

## Research Paper

# Fermentation quality and aerobic stability of Napier grass ensiled with citric acid residue and lactic acid bacteria

## *Calidad fermentativa y estabilidad aeróbica del pasto Napier ensilado con residuo de ácido cítrico y bacterias ácido-lácticas*

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### Abstract

This study was conducted to investigate the effects of adding citric acid residue (CAR) with or without lactic acid bacteria (LAB) to Napier grass (*Cenchrus purpureus*; syn. *Pennisetum purpureum*) cv. Sumu No. 2 at ensiling on the fermentation quality and aerobic stability of the resulting silage. Treatments included: Control (Napier grass forage without additives); and Napier grass inoculated with lactic acid bacteria (*Lactobacillus plantarum* and *L. buchneri*) at  $1 \times 10^6$  cfu/g fresh weight (FW) forage (LAB) or 36 g citric acid residue/kg FW forage (CAR) or a mixture of CAR and LAB (CL). Forty-five days after ensiling the silages were tested for chemical and microbial composition and an aerobic stability test was conducted. The addition of CAR with or without LAB increased the DM and lactic acid concentrations in silage and decreased pH plus acetic acid, ammonia nitrogen (NH<sub>3</sub>-N), neutral detergent fiber and cellulose concentrations relative to Control. The pH in LAB silage was lower than in Control, while lactic acid concentration was higher. During the first 2 days of aerobic exposure, all additives increased the water-soluble carbohydrate (WSC) and lactic acid concentrations and decreased pH plus NH<sub>3</sub>-N and acetic acid concentrations. Moreover, CL silages had the highest WSC and the lowest NH<sub>3</sub>-N and acetic acid concentrations during aerobic exposure. However, all additives failed to improve the aerobic stability of the silage. While CAR with or without LAB inoculant improved the fermentation quality of silage made from Napier grass, more studies are warranted to identify additives which can improve aerobic stability of the silage after opening.

**Keywords:** Aerobic deterioration, antibacterial effect, *Cenchrus purpureus*, ensilage, silage additives.

### Resumen

En Nanjing, provincia de Jiangsu, China, se investigaron los efectos de la adición de residuo de ácido cítrico con o sin bacterias ácido-lácticas, al ensilar pasto Napier (*Cenchrus purpureus*; sin. *Pennisetum purpureum*) cv. Sumu No. 2, en la calidad fermentativa y estabilidad aeróbica del ensilaje resultante. Los tratamientos incluyeron: Testigo (pasto sin aditivos); y pasto inoculado con bacterias ácido-lácticas (*Lactobacillus plantarum* y *L. buchneri*) a una concentración de  $1 \times 10^6$  ufc/g de peso fresco del forraje (LAB) o 36 g de residuo de ácido cítrico/kg de forraje (CAR) o una mezcla de CAR y LAB (CL). Cuarenta y cinco días después del ensilado, se analizaron la composición química y microbiana de los ensilajes y se realizó una prueba de estabilidad aeróbica. En relación con el Testigo, la adición de CAR con o sin LAB aumentó las concentraciones de materia seca y ácido láctico en el ensilaje y disminuyó el pH, las concentraciones de ácido acético, nitrógeno amoniacal (NH<sub>3</sub>-N), fibra detergente neutro y celulosa. El pH en el ensilaje LAB fue menor que en el Testigo, mientras que la concentración de ácido láctico fue mayor. Durante los primeros dos días de exposición aeróbica, todos los aditivos aumentaron las concentraciones de carbohidratos solubles en agua (WSC) y ácido láctico, y disminuyeron el pH y las concentraciones de NH<sub>3</sub>-N y ácido acético. Además, los ensilajes CL presentaron las concentraciones más altas de WSC y las concentraciones más bajas de NH<sub>3</sub>-N y ácido acético durante la exposición aeróbica. Sin embargo, los aditivos no mejoraron la estabilidad aeróbica del

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ensilaje del pasto Napier. Si bien el CAR con o sin inoculante LAB mejoró la calidad fermentativa del ensilaje, se necesitan más estudios para identificar aditivos que mejoren la estabilidad aeróbica después de la apertura del silo.

