

Nictemeral composition of corn plants: afternoon harvest decreases dry matter recovery and increases silage starch content and *in vitro* **degradation**

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ABSTRACT: Plants' physiology changes through the day because photoassimilates can increase water-soluble carbohydrate concentration in the afternoon compared to the morning. This study evaluated the harvest time effect on whole-plant corn silage morphological composition, particle size, fermentation profile, chemical composition, *in vitro* degradation, and estimated milk yield. A two-year agronomic assay was performed in a completely random design, and one experimental silo was produced by each parcel $(n = 16)$. The afternoon harvest increased $(P < 0.01)$ dry matter content compared to the morning harvest. Harvest time did not affect $(P \ge 0.32)$ corn grain, stalk, leaf proportion, and silage particle size. However, the morning harvest increased dry matter recovery ($P = 0.01$) and had no effect ($P \ge 0.10$) on silage pH and concentrations of lactic and acetic acid compared to the afternoon harvest. In addition, afternoon harvest instead of morning harvest increased (P ≤ 0.05) silage starch, water-soluble carbohydrates, acid detergent lignin, and ether extract content and dry matter *in vitro* degradation. Controversially, treatments showed no effect ($P \ge 0.14$) on silage energy concentration, estimated energy content, and milk yield. Thus, the morning harvest produces more silage dry matter, but the afternoon harvest improves corn silage's nutritional value with no impact on estimated milk yield.

Key words: chemical composition, fermentation profile, morphological composition, water-soluble carbohydrate.

Composição química de plantas de milho: **colheita da tarde diminui recuperação de matéria seca e aumenta teor de amido na silagem e degradação** *in vitro*

RESUMO: A fisiologia das plantas muda ao longo do dia porque os fotoassimilados podem aumentar a concentração de carboidratos solúveis em água à tarde em comparação com a manhã. Este estudo teve como objetivo avaliar o efeito do horário de colheita na composição morfológica da silagem de milho de planta inteira, tamanho de partícula, perfil de fermentação, composição química, degradação *in vitro* e estimativa da produção de leite. Um ensaio agronômico de dois anos foi realizado em delineamento inteiramente casualizado, e um silo experimental foi produzido por cada parcela (n = 16). A colheita da tarde aumentou (P < 0,01) o teor de matéria seca em relação à colheita da manhã. A época de colheita não afetou (P \geq 0,32) a proporção de grãos, colmos e folhas do milho e o tamanho das partículas da silagem. Entretanto, a colheita da manhã aumentou ($P = 0.01$) a recuperação de matéria seca e não teve efeito ($P > 0.10$) no pH da silagem e nas concentrações de ácido láctico e acético em comparação à colheita da tarde. Além disso, a colheita da tarde, em vez da colheita da manhã, aumentou (P ≤ 0,05) o amido da silagem, os carboidratos solúveis em água, a lignina detergente ácida e o teor de extrato etéreo e a degradação *in vitro* da matéria seca. Controversamente, os tratamentos não mostraram efeito ($P \ge 0,14$) na concentração de energia da silagem, no conteúdo energético estimado e na produção de leite. Assim, a colheita da manhã produz mais matéria seca da silagem, mas a colheita da tarde melhora o valor nutricional da silagem de milho sem impacto na produção estimada de leite.

Palavras-chave: carboidratos solúveis em água, composição química, composição morfológica, perfil fermentativo.

INTRODUCTION

Plants use solar energy in endergonic reactions to accumulate chemical energy such as glucose, fructose, and sucrose (TAIZ & ZIEGER, 2013). This chemical energy, produced mainly in leaves and stems, is transported through the plant to support cell growth or energy storage (HOPKINS & HÜNER, 2011). Diurnal photosynthesis and nocturnal respiration of plants regulate the synthesis, accumulation, and degradation of synthesized carbohydrates (O'LEARY et al., 2017). These

dynamics result in a nictemeral variance in the chemical composition of plants (WEINERT-NELSON et al., 2022). Higher levels of photoassimilates could improve palatability and nutrient digestibility in the afternoon (FISHER et al., 1999; YARI et al., 2014). According to BRITO et al. (2009), alfalfa harvested in the afternoon has higher soluble carbohydrates and starch content compared to the morning harvest. Other authors reported increased crude protein and reduced fiber content in orchard grass and festuca harvested in the afternoon (FISHER et al., 1999; MAYLAND, 2005).

