

Effects of cutting height and bacterial inoculant on corn silage aerobic stability and nutrient digestibility by sheep

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ABSTRACT - In this study, we aimed to determine the effects of cutting height (25 or 40 cm above ground) and bacterial inoculation (a combined inoculant of *Lactobacillus plantarum* and *Propionibacterium acidipropionici*) on the chemical and microbial compositions, fermentative profile, and aerobic stability of whole-plant corn silage and nitrogen balance, intake, and apparent nutrient digestibility by sheep. To evaluate silage characteristics and sheep metabolism, we performed analyses based on a completely randomized block design with a 2×2 factorial arrangement (two cutting heights, with or without bacterial inoculant). We evaluated the chemical and microbial compositions, pH, fermentation end-products, and aerobic stability of silage. To examine nutrient digestibility of silage, we used 24 male sheep over a 21-day period. We found that the aerobic stability did not differ among the silages. Sheep fed silages produced from corn harvested at 40 cm had increased intakes of crude protein, non-fiber carbohydrate, and total digestible nutrients, whereas the non-fiber carbohydrate intake of inoculated corn silages was found to be higher than that of uninoculated silage. Furthermore, the amounts of nitrogen retained by sheep fed silage produced from corn harvested at 40 cm were higher than those of sheep fed silage produced from corn harvested at 25 cm. Collectively, our findings indicate that, despite the observed effects, a difference of 15 cm in cutting height results in relatively small changes in the chemical composition of corn silage and a limited effect on the nutrient intake and nitrogen balance of animals fed this silage. Moreover, although bacterial inoculation promotes an efficient fermentation, it has no marked effects on the aerobic stability of silage.

Keywords: ensiling, feed-out phase, intake, *Lactobacillus plantarum*, *Propionibacterium acidipropionici*

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Received: November 1, 2019

Accepted: July 14, 2020

How to cite: Mendonça, R. C. A.; Cardoso, M. V. S. B.; Pantoja, S. O. S.; Souza, M. S.; Domingues, F. N.; Faturi, C.; Silva, T. C. and Rêgo, A. C. 2020. Effects of cutting height and bacterial inoculant on corn silage aerobic stability and nutrient digestibility by sheep. Revista Brasileira de Zootecnia 49:e20190231.
<https://doi.org/10.37496/rbz4920190231>

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1. Introduction

With a view toward improving the characteristics of silage, and consequently the utilization of nutrients by animals (Caetano et al., 2011; Nigon et al., 2016; Muck et al., 2018), the effects of a number of different management practices (e.g., cutting height and kernel processing) applied during the ensiling process have been examined. During harvest, adjusting the height at which the corn crop is cut can modify the fermentation profile in silo and the nutritive value of silage (Lewis et al., 2004; Aoki et al., 2013). Furthermore, harvesting forage at a high cutting height has been found to increase the residual forage mass in the field, thereby promoting increased nutrient cycling in soil and contributing to reductions in fertilizer usage (Jobim et al., 2013).

Overall, increasing the height at which the corn crop harvested increases the proportion of grain of the forage, thereby increasing the nutritive value of silage (Ferraretto et al., 2018). This practice may be particularly suitable in regions with hot climates, as corn grown under higher temperatures tends to be more fibrous and accumulates less starch (Daniel et al., 2019). However, corn silage with a higher nutritive value can be susceptible to more pronounced aerobic deterioration (Tabacco et al., 2011). According to Borreani et al. (2018), the preservation potential of forage is reduced during the feed-out phase, as yeasts and molds can assimilate lactic acid under aerobic conditions, thereby contributing to the deterioration.

To overcome this problem, bacteria that produce lactic and antifungal acids can be used as inoculants to control fermentative profiles and aerobic deterioration (Filya et al., 2006). Lactic and propionic acids are fermentation end-products that have beneficial effects on the fermentative process and can enhance the aerobic stability of silage, respectively. This can contribute to reducing losses and, indirectly, have a positive effect on animal production (Muck et al., 2018). Accordingly, it is important to determine the effects of inoculants on the characteristics of silage through studies on animal assays.

Therefore, in this study, we aimed to determine the effects of cutting height (25 or 40 cm) and inoculation with a co-culture of *Lactobacillus plantarum* and *Propionibacterium acidipropionici* on the chemical and microbial compositions, fermentative profile, and aerobic stability of whole-plant corn silage and nitrogen balance, intake, and nutrient digestibility by sheep.

2. Material and Methods

2.1. Ethical declaration

All procedures and animal manipulations performed in this study were approved by the institutional Ethics Committee on the Use of Animals (CEUA) in Belém, Pará, Brazil (case no. 029/2014 and 23084.017610/2014-79).

Experiment I: Fermentative, microbial, and chemical compositions and aerobic stability of silage

2.2. Experimental design and ensiling process

In February 2015, the corn hybrid DKB 177 was planted at 55,000 plants ha⁻¹ on a farm located in Paragominas, PA, Brazil (farm coordinates: 02°59'45" S, 47°21'10" W; altitude 90 m). The soil at this site is classified as an Oxisol, and fertilizer was applied in accordance with the results of a soil test. The regional climate is classified as Aw (Alvares et al., 2013), with two well defined seasons, namely, a rainy season from December to May and a dry season from June to November.

Corn plants with a dry matter (DM) content of approximately 350 g kg⁻¹ were harvested using a pull-type harvester adjusted to the desirable cutting height (25 or 40 cm above ground) and with a theoretical length of cut of 20 mm. Fresh corn was inoculated with mixed bacterial inoculant containing 1×10⁵ cfu g⁻¹ fresh forage of *L. plantarum* MA 18/U5 and *P. acidipropionici* MA 26/4U. The inoculant was diluted in distilled water at ambient temperature, applied by spraying at rate of 5 mL kg⁻¹ fresh forage, and subsequently mixed with the forage. Samples of uninoculated forage were used as controls.