**Palabras clave:** Aditivos de ensilaje, *Cenchrus purpureus*, deterioro aeróbico, efecto antibacteriano, ensilado.

## Introduction

Napier grass [*Cenchrus purpureus* (Schumach.) Morrone; syn. *Pennisetum purpureum* Schumach.] is a fast-growing forage widely cultivated in tropical areas due to its high potential dry matter (DM) yield (Bureenok et al. 2012). The forage is an important crop for biofuel and animal feed production and is routinely stored by ensiling for feeding ruminants year-round. However, high quality silage of Napier grass is difficult to produce because the coarse and stemmy structure of the forage leads to poor compaction during silage preparation (Desta et al. 2016). The presence of excess air in forage mass at ensiling encourages the growth of undesirable microorganisms during the initial stages of ensiling, inducing abundant loss of nutrients. Thus, various biological and chemical additives have been developed to improve the fermentation quality of Napier grass (Ferreira et al. 2013; Desta et al. 2016; Khota et al. 2018).

Organic acids, e.g. formic, acetic and propionic, are common additives at ensiling that cause a rapid reduction in pH and suppression of undesirable bacteria, thereby improving silage quality (Muck et al. 2018; Wilkinson and Rinne 2018). However, the use of organic acids increases the cost of silage making. Citric acid residue (CAR) is the main by-product of citric acid production, which contains some citric acid, crude protein and other nutrients. Citric acid is widely used in food preparation owing to its safety and antibacterial properties (Bou et al. 2017). With the rapid growth in demand for citric acid in the food industry, the amount of CAR generated from citric acid production has increased in recent years (Zhang et al. 2014). A large amount of this CAR is merely discarded, which is a wasted resource and might cause environmental pollution. Previous studies reported that citric acid improved the fermentation quality of alfalfa silage, limiting proteolysis and improving polyunsaturated fatty acid composition during ensiling (Ke et al. 2017; 2018). In addition, feeding trials confirmed that citric acid increased feed digestion and utilization in the diet of steers (Wang et al. 2009). While CAR might have similar effects on the fermentation quality of silage, literature on the incorporation of CAR during silage making is scarce, and further investigation is needed.

Lactic acid bacteria (LAB) are also commonly applied during silage making, based on ensuring the presence of enough efficient LAB during ensiling to enhance lactic fermentation (Moselhy et al. 2015). Citric acid can be

utilized by some LAB strains, which might promote the growth of these LAB (McDonald et al. 1991). Therefore, inoculating CAR-treated silage with LAB might have a synergistic effect on fermentation quality. The objective of this study was to investigate the effects of adding CAR with or without LAB inoculant at ensiling on fermentation quality and aerobic stability of Napier grass silage.

## Materials and Methods

### *Silage preparation*

Napier grass cv. Sumu No. 2 was cultivated in an experimental field of Nanjing Agricultural University, located in Nanjing, Jiangsu, China (32°03' N, 118°88' E; 20 masl). The grass was harvested at the heading stage (approximately 2.5 m tall) and chopped into lengths of about 2–3 cm with a forage cutter (93ZT-300, Xingrong Co. Ltd, Guangzhou, China). LAB inoculant and CAR were used as additives in the experiment. The LAB inoculant was a mixture of *Lactobacillus plantarum* MTD-1 (Ecosyl Products Ltd, Stokesley, UK) and *Lactobacillus buchneri* 40788 (Lallemand Animal Nutrition, Milwaukee, WI, USA) at a ratio of 1:1. The chemical composition of CAR (Jiangsu Guoxin Union Energy Co. Ltd, Yixing, China) is shown in Table 1. The chopped Napier grass was treated in various ways to form the different treatments: (1) Napier grass without additives (Control); (2) Napier grass with LAB inoculant (LAB); (3) Napier grass with 36 g CAR/kg fresh weight (CAR); and (4) Napier grass with 36 g CAR/kg fresh weight + LAB inoculant (CL). The LAB inoculant was dissolved in sterile distilled water and sprayed on each replicate of LAB and CL treatments (5 mL/kg fresh weight) to give an equivalent of  $1 \times 10^6$  colony-forming units (cfu)/g fresh weight before thorough mixing with the chopped forage. The CAR was added manually to chopped forage for each replicate of CAR and CL treatments and mixed thoroughly. The same amount of sterile distilled water was applied to the Control and CAR treatments. Approximately 3.2 kg treated forage was packed into 5 L laboratory silos (polyethylene bottles with a diameter of 17.3 cm and a height of 26.5 cm; Lantian Biological Experimental Instrument Co. Ltd, Jiangsu, China). The silos were stored at ambient temperature (17–22 °C) after being sealed with screw tops and plastic tape. Five silos for each treatment were opened after 45 days of ensiling for subsequent analyses.

