Research, Society and Development, v. 12, n. 7, e16512742666, 2023
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v12i7.42666

Effect of different alternative additives on nutritional value, in vitro digestibility, and carbohydrate fractionation of whole-plant sorghum silage

Efeito de diferentes aditivos sobre o valor nutricional, digestibilidade in vitro e fracionamento de carboidratos de silagem de planta inteira de sorgo

Efecto de diferentes aditivos sobre el valor nutricional, digestibilidad in vitro y fraccionamiento de carbohidratos del ensilaje de plantas enteras de sorgo

Received: 07/12/2023 | Revised: 07/25/2023 | Accepted: 07/26/2023 | Published: 07/30/2023

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 impact of different additives on nutritional value, in vitro digestibility, and fractionation of carbohydrates of sorghum silage. The experimental design was completely randomized with four replications and seven treatments: 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 sorghum was cut 111 days after emergence, in the milky-floury stage, and ensiled in polyvinyl chloride silos, adapted with a Bunsen valve. After 35 days of storage, the silos were opened, and samples were collected for the respective analyzes. The additives only presented differences for dry matter, mineral matter, organic matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, total carbohydrates, and A + B1 fraction of the carbohydrates. In the present study, microbial inoculants, brown sugar, whey, and their interactions were effective in improving the chemical profile as well as the soluble carbohydrate fraction of whole-plant sorghum silage.
Keywords: Brown sugar; Bromatology; Fermentation; Microbial inoculant; Whey.

Resumo
Neste estudo, objetivou-se avaliar o impacto de diferentes aditivos sobre o valor nutricional, a digestibilidade in vitro e o fracionamento de carboidratos da silagem de sorgo. O delineamento experimental foi inteiramente casualizado com quatro repetições e sete tratamentos: 1) 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 planta-inteira foi cortado aos 111 dias após a emergência, no estágio de grão leitoso – farináceo e ensilado em silos de policloreto de vinil, adaptados com válvula de Bunsen. Após a armazenagem de 35 dias, os silos foram abertos e amostras foram coletadas para análises. Os aditivos utilizados apresentaram diferenças somente para as variáveis matéria seca, matéria mineral, matéria orgânica, proteína bruta, extrato etéreo, fibra em detergente neutro, fibra em detergente ácido, carboidratos totais e fração A + B1 dos carboidratos. No presente estudo, inoculantes microbianos, açúcar mascavo, soro de leite e suas interações foram eficazes em melhorar o perfil químico, bem como a fração de carboidratos solúveis da silagem de sorgo planta-inteira.
Palavras-chave: Açúcar mascavo; Bromatologia; Fermentação; Inoculante microbiano; Soro de leite.

Resumen

Este estudio tuvo como objetivo evaluar el impacto de diferentes aditivos en el valor nutricional, digestibilidad in vitro y fraccionamiento de carbohidratos en ensilaje de sorgo. El diseño experimental fue completamente al azar con cuatro repeticiones y siete tratamientos: 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 de planta entera se cortó a los 111 días después de la emergencia, en la etapa de grano lechoso - farináceo y se ensiló en silos de policloruro de vinilo, equipados con una válvula Bunsen. Después de 35 días de almacenamiento, se abrieron los silos y se tomaron muestras para su análisis. Los aditivos utilizados mostraron diferencias solo para las variables materia seca, materia mineral, materia orgánica, proteína cruda, extracto etéreo, fibra detergente neutro, fibra detergente ácido, carbohidratos totales y fracción A+B1 de carbohidratos. En el presente estudio, los inoculantes microbianos, el azúcar muscovado, el suero de leche y sus interacciones fueron efectivos para mejorar el perfil químico y la fracción de carbohidratos solubles del ensilaje de sorgo de planta entera.
Palabras clave: Azúcar moreno; Bromatologia; Fermentación; Inoculante microbiano; Suero de leche.

