STATE UNIVERSITY OF MARINGÁ
AGRICULTURAL SCIENCES CENTER

EFFECT OF CHEMICAL ADDITIVES ON THE CONSERVATION OF TROPICAL GRASS SILAGE

Author: Ana Luiza Mendonça Gomes
Supervisor: Prof. Dr. João Luiz Pratti Daniel

MARINGÁ
State of Paraná
October – 2020

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Thesis presented to the Graduate Program in Animal Science of the State University of Maringá in partial fulfillment of requirements for MASTER OF SCIENCE degree IN ANIMAL SCIENCE, mainly: Animal Production.

MARINGÁ
State of Paraná
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Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

G633e

Gomes, Ana Luiza Mendonça
Effect of chemical additives on the conservation of tropical grass silage / Ana Luiza Mendonça Gomes. -- Maringá, PR, 2021.
52 f.figs., tabs.

Orientador: Prof. Dr. João Luiz Pratti Daniel.
Coorientador: Prof. Dr. Cloves Cabreira Jobim.
Dissertação (Mestrado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Zootecnia, Programa de Pós-Graduação em Zootecnia, 2021.

1. Digestibilidade. 2. Nitrito de sódio. 3. Capim tropical - Silagem. 4. Aditivos químicos. 5. Nutrição animal. I. Daniel, João Luiz Pratti, orient. II. Jobim, Cloves Cabreira, coorient. III. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. IV. Título.

CDD 23.ed. 636.085

Rosana de Souza Costa de Oliveira - 9/1366

UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

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Autora: Ana Luiza Mendonça Gomes
Orientador: Prof. Dr. João Luiz Pratti Daniel

TITULAÇÃO: Mestre em Zootecnia - Área de Concentração Produção Animal

APROVADA em 29 de outubro de 2020.

Prof. Dr. Thiago Fernandes Bernardes	Prof. Dr. Thiago Carvalho da Silva

Prof. Dr. João Luiz Pratti Daniel
Orientador

To my parents and sisters. My greatest examples and my foundation.

Love unconditional and eternal gratitude.

AKNOWLEDGEMENTS

To God for always guiding my steps and allowing me to accomplish and unimaginable achievements.

To my parents Sueli de Fátima Mendonça Gomes and Gercio Grosso Gomes for support and guide me throughout all my life. Thank for all their love and encouragement. You are everything for me!

To my sisters, Jaqueline and Maria Clara, for their immense companionship and for being light in my life.

To Egídia, my grandmother and my treasure. Thank you for always believing in me and for all the prayers.

To my supervisor Prof. Dr. João Luiz Pratti Daniel, for sharing with me knowledge and experiences. Thank you for your patience and friendship, for contributing with my professional and personal development.

To Prof. Dr. Clóves Cabreira Jobim and the Silage Study Group and Hay, which helped me so much in this work.

To Iguatemi's Experimental Farm employees and the technicians of LANA laboratory for all help.

To the CNPq foundation (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the master’s scholarship,

BIOGRAFY

ANA LUIZA MENDONÇA GOMES, daughter of Gercio Grosso Gomes and Sueli de Fátima Mendonça Gomes, was born on May 16, 1995.

Born in Araçatuba, in the interior of São Paulo state, she spent part of his childhood and teenager in Penápolis, SP.

In 2012, he finished high school at Centro Educacional SESI - 349, together with the Technical Course in Electro-electronics by the SENAI school, in Araçatuba - SP.

In 2018 she earned her bachelor's degree in Animal Science, at the State University of Maringá, Maringá - Paraná.

In 2020, she submitted to the examining board in order to receive the title of Master's in Animal Science.

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ABSTRACT

As producing tropical grass silage with high feeding value is still a challenge, we examined the effectiveness of sodium nitrite-based additives on guinea grass (*Megathyrsus maximum* cv. Mombaça) silage quality. The forage was mechanically harvested from four 3ha fields and divided into 5 piles per field to receive one of the following treatments (fresh matter basis): no additive (control), soybean hulls (100 g/kg; SH), sodium nitrite (1 g/kg; NIT), sodium nitrite (1 g/kg + hexamine (0.65 g/kg; NIT+HEX), and formic acid (85%) (4 mL/kg; FA). The SH and FA were included as positive controls. Sodium nitrite-based additives (sodium nitrite applied alone or in combination with hexamine) and FA were capable of decreasing clostridial development, resulting in lower concentrations of NH_3-N and n-butyric acid and reduced dry matter (DM) loss during fermentation, whereas protein quality and hygienic quality (reflected by lower *Clostridium* counts) were improved. A strong linear relationship was detected between the concentrations of butyric and valeric acids and DM losses during fermentation ($R^2 = 0.87$, $P < 0.01$). Addition of SH improved DM digestibility and slightly decreased fermentation losses, but it did not lead to butyric-free silages. The use of sodium nitrite-based additives was effective to improve the fermentation quality of tropical grass silage, and its combination with hexamine was superior to the sole use of sodium nitrite. All treatments improved *in vitro* dry mater digestibility over untreated silage. Only NIT, NIT+HEX and FA increased the concentration of rumen-undegradable protein, with NIT+HEX and FA outperforming NIT.

I. INTRODUCTION

1. Literature Review

1.1. Tropical grass silage

Tropical grasses are perennial or semi-perennial crops with high dry matter (DM) yield (e.g., 20–30 t/ha) and great potential as silage source (Pereira, 2004; Daniel et al., 2019). However, harvesting tropical grass with high nutritive value may result in excess of moisture and low soluble carbohydrates content and can impair the ensiling process (Bernardes et al., 2018; Silva et al., 2019). The mal fermentation may result in silages with high fermentative losses and low hygienic quality (McDonald et al, 1991).

Wilting is an interesting strategy to increase DM and improve silage fermentability in short and thin-stemmed crops, but wilting tall and thick-stemmed grasses is a challenge. Addition of absorbent ingredients (e.g. soybean hulls) also are used as way to decrease moisture content and supply nutrients to promote the development of beneficial -microorganisms and to enhance fermentation (McDonald et al., 1991).

1.2. Fermentability coefficient

Weissbach et al. (1974) coined the concept of fermentability coefficient (FC), that considers the contents of DM, soluble carbohydrates (SC) and buffering capacity (BC) of the pre-ensiled material [FC = DM (g/kg FM) + 80 × SC (g/kg DM) / BC (g/kg DM)]. Based on this model, forages with FC above 450 might result in well-fermented silages, while forages with FC below 350 are prone to fermentation problems.

Nonetheless, in a further development of models to predict the run of fermentation, Weissbach and Honig (1996) reported that 51% of silages with FC > 350 underwent butyric fermentation, which indicate that the FC as a sole index is not capable of predicting the run of fermentation. Yet, according the authors, LAB counts and nitrate concentration in the fresh crop also have a great effect on crop ensilage process. They inferred that materials with LAB counts below 10^5 LAB/g fresh matter (FM) and nitrate content below 0.5 g NO_3/kg DM have a high risk of clostridium development, regardless the FC. Under extensive farming management (low fertilizer input), Weissbach et al. (1993) also reported that 77% of grass silages produced with FC > 350 underwent butyric fermentation, regardless of LAB count. Those findings indicate that nitrate concentration at ensiling has a pronounced effect on the run of fermentation, as will be discussed below.

1.3. Nitrate and grass silage fermentation

Nitrate occurs naturally in green forages and its concentration depends of the vegetal organs of plants, growth conditions and N fertilization level (Weiss et al., 2006). Nitrate has important significance on the silage fermentation process (Wieringa, 1966) and it is necessary to be considered to estimate the ensiling potential of grasses. Nitrate acts as a natural inhibitor of clostridia, mainly during early phases of the fermentation process (Spoelstra, 1985). Optimum nitrate levels range from 4.4 to 13 g NO_3/kg DM (Wieringa, 1966; Kaiser and Weiss, 2004). Lower levels may not be enough to inhibit undesirable microorganisms (e.g. clostridia), whereas excessively high levels might buffering the silage and impair pH drop. When nitrate is present in grass within such optimal range, the prediction of minimal DM content [DMmin (g/kg FM) = 450 – 80 × SC/BC] developed by Weissbach et al. (1974) to prevent butyric fermentation is still valid. However, in grasses harvested with lower nitrate levels, the FC estimated by Weissbach et al (1974) is not suitable for rating the ensiling potential.