**Table 1.** Chemical composition of citric acid residue.

Parameter	Value
pH	2.50
Concentrations (% FW)	
Dry matter	44.0
Citric acid	6.00
Crude protein	11.5
Crude fiber	24.3
Water soluble carbohydrate	ND
Ether extract	1.20
Crude ash	0.80

FW - fresh weight; ND - not detected.

### *Aerobic stability*

Two kg of silage was placed loosely in 5 L plastic buckets (5 replicates for each treatment) without sealing to monitor aerobic stability. Each bucket was covered with a layer of cheesecloth to avoid contamination but allow air flow. Thermocouple wires were placed at the center of the silage mass and connected to a data logger (SMOWO MDL-1048A, Tianhe Automation Instrument Co. Ltd, Shanghai, China) that measured the temperature every 30 min for 6 days. When the temperature of the silage increased by 2 °C above ambient temperature (17–22 °C), the silage was considered to be undergoing aerobic deterioration. Subsamples of the air-exposed silages (100 g) were removed from each plastic bucket after 2, 4 and 6 days to quantify the levels and rates of change of chemical and microbial compositions.

### *Chemical and microbial analyses*

At silo opening, a cold-water extract was prepared by blending a 60 g sample of silage with 120 mL distilled water and stored in a refrigerator at 4 °C for 24 h. The extracts were then filtered through 2 layers of cheesecloth and Whatman filter paper (11 µm pore size, Xinhua Co., Hangzhou, China) and the pH of the filtrate was measured immediately with a pH meter (HANNA pH 211, Hanna Instruments Italia Srl, Villafranca Padovana, Italy). The filtrate was stored at -20 °C for subsequent determination of ammonia nitrogen (NH<sub>3</sub>-N) and organic acids. After thawing, the filtrate was centrifuged for 10 min at 4 °C (10,000 × G) and filtered through a microfilter (0.22 µm) for determination of organic acids and ethanol, which was carried out using Agilent 1260 HPLC system (Agilent Technologies Inc., Waldbronn, Germany) equipped with a refractive index detector (column: Carbomix® H-NP5, Sepax Technologies Inc., Newark, DE, USA; eluent: 2.5 mmol/L H<sub>2</sub>SO<sub>4</sub>, 0.5 mL/min; temperature: 55 °C). The NH<sub>3</sub>-N was determined by the phenol-hypochlorite reaction (Broderick and Kang 1980), while buffering capacity was determined according to the method described by Playne and McDonald (1966).

The dry matter (DM) concentrations of fresh material and silages were determined by drying samples in a forced-draft oven to a constant weight at 60 °C for 72 h, and then ground through a 1 mm screen in a laboratory knife mill (FW100, Taisite Instrument Co. Ltd, Tianjin, China). The ground samples were analyzed for: water-soluble carbohydrates (WSC) by colorimetry after reaction with anthrone reagent (Arthur Thomas 1977); total nitrogen (TN) by a Kjeldahl nitrogen analyzer (Kjeltec 8200, FOSS, Hillerød, Denmark); and neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Van Soest et al. (1991), using the ANKOM filter bag technique with an ANKOM 200i fiber analyzer (ANKOM Technologies Inc., Fairport, NY, USA). Crude protein (CP) was calculated as TN% × 6.25. Hemicellulose concentration was calculated as NDF minus ADF, and cellulose concentration as ADF minus ADL.

