SCIENTIA PLENA VOL. 20, NUM. 12 2024
www.scientiaplena.org.br doi: 10.14808/sci.plena.2024.120201

Changes in the nutritional composition of corn silage with the use of chemical additives and microbial inoculants

Alterações na composição nutricional na silagem de milho com o uso aditivos químicos e inoculantes microbianos

J. B. Zanella[1]*; A. A. Rommel[1]; A. Turmina[1]; M. R. Rosin[1]; M. F. Silveira[2]; J. Cattelam[1]

[1]*Universidade Federal da Fronteira Sul, 85770-000, Realeza-PR, Brasil*
[2]*Universidade Tecnológica Federal do Paraná, 85660-000, Dois Vizinhos-PR, Brasil*

**jaquelinebiazanella@gmail.com*
(Recebido em 29 de junho de 2023; aceito em 22 de dezembro de 2024)

This work aimed to evaluate the use of different additives in the bromatological composition of corn silage. The experimental design was completely randomized with five additives evaluated: no additive; silage added with urea (3.0%); silage added with limestone (3.0%); silage added with granulated sugar (3.0%); and silage with bacterial inoculant. After 60 days of ensiling, the chemical-bromatological composition of the produced silages was evaluated. Lime-added silage had higher dry matter contents (not differing from silage with no additive), mineral matter, and lignin. Silage with urea had higher values of crude protein (19.55%) and lower levels of total carbohydrates (74.29%) and non-fiber carbohydrates (27.13%). Silage with no additive had lower levels of ADF (38.99%) and NDF (19.79%) and a higher content of total digestible nutrients (73.98%). Using additives in corn silage promotes higher concentrations of acid or neutral detergent fiber, cellulose, and hemicellulose and lower TDN levels in its bromatological composition. The addition of urea provides an increase in protein levels, while limestone increases mineral material levels.
Key-words: ensilage, chemical composition, inoculant.

Objetivou-se avaliar o uso de diferentes aditivos na composição bromatológica da silagem de milho. O delineamento experimental foi inteiramente casualizado com cinco aditivos avaliado: sem aditivo; silagem aditivada com ureia (3,0%); silagem aditivada com calcário (3,0%); silagem aditivada com açúcar cristal (3,0%); e silagem com inoculante bacteriano. Após 60 dias de ensilagem realizou-se avaliação da composição química-bromatológica das silagens produzidas. A silagem aditivada com calcário apresentou maiores teores de matéria seca (não diferindo da silagem sem aditivo), matéria mineral e lignina. A silagem com ureia apresentou maiores valores de proteína bruta (19,55%) e menores teores de carboidratos totais (74,29%) e carboidratos não fibrosos (27,13%). A silagem sem aditivo apresentou menores teores de FDA (38,99%) e FDN (19,79%), e maior teor de nutrientes digestíveis totais (73,98%). O uso de aditivos na ensilagem de milho promove maiores concentrações de fibra em detergente ácido ou neutro, celulose e hemicelulose, e menores teores de NDT nas silagens produzidas. A adição de ureia proporciona aumento nos teores de proteína enquanto o calcário eleva os teores de material mineral.
Palavras-chave: ensilagem, composição química, inoculante.

1. INTRODUCTION

Corn silage produced from the whole plant is widely used in cattle feed. It is one of the main components of ruminant diets and is relevant to the production sector [1]. The objective of the ensilage technique is to preserve the forage's nutrients immediately after harvest, ensuring that the final silage maintains good quality for animal feed. However, problems during preparation or failures in the fermentation process can lead to loss of dry matter and nutrients, compromising the aerobic stability and quality of the silage [2].

In order to improve silage nutritional value, several studies have sought alternatives to modify the physical and chemical characteristics of corn silage and maximize the farmers' profitability [1]. The use of chemical and microbiological additives has been a promising approach, as these additives can contribute to the fermentation process, preserving nutrients and improving forage conservation. They help to increase the population of desirable bacteria, accelerate fermentation, reduce losses of dry matter and nutritional principles, and improve the aerobic stability of silage [3, 4].

Although several additives are available for corn silage, there are still gaps in the literature regarding their efficiency. Microbial inoculants, composed of homofermentative or heterofermentative bacteria or their combination, are widely used to promote efficient fermentation, reducing losses of dry matter and energy and altering the chemical composition of silage [4, 2]. These additives accelerate lactic acid production, rapidly reducing pH and improving the fermentation process. However, their effects vary, with reports of positive, neutral, or absent impacts highlighting the need for more robust comparative studies to evaluate different combinations and additive strategies [5]. Although this subject is widely explored in the scientific community, the number of studies that compare the adequate exploration of the effects of microbial additives can still be considered scarce, and the results are generally insufficient to establish conclusive positions on this topic.

In this sense, in view of improving the quality of ensiled forage, chemical additives such as urea (CH_4N_2O) have been used to increase the protein level of silage. Studies show that adding urea to sugarcane silage increases dry matter and crude protein contents, reduces neutral detergent fiber (NDF), and improves digestibility [6, 7]. Limestone or calcium carbonate ($CaCO_3$) has shown potential to reduce cell wall constituents and preserve nutrients after the silo opening, especially in sugarcane silage [8]. It may also become an interesting option for corn silage.

Other additives, such as fermentation stimulants, including sugar sources such as molasses or crystal sugar, promote the growth of lactic acid bacteria responsible for the rapid decrease in pH, which helps minimize proteolysis and the proliferation of undesirable microorganisms [9]. However, studies on the use of sugar in corn silage are still scarce, especially in Brazil, which justifies the need for research on the effects of this additive.

