

RESEARCH

Forage and Grazinglands

Effects of commercial and farm-made inoculants on the chemical composition and fermentation of kikuyu grass silages

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Abstract

Ensiling allows for more efficient pasture utilization in kikuyu (*Cenchrus clandestinus* Hochst. ex Chiov.)-based forage systems. However, the chemical composition of kikuyu challenges this process. Additives can improve the quality of kikuyu silage. This study aimed to evaluate how the organoleptic, fermentative, and nutritive traits of kikuyu (90-d regrowth) were affected by three inoculants [farm-made inoculants (FMI), Efficient Microorganisms (EMI), and a commercial inoculant] compared with a control. We hypothesized that FMI and EMI would perform similarly to the commercial inoculant on kikuyu silage. The control treatment had lower scores for odor, but texture, moisture and color were adequate and similar among treatments. Kikuyu silage had similar concentrations of ammonia nitrogen (2.72–3.23%) among treatments, with the pH (5.04–5.20) and buffering capacity [95–111 meq NaOH 100 g⁻¹ dry matter (DM)] indicating resistance to acidification. The DM of kikuyu silage (20.7–21.0%) was similar to the forage prior to ensiling. The crude protein (8.13–8.69%) and ash (15.5–16.8%) concentrations indicated the forage's advanced maturity and were not affected by the inoculants. Mean neutral detergent fiber (NDF) was similar for FMI and EMI (54.8 and 54.8%) compared with the control and commercial inoculant (58.5 and 62.8%), and kikuyu silage had lower concentrations than fresh forage. In vitro DM digestibility was similar among treatments (55.7–59.0%). There was no clear advantage of using inoculants to ensile kikuyu grass, but the variation in NDF and hemicellulose concentrations in the control treatment suggests that kikuyu silages may benefit from inoculants.

1 | INTRODUCTION

Kikuyu grass (*Cenchrus clandestinus* Hochst. ex Chiov.) is a widely propagated species that constitutes the pasture base of beef and dairy grazing systems in costal Australia, and dairy regions in New Zealand, South Africa, Colombia, and Costa

Rica (García et al., 2014). It is a deep-rooted grass that withstands high-frequency and highly intensive grazing management (e.g., strip grazing), and is also characterized by its high yield potential, excellent response to fertility, and persistence (García et al., 2014).

The months of greater waste (i.e., low utilization) in perennial pastures are normally those with greater biomass (Piltz et al., 2000; Villalobos et al., 2013); which turns increased pasture utilization into one of the main challenges for kikuyu-based systems (García et al., 2014). The surplus biomass

Abbreviations: ADF, acid detergent fiber; CP, crude protein; DM, dry matter; EMI, Efficient Microorganisms; FMI, farm-made inoculant; IVDMD, in vitro dry matter digestibility; NDF, neutral detergent fiber; NEL, net energy of lactation; NFC, nonfibrous carbohydrates.

could be preserved for later use (Kaiser et al., 2000), which allows to harvest forage with greater nutritive value for subsequent feeding (Harrison et al., 1994). Preserved forages may allow producers to cope with changes in the nutritive value of grass throughout the growing season, as well as reducing the need for purchased feeds (Hanrahan et al., 2018). Likewise, preserved forages may fill the gaps of forage shortage during months when forage intake drops due to restricted grazing conditions such as drought, waterlogged pastures, and trampling (Villalobos et al., 2013).

Preservation of kikuyu as silage can be integrated into a management system in order to maintain the grass at the vegetative stage (Kaiser et al., 2000). However, kikuyu grass is typically low in fermentable carbohydrates compared with temperate pasture species (García et al., 2014), which limits its use as silage in tropical and subtropical regions (De Figueiredo & Marais, 1994; Kaiser et al., 2001). Moreover, the low concentration of dry matter (DM) typically found in kikuyu grass imposes challenges for its conservation as silage (De Figueiredo & Marais, 1994; Piltz et al., 2000), particularly in regions where a forage biomass surplus occurs during times of the year with greater rainfall.

Silage additives can improve the fermentation of preserved forages (Kung et al., 2018), thus maintaining nutritive value and reducing the losses in the feed-out phase (Merry et al., 2000). Among the different types of additives used in silage, microbial inoculants consist of a blend of microorganisms (mostly bacteria of the genus *Lactobacillus*) that are applied to the forage to be ensiled. Research indicates that the use of microbial inoculants and a supply of fermentable carbohydrates can improve the fermentation process of kikuyu grass silage (De Figueiredo & Marais, 1994; Piltz et al., 2000).

Forage preservation techniques have not yet been broadly adopted in tropical and subtropical regions, which means that silage additives such as microbial inoculants tend to be rarely used by producers. Given that the materials used to make biological additives may be accessible in dairy farms, we aimed to evaluate a farm-made additive and compare it with two commercial inoculants. Our objective was to evaluate the effect of type of inoculum on nutritional and fermentative traits of kikuyu grass silage. We hypothesized that fermentation and nutritional composition would be similar among inoculated silages, and that pH and ammonia nitrogen concentrations would be lower in inoculated treatments compared with noninoculated control silage.