To evaluate the chemical and microbial compositions, fermentative profile, and aerobic stability of silage, we used a completely randomized design with a 2×2 factorial arrangement of two harvest heights (25 or 40 cm above ground) and two inoculation treatments (with or without the application of a bacterial inoculant), and six replicates per treatment. Accordingly, we used a total of 24 plastic drums (200 L) as experimental silos, into each of which an average 120 kg of forage was packed manually to give a density of approximately 600 kg m⁻³. Prior to ensiling, four samples (~300 g) of fresh corn plants were collected to determine the chemical composition of the harvested forage (Table 1).

Table 1 - Chemical composition of corn plants harvested at two cutting heights prior to ensiling

Item	25 cm height	40 cm height
Dry matter ¹	323.9	360.1
Organic matter ²	967.1	970.9
Ash ²	32.9	29.1
Crude protein ²	61.4	71.9
Ether extract ²	22.4	21.5
NDF ²	485.5	486.3
ADF ²	263.7	259.8
Hemicellulose ²	221.8	226.5
Lignin ²	31.8	28.2

NDF - neutral detergent fiber assayed using heat-stable amylase; ADF - acid detergent fiber.

¹ g kg⁻¹ on a fresh basis.

² g kg⁻¹ dry matter.

The silos were covered and stored for 45 days at ambient temperature (26.2±1.2 °C). On the mornings of each day during the course of the ensiling process, a layer of silage (30 cm) was manually removed from the top of the experimental silos and used to feed sheep to evaluate the intake, nutrient digestibility, and chemical composition. The pH, aerobic stability, nitrogen balance, microbial counts, ammonia nitrogen, and organic acids were determined in samples obtained when the contents of the silos had reached half the ensiled mass.

2.3. Determination of chemical composition

To evaluate the nutrient contents of silage, Orts, and feces, samples were weighed and dried in a forced-air circulation oven at 55 °C for 72 h, after which they were ground in a Wiley mill equipped with a 1-mm sieve. The DM, organic matter (OM), and crude protein (CP) concentrations were determined according to methods (934.01, 923.03, and 978.04, respectively) described by the Association of Official Analytical Chemistry (AOAC, 1990). Ether extract (EE) was determined using a fat extraction system (model XT10-Ankom® 2009).

Analysis of neutral detergent fiber (NDF) was performed according to AOAC method 973.18 (AOAC, 1990), using a thermostable alpha-amylase enzyme without sodium sulfite, and subsequently corrected for ash and protein. Acid detergent fiber (ADF) and lignin contents were determined using AOAC method 973.18 (AOAC, 1990).

The concentrations of total carbohydrate and non-fibrous carbohydrates (NFC) were calculated as described by Sniffen et al. (1992) and Detmann and Valadares Filho (2010), respectively, and the particle size of silage was evaluated as previously described by Kononoff and Heinrichs (2003).

2.4. Microbial counts and fermentation characteristics

To quantify the microbial populations of silage, we prepared an aqueous suspension of silage (25 g) in 225 mL of peptone water, which was homogenized manually for 3 min. Subsequently, eight-fold dilutions were prepared to determine the yeast and mold counts via spreading on potato dextrose agar plates (Sigma-Aldrich, Brazil LTDA). Counts of yeasts and molds, the colonies of which were identified based on micromorphological characteristics, were performed after incubation at 26 °C for three and five days, respectively.

Silage pH values were determined from an aqueous extract prepared from 9 g of silage in 60 mL of distilled water (Silva and Queiroz, 2002). The sample was homogenized and allowed to stand for 30 min, prior to taking reading using a benchtop pH meter (Tekna T-1000). Ammonia nitrogen (N-NH₃) concentrations were determined according AOAC method 941.04 (AOAC, 1990).

Fermentation end-products (lactic, acetic, propionic, and butyric acids, ethanol, and 1,2-propanediol) were analyzed using high-performance liquid chromatography. For determinations, we prepared an aqueous extract containing 30 g of silage in 270 mL of distilled water. The samples were homogenized for 4 min at 200 rpm using a Stomacher bag (Stomacher® 400; Seward). Aliquots (2.0 mL) of the aqueous extracts were placed in Eppendorf tubes containing 0.01 mL of a 50 mL L⁻¹ solution of sulfuric acid and subsequently centrifuged three times for 10 min at 10,000 rpm, filtered, and injected. The HPLC system (Shimadzu, Corp., Tokyo, Japan) was equipped with a dual detection system comprising an ultraviolet detector (UV-Vis) and a refractive index detector (RID; 10A SPD-10Ai) and incorporated to two columns [an ion exclusion column (SUPELCO-SUPELCOGEL 8H; 30 cm × 7.8 mm) and a pre-column (SUPELCO-SUPELCOGEL 8H; 5 cm × 4.6 mm)] operating at 30 °C. The mobile phase consisted of water and 0.005 M sulfuric acid with a flow of 0.6 mL min⁻¹. The organic acids were detected based on their UV absorbance at 210 nm, and the alcohols were identified according to their refractive index. Total organic acids were determined by summing the values obtained for each of the acids evaluated. The lactic:acetic acid ratio was calculated by dividing the concentration of lactic acid by that of acetic acid.

2.5. Aerobic stability

The aerobic stability of silages was assessed over a 12-day period. A sample of silage (~3 kg) from each silo was placed in a plastic container (20 L) and kept at 21 °C in a controlled-temperature room. The ambient and silage temperatures were determined at half-hourly intervals using dataloggers (Escort, MX-ST-S-8-L, Cryopak®), which were placed at the geometric center of the silage mass. Aerobic stability was defined as the number of hours that the silage remained stable prior to reaching a temperature of 2 °C above the ambient temperature. The maximum temperature and time to reach the maximum temperature were calculated from the data obtained from the aerobic stability assay. Amplitude was calculated as the difference between the maximum and minimum temperatures of the silages.