1. Introduction

Forage storage is currently seen as an important and indispensable technique for livestock production systems, especially for ruminants, which demand a high amount of dietary roughage. Ensiling is an on-farm conservation option in which forage is stored and, based on an adequate fermentation process, maintains its nutritional quality, and remains viable for animal feeding for a long period (Grant & Adesogan, 2018). Among the most used whole plants for silage, corn (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] can be mentioned. Likewise, whole-plant corn and whole-plant sorghum have desirable characteristics for their ensilage, such as adequate dry matter and soluble carbohydrates content, high yield, and low buffering power (Rodrigues et al., 2020).

Ensiling is a rigorous process with several steps and, the proper performance of this process may provide a quality feed outcome. For a long time, farmers have had additives to improve silage preservation. This strategy improves fermentation and stability and accelerates the pH reduction, favoring the fermentation process and, indirectly, leading to silage quality (Muck et al., 2018), keeping its composition as close as fresh sorghum composition. Among these additives, microbial inoculants and natural products, such as whey and sugar, may be mentioned (Cajarville et al., 2012; Fallah, 2019; Muck et al., 2018; Zanette et al., 2012).

The principle of the microbial inoculant, based on homofermentative and heterofermentative, is to increase the number of lactic acid-producing bacteria by accelerating fermentation, leading to a sudden drop in pH, improving thus the material conservation. In addition, heteroferments ferment pentoses, producing in addition to lactic acid, acetic acids, through the phosphoketolase enzyme. This heterofermentative bacteria aims also to preserve the material's nutritional values after the silo opening. Whey is a cheap and highly available by-product from dairy agricultural waste. It has high levels of soluble

carbohydrates (lactose), in addition to being a great source of lactic acid bacteria (González Siso, 1996). Altogether, these factors may favor the fermentation process and keep the nutritional value of forage silages (Fallah, 2019). Brown sugar, like any sugar, maybe a readily available source of carbohydrates, which favors fermentation and quick pH drop, as lactic acid bacteria may use it as the main source of substrate (Zanette et al., 2012).

Therefore, the present study aimed to evaluate the nutritional value, *in vitro* digestibility, and carbohydrate fractions of BRS 658 sorghum silage by using a blend of homofermentative and heterofermentative microbial inoculant, whey, brown sugar, and their combinations as additives.

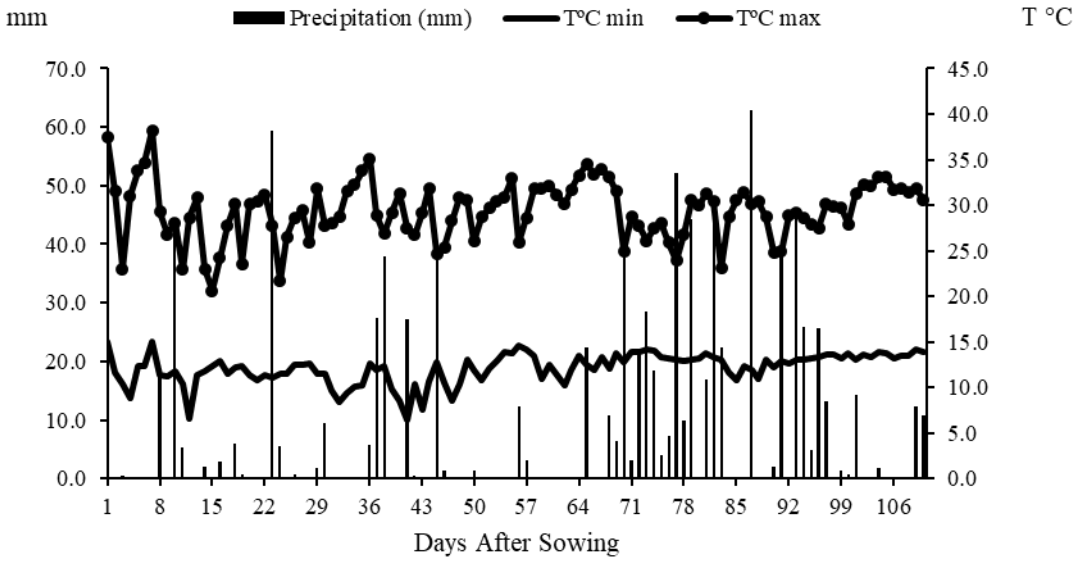
2. Methodology

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, an 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). Before 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.

