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Fermentative characteristics, fungal population, and losses of forage sorghum silage BRS 658 treated with different additives

Caracteristícas fermentativas, população fúngica e perdas de silagem de sorgo forrageiro BRS 658 tratadas uso com diferentes aditivos

Características fermentativas, población de fongos y pérdidas del ensilaje de sorgo forrajero BRS 658 utilizando distintos aditivos

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Claudia Anete Führ
ORCID: https://orcid.org/0000-0002-5630-9942
State University of the West of Paraná, Brazil
E-mail: furhca@gmail.com
Marcela Abbado Neres
ORCID: https://orcid.org/0000-0003-3221-4030
State University of the West of Paraná, Brazil
E-mail: mabaneres@gmail.com
Magno Daniel Porfirio
ORCID: https://orcid.org/0000-0002-0755-0254
State University of the West of Paraná, Brazil
E-mail: magnoporfirio@gmail.com
Iuli Caetano da Silva Brandão Guimarães
ORCID: https://orcid.org/0000-0003-1375-1148
University of Sao Paulo, Brazil
E-mail: iulivet3@gmail.com
Thiago Henrique da Silva
ORCID: https://orcid.org/0000-0002-0028-1127
University of Sao Paulo, Brazil
E-mail: silvath@usp.br

Abstract
This study aimed to evaluate the fermentative profile, losses, dry matter recovery, and fungi population in forage sorghum silage treated with different additives. The design was completely randomized, with four replications and seven treatments, which were: 1) Control (C), whole-plant sorghum with no additives; 2) Microbial (M), whole-plant sorghum + microbial additive (0.2% of original matter); 3) Sugar (S), whole-plant sorghum + brown sugar (4.0% of original matter); 4) Microbial plus sugar additives (MS), whole-plant sorghum + microbial additive (0.2% of original matter) + brown sugar (4.0% of original matter); 5) Whey (W), whole-plant sorghum + whey (3% of original matter); 6) Microbial plus whey additives (MW), whole-plant sorghum + microbial additive (0.2% of original matter) + whey (3% of original matter); 7) Microbial plus sugar plus whey (MSW), whole-plant sorghum + microbial additive (0.2% of original matter) + brown sugar (4.0% of original matter) + whey (3% of original matter). Sorghum was cut 111 days after emergence and ensiled in polyvinyl chloride silos. The storage lasted 35 days, after that period, the silos were opened and submitted to analysis. The inclusion of additives in sorghum silage has shown no significant difference in fungal population, lactic acid production, and losses by gases and effluents. The use of microbial inoculant + brown sugar in the sorghum silage BRS 658 decreased the values of ammoniacal nitrogen, pH, propionic, and butyric acid. The inclusion of brown sugar in the sorghum silage promoted an increase in soluble carbohydrates and greater recovery of dry matter.
Keywords: Brown sugar; Fermentation; Microbial inoculant; Whey.

Resumo
Objetivou-se com este trabalho avaliar o perfil fermentativo, as perdas, a recuperação de matéria seca e a população de fungos na silagem de sorgo forrageiro com diferentes aditivos. O delineamento foi inteiramente casualizado, com quatro repetições e sete tratamentos, que foram1) controle (C), sorgo planta-inteira sem aditivos; 2) aditivo microbiano (M), sorgo planta-inteira + aditivo microbiano (0,2% da matéria original); 3) açúcar mascavo (S), sorgo planta-inteira + açúcar mascavo (4,0% da matéria original); 4) aditivo microbiano mais açúcar (MS), planta-inteira + aditivo microbiano (0,2% da matéria original) + açúcar mascavo (4,0% da matéria original); 5) soro de leite (W), sorgo planta-inteira + soro de leite (3% da matéria original); 6) aditivo microbiano mais soro de leite (PM), sorgo planta-inteira + aditivo microbiano (0,2% da matéria original) + soro de leite (3% da matéria original); 7) aditivo

microbiano mais açúcar mascavo mais soro de leite (RSM), sorgo planta-inteira + aditivo microbiano (0,2% da matéria original) + açúcar mascavo (4,0% da matéria original) + soro de leite (3% da matéria original). O sorgo foi cortado aos 111 dias após a emergência e ensilado em silos de policloreto de vinil. O armazenamento teve duração de 35 dias. Após esse período, os silos foram abertos e submetidos a análises. A inclusão de aditivos na silagem de sorgo não apresentou diferença significativa para população fúngica, produção de ácido láctico, perdas por gases e efluentes. A utilização de inoculante microbiano + açúcar mascavo na silagem de sorgo BRS 658 diminuiu os valores de nitrogênio amoniacal, pH, ácido propiônico e butírico. A inclusão de açúcar mascavo na silagem de sorgo, promoveu aumento nos carboidratos solúveis e maior recuperação de matéria seca.
Palavras-chave: Açúcar mascavo; Fermentação; Inoculante microbiano; Soro de leite.