Experiment II: Ingestion and digestibility in sheep

2.6. Management and animal feed

Evaluations of silage intake, nutrient digestibility, and nitrogen balance were performed using 24 ten-month-old non-castrated male Santa Inês sheep with an average initial body weight of 27.3 kg, which were assessed in a randomized block design with a 2×2 factorial arrangement of two harvest heights (25 or 40 cm above ground) and two inoculation treatments (with or without bacterial inoculant). The silages were the sole feed sources in the sheep diets. Each animal was assigned to one of the 24 silos, and fed exclusively from the silage in that silo until the end of the experimental period. The diets, to each of which six sheep were assigned, were as follows: corn silage harvested at a cutting height of 25 cm and ensiled without inoculant; corn silage harvested at a cutting height of 25 cm and ensiled with *L. plantarum* and *P. acidipropionici* inoculant; corn silage harvested at a cutting height of 40 cm and ensiled without inoculant; and corn silage harvested at a cutting height of 40 cm and ensiled with *L. plantarum* and *P. acidipropionici* inoculant.

The 24 sheep were divided into two groups according to body weight (BW) [group 1 = animals with a BW greater than 27.3 kg; group 2 = animals with a BW smaller than 27.3 kg]. The animals were divided into two blocks, with three animals from group 1 and three animals from group 2 being placed in each block to give an average BW of 27.3 kg. The animals were maintained in wooden metabolic cages (0.79 m²) equipped with a slatted floor and a water fountain, and were given one of the four diets.

The experimental period was 21 days, in which the first 14 days the animals were allowed to adapt to the ambient conditions, management, diet, and intake adjustments. In the subsequent seven days, we collected orts, feces, and total urine. The feed was offered *ad libitum*, twice a day at 08:00 and 17:00 h, and the animals had free access to water throughout. The DM and nutrient intake, digestibility, and nitrogen balance were determined from the chemical compositions of the samples.

Determination of animal intake

Animal intake was assessed for the feed supplied between days 14 and 21 of the experimental period, and orts were assessed between days 15 and 22. During the sampling period, silage samples (~300 g) and orts (maximum of 10 g kg⁻¹) were collected. The samples were placed in plastic bags, labeled, and stored at -20 °C for subsequent determination of the chemical composition.

Nutrient intake was calculated as the difference between feed intake and orts divided by feed intake. The intake of total digestible nutrients (TDN) was determined as described by Sniffen et al. (1992).

Determination of apparent digestibility

The apparent digestibility of silage was estimated from fecal samples collected from days 15 to 22 of the experimental period. The feces from each animal were collected into plastic bowls, weighed daily, and homogenized individually to obtain fecal samples. The samples were placed in plastic bags, labeled, and stored at -20 °C for subsequent determinations of chemical composition.

The apparent digestibility of nutrients was calculated by the difference between feed intake and feces, divided by feed intake, and multiplied by 100. The TDN were determined as described by Sniffen et al. (1992).

Nitrogen balance

The nitrogen balance was calculated based on nitrogen concentrations in the feed, orts, feces, and urine. Urine was collected in a plastic bucket equipped with a filter at the top that separated feces from urine. To prevent fermentation and ammonia losses by volatilization, 10 mL of sulfuric acid (10 mL L⁻¹) was placed inside the plastic receptacles. Urine samples were placed in glass bottles, labeled, and stored at -20 °C.

The nitrogen concentration in urine samples was determined using AOAC method 994.19 (AOAC, 1990) to determine the nitrogen balance. The nitrogen fixed (NF), nitrogen absorbed (NA), and nitrogen intake (NI), expressed as g animal day⁻¹ and metabolic weight, g MW⁻¹ (BW^{0.75}), were calculated according to equations 1, 2, and 3:

$$N \text{ (g) fixed} = N \text{ (g) intake} - (N \text{ (g) feces} + N \text{ (g) urine}) \quad (1)$$

$$N \text{ (g) absorbed} = N \text{ (g) intake} - N \text{ (g) feces} \quad (2)$$

$$N \text{ (g) intake} = N \text{ (g) offered} - N \text{ (g) orts} \quad (3)$$

2.7. Statistical analysis

Microbial count data were log₁₀-transformed. All variables were analyzed based on the 2×2 factorial arrangement, using the PROC GLM procedure of SAS (Statistical Analysis System, version 9.2), considering the effects of height (H), inoculation (I), and their interaction (H × I).

To analyze the effects of pH on aerobic stability, a split-plot arrangement was used, in which the factors of the plots were the silage in the different treatments and the time of exposure to the air was the subplot. The effects of height, inoculation, exposure time (T), and their interaction were determined. The pH data with respect to cutting height (25 or 40 cm) were subjected to polynomial regression analysis. Average values were compared using a *t* test ($\alpha = 0.05$).

The statistical model used to evaluate chemical and microbial compositions, fermentative profile, and aerobic stability of whole-plant corn silage was as follows:

$$Y_{ijr} = \mu + A_i + I_j + IA_{ij} + E_{ijr},$$

in which Y_{ijr} , μ , A_i , I_j , IA_{ij} , and E_{ijr} represent the analyzed variable, the overall mean, the fixed effect of height, the fixed effect of inoculation, the fixed effect of the interaction height \times inoculation, and the residual error, respectively.

The statistical model used to evaluate the pH data collected during 12 days of aerobic exposure of whole-plant corn silage was as follows:

$$Y_{ijer} = \mu + A_i + I_j + T_e + IA_{ij} + IAT_{ie} + IIT_{je} + IAIT_{ije} + E_{ijer},$$

in which Y_{ijer} , μ , A_i , I_j , T_e , IA_{ij} , IAT_{ie} , IIT_{je} , $IAIT_{ije}$, and E_{ijer} represent the analyzed variable, the overall mean, the fixed effect of height, the fixed effect of inoculation, the fixed effect of time, the fixed effect of the interaction height \times inoculation, the fixed effect of the interaction height \times time, the fixed effect of the interaction inoculation \times time, the fixed effect of the interaction height \times inoculation \times time, and the residual error, respectively.