Therefore, to contribute to reducing the lack of studies in this area, this study aimed to evaluate the chemical and bromatological composition of corn silage made with different additives. The main contribution of this study lies in the comparative evaluation of widely used additives (such as urea and bacterial inoculants) and less explored alternatives (such as crystal sugar and limestone). These data provide important support for technical decision-making in production systems, helping farmers choose additive strategies appropriate to the specific demands of nutritional improvement and silage conservation. Thus, it contributes to maximizing feed efficiency in ruminants and to the sustainability and profitability of animal production systems.

2. MATERIAL AND METHODS

The study was conducted in Realeza, state of Paraná, southern Brazil (25°46′ S, 53°31′ W, and altitude of 520 m), from 2019 to 2020 in the experimental area of the Federal University of Fronteira Sul (UFFS), Realeza campus. The climate of the experimental area was humid subtropical (Cfb type) according to Köppen Climate Classification [10]. The soil was a typical Dystroferric Red Latosol with a clayey texture [11]. Chemical properties and textural characteristics of 0-0.2m soil layer at the beginning of the experiment in July 2019 were pH ($CaCl_2$) = 4.70, organic matter (OM)= 26.23 g dm^{-3}, Mehlich^{-1} P available= 11.25 mg dm^{-3}, Mehlich^{-1} K available= 0,49 cmolc dm^{-3}, Ca =3.66 cmolc dm^{-3}, Mg= 1.58 cmolc dm^{-3}, $H^{+}Al^{3+}$= 7.20 cmolc dm^{-3}, Al^{3+} =2.86 cmolc dm^{-3}; base saturation= 44.32% and cation exchange capacity = 12.93 cmolc dm^{-3}, clay = 650 g kg^{-1}; silt = 170 g kg^{-1}; and sand = 175 g kg^{-1}.

The area used for the experiment was composed of 300 m². In the first year of evaluation, corn was planted on September 30th. Before its establishment, the area was plowed and 3.0 Mg ha^{-1} of limestone was applied. In the second year of evaluation, the implantation of the crop took place on November 6th. In both years, the base fertilization comprised 10 Mg ha^{-1} of chicken litter. It was not used seeds of genetically modified corn hybrids for planting. Chemical herbicides, insecticides, and fungicides were not applied. Therefore, the cultivation was carried out organically.

Harvest was carried out when plants reached the point for silage, with grains in the farinaceous stage. Prior to harvest, an evaluation was performed to characterize the tillage: number of plants (counting the plants in two linear meters in 15 distinct points in the tillage); green matter (GM) production (estimated by cutting ten bundles, each with five plants cut at 25 cm from the ground;

they were randomly harvested and weighed, and their weight multiplied by the plant population). In addition, 50 plants were randomly selected to perform the following measurements: plant height, straw diameter, and leaf count. The evaluated plants were cut, sent to the UFFS laboratory, and separated into their physical components: stem, leaves, grains, and others (bract, cob, and panicle). The physical components of the plants were separately weighed in order to know the participation of each component in the green matter ensiled. After separation, the components were placed separately in paper bags, weighed, and placed in a forced air oven at 55 ºC until reaching constant weight to estimate the participation of components in the dry matter of the material to be ensiled. The productivity of the crop and the physical composition of the ensiled material are presented in Table 1.

Table 1. Corn crop productivity, physical composition of the ensiled material, and dry matter of the ensiled forage.

Variables	**Value**	**Standard error**
Number of plants per hectare	56,800	1,009.2
Green matter production, kg ha $^{-1}$	51,458.7	1,479.5
Dry matter production, kg ha $^{-1}$	17,660.4	387.9
Plant height, m	2.32	0.02
Stem diameter, mm	23.41	0.4
Number of green and dry leaves	12.42	0.27
Stem, % MS	35.19	1.08
Leaves, % MS	13.17	0.4
Grain, % MS	33.45	1.82
Others (Bract, Sap, panicle), %	18.15	0.83
DM at silo closing, %	34.65	0.01

After tillage evaluation, all plants were harvested manually, considering a height of 25 cm from the ground level, and fragmented in a forage harvester coupled to the tractor. At the time of ensiling, samples of the ground material were collected to estimate dry matter (DM), which were stored in paper bags, weighed, and placed in a forced air oven at 55 ºC until constant weight.

The ground material was stored in silage bags using a silage packing and compacting machine at a density equivalent to 550 kg m^{-3}. All bags were hermetically sealed utilizing plastic seals. During the packaging process, different additives were added to the chopped material: urea, crystal sugar, limestone, microbial additive, or no additive. The additives urea, commercial crystal sugar, and limestone were incorporated in the proportion of 3.0% of the green matter weight to be ensiled. The bacterial inoculant used was the commercial inoculant Total Silo®. The inoculant was incorporated in the proportion of 1.0 liter of the additive diluted in 100 liters of water per ton of green material for silage. For each additive evaluated, eight silage bags were produced, of which three were weighed to obtain the weight of the silage bags at silo closing. Three bags of silage were produced for each additive evaluated.

After storing the silage bags for about 60 days, they were opened. A sample of approximately 500 g of the central material was collected in each silage bag for bromatological analysis. The collected sample was stored in paper bags, weighed, and sent to a forced air oven at 55 ºC until it reached a constant weight. After this period, the sample was weighed again and subjected to grinding in a Wiley mill, half ground on a 1.0-millimeter sieve and the rest on a 2.0-mm sieve.