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During the ensiling process, lactic acid bacteria (LAB) ferment water-soluble carbohydrates (WSC) to produce organic acids (MCDONALD et al., 1991). Forage with higher WSC content showed a better fermentation profile and improved *in vitro* digestibility than low-WSC silage (AMER et al., 2012). However, increased WSC content could increase proteolysis and harm the recovery of silage dry matter (DM) (KUNG JR. et al., 2018). The increased fermentability obtained by facultative heterofermentative LAB inoculation shows no positive effects on whole-plant corn silage fermentation profile and chemical composition (OLIVEIRA et al., 2017). However, to our knowledge, no study has evaluated the effect of whole-plant harvest time on silage DM recovery and nutritional value.

Therefore, we hypothesized that afternoon harvest, instead of morning harvest, increases leave proportion and DM recovery, reduces silage fiber content, and improves silage *in vitro* DM degradation. This study evaluated the harvest time effect on wholeplant corn silage morphological composition, particle size, fermentation profile, chemical composition, *in vitro* degradation, and estimated milk yield.

MATERIALS AND METHODS

The trial was performed between December 2016 and June 2018 at the Universidade Federal de Santa Maria (UFSM, Santa Maria, Brazil, 29,43'51'' S latitude, 53.43'05'' W longitude, and 113 m asl).

Treatments and experimental design

A two-year agronomic assay was conducted during the 2016/2017 and 2017/2018 crop years. Every year, eight plots were used in a completely randomized design to evaluate the following treatments: 1) morning: corn whole-plant harvest at 07:00; and 2) afternoon: corn whole-plant harvest at 18:00. Harvest was performed on sunny days. The agronomic plot had 2.25×6.00 m (13.5) m2). An experimental silo (8 kg of fresh plants was compacted by a 75 kg person for 5 min. in a tube of 0.30 m diameter and 0.32 m height) was produced from each experimental parcel to evaluate the harvesttime effect on ensiling parameters.

Agronomic management

The experimental area (2000 m²) of Dystrophic Red Argisol soil (SANTOS, 2023) received dolomitic limestone to increase pH to 5.5 and eliminate soil Al^{++} . No soil preparation was performed, and seedings occurred on December

02, 2016, and November 06, 2017. The area was desiccated 30 days before planned sowing with the application of 3.0 liters' ha⁻¹ (1440 g/ha⁻¹) of glyphosate and 0.5 liters' ha-1 of mineral adjuvant. Soil fertilization was defined as considering 18 tons. DM/ ha⁻¹ using $P_2O_5(41\% \text{ of P and } 12\% \text{ of Ca})$ and KCL (60% of K_2 O) mixture according to the COMISSÃO DE QUÍMICA E FERTILIDADE DO SOLO **-** RS/SC (2016) recommendations. During the development of the crop (V4), glyphosate plus mineral adjuvant was applied to the corn crop to control weeds, and chlorpyrifos (V4 and V8) as an insecticide to control the fall armyworm. Top dressing fertilization with nitrogen (N) was applied, divided into stages V4 and V8, totaling 220 kg ha⁻¹ of N, using commercial urea (45% of N).

A semi**-**toothed precocious corn hybrid (AG 8690 VT PRO 3TM, Agroceres, Rio Claro, Brazil) was used. The seeding rate was defined to meet 65.000 plants/ha in each parcel and 0.5 m between rows. Area weather was classified as Cfa, and the National Institute of Meteorology (INMET) weather station recorded precipitation and temperature data (Figure 1).

Ensiling and sampling

Harvests were performed on March 25, 2017, and March 10, 2018. The plants of the central parcel row were manually harvested at 10 cm height, and mass production was recorded using a 1 g sensitive scale. Five plants were sampled from each parcel to evaluate morphological composition. Plants were divided as follows: leaves, stalk, senescent material, flower, straw, kernels, and cob, according to KELLER (1942).