The statistical model used to evaluate intake, apparent nutrient digestibility, and nitrogen balance of whole-plant corn silage was as follows:

$$Y_{pijr} = \mu + B_p + A_i + I_j + IA_{ij} + E_{pijr},$$

in which Y_{pijr} , μ , B_p , A_i , I_j , IA_{ij} , and E_{pijr} represent the analyzed variable, the overall mean, the fixed effect of block (weight), the fixed effect of height, the fixed effect of inoculation, the fixed effect of the interaction height \times inoculation, and the residual error, respectively.

3. Results

Experiment I: Fermentative, microbial, and chemical compositions and aerobic stability of silages

3.1. Microbial counts and silage fermentative and chemical characteristics

With respect to yeast counts, we found that a higher cutting height reduced yeast numbers ($P < 0.05$; Table 2). Silage derived from corn harvested at a cutting height of 40 cm was found to contain $8.06 \log_{10} \text{ cfu g}^{-1}$ of silage, whereas the silage produced from corn harvested at a cutting height of 25 cm contained $8.95 \log_{10} \text{ cfu g}^{-1}$ of silage. In contrast, we detected no interactive effect between cutting height and inoculant treatment ($P > 0.05$) on yeast counts.

We found that inoculated silages prepared from corn harvested at a cutting height of 25 cm had higher concentrations of lactic acid and total organic acids than uninoculated silage prepared from corn

Table 2 - Microbial composition and fermentation profile of silages prepared from corn plants harvested at two cutting heights ensiled with and without bacterial inoculant

Item	25 cm height		40 cm height		SEM	P-value		
	Control	WI	Control	WI		H	I	H \times I
Microbial profile								
Molds ¹	<2.0	<2.0	<2.0	<2.0	-	-	-	-
Yeasts ¹	9.53	8.38	7.98	8.13	0.43	0.041	0.262	0.151
Fermentation profile								
pH	3.64	3.62	3.66	3.69	0.04	0.291	0.892	0.572
Lactic acid ²	51.2b	83.5a	72.2a	52.1b	0.64	0.511	0.442	0.003
Acetic acid ²	4.3	2.1	0.8	1.4	0.14	0.011	0.312	0.083
TOA ²	55.5b	85.6a	73.0a	53.5b	0.63	0.351	0.512	0.004
Ammonia-N ³	52.9a	28.9b	27.1b	27.3b	0.40	0.011	0.012	0.002

cfu - colony forming units; TOA - total organic acids; Control - without inoculant; WI - with inoculant; H - cutting height; I - inoculation; H \times I - interaction (cutting height \times inoculation).

¹ cfu g⁻¹ of fresh silage.

² g kg⁻¹ dry matter.

³ g kg⁻¹ total nitrogen.

Means in the same row followed by different letters are different by the Tukey test ($P < 0.05$).

harvested at the same cutting height. However, we detected no comparable difference for the silages prepared from corn harvested at a cutting height of 40 cm. When inoculated, these silages contained less lactic acid and total organic acids than uninoculated silages. However, when corn was cut at 25 cm, uninoculated silage was found to contain less lactic acid and total organic acids than when corn was harvested at 40 cm. We also detected interactive effects between cutting height and inoculant treatment on the concentrations of lactic acid ($P < 0.05$) and total organic acids ($P < 0.05$), whereas no similar effect was observed with respect to silage pH (Table 2).

Silages prepared from corn harvested at the lower cutting height (25 cm) were found to have higher ($P < 0.05$) concentrations of acetic acid than those prepared from corn harvested at 40 cm (Table 2). However, we detected no interactive effect between cutting height and inoculant treatment with respect to acetic acid concentrations ($P > 0.05$). None of the silages examined were found to contain propionic or butyric acids. Inoculated silages prepared from corn harvested at a cutting height of 25 cm had a higher $N-NH_3$ concentration than inoculated silages prepared from corn harvested at the same cutting height (Table 2). Conversely, we detected no significant differences between inoculated and uninoculated silages at a cutting height of 40 cm. Regardless of inoculation status, silages prepared from corn harvested at a cutting height of 40 cm contained a low $N-NH_3$ concentration compared with uninoculated silages prepared from corn harvested at a cutting height of 25 cm, although it did differ from the same silage that had been inoculated. Furthermore, we detected an interactive effect between cutting height and inoculation treatment ($P < 0.05$) with respect to $N-NH_3$ concentration (Table 2).

For a harvest cutting height of 25 cm, we found that inoculated silages had higher CP concentrations than uninoculated silages did, whereas when corn was harvested at 40 cm, inoculated and uninoculated silages had similar CP concentrations (Table 3). There was also an interactive effect of between cutting height and inoculation treatment ($P < 0.05$) with respect to CP concentration.

Table 3 - Chemical composition and particle size of silages prepared from corn plants harvested at two cutting heights ensiled with and without bacterial inoculant

Item	25 cm height		40 cm height		SEM	P-value		
	Control	WI	Control	WI		H	I	H × I
Chemical composition								
Dry matter ¹	319.5	325.0	348.1	344.0	0.33	0.011	0.832	0.175
Organic matter ²	968.7	969.0	969.7	969.0	0.07	0.501	0.742	0.501
Ash ²	31.2	31.0	30.3	30.2	0.07	0.501	0.742	0.501
Crude protein ²	62.5c	69.1b	73.7a	73.9a	0.09	0.001	0.001	0.012
Ether extract ²	26.4b	26.1b	25.2b	29.7a	0.10	0.251	0.052	0.022
NDF ²	459.0	432.2	408.1	397.7	0.87	0.001	0.041	0.355
NDFap ²	459.0	419.3	386.4	369.3	0.87	0.001	0.001	0.211
ADF ²	248.9	229.6	211.5	217.1	0.65	0.001	0.311	0.074
Hemicellulose ²	214.2	209.1	199.5	181.5	0.57	0.001	0.061	0.273
NFC ²	420.8	441.7	462.6	467.7	0.90	0.011	0.141	0.351
Lignin ²	27.1	20.0	17.4	24.3	0.38	0.491	0.981	0.081
% Particle size retained								
>1.91 cm	14.86		15.37		2.20	0.822	-	-
0.79–1.91 cm	76.08		73.66		2.19	0.442	-	-
<0.79 cm	9.06		10.97		1.98	0.342	-	-

NDF - neutral detergent fiber assayed using heat-stable amylase; NDFap - neutral detergent fiber assayed with the heat-stable amylase and expressed exclusive of ash and protein; ADF - acid detergent fiber; NFC - non-fibrous carbohydrate; Control - without inoculant; WI - with inoculant; H - cutting height; I - inoculation; H × I - interaction (cutting height × inoculation).