Resumen

Este trabajo tuvo como objetivo evaluar el perfil fermentativo, pérdidas, recuperación de materia seca y población fúngica en ensilaje de sorgo forrajero con diferentes aditivos. El diseño fue completamente al azar, con cuatro repeticiones y siete tratamientos, los cuales fueron: 1) control, planta entera de sorgo sin aditivos; 2) aditivo microbiano (M), sorgo de planta entera + aditivo microbiano (0,2% de materia original); 3) muscovado (S), sorgo integral + azúcar muscovado (4,0% de la materia original); 4) aditivo microbiano más azúcar muscovado (MS), planta entera + aditivo microbiano (0,2% de materia original) + azúcar muscovado (4,0% de materia original); 5) suero (W), planta entera de sorgo + suero de leche (3% de materia original); 6) aditivo microbiano más suero (MW), sorgo de planta entera + aditivo microbiano (0,2% de materia original) + suero de leche (3% de materia original); 7) aditivo microbiano más azúcar muscovado más suero de leche (MSW), sorgo de planta entera + aditivo microbiano (0,2% de la materia original) + azúcar muscovado (4,0% de la materia original) + suero de leche (3% del artículo original). El sorgo se cortó a los 111 días después de la emergencia y se ensiló en silos de policloruro de vinilo. El almacenamiento duró 35 días. Después de este período, los silos fueron abiertos y sometidos a análisis. La inclusión de aditivos en el ensilaje de sorgo no mostró diferencias significativas para la población de hongos, producción de ácido láctico, pérdidas de gases y efluentes. El uso de inoculante microbiano + panela en el ensilaje de sorgo BRS 658 disminuyó los valores de nitrógeno amoniacal, pH, ácido propiónico y butírico. La inclusión de panela en el ensilaje de sorgo promovió un aumento de carbohidratos solubles y una mayor recuperación de materia seca.
Palabras clave: Azúcar moreno; Fermentación; Inoculante microbiano; Suero de leche.

1. Introduction

The nutritional value of silage is of paramount importance for better animal consumption toward their maximum yield. However, it is not only the quality that must be taken into account when looking for the efficiency of the forage conservation system. The losses that occur from planting to silo opening are important and are closely related to management misconduct and silage fermentation issues, which jeopardize livestock profitability (Zanette et al., 2012).

The basis of the forage fermentation process is soluble carbohydrate metabolism, which leads to acetic and lactic acid production. Hence, there is pH reduction and, due to the maintenance of anaerobiosis, it is possible to achieve microbiological stability and produce a stable feed with a high recovery of nutrients (Kung et al., 2018). Even having specific functions, organic acids may promote mass deterioration and negatively interfere with silage aerobic stability (Santos et al., 2006). In a scenario of losses during silage fermentation and aerobic stability, the association of additives in silage has been studied (Muck et al., 2018).

Overall, additives must modulate silage fermentation, reducing losses along with increasing nutrient recovery (Neumann et al., 2007). Among the commercially used additives, it is possible to mention microbial inoculants, which have become commonly used by producers (Bolsen et al., 1992; Muck et al., 2018). These additives aim to accelerate the fermentation process, promote quick pH reduction, and inhibit undesirable microorganisms' growth (Peng, Sun, Dong, Zhao, & Hao, 2021). Other less conventional additives, which may be used as silage additives are sugar and whey (Fallah, 2019; Santos et al., 2006; Zanette et al., 2012). They have higher levels of soluble carbohydrates for lactic acid bacteria growth, which are essential for the beginning of the fermentation process (Muck et al., 2018).

In this context, we hypothesized that microbial inoculant, brown sugar, whey, and their interactions would improve the fermentation profile and dry matter recovery, decreasing the fungal population and losses by increasing the aerobic stability of whole-plant sorghum silage. The objective of the present study was to evaluate the addition of microbial inoculant, brown

sugar, whey, and their associations in whole-plant BRS 658 sorghum silage on the fermentative profile, fungal population, losses, dry matter recovery, and aerobic stability of the silage.

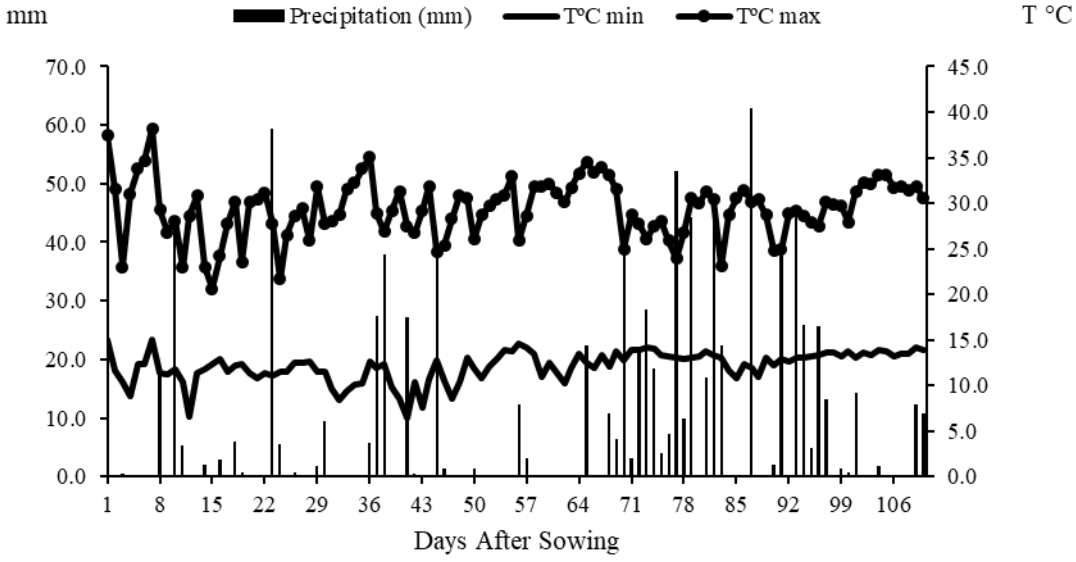
2. Methodology

The methodologic approach herein used may be classified as a quantitative method. Quantitative or numerical data is collected due to the use of measurements of quantities, which is obtained through metrology (numbers with their respective units; Yin, 2015).