Considering the relatively high proportion of grasses with an enough FC (>350) that occasionally underwent butyric fermentation, Kaiser et al. (2002) developed new models to estimate the ensiling potential of temperate forages with low nitrate content. In their models, the minimum DM to obtain butyric-free silages was define as follows:

crops with low clostridial contamination at ensiling (<10^3 cfu/g FM), DM_{min} (g/kg FM) = 620 – 71 × SC/BC; and crops with high clostridial contamination at ensiling (≥10^3 cfu/g FM), DM_{min} (g/kg FM) = 890 – 130 × SC/BC. From those models, the DM required to prevent mal fermentation would exceed the acceptable values (e.g. 400 to 600 g/kg FM) to store forage as silage without increase the risk of aerobic deterioration, especially in horizontal-unwalled silos (drive over silos). Therefore, the adoption of chemical additives or the combination of wilting and chemical additives might be a reasonable strategy to prevent storage losses and produce grass silages with high feeding value.

1.4. Fermentation improvers

The main action of chemical additives is through the suppression or inhibition of undesirable microorganisms, such as clostridium, enterobacteria and listeria (Auerbach and Nadeau, 2019). The effect of formic acid, nitrate and hexamine will be discussed below.

1.4.1. Formic acid

During silage conservation, formic acid is used as dual purpose. It causes a drop in pH by direct acidification and has action on suppression of undesired spoilage bacteria (Auerbach and Nadeau, 2019). The immediate reduction in pH restricts activities of acid-sensitive epiphytic bacteria such as enterobacteria and aerobes, thus establishing conditions for LAB to develop quickly and dominate the silage (Kung et al., 2003). Additionally, the non-dissociated molecule of formic acid has a specific inhibitory effect, penetrating cell membrane by diffusion, releasing H + and acidifying the cytoplasm (Wooldford, 1975, Krebs et al., 1983, Lambert and Stratford, 1999, Warth, 1991).

Formic acid has been used as an additive in crops with low DM and sugar concentrations, in order to decrease pH rapidly (<4.2) and prevent clostridia development (Nadeau et al., 2000). As a result, formic acid induces water-soluble

carbohydrates and true protein preservation during ensiling and increase microbial protein synthesis in the rumen (Jaakkola et al., 2006, Muck et al., 2018). The intensity of declining pH post formic acid application is heavily dependent of dose level, water concentration and buffering capacity of forage crops (Kung et al., 2003). Application of a dose around 4 L/t FM of formic acid 85% has been common in countries with tradition in chemical additives (Auerbach and Nadeau, 2019).

Nowadays, the use of formic acid has decreased due to corrosive potential, which may cause occupational issues such as skin, eyes and lung irritations. In addition, it can occur damage to the machinery used in the silage process (Kung et al., 2003; Auerbach and Nadeau, 2019).

1.4.2. Sodium nitrite

Sodium nitrite ($NaNO_2$) is a reactive molecule with bacteriostatic and bactericidal activity. The main function of sodium nitrite is the suppression of undesirable spoilage bacteria, particularly at low pH levels (Woolford, 1975).

The antimicrobial action of nitrite is attributed to reactions associated with the generation of nitric oxide (NO) and probably peroxynitrite ($ONOO^-$) and peroxynitrous acid (ONOOH), which are strong oxidizing species in vivo (Majou and Christieans, 2018). The latter two compounds may oxidase zinc fingers, protein thiols, membrane lipids, cysteine and arginine biosynthesis and iron-sulfur proteins, and DNA bases, producing DNA strand breaks, which results in NAD+ and ATP consumption. The phosphoroclastic system is a major pathway to ATP synthesis in many clostridia and depends on the activity of two iron-sulfur enzymes, ferredoxin and pyruvateferredoxin oxidoreductase. These enzymes act in the transport of electrons linked to the ATP production from pyruvate. Nitric oxide inhibits the iron-sulfur enzymes (which are associated to electrons transport and ATP production) due to the formation of iron-NO complexes such as catalase, ferrochelatase and aconitase. The inhibitory action in the clostridium development is for the commitment of respiratory activity, inhibiting them (Carpenter et al, 1987; Majou and Christieans, 2018). Sodium nitrite has a wide antimicrobial spectrum and showed a marked increase in activity with a reduction in

pH. On the other hand, yeasts demonstrated a high resistance to it, even at pH 4 and 3 (Wooldford, 1975).

1.4.3. Hexamine

Hexamethylene tetramine (hexamine) is a bacteriostatic agent and in acidic conditions, gradually decomposes in ammonia and formaldehyde (Restani et al., 1992). The formaldehyde reduces protein degradability by forming crosslinks between protein chains and have antimicrobial properties due to the ability to inactivate certain macromolecules, such as protein and nucleic acids (Woolford, 1975, Aurelli et al., 2011).

1.4.4. Combination of sodium nitrite and hexamine

The aim of combining nitrite and hexamine is to protect all fermentation phases. Nitrite has an action during early phases whereas the formaldehyde derived from hexamine has an antimicrobial action posteriorly, after pH drops. Nitrite plus hexamine improves the silage quality when compared with use of sole sodium nitrite, and some trials showed a synergetic effect of hexamine and sodium nitrite (Hellberg, 1967, Auerbach and Nadeau, 2019). The combination of nitrite and hexamine have the potential to improve the fermentation pattern, reducing butyric acid production and proteolysis during fermentation, resulting in silages with lower dry matter losses, desirable hygienic quality and feeding value (Weissbach and Auerbach, 2012).

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II. Effect of sodium nitrite-based additives on the conservation and nutritive value of guinea grass silage

(Manuscript formatted according to Animal Feed Science and Technology)

Abstract

As producing tropical grass silage with high feeding value is still a challenge, we examined the effectiveness of sodium nitrite-based additives on guinea grass (*Megathyrsus maximum* cv. Mombaça) silage quality. The forage was mechanically harvested from four 3-ha fields and divided into 5 piles per field to receive one of the following treatments (fresh matter basis): no additive (control), soybean hulls (100 g/kg; SH), sodium nitrite (1 g/kg; NIT), sodium nitrite (1 g/kg + hexamine (0.65 g/kg; NIT+HEX), and formic acid (85%) (4 mL/kg; FA). The SH and FA were included as positive controls. Sodium nitrite-based additives (sodium nitrite applied alone or in combination with hexamine) and FA were capable to increase curtailing clostridial development, resulting in lower concentrations of NH_3-N and n-butyric acid and reduced dry matter (DM) loss during fermentation, whereas protein quality and hygienic quality (reflected by lower *Clostridium* counts) were improved. A strong linear relationship was detected between the concentrations of butyric and valeric acids and DM losses during fermentation ($R^2 = 0.87$, $P < 0.01$). Addition of SH improved DM digestibility and slightly decreased fermentation losses, but it did not lead to butyric-free silages. The use of sodium nitrite-based additives was effective to improve the fermentation quality of tropical grass silage, and its combination with hexamine was superior to the sole use of sodium nitrite. All treatments improved *in vitro* dry mater digestibility over untreated silage. Only NIT, NIT+HEX and FA increased the concentration of rumen-undegradable protein, with NIT+HEX and FA outperforming NIT.