¹ g kg⁻¹ on a fresh basis.

² g kg⁻¹ dry matter.

Means in the same row followed by different letters are different by the Tukey test ($P < 0.05$).

We detected no significant differences in the EE concentrations of inoculated and non-inoculated silages when corn was harvested at 25 cm, whereas inoculated silages had higher EE concentrations than uninoculated silages when the cutting height was raised to 40 cm. In addition, the EE concentrations of non-inoculated silages when corn was harvested at 25 cm and 40 cm were similar. In the case of inoculated silages, however, harvesting at 40 cm resulted in higher silage EE concentrations than harvesting at 25 cm. There was an interactive effect between cutting height and inoculation treatment ($P < 0.05$) for EE concentration.

A cutting height of 40 cm resulted in increases in silage DM (346.1 vs. 322.2 g kg⁻¹ DM; $P < 0.05$) and NFC (465.1 vs. 431.2 g kg⁻¹ DM; $P < 0.05$) concentrations and reductions in NDF (402.9 vs. 445.6 g kg⁻¹ DM; $P < 0.05$), hemicellulose (190.5 vs. 211.7 g kg⁻¹ DM), and ADF (214.3 vs. 239.2 g kg⁻¹ DM) concentrations, compared with the silages obtained from corn harvested at a cutting height of 20 cm. Furthermore, we found that inoculated silage had a lower NDF concentration (414.9 vs. 433.6 g kg⁻¹ DM; $P < 0.05$) compared with uninoculated silages. There was, however, no effect ($P > 0.05$) of cutting height on silage particle size (Table 3).

3.2. Aerobic stability

At the opening of all silos, the silage temperature was 2 °C higher than the ambient temperature. However, inoculated silages prepared from corn harvested at a cutting height of 40 cm needed approximately 89 h of aerobic exposure to reach the maximum temperature, which was significantly different ($P < 0.05$) compared with the uninoculated silages. Neither cutting height nor inoculation temperature appeared to have any discernable effects on the maximum temperature ($P > 0.05$) or silage amplitude ($P > 0.05$) (Table 4).

The interaction between cutting height and time in aerobic exposure ($P < 0.05$) revealed that the pH of silage was altered as a function of time (Figure 1). During the initial five days of ensiling, the pH of silages prepared from corn harvested at a cutting height of 40 cm (average pH = 4.91) was lower than that of silages prepared from corn harvested at a cutting height of 25 cm (average pH = 5.40) during the same period. However, we noted that silage pH (average pH = 6.23) tended to stabilize from the sixth day of evaluation.

Experiment II: Ingestion and digestibility in sheep

3.3. Intake, digestibility, and nitrogen balance

We observed that a cutting height of 40 cm resulted in higher intakes of CP ($P < 0.05$), NFC ($P < 0.05$), TDN ($P < 0.05$), and ADF ($P < 0.05$) of 2.0, 13.7, 18.8, and 5.3 g kg⁻¹ BW, respectively, compared with the corresponding intakes of 1.3, 12.5, 17.0, and 4.7 g kg⁻¹ at a cutting height of 25 cm. Furthermore, inoculation of corn silages resulted in a higher ($P < 0.05$) NFC intake (13.9 g kg⁻¹ BW) compared with uninoculated silages (12.3 g kg⁻¹ BW). In contrast, there was no significant difference in the intake of OM among the different silages. Similarly, we detected no interactive effect of cutting height and inoculation treatment ($P > 0.05$) with respect to either DM or nutrient intake (g kg⁻¹ BW) (Table 5).

Table 4 - Temperature of silages produced from corn plants harvested at two cutting heights ensiled with and without bacterial inoculant over 12 days of aerobic exposure

Item (°C)	25 cm height		40 cm height		SEM	P-value		
	Control	WI	Control	WI		H	I	H × I
MT	39	37	37	35	1.65	0.171	0.171	0.782
THM	49b	37b	37b	90a	12.7	0.131	0.131	0.022
AMP	20	18	18	16	1.64	0.171	0.171	0.782

MT - maximum temperature; THM - times in hours to reach the maximum temperature; AMP - amplitude (the difference between the maximum and minimum temperatures of the silages); Control - without inoculant; WI - with inoculant; H - cutting height; I - inoculation; H × I - interaction (cutting height × inoculation); SEM - standard error of the means.

Means in the same row followed by different letters are different by the Tukey test ($P < 0.05$).

The CP digestibility of uninoculated silages prepared from corn harvested at a cutting height of 25 cm was found to be higher than that of inoculated corn silages at the same cutting height. Conversely, at a cutting height of 40 cm, the CP digestibility of silages was similar, regardless of inoculation. Similarly,