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 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, and 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-1of 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 m^2 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 10^3 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 Chemical-nutritional analysis

After 35 d of storage, the minisilos were opened and about 5 cm from the top and bottom layers were discarded. The remaining material was sampled and placed into paper bags and submitted to the laboratory for chemical analysis, carbohydrate fractioning, and *in vitro* digestibility.

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 obtain 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. 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 detergente fiber	732.30
Acid detergente 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

Source: Authors

In vitro dry-matter digestibility and IVNDFD were determined in conformance with Tilley and Terry (1963). Filter bags (F-57; 50 × 55 mm; ANKOM Technology Corporation, Macedon, NY, USA) were identified and weighed. In the formula below, the bag was considered the inoculum without sample. Approximately 0.5 g of forage samples previously oven-dried and ground were weighed and inserted into filter bags in duplicates. These bags were put into test tubes. Into test tubes, 40 ml of McDougall solution (artificial saliva) was added to 10 ml of rumen inoculum from 1 castrated Jersey steer that had been kept at pasture grazing *Brachiaria decumbens* and then supplemented with 3.0 kg of maize silage (DM basis) and *ad libitum* mineral salt. Tubes were sealed with rubber corks containing a Bunsen gas release valve, immediately after flushing out with CO^2, and incubated in oven for 48 h under controlled temperature (39°C). They were agitated a minimum of 3 times during fermentation. The second IVDMD phase occurred after discarding the liquid solution. A pepsin solution (1:10.000) at 0.2% (50 ml) was added to each tube, followed by agitation at 39°C for another 48 hr. After washing, drying, and weighing the bags, calculations were performed as the formula below:

$$\text{IVDMD} = 100 \text{ x} \left[\frac{[\text{g DM sample} - (\text{g of residual DM} - \text{g of DM of inoculum without sample})]}{\text{g of DM sample}} \right]$$

The same procedure was performed in IVOMD and IVNDFD analyses, except that the bags were submitted to aNDFom washing procedures after incubation. Water-soluble carbohydrate was determined according to (Johnson et al., 1964).

Total carbohydrates (TC) were obtained by the following equation: TC = 100 – (%CP + %EE + % ashes) according to Sniffen et al. (1992). For carbohydrate fractionation, the C fraction (indigestible carbohydrates) was estimated using the formula: 100 * [NDF (%DM) * 0.01 * lignin (%NDF) * 2.4] / TC (%DM), the fraction B2 (slowly degrading carbohydrates) was obtained by the equation: 100 * [(NDF (%DM) – PIDN (%PB) * 0.01 * CP (%DM)) – (NDF (%DM) * 0.01 * lignin (%NDF) * 2.4)] / CT (%MS). Carbohydrate fractions with rapid rumen degradation rate (A + B1 fraction) were determined by the difference between 100 – (C + B2 fraction).

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 the Tukey test, and the significance level was set at $P \leq 0.05$.

3. Results

3.1 Chemical analysis

The effect of treatment on chemical parameters of whole-plant sorghum silage is presented in Table 2. Briefly, there were differences for dry matter content, ash, organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) among treatments. The DM content was higher (P<0.05) for S treatment (271.27 $g.kg^{-1}$) compared to other treatments. The treatments C, M, and MS presented intermediate-high DM content (262.82 g kg^{-1}, 258.19 g kg^{-1}, 256.23 g kg^{-1}, respectively). The treatments W and MW presented intermediate-low DM content (249.41 $g.kg^{-1}$, 238.26 $g.kg^{-1}$, respectively). Finally, the treatment MSW presented the lowest DM content (235.45 g kg^{-1}). Regarding ash, and consequently OM, there was difference (P<0.05) among treatments. The treatments C, M, MS, MW, and MSW had similar ash (OM) content, but higher results when compared to S and W treated silages (P<0.05). For CP content, the MW treatment had the highest value when compared to other treatments (P<0.05). The treatments MS, W, and MSW had intermediate CP content (P<0.05), and the treatments C, M, S had the lowest values (P<0.05). The MW treatment had the highest EE content when compared to other treatments (P<0.05). The treatments M, S, and MSW presented intermediate-high EE content (P<0.05), and the treatment C and W had intermediate-low EE content (P<0.05). The lowest EE content was detected for MS treatment. The NDF content was higher for C compared to other treatments (P<0.05). Intermediate NDF content was detected in M, W, MW, and MSW treatments (P<005). The lowest NDF content was detected in S and MS treatments (P<0.05). The C treatment had the highest ADF content (P<0.05). The treatments M, MS, W, MW, and MSW had intermediate ADF content (P<0.05). Finally, treatment S had the lowest ADF content (P<0.05).