After plant sampling for morphological evaluation, harvested plants were processed in a stationary mill (GTM 2001®, Garthen, Navegantes, Brazil). One sample (200 g) of processed corn was obtained to access DM content and productivity. It produced one experimental silo per parcel. Silage was produced in plastic tubes (0.38 m height and 0.32 m i.d.) using three 200 μ m plastic bags to obtain fermentation losses. One bag contained 2 kg of dried sand to absorb effluent. The other bag contained silage (6 kg). Another bag encompassed previously described bags and was used to avoid air penetration. The silage bag's lower layer and the sandbag's upper layer had pores and a non-decided woven layer to separate materials. Silos were weighed and stored for 104 days.

Before the opening, silos were weighed, and volume was measured to assess silage density.

After opening, silage was removed from the bags, homogenized, and sampled. One 200 g sample was squeezed in a hydraulic press (Model C, Carver Inc., Wabash, IN, USA) to access silage pH using a bench pH meter (LUCA**-**210®, Lucadema, São José do Rio Preto, Brazil). Another sample (200 g) was collected for chemical analysis. One more silage sample (almost 800 g) was collected to assess particle size using a Penn State separator (MAULFAIR et al., 2011).

Chemical analysis and in vitro assay

Silage and fresh corn samples were dried at 55 ºC for 72 h in a forced-air oven. Later, samples were ground using a 1 mm sieve knife mill (MA340, Marconi Equipment for Laboratories, Piracicaba, Brazil). Samples (silage and fresh corn) were analyzed for DM (method 950.15, AOAC, 2000). In addition, we evaluated silage ash (method 942.05), crude protein (N \times 6.25; Kjeldahl method 984.13), ether extract (method 920.39), acid detergent fiber (ADF), and lignin (method 973.18) according to AOAC (2000). Ash corrected-NDF was evaluated using α-amylase (VAN SOEST et al., 1991). Water-soluble carbohydrates (WSC) were analyzed by ethanol extraction and phenol-sulfuric acid evaluation of glucose (HALL, 2000). To access starch concentration, samples were digested by enzymes [amylase (Termamyl 120L®, Novozymes, Ararucária, Brazil), glucoamylase (AMG 300L®, Novozymes), and proteases (Flavourzyme 500L®, Novozymes)] for 18 h. Starch was calculated by the increase of glucose concentration after enzymatic digestion. Glucose concentration was evaluated using a glucose oxidaseperoxidase (GOP) kit and read in a spectrophotometer at 505 nm (WALTER et al., 2005).

Organic acid concentration was evaluated by gas chromatography. Samples (1 g) were mixed with acetonitrile (5 mL) and centrifuged. Supernatants were used to access acetic, butyric, and propionic acid concentrations. Lactic acid was evaluated after its derivation, performed according to OMS-OLIU et al. (2011). A gas chromatograph was used (Varian Star 3400CX, Santa Clara, CA, USA) equipped with a polar capillary column (50 m \times 0.25 mm i.d. \times 0.25 μm of film; CP-WAX 52 CB®, Chrompack, São Paulo, Brazil). The oven temperature started at 50 °C for 1 min. Moreover, the latter increased at 5 ºC/min. up to 110 ºC, and at 15 ºC/min. until 250 ºC. Then, the temperature was maintained for 10 min. The detector temperature was maintained at 230 ºC. Lactic acid was evaluated using a similar assay and another column (30 $m \times 0.22$ mm i.d. $\times 0.25$ µm of film thickness, SGE-BPX 5®, BGB Analytik, Boeckten, Switzerland). The method was calibrated using internal markers (Sigma-Aldrich, St. Louis, MO, USA).

Dry matter and NDF *in vitro* degradation were evaluated according to TILLEY & TERRY

(1963), modified by HOLDEN (1999). Samples were processed in a knife mill using a 2 mm sieve and placed in 41µm pores polyester bags. The bags (three per sample) were incubated for 48 h at 39 °C in an *in vitro* incubator (TE150®, Tecnal, Santa Maria, Brazil). Each vial received 1.6 l of MCDOUGALL (1948) buffer and 0.4 l of fresh ruminal fluid sampled from two Angus steers (700 kg body weight) maintained in a pasture without concentrate. At the end of incubations, bags were washed in running tap water, and NDF was analyzed as previously described.