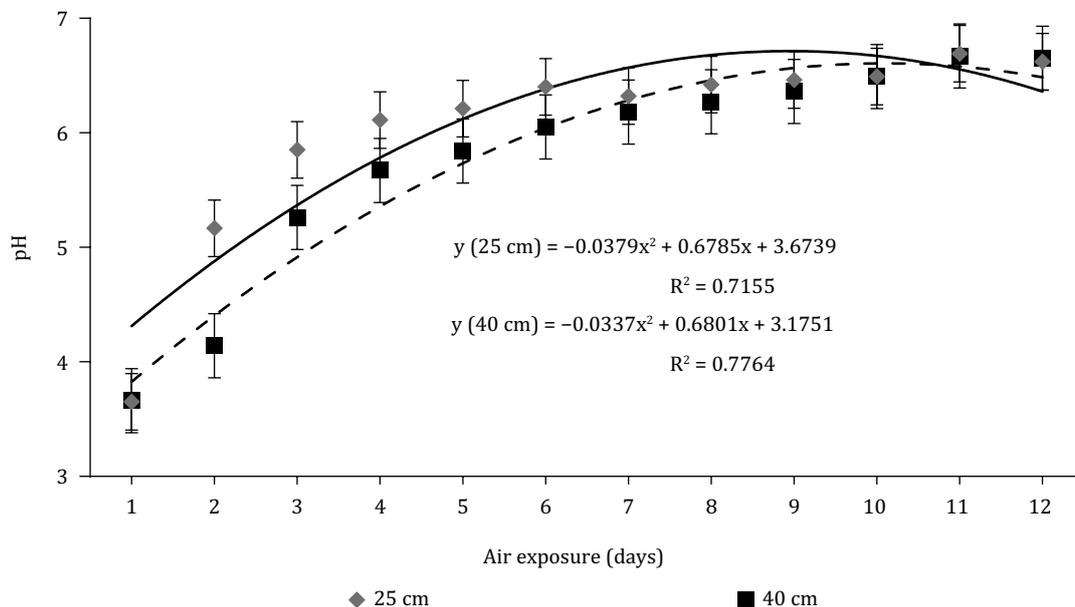


Figure 1 - Changes in the pH of silages prepared from corn plants harvested at two different cutting heights during aerobic exposure for 12 days. $P > 0.05$.

Table 5 - Nutrient intake of sheep fed corn silages prepared from plants harvested at two cutting heights ensiled with and without bacterial inoculant

Item	25 cm height		40 cm height		SEM	P-value		
	Control	WI	Control	WI		H	I	H × I
	g kg ⁻¹ of body weight							
DM	23.7	25.2	26.5	26.7	0.11	0.071	0.472	0.542
OM	23.0	24.4	25.7	25.8	0.10	0.082	0.471	0.561
CP	1.7	1.6	2.0	2.1	0.06	0.001	0.891	0.692
EE	0.6	0.6	0.7	0.7	0.03	0.132	0.611	0.612
NDFap	9.2	8.7	10.0	8.7	0.05	0.402	0.101	0.451
ADF	5.0	4.5	5.2	5.4	0.03	0.041	0.661	0.181
NFC	11.6	13.4	13.0	14.4	0.06	0.041	0.001	0.632
TDN	16.7	17.3	18.5	19.2	0.08	0.041	0.441	0.992
	kg d ⁻¹							
DM	0.663	0.656	0.683	0.681	43.4	0.611	0.912	0.961
OM	0.643	0.636	0.660	0.660	42.1	0.632	0.941	0.942
CP	0.047	0.043	0.052	0.053	2.5	0.031	0.622	0.433
EE	0.018	0.016	0.017	0.017	1.0	0.722	0.201	0.484
NDFap	0.260	0.226	0.256	0.223	17.8	0.941	0.092	0.922
ADF	0.140	0.117	0.134	0.138	9.73	0.452	0.371	0.171
NFC	0.322	0.349	0.334	0.367	22.26	0.501	0.182	0.922
TDN	0.467	0.451	0.478	0.492	33.85	0.462	0.991	0.683

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDFap - neutral detergent fiber assayed using heat-stable amylase and expressed exclusive of ash and protein; ADF - acid detergent fiber; NFC - non-fibrous carbohydrate; TDN - total digestible nutrients; Control - without inoculant; WI - with inoculant; H - cutting height; I - inoculation; H × I - interaction (cutting height × inoculation).

we detected comparable CP digestibilities between uninoculated silages at cutting heights of 25 and 40 cm, whereas the CP digestibility of inoculated silages prepared from corn harvested at a cutting height of 25 cm was lower than that when corn was harvested at 40 cm.

For a harvest cutting height of 25 cm, the EE digestibility of uninoculated corn silages was found to be higher than that of inoculated corn silages, whereas no differences were observed in the EE digestibilities of inoculated and uninoculated silages when corn was harvested at a cutting height of 40 cm. For a cutting height of 25 cm, the digestibility of the ADF content of uninoculated corn silages was found to be higher than that of inoculated silages. However, when the cutting height was raised to 40 cm, inoculated corn silages were found to have a higher ADF digestibility than uninoculated silages harvested at the same cutting height. We also detected interactive effects between cutting height and inoculant treatment for digestibility of CP ($P < 0.05$), EE ($P < 0.05$), and ADF ($P < 0.05$) (Table 6).

Cutting height was also found to have an influence on the NDF digestibility of silage, being significantly lower when the corn was harvested at 40 cm ($P < 0.05$). However, the use of inoculants did not alter the digestibility variables (Table 6). Similarly, the nitrogen intake and fixed and absorbed nitrogen in sheep fed corn silage were only affected by cutting height (Table 7). Overall, we found that silages prepared from corn harvested at a cutting height of 40 cm provided a better nitrogen balance in sheep. When corn was harvested at 25 cm, the average nitrogen intake of sheep fed the resulting silages was 0.60 g MW^{-1} , which was significantly lower ($P < 0.05$) than that of sheep fed silages prepared from corn

Table 6 - Apparent digestibility of nutrients and total digestible nutrients from corn silage harvested at two cutting heights ensiled with and without bacterial inoculant

Item (g kg^{-1} dry matter)	25 cm height		40 cm height		SEM	P-value		
	Control	WI	Control	WI		H	I	H × I
DM	586.0	545.6	559.8	606.7	2.52	0.472	0.891	0.082
OM	653.6	639.6	633.8	652.1	1.45	0.792	0.881	0.262
CP	455.7a	368.0b	453.6a	494.9a	3.23	0.071	0.233	0.021
EE	833.7a	771.4b	829.9a	858.2a	1.57	0.011	0.272	0.001
NDFap	557.9	482.9	478.8	453.3	2.74	0.041	0.071	0.351
ADF	513.1a	417.5b	424.3b	514.3a	3.47	0.901	0.931	0.011
NFC	747.3	778.8	766.5	784.7	1.82	0.352	0.273	0.972
TDN	723.3	710.7	698.7	718.3	1.41	0.702	0.851	0.221

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDFap - neutral detergent fiber assayed using heat-stable amylase and expressed exclusive of ash and protein; ADF - acid detergent fiber; NFC - non-fibrous carbohydrate; TDN - total digestible nutrients; Control - without inoculant; WI - with inoculant; H - cutting height; I - inoculation; H × I - interaction (cutting height × inoculation). Means in the same row followed by different letters are different by the Tukey test ($P < 0.05$).