2 | MATERIALS AND METHODS

2.1 | Forage

Mature kikuyu grass (90-d regrowth) was harvested using a machete from a perennial pasture (2,500 m²) normally

Core Ideas

- Kikuyu grass is grown in specialized dairy farms in tropical and subtropical conditions.
- Wilting of kikuyu grass did not increase dry matter concentration before ensiling.
- Fermentation indicators were similar among inoculation treatments in kikuyu silage.
- Inoculants allow for less variation in cell wall components in kikuyu silage.
- Kikuyu silage's nutritive value indicates its potential as a source of fiber for dairy cows.

under cut-and-carry management at a commercial dairy farm located in the province of Cartago, Costa Rica (09°54'N, 83°49'W, elevation 2,600 m). Either under grazing management (30–35 d of regrowth) or stockpiled for preservation, kikuyu grass in this farm is typically fertilized at a rate of 200 kg N ha⁻¹ split into four to five applications throughout the year. The 90-d regrowth period of kikuyu grass was chosen to allow for greater production of biomass per hectare and the concentration of DM. The forage was chopped prior to wilting with a stationary chopper. The chopped length of the wilted kikuyu forage used for our experimental silos was estimated by using the Penn State particle size separator (Heinrichs & Kononoff, 1996) with 2.5, 9.5, and 88.0% on screens of <0.8, 1.9–0.8, and >1.9 cm, respectively.

2.2 | Additives and treatments

The kikuyu forage was harvested and wilted under average weather conditions for this study region of this (humidity, 88%; temperature, 11.5–15.4 °C; maximum solar radiation, 0.75 kW m²) for 24 h to reduce moisture (21.5–22.0% DM) before chopping. Samples of wilted forage were collected for analysis prior to ensiling. Chopped kikuyu was mixed with sugarcane (*Saccharum officinarum* L.) molasses at 30 ml kg⁻¹ forage regardless of the experimental treatment. Treatments consisted of: (a) the control without inoculant, (b) a farm-made inoculant (FMI) applied at the rate of 1 ml kg⁻¹ forage (Villalobos & Arce, 2016), (c) Efficient Microorganisms EM (EMI), and (d) a commercial inoculant (CEN-sile), the two latter applied at the rates recommended in their respective commercial labels (Table 1). The FMI was prepared as indicated by (Villalobos & Arce, 2016). Briefly, a solution consisting of 50, 25, and 25% sour milk, whey, and sugarcane molasses, respectively, was evenly mixed and stored at room temperature (24 °C) for 48 h. A final dilution was then prepared, which consisted of 0.5% of the first solution, 2%

TABLE 1 Description of treatments and microbial inoculums applied

No	Treatment	Inclusion rate of inoculant) ml kg ⁻¹ fresh forage	Inoculum composition	Microbial concentration CFU g ⁻¹
1	Control	—	Sugarcane molasses	—
2	Farm-made inoculant	1	Sour milk, whey and sugar-cane molasses	<i>Lactobacillus</i> sp., 8×10^6
3	Efficient Microorganisms	5	Phototropic and lactic acid bacteria, and yeast	<i>Lactobacillus casei</i> , 1×10^4 ; <i>Rhodopseudomonas polistris</i> , 1×10^3 ; <i>Saccharomyces cerevisiae</i> , 1×10^3
4	Commercial inoculant	1	Cen-sile	<i>Lactobacillus acidophilus</i> , 5×10^6 ; <i>Lactobacillus plantarum</i> , 5×10^6 ; <i>Streptococcus faecium</i> , 5×10^6 ; <i>Pediococcus acidilati</i> , 5×10^6

Note. All the treatments were dosed with 30 g kg⁻¹ of sugarcane molasses. CFU, colony-forming units.

sugarcane molasses, and 97.5% distilled water. The microbial concentrations shown in Table 1 are those reported by the manufacturer for EMI and the commercial inoculant. For the FMI, concentration of *Lactobacillus* sp. was analyzed at the Agronomic Research Center of the University of Costa Rica.

2.3 | Ensiling process

Experimental microsilos were prepared according to the methodology described by Johnson et al. (2005). Forage was ensiled with the corresponding additives, according to the experimental treatments, and were placed into vacuum polyethylene bags (0.06 mm thick), the air was extracted with a vacuum pump and each bag was sealed to maintain the vacuum. Six replicates of 1.5 kg per treatment were prepared, for a total of 24 silos. The silos were stored at room temperature (24 °C) and protected from weather for 60 d.

After 60 d of ensiling, all the silos were opened, and the top 4 cm layer was discarded to improve the representativeness of the samples.

2.4 | Organoleptic, nutritional, and fermentative analyses

Two samples of forage per treatment were collected and composited for chemical analysis of kikuyu grass prior to ensiling. After the 60-d ensiling period, one sample was collected from each silo for chemical analysis. All samples were dried at 60 °C in a forced-air oven (Heratherm, Thermo Scientific) and then ground to pass a 1-mm screen in a Wiley mill (Model No. 2, Arthur H. Thomas Co.) for further analyses. These samples

were analyzed for DM (Method 930.15) (AOAC International, 1990), crude protein (CP)(Method 990.03) (AOAC International, 1990), ether extract (Method 920.85) (AOAC International, 1990), and ash (Method 942.05) (AOAC International, 1990). Concentrations of neutral detergent fiber (NDF), NDF corrected by protein, acid detergent fiber (ADF), cellulose, hemicellulose, and lignin were analyzed with the method adapted for the Ankom²⁰⁰ Fiber Analyzer (Ankom Technology)(Van Soest et al., 1991) and in vitro DM digestibility (IVDMD) according to Van Soest and Robertson (1985). Neutral detergent-insoluble CP was isolated by gravimetric determination, followed by CP analysis (Method 990.03)(AOAC International, 1990) and used to calculate the NDF corrected by protein. Moreover, lignin concentration was analyzed as acid detergent lignin according to Van Soest (1973). The concentration of nonfibrous carbohydrates (NFC) and the net energy of lactation (NEL) were estimated according to Van Soest et al. (1991) and Weiss et al. (1992), respectively, for the fresh forage and the kikuyu silages.