The material sampled from the silage bags, ground to 1.0 mm, dry matter (DM) analysis was performed, determined by drying the sample in an oven at 105 ºC for 16 hours. Mineral matter (MM) was determined by combustion at 550 °C for two hours, and the organic matter (OM) by mass difference. Total nitrogen was determined by the Kjeldahl method [12], modified by Kozloski et al. (2003) [13]. The acid detergent lignin contents were determined according to Robertson and Van Soest (1981) [14]. The determinations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed in polyester sachets [15]. Acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) were analyzed according to Licitra et al. (1996) [16]. Ethereal extract (EE) was determined in a reflux system with ethyl ether at 180 ºC for two hours [12]. The total digestible nutrients (TDN) content was calculated according to Weiss et al. (1992) [17]. Samples ground on 2.0 mm sieves were used to evaluate in vitro digestibility DM (IVDDM), according to Tilley and Terry (1963) [18].

Total carbohydrate (TC) and non-fibrous carbohydrate (NFC) contents were estimated according to Sniffen et al. (1992) [19] as follows: TC = 100 - (CP% + EE% + MM%); and NFC= 100 - (NDF% - CP% - MM%). Hemicellulose content was calculated as the difference between neutral detergent fiber and acid detergent fiber: HEM = NDF - ADF. Cellulose (CEL) content was calculated by the equation: CEL = ADF - lignin.

The experimental design used was completely randomized. Data were subjected to analysis of variance by the following mathematical model:

$$Yij = \mu + Ai + Tj + \varepsilon ij$$

Where: Yij represents the dependent variables; μ is the overall mean of the observations; The year of evaluation effect was used as a covariate; Tj is the effect of the additive used; and εij is the random residual error.

Data were submitted to analysis of variance using the "F" test (ANOVA). When significance was observed in the evaluated parameter, means were compared using the Student "t" test, with $\alpha = 0.05$. For the orthogonal contrasts analysis, the F test was applied considering $\alpha = 0.05$, in which silage with no additive was compared to silages with the inclusion of additives (bacterial inoculant, sugar, limestone, or urea). The orthogonal contrast of bacterial inoculant use versus the other chemical additives evaluated (sugar, limestone, or urea) was also carried out. Analyzes were performed using the SAS statistical program version 9.2 [20].

3. RESULTS AND DISCUSSION

Silage bromatological composition results are presented in Table 2. Information concerning the fermentation process, pH values, and concentration of organic acids in this research was published by Zanella et al. (2022) [21]. Dry matter (DM) contents were lower for silage added with urea (23.56%) and higher for silages with no additive (26.94%) or limestone (27.00%), with intermediate values for silages produced with sugar or microbial additive. At the time of closing silo bags, the ground forage presented DM means of 34.65%, and at the opening, values lower than 30% were verified for all treatments, with a reduction of DM during the fermentation process. According to Borreani et al. (2018) [22], DM losses that occur during the fermentation process are mainly associated with carbon dioxide production, and the amount of DM loss in fermentation depends on the dominant microbial species and the fermented substrates. The losses of DM and gross energy during forage fermentation processes are directly related to the type of microorganisms that develop in the plant matrix and the availability of specific substrates. Some products generated by fermentation have a higher energy value than the original substrates, which can lead to more significant DM losses than gross energy losses [23].

Without the use of additives in silage, the fermentation process occurs through the action of epiphytic microorganisms naturally present in the plant material. The main microbial groups involved are lactic acid bacteria (LAB), enterobacteria, clostridia, and yeasts. The composition and quantity of these microorganisms in plants vary according to the plant species, cultivation conditions, and environmental factors during wilting [23]. In this study, the corn plants may have had a balanced epiphytic population and characteristics that were favorable to fermentation by LAB, which may have ensured efficient fermentation with low dry matter (DM) losses.

Although the use of inoculants is widely practiced in corn silages, aiming to improve the efficiency of the fermentation process and reduce DM losses [24], this effect was not observed in the present study. The results obtained were contrary to those reported by Silva and Machado Junior (2014) [24], who found a significant increase in DM levels with the use of microbial inoculants, and by Fasolo and Carvalho (2021) [25], who observed better results in treatments with *Lactobacillus buchneri* associated with *Lactobacillus plantarum* or *Lactobacillus plantarum* combined with *Propionibacterium acidipropionici*. These findings indicate that, in this case, adding inoculants did not bring benefits.

Table 2. Chemical composition and in vitro digestibility of corn silages produced with the addition of different additives, Realeza (PR).

Variables	Additive					Standard error	P-value	Contrast	
	Sugar	Limestone	Bacterial inoculant	No additive	Urea		Additives	No additive x Additives	Bacterial inoculant x Additives
DM (g kg[1])	25.87 b	27.00a	25.39 b	26.94a	23.56 c	0.32	<0.0001	0.0001	0.8284
MM (g kg[1])	3.87 b	10.16 a	3.66 b	3.51 b	3.78 b	0.34	<0.0001	<0.0001	<0.0001
OM (g kg[1])	96.12 a	89.83 b	96.34 a	96.49 a	96.22 a	0.34	<0.0001	<0.0001	<0.0001
CP (%)	7.83 bc	8.26 b	7.21 bc	6.62c	19.55 a	0.42	<0.0001	<0.0001	<0.0001
ADF (%)	24.43 a	23.89 a	24.40 a	19.79 b	24.46 a	0.44	<0.0001	<0.0001	0.7484
NDF (%)	47.16 a	43.83 b	45.36 ab	38.99 c	47.16 a	0.81	<0.0001	<0.0001	0.4700
ADIN (%)	2.17	2.18	1.70	1.86	1.95	0.19	0.3541	0.5127	0.0762
NDIN (%)	2.05 ab	2.02 ab	1.27 c	1.67 bc	2.67 a	0.25	0.0050	0.2508	0.0015
CEL (%)	20.11 bc	19.70 c	21.24 ab	17.56 d	22.38 a	0.47	<0.0001	<0.0001	0.3569
HEM (%)	22.72 a	19.94 bc	20.96 b	19.20 c	22.69 a	0.51	<0.0001	<0.0001	0.1682
LIG (%)	4.32 a	4.18 a	3.15 b	2.23 c	2.08 c	0.20	<0.0001	<0.0001	0.1284
EE (%)	2.60 b	1.77 d	2.61 b	2.99 a	2.36 c	0.07	<0.0001	<0.0001	<0.0001
TC (%)	85.68 a	79.79 b	86.50 a	86.87 a	74.29 c	0.55	<0.0001	<0.0001	<0.0001
NFC (%)	38.52 bc	35.96 c	41.14 b	47.87 a	27.13 d	1.09	<0.0001	<0.0001	<0.0001
TDN (%)	70.73 b	71.11 b	70.75 b	73.98 a	70.71 b	0.31	<0.0001	<0.0001	0.7840
IVDDM (%)	74.60 ab	67.58 b	71.34 b	78.26 a	77.69 a	1.87	0.0022	0.0152	0.3752