Keywords: fermentation quality, formic acid, hexamine, sodium nitrite, soybean hulls, tropical grass

Abbreviations: ADF, acid detergent fiber expressed inclusive of residual ash; aNDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; BC, buffering capacity; CFU, colony-forming units; CON, control; CP, crude protein; DM, dry matter; DM_{min}, minimum dry matter; FA, formic acid; FC, fermentability coefficient; NH_3-N, ammonia nitrogen; NH_3-N_{corr}, ammonia nitrogen corrected for addition of nitrogen by additives; LAB, lactic acid bacteria; NIT: sodium nitrite; NIT+HEX: sodium nitrite plus hexamine; RDP: rumen degradable protein; RUP: rumen undegradable protein; SC: soluble carbohydrates; SD, standard deviation; SE, standard error of the mean; SH, soybean hulls.

Introduction

Tropical grasses are perennial or semi-perennial crops with high dry matter (DM) yield (e.g., 20-30 t/ha per year) high regrowth vigor and high adaptation to different climate and soil conditions resulting in lower agronomical risks (Da Silva et al., 2009; Jank et al., 2010). Therefore, tropical grasses have huge potential as ensiled forage source. Although research on silage production from tropical grasses is not recent (Condé, 1970), achieving high quality tropical grass silage at farm level remains a challenge (Daniel et al., 2019).

In Brazil, approximately 24% of dairy farms (Bernardes and do Rêgo, 2014) and 9% of beef feedlots utilize tropical grass silage (Pinto and Millen, 2018), with *Megathyrsus* and *Urochloa* genera predominating. In the meantime, consultants have claimed that tropical grass silage usage has declined in the last decade, in some way because of the struggles faced during mechanical harvesting but mainly due to high storage losses and low feeding value caused by undesirable fermentations, and too a late harvest beyond the optimal stage of maturity.

Although tropical grass silages can be used as a second forage source in addition to whole-plant corn silage in dairy diets (Daniel et al., 2019), only very recently has its use attracted attention by beef farmers, mainly due to the backgrounding intensification (from weaning until the start of the finishing period). In growing rations, the use of tropical grass silage enables nutritionists to formulate diets that balances energy intake, thereby avoiding that cattle gain weight too quickly, which negatively impact carcass weight as animals reach their mature size (Owens et al., 1995; Taylor et al., 2015). Also, dairy heifers might be raised under controlled energy diets, which will reduce fat deposition and avoid negative effects on the future lactation performance (Drackley,

2008). Therefore, this demand now requires resumption of research to find effective strategies to solve the problem of poor fermentation quality in tropical grass silage.

Current strategies used to improve the fermentation in tropical grass silages have not led to consistent results. Although feasible, wilting is challenging in tall and thick-stemmed tropical grasses during the summer. Addition of absorbents consistently decreases effluent formation but turns the ensiling process more complex in large operations. Moreover, improving crop ensilage process through a sole increase in DM content has not always guaranteed good silage preservation (Weissbach et al., 1993; Weissbach and Honig, 1996; Kaiser et al., 2002). Inoculation with lactic acid bacteria (LAB) have improved fermentation in some studies (Santos et al., 2014), but their benefits are inconsistent mainly when the crop had a high moisture content at ensiling (Tomaz et al., 2018; Gouvea et al., 2020).

Chemical additives are widely used for silage making from temperate grasses. For a long time since the mid 20th century, the use of formic acid dominated in countries with long tradition in silage making, e.g. Scandinavian countries, but its corrosivity on metal, skin and eyes damages machinery and poses serious health risks to workers, leading to a decline in popularity (Auerbach and Nadeau, 2019). Alternatively, chemical additives based on sodium nitrite were introduced to the market place in the mid 1980ies, which have the ability to inhibit butyric fermentation without the concerns reported for formic acid (Weissbach et al., 1989a; Weissbach and Auerbach, 2012). However, to the best of our knowledge there is no information available about the effects of sodium nitrite-based additives in tropical grass silage. Thus, the objective of this study was to examine the effectiveness of sodium nitrite-based additives on fermentation, aerobic stability and nutritive value of guinea grass silage. We hypothesized that, as known for temperate grasses, sodium nitrite-based additives would elucidate the same effects as formic acid

also in tropical grasses and could therefore be a useful and a practical management tool to ensure high silage quality.

Material and Methods

Ensiling and sampling

Four 3-ha fields of guinea grass (*Megathyrsus maximus* cv. Mombaça), which had been in use for three years at the Estância Independente Farm (Mandaguari, PR, Brazil), were harvested with a double-chop flail pull-type forage harvester (CFC-1800, Casale, São Carlos, Brazil) on February of 2019, after 45 d of regrowth. At harvest, canopy and stubble heights were 120 cm and 15 cm, respectively. The fields received annual nitrogen fertilization by urea at 200 kg/ha and by cattle slurry (30 to 40 m^3/ha). The chemical and microbiological composition of guinea grass at harvest is shown in Table 1.

Direct cut chopped forage from each of the four field replicates was divided into five piles (1.5 kg per pile). Subsequently, each pile received one of the following treatments (fresh matter basis): no additive (control; CON), soybean hulls (100 g/kg; SH), sodium nitrite (1 g/kg; NIT), sodium nitrite (1 g/kg) + hexamine (0.65 g/kg; NIT+HEX), and formic acid (85%, 4 mL/kg; FA). The SH and FA treatments were included as positive controls, as soybean hulls have been widely used to increase DM content of tropical grass at ensiling, and FA has been considered a standard additive to prevent *Clostridium* development, to preserve protein and to secure the hygienic quality of temperate grass silages. All dilutions were made using the same volume of water (15 mL/kg) to avoid that the chemical additive use does not affect the DM content at ensiling. Control and SH treatments also received the same volume of water. Samples

from each pile were collected for analysis of DM, soluble carbohydrates (SC), and buffering capacity (BC).

Treated forage was then ensiled in vacuum sealed nylon-polyethylene bags (33 × 45 cm, 160 μm thickness), with 1 kg per bag and with 4 replicates per treatment, totaling to 20 silage bags. After 101 d of storage in a closed barn (16 to 32°C), silos were weighed to calculate the fermentation losses. Gas loss was computed as the mass loss in proportion of the DM ensiled, whereas DM loss was calculated as the difference between the amount of DM ensiled and DM recovered in proportion of DM ensiled. Silage samples (170 g) were collected for drying at 60°C for 72 h and to prepare aqueous extracts (30 g of silage + 270 g of distilled water, blended for 2 min and filtered through four layers of cheese cloth). The remaining material (800 g) was used to determine aerobic stability.

For the aerobic stability test, silages were transferred to 3-L plastic buckets with a data logger placed in the center of the silage mass. Two additional data loggers were set to record room temperature (25 ± 1.8°C). The data loggers were programmed to record the temperature of the room and the silages every 15 min for 10 d. Aerobic stability based on temperature rise was defined as the time elapsed until silage temperature reached 3°C above the room temperature (Honig, 1990). On each morning of the 10 d aeration period, sub-samples (10 g) were collected from each bucked, approximately 12 cm from the top surface, for measuring silage pH during the aerobic exposure. The pH was measured in aqueous extract prepared with 10 g of silage + 90 g of distilled water blended for 2 min and filtered through four layers of cheese cloth. Aerobic stability based on pH rise was defined as the time elapsed until silage pH increased by 0.5 unit.

Laboratorial analysis

Microbial counts (yeasts and molds, lactic acid bacteria (LAB) and clostridia) were evaluated in a serial dilution of the aqueous extract. Microorganisms were enumerated in Petri dishes with selective media. Malt Extract Agar (M137, Himedia®, 632 Mumbai, India) acidified to pH 3.5 with lactic acid was used for enumeration of yeasts and molds, and De Man Rogosa and Sharp (7543A, Acumedia®, Lansing, Michigan, USA) was used for enumeration of LAB. The plates were incubated aerobically at 30°C for 48 h before counting yeasts and LAB and for 72 h before counting molds. Reinforced Clostridium Agar (M154, Himedia®, 632 Mumbai, India) supplemented with neutral red and D-cycloserine (Jonsson, 1990) was used for enumeration of clostridia after 5 d of incubation at 37°C. Colony-forming units (CFU) were counted and expressed as log10.