Additionally, at the end of experimental ensiling period, all silos were evaluated for organoleptic traits (moisture, texture, color, and odor) according to Esperance and Ojeda (1997), and one sample was obtained from each silo for evaluating the buffering capacity and pH (McDonald Henderson, 1962) and ammonia nitrogen (AOAC International, 1990).

2.5 | Experimental design and data analysis

Data for fermentative and nutritional traits were analyzed as a completely randomized design via the GLIMMIX procedure in SAS (SAS Institute 2011). Least significant differences ($P < .05$) were estimated for multiple comparisons via Tukey's test.

TABLE 2 Organoleptic evaluation of the kikuyu silage under four inoculation treatments

Treatment	Texture	Moisture	Color	Odor
Control	Good	Moist	Green-yellow	Regular
Farm-made inoculant	Good	Moist	Green-yellow	Pleasant
Efficient Microorganisms	Good	Moist	Green-yellow ^a	Pleasant ^b
Commercial inoculant	Good	Moist	Green-yellow ^a	Pleasant

Note. Good texture was indicated by well-defined edges. Moist samples produced residual moisture when pressed. Regular odor was strong acidic to acetic; pleasant odor was slight acidic to acetic.

^aMold and yeast growth appeared in one of the silos.

^bTwo samples had an unpleasant odor at the bottom of the silo.

3 | RESULTS

3.1 | Organoleptic characteristics of kikuyu silage

The kikuyu silages had a good texture and color (Table 2), with well-defined edges, and undesirable colors were not found. One replicate of both EMI and commercial inoculant presented moldy spots at the upper level of the silo bags, which was discarded.

Moisture was qualitatively evaluated by grabbing a handful of silage from each replicate and squeezing by hand. No silage dripped when squeezed, and only residual moisture was left on the hands after this procedure.

3.2 | Fermentative characteristics of kikuyu silage

The average pH values (5.12) for kikuyu silos were similar ($P = .16$) among experimental treatments in our study (Figure 1a).

The concentration of ammonia nitrogen was low and similar ($P = .15$) among all the treatments (2.72–3.23%). The buffering capacity of the commercial inoculant treatment was significantly lower ($P = .02$) than that of the control but similar to those of FMI and EMI. The latter tended to reduce the buffering capacity of kikuyu silage compared with the control treatment.

3.3 | Chemical composition of kikuyu silage

We evaluated the nutritional value of kikuyu grass prior to ensiling to have a baseline of the forage and assess the nutrient losses during the process. Prior to ensiling, the chemical composition of forage averaged 21.8% DM, 8.73% CP, 13.8% ash, 1.51% ether extract, 68.8% NDF, 35.9% ADF, 3.10% lignin, and 7.23% nonfibrous carbohydrates. The IVDMD was 57.1% and its estimated NEL averaged 1.18 Mcal kg⁻¹ forage.

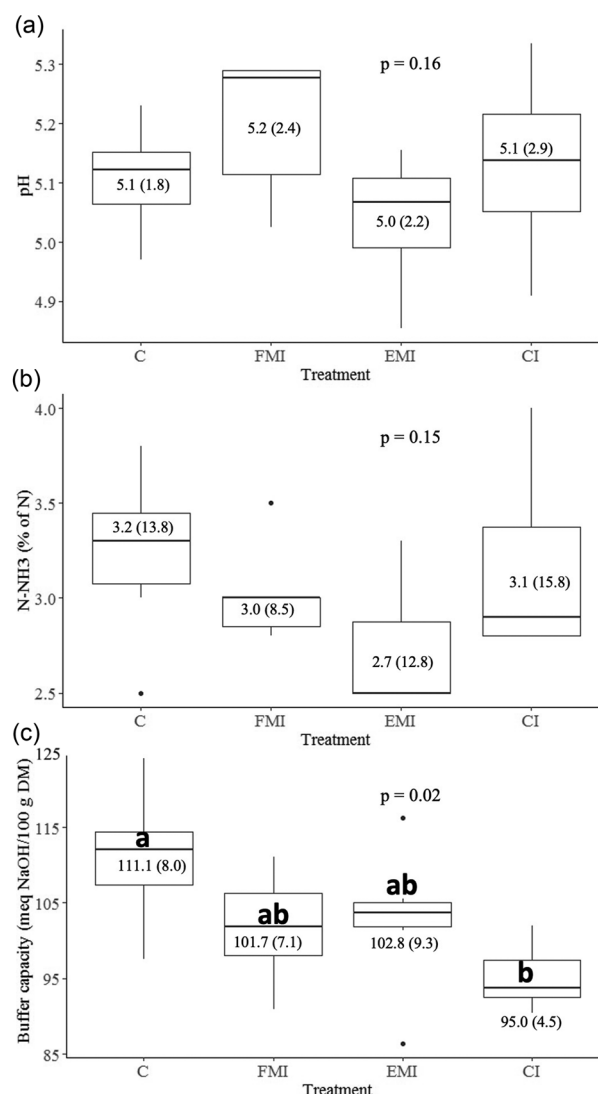


FIGURE 1 Fermentative characteristics of kikuyu silage under four inoculation treatments: (a) pH; (b) ammonia nitrogen; (c) buffering capacity. Bold horizontal bars represent the median of each treatment, and the boxes correspond to the lower and upper quartiles. Treatment least square means are indicated with the CV in parentheses. Different letters within the same panel are indicative of statistical differences among treatment means. C, control; FMI, farm-made inoculant; EMI, Efficient Microorganisms; CI, commercial inoculant

TABLE 3 Dry matter, crude protein, ether extract and ash concentrations of kikuyu silage under four inoculation treatments

Variable	Control	Farm-made inoculant	Essential Microorganisms	Commercial inoculant	SEM	<i>P</i> value
Dry matter (%)	21.0	20.7	20.6	20.7	0.1	.42
Crude protein (%)	8.3	8.1	8.5	8.7	0.2	.18
Ash (%)	16.6	15.5	15.5	16.8	0.7	.02
Ether extract (%)	3.2	3.5	3.1	3.0	0.4	.59

The DM concentration of kikuyu forage was less than 25%. Overall, the forage prior to ensiling was low in CP, high in NDF and ash concentrations, and had low IVDMD, being all indicators of the mature stage at which it was harvested.