*DM = dry matter; MM = mineral matter; OM = organic matter; CP= crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; ADIN= acid detergent insoluble N; NDIN= neutral detergent insoluble N; CEL=cellulose; HEM=hemicellulose, LIG=lignin; EE= ether extract; TC= total carbohydrates; NFC = non-fibrous carbohydrates; TDN = total digestible nutrients; IVDDM = in vitro digestible dry matter; **a, b, c Means followed by different letters on the row differ with $P<0.05$ by Student's "t" test.

Similarly, the absence of a positive effect was observed for the use of crystal sugar, presenting results contrary to those of Führ et al. (2023) [26]. These authors reported that the inclusion of brown sugar in sorghum silage increased dry matter content due to the higher concentration of soluble carbohydrates, which favor microbial activity during fermentation. In contrast, Rodrigues Neto et al. (2001) [27] evaluated the use of sugar in silages made from pupunha by-products and found the opposite effect. The authors did not identify significant changes in dry matter or the final quality of the silage.

Regarding mineral and organic matter contents, there was a significant difference ($P<0.05$) between the evaluated silages. The lower organic matter content observed in corn silage with limestone as additive results from the higher concentration of mineral matter that the additive provided. Similar results were found by Romão et al. (2018) [28], in which the addition of CaO promoted an increase in mineral matter content in sugarcane silages. This increase in ash values is related to the fact that the additive is of mineral origin and presents a large proportion of this fraction in its totality [29]. The other silages produced had an organic matter content above 96.0%, which according to Ashbell (1995) [30], indicates adequate forage conservation because when fermentation is inadequate, losses of organic material occur.

Considering crude protein concentration, silages with no additive had the lowest CP contents (6.62%), not differing from silages added with inoculant (7.21%) or sugar (7.83%), which were similar to silage with the addition of limestone (8.26%). However, the inclusion of urea promoted a significant increase in the CP content compared with the other additives ($P<0.05$). This increase can be explained by urea being a non-protein nitrogen source [31]. In this way, adding urea in the ensiling process can be an alternative to correct the low protein content of corn silage. The increase in protein content was also observed by Dos Santos et al. (2021) [32], in which the inclusion of 0.5; 1.0; 1.5, or 2.0% in corn silage promoted a linear increase in CP values, which were 108.98; 111.51; 139.93 and 143.18 g kg^{-1}MS, respectively, while the silage without additive had a content of 82.15 g kg^{-1}MS.

Pasandi et al. (2012) [33] and Fallah (2019) [34] reported that the addition of molasses to corn silage resulted in a significant increase in crude protein (CP) contents. The authors suggest that this increase is related to the reduction in proteolytic activity during fermentation, which contributes to preserving the original protein fractions of the silage. On the other hand, Führ et al. (2023) [26] found no significant effect of adding brown sugar on the protein content in sorghum silage, suggesting that the impact of this additive may vary depending on the plant and fermentation conditions. Zanette et al. (2012) [35] evaluated the inclusion of sugar or microbial inoculants in corn silages. They observed that silages with no additives (5.4%) and sugar (4.77%) had higher CP contents compared to those treated with microbial inoculant (4.06%).

Regarding the fibrous fraction, differences were observed in the acid detergent fiber (ADF) and neutral detergent fiber (ADF) values of silages. The silage with no additive presented the lowest values, 19.79% for ADF and 38.99% for NDF, differing statistically from the other treatments, which presented a mean of 24.30% for ADF. The increase in these silage fractions produced using additives is probably related to the consumption of soluble carbohydrates during fermentation. According to Balieiro Neto et al. (2007) [36], the consumption of soluble carbohydrates by microorganisms causes a proportional increase in the fibrous fraction and reduces the nutritive value of the silage. In the present study, the increase in fibrous fractions provided a reduction in TDN, observed through its negative correlation with ADF ($r = -0.9998$) and NDF ($r = -0.9726$), both with $P<0.0001$. For Van Soest (1994) [37], values above 55 to 60% for NDF are negatively correlated with the consumption of dry mass by the animal.

For variables related to fibrous fractions, the use of inoculant was expected to have a positive impact, reducing components such as NDF, ADF, cellulose, and hemicellulose, as observed in previous studies. Silva et al. (2005) [38] reported that microbial inoculants were effective in corn silage, promoting the reduction of these fibrous components, which resulted in greater *in vitro* digestibility of dry matter and, consequently, higher potential nutritional value. Similarly, Silva and Machado Junior (2014) [24] found that the use of microbial inoculant in corn silage led to a decrease in neutral detergent fiber (NDF) values, although there was no significant change in crude protein levels. However, such results were not observed in this study, indicating that the effect of inoculants may vary depending on the specific conditions of each ensilage.