2.1 Experimental site

The study was carried out at the experimental field of the State University of the West of Paraná (UNIOESTE) Research Farm: 24º31'55'' S, 54º01'05'' W; altitude 396 m), located in Western Paraná, Marechal Cândido Rondon, PR, Brazil. The study was carried out from October 2017 to January 2018. The area has an arid to humid mesothermal (subtropical) climate (type Cfa according to Köppen) with 30-year average annual precipitation of 1752 mm, annual maximum average temperature of 27.3ºC, and a minimum average temperature of 17.8 ºC. The experiment was conducted on a eutrophic red latosol (Oxisol). Prior to seeding, soil samples from each plot were taken from the top 20 cm of soil to test its background nutritional level. The chemical properties of the soil were: pH ($CaCl_2$) = 5.9; P (Mehlich) = 25.5 mg.dm^{-3}; K (Mehlich) = 0.7 cmolc.dm^{-3}; Ca++ (KCl 1 mol.L^{-1}) = 4.4 cmolc.dm^{-3}; Mg^{++} (KCl 1 mol.L^{-1}) = 3.1 cmolc.dm-3; Al^{+++} (KCl 1 mol.L^{-1}) = 0.0 cmolc.dm^{-3}; H+Al (pH 7.5) = 4.96 cmolc.dm^{-3}; Base saturation = 8.15 cmolc.dm^{-3}; Cation-exchange capacity = 13.1 cmolc.dm^{-3}; Saturation point = 62.2 %; Organic matter = 24.6 g.dm^{-3}; Cu = 6.5 mg.dm^{-3}; Zn = 8.3 mg.dm^{-3}; Mn = 56.0 mg.dm^{-3}, and Fe = 24.5 mg.dm^{-3}. The average daily precipitation and temperature obtained from an automated weather station device, located at the research farm, are presented in the Figure 1.

Figure 1 - Total precipitation (mm) and maximum/minimum temperature (ºC) from October 2017 to January 2018, at the State University of Western Paraná (UNIOESTE) Research Farm. Data were obtained from an automated weather station device.



Source: Authors.

According to the Figure 1, the precipitation and temperatures were homogeneously distributed through the study period. It is important since the environment favored the crop growth satisfactorily, providing a high-quality forage to be studied.

2.2 Experimental design

This study was performed using plastic buckets as minisilos. The treatments were arranged in a completely randomized design with 7 treatments using 4 replicates (buckets) each. The treatments were: 1) Control (C), whole-plant sorghum with no additives; 2) Microbial (M), whole-plant sorghum + microbial additive (0.2% of original matter); 3) Sugar (S), whole-plant sorghum + brown sugar (4.0% of original matter); 4) Microbial plus sugar additives (MS), whole-plant sorghum + microbial additive (0.2% of original matter) + brown sugar (4.0% of original matter); 5) Whey (W), whole-plant sorghum + whey (3% of original matter); 6) Microbial plus whey additives (MW), whole-plant sorghum + microbial additive (0.2% of original matter) + whey (3% of original matter); 7) Microbial plus sugar plus whey (MSW), whole-plant sorghum + microbial additive (0.2% of original matter) + brown sugar (4.0% of original matter) + whey (3% of original matter).

The microbial additive (Silotrato®, SLO Biotechnology, Cambé, PR, Brazil) was applied following the manufacturer's instructions. First, a flask of 250 g was diluted into 100 L of water. Then, the solution was applied at doses of 2 mL for each kg of forage on the original matter base. The product was composed of *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Pediococcus acidilactici*, *Enterococcus faecium*, *Lactobacillus buchneri*, *Lactococcus lactis*, and *Propionibacterium acidipropionici* at concentrations of 1.0 x 10^{10} CFU.g^{-1}). The brown sugar had 944 g.kg^{-1} of total carbohydrates, 7.6 g.kg^{-1} of protein, 1.38 g.kg^{-1} of ash, 0.9 g.kg^{-1} of fat. It was applied at doses of 40 g.kg^{-1} of forage directly onto the material. The liquid whey was used within the 30-hour shelf life, being kept at a temperature of 8.0 to 10.0°C. The average physicochemical parameters of the serum were: pH from 6.0 to 6.7, defatted dry extract (DDE) ranging from 45 to 64 g.kg^{-1}, acidity ranging from 9.0 to 12.0 (ref. g.kg^{-1} of DDE), 6 g.kg^{-1} of fat, 64.1 g.kg^{-1} of soluble carbohydrates, 22.37 g.$kg^{-1}$of protein, 33.50 g.kg^{-1} of lactose. The whey was provided by the SOORO® company (Marechal Cândido Rondon, PR, Brazil) and about 30 mL per kg of forage (as original matter) were applied. All additives were applied onto the material (according to the treatment) and subsequently were manually homogenized.