Fresh crop samples, dried and ground at 1-mm, were used for measuring the concentration of soluble carbohydrates (SC) by the phenol-sulfuric method (Hall, 1999), buffering capacity (BC, g lactic acid/ kg DM; Weissbach, 1967), and nitrate (Beutler et al., 1986). Fermentability coefficient (FC) and minimum DM content (DMmin) required to obtain butyric acid-free silage were calculated as follows: FC = DM (g/100 g FM) + 8 × SC/BC (Weissbach et al., 1974); DMmin Weissbach (g/kg) = 450 – 80 × SC/BC (Weissbach et al., 1974); DMmin Kaiser (g/kg) = 620 – 71 × SC/BC (Kaiser et al., 2002; for crop with ≤1 g NO3/kg DM and low count of clostridium spores).

Silage sub-samples were dried and ground (1-mm, Wiley mill) and analyzed for DM (AOAC, 1990), ash (AOAC, 1990), crude protein (CP; AOAC, 1990), neutral detergent fiber (aNDF; assayed with a heat stable amylase and sodium sulfite and expressed inclusive of residual ash; Mertens, 2002), acid detergent fiber (ADF; assayed sequentially and expressed inclusive of residual ash; Van Soest, 1973), soluble

carbohydrates (Hall, 1999), soluble protein (Krishnamoorthy et al. 1982) and acid-detergent insoluble nitrogen (ADIN) and neutral-detergent insoluble nitrogen (NDIN) contents (Licitra et al., 1996). Nitrogen fractionation was determined according to CNCPS v. 6.5 (A1, A2, B1, B2 and C fractions) (Van Amburgh et al., 2015). From nitrogen fractionation, the proportion of rumen degraded protein (RDP) and rumen undegraded protein (RUP) were calculated using the first-order approach [kd/(kd + kp)] for a mid-lactation dairy cow (Van Amburgh et al., 2015). Fractional passage rates (liquid, concentrate and forage) were estimated assuming 20 kg/d DM intake, 50% dietary forage level and 600 kg shrunk body weight (Tylutki et al., 2008).

A sample of undiluted aqueous extract was centrifuged (10,000 x g for15 min) and the supernatant was used for analyses of fermentation end-products. The contents of lactic acid (Pryce, 1969) and ammonia (Chaney and Marbach, 1962) were determined by colorimetry. For treatments containing nitrite-based additives, ammonia concentration was corrected considering that the conversion of nitrite and hexamine to ammonia, were 50 and 90%, respectively. The other fermentation products were analyzed by gas chromatography (GCMS 628 QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column Stabilwax, Restek, 629 Bellefonte, PA; 60 m, 0.25 mm ø, 0.25 μm crossbond arbowax polyethylene glycol). The DM content was corrected for loss of volatile compounds during oven drying (Weissbach and Strubelt, 2008).

Statistical analysis

Statistical analyses were performed using the MIXED procedure of SAS (version 9.4, SAS Institute, Cary, NC, USA). The model included a random effect of field and a fixed effect of treatment. Treatment means were compared by the Tukey-Kramer test ($\alpha = 0.05$ and $\alpha = 0.10$). Silage pH during aerobic exposure was analyzed as

repeated measures. The interaction between treatment and silo was used as error term. The covariance structure based on the lowest corrected Akaike information criterion was unstructured (UN). The relationships between silage characteristics were evaluated using the REG procedure of SAS.

Results

Forage characteristics

Fermentability traits of the forages after additive application are shown in Table 2. The inclusion of SH increased the DM content compared with the other treatments. The SC content was similar among treatments, whereas BC was highest for NIT+HEX followed by NIT. The fermentability coefficient was greatest for SH treatment. The DM_{min} required to obtain butyric acid-free silage estimated by the equations of Weissbach et al. (1974) and Kaiser et al. (2002) was highest for NIT and NIT+HEX but they did not differ from CON and SH, whereas FA application resulted in the lowest value of DM_{min}.

Silage characteristics

Silage microbial counts, fermentation profile and aerobic stability are shown in Table 3. The counts of LAB and yeasts were lowest in untreated guinea grass silage and the addition of NIT, NIT+HEX and FA resulted in lower *Clostridium* counts when compared with CON and SH. Molds were always below the detection limit of log 2 CFU/g, regardless of additive treatment.

The pH was higher in SH and NIT+HEX than in FA silage, and no differences were detected between NIT+HEX and NIT and CON. The FA application led to the lowest pH, which did not differ from CON. All fermentation products were affected by

additive treatment, except ethyl acetate,1,2-propanediol, ethyl lactate and propyl acetate. When compared with CON, total NH_3-N was decreased by SH and chemical additives, with the lowest value observed in FA silage. Correction for nitrogen applied with the sodium nitrite-containing additive resulted in the treatment NIT+HEX with the lowest concentration of NH_3-N_{corr}. Silage treated with FA and NIT+HEX had the highest concentrations of lactic acid, whereas NIT had an intermediate value and did not differ from SH. The control had the lowest concentration of lactic acid, but it was similar to SH. The use of FA decreased the acetic acid concentration when compared with CON, SH and NIT.

The n-butyric acid concentration was greater in CON followed by SH. Silages treated with NIT+HEX, FA and NIT had low concentrations of this compound (<3 g/kg DM). The 2,3-butanediol and propionic acid were higher in CON followed by SH, whereas the chemical additives depressed the formation of those compounds. Greater concentrations of i-butyric, i-valeric and n-valeric acids were found in SH followed by CON. The NIT+HEX and FA were the only treatments capable of suppressing the formation of n-propanol. Untreated silage had the highest undissociated VFA concentration, followed by NIT and SH, whereas FA and NIT+HEX resulted in the lowest undissociated VFA concentration.

When compared with CON, all additives reduced gas loss, with lower values for chemical additives than SH, and NIT+HEX being more effective than FA and NIT. Untreated silage showed the highest DM loss during fermentation and the use of NIT+HEX caused the largest reduction of all additives. A positive linear relationship ($R^2 = 0.87$, $P < 0.01$) was detected between the sum of i-butyric, n-butyric, i-valeric and n-valeric acids and the DM loss during fermentation (Figure 2). Including the traits n-propanol, 2,3-butanediol and propionic acid in the regression model in addition to

198 butyric and valeric acids improved the relationship ($R^2 = 0.88$, $P < 0.01$, RMSE = 10.0,
199 $Y = 47.2 + 3.08 \times X$). No temperature increase was observed in any of the silages
200 during the entire duration of the aerobic stability test of 10 d. However, in silages
201 treated with NIT+HEX, NIT and FA pH increased after 6 or 7 d of aerobic exposure
202 (Figure 1). A positive linear relationship ($R^2 = 0.55$, $P < 0.01$) was detected between the
203 ratio of total undissociated VFA concentration and the sum of SC and lactic acid
204 content and the aerobic stability based on pH rise (Figure 3).
205 Data about chemical composition and nutritive value of guinea grass silage is
206 presented in Table 4. Treatment SH showed the highest DM and the lowest ash
207 concentrations. Treatment with FA decreased aNDF content, but this did not differ from
208 SH, and a trend was observed for lower NDF concentration in FA silage when
209 compared with CON ($P = 0.07$). Regardless of additive type, all chemically treated
210 silages had a lower ADF content when compared with CON and SH. The SC
211 concentration was higher in silages treated with chemical additives, with the highest
212 value observed in FA followed by NIT+HEN and then NIT. Untreated silage had the
213 lowest CP content, whereas SH addition increased it over all treatments. Chemical
214 additives decreased the proportion of soluble CP, with the lowest values detected in
215 NIT+HEX and FA. The N fractionation revealed that CON had a greater proportion of
216 A1 and the lowest proportions of B2 and C fractions among all treatments. Addition of
217 SH increased the proportion of A2 (non-ammonia soluble protein) over silages treated
218 with sodium nitrite-containing additives. In addition, it also reduced the proportions of
219 B1 when compared with NIT and of B2 (insoluble potentially digestible protein) over
220 NIT, NIT+HEX and FA. The highest proportion of fraction C (indigestible protein) was
221 detected in NIT+HEX silages. The proportion of RDP was greatest in CON, followed

by SH and NIT, whereas the use of NIT+HEX and FA resulted in the largest proportion of RUP.