Approximately 10 g of fresh material or silage was serially diluted 10-fold with sterilized saline solution (0.85% sodium chloride). The LAB were enumerated on deMan, Rogosa and Sharp agar medium after incubation in an anaerobic incubator at 37 °C for 2 days. Yeasts and molds were enumerated on potato dextrose agar with 0.25% chloramphenicol (Sincere Biotech Co. Ltd, Shanghai, China) after incubation at 30 °C for 3 days.

### *Statistical analyses*

The microbial data were converted to log<sub>10</sub>. Since the experiment had a completely randomized design, all data were analyzed using the General Linear Model (GLM) procedure of SPSS 22 software. Effects of additives on fermentation characteristics and microbial composition during ensiling were subjected to one-way ANOVA with the model:  $Y_{ij} = \mu + T_i + E_{ij}$ , where:  $\mu$  is general mean;  $T_i$  is the fixed effect of treatment; and  $E_{ij}$  is experimental error. The aerobic stability parameters, including pH, plus WSC, NH<sub>3</sub>-N, lactic and acetic acid concentrations and the counts of yeasts and molds, were analyzed via repeated measures ANOVA in a GLM with additives, days of air exposure and their interaction in the model. Means of different treatments were compared for significance by Duncan's multiple range test and significance was declared at  $P < 0.05$ .

### **Results**

As shown in Table 2, the DM concentration of fresh Napier grass was 249 g/kg FW, while chemical composition was (g/kg DM): CP 64.2; WSC 58.5; NDF 674; and ADF 416. Buffering capacity was 49.7 meq/kg DM and numbers of LAB and yeasts plus molds were 5.08 and 4.38 log cfu/g FW, respectively.

**Table 2.** Chemical and microbial composition of fresh Napier grass at ensiling.

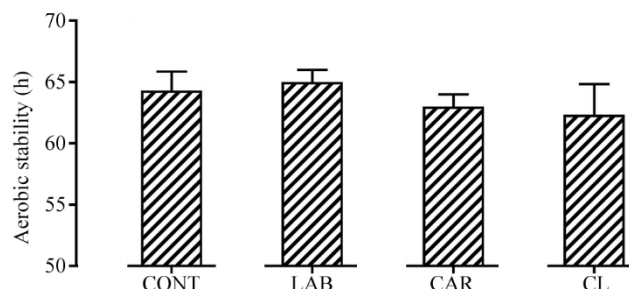
Parameter	Concentration
Dry matter (g/kg FW)	249
Crude protein (g/kg DM)	64.2
Water soluble carbohydrate (g/kg DM)	58.5
Neutral detergent fiber (g/kg DM)	674
Acid detergent fiber (g/kg DM)	416
Buffering capacity (meq/kg DM)	49.7
Lactic acid bacteria (log cfu/g FW)	5.08
Yeasts and molds (log cfu/g FW)	4.38

FW - fresh weight; DM - dry matter; cfu - colony-forming units. Data presented represent means of 5 replicates.

At 45 days after ensiling, all silages containing additives had lower pH and counts of yeasts and molds but higher lactic acid concentrations than the Control (Table 3). Addition of CAR significantly increased ( $P < 0.05$ ) DM concentration and decreased ( $P < 0.05$ ) acetic acid, propionic acid,  $\text{NH}_3\text{-N}$ , NDF and cellulose concentrations compared with Control. Concentrations of WSC in silage containing only LAB were lower than those of the Control silage but the difference just failed to reach significance ( $P = 0.054$ ). Adding LAB at ensiling increased the numbers of LAB in silage ( $P = 0.049$ ) compared with the Control, while CAR silage was intermediate. Adding LAB plus CAR at ensiling significantly decreased ( $P < 0.05$ ) hemicellulose concentration compared with the Control.

