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Storage length interacts with maturity to affect nutrient availability in unprocessed flint corn silage

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ABSTRACT - Our objective was to determine the effects of maturity and storage length on the fermentation pattern, ruminal disappearance of nutrients, and recovery of digestible nutrients in flint corn (*Zea mays* L.) silage harvested with a pull-type machine without a kernel processor. Corn plants were harvested at 300 g kg^{-1} DM (low dry matter, LDM) or 400 g kg^{-1} DM (high dry matter, HDM) and stored for 0, 7, 14, 30, 60, 90, 120, and 180 d in laboratory silos. Corn harvested at HDM had a higher proportion of long particles (>19 mm), more intact kernels, and lower kernel processing score. There was an interaction between maturity and storage length for several fermentation end-products, DM recovery, ruminal disappearance of nutrients, and recovery of digestible DM. Overall, the concentrations of fermentation end-products were higher in LDM than in HDM silage. The DM recovery decreased over time, mainly for LDM silage. Ruminal disappearance of nutrients (starch, CP, and DM) increased with storage length, with greater values for LDM silage at shorter storage, but closer values among LDM and HDM at longer storage periods. The recovery of digestible DM (DM recovery × ruminal disappearance) slightly increased in LDM silage up to 26 d of fermentation, but it markedly increased in HDM silage, mainly up to 60 d of storage. Storing for longer periods is a strategy to partially offset the negative impact of maturity on the digestibility of flint corn silage harvested with pull-type machines without kernel processor.

Keywords: digestibility, fermentation, proteolysis, starch

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Introduction

In Brazil, pull-type forage harvesters without a kernel processor are still widely used by farmers (Bernardes and Do Rêgo, 2014; Daniel et al., 2019). Pull-type forage harvesters typically result in poor kernel processing and, in turn, lower starch digestibility (Salvati et al., 2017; Ferraretto et al., 2018). Additionally, corn hybrids grown in tropical areas predominantly have a greater proportion of vitreous endosperm, which is also associated with lower starch digestibility (Correa et al., 2002). Consequently, many Brazilian farmers are harvesting corn crop earlier than desirable (< 300 g kg^{-1} of DM), which result in lower DM yield and silages with lower starch content (Daniel et al., 2019). Meanwhile, ensiling wetter crops are also associated with higher fermentative losses (McDonald et al., 1991) and greater formation of volatile organic compounds (e.g. volatile fatty acids, alcohols, esters, aldehydes, ketones), which has been claimed to have negative impacts on animal performance (Weiss, 2017) and the environment (Howard et al., 2010; Hafner et al., 2013).

Several studies have shown that the negative impact of the protein matrix surrounding starch granules in corn vitreous endosperm is attenuated over the ensiling period (Hoffman et al., 2011; Der Bedrosian et al., 2012), mainly due to the proteolytic activity of bacteria and plant enzymes (Junges et al., 2017). Hence, storing corn silages (whole crop or grain) for longer periods typically increases starch and protein digestibility, with minor or no effect on fibre digestibility (Benton et al., 2005; Daniel et al., 2015; Kung Jr. et al., 2018). However, the extension of this benefit is unknown in corn hybrids with a high proportion of vitreous endosperm when harvested with forage harvesters without a kernel processor.

The main objective of our study was to determine the effects of maturity and storage length on the fermentation pattern, ruminal disappearance of nutrients, and recovery of digestible DM in flint corn silage harvested with a pull-type machine without a kernel processor. We hypothesised that although a more mature crop may lead to a less intense fermentation, it would achieve similar levels of ruminal DM disappearance of the early-harvested corn at longer storage periods. A second objective was to verify if the DM content at harvesting affects the recommendation of minimal storage length to improve DM digestibility.

Material and Methods

On October 15, 2015, a corn hybrid with flint endosperm (2B877PW, Dow Agrosciences, São Paulo, Brazil) was sown in four plots (10×18 m) in the municipality of Maringá, (23°25'38" S and 51°56'15" W), state of Paraná, Brazil. Five seeds were distributed per linear meter (62,500 plants per ha). Each plot contained 12 rows with a spacing of 80 cm between rows. To reduce border effects, only the six central rows were used. At seeding, 250 kg ha^{-1} of a commercial NPK (19:15:16) fertilizer was applied in the furrow. Four weeks after seeding, 150 kg ha^{-1} of urea, and 100 kg ha^{-1} of KCl were spread over the plots.

Two weeks after silking, the DM content was monitored once a week. At 91 d after seeding, plants achieved approximately 300 g kg^{-1} of DM (LDM), then three of those six central rows in each plot were mechanically harvested. The remaining three central rows were harvested 12 d later, at 400 g kg^{-1} of DM (HDM). In both maturities, plants were harvested at 25-cm cut height with a pull-type forage harvester set for a 7-mm theoretical cut length and without a kernel processor (JF192C12 single row, JF Máquinas Agrícolas, Itapira, Brazil).

In each maturity stage (LDM and HDM), chopped forage from each plot was placed in eight nylon-polyethylene bags (33×45 cm, 160 µm) without any additive, subsequently sealed under vacuum (~1.8 kg per mini-silo), and stored for 0 (opened immediately after sealing), 7, 14, 30, 60, 90, 120, and 180 d. A total of 64 silos were prepared [four replicates (blocks) per maturity per storage time]. At ensiling and at opening, silos were weighed and sampled. Dry matter recovery was determined by DM weight change, as a fraction of ensiled DM mass. Silage samples were frozen at −20 °C for further analysis.