The IVDMD was increased by SH addition and all chemical additives. Recovery of digestible DM was improved over CON by SH and NIT, and the highest value was detected in silage treated with NIT+HEX and FA.

Discussion

Crop characteristics, fermentation pattern, dry matter loss and aerobic stability of guinea grass silage

Based on the evaluated chemical and microbiological traits of guinea grass at ensiling, the forage can be considered of typical composition. Our DM data at ensiling and nutrient composition substantiate previous observations by Kotha et al. (2018), and by Tomaz et al. (2018), who monitored the chemical changes in guinea grass as affected by sward heights. The FC, which is used mainly to predict the risk of poor fermentation quality caused by clostridia (Weissbach et al., 1974), in the untreated forage and in the forage that received chemical additives were similar to the values reported by Tomaz et al. (2018) for the sward height used in our study. The increased FC by SH inclusion can be explained by the higher DM caused by this treatment. Although the FC increase induced by SH (from 34 to 39.5) slightly reduced DM losses, such improvement in FC was not enough to prevent clostridial fermentation.

Beyond the FC, forage nitrate concentration plays a major role in inhibiting clostridia during the early fermentation stages due to its microbial degradation into nitrite and NO_x (nitric and nitrous oxides), which are potent clostridial inhibitors (Wieringa, 1966; Spoelstra, 1985; McDonald et al., 1991). Under extensive farming

management, with low input of fertilizers and, in turn, low nitrate content in crop, Weissbach et al. (1993) reported that 77% of grass silages with FC > 35 underwent butyric fermentation, regardless of LAB count.

More evidence, however, suggests an interaction between nitrate and epiphytic LAB number based on silages produced from 244 different forages (including 195 from grasses produced at different N fertilization levels) grown in a temperate climate (Weissbach and Honig, 1996). These authors showed that the highest risk of butyric acid formation (78% frequency of butyric acid-containing silages) existed when forage had $<10^5$ cfu epiphytic LAB/g and <0.5 g NO_3/kg at ensiling. When each factor was considered individually, 26% of the silages made from forage high in nitrate (> 1 g/kg DM) and 4% of the silages produced from forage with high epiphytic LAB count (> 10^6 cfu/g) were free of butyric acid. As the forage used in our study had an epiphytic LAB count in excess of 10^6 cfu/g, the risk of poor fermentation quality should have been low given that the observations from temperate grasses are also applicable to tropical species. However, this was not the case, as untreated silages clearly showed characteristics typical for clostridial fermentations. Namihira et al. (2010) showed that nitrate levels in guinea grass depended on N fertilization rate and that forage nitrate concentration was strongly negatively related with n-butyric acid formation in the silage (r = -0.97).

Despite the high epiphytic LAB count, which was much higher than found in guinea grass and Napier grass by Khota et al. (2018), CON and SH silages were prone to *Clostridium* development, as reflected by the presence of n-butyric acid at significant concentrations. Even higher butyric acid levels exceeding 30 - 50 g/kg DM in guinea grass silages have been reported previously but forage epiphytic LAB counts were not presented (Namihira et al., 2010; Tomaz et al., 2018). Likely, epiphytic LAB population

may not have a sufficiently high proportion of species that can convert plant sugar efficiently into lactic acid (homofermentative LAB), thereby reducing the pH rapidly and significantly preventing clostridial development. Additionally, LAB present on the crop may not have been capable of thriving competitively due to excessively high moisture content (Pahlow and Weissbach, 1999). Nevertheless, our findings are in line with the lack of consistency with the application of inoculants containing LAB in tropical grass silages (Igarasi, 2002; Ribeiro et al., 2009; Tomaz et al., 2018).

Models developed by Kaiser et al. (2002) for predicting the ensiling potential of temperate grasses indicate that for crops with low nitrate content (≤ 1 g/kg DM), the minimum DM content required to obtain butyric acid-free silages is much higher than that with adequate nitrate content. In our study, the guinea grass with a low nitrate content (0.173 g/kg DM) would have needed a $DM_{min} \geq 530$ g/kg to prevent *Clostridium* development (Kaiser et al., 2002), which is challenging to attain in most practical conditions with tall and thick stemmed tropical grass silages. Moreover, such high DM level might be not recommended for practical silage making, due to the increased risk of loss by aerobic deterioration (Wilkinson and Davies, 2013), mainly when silage is intended to be stored in horizontal silos, especially in unwalled horizontal silos.

In addition to high concentrations of butyric and valeric acids, untreated silages showed traits that are characteristic for *Clostridium* growth in silage made from tropical and temperate grasses as well as from alfalfa, e. g. low concentrations of lactic acid and relatively high acetic acid content as well as an elevated proportion of NH_3-N (Namihira et al. 2010; Tomaz et al., 2018; Auerbach et al., 2016). Although we only measured fermentation characteristics after an extended storage period, it is likely that, due to the very low nitrate concentration in the forage, *Clostridium* proliferation was supported

297 already from the early stages of fermentation, as was shown by Namihira et al. (2010).
298 Although Tomaz et al. (2018) showed good effects of using an absorbent (i.e., citrus
299 pulp) to improve guinea grass fermentation, the SH addition did not result in
300 satisfactory silage quality in our study. This finding, in addition to challenges to the
301 ensiling management, leads us to question the feasibility of this strategy to enhance
302 silage fermentation quality, especially for larger farm operations.

303 To our best knowledge for the first time on tropical grass, we confirmed
304 observations from temperate forages that the application of sodium nitrite-containing
305 additives and formic acid are successful to suppress clostridial development for an
306 extended storage period with no difference between NIT+HEX and FA. Weissbach et
307 al. (1989b) and Reuter and Weissbach (1991) showed in a total of 143 trials performed
308 between 1984 and 1989 that the combination of sodium nitrite (900 g/t) and hexamine
309 (600 g/t) was as effective as 4 L/t of formic acid (85%) in reducing the *Clostridium*
310 spore load and in restricting the metabolic activity of clostridia during fermentation.
311 Obviously, in our study the combination of NIT+HEX was superior to NIT in terms of
312 fermentation process efficiency as reflected by DM losses and the extent of proteolysis
313 despite no, or not biologically relevant, differences in the concentrations of butyric and
314 valeric acids. Moreover, based on 14 trials with a total of 42 observations, Weissbach
315 (personal communication) showed that the individual use of sodium nitrite (1 g/kg) or
316 hexamine (0.6 g/kg) produced silage with higher mean butyric acid content and a lower
317 frequency of butyric acid free silages than was found for the combination of both
318 chemicals used at the given application rate. This contradicts observations from one trial
319 by König et al. (2019) who found no additional benefit of combining sodium nitrite with
320 graded doses of hexamine, but their forage had a high nitrate content (3.8 g/kg DM),

resulting in the formation of only small butyric acid concentrations (< 3 g/kg DM) even when the forage did not receive any additive treatment.