3.2 In vitro digestibility and carbohydrate fractioning

The effects of additives on *in vitro* DM and NDF digestibility as well as the carbohydrate fractions are presented in Table 3. The additives did not affect (P<0.05) the *in vitro* DM and NDF digestibility (605.86 $g.kg^{-1}$ and 512.38 $g.kg^{-1}$, respectively). In addition, no effects were detected on B2 (potentially degradable fraction) and C (undegradable cell wall fraction) carbohydrate fractions (P>0.05). Otherwise, the total carbohydrate (TC) and the A+B1 fraction (non-fiber carbohydrates) had several differences among treatments. The treatments C, M, S, MS, and W had the highest CT content compared to MW (P<0.05); however, these treatments did not differ from MSW-treated silage (P>0.05).

Regarding the fraction A+B1, the S-treated silage had the highest value, in which the treatments M and MS had the intermediate-high values (P<0.05). In addition, the treatments W and MSW had intermediate-low values (P<0.05, while the treatments C and MW had the lowest A+B1 values (P<0.05).

Table 2 - Chemical composition of whole-plant sorghum silage treated with different additives (g.kg^{-1} DM, otherwise stated).

	Treatments									
Item	C	M	S	MS	W	MW	MSW	Mean	SEM	P-value
DM (g kg^{-1})	262.82^{ab}	258.19^{ab}	271.27^{a}	256.23^{b}	249.41^{bc}	238.26^{cd}	235.45^{d}	253.09	2.5060	<0.0001
Ash	65.44^{ab}	63.88A^{b}	61.90^{b}	63.91^{ab}	61.96^{b}	70.39^{a}	66.83^{ab}	64.90	0.8022	0.0377
OM	936.56^{ab}	936.12^{ab}	938.11^{a}	936.09^{ab}	938.04^{a}	929.61^{b}	933.17^{ab}	935.10	0.8022	0.0377
CP	63.76^{b}	65.50^{b}	64.13^{b}	68.09^{ab}	70.75^{ab}	74.94^{a}	72.12^{ab}	68.47	1.0082	0.0034
EE	18.19^{cd}	21.73^{ab}	20.00^{bc}	15.59^{d}	17.26^{cd}	23.57^{a}	19.53^{bc}	19.41	0.5370	<0.0001
NDIP (g kg^{-1} CP)	359.95	350.32	388.26	361.18	345.35	328.69	329.46	351.89	6.7933	0.2206
ADIP (g kg^{-1} CP)	259.39	246.86	267.00	262.40	248.35	246.15	211.85	248.86	5.6240	0.1552
NDF	732.54^{a}	710.61^{ab}	692.53^{b}	698.69^{b}	724.71^{ab}	720.68^{ab}	713.32^{ab}	713.30	3.5053	0.0103
ADF	484.78^{a}	471.16^{ab}	454.72^{b}	461.82^{ab}	471.88^{ab}	475.70^{ab}	475.91^{ab}	470.85	2.6649	0.0412
LIG	123.78	117.52	117.39	113.09	114.37	117.16	125.61	118.42	1.6678	0.3767
CEL	413.80	434.73	466.91	524.79	488.74	528.55	446.46	471.99	17.5630	0.5259
HEM	247.76	239.46	237.82	236.88	252.83	244.99	237.40	242.45	2.3783	0.4777

C = control; M = microbial; S = brown sugar; MS = microbial + brown sugar; W = whey; MW = microbial + whey; MSW = microbial + brown sugar + whey. DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDIP = neutral detergent insoluble protein; ADIP = acid detergent insoluble protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; LIG = lignin; CEL = cellulose; HEM = hemicellulose. SEM = standard error of the mean. Within a row, means without a common lowercase letter superscript differ by the Tukey test ($^{a-d}P < 0.05$). Source: Authors

Table 3 - *In vitro* dry matter digestibility (IVDMD), *in vitro* neutral detergent fiber digestibility (IVNDFD) and carbohydrate fractions of whole-plant sorghum silage treated with different additives (g.kg^{-1} DM, otherwise stated).