The average concentrations of DM and CP in kikuyu silage were similar to those of the forage prior to ensiling (21.8 vs. 20.8% and 8.72 vs. 8.39%, respectively). Ash and ether extract concentrations were, on average, greater in the silage than prior to ensiling (13.8 vs. 16.1% and 1.50 vs. 3.21%, respectively), which may be a result of the nutrients supplied by the ingredients of the inoculants, partial degradation of other chemical components of forage during the ensiling process, or even contamination of the forage with soil particles at the moment of harvest. No significant differences ($P > .05$) were found in DM ($P = .42$), CP ($P = .18$), ether extract ($P = .59$), and ash ($P = .02$) concentrations in kikuyu silage among the treatments evaluated (Table 3).

The cell wall components represented by NDF (68.8 vs. 57.7%), ADF (35.9 vs. 34.3%), and lignin (3.10 vs. 2.09%) were lower in kikuyu silage (Figure 2) than in forage prior to ensiling. The FMI and EMI treatments had lower NDF concentrations ($P < .01$) than the commercial inoculant but were similar to the control (Figure 2a). Conversely, the ADF concentration of the commercial inoculant treatment was the lowest with respect to the three other treatments (Figure 2b). Lignin concentration was significantly ($P < .01$) lower in the FMI and EMI treatments, whereas the control and the commercial inoculant treatment had greater and similar concentrations (Figure 2c), showing a similar trend to that previously mentioned for the concentrations of NDF. The concentrations of cellulose and hemicellulose in kikuyu silage followed the same trends shown by ADF and NDF, respectively, for all the treatments evaluated (Figure 2 and Figure 3). The commercial inoculant treatment had a significantly ($P < .01$) lower cellulose concentration than the other treatments (Figure 3a), though for hemicellulose, it was the treatment with the greatest concentration (Figure 3b).

The NDF corrected by protein showed the same differences among the treatments (Figure 3c) as normal NDF (Figure 2a). On average, the NDF corrected by protein showed differences of 14.1 and 3.02 percentage units less than forage prior

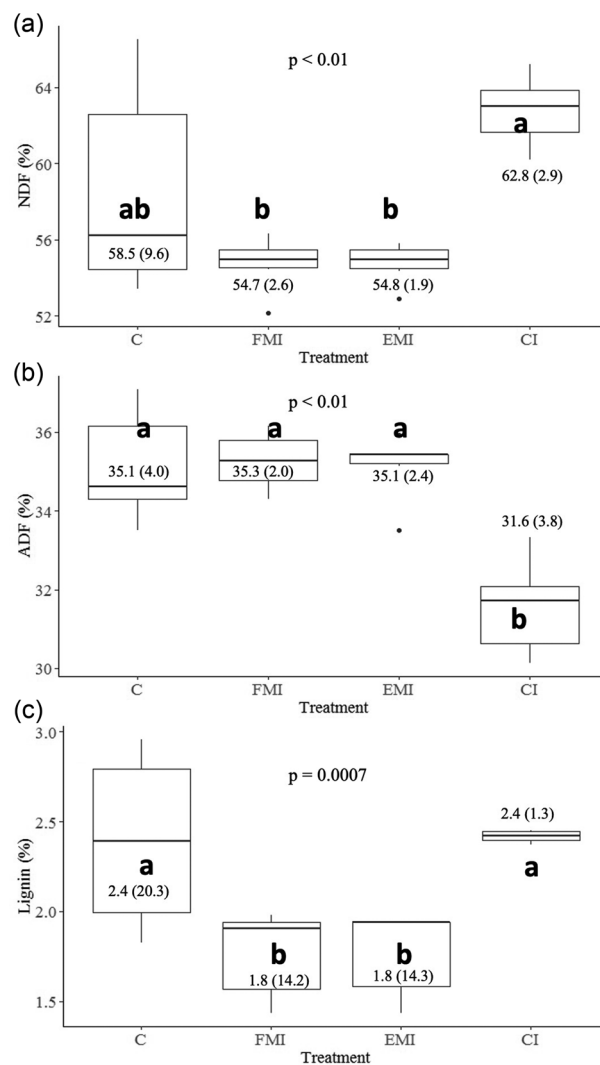


FIGURE 2 Concentration of cell wall components of kikuyu silage under four inoculation treatments: (a) neutral detergent fiber (NDF); (b) acid detergent fiber (ADF); (c) lignin. Bold horizontal bars represent the median of each treatment, and the boxes correspond to the lower and upper quartiles. Treatment least square means are indicated with the CV in parentheses. Different letters within the same panel are indicative of statistical differences among treatment means. C, control; FMI, farm-made inoculant; EMI, Essential Microorganisms; CI, commercial inoculant