The effects of treatments on aerobic stability of Napier grass silages are shown in Figures 1 and 2. All silages remained stable for more than 60 h after being exposed to

the atmosphere. However, none of the additives was able to improve the aerobic stability. The additive treatments, days of aerobic exposure and their interactions had significant ( $P < 0.05$ ) effects on pH, concentrations of WSC,  $\text{NH}_3\text{-N}$ , lactic acid and acetic acid, plus counts of yeasts and molds during exposure to air. All silages showed an increase in pH and decreases in lactic acid and acetic acid concentrations with the progression of exposure to air. This effect was most pronounced between Days 2 and 4 of aerobic exposure. Associated with these changes was a marked increase in  $\text{NH}_3\text{-N}$  concentrations, except in the silage containing both LAB and CAR. The WSC concentrations remained basically stable in silages containing LAB but declined in both CAR and Control, with the greatest decline in Control ( $P < 0.05$ ). Counts of yeasts and molds increased rapidly in all silages during the first 4 days of exposure but then plateaued.



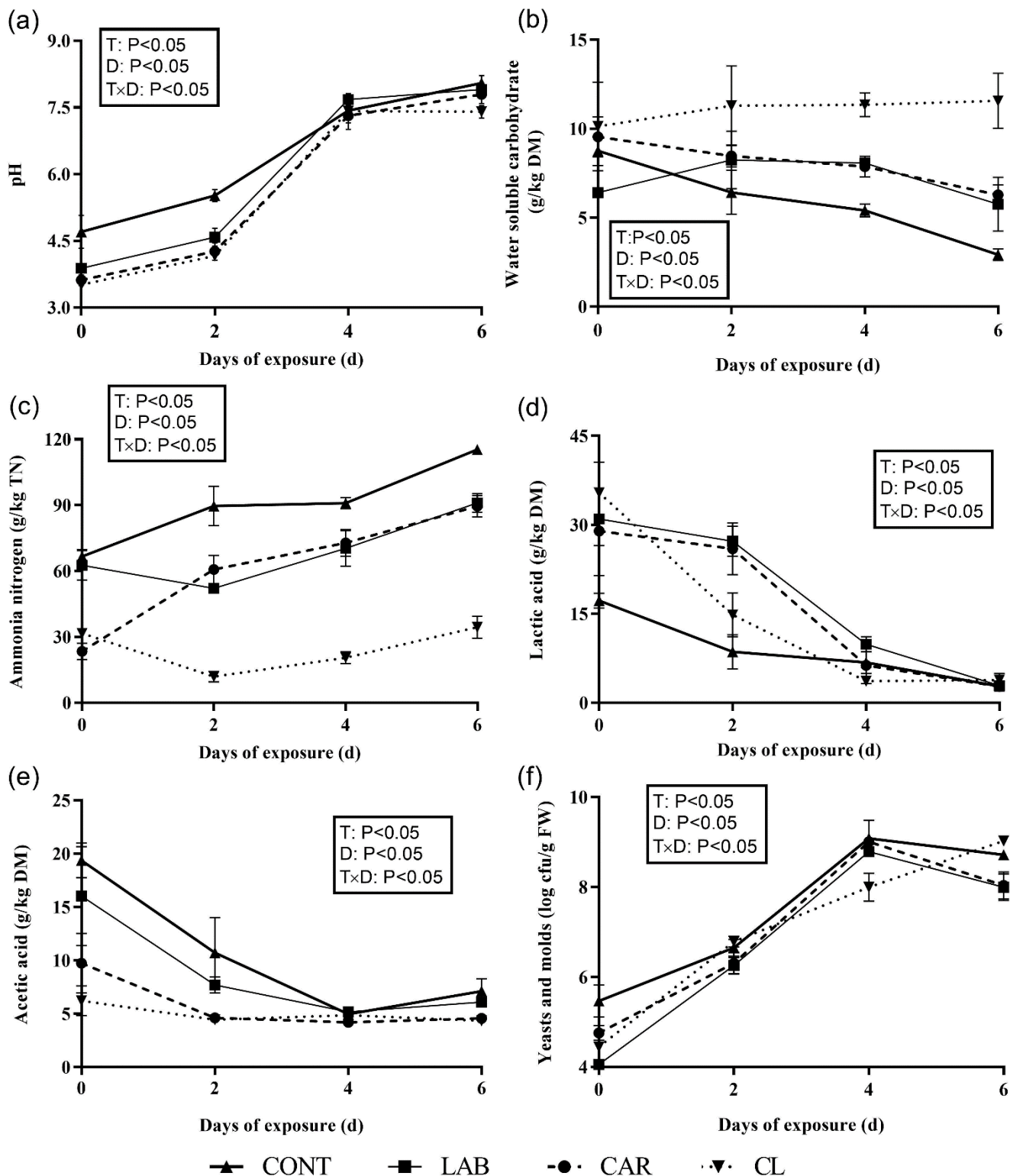
**Figure 1.** Effect of adding lactic acid bacteria and citric acid residue at ensiling on aerobic stability of Napier grass silages. CONT - Control; LAB -  $1 \times 10^6$  log cfu/g lactic acid bacteria (*Lactobacillus plantarum* + *L. buchneri*) added at ensiling; CAR - 36 g/kg citric acid residue added at ensiling; CL - CAR + LAB. There was no significant difference between treatments.