Regardless of treatment, yeast numbers were higher at silo opening (> log 4.3 cfu/g) than at ensiling (log 3.1 cfu/g) indicating a development during storage. This observation was unexpected due to the presence of a high concentration of total VFA acids with antifungal activity (C_2 to C_5) in all silages although the composition of total VFA differed between treatment, with CON and SH only containing significant quantities of butyric and valeric acids. According to Woolford (1975), the inhibitory effect on yeasts increases with increasing chain length. We can only speculate why yeasts could develop and survive during storage because there is no information available when those acids were produced, either already during the initial stages of fermentation by conversion of plant sugar, or during the later phase by utilizing lactic acid. Despite the relatively high yeast counts compared with the threshold value of 10^5 cfu/g signifying a high risk of aerobic instability (Jonsson and Pahlow, 1984), all silages remained stable throughout the 10 d of air exposure when aerobic stability was evaluated using the temperature method by Honig (1990). As stated by Jonsson and Pahlow (1990), strictly anaerobically stored silages may not heat-up even in the presence of high yeast numbers when lactate-utilizing species were absent, which we did not test. In support of this, da Silva et al. (2020) found a positive effect of a chemical silage additive with antimycotic action on aerobic stability despite similar yeast counts than that of untreated whole-plant corn silage, which received air treatment for 2 h/wk over 63 d of storage. They explained this finding with the additive effect on the yeast population composition, causing a shift from non-lactate utilizing *Candida pumulis* in untreated silage to lactate-assimilating *Pichia kudriavzevii* in treated silage. Additionally, more heat is required in wet silage than in drier silage (Wilkinson and

346 Davies, 2013), which, in turn, requires high concentrations of utilizable substrate
347 (mainly sugar and lactic acid) to produce enough heat to detect a temperature increase.
348 Recently, Auerbach and Nadeau (2020) found a strong positive relationship ($R^2 = 0.67$,
349 $P < 0.01$) between the total concentration of water-soluble carbohydrates and lactic acid
350 at silo opening and the extent of aerobic deterioration (based on cumulated
351 temperature).

352 Although temperature remained unchanged during the aeration in all silages,
353 changes in another indicator for aerobic microbial activity (i.e., pH) were observed in
354 treatments NIT, NIT+HEX and FA when compared with CON, resulting in at least 2.25
355 d earlier onset of aerobic instability based on a pH increase of ≥ 0.5 units. Previous
356 studies also reported the occurrence of aerobic deterioration without detection of heat
357 production in tropical grass silage (Bernardes et al., 2007). Despite being undetectable
358 at silo opening, molds may have developed during air exposure due to its higher
359 resistance to antimycotic VFA compared with yeasts (Woolford, 1975), but it cannot be
360 ruled out that also aerobic bacteria have played a role. The generally slower growth of
361 molds compared with yeasts may explain why it took at least 6 to 7.75 d (treatments
362 NIT, NIT+HEX and FA) to reach a pH, which was at least 0.5 units higher than that
363 measured at silo opening. Unfortunately, we did not measure mold counts during
364 aerobic exposure. More studies on tropical grass silage are warranted to better
365 understand the process of aerobic instability and aerobic deterioration, respectively, and
366 the microorganism causing pH increases without heat detection. The pH difference used
367 can help signifying aerobic spoilage in tropical grass silage and may be a more suitable,
368 more reliable and more robust indicator for aerobic microbial activity than is heat
369 production. The power of the relationship between the ratio of the total concentration of
370 undissociated VFA and utilizable substrate (soluble carbohydrates and lactic acid) and

371 the time elapsed to attain a pH increase by 0.5 unit offered a plausible explanation for
372 the findings in our study. The higher the pH-dependent concentration of antimycotic
373 VFA and the lower the quantity of substrates utilizable by fungi, the faster a pH
374 increase is detected. At a given concentration of undissociated VFA, a higher substrate
375 concentration will result in a smaller ratio and, thus, in a faster onset of aerobic
376 deterioration, likely leading to a greater extent of spoilage. More studies are needed to
377 confirm whether this index [the ratio of the total concentration of undissociated VFA
378 and utilizable substrate (soluble carbohydrates and lactic acid)] is suitable to predict
379 aerobic stability in other silage types (e.g., whole-plant corn silage, sorghum silage,
380 sugarcane silage, legume silages, high moisture corn, etc.).
381
382 *Nutritive value of guinea grass silage*
383 As the course of the fermentation process of tropical grass silage has been
384 unpredictable under research and farm conditions (Daniel et al., 2019), there is a high
385 risk of producing poorly fermented forages leading to poor silage intake and animal
386 performance (Krizsan and Randby, 2007; Restle et al., 2003; Auerbach et al., 2012;
387 Santos et al., 2016). In this study, we demonstrated that the IVDMD of guinea grass
388 silage was improved by SH addition over untreated silage. However, it should not be
389 ignored that digestible nutrients were added to the forage before ensiling by SH
390 supplementation. Considering the SH inclusion rate of 100 g/kg and an average of 80%
391 IVDMD in SH (Mohammadzadeh et al., 2007), the improvement of IVDMD over
392 untreated silage (4.9 percentage units) can be explained simply by the SH addition.
393 Thus, the degradation extent of digestible nutrients process in the forage fraction of SH
394 treatment was similar to that of untreated silage. On the contrary, all chemical additives
395 improved IVDMD and SC, which can be attributed to the protection of readily available

nutrients from degradation and the lower ADF concentration (Mills and Kung, 2012). This assumption is supported by the much higher recovery of digestible DM after long storage, especially in treatments NIT+HEX and FA.

Although CP concentration in tropical grasses is lower than in temperate species, it can still be important and valuable to contribute to meeting the dietary protein requirements of ruminant categories (e.g., growing cattle). Silva et al. (2009) showed that N fractions in guinea grass depended on N fertilization rate and cutting heights, with protein soluble fraction A decreasing with increasing N fertilizer application and cutting heights, fraction B_1 remaining unaffected, and other fractions being altered at varying extent. To our knowledge, this is the first study to show the effects of additives on N fractions in guinea grass silage. Of all chemical additives, treatments NIT+HEX and FA reduced the RDP concentration simultaneously increased the RUP fraction by about 61-62 g/kg CP, which could have a significant effect on diet formulation and diet-related feeding costs. In support of studies about positive effects of chemical additives on N fractions in silages from temperate grasses (Broderick et al., 2007; Nadeau et al., 2014; Nadeau et al., 2015), these changes associated with the use of chemical additives may have the potential to improve animal performance, but *in vivo* trials are warranted to test this hypothesis and are considered of great scientific and commercial merit. Although additional costs are incurred by silage additive use, a return-on-investment scenario could prove the use of chemical additives economically feasible. Considering the large reduction of DM losses by NIT+HEX compared with untreated (71 g/kg DM), it seems that there is an opportunity by using additives such as NIT+HEX for ensiling tropical grasses. More so, the effect of additives on protein quality (e.g., RUP) in guinea grass silage must be considered. Improved silage protein quality by additive use may enable farmers to partially replace protein meals, and thus save feed costs. Overall, reducing

DM losses and improving protein quality may likely save more feed cost than the additive cost.

Conclusion

Formic acid and additives containing sodium nitrite alone or in combination with hexamine were efficient in controlling clostridial fermentations in tropical grass silage, reducing the fermentation losses, improving the nutritive value and securing the hygienic quality of silages, whereas soybean hulls only slightly improved fermentation and did not control *Clostridium* development. More studies on the effects of chemical additives on tropical grass fermentation are warranted and animal studies are required to evaluate their potential to reduce feed costs by partially replacing protein meals in ruminant diets.

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637 **Tables**

638

639 **Table 1** Chemical and microbiological composition of fresh guinea grass at harvest
640 before additive application (n = 4)

Item	Mean	SD
DM[2], g/kg	247	4.8
Crude protein, g/kg DM	77.1	1.16
Soluble CP, g/kg CP	21.4	2.36
aNDF[3], g/kg DM	711	6.0
ADF[4], g/kg DM	385	5.1
Ash, g/kg DM	109	1.7
Soluble carbohydrates, g/kg DM	44.0	3.58
Buffering capacity, g/kg DM	43.9	1.00
Nitrate, g/kg DM	0.173	0.0058
Lactic acid bacteria, log cfu/g	6.11	0.032
Clostridia, log cfu/g	2.48	0.231
Yeasts, log cfu/g	3.10	0.128
Molds, log cfu/g	3.12	0.239

641 [1]Standard deviation.
642 [2]Dry matter.
643 [3]Neutral detergent fiber.
644 [4]Acid detergent fiber.