Calculations and statistical analysis

Dry matter recovery (DMR) was calculated by the following equation (JOBIM et al., 2007):

 (1) Where: DM_{op} is the whole silage dry matter at opening; and *DM*_n is the whole silage DM at ensiling.

Milk productivity (kg/kg DM or kg/ha) was calculated using the Milk (2006) model, According to SHAVER & LAUER (2006) the parameters DM, CP, aNDF, *in vitro* digestibility of NDF (IVNDFD), starch, waste ash, EE and DM production per hectare were used as input data in the model. Total digestible nutrients (TDN) were obtained using the NRC (2001) model:

TDN (g/kg) = NFCdig + CPdig + (EEdig \times 2,25) + NDFdig) -7

Where: NFCdig is the digestible non-fibrous carbohydrate (g/kg); CPdig is the digestible crude protein (g/kg); EEdig is the ethereal extract digestible (g/kg); NDFdig is the digestible neutral detergent fiber (g/kg).

Data was analyzed using PROC MIXED of SAS (9.2 version, SAS Institute, Cary, NC) and the

following model:
 $Y_{ijk} = \mu + H_i + C_j + H \times C_{ij} + e_{ijk}$ (2) with $e_{ijk} \sim N(0, \sigma_e^2)$, where: Y_{iik} is the observed value of dependent variable; μ is the overall mean; H_i is the fixed effect of harvest time $(i = \text{ morning - afternoon}); C$ is the fixed effect of crop year $(j = \text{moving} - \text{afternoon})$; $H \times C_{ii}$ is the fixed effect of harvest time and crop year interaction; e_{ijk} is the random residual error; *N* stands for Gaussian distribution; σ_e^2 is the random residual variance. Significance was defined at 5% of probability.

RESULTS

Crop year productivity, morphological composition, and silage density

In general, the afternoon harvest increased $(P \le 0.01)$ dry matter content compared to the morning harvest (Table 1). However, a crop year and harvest

Table 1 - Dry matter content, productivity, morphological composition, and density at ensiling of whole-plant corn silage harvested at different hours of the day.

Item	$---$ Harvest time ¹ -----		Crop $Year^2$		SEM ³	$-Probabilities4-----$		
	Morning	Afternoon		\mathbf{I}		H	\mathcal{C}	$H \times C$
Dry matter, g/kg as-fed	340	390	335	396	7.63	${}_{0.010}$	${}_{0.010}$	${}_{0.010}$
Productivity, ton. DM/ha	16.9	15.0	19.0	12.8	0.43	0.071	${}_{0.011}$	0.071
-------------Morphological composition, g/kg DM---------------------------								
Grain	419	401	345	476	22.0	0.322	${}_{0.011}$	0.282
Stalk	267	270	335	202	7.60	0.760	${}_{0.013}$	${}_{0.010}$
Leaf	128	122	148	103	7.31	0.393	${}_{0.011}$	0.551
Cob	93.5	96.7	90.6	99.6	4.50	0.745	0.382	0.282
Straw	55.0	56.6	59.6	51.9	1.70	0.621	0.033	0.183
Senescent	39.5	37.9	24.0	53.3	4.90	0.821	${}_{0.010}$	0.832
Flower	7.50	11.0	8.00	10.5	0.70	0.020	0.051	0.490
Silage density, $kg/m3$	563	533	593	503	10.3	0.050	${}_{\leq 0.010}$	0.011

1 Harvest time: morning: corn whole-plant harvest at 07:00; and afternoon: corn whole-plant harvest at 18:00.

2 Crops year: I: 2016/2017; and II: 2017/2018.

³SEM: standard error of mean.

⁴ Probabilities: H: Harvest time (morning vs. afternoon harvest); C: crop year effect (2016/2017 vs. 2017/2018); H \times C: harvest time and crop year interaction effect.