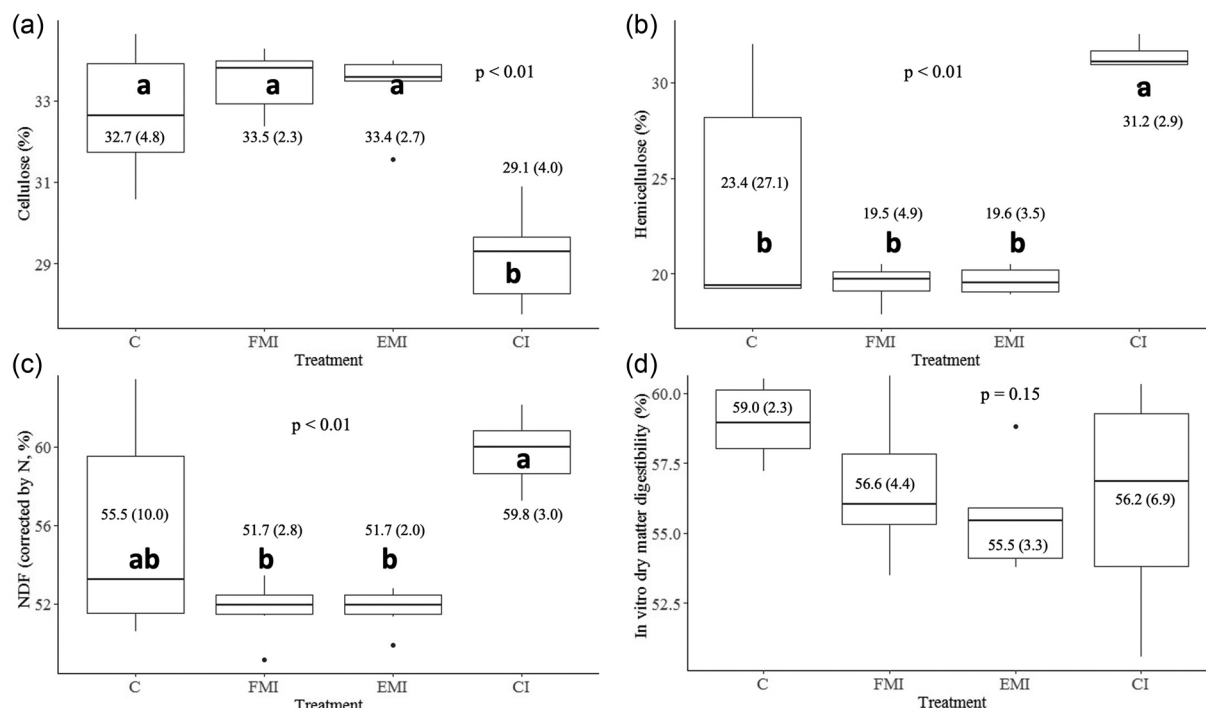


FIGURE 3 Concentration of cell-wall components and digestibility of kikuyu silage under four inoculation treatments: (a) cellulose; (b) hemicellulose; (c) neutral detergent fiber (NDF) corrected by nitrogen; (d) in vitro dry matter digestibility (IVDMD). Bold horizontal bars represent the median of each treatment, and the boxes correspond to the lower and upper quartiles. Treatment least square means are indicated with the CV in parentheses. Different letters within the same panel are indicative of statistical differences among treatment means. C, control; FMI, farm-made inoculant; EMI, Essential Microorganisms; CI, commercial inoculant

to ensiling and silage, respectively. The IVDMD in kikuyu silage was, on average, similar to the forage prior to ensiling (56.8 vs. 57.1%, respectively). Digestibility was neither affected ($P = .15$) by the treatments (Figure 3d) nor by the differences in the cell wall components mentioned above (Figure 2 and Figure 3).

The NFC concentration in kikuyu silage was significantly ($P < .01$) greater for the FMI and EMI treatments than the commercial inoculant (Figure 4a), whereas the control had values intermediate and similar to both groups. Kikuyu silage had, on average, a numerically greater NFC concentration than forage prior to ensiling (17.6 vs. 7.23%).

Energy concentration in kikuyu silage was, on average, slightly greater than in forage prior to ensiling (1.18 vs. 1.28 Mcal kg⁻¹ DM, Figure 4b). The FMI and EMI treatments had significantly ($P < .01$) greater NEL than the commercial inoculant and the control, showing a pattern inversely proportional to that of lignin (Figure 2c).

4 | DISCUSSION

4.1 | Organoleptic evaluation of kikuyu silage

The organoleptic evaluation of the silage in this study helped explain the results of the wet chemistry analyses as well as

the relative success of the fermentation process. The texture of kikuyu grass was not affected by the ensiling process (Table 2). Because neither irregular edges nor plant parts that were not recognizable were found in our silages, we could not find any evidence that the fermentation process affected the texture of the forage. None of the silages had an accumulation of liquid that would be an indicator of nutrient loss.

Even though no inoculants were added to the control treatment, it had good organoleptic scores. Usually, a yellow color in silage is referred as a sign of acetic fermentation in wet silage (Kung et al., 2018), as was the case in our study for kikuyu silage. Although the color of silage is inevitably a result of the forage ensiled, as well as other additives applied (e.g., molasses), we could not find silage with a dark brown to black color, which is common when heat damage occurs during fermentation (Kung et al., 2018).