2.3 Cultural practices

On October 11th, 2017, after conventional seedbed management, a manual 1-row planter (Earthway, 1001-B) was used to plant sorghum at a depth of 5 cm. Each plot consisted of 6 m (length) x 5 m (width) performing a total area of 30 m2 per plot. A late relative maturity forage sorghum [Sorghum bicolor (L.) Moench; 110 d-115d to soft dough stage; BRS 658 – Embrapa] was planted at a row spacing of 1.0 m and at a planting population of 100 x 103 plants.ha-1. A gap of two meters was considered between adjacent main plots. The fertilizer rate was maintained according to soil test recommendations [at sowing: 30, 45, 45 kg of N, P, and K per hectare, respectively; and 35 days after sowing (DAS): 20, 40, 40 kg of N, P, and K per hectare, respectively]. Plots were manually weeded twice, once 15 days after planting and the second time when plants were 30 cm in height. Seed treatment was performed using the insecticide fipronil plus the fungicides plyraclorstrobin and thiophanate-metyl (100 mL.kg-1 of seeds). Prophylactic pest management was performed at 21 DAS and 40 DAS using the insecticide Lufenuron (150 mL.ha -1).

2.4 Harvest, ensiling procedure, and storage

At harvest (February 1st, 2018; 111 DAS; at the soft-dough stage), the whole plots were hand cut, leaving 10 cm of stubble in a 2-m continuous section. Thereafter, the sorghum plants were transported to the laboratory, where they were

immediately chopped (JFTM, model C-120, Itapira, SP, Brazil) to obtain a mean theoretical length of cut = 14 mm. Then, the material was placed into a sterile polyvinyl chloride (PVC) plastic bucket (10 cm diameter by 40 cm length) equipped with a Bunsen valve for gas elimination (minisilos).

About 0.3 kg of sterilized sand was placed in the bottom of the minisilos and, over this, a layer of cotton liner was placed to avoid contact between silage and sand. These components (sand + cotton liner) were added to drain possible generated liquids. The compaction was performed using a wooden stick and the lids were closed with adhesive tape to prevent air into the silo. The minisilos storage was performed under room temperature and was protected from sunlight and rain. About 1.8 kg of whole-plant sorghum (as original matter) was ensiled.

2.5 Microbiological and chemical analyses

For the microbiological analysis, 450 mL of sterile distilled water was added to 50 g of the sample under agitation. From the solution obtained, 1 mL or 0.1 mL was removed through a pipette to provide successive dilutions of 10^1 to 10^7 in test tubes containing 9 mL of distilled water. After this, 0.1 mL of the diluted extracts were placed onto each Petri dish. Three Petri dishes were used for each culture and dilution medium.

For fungi quantification in the silage, the diluted extracts of each sample were inoculated on the surface of Potato Dextrose Agar (PDA), acidified with 10% tartaric acid, where the antibiotic was also added to avoid bacterial contamination. After inoculation, the plates remained incubated at 28ºC ± 1ºC for 7 days (de Souza et al., 2012). The microorganisms were quantified by using a "Quebec" colony counter. Only plates containing 30 to 300 colonies, expressed in CFU (Colony Forming Unit).g^{-1}, were evaluated. Yeasts and filamentous fungi were distinguished by the physical characteristics of the colonies. Results were obtained through the average from Petri plates, for the dilutions selected, and expressed as log.

The samples were dried in a forced air oven at 55◦C for 72 h and ground in a 1-mm screen Willey mill (MA340, Marconi, Piracicaba, SP, Brazil). Dry matter (DM, AOAC 950.15), ether extract (EE, AOAC 920.39), and total N (AOAC, 984.13) contents were analyzed in all samples according to the methods described by (AOAC, 2000). Organic matter (OM) was determined by the difference between DM and ash content. Neutral detergent insoluble protein (NDIP) was determined according to (Licitra et al., 1996). The NDF, ADF, and lignin contents were assessed according to Van Soest et al. (1991) using a fiber analyzer (TE-149, Tecnal Equipment for Laboratory Inc., Piracicaba, SP, Brazil). In addition, the NDF samples were treated with amylase and sodium sulfite. Afterward, the NDF samples were corrected for ashes to obtains the aNDFom (Mertens et al., 2002). Hemicellulose and cellulose content were obtained by the difference between aNDFom and ADF and between ADF and lignin, respectively.

Water-soluble carbohydrate was determined according to (Johnson et al., 1964). Ammonia nitrogen (NH_3-N) was determined according to Bolsen et al. (1992). Buffering capacity was determined using a 10 to 20 g fresh sample, expressed in milligram equivalent (e.mg) of alkali, required to raise the pH from 4.0 to 6.0 per 100 g dry matter (Playne & McDonald, 1966). The chemical composition of fresh whole-plant sorghum is presented in Table 1.

Table 1 - Chemical composition of fresh whole-plant sorghum (g.kg^{-1} DM, otherwise stated).

Item	Average
Dry matter (g kg^{-1})	265.81
Ash	62.34
Organic matter	937.66
Crude protein	69.40
Ether extract	16.69
Neutral detergent fiber	732.30
Acid detergent fiber	493.22
Lignin	207.33
Cellulose	477.04
Hemicellulose	239.08
In vitro dry matter digestibility	552.99
In vitro neutral detergent fiber digestibility	482.54
Total carbohydrates	851.57
Fungal population (log CFU g^{-1})	1.03
Soluble carbohydrate	73.60
Ammonia-nitrogen	4.50
pH	5.70

CFU – Colony Forming Unit. Source: Authors.