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time interaction effect ($P = 0.01$) was on DM content. Afternoon harvest increased by 10.7 g/kg DM content in crop year I and 88.9 g/kg in crop year II (Figure 2).

There was a harvest time and crop year interaction effect ($P < 0.01$) on stalk proportion. The afternoon harvest (377 g/kg) in the 2017/2018 crop year had higher $(P = 0.03)$ stalk content than the morning harvest (297 g/kg). The afternoon harvest increased $(P = 0.02)$ flower content of corn plants compared to the morning harvest, regardless of $(P =$ 0.49) crop year. Furthermore, there was no harvest time effect ($P \ge 0.32$) on the leaf, senescent, straw, grain, and cob content regardless ($P \ge 0.18$) of the crop year. There was a harvest time and crop year interaction effect $(P = 0.01)$ on silage density. In general, the morning harvest had a lower ($P \le 0.04$) density than the afternoon harvest. Only in crop year II did the afternoon harvest decrease ($P = 0.01$) silage density compared to the morning harvest (Figure 2).

Particle size and fermentation profile and DM losses Harvest time showed no effect ($P \ge 0.22$) on silage particle size regardless ($P \ge 0.08$) of the

evaluated crop year (Table 2). In addition, harvest time did not affect the fermentation profile ($P \ge 0.10$). However, the afternoon harvest reduced $(P = 0.01)$ by 0.41% DM recovery compared to the morning harvest.

Chemical composition and in vitro degradation

In general, the afternoon harvest increased $(P \le 0.05)$ silage DM content, ether extract, acid detergent lignin, WSC, starch, and DM *in vitro* degradation compared to the morning harvest (Table 3). However, a harvest and crop year interaction effect (P ≤ 0.01) was on silage DM and energy content. Only in crop year II did the afternoon harvest increase (P $= 0.02$) silage DM content (Figure 2). In addition, the harvest did not affect ($P \ge 0.14$) silage CP, NDF, ADF, and energy content regardless ($P \ge 0.08$) of the evaluated crop year.

DISCUSSION

Rainfall varied significantly between the two crop years evaluated in this study (Figure 1). Consequently,

Table 2 - Particle size and fermentation profile of whole-plant corn silage harvested at different hours of the day.

¹Harvest time: morning: corn whole-plant harvest at 07:00; and afternoon: corn whole-plant harvest at 18:00.

2 Crops year: I: 2016/2017; and II: 2017/2018.

3 SEM: standard error of mean.

4 Probabilities: H: Harvest time (morning *vs.* afternoon harvest); C: crop year effect (2016/2017 vs. 2017/2018); H × C: harvest time and crop year interaction effect.

⁵Theorical particle size.

the results between crops varied significantly in terms of productivity, the morphological participation of the plants in the silage, and, consequently, the nutritional value of the corn silage. In crop year II, the lack of precipitation altered the physiological conditions of the plants, increasing leaf senescence. Plants reported a higher DM content due to the lower ability to maintain internal water content and draining compounds to maximize grain formation (XUE et al., 2020).

We hypothesized that afternoon harvest increases leaf proportion and DM recovery, reduces silage fiber content, and improves silage *in vitro* DM degradation compared to morning harvest. Although the afternoon harvest showed lower DM recovery and higher *in vitro* DM degradation than the morning harvest, there was no harvest effect on the leaf content of fresh corn and silage fiber concentration.

In the present study, the afternoon harvest increased fresh corn DM content compared to the morning harvest, especially in crop year II. YARI et al. (2014) also observed higher DM content when performing afternoon harvest. Although plants could attenuate water losses during the day, higher relative humidity during the morning favors intracellular water accumulation (TAIZ & ZIEGER, 2013).

ZOPOLLATTO et al. (2009) studied corn plants' physiological stage and morphological composition. According to these authors, plants

show progressively improved cobs and kernel content as stages increase, whereas leaves and stems progressively decrease. However, harvest time did not affect most morphological variables in the present study. The short-time effect seems less critical to these variables.