Only two silages in our study showed evidence of mold and yeast growth; however, no moldy smell was perceived to come from these. Because *Saccharomyces cerevisiae* was part of the EMI treatment (Table 1), one could suggest that yeast growth was feasible; however, this would not be the case for the commercial inoculant treatment. Poor air exclusion may have been more likely, as the mold and yeast were located at the upper section of the silo bags. Silages with low aerobic stability are related to high counts of yeasts and molds brought from the field at the time of harvest (Wilkinson & Davies,

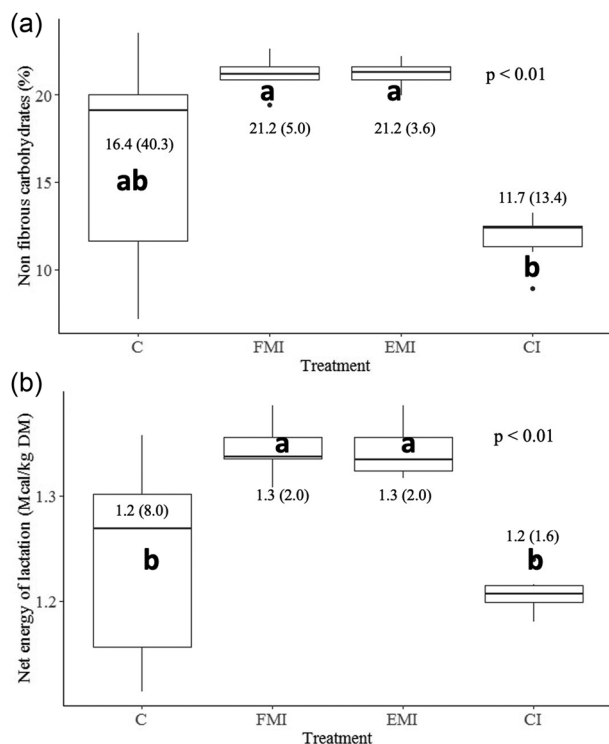


FIGURE 4 Concentration of (a) nonfibrous carbohydrates (NFC) and (b) net energy of lactation (NEL) of kikuyu silage under four inoculation treatments. Bold horizontal bars represent the median of each treatment, and the boxes correspond to the lower and upper quartiles. Treatment least square means are indicated with the CV in parentheses. Different letters within the same panel are indicative of statistical differences among treatment means. C, control; FMI, farm-made inoculant; EMI, Essential Microorganisms; CI, commercial inoculant

2013) and indicate extensive aerobic deterioration (Wilkinson & Davies, 2013). The low presence of both yeast and mold in kikuyu silage may reflect a good aerobic stability overall.

The odor was considered pleasant in all treatments except the control, in which only molasses was applied to kikuyu forage prior to ensiling (Table 2). The application of molasses in all treatments was intended to provide a substrate of readily available carbohydrates for bacteria to grow, consequently creating acidic conditions for preservation (Kung Jr et al., 2018). Kung et al. (2018) indicated that the nearly odorless lactic acid is the desired organic acid in silages, but most silages have a mild odor of vinegar caused by the greater volatility of the acetic acid produced. Although organoleptic indicators should not be taken as conclusive, they could give some insight into the effectiveness of the fermentation process, and they are practical for producers to use to assess moisture concentrations in kikuyu forage prior to ensiling.

4.2 | Fermentative traits of kikuyu silage

The pH values found in this study were greater than those recommended by Kung et al. (2018) for grass silages (pH = 4.3–4.7) but similar to those reported by De Figueiredo and Marais (1994) for kikuyu silages with the three bacterial inoculants (5.36–6.42) and the control without an inoculant (6.6). The same authors reported lower values (pH = 4.70–5.08) when microbial inoculants were applied in combination with molasses. Piltz et al. (2000) reported lower pH values (3.85–4.45) when fast wilting was applied to kikuyu grass. These pH values were attained when the dosage of molasses was greater than 4% (40 kg t⁻¹ fresh forage).

In our study, the DM concentration of kikuyu silages was lower (Table 3) than recommended for grass silages (25–35%) (Kung et al., 2018), which could have affected the decline in pH during fermentation. When low-DM forages are ensiled, the metabolic water available allows for a more rapid decrease in pH (Kung et al., 2018); however, some studies reported gradual decreases in pH over time over extended periods of storage (Der Bedrosian et al., 2012; Windle et al., 2014).

The kikuyu silages had pH values that were roughly one unit greater (Figure 1a) than those reported by Dawson et al. (1999) for temperate grasses (pH = 3.8–4.4) but were similar to the pH levels of legume silages (Kung et al., 2018). The three inoculants applied to kikuyu grass did not affect the pH in the silages compared with the control treatment (Figure 1a). Han et al (2015) reported greater pH values in untreated silages of sorghum-sudangrass [*Sorghum × drummondii* (Nees ex Steud.) Millsp. & Chase] compared with those that received fermentation enhancers (propionic acid and sodium metabisulphite). Piltz et al. (2000) reported that wilting speed affects the final pH of kikuyu silages, showing lower values for a fast method (3.85–4.87) than for a slow one (3.74–5.51).

Ammonia nitrogen is a measure of protein loss during the ensiling process (De Figueiredo & Marais, 1994; Kaiser et al., 2001). Ammonia nitrogen in kikuyu silages (Figure 1b) was similar and lower than the values reported for corn (*Zea mays* L.) silage (5–7%) and grass silage (8–12%) (Dawson et al., 1999; Kung et al., 2018). In the current study, ammonia nitrogen was much lower than that reported for high-moisture corn silage (>10%) and at least half that reported for kikuyu silages with molasses and microbial inoculants (4.98–8.62%) (De Figueiredo & Marais, 1994; Kung et al., 2018).

Piltz et al. (2000) reported greater ammonia nitrogen in kikuyu silages that were either wilted fast (9.3–45.3%) or slow (13.7–43.6%). Likewise, De Figueiredo and Marais (1994) found greater ammonia concentrations in kikuyu silages (9.44–25.6%), with the control treatments (0% molasses) having the highest values of ammonia nitrogen. The low values of ammonia nitrogen in this study could be partly explained

by the low CP concentration in the kikuyu forage prior to ensiling.