Alamoti et al. (2004) [39] reported that adding molasses and bacterial inoculant reduced ADF levels in millet silage, indicating its fibrous quality improved. Similarly, Fallah (2019) [34] found that the inclusion of molasses in corn silage resulted in significant decreases in ADF and NDF levels, reinforcing the positive role of molasses as an additive in improving the fibrous profile of silage. Complementing these results, Führ et al. (2023) [26] demonstrated that brown sugar also reduced fiber levels in sorghum silage, corroborating the effectiveness of soluble carbohydrates in modulating the fibrous composition of different types of silage. However, in the present study, the addition of crystal sugar did not significantly impact fiber levels, suggesting that the effects may vary depending on the carbohydrate source and the conditions of the ensiling process.

Similarly, NDF levels were not lower in silages supplemented with urea than in silages with no supplementation. These results corroborate those obtained by Dos Santos et al. (2020) [40], who did not observe significant changes in NDF levels when evaluating different additives in corn silage. The expectation was that silages supplemented with urea or its combination with microorganisms would present a lower NDF concentration due to the alkaline effect of the ammonia released. However, the authors suggest that the absence of this effect may be related to the low dose of urea used (1.0%) since much of the ammonia produced was probably consumed to form salts by binding to fermentation acids, not promoting alkaline hydrolysis of the fiber, as initially predicted. In the present study, despite using a higher dose of 3%, no significant impact on NDF levels was observed.

Additives did not have an effect on the levels of acid detergent insoluble nitrogen (ADIN), although they affected neutral detergent insoluble nitrogen (NDIN) ($P<0.05$). The levels of NDIN in silage with urea were higher than in silages with inoculant or without additive, presenting intermediate behavior in silages added with limestone or sugar. Neutral detergent insoluble nitrogen represents the fraction of nitrogen present in the fiber that is slowly degraded by microorganisms in the rumen, while ADIN corresponds to the fraction of nitrogen present in the cell wall that is unavailable for absorption, which is resistant to microbial enzymes and indigestible throughout the gastrointestinal tract [16].

There was a significant difference in cellulose and hemicellulose contents ($P<0.05$). Cellulose contents in silage with urea were higher (22.38%), without differing from the silage with inoculant (21.24%), with a smaller participation of this fraction in the silage that did not receive additives (17.56%). These results corroborate the data of Vieira et al. (2004) [41], who reported higher cellulose contents in silages of sorghum hybrids BR701 and BR601 treated with pure urea, urea associated with $CaCO_3$ or inoculants when compared to the control treatment. Cellulose is the structural component that represents most of the ADF, and its use by the animal depends on the degree of lignification of this fraction [37]. The highest levels of hemicellulose (Table 2) were observed in silages with urea or limestone due to the higher concentration of fiber in the cell wall, as hemicellulose was obtained from the difference in NDF and ADF [36]. For lignin, which represents the non-digestible fraction, the silage with no additive or with urea had the lowest levels, 2.23 and 2.08%, respectively, and differed in terms of its participation in the other silages. These values are lower than those found by Faria et al. (2020) [42], who found lignin contents between 5.5 and 3.0%.

The application of additives reduced the ether extract concentrations in the silages, with a concentration of 2.99% in the silage with no additive. The values obtained are within the range that Ortiz et al. (2021) [43] verified, ranging from 1.39 to 3.49%. According to Khan et al. (2012) [44], values from 2.1 to 3.8% are considered adequate for producing quality corn silage. Attention must be paid to the ether extract content in silage when the total diet is supplemented with the concentrate fraction, as levels above 6.0 to 7.0% of fat in the diet can lead to reductions in ruminal fermentation, fiber digestibility, and passing rate [45].

The total carbohydrate content was reduced with the addition of urea or limestone additives, with values of 74.29% for urea and 79.79% for limestone, while silages using sugar (85.68%), inoculant (86.50%) or without additive (85.68%) were similar to each other. Melo et al. (2016) [31] also observed a reduction in the total carbohydrate content when additives in grass silage were used and associated this reduction with the gradual and simultaneous increase in the ether extract and ash contents. In this research, mineral matter content was inversely correlated with total carbohydrate content ($r = -0.3360$; $P=0.0087$).

Non-fibrous carbohydrate (NFC) content was lower in silages with urea or limestone ($P<0.05$), probably due to the greater production of lactic acid during the fermentation process [21]. Since both are alkaline additives, carbohydrates are used as substrates in the production of organic acids [46]. It was expected that adding sugar would increase NFC; however, this was not verified in the results. Therefore, crystal sugar may be a substrate that fermentative microorganisms do not prefer. The superiority of NFC content found in silages without additive (47.87%) indicates that this treatment will probably present a higher energy value, and in the present research, the TDN was directly related to the non-fibrous carbohydrate content ($r = 0.8468$; $P<0.0001$).

The TDN content was 73.98% for corn silage with no additive, higher than the participation of it in the other silages produced, which presented a concentration of 71.11% (limestone), 70.75% (inoculant), 70.73% (sugar), and 70.71% (urea). The higher TDN for silage produced without additive may be associated with the fact that the additives used raised ADF and NDF. However, although TDN in silage with additives is lower than in silage with no additive, their values are 67.19 to 72.99% of TDN reported by Ortiz et al. (2021) [43]. It should be noted that the higher the TDN, the better the digestibility and, consequently, the consumption of silages. According to Nussio (1993) [47], the energy value of the silage is related to the proportion of grains present in dry matter, and the author recommends that quality silage should contain from 40 to 50% grains in the ensiled DM. In this research, ensiled material presented a proportion of 33.45% in DM grains (Table 1). Although below the indicated by the literature, the produced silage presented considerable TDN content.