Table 7 - Nitrogen balance of sheep fed silages from corn plants harvested at two cutting heights ensiled with or without bacterial inoculant

	25 cm height		40 cm height		SEM	P-value		
	Control	WI	Control	WI		H	I	H × I
	g d^{-1}							
Nitrogen intake	7.34	6.57	8.14	8.92	0.45	0.071	0.762	0.561
Nitrogen fixed	3.09	3.31	3.47	3.71	0.29	0.031	0.411	0.151
Nitrogen absorbed	4.26	3.26	4.67	5.20	0.29	0.031	0.541	0.131
	g MW^{-1}							
Nitrogen intake	0.62	0.57	0.73	0.76	0.02	0.001	0.783	0.921
Nitrogen fixed	0.33	0.26	0.38	0.39	0.02	0.001	0.531	0.152
Nitrogen absorbed	0.36	0.29	0.42	0.44	0.02	0.001	0.742	0.122

MW - metabolic weight ($\text{BW}^{0.75}$); Control - without inoculant; WI - with inoculant; H - cutting height; I - inoculation; H × I - interaction (cutting height × inoculation).

harvested at 40 cm (0.75 g MW^{-1}) (Table 7). This difference was reflected in the amount of nitrogen fixed by sheep, which was significantly higher in sheep fed silages prepared from corn harvested at 40 cm (0.39 g MW^{-1}) ($P < 0.05$) than that in sheep fed silages prepared from corn harvested at 25 cm (0.32 g MW^{-1}).

4. Discussion

A notable finding of the present study is that all silages had elevated yeast counts. According to Woolford (1990), silages containing yeast populations greater than $\log 1 \times 10^5 \text{ cfu/g}$ of forage are more prone to aerobic deterioration. We found, however, that silages prepared from corn harvested at the lower cutting height (25 cm) were characterized by higher yeast populations compared with those silages prepared from corn harvested at the higher cutting height (40 cm). According to McDonald et al. (1991), epiphytic microorganisms tend to congregate in the basal portions of forage plants to gain protection against sunlight. Consequently, forage harvested at a lower height would presumably have an inherently higher yeast count than that harvested at a higher level. Furthermore, in the present study, we determined the chemical composition as well as the fermentative characteristics and aerobic stability of samples of silage collected from the silo face, which is comparable to farm conditions. Accordingly, it might be expected that our findings would differ from those obtained in other studies, which commonly evaluate silages at the time of silo opening in laboratory silos without prior exposure to air.

The pH and lactic acid concentration of silage produced in the present study were indicative of good fermentation within the experimental silos (Kung Jr. et al., 2018). We can accordingly assume that the ambient conditions and substrates were conducive to lactic acid bacteria development, resulting in sufficient lactic acid production to reduce the pH to between 3.8 and 4.2, which can inhibit the growth of undesirable microorganisms. This is commonly observed in corn silage, as corn forage has desirable characteristics at harvest that contribute to enhancing the anaerobic fermentative process and forage preservation.

We found that the use of bacterial inoculants during the ensiling of corn harvested at a cutting height of 25 cm resulted in high lactic acid production, and in this regard, we suspect that the use of additive may have promoted rapid colonization by *L. plantarum* in the ensiled forage, thereby accelerating homofermentative fermentation, and consequently, increasing lactic acid production and reducing silage pH. Interestingly, however, the lactic acid concentrations in inoculated silages produced from corn harvested at a cutting height of 40 cm were lower than those in uninoculated silages. We believe that the observed differences in the fermentation rate of silages can be explained in terms of differences in the DM contents of the cut forage (Kung Jr. et al., 2018).

Regardless of the height at which the corn forage was cut, all silages were found to have elevated yeast counts greater than $6 \log_{10} \text{ cfu}$ of yeasts g^{-1} (Kung Jr. et al., 2018). Nevertheless, the silages produced from corn harvested at a cutting height of 25 cm were characterized by higher yeast populations than those produced from corn harvested at 40 cm. We found that acetic acid production was increased in corn silages when forage was harvested at a cutting height of 25 cm and suspect this could be attributable to the presence of acetic acid bacteria, which often grow together with yeasts and increase the acetic acid concentration in silage (Muck, 2010). Compared with the concentrations of lactic acid, however, the corn silages were characterized by relatively low concentrations of acetic acid (Kung Jr. et al., 2018).

Contrary to expectations, we failed to detect propionic acid in any of the experimental silages. We had anticipated that inoculated silage would contain higher amounts of this acid, owing to the combined presence of *L. plantarum* and *P. acidipropionici* in the inoculants. However, it is conceivable that high growth of the lactic acid bacterium *L. plantarum* promoted rapid ambient acidification, thereby inhibiting the growth of *P. acidipropionici*, which has a tendency to exhibit restricted growth at a low pH (Filya et al., 2006). Similarly, we detected no butyric acid in any of the corn silages. According to Tomich et al. (2003), a butyric acid concentration of less than 3 g kg^{-1} is indicative of minor energy

and DM losses in silages during the fermentative process, which are characteristics of silage with an excellent fermentative profile.

Furthermore, the low N-NH₃ concentrations in inoculated silages and in those produced from corn harvested at a cutting height of 40 cm are indicative of slow proteolytic activity, as characterized by reductions in pH and ambient acidification (McDonald et al., 1991). This was also observed in inoculated silages when the corn forage was cut at a height of 25 cm.