	Treatments									
Item	C	M	S	MS	W	MW	MSW	Mean	SEM	P-value
IVDMD	598.40	626.58	609.09	621.41	581.16	602.61	601.75	605.86	5.6745	0.4387
IVNDFD	476.87	502.51	511.72	542.81	499.91	539.67	513.18	512.38	6.7892	0.1084
TC	852.60^{a}	848.88^{a}	853.90^{a}	852.40^{a}	850.04^{a}	831.09^{b}	841.52^{ab}	847.22	1.8424	0.0009
A + B1 (g kg^{-1} CT)	169.62^{c}	189.91^{abc}	218.17^{a}	209.03^{ab}	176.21^{bc}	162.45^{c}	180.59^{bc}	186.56	4.3934	0.0002
B2 (g kg^{-1} CT)	482.22	477.83	451.77	472.53	500.90	499.09	461.08	477.91	5.5897	0.1519
C (g kg^{-1} CT)	348.16	332.26	330.06	318.44	322.89	338.46	358.32	335.51	4.7747	0.2918

C = control; M = microbial; S = brown sugar; MS = microbial + brown sugar; W = whey; MW = microbial + whey; MSW = microbial + brown sugar + whey. IVDMD = in vitro dry matter digestibility; IVNDFD = in vitro neutral detergent fiber; TC = total carbohydrates; A + B1 = non-fiber carbohydrates; B2 = potentially degradable fraction; C = indigestible cell wall fraction. SEM = standard error of the mean. Within a row, means without a common lowercase letter superscript differ by the Tukey test ($^{a-d}P < 0.05$). Source: Authors.

4. Discussion

4.1 Chemical analysis

Regarding the treatments using whey, the DM contents were lower than others, which was caused by the high moisture content present in these treatments. Santos et al. (2006), evaluating the whey inclusion on tropical grass silage (*Pennisetum purpureum*), observed lower DM content compared to C silage, explaining that whey inclusion into ensiling procedure increases moisture, generating losses by effluents, resulting in losses of DM contents Zanette et al. (2012), including sugar or microbial inoculant in corn silage, did not observe a significant difference between treatments for DM content, with mean values of 331.1 $g.kg^{-1}$. Ribas (2018), evaluating different bacteria strains in corn silage, did not detect difference between microbial inoculant and control treatment, with an average DM content of 388.2 $g.kg^{-1}$.

The ash contents increased in most treatments compared to what was verified for fresh sorghum plant (data not shown). The additives may be interfered on this variable due to possible environmental contamination at harvest or ensiling procedure since soil contamination can increase silage ash content. The results obtained did not corroborate Santos (2014), who observed similar ash content in control treatments and those with whey and *Lactobacillus spp*. addition. Ribas (2018), evaluating the inclusion of different bacteria strains in corn silage, also did not confirm difference in ash values in comparison to control silage. The results herein presented regarding ashes mirrored OM content, which followed a similar pattern among treatments. The OM losses signal losses that occur in the nutritional value of feedstuff, including protein, carbohydrates, and lipids contents, thus decreasing their energy value. The greatest loss was observed for sorghum silage with MW (929.61 $g.kg^{-1}$), differing only for sorghum silage treated with whey and brown sugar.