645 **Table 2** Fermentability traits of guinea grass forage after additive application (n = 4)

Item	Treatment[1]					SEM[2]	*P*-value
	CON	SH	NIT	NIT+HEX	FA		
DM[3], g/kg	247^{b}	307^{a}	245^{b}	239^{b}	240^{b}	3.3	<0.01
SC[4], g/kg DM	43.9	42.3	42.2	43.2	48.2	1.96	0.23
BC[5], g/kg DM	37.6^{c}	38.2^{c}	41.3^{b}	45.0^{a}	38.2^{c}	0.56	<0.01
SC:BC ratio	1.17^{ab}	1.11^{ab}	1.02^{b}	0.96^{b}	1.26^{a}	0.050	<0.01
Fermentability coefficient[6]	34.0^{b}	39.5^{a}	32.6^{b}	31.6^{b}	34.1^{b}	0.59	<0.01
$DM_{min\ Weissbach}$[7], g/kg	356^{ab}	361^{ab}	368^{a}	373^{a}	349^{b}	4.0	<0.01
$DM_{min\ Kaiser}$[8], g/kg	537^{ab}	541^{ab}	548^{a}	552^{a}	530^{b}	3.5	<0.01

646 [1]CON: without additive, SH: soybean hulls at 100 g/kg, NIT: sodium nitrite at 1 g/kg, NIT+HEX: Sodium
647 nitrite at 1 g/kg + Hexamine at 0.65 g/kg, FA: Formic acid 85% at 4 mL/kg.
648 [2]Standard error of the mean.
649 [3]Dry matter.
650 [4]Soluble carbohydrates.
651 [5]Buffering capacity.
652 [6]FC = DM (g/100 g) + 8 × SC/BC.
653 [7]Minimum DM content to prevent butyric fermentation according to Weissbach et al. (1974; $DM_{min\ Weissbach}$ = 450 - 80 × SC/BC).
655 [8]Minimum DM content to prevent butyric fermentation according to Kaiser et al. (2002; $DM_{min\ Kaiser}$ = 620 - 71 × SC/BC; for crop with ≤1 g NO_3/kg DM and low count of clostridium spores).
657 [a,b,c] Tukey test ($\alpha = 0.05$).

Table 3 Microbial counts, fermentation profile, aerobic stability and losses of guinea grass silages stored for 101 d (n = 4)

	Treatment[1]						
Item	CON	SH	NIT	NIT+HEX	FA	SEM[2]	*P*-value
Lactic acid bacteria, log cfu/g	6.71^{b}	7.55^{a}	8.17^{a}	8.12^{a}	8.20^{a}	0.172	<0.01
Clostridia, log cfu/g	4.43^{a}	4.13^{a}	2.47^{b}	2.51^{b}	2.73^{b}	0.145	<0.01
Yeasts, log cfu/g	4.27^{b}	5.15^{a}	5.20^{a}	5.19^{a}	5.46^{a}	0.146	<0.01
Molds, log cfu/g	< 2	< 2	< 2	< 2	< 2	-	-
pH	4.60^{bc}	4.89^{a}	4.66^{b}	4.79^{ab}	4.44^{c}	0.053	<0.01
NH_3-N, g/kg N	251^{a}	180^{b}	174^{b}	185^{b}	103^{c}	12.5	<0.01
NH_3-N_{corr}[3], g/kg N	251^{a}	180^{b}	138^{bc}	70.6^{d}	103^{cd}	12.4	<0.01
Lactic acid, g/kg DM[4]	2.03^{c}	9.15^{bc}	17.2^{b}	29.9^{a}	37.1^{a}	3.23	<0.01
Acetic acid, g/kg DM	22.0^{ab}	15.9^{bc}	25.7^{a}	15.9^{bc}	10.8^{c}	1.56	<0.01
n-Butyric acid, g/kg DM	16.9^{a}	11.2^{b}	2.33^{c}	1.95^{c}	2.23^{c}	1.164	<0.01
Ethanol, g/kg DM	3.27^{a}	2.67^{a}	1.23^{b}	1.98^{ab}	1.15^{b}	0.327	<0.01
2,3-Butanediol, mg/kg DM	2340^{a}	1531^{b}	527^{c}	225^{c}	826^{c}	164.1	<0.01
Propionic acid, mg/kg DM	1838^{a}	1451^{a}	530^{b}	301^{b}	325^{b}	140.2	<0.01
i-Butyric acid, mg/kg DM	571^{b}	1613^{a}	240^{bc}	56.7^{c}	253^{bc}	113.08	<0.01
i-Valeric acid, mg/kg DM	135^{b}	243^{a}	61.8^{bc}	44.5^{c}	36.5^{c}	19.02	<0.01
n-Valeric acid, mg/kg DM	102^{b}	208^{a}	49.8^{bc}	41.5^{bc}	34.0^{c}	16.15	<0.01
n-Propanol, mg/kg DM	89.3^{a}	92.3^{a}	80.5^{a}	39.5^{b}	23.3^{b}	10.94	<0.01
Ethyl acetate, mg/kg DM	14.3	16.5	13.5	19.0	13.0	3.87	0.80
1,2-Propanediol, mg/kg DM	2.50	2.67	2.25	2.25	2.50	0.781	0.99
Ethyl lactate, mg/kg DM	1.75	1.25	1.00	2.00	1.50	0.446	0.58
Propyl acetate, mg/kg DM	1.00	1.00	1.75	1.50	1.50	0.423	0.65
Undissociated VFA[5], g/kg DM	25.3^{a}	13.6^{bc}	16.1^{b}	8.70^{c}	9.27^{c}	1.331	<0.01
Gas loss, g/kg DM	90.9^{a}	69.6^{b}	57.6^{c}	46.0^{d}	54.4^{cd}	2.43	<0.01
DM loss, g/kg DM	119^{a}	98.9^{b}	63.2^{c}	48.1^{e}	56.7^{d}	1.94	<0.01
Aerobic stability T[6], d	> 10	> 10	> 10	> 10	> 10	-	-
Aerobic stability pH[7], d	> 10^{a}	9.25^{ab}	7.75^{b}	6.25^{b}	6.00^{b}	0.552	<0.01

[1]CON: without additive, SH: soybean hulls at 100 g/kg, NIT: sodium nitrite at 1 g/kg, NIT+HEX: sodium nitrite at 1 g/kg + hexamine at 0.65 g/kg, FA: formic acid (85%) at 4 mL/kg.

[2]Standard error of the mean.
[3]NH_3-N corrected for addition of nitrogen by additives.
[4]Dry matter.
[5] Sum of undissociated acetic, propionic, i-butyric, n-butyric, i-valeric and n- valeric acids.
[6]Aerobic stability based on temperature rise (+2°C).
[7]Aerobic stability based on pH rise (+0.5).
[a,b,c,d]Tukey test ($\alpha = 0.05$).