The ammonia nitrogen in the kikuyu silages was not affected by the inoculants, showing similar values to the control (Figure 1b). Other studies have shown lower values of ammonia nitrogen in silages that were chemically inoculated (Han et al., 2015). Regardless of the wilting speed, Piltz et al. (2000) saw consistent reductions in ammonia nitrogen when molasses was applied to kikuyu silages, implying that a source of readily available sugars enhances the fermentation process.

Kikuyu silage had buffering capacity values that were roughly four- and two-fold the values recommended for corn (20–25 mEq NaOH 100 g⁻¹ DM) and legume silages (50–55 mEq NaOH 100 g⁻¹ DM), respectively (Kung et al., 2018). High buffering capacity is related to silages with pH values greater than normal resulting from high protein and ash concentrations (Kung et al., 2018), which was the case in this study both for forage prior to ensiling and silage (Table 3) and could be attributed to the advanced stage of maturity of kikuyu in this study.

The buffering capacity in the commercial inoculant treatment was lower than in the control (Figure 1c) but was not different from the FMI and EMI treatments. High buffering capacities are common in forages with low DM concentrations, especially when wilting may not be feasible or ineffective because of weather conditions (Conaghan et al., 2012). Kaiser et al. (2001) reported that kikuyu grass needs to be wilted to DM levels greater than 30% in order to obtain low buffering capacities (22–49 mEq NaOH 100 g⁻¹ DM). On the other hand, Wilkinson and Davies (2013) indicated that highly buffered forages tend to have greater aerobic stability, which may be a benefit of making silage with kikuyu at an advanced stage of maturity.

4.3 | Chemical composition of kikuyu silage

The nutritional value of the kikuyu forage prior to ensiling in this experiment was low as a result of the advanced stage of maturity (90 d of regrowth). The nutritive value of a silage is the result of the quality of the crop at ensiling, as well as the conditions during the preservation phase (De Figueiredo & Marais, 1994). Kaiser et al. (2000) indicated that kikuyu grass needs to be harvested with 20 to 50 d of regrowth in order to obtain a silage with 60 to 70% organic matter digestibility; however, the authors mentioned that this may change because of local environmental conditions.

In a previous experiment, Villalobos and Arce (2016) found greater DM concentration in kikuyu silages than in perennial ryegrass (*Lolium perenne* L.) and reed canarygrass (*Phalaris arundinacea* L.) with 60 and 70 d of regrowth, respectively. Although this was partly the reason for choosing kikuyu for this experiment, wilting was still necessary to increase the

DM for silage. In spite of having wilted kikuyu trying to reach 25% DM, the decrease in moisture was not as expected in this study. Wilting under poor drying conditions may not necessarily increase the DM concentration of the forage to be ensiled (Conaghan et al., 2010). Titterton and Bareeba (2001) recommended not wilting tropical grasses for more than 12 h; however, this study contradicts that statement and indicates that the weather conditions may be more relevant than the number of hours.

Low DM concentrations have been reported for ryegrass silages under wet weather conditions (Dawson et al., 1999) even after wilting (Conaghan et al., 2012). Different drying and wilting methods applied to kikuyu forage prior to ensiling have agreed on prevailing weather conditions as the main factor influencing its success (De Figueiredo & Marais, 1994; Piltz et al., 2000). Kaiser et al. (2001) analyzed kikuyu silages from the east coast of Australia and found that they were typically low in DM (10–16%), having concentrations similar or greater than 30% only under drought conditions.

Increasing the DM concentration prior to ensiling has been proven to be a measure to enhance the fermentative process in high-moisture forages (Conaghan et al., 2012; Villalobos & Arce, 2016). The DM of kikuyu silage in this study averaged 20% with a less intensive method and it was not increased to the levels expected (25–30%) after wilting for 24 h. The weather conditions where kikuyu grass has adapted to tropical regions (Andrade, 2006; Quesada, 2007) may impair the decrease in moisture, implying that more intensive systems of wilting may be required in order to reduce moisture prior to ensiling kikuyu forage under the conditions of this experiment (Conaghan et al., 2012).

The inoculants evaluated in this study did not affect the DM concentration of kikuyu silage (Table 3). Conaghan et al. (2012) evaluated inoculants of lactic acid bacteria in low DM grass (107–132 g kg⁻¹ DM) and found that these were ineffective at improving silage fermentation, with high losses of DM in the effluents. In our study, the DM concentration of kikuyu silage was intermediate (De Figueiredo & Marais, 1994; Kaiser et al., 2000); however, the effect of microbial inoculants may be enhanced with DM concentrations between 25 and 30% (De Figueiredo & Marais, 1994). Both the use of microbial inoculants and a source of readily available carbohydrates are recommended tools to enhance the fermentation process when wilting is not effective at reducing moisture (Mühlbach, 2001).

Sugarcane molasses is one of the most common additives applied to silages in the tropics because of its high DM concentration (>70%), which offsets the high moisture typical of tropical grasses (Mühlbach, 2001). The carbohydrates contained in molasses are also aimed to amend the low carbohydrate concentration in tropical grasses (De Figueiredo & Marais, 1994) by providing a substrate for microorganisms to grow (Piltz et al., 2000). Garcés-Molina et al. (2006) increased

the DM concentration in kikuyu silages from 13.3 up to 28.6% by using increasing rates (0–30% on a DM basis) of sugarcane molasses prior to ensiling.

The kikuyu silage had low protein concentrations in all treatments (Table 3), showing values close to the minimum required for ruminants to maintain adequate ruminal fermentation (National Research Council, 2001). Titterton and Bareeba (2001) recommend harvesting forages at an early stage when high protein and digestibility foster the growth of lactic acid bacteria in the silage.

