-17.

Oude Elferink, S. J., Krooneman, J., Gottschal, J. C., Spoelstra, S. F., Faber, F., & Driehuis, F. (2001). Anaerobic conversion of lactic acid to acetic acid and 1, 2-propanediol by *Lactobacillus buchneri*. *Applied and Environmental Microbiology, 67*(1), 125-132.

Pahlow, G., Muck, R. E., Driehuis, F., Elferink, S. J. W. H. O., & Spoelstra, S. F. (2003). Microbiology of Ensiling. In D. R. Buxton, R. E. Muck, & J. H. Harrison (Eds.), *Silage science and technology.* (pp. 31-93). Madison, WI, USA: Agronomy Publication.

Playne, M. J., & McDonald, P. (1966). The buffering constituents of herbage and of silage. *Journal of the Science of Food and Agriculture, 17*.

Rooke, J. A., & Hatfield., R. D. (2003). Biochemistry of ensiling. In K. A. Barbarick, J. J. Volenec, & W. A. Dick (Eds.), *Silage Science and Technology* (pp. 95-139). Madison, WI, USA: Agronomy Publication.

Salvetti, E., Torriani, S., & Felis, G. E. (2012). The Genus Lactobacillus: A Taxonomic Update. *Probiotics and Antimicrobial Proteins*.

Tabacco, E., Righi, F., Quarantelli, A., & Borreani, G. (2011). Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as

influenced by different lactic acid bacteria inocula. *Journal of Dairy Science, 94*, 1409-1419.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*, 3583-3597.

Weatherburn, M. W. (1967). Phenol-Hypochlorite Reaction for Determination of Ammonia. *Analytical Chemistry, 39*(8), 971-974.

Weinberg, Z. G., Ashbell, G., Hen, Y., Azrieli, A., Szakacs, G., & Filya, I. (2002). Ensiling whole-crop wheat and corn in large containers with *Lactobacillus plantarum* and *Lactobacillus buchneri*. *Journal of Industrial Microbiology & Biotechnology, 28*, 7-11.

Wilkinson, J. M. (2005). *Silage*. Painshall, Church Lane, Welton, Lincoln, UK: Chalcombe Publications.