The corn silages remained stable from the beginning of the aerobic stability assay at 2 °C above the ambient temperature. This can be ascribed to the fact that the assay was not initiated at the time of silo opening and because the high temperature in the region (average 28.8 °C) may have facilitated the growth of spoilage microorganisms. Conversely, corn silages inoculated with a combination of *L. plantarum* and *P. acidipropionici*, even those sampled during the feed-out phase, would be anticipated to show good aerobic stability during the assay due to the production of propionic acid (Filya et al., 2006). However, we found that this acid was not produced in sufficient quantities during fermentation. In a similar study, Filya et al. (2006) observed that rapid silage acidification inhibited the growth of propionic acid bacteria, resulting in low acetic and propionic acid production, thereby contributing to the instability of silages under aerobic conditions.

Although we found that silages were not stable at ambient temperatures, we observed that inoculated silages produced from corn harvested at a cutting height of 40 cm required a prolonged exposure to aerobic conditions to reach the maximum temperature, which could be attributable to the low yeast counts in these silages. In this regard, determinations of pH enable an evaluation of silage quality during aerobic exposure. According to Tabacco et al. (2011), pH increases during aerobic deterioration due to the degradation of lactic acid by yeasts, providing opportunities for microbial growth in silages. In the present study, we observed a delay in the increase of pH, and thus initiation of the deterioration process, in silages produced from corn harvested at a cutting height of 40 cm, which can probably be attributed to the low yeast counts in these silages.

The use of inoculants containing homofermentative bacteria can improve the fermentative process and reduce nutrient losses (Kamarloiy and Yansari, 2008), and in the present study, we found that inoculated silages produced from corn harvested at a cutting height of 25 cm were characterized by reduced CP losses, thereby enhancing the nutrient concentration in silages. Similarly, when the forage cutting height was 40 cm, inoculated corn silages were found to have high EE concentrations.

Treating silage with bacterial inoculants was found to result in low NDF contents due to the tendency to reduce the concentrations of hemicellulose ($P>0.05$), which are generally hydrolyzed in an acidic environment during efficient fermentation (Kamarloiy and Yansari, 2008). Increasing the height at which the corn is cut contributes to increasing the DM concentration of forage, owing to the higher proportion of ears and corn kernels in the ensiled mass, which are generally dryer than the vegetative proportion of forage (Neylon Kung Jr., 2003; Kung Jr. et al., 2008; Rezende et al., 2015).

A higher cutting height also contributes to enhancing silage quality and chemical composition, as it results in increased NFC concentrations and reductions in the concentrations of cell wall components, such as NDF and hemicellulose, which, again, can be attributed to the higher proportion of kernels in the final forage weight and lower fractions of stems and senescent leaves (Pedó et al., 2009; Oliveira et al., 2011).

Consistent with the absence of an interactive effect between cutting height and inoculant treatment on fermentative characteristics and chemical composition, we detected no interactive effects with respect to the intake of DM and nutrients. Inoculated corn silages tended to be associated with a higher NFC intake, owing to the low NDF and high NFC concentrations in silages. Furthermore, a higher cutting height yielded silages with higher intakes of CP, NFC, TDN, and ADF, which reflects the higher nutrient concentrations in forage harvested at a cutting height of 40 cm. Similar results have been reported by Kung Jr. et al. (2008), who showed improvements in the nutrient composition of corn silages, although these were not sufficient to increase the intake and DM digestibility of animals.

In the present study, we detected an atypical pattern in the nutrient digestibility of animals fed silages produced from corn harvested at a cutting height of 25 cm. Consistent with the findings of Rowghani and Zamiri (2009), we observed reductions in the digestibility of EE and CP. The higher NDF digestibility of silages produced from corn harvested at the low cutting height can be attributed to the higher nutrient concentration of the silage, as NDF digestibility tends to increase linearly with NDF concentrations in the diet (Araújo, 1998).

We found that a diet consisting entirely of corn silage had a positive effect on the nitrogen balance of sheep, indicating that the animals obtained a balance supply of nitrogen compounds, even without protein supplementation, which we specifically withheld in the present study to avoid a potential dilution of the effects of cutting height and bacterial inoculation on silage characteristic. Harvesting corn at a cutting height of 40 cm enhanced the nitrogen balance of the sheep as a function of the higher protein and energy concentration in the resulting silages, as this cutting height increased the CP and TDN intake in animals.

5. Conclusions

In this study, we demonstrated that different forage cutting heights (25 and 40 cm) and the use of a bacterial inoculant containing *L. plantarum* and *P. acidipropionici* have different effects on the fermentation profile of the resulting silages, but no marked effects on the aerobic stability and nutritive value of corn silage. The difference between cutting heights (15 cm) results in small changes in the chemical composition of silage, with a limited effect on nutrient intake and nitrogen balance in sheep, and no effect on digestibility. Bacterial inoculation was found to improve the fermentation process during ensiling, without affecting the feed-out phase.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: R.C.A. Mendonça, M.V.S.B. Cardoso, S.O.S. Pantoja, F.N. Domingues, T.C. Silva and A.C. Rêgo. Data curation: R.C.A. Mendonça, M.V.S.B. Cardoso, S.O.S. Pantoja and M.S. Souza. Formal analysis: R.C.A. Mendonça, M.V.S.B. Cardoso and M.S. Souza. Funding acquisition: R.C.A. Mendonça and A.C. Rêgo. Investigation: R.C.A. Mendonça and S.O.S. Pantoja. Methodology: R.C.A. Mendonça, M.V.S.B. Cardoso, S.O.S. Pantoja and M.S. Souza. Project administration: A.C. Rêgo. Resources: A.C. Rêgo. Software: C. Faturi. Supervision: F.N. Domingues, C. Faturi and A.C. Rêgo. Visualization: T.C. Silva. Writing-original draft: R.C.A. Mendonça. Writing-review & editing: R.C.A. Mendonça, F.N. Domingues, C. Faturi, T.C. Silva and A.C. Rêgo.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors thank the Universidade Federal Rural da Amazônia for the financial support and the undergraduate and graduate students from the Grupo de Estudo em Ruminantes e Forragicultura da Amazônia (GERFAM) for growing and harvesting the crop. We would like to thank the Multirão farm for the availability of the corn crop.

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