Silages produced with urea or without additives showed in vitro digestible DM (IVDDM) higher than those added with limestone or inoculant, with an intermediate behavior for silage produced with sugar. The reduction in digestibility with the application of the additives mentioned above differs from that observed by some authors, such as Balierio-Neto et al. (2007) [36], who observed greater digestibility with increasing doses of calcium oxide in sugarcane silage and Silva et al. (2005) [38], who found higher IVDDM values in silage treated with inoculant compared to silage without additives. In this study, including additives promoted an increase in fibrous components and, consequently, reduced digestibility.

The results of this experiment are essential for comparing silages treated with additives to those without additives. The contrast analysis showed that the crude protein content increased significantly with the addition of additives ($P<0.0001$), evidencing a positive effect in all formulations compared to the control silage. However, the total carbohydrate (TC) contents in silages with additives were significantly lower than those observed in silage without additives ($P<0.0001$).

Furthermore, the contrast analysis indicated that the means of silages without additives exceeded the means of all treatments with additives ($P<0.01$) in terms of *in vitro* digestibility. This reduction in digestibility can be attributed to the increase in fibrous components resulting from the action of the additives. The means of dry matter (DM) were also higher in the silages without additives than those with additives (0.0001).

These results highlight that, although additives improve parameters such as crude protein content, they can adversely affect other nutritional and fermentative aspects, such as digestibility and total carbohydrates. Therefore, it is essential to consider the balance between the benefits and the possible negative impacts when evaluating the application of additives in silage production. In addition, other factors, such as the type of additive and silage management, can interfere with these results, requiring additional studies.

When comparing additives, although bacterial inoculants have been widely used in corn silage for a long time, their effects remain inconclusive. Some studies report benefits in silage's chemical composition [38], while others show neutral effects [48]. In the present study, bacterial inoculants did not provide significant positive changes in the chemical composition variables of the ensiled forage.

Urea, widely used as an additive in crops such as sugarcane, showed similar effects on corn silage, especially in increasing crude protein (CP) content. This effect is related to the ability of urea to provide non-protein nitrogen (NPN), which contributes to improving the protein value of the ensiled material. However, the results of other nutritional composition variables, such as fibrous fractions (NDF and ADF) and digestibility, indicate that the benefits are limited or absent.

In addition, it is essential to be cautious regarding the dosage used since inadequate inclusion may compromise fermentation or acceptability by ruminants. Therefore, it is suggested that new studies be carried out to investigate and determine the ideal concentration of urea, which optimizes the fermentation process and improves silage quality without negatively impacting animal consumption. A comprehensive study evaluating interrelated parameters, such as fermentation, nutritional composition, and animal consumption, is necessary to establish safe and effective guidelines for the use of urea.

Regarding the addition of sugar, no improvements were observed in corn silage's nutritional composition compared to the control treatment's silage. Although some studies on sorghum silages have demonstrated nutritional benefits with brown sugar addition, such as increased dry matter and total carbohydrates [26], the results are inconsistent. Some studies, such as Zanette et al. (2012) [35], indicate a lower recovery of dry matter, suggesting that the effects may vary depending on the conditions and type of forage used.

Finally, calcium carbonate also did not cause significant changes in the overall chemical composition of the silage in this study, suggesting that its inclusion could represent only an additional cost to the production system. However, it can serve as a source of calcium, especially in crops deficient in this nutrient, such as corn [49]. Limestone has the advantage of supplying part of the calcium (Ca) requirements in the animal diet, which can positively impact feeding. However, it is essential to adequately balance the proportions of calcium and phosphorus in the diet, as recommended by the NRC (2000) [50], which suggests a 2:1 ratio in order to avoid imbalances. High calcium levels, when combined with low phosphorus levels, can result in greater urinary excretion of calcium due to the lack of phosphorus for efficient bone deposition [51]. Since calcium content was not evaluated in this study, it is essential to include it in future studies to adjust supplementation and avoid negative impacts on the calcium-phosphorus ratio.

4. CONCLUSION

The use of different additives in corn silage has distinct impacts on the silage's chemical composition, with variable effects depending on the additive used. Urea significantly increases the crude protein content, with a 295% increase in the protein content of whole-plant corn silage, constituting an effective strategy to correct the low protein content characteristic of this type of silage. The use of limestone increases the mineral matter and lignin contents without improving fiber digestibility.

The administration of additives in corn silage results in higher concentrations of acid and neutral detergent fiber, cellulose, and hemicellulose, followed by a reduction in the energy value (TDN). These results indicate that the use of additives at the tested dose does not significantly impact the nutritional value of corn silage.

5. ACKNOWLEDGMENTS

To Postgraduate Programme in Saúde, Bem-estar e Produção Animal Sustentável na Fronteira Sul of Universidade Federal da Fronteira Sul campus Realeza, Paraná, (UFFS), for the opportunity to carry out this study; and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the scholarship.

6. REFERENCES

1. Ferraretto LF, Shaver RD, Luck BD. Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. J Dairy Sci. 2018;101(5):3937-51. doi: 10.3168/jds.2017-13728
2. Kung Jr L. Silage fermentation and additives. Arch latinoam prod anim. 2018;26(3-4):61-6.
3. Yitbarek MB, Tamir B. Silage additives: Review. Open J Appl Sci. 2014;4(5):258-74. doi: 10.4236/ojapps.2014.45026