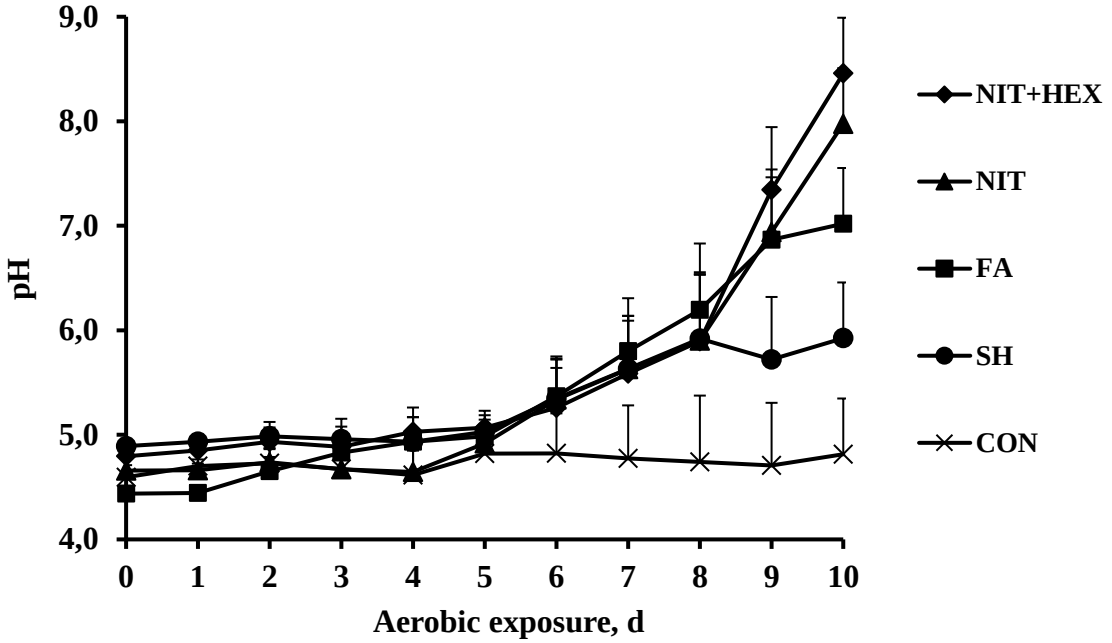
668 **Table 4** Chemical composition and nitrogen fractions of guinea grass silages stored for
669 101 d (n = 4)

Item	Treatment[1]					SEM[2]	*P*-value
	CON	SH	NIT	NIT+HEX	FA		
DM[3], g/kg	211^{c}	282^{a}	226^{bc}	226^{bc}	234^{b}	4.15	<0.01
Ash, g/kg DM	119^{a}	108^{b}	114^{a}	115^{a}	116^{a}	1.5	<0.01
aNDF[4], g/kg DM	717^{x}	708^{xy}	714^{xy}	709^{xy}	701^{y}	4.0	0.08
ADF[5], g/kg DM	425^{a}	419^{a}	407^{b}	404^{b}	398^{b}	1.7	<0.01
SC[6], g/kg DM	8.92^{d}	8.85^{d}	11.3^{c}	13.8^{b}	19.0^{a}	0.62	<0.01
CP[7], g/kg DM	64.2^{c}	83.3^{a}	75.5^{b}	78.2^{ab}	77.6^{ab}	1.78	<0.01
Soluble CP, g/kg CP	525^{a}	526^{a}	419^{b}	362^{c}	397^{bc}	9.4	<0.01
IVDMD[8]	0.562^{b}	0.611^{a}	0.596^{a}	0.608^{a}	0.615^{a}	0.006	<0.01
RdDM[9], g/kg DM	495^{c}	551^{b}	558^{b}	583^{a}	580^{a}	5.6	<0.01
N fractionation[10], g/kg N							
A1	251^{a}	180^{b}	174^{b}	185^{b}	103^{c}	12.5	<0.01
A2	274^{ab}	346^{a}	245b^{c}	177^{c}	293^{ab}	18.0	<0.01
B1	240^{ab}	230^{b}	277^{a}	243^{ab}	213^{b}	9.9	<0.01
B2	151^{c}	154^{c}	212^{b}	287^{a}	297^{a}	10.4	<0.01
C	83.4^{b}	89.9^{ab}	92.2^{ab}	109^{a}	93.2^{ab}	4.57	0.02
RDP[11], g/kg CP	749^{a}	733^{b}	716^{c}	687^{d}	686^{d}	3.7	<0.01
RUP[12], g/kg CP	251^{d}	267^{c}	284^{b}	313^{a}	314^{a}	3.7	<0.01

670 [1]CON: without additive, SH: soybean hulls at 100 g/kg, NIT: sodium nitrite at 1 g/kg, NIT+HEX: sodium
671 nitrite at 1 g/kg + hexamine at 0.65 g/kg, FA: formic acid (85%) at 4 mL/kg.
672 [2]Standard error of the mean.
673 [3]Dry matter.
674 [4]Neutral detergent fiber.
675 [5]Acid detergent fiber.
676 [6]Soluble carbohydrates.
677 [7]Crude protein.
678 [8]*In vitro* dry matter digestibility.
679 [9]Recovery of digestible dry matter.
680 [10]Nitrogen fractionation according to CNCPS v. 6.5.
681 [11]Rumen degradable protein (calculated for growing cattle).
682 [12]Rumen undegradable protein (calculated for growing cattle).
683 [a,b,c,d]Tukey test ($\alpha = 0.05$).
684 [x,y]Tukey test ($\alpha = 0.10$).

685 **Figure**

686



687

688 **Figure 1** Development of pH in guinea grass silage during aerobic exposure for 10 d.
689 CON: without additive, SH: soybean hulls at 100 g/kg, FA: formic acid (85%) at 4
690 mL/kg, NIT: sodium nitrite at 1 g/kg, NIT+HEX: sodium nitrite at 1 g/kg + hexamine at
691 0.65 g/kg. Bars indicate the standard error of the mean. $P < 0.01$ for interaction between
692 additive treatment and day of air exposure.

693

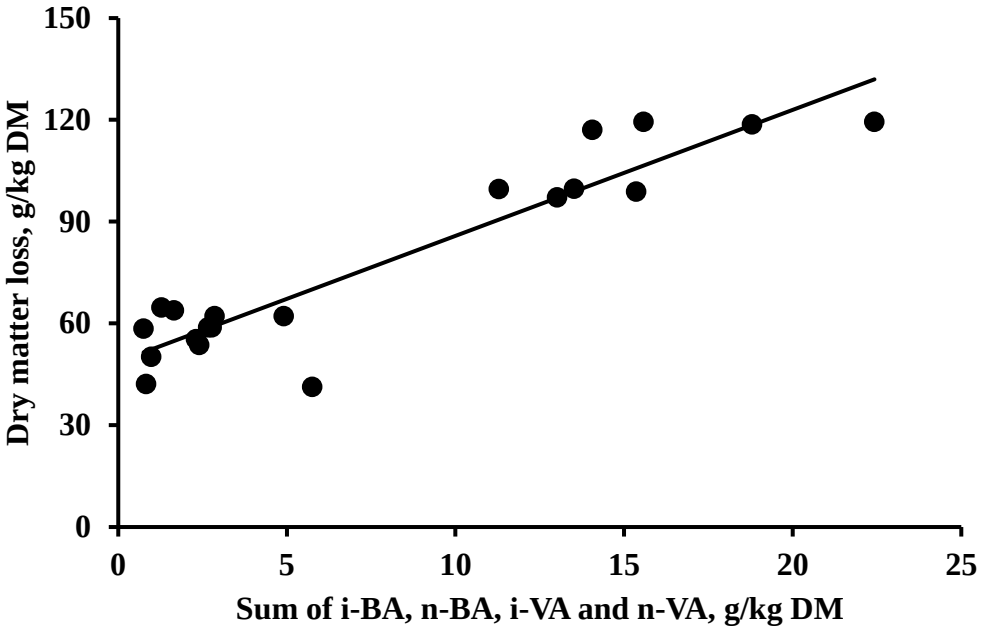


Figure 2 Relationship between the sum of i-butyric (i-BA), n-butyric (n-BA), i-valeric (i-VA) and n-valeric (n-VA) acids (BVA, g/kg DM) and the dry mater loss (DML, g/kg DM) during fermentation in guinea grass silage after 101 d of storage. DML = 48.7 + 3.71 × BVA, $R^2 = 0.87$, RSME = 10.4, $P < 0.01$.