In the present experiment, W-treated silage had the highest CP content, which can be attributed to the additive itself, as it has high CP concentration and/or other nutrients, which provided better conditions for bacteria development and proper fermentation, avoiding nutritional losses and protein hydrolysis. In addition, reduced proteolysis may be present in W-treated silage as reported by Fallah (2019). Unlike, Zanette et al. (2012), evaluating sugar inclusion or microbial inoculant in corn silage, observed higher CP levels for control (54 $g.kg^{-1}$ DM) and sugar (47.7 g kg^{-1} DM) treated silages compared to microbial inoculant treated ones (40.6 g kg^{-1} DM). On the other hand, Santos et al. (2006), studying tropical grass silage (*Pennisetum purpureum*), observed lower CP contents in comparison to control silage.

Ether extract is composed of fats, oils, and other similar components that are extracted when ether is heated during chemical analysis. Our EE results ranged from 15.59 to 23.57 $g.kg^{-1}$ DM; however, all silages are below 60.0 g kg-1 DM, which is the maximum threshold content for ruminant animals with no negative impact on voluntary intake (Souza et al., 2009). Corroborating, Paviz et al. (2011) evaluated the addition of molasses or microbial inoculant to sorghum silage and observed that EE concentration was affected by the bacterial inoculant compared to the control and molasses silage. Counterpart, in an experiment adding whey and *Lactobacillus buchneri* to tropical grass silage (*Pennisetum purpureum* cv. Pioneiro), Santos, (2014) did not observe difference in the EE content between treatments. The silage EE content after the fermentation process is associated to effluent losses, which may carry out substances soluble in ether. Thus, we may conclude that ensiling practices and/or additives that reduce losses by effluents may maintain the EE content of silages close to fresh plants (Miller et al., 1962).

Neutral detergent fiber content may negatively influence feed intake by ruminants, as it interferes with rumen filling capacity. Therefore, the higher the NDF content, the lower the feed intake (Bonfá et al., 2015). All treatments showed high levels of NDF; however, according to Contreras-Govea et al. (2010), this is a common characteristic of sorghum crops. The sorghum evaluated in the present experiment, despite being a forage hybrid, was tall and had a large proportion of stems, which may negatively influence the fibrous fraction digestibility, although we did not detect differences in lignin, cellulose, and hemicellulose contents.

The results obtained in the present study showed positive effects of brown sugar treatments, reducing NDF and ADF contents when compared to control silage. In view of this, the greater availability of sugars for the fermentation process of grain-rich silages is not always positive (Rodrigues et al., 2020). There is a possibility that these soluble sugars also favor alcohol production by yeasts, which increases both DM losses and putrefactive capacity of silages after silo opening (Baytok et al., 2005). In a study evaluating sugar or microbial inoculant in corn silage, Zanette et al., (2012) found no difference for NDF and ADF. Corroborating, Santos (2014) evaluating levels of whey with or without *Lactobacillus buchneri*, observed that gradual inclusion of whey reduced the NDF and ADF contents of tropical grass silage. Therefore, the inclusion of microorganisms or substrates which facilitate the silage fermentation process may improve the cell wall degradation, solubilizing undigestible carbohydrates fractions and increasing soluble carbohydrate fractions, as reported by Santos (2014) who detected that whey addition helped to reduce cellulose and lignin content.

4.2 *In vitro* digestibility and carbohydrate fractioning

It is hypothesized that TC reduction in MW-treated silages can be explained by the higher proportion of proteins and ether extract verified for this treatment. According to Sniffen et al. (1992), the TC levels should be between 500 and 800 g kg^{-1} DM; however, all silages have levels above the recommended levels. Regarding the fractionation of carbohydrates, fraction A corresponds to soluble sugars with rapid ruminal degradation and fraction B1 represents starch, pectin, and glucans. As previously mentioned, the sorghum silage treated with brown sugar had a higher A+B1 content compared to the other treatments, this can be explained by the fact that sugar is a soluble carbohydrate, adding its value to the silage. Sugar inclusion in silage is intended to increase the soluble carbohydrate content of the ensiled mass, increasing carbohydrate concentration after acetic fermentation for lactic fermentation and thus optimizing lactic acid production.