At the moment of silos opening, samples were collected to assess the organic acids (lactic, acetic, propionic, and butyric). To obtain the juice, a hydraulic press was ulsed and, after collection, the material was kept frozen for later analysis by using high-performance liquid chromatography (HPLC). The capillary used was an Aminex HPX-87H with 300 x 7.8 mm (Bio-Rad) and the oven was kept at 50ºC. The mobile phase consisted of a 0.005 mol.L^{-1} sulfuric acid solution with 3% acetonitrile, with a constant flow rate of 0.8 mL per minute, and the injection volume was 20 µL with a wavelength of 210 nm. The time for the analysis of each sample was 25 minutes.

Losses caused by gases, effluents, and dry matter recovery were estimated by equations proposed by Jobim et al. (2007), as described below.

- Gas losses (GL) were estimated using the formula: GL (% DM) = [(SWf - SWo) / (FMf x DMf)] x 100, where SWf = silo weight at ensilage (kg); SWa = silo weight at opening (kg); FMe = forage mass at ensilage (kg); DMf = DM content of fresh plant (%).
- Effluent losses (EL) were estimated using the formula: EL (kg t MV) = (Ws x 1000) / FMf, where Ws = weight of the set (silo + sand + cloth) at opening (kg) - weight of the set at opening silage (kg); FMe = forage mass at ensilage (kg).
- Dry matter recovery (DMR) was estimated using the formula: DMR (% DM) = [(FMo x DMo) / (MFfe x MSfe)] x 100, where FMo= forage mass at opening (kg); DMo = DM content of silage at opening (%); FMe = forage mass at ensilage (kg); DMe = DM content at ensilage (%).

The specific mass (EM) (kg fresh material.m^{-3}) of the silages was determined by the relationship between the ensiled forage mass (kg fresh material) and the volume of the experimental silos (m^{-3}).

2.6 Statistical analysis

All statistical analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC). For the normality of residuals, the data were evaluated using the Shapiro-Wilk test. Analysis of variance was performed according to the following model:

$$y_{ij} = \mu + t_i + e_{ij}$$

Where: y_{ij} = dependent variable observation; μ = overall mean; t_i = fixed effect of the i^{th} treatment; e_{ij} = random error. Differences among treatments were analyzed by the method of least square means using Tukey test, and significance level was set at $P \leq 0.05$.

3. Results

Ammonia-N, soluble carbohydrates (CHO), and pH differed among treatments (P<0.05). Otherwise, the fungal population was not different among treatments (average 0.78 log CFU g^{-1}; Table 2). The highest NH_3-N concentration was detected for control treatment and the lowest NH_3-N concentration was detected for microbial inoculant + brown sugar-treated sorghum silage (11.22 g kg^{-1} TN). All the other treatments had intermediate NH_3-N concentrations. Brown sugar-treated silage showed the highest CHO value (22.98 g kg^{-1} DM), differing from all other treatments (P<0.05). The pH values were different among treatments (P<0.05). The control silage had the highest pH value (3.74), differing only from microbial inoculant and microbial inoculant + brown sugar treated silage, which had a pH of 3.66 and 3.66, respectively.

The effect of treatments on organic acid production is presented in Table 3. All organic acids presented differences among treatments (P<0.05), except for lactic acid, which presented an average value of 27.38 $g.kg^{-1}$. Acetic acid concentration was reduced (P<0.05) in whey and whey + microbial inoculant treated silages, presenting average values of 1.78 $g.kg^{-1}$ and 1.60 $g.kg^{-1}$, respectively. The treatment whey + brown sugar had an intermediate acetic acid concentration of 2.66 $g.kg^{-1}$. For propionic acid, microbial inoculant and brown sugar-treated silage had the highest values (10.78 $g.kg^{-1}$ and 9.19 $g.kg^{-1}$, respectively), differing from all the other silages (P<0.05). The highest butyric acid production was detected for whey + brown sugar + microbial-treated sorghum silage (6.81 $g.kg^{-1}$; P<0.05) when compared to other treatments. The whey + microbial inoculant treated silage had an intermediate butyric acid concentration compared to other treatments (P<0.05). All remaining treatments had lower butyric acid concentrations but did not differ among them (P>0.05). It was not possible to detect any butyric acid concentration (ND – not detected) in the whey-treated silage.

The silage losses either for gases or effluents were not different among treatments (P>0.05; Table 4). The highest dry matter recovery (DMR) occurred in brown sugar-treated silages (97.06 g kg-1 DM; P<0.05), control silage (94.64 g kg-1 DM; P<0.05), and microbial inoculant treated silage (92.75 g kg-1 DM; P<0.05). The specific mass (SM) was different among treatments (P<0.05). The whey + brown sugar + microbial inoculant treated silage had the highest SM value (607.28 kg $DM.m^3$), differing only from microbial inoculant treated silage (547.84 kg $DM.m^3$), which had the lowest value. The other remaining treatments did not differ from each other (P>0.05).

Table 2 - Ammonia (NH3-N), soluble carbohydrates (CHO), pH e fungal population of whole-plant sorghum silage treated with different additives.