In crop year II, plants had higher DM content, and the afternoon harvest increased stalk plant concentration than the morning harvest compared to crop year I. According to XUE et al. (2020), plants tend to reduce the mass of stems with the advancement of the maturity stage due to the translocation of carbon from the tissues of the leaves and stems to the physiological maturation of the grains. However, the increase in DM content in the afternoon harvest demonstrated that this change did not occur within a few hours and that other physiological mechanisms associated with water stress caused an increase in stems of plants harvested in the afternoon, probably blocking carbon translocation to the grains.

In the present study, harvest time did not affect silage particle size, although it affected silage DM content. According to WEIRICH NETO et al. (2013), DM content slightly affects corn silage particle size. In addition, this effect is associated with plant genotype. The absence of harvest time on particle size shows the consistency of forage processing in the present study. However, the afternoon harvest decreased silage-specific weight

Table 3 - Chemical composition, in vitro degradation, and energy content of whole-plant corn silage harvested at different hours of the day.

¹Harvest time: morning: corn whole-plant harvest at 07:00; and afternoon: corn whole-plant harvest at 18:00.

2 Crops year: I: 2016/2017; and II: 2017/2018.

³SEM: standard error of mean.

4 Probabilities: H: Harvest time (morning vs. afternoon harvest); C: crop year effect (2016/2017 vs. 2017/2018); H × C: interaction effect.

and DM recovery compared to the morning harvest. Dry matter content is positively associated with silage porosity and negatively associated with silage density (RICHARD et al., 2004; BORREANI et al., 2018). Both characteristics of afternoon harvest silages (higher porosity and lower density) result in higher oxygen at ensiling, increasing the aerobic phase, when plant enzymes and aerobic microorganisms respire and use available carbohydrates (BORREANI et al., 2018). CARVALHO et al. (2021) reported an inverse relationship between silage density and DM losses. Besides, DM concentration and air infiltration affect fermentation losses more than particle size (MCENIRY et al., 2007).

In the present study, the afternoon harvests increased silage WSC and starch compared to the morning harvest. Water-soluble carbohydrates are the primary substrate for lactic acid bacteria growth (KUNG JR. et al*.*, 2018). Increased organic acids and decreased silage pH were expected with higher WSC (YANG et al., 2006). However, increased DM content, also observed in afternoon harvested silage,

inhibits the growth of bacteria by reducing water activity (ÁVILA & CARVALHO, 2020). This reduced fermentability explains higher starch concentration even with higher DM losses in the afternoon harvest compared to the morning harvest in the current study. KRÜGER et al. (2020) and SHOKRIPOOR et al. (2021) have already reported positive effects of afternoon harvest on fats and non-fiber carbohydrate content (NFC). Increased NFC and similar NDF levels may be associated with the decrease in the soluble fiber of silages from maize plants harvested in the afternoon, as observed by increased lignin content (HALL, 2000).

Increased starch and WSC observed in the afternoon harvest related to higher *in vitro* degradation observed in the present study. This occurs because starch is more digestible than other components of corn silage (FERRARETTO et al.*,* 2018). However, the harvest period did not affect silage energy content and estimated milk yield. Estimated values were numerically higher in the afternoon harvest compared to the morning harvest. However, the energy

estimation method did not consider starch content, which limited the statistical power of comparison in the present study.

In practical farm conditions, silage from corn plants harvested in the afternoon has more potential for animal feed conversion. This is because increasing DM content due to higher WSC, mainly starch, is an essential factor for increasing the energy density of the silage (BORREANI et al., 2018). Another crucial practical factor is to alert researchers to evaluate the nutritional quality of silage. Experimental silage should ideally be carried out during a specific time, either in the morning or afternoon. This avoids inadequate comparative results and incorrect conclusions, especially in research evaluating corn silage's DM digestibility, starch, and energy content.

CONCLUSION

The afternoon harvest increases corn silage DM, WSC, starch, and DM *in vitro* degradation more than the morning harvest. Nevertheless, increasing DM content hampers silage compaction and decreases DM recovery.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflicts of interest in the current manuscript.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

Procedures were previously approved by the UFSM Animal Ethics Committee (approval number 5439180417).

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