In contrast to previous studies under intensive grazing management in Costa Rica (Andrade, 2006; Peters, 2008; Villalobos & WingChing-Jones, 2020), the kikuyu grass used in this experiment had nutritional traits typical of grasses at an advanced stage of maturity. The inoculants affected the NFC concentration of kikuyu silage (Figure 2a), which was 11 percentage units lower than that of fresh forage. In grass–legume silage, Harrison et al. (1994) found a reduction in the fibrous components of forage when lactic acid bacteria and/or cell wall degrading enzymes were used.

Although the commercial inoculant treatment had the greatest hemicellulose and lowest cellulose concentrations (Figure 3a,b), this pattern was not found for lignin concentration among the treatments evaluated (Figure 2c), which could indicate that the cell wall components showed different responses to the fermentation process. The NDF corrected by protein had the same differences among treatments as NDF (Figure 2a and Figure 3c), which is attributed to the similar concentrations of nitrogen bound to the cell wall (0.46–0.50%) that were subtracted from this component (Licitra et al., 1996). Fiber must ensure rumen function without reducing the nutritional value of the preserved forage (Titterton & Bareeba, 2001).

Neither the inoculants added to kikuyu nor the reduction in NDF affected the digestibility of kikuyu silage. Perhaps the advanced maturity at harvest, compared with Kaiser et al. (2001)'s recommendations, limited the effect of inoculants, possibly through the lower concentration of soluble components and the digestibility of the fiber (Harrison et al., 1994). The importance of having an adequate supply of carbohydrates in kikuyu silages has been reported by De Figueiredo and Marais (1994), who found greater *in vitro* digestibility when a mixture of molasses and microbial inoculants was applied at the moment of ensiling than with microbial inoculants only. The *in vitro* digestibility of kikuyu silage in our study was greater than the values reported by these authors, but it may have been enhanced through the harvest of more immature forage.

Prior to ensiling, the kikuyu grass used for this experiment had an average NFC concentration of 7.23% but the NFC after ensiling ranged from 11.74 to 21.20% across treatments. This may be attributed to several factors, including the supply of nutrients by both the molasses and the inoculants (De

Figueiredo & Marais, 1994). Kaiser et al. (2000) mentioned that kikuyu grass with 20 to 50 d of regrowth tends to be low in water-soluble carbohydrates (23.4–68.4 g kg⁻¹ DM) but it contains an appreciable quantity of starch (14.2–57.8 g kg⁻¹ DM). Although starch is not fermented by silage bacteria, the hydrolysis processes that occur during wilting and prior to the establishment of anaerobic conditions may boost the supply of sugars available for fermentation (Kaiser et al., 2000). The differences in cell wall components mentioned above also affected the NFC concentration in kikuyu silage, with the FMI and EMI treatments having greater concentrations than commercial inoculant, which is consistent with the differences found in NDF and lignin (Figure 2a,c), suggesting a greater fermentation of the NFC fraction when the commercial inoculant was used rather than FMI and EMI, but it was not different from control without inoculum.

The increase in NEL in kikuyu silage with respect to the forage prior to ensiling may be explained by the factors mentioned above related to nutrient supply by the additives as well as the degradation process of cell wall components as a result of the inoculants (Harrison et al., 1994). Either as forage prior to ensiling or as silage, kikuyu had energy concentrations greater than *Digitaria decumbens* Stent cv. 'Transvala' hay (1.02 Mcal kg⁻¹ DM), which is the typical preserved forage purchased by livestock producers in Costa Rica (WingChing-Jones & Retana, 2009). Cubero et al. (2010) ensiled corn with a bacterial inoculant in Costa Rica and achieved a NEL similar (1.29 Mcal kg⁻¹ DM) to that of kikuyu silage, and the FMI and EMI treatments' energy concentrations were slightly greater (1.34 Mcal kg⁻¹ DM).

An interesting outcome of our data was the observed within-treatment variation for the chemical composition of silage; specifically for cell wall-related variables and NFC (Figure 2 and Figure 4). Besides the differences among treatments that were previously discussed for these variables, a larger CV was observed for the means of the control treatment than for the three other treatments where inoculants were used during the ensiling process. Although it is not conclusive, a smaller variation around the mean values of inoculated silages may suggest a possible effect on fermentation, suggesting that the more homogeneous composition of silage was reflected by the lower CV for NFC and cell wall-related variables when an inoculant was used during the ensiling process.

5 | CONCLUSIONS

The fermentation indicators evaluated in kikuyu silage showed similar results for the FMI and the commercial inoculants. Moreover, there was no clear advantage of using inoculants during the ensiling process in comparison with the non-inoculated control treatment. Factors such as forage maturity

and the efficiency of wilting process may have influenced the results obtained in our experiment.

Lower variation in the chemical composition of kikuyu silage treated with inoculants suggests a possibly more controlled fermentative process when inoculants are used; however, the implications of this are unknown.

Further research will elucidate the feasibility of using inoculants during the ensiling process of kikuyu grass and the conditions that allow for an adequate conservation process. Kikuyu harvested after fewer days of regrowth than those used in this experiment has the potential to provide forage with a greater nutritive value that may encourage greater adoption by producers in tropical and subtropical regions of the world.

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AUTHOR CONTRIBUTIONS


Luis A. Villalobos-Villalobos: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. J. A. Arce-Cordero: Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders (Research Provost Office at the University of Costa Rica) had no role in the design of the study nor the collection of data, the analyses performed, the interpretation of the results, the writing of the manuscript, or the decision to publish the results.

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