4. Muck RE, Nadeau EMG, McAllister TA, Contreras-Govea FE, Santos MC, Kung Jr L. Silage review: Recent advances and future uses of silage additives. J Dairy Sci. 2018;101(5):3980-4000. doi: 10.3168/jds.2017-13839
5. Serva L. A comparative evaluation of maize silage quality under diverse pre-ensiling strategies. Plos One. 2024;19(9):e0308627. doi: 10.1371/journal.pone.0308627
6. Dias AM, Ítavo LCV, Ítavo CCBF, Blan LR, Gomes ENO, Soares CM, et al. Ureia e glicerina bruta como aditivos na ensilagem de cana-de-açúcar. Arq Bras Med Vet Zootec. 2014;66(6):1874-82. doi: 10.1590/1678-7349
7. Sousa-Alves W, Rigueira JPS, Almeida-Moura MM, de-Jesus DLS, Monção FP, Rocha-Júnior VR, et al. Fermentative characteristics and nutritional value of sugarcane silage added with two types of urea. Rev Colomb Cienc Pecu. 2019;33(3):182-94. doi: 10.17533/udea.rccp.v33n3a02
8. Rabelo FHS, Rezende AV, Rabelo CHS, Nogueira DA, Silva WA, Vieira PF, et al. Consumo e desempenho de ovinos alimentados com silagens de cana-de-açúcar tratadas com óxido de cálcio e cloreto de sódio. Arq Bras Med Vet Zootec. 2013;65(4):1158-64. doi: 10.1590/S0102-09352013000400003
9. Woolford MK, Pahlow G. The silage fermentation. In: Wood BJB, editor. Microbiology of fermented foods. Boston (MA): Springer; 1998. p. 73-102.
10. Alvares CA, Stape JL, Sentelhas PC, De Moraes Gonçalves JL, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Z. 2013;22(6):711-28. doi: 10.1127/0941-2948/2013/0507
11. Bognola IA, Fritzsons E, Grabias J, Aguiar AV. Caracterização dos solos em áreas experimentais em grevílea, no estado do Paraná. Colombo: Embrapa; 2011.
12. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. Official methods of analysis. 12th ed. AOAC, Washington, D.C. 1995.
13. Kozloski GV, Perottoni J, Rocha JBT, Raiser AG, Sanchez LMB. Potential nutritional assessment of dwarf elephant grass *(Pennisetun purpureum*, Schum. Mott) by chemical composition, digestion and net portal flux of oxygen in cattle. Anim Feed Sci Technol. 2003;104(1-2):29-40. doi: 10.1016/S0377-8401(02)00328-0
14. Robertson JB, Van Soest PJ. The detergent system of analysis. In: James WPT, Theander O, editors. The analysis of dietary fiber in food. New York (US): Marcel Dekker; 1981. p. 123-58.
15. Komarek AR. A fiber bag procedure for improved efficiency of fiber analyses. J Dairy Sci. 1993;76(6):250-9.
16. Licitra G, Hernandez TM, Van Soest PJ. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Technol.1996;57(4):347-58.
17. Weiss WP, Conrad HR, Pierre NR. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim Feed Sci Technol. 1992;39(1-2):95-110.
18. Tilley JMA, Terry RA. A two stagee technique for the in vitro digestion of forage crops. J Br Grassl Soc. 1963;18(2):104-11.
19. Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG, Russell JB. A net carbohydrate and protein system for evaluating cattle diets: carbohydrate and protein availability. J Anim Sci. 1992;70(12):3562-77. doi: 10.2527/1992.70113562x
20. SAS/STAT user's guide: statistics. 2. ed. Version 9.2. Cary (NC): SAS Institute; 2009.
21. Zanella JB, Rommel AA, Turmina A, Kailer EK, Rosin MR, Schlichmann F, et al. Fermentative profile of corn silages with the inclusion of chemical additives or bacterial inoculant. Arq Bras Med Vet Zootec. 2022;74(6):1143-50. doi: 10.1590/1678-4162-12760
22. Borreani G, Tabacco E, Schmidt RJ, Holmes BJ, Muck RE. Silage review: Factors affecting dry matter and quality losses in silages. J Dairy Sci. 2018;101(5):3952-79. doi: 10.3168/jds.2017-13837
23. Wróbel B, Nowak J, Fabiszewska A, Paszkiewicz-Jasińska A, Przystupa W. Dry matter losses in silages resulting from epiphytic microbiota activity—A comprehensive study. Agronomy. 2023;13(2):450.
24. Silva CO, Machado Junior PC. Efeito da adição de inoculante bacteriano sobre a qualidade da silagem de milho (*Zea mays* L.). Rev Cient MediVet UNICEPLA. 2014;1(1):31-45.
25. Fasolo DJ, Carvalho AFG. Uso de diferentes inoculantes bacterianos isolados e em associação para silagem de milho. Revista Técnico-Científica do CREA-PR. 2021;27(Edição Especial):1-20.
26. Führ CA, Neres MA, Porfirio MD, Guimarães ICDSB, Da Silva TH. Effect of different alternative additives on nutritional value, in vitro digestibility, and carbohydrate fractionation of whole-plant sorghum silage. Res Soc Dev. 2023;12(7):e16512742666. doi: 10.33448/rsd-v12i7.42666
27. Rodrigues Neto AJ, Bergamaschine AF, Isepon OJ, Alves JB, Hernandez FBT, Macedo M P. Efeito de aditivos no valor nutritivo de silagens feitas com subproduto da extração do palmito de pupunha (*Bactris gasipaes HBK*). Rev Bras Zootec. 2001;30(4):1367-75. doi: 10.1590/S1516-35982001000500033