According to Neumann et al. (2007), during the ensiling period, there may be a reduction in soluble carbohydrates contents (fraction A), due to consumption by the fermentation process, resulting in increased ADF contents due to the dilution effect. In the present study, an association may be observed between ADF contents compared to soluble carbohydrates, where the C silage presented high ADF contents (484.78 g.kg^{-1} DM) and lower A + B1 carbohydrate fraction (169.62 g.kg^{-1} TC), whereas the S silage showed inverse levels, with lower ADF content (454.72 g kg^{-1} DM) and higher A + B1 fraction content (218.17 g kg^{-1} TC).

5. Conclusion

In the present study, inoculants composed of homo and heterofermentative microbes, brown sugar, whey, and their interactions were effective in improving the chemical profile as well as the soluble carbohydrate fraction of whole-plant sorghum silage. 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.

Acknowledgments

The authors thank the State University of the West of Paraná (UNIOESTE), for providing the facilities and staff necessary for this experiment. Also, the authors express appreciation to the Brazilian funding agency CAPES (“Coordenação de Aperfeiçoamento de Pessoal de Nível Superior”) and CNPq (“Conselho Nacional de Desenvolvimento Científico e Tecnológico”) for providing the fellowships.

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.

Baytok, E., Aksu, T., Karsli, M. A., & Muruz, H. (2005). The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation characteristics in sheep. *Turkish Journal of Veterinary and Animal Sciences*, 29(2), 469–474.

Bonfá, C. S., Castro, G. H. F., Villela, S. D. J., Santos, R. A., Evangelista, A. R., Jayme, C. G., & Barbosa, J. A. S. (2015). Silagem de capim-elefante adicionada de casca de maracujá. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*, 67(3), 801–808. https://doi.org/10.1590/1678-4162-7982

Cajarville, C., Britos, A., Garciarena, D., & Repetto, J. L. (2012). Temperate forages ensiled with molasses or fresh cheese whey: Effects on conservation quality, effluent losses and ruminal degradation. *Animal Feed Science and Technology*, 171(1), 14–19. https://doi.org/10.1016/j.anifeedsci.2011.09.016

Contreras-Govea, F. E., Marsalis, M. A., Lauriault, L. M., & Bean, B. W. (2010). Forage Sorghum Nutritive Value : A Review Forage and Grazinglands Forage and Grazinglands, (October 2009). https://doi.org/10.1094/FG-2010-0125-01-RV.Introduction

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). Retrieved from https://doi.org/10.33259/jlivestsci.2019.91-96

González Siso, M. I. (1996). The biotechnological utilization of cheese whey: A review. *Bioresource Technology*, 57(1), 1–11. https://doi.org/10.1016/0960-8524(96)00036-3

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.

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

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. Retrieved from https://doi.org/10.1093/jaoac/85.6.1217

Miller, W. J., Clifton, C. M., & Cameron, N. W. (1962). Nutrient Losses and Silage Quality as Affected by Rate of Filling and Soybran Flakes. *Journal of Dairy Science*, 45(3), 403–407. Retrieved from https://doi.org/10.3168/jds.S0022-0302(62)89405-3

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. Retrieved from 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). Retrieved from https://doi.org/10.1590/s1516-35982007000600024

Paviz, M. M., Ghoorchi, T., & F., G. (2011). 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.

Ribas, T. (2018). *Efeito da Inclusão De Inoculante Bacteriano Na Silagem De Milho Sobre As Características Quimiofermentativas E Desempenho Animal*.

Rodrigues, P. H. M., Pinedo, L. A., Meyer, P. M., da Silva, T. H., & Guimarães, I. C. da S. B. (2020). Sorghum silage quality as determined by chemical–nutritional factors. *Grass and Forage Science*, 75(4), 462–473. Retrieved from https://doi.org/10.1111/gfs.12495

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*.

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science*, 70(11), 3562–3577. Retrieved from https://doi.org/10.2527/1992.70113562x

Souza, A. R. D. L., de Medeiros, S. R., da Graça Morais, M., Oshiro, M. M., & de Almeida Torres Júnior, R. A. (2009). Dieta com alto teor de gordura e desempenho de tourinhos de grupos genéticos diferentes em confinamento. *Pesquisa Agropecuaria Brasileira*, 44(7), 746–753. Retrieved from https://doi.org/10.1590/S0100-204X2009000700015

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. Retrieved from 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. Retrieved from 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