**Table 3.** Final composition of Napier grass silage 45 days after ensiling.

Parameter	CONT	LAB	CAR	CL	s.e.m.	P value
DM (g/kg FW)	232b	241ab	253a	260a	3.99	0.033
pH	4.70a	3.88b	3.78b	3.51b	0.149	<0.001
Lactic acid (g/kg DM)	17.2b	30.9a	28.9a	35.4a	2.36	0.011
Acetic acid (g/kg DM)	19.3a	16.0a	9.75b	6.22b	1.71	0.025
Propionic acid (g/kg DM)	0.42a	0.61a	0.07b	0.17b	0.069	0.001
Butyric acid (g/kg DM)	0.41	0.26	0.35	0.15	0.046	0.195
Ethanol (g/kg DM)	26.1	15.9	21.5	20.5	1.57	0.142
WSC (g/kg DM)	8.76	6.41	9.54	10.1	0.553	0.054
$\text{NH}_3\text{-N}$ (g/kg TN)	66.6a	62.6a	23.4c	31.6b	5.77	<0.001
LAB (log cfu/g FW)	7.55b	8.40a	7.97ab	8.35a	0.132	0.049
Yeasts and molds (log cfu/g FW)	5.47a	4.06c	4.76b	4.45bc	0.178	0.006
NDF (g/kg DM)	685a	674a	625b	619b	9.39	<0.001
ADF (g/kg DM)	428	418	391	398	6.10	0.084
ADL (g/kg DM)	52.3	51.4	46.8	59.7	1.57	0.974
Hemicellulose (g/kg DM)	257a	256a	233ab	221b	5.72	0.018
Cellulose (g/kg DM)	376a	366ab	344bc	338c	5.47	0.023

DM - dry matter; FW - fresh weight; WSC - water soluble carbohydrate; LAB - lactic acid bacteria; NDF - neutral detergent fiber; ADF - acid detergent fiber; ADL - acid detergent lignin; CONT - Control; LAB -  $1 \times 10^6$  log cfu/g lactic acid bacteria (*Lactobacillus plantarum* + *L. buchneri*) added at ensiling; CAR - 36 g/kg citric acid residue added at ensiling; CL - CAR + LAB. Data presented represent means of 5 replicates. Values in the same row without common letters are significantly different ( $P < 0.05$ ).