28. Romão C, De Carvalho GGP, Tosto MSL, Santos SA, Pires AJV, Maranhão CMA, Rufino LMdA, et al. Nutritional profiles of three genotypes of sugarcane silage associated with calcium oxide. Grassl Sci. 2018;64(1):16-28. doi: 10.1111/grs.12175
29. Amaral RCD, Pires AV, Susin I, Nussio LG, Mendes CQ, Gastaldello Junior AL. Cana-de-açúcar ensilada com ou sem aditivos químicos: fermentação e composição química. Rev Bras Zootec. 2009;38(8):1413-21. doi: 10.1590/S1516-35982009000800004
30. Ashbell G. Basic principles of preservation of forage, by-products and residues as silage or hay. Bet Dagan (IL): Agricultural Research Organization; 1995.
31. Melo MJAF, Backes AA, Fagundes JL, Melo MT, Silva GP, Freire APL. Características fermentativas e composição química da silagem de capim Tanzânia com aditivos. Bol Ind Anim. 2016;73(3):189-97. doi: 10.17523/bia.v73n3p189
32. Dos Santos APM, Santos EM, De Oliveira JS, De Carvalho GGP, De Araújo GGL, Zanine AM, et al. Effect of urea on gas and effluent losses, microbial populations, aerobic stability and chemical compositivo of corn (*Zea mays* L.) silage. Rev Fac Ci Agrar. 2021;53(1):309-19. doi: 10.48162/rev.39.030
33. Pasandi M, Kamali R, Kavian A. The use of molasses to improve the fermentation of sweet corn Stover silage. Anim Sci J (Pajuhesh and Sazandegi). 2012;25:27-32.
34. Fallah, R. Effects of adding whey and molasses on corn silage quality, growth performance and health of Simmental fattening calves. J Livestock Sci. 2019;10(2):91-6. doi: 10.33259/JLivestSci.2019.91-96
35. Zanette PM, Neumann M, Faria MV, Ueno RK, Marafon F, Durman T. Valor nutricional e perdas durante a fermentação de silagens de milho (*Zea mays* L.) com açúcar ou inoculante. Rev Bras Milho e Sorgo. 2012;11(2):178-89. doi: 10.18512/1980-6477/rbms.v11n2p178-189
36. Balieiro Neto G, Siqueira GR, Reis RA, Nogueira JR, Roth MTP, Roth APT. Óxido de cálcio como aditivo na ensilagem de cana-de-açúcar. Rev Bras Zootec. 2007;36(5):1231-9. doi: 10.1590/S1516-35982007000600003
37. Van Soest PJ. Nutrition and ecology of the ruminant. 2. ed. Ithaca: Cornell University Press; 1994.
38. Silva AV, Pereira OG, Garcia R, Valadares Filho SDC, Cecon PR, Ferreira CLDLF. Composição bromatológica e digestibilidade in vitro da matéria seca de silagens de milho e sorgo tratadas com inoculantes microbianos. Rev Bras Zootec. 2005;34(6):1881-90. doi: 10.1590/S1516-35982005000600011
39. Alamoti AA, Alikhani M, Ghorbani G, Samie A. Effects of different additives on fermentation quality of millet silage in laboratory silos. J Crop Prod Process. 2024;8(3):149-61.
40. Dos Santos APM, Santos EM, De Araújo GGL, De Oliveira JS, Zanine AM, Pinho RMA, et al. Effect of inoculation with preactivated Lactobacillus buchneri and urea on fermentative profile, aerobic stability and nutritive value in corn silage. Agriculture. 2020;10(8):335. doi: 10.3390/agriculture10080335
41. Vieira FAP, Borges I, Stehling CAV, Gonçalves LC, Coelho SG, Ferreira MIC, et al. Qualidade de silagens de sorgo com aditivos. Arq Bras Med Vet. 2004;56(6):764-72. doi: 10.1590/S0102-09352004000600011
42. Faria TFR, Pinese F, Gimenes FMA, Demarchi JJAA, Campos FP, Premazzi LM, et al. Chemical composition of commercial corn silage produced in Brazil. Arch Zootec. 2020;69(265):156-63. doi: 10.21071/az.v69i266.5110
43. Ortiz S, Martin T, Fulaneti FS, Valdovino VC. Silos experimentais e a composição bromatológica de silagem de milho e sorgo. Rev Vivências. 2021;17(33):229-42. doi: 10.31512/vivencias. v17i33.462
44. Khan NA, Cone JW, Fievez V, Hendrik WH. Causes of variation in fatty acid content and composition in grass and maize silages. Anim Feed Sci Technol. 2012;174(1-2):36-45. doi: 10.1016/j.anifeedsci.2012.02.006
45. National Research Council (NRC). Nutrient requirements of small ruminants. Washington (US): National Academy Press; 2007.
46. Muck RE. Silage microbiology and its control through additives. Rev Bras Zootec. 2010;39:183-91. doi: 10.1590/S1516-35982010001300021
47. Nussio LG. Milho e sorgo para a produção de silagem. In: Peixoto AM, De Moura JC, De Faria VP, organizers. Volumosos para bovinos. Piracicaba (SP): FEALQ; 1993. p. 75-174.
48. Tavares QG, Carvalho WTV, Pereira RV G, Minighin DC, Silva LV, De Carvalho CAO, De Melo NN. Nutritional evaluation of corn and sorgo silages inoculated with *Lactobacillus plantarum*. PUBVET. 2020;14(3):NA2536. doi: 10.31533/pubvet.v14n3a536.1-9
49. McDonald P, Henderson AR, Heron SJE. The biochemistry of silage. 2. ed. Marlow: Chalcombe Publications; 1991.
50. National Research Council (NRC). Nutrient requerements of beef cattle.Washington (US): National Academy of Science; 2000.

51. Gionbelli MP, Marcondes MI, Valadares Filho SC, Prados LF. Exigências nutricionais de minerais para bovinos de corte. In: Valadares Filho SC, Marcondes MI, Chizzotti ML, Paulino PVR, editors. Exigências nutricionais de zebuínos puros e cruzados. BR-CORTE. 2. ed. Viçosa (MG): UFV; 2010. p. 135-74.