	Treatments							Mean	SEM	P-values
	C	M	S	MS	W	MW	MSW			
NH_3-N (g.kg-^{1} TN)	16.17^a	13.00bc	12.15bc	11.22c	12.97bc	14.60ab	13.62bc	13.39	0.3426	<0.0001
CHO (g.kg-^{1} DM)	10.42d	18.52b	22.98a	18.85b	17.79b	10.98cd	14.86bc	16.10	0.8505	<0.0001
pH	3.74^a	3.66b	3.68ab	3.66b	3.67ab	3.73ab	3.71ab	3.69	0.0078	0.0039
Fungal (log CFU g-1)	0.79	0.74	0.92	0.68	0.75	0.73	0.83	0.78	0.0343	0.7003

TN – Total nitrogen; CFU – Colony Forming Unit; SEM – standard error of the mean; Means in rows within each variable not sharing a common superscript are different (P<0.05) by Tukey's test. Source: authors

Table 3 - Organic acid production (g.kg^{-1} DM) of whole-plant sorghum silage treated with different additives.

Item	Treatments							Mean	SEM	P-values
	C	M	S	MS	W	MW	MSW			
Acetic	3.48^a	3.06^a	3.55^a	2.66^b	1.78^c	1.60^c	3.05^a	2.74	0.2001	0.0211
Propionic	ND	10.78^a	9.19^a	2.71^b	2.99^b	3.08^b	2.08^b	5.14	0.7753	<0.0001
Butyric	0.25^c	0.93^c	0.45^c	0.14^c	ND	2.45^b	6.81^a	1.83	0.4998	<0.0001
Lactic	30.3	30.8	29.1	24.5	22.1	27.1	27.8	27.3	1.2127	0.4574

C = control; M = microbial; S = brown sugar; MS = microbial + brown sugar; W = whey; MW = microbial + whey; MSW = microbial + brown sugar + whey; ND – not detected; SEM – standard error of the mean; Means in rows within each variable not sharing a common superscript are different (P<0.05) by Tukey's test. Source: Authors.

Table 4 - Gas losses (GL), effluent losses (EL), dry mater recovery (DMR), specific mass (SM) of whole-plant sorghum silage treated with different additives.

	Treatments							Mean	SEM	P-values
	C	M	S	MS	W	MW	MSW			
GL (g.kg^{-1} DM)	0.03	0.03	0.05	0.03	0.02	0.04	0.04	0.03	0.003	0.0529
EL (kg.ton^{-1} NM)	34.92	37.20	34.93	37.62	38.84	40.37	38.57	37.5	0.634	0.1779
DMR (g.kg^{-1} DM)	94.64^{ab}	92.75^{abc}	97.06^a	91.80^{bc}	89.52^{cd}	85.04^{de}	84.16^e	90.7	0.927	<0.0001
SM (kg DM.m^{-3})	596.7^{ab}	547.8^b	580.0^{ab}	583.0^{ab}	586.3^{ab}	601.1^{ab}	607.3^a	583.5	5.093	0.0364

C = control; M = microbial; S = brown sugar; MS = microbial + brown sugar; W = whey; MW = microbial + whey; MSW = microbial + brown sugar + whey; ND – not detected; NM – natural matter; SEM – standard error of the mean. Means in rows within each variable not sharing a common superscript are different (P<0.05) by Tukey's test.
Source: Authors.

4. Discussion

The results obtained for NH_3-N demonstrate that the additives and their combination were efficient in reducing its concentration in sorghum silage, mainly in brown sugar-treated silage. It is believed that the additives contributed to the rapid pH drop, which is beneficial during the fermentation period, preserving the ensiled material (Kung et al., 2018). According to Kung and Shaver (2004), levels below 100 g kg^{-1} of NH_3-N indicate that the material has been preserved, with low protein degradation, resulting in good-quality silage. In this context, all treatments are below the threshold limit, indicating that the preparation, sealing, and fermentation process occurred as expected. The NH_3-N production in silages is linked to the presence of undesired proteolytic microorganisms (Kung et al., 2018). Santos (2014) evaluating levels of whey in grass silage, observed higher levels of NH_3-N in control silage compared to whey-treated silages.

Brown sugar-treated silage had the highest content of soluble carbohydrates. This result can be justified by the fact that brown sugar is a source of available carbohydrates, contributing to higher levels of this nutrient in the silage. The inclusion of sugar in silage aims to increase its soluble carbohydrate content at the moment of ensiling process. However, an increase in acetic acid concentration may occur through saccharolytic clostridia, which utilize soluble sugars or organic (lactic) acids to produce acetic acid (Kung et al., 2018).

The mean pH for all treatments together herein was 3.69. According to (McDonald et al., 1991) pH values should be between 3.8 to 4.2, which promotes the conservation of the material and prevents the proliferation of undesirable microorganisms. The values verified for all silages are below the appropriate range, which indicates that there was a rapid development of lactic acid bacteria and a consequent rapid drop in pH. The decrease in pH is directly related to the activity of lactic acid bacteria. Therefore, other undesirable microorganisms (enterobacteria, fungi, and yeasts) are inhibited, reducing losses in silage quality (Kung et al., 2018; McDonald et al., 1991). In addition, in this study, we found that all additives were effective in reducing silage pH compared to the control treatment. Zanette et al. (2012) evaluated sugar or bacterial inoculant inclusion in corn silage and did not detect differences in pH. Otherwise, Paviz et al. (2010) evaluating molasses or bacterial inoculant inclusion in sorghum silage observed a higher pH silage pH in inoculant treated silage (4.66); however, for all silages, pH was similar to or less than 4, with an average value of 4.39.