**Figure 2.** Changes in: (a) pH; (b) water soluble carbohydrate; (c) ammonia nitrogen (NH<sub>3</sub>-N); (d) lactic acid; (e) acetic acid; and (f) yeasts and molds in Napier grass silage during aerobic exposure. FW - fresh weight; DM - dry matter; NH<sub>3</sub>-N - ammonia nitrogen; TN - total nitrogen; cfu - colony-forming units; CONT - Control; LAB - 1 × 10<sup>6</sup> log cfu/g lactic acid bacteria (*Lactobacillus plantarum* + *L. buchneri*) added at ensiling; CAR - 36 g/kg citric acid residue added at ensiling; CL - CAR + LAB. Data presented represent means of 5 replicates. T - effect of treatment; D - effect of length of air exposure; T × D - interaction between treatment and length of exposure (P<0.05).

## Discussion

### *Fermentation quality*

As a tropical forage, Napier grass has a low DM and high fiber concentration (at certain stages of harvesting and rainfall conditions), which is beneficial for the growth of undesirable bacteria like clostridia during ensiling ([Khota et al. 2018](#)). Achieving low pH during the initial stage of ensiling could effectively inhibit the activity of undesirable bacteria and decrease the loss of nutrients ([McDonald et al. 1991](#)). In addition, wilting to reduce the moisture concentration in forage prior to ensiling is an option to inhibit the activity of undesirable bacteria in silage. In this study, all additives had a positive effect on the fermentation quality of Napier grass, as indicated by the lower pH and higher lactic acid concentrations. In detail, CAR-treated silages had lower pH plus acetic acid and NH<sub>3</sub>-N concentrations and higher DM and lactic acid concentrations than silages without CAR, indicating that CAR had a positive effect in improving fermentation quality of Napier grass. In agreement with our results, Ke et al. ([2017](#)) found that treating alfalfa with 0.1% or 0.5% citric acid (0.22% in this study) at ensiling improved fermentation quality and limited proteolysis, which might be related to the antibacterial properties of citric acid and the direct acidification it produces ([Bou et al. 2017](#)). The antibacterial activity of citric acid during ensiling was confirmed by Lv et al. ([2020](#)), who found that application of citric acid to *Amomum villosum* at ensiling decreased the number of undesirable bacteria, such as *Enterobacter*, *Escherichia*, *Shigella* and *Pantoea*. In addition to containing citric acid, CAR is rich in crude fiber, crude protein and other nutrients that could provide additional substrates and thereby increase DM% in CAR-treated silages. Furthermore, by limiting the activity of undesirable bacteria, adding CAR at ensiling reduced substrate losses in these silages, as evidenced by the higher DM concentration in silages containing CAR.

Compared with the Control silage, the higher lactic acid and lower acetic acid concentrations, observed in CAR-treated silages, indicate that adding CAR to forage at ensiling favors lactic fermentation. Besides, more substrates would have been converted to lactic acid by LAB, because CAR inhibited the activity of undesirable bacteria. The results of Ke et al. ([2018](#)) showed that treating alfalfa with citric acid with or without LAB at ensiling increased lactic acid concentration in the resulting silage compared with that of the Control. Inoculation with LAB also increased the lactic acid concentration due to the predominant population of LAB during the initial stages of ensiling. However, a combination of CAR and LAB had no synergistic effects on lactic acid production, which is probably because the low pH caused by CAR limited the effects of LAB inoculant. Compared with

the Control silages, ethanol concentrations in silages with additives were relatively low. This would probably be due to the lower yeast counts in these silages. High concentrations of ethanol in silages are often associated with high populations of yeast ([Kung et al. 2018](#)), and ethanol is considered to be the main fermentation product of yeasts.

During ensiling, substantial amounts of forage proteins are degraded into peptides and amino acids, the latter being further deaminated to NH<sub>3</sub>-N and decarboxylated to amines ([Rooke and Hatfield 2003](#); [Scherer et al. 2019](#)). In general, plant enzymes play a major role in proteolysis, and low pH can inhibit their activity ([Ding et al. 2013](#)). In the present study, lower NH<sub>3</sub>-N concentrations in CAR and CL silages were mainly attributed to the lower pH caused by the acidification produced by CAR and the accumulation of lactic acid. It is consistent with the results of Lv et al. ([2020](#)), that a decrease of NH<sub>3</sub>-N concentration was observed in silage treated with citric acid.