The organic acids produced during the fermentation process demonstrate how was the microbial activity and demonstrate how stable is the silage at the end of all procedures (McDonald et al., 1991). Several organic acids can be produced during silage fermentation, among them acetic, propionic, butyric, and lactic acid. The most important is lactic acid, which is produced by lactic acid bacteria and may be homo or heterofermentative. Its importance is because it is about 10 to 12 times stronger in decline pH than any of the other major acids found in silages, allowing better silage conservation (Kung et al., 2018). In this study, acetic acid concentration was intermediate-low for MS and the lowest values were detected for W and MW treatments compared to other treatments with no biological explanation. Perhaps, soluble carbohydrates from brown sugar or whey associated with microbial inoculant led to this effect, but it is not true for MSW association in this study. However, low acetic acid production may lead to aerobic unstable silage since this end-product is responsible for inhibiting lactate-assimilating yeasts (Kung et al., 2018).

The propionic acid was higher for M and S treatment. Silages treated with *L. buchneri* may present high concentrations of propionic acid since 1,2 propanediol, an intermediate product of *L. buchneri* bacteria may be converted to propionic acid by *Lactobacillus diolivorans* (Krooneman et al., 2002). The microbial additive herein utilized had *L. buchneri* in its composition, explaining such findings. Regarding brown sugar silage, we hypothesized that the glucose availability may increase the intermediate products abovementioned, but their interaction, as well as the whey inclusion, did not follow similar results.

According to Kung et al. (2018), the maximum content of butyric acid in silages is 1 $g.kg^{-1}$. Therefore, only whey + microbial inoculant and whey + brown sugar + microbial inoculant treated silages exceeded the established limit. This fact may occur due to the high moisture content of the additives used (whey and microbial inoculant) which were diluted in water, increasing the moisture of silage (DM not shown). In addition, whey contains lactose, soluble proteins, and salts, requiring a high biological demand for oxygen, which facilitates the proliferation of bacteria of the genus *Clostridium* (Santos et al., 2006).

Gas losses (GL) are associated with the type of fermentation that occurred inside the silo. If bacteria are homolactic, they may use glucose as a substrate for lactic acid production. Otherwise, if the fermentation is performed by heterofermentative bacteria (clostridia and enterobacteria), CO_2 production occurs, which facilitates the occurrence of losses caused by gases (McDonald et al., 1991). Santos (2014), adding whey and/or inoculant to grass silage, observed an increase in effluent losses as increasing whey level. In this case, the effluent production may be linked to increased levels of soluble carbohydrates and increased humidity. Altogether, may allow the multiplication of bacterial groups, which, due to their metabolic routes, increase DM losses through gas and/or effluents.

Evaluating cheese whey levels in grass silage, Santos et al. (2006) observed that gas losses were reduced compared to the control silage. This reduction was attributed to lactic fermentation which reduced secondary fermentations. However, in this experiment, it was not possible to observe this positive difference for GL in whey-treated silages. In addition, Santos et al. (2006) demonstrated the relationship between additive moisture and higher effluent losses in silage; however, the authors did not detect any difference in DMR. Corroborating, Santos (2014) observed quadratic behavior for DMR with a maximum recovery point in the control silage and minimum recovery in the treatment with 10% whey. The same was observed in the study since the lowest DMR values occurred in whey-treated silages.

Regarding the SM, the higher its value, the lower the oxygen concentrations at the beginning of the fermentation process, before and after opening the silo. In this context, SM is directly linked to the forage compaction efficiency at the time of ensiling (Kruger, 2012). In an experiment evaluating microbial inoculants with different strains of bacteria in corn silage, Zanette et al. (2012) did not observe difference for SM when silages were treated with additives. In the present study, the inoculant treatment had lower SM compared to other treatments, demonstrating that this additive was not efficient in interfering with the silage SM, since silage compaction was similar in all treatments.

5. Conclusion

Microbial inoculant + brown sugar in BRS 658 sorghum silage decreased NH_3-N, pH, propionic, and butyric acid values. The inclusion of brown sugar in sorghum silage promoted higher levels of soluble carbohydrates and higher dry matter recovery. However, the additives herein presented did not influence the losses of whole-plant sorghum silages. More studies are needed to elucidate the impact of using alternative additives on sorghum ensilage and other crops, so that we can improve the understanding of this important topic in forage quality to ruminant nutrition.

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References

AOAC. (2000). Official Methods od Analysis - Animal Feed. In *Official Methods of Analysis* (17th ed.). Gaithersburg, MD, USA: The Association of Official Analytical Cheimists.

Bolsen, K. K., Lin, C., Brent, B. E., Feyerherm, A. M., Urban, J. E., & Aimutis, W. R. (1992). Effect of Silage Additives on the Microbial Succession and Fermentation Process of Alfalfa and Corn Silages. *Journal of Dairy Science*, 75(11), 3066–3083. https://doi.org/10.3168/jds.S0022-0302(92)78070-9

Cherney, D. J. R., Cherney, J. H., & Cox, W. J. (2004). Fermentation characteristics of corn forage ensiled in mini-silos. *Journal of Dairy Science*, 87(12), 4238–4246. https://doi.org/10.3168/jds.S0022-0302(04)73569-9

de Souza, L. C., Zambom, M. A., Pozza, M. S. dos S., Neres, M. A., Radis, A. C., Borsatti, L., & Gundt, S. (2012). Development of microorganisms during storage of wet brewery waste under aerobic and anaerobic conditions. *Revista Brasileira de Zootecnia*, 41(1), 188–193. https://doi.org/10.1590/S1516-35982012000100027

Driehuis, F., Oude Elferink, S. J. W. H., & Van Wikselaar, P. G. (2001). Fermentation characteristics and aerobic stability of grass silage inoculated with Lactobacillus buchneri, with or without homofermentative lactic acid bacteria. *Grass and Forage Science*, 56(4), 330–343. https://doi.org/10.1046/j.1365-2494.2001.00282.x

Fallah, R. (2019). Effects of adding whey and molasses on corn silage quality, growth performance and health of Simmental fattening calves. *Journal of Livestock Science*, 10(2). https://doi.org/10.33259/jlivestsci.2019.91-96

Jobim, C. C., Nussio, L. G., Reis, R. A., & Schmidt, P. (2007). Avanços metodológicos na avaliação da qualidade da forragem conservada Methodological advances in evaluation. *Revista Brasileira de Zootecnia*, 36, 101–119.

Johnson, R. R., Balwani, T. L., Johnson, L. J., Mcclure, K. E., & Dehority, B. A. (1964). Corn Plant Maturity. II. Effect on in vitro cellulose digestibility and soluble content. *Journal of Animal Science*, 25(3), 617–623.

Krooneman, J., Faber, F., Alderkamp, A. C., Oude Elferink, S. J. H. W., Driehuis, F., Cleenwerck, I., & Vancanneyt, M. (2002). Lactobacillus diolivorans sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. *International Journal of Systematic and Evolutionary Microbiology*, 52(2), 639–646. https://doi.org/10.1099/00207713-52-2-639

Kruger, A. M. (2012). *Massa específica e suas implicações na conservação e valor nutricional em silagens de milho*. Universidade Estadual de Maringá.

Kung, L, & Shaver, R. (2004). Interpretation and use of silage fermentation analysis reports, 3(13), 1–5.

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

Licitra, G., Hernandez, T. M., & Van Soest, P. J. (1996). Feedbunk management evaluation techniques. *Animal Feed Science Technology*, 57, 347–358.

McDonald, P., Henderson, A. R., & Heron, S. J. E. (1991). *The Biochemistry of Silage* (Chalcomb). Marlow.

Mertens, D. R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., & Wolf, M. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *Journal of AOAC International*, 85(6), 1217–1240. https://doi.org/10.1093/jaoac/85.6.1217

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., & Kung, L. (2018). Silage review: Recent advances and future uses of silage additives. *Journal of Dairy Science*, 101(5), 3980–4000. https://doi.org/10.3168/jds.2017-13839

Neumann, M., Mühlbach, P. R. F., Nörnberg, J. L., Restle, J., & Ost, P. R. (2007). Efeito do tamanho de partícula e da altura de colheita das plantas de milho (Zea mays L.) sobre as perdas durante o processo fermentativo e o período de utilização das silagens. *Revista Brasileira de Zootecnia*, 36(5), 1395-1405 (in portuguese). https://doi.org/10.1590/s1516-35982007000600024

Paviz, M. M., Ghoorchi, T., & Ghanbari, F. (2010). Effects of molasses and bacterial inoculant on chemical composition and aerobic stability of sorghum silage. *Asian Journal of Animal and Veterinary Advances*, 6(4), 385–390. https://doi.org/10.3923/ajava.2011.385.390

Peng, C., Sun, W., Dong, X., Zhao, L., & Hao, J. (2021). Isolation, identification and utilization of lactic acid bacteria from silage in a warm and humid climate area. *Scientific Reports*, 11(1), 1–9. https://doi.org/10.1038/s41598-021-92034-0

Playne, M. J., & McDonald, P. (1966). The buffering constituents of herbage and of silage. *Journal of the Science of Food and Agriculture*, 17(6), 264–268. https://doi.org/10.1002/jsfa.2740170609

Santos, E. M., Zanine, A. de M., Ferreira, D. de J., Oliveira, J. S. de, Pereira, O. G., & Almeida, J. C. de C. (2006). Efeito da adição do soro de queijo sobre a composição bromatológica, fermentação, perdas e recuperação de matéria seca em silagem de capim-elefante. *Ciência Animal Brasileira*, 7, 235–239.

Santos, T. M. (2014). *Características químicas, fermentativas, estabilidade aeróbia e perdas do capim pioneiro (Pennisetum purpureum Schum.) ensilado com permeado de soro de leite e Lactobacillus buchneri*.

Tilley, J. M. A., & Terry, R. A. (1963). A two-stage technique for the in vitro digestion of forage crops. *Grass and Forage Science*, 18(2), 104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science*, 74(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Yin, R. K. (2015). *O estudo de Caso* (5th ed.). Bookman.

Zanette, P. M., Neumann, M., Faria, M. V., Ueno, R. K., Marafon, F., & Durman, T. (2012). Valor Nutricional e Perdas Durante a Fermentação de Silagens de Milho (Zea mays L.) com Açúcar ou Inoculante. *Revista Brasileira de Milho e Sorgo*, 11(2), 178–189. https://doi.org/10.18512/1980-6477/rbms.v11n2p178-189