To improve fiber digestibility in ruminant diets, interest in improving degradation of structural carbohydrates (especially lignin and ADF, which cannot be fermented by the ruminal microflora) of silage has increased in recent years ([Lynch et al. 2014](#)). Acidolysis, enzymatic action and microbial activity are considered the primary factors influencing degradation of structural carbohydrates ([Zhao et al. 2018](#)). In the present experiment, CAR-treated silages showed lower NDF, cellulose and hemicellulose concentrations than the Control. It is possible that CAR and organic acids added during ensiling have a hydrolyzing effect on the structural carbohydrates. A previous study reported that treating Napier grass with formic acid at ensiling reduced structural carbohydrates and increased WSC concentrations in silage relative to silage without additives ([Desta et al. 2016](#)). Thus, the relatively high WSC concentrations in CAR-treated silages might be due to the degradation of structural carbohydrates.

### *Aerobic stability*

When silos are opened for feeding, the silage is exposed to air. Under the aerobic conditions, undesirable bacteria, which remain dormant in the absence of air, multiply, resulting in a deterioration of the silage ([McDonald et al. 1991](#)). This deterioration is usually manifested as a rise in temperature. In this study, none of the additives was able to delay the aerobic deterioration of Napier grass silage (Figure 1).

During the first 4 days of aerobic exposure, the rapid increase in pH and counts of yeasts and molds plus the sharp decrease in lactic and acetic acid concentrations, observed in all silages, indicated that all silages underwent aerobic deterioration during this period. However, silages treated with additives (especially CAR combined with LAB) had lower pH plus NH<sub>3</sub>-N and acetic acid concentrations and

higher WSC and lactic acid concentrations than Control during the first 2 days of exposure to conditions, which suggested that these additives can preserve stability of silage for the first 2 days of exposure to air. These results provide a valuable guide for use when opening silos. Generally, yeasts are considered to play the main role in aerobic deterioration (Pahlow et al. 2003). The lactic acid and WSC, which remain in silages when opened, are potential sources of readily available substrates for the growth of yeasts, when the silages are exposed to air (Wilkinson and Davies 2013). Application of CAR at ensiling of the forage failed to improve aerobic stability of the resulting silage, which could be related to the relatively high WSC concentration in the CAR-treated silages. In the study of Adesogan and Salawu (2002), addition of formic acid at ensiling increased residual WSC concentration and improved aerobic stability in pea-wheat bi-crop silages. The reason for the different findings may relate to the different forage species involved. Ke et al. (2017) reported that adding citric acid to alfalfa at ensiling promoted the growth of yeasts in the resulting silage, which might explain the comparable aerobic stability times displayed by the CAR-treated and Control silages. Heterofermentative LAB like *Lactobacillus buchneri* are commonly added to forage at ensiling to improve aerobic stability of silage, although inoculation of forage with LAB had no positive effects on aerobic stability of the resulting silage in this study. This is possibly related to the lower acetic acid concentrations in the silage. The LAB inoculant might have specificity for different forage species. It is interesting that aerobic stability of all silages in the study exceeded 60 hours, even though growth of yeasts and molds occurred rapidly in all silages during the first 2 days of aerobic exposure. An explanation for this phenomenon could be that high moisture levels in ensiled material required more energy for heating (Tomaz et al. 2018). The low DM concentrations (maximum 26%) in all silages are likely to have delayed the rise of temperature.

## Conclusions

The results of this study have demonstrated the benefits of using CAR as an additive when ensiling Napier grass. While addition of CAR alone or in combination with LAB improved the fermentation quality of the silage, no positive effects on aerobic stability of the silage were observed. Further investigations of the effects of CAR combined with other inhibitors of aerobic deterioration in silage making seem warranted, as the search for additives to improve aerobic stability of silages continues.

## Competing interests

The authors have declared that no competing interests exist.

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(Note of the editors: All hyperlinks were verified 10 January 2020.)

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