Additionally, fresh chopped forage from each plot were sampled for measuring particle size distribution using the Penn State Particle Separator (Three-sieve model with 1.18-mm sieve). The mean particle length and geometric standard deviation were calculated according to Kononoff et al. (2003). The number of intact kernels per 250 g of fresh forage (including whole and slightly damaged kernels) and the length of ten longest particles were recorded. A subsample of chopped forage from each plot was dried at 55 °C for measuring the kernel processing score (KPS), which represents the proportion of starch passing through a 4.75-mm sieve (Ferreira and Mertens, 2005; Mertens, 2005).

For the *in situ* ruminal degradability, approximately 40 g of fresh thawed samples were placed into polyamide bags (15×35 cm, 60 µm porosity) and incubated for 24 h in the rumen ventral sac of two rumen-cannulated non-lactating Holstein cows fed corn silage (*ad libitum*) and concentrate (2 kg d^{-1}). One sample from each silo was incubated in each cow. Immediately after retrieving, bags were submerged in cold water (0 °C) for 5 min and washed in a washing machine (three cycles, followed

by a final spin). Washed bags were dried in a forced-air oven at 55 °C for 72 h and weighed to calculate DM disappearance.

Fresh subsamples of silages were used to prepare an aqueous extract (Kung Jr. et al., 1984). Aqueous extracts were used for measuring pH and fermentation end-products. Lactic acid (Pryce, 1969) and NH_3-N (Chaney and Marbach, 1962) were analysed by colorimetric methods. Volatile fatty acids, alcohols, and esters were determined by gas chromatography-mass spectrometry (GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, PA; 60 m, 0.25 mm ø, 0.25 µm crossbond carbowax polyethylene glycol). Compounds were identified based on their retention time and mass spectra and quantified with external standards.

Dried samples were ground in a Wiley mill through 1-mm screen and analysed for crude protein (CP; AOAC, 1990; method 984.13), neutral detergent fibre (NDF; assayed with a heat stable α-amylase and sodium sulphite, and expressed inclusive of residual ash; Mertens, 2002), starch (Hall, 2015), and dry matter (DM_{oven}; AOAC, 1990; method 934.01). The DM_{oven} was corrected for volatiles as follows (Weissbach, 2009): DM (g kg^{-1} FM) = DM_{oven} (g kg^{-1} FM) + n-alcohols (g kg^{-1} FM) + i-propyl alcohol (g kg^{-1} FM) + 2,3-butanediol (g kg^{-1} FM) + esters (g kg^{-1} FM) + 0.95 × volatile fatty acids (g kg^{-1} FM) + 0.77 × 1,2-propanediol (g kg^{-1} FM) + 0.08 × lactic acid (g kg^{-1} FM). The n-alcohols included methanol, ethanol, and propanol; esters included ethyl lactate, ethyl acetate, and propyl acetate; and volatile fatty acids included acetic, propionic, i-butyric, butyric, i-valeric, and valeric acids.

Residues from *in situ* incubation were ground in a Wiley mill through 1-mm screen and analysed for NDF, CP, and starch to calculate the ruminal disappearance of those fractions. The recovery of digestible DM was calculated by multiplying DM recovery in the silo by DM disappearance in the rumen.

Data were analysed using the Mixed procedure of SAS (Statistical Analysis System, version 9.4). Forage processing outcomes (particle size, KPS, intact kernels) were analysed with a model including fixed effect of maturity and random effect of plot.

Fermentation pattern, chemical composition and *in situ* disappearance were analysed as a split-plot arrangement (two maturities × eight storage periods) with the same model described above including the effects of storage length and its interaction with maturity. The interaction between plot and maturity was defined as subject. Covariance structures were chosen for each outcome based on the smaller value for the corrected Akaike Information Criterion.

Ruminal disappearance of DM, starch, and protein and recovery of digestible DM were modelled with a segmented regression (first segment quadratic, second segment linear) to define the curve breakpoint and infer on the minimal length of storage required to improve those responses (Robbins et al., 2006; Daniel et al., 2015).

Results

Forage harvested at HDM had greater proportion of particles >19 mm and tended to have more fine particles (retained on 1.18-mm sieve and pan). The LDM forage had a higher proportion of particles retained on the 8-mm sieve. The mean particle length was similar between treatments, whereas the geometric standard deviation was higher in HDM than in LDM forage. Corn harvested at HDM had more intact kernels, lower KPS, and greater proportion of coarse particles than LDM corn (Table 1).

For the outcomes measured across fermentation, when the interaction between maturity and storage length or the main effect of maturity was significant (Table 2), data were presented in graphic form. For the remaining few responses, means were described along the text.

There was an interaction between maturity and storage length for pH, lactic acid, 1,2-propanediol, and DM recovery, whereas NH_3-N, acetic acid, propionic acid, methanol, ethanol, 1-propanol, and ethyl esters were affected by the main effects of maturity and storage length. Propyl acetate was only affected by maturity.

Table 1 - Particle size distribution and kernel processing score in corn forage harvested with a pull-type forage harvester without kernel processor

Item	Maturity[1]		SEM	P
	LDM	HDM		
Retention				
>19 mm (g kg^{-1} FM)	74.5	113	8.62	0.02
8 to 19 mm (g kg^{-1} FM)	500	388	13.5	<0.01
1.18 to 8 mm (g kg^{-1} FM)	412	456	14.8	0.08
0 to 1.18 mm (g kg^{-1} FM)	13.0	43.6	9.96	0.07
Mean particle length (mm)	8.30	8.00	0.42	0.67
Geometric standard deviation (mm)	2.40	3.10	0.06	<0.01
Length of longest particles (mm)	149	192	10.2	0.02
Intact grains (n 250 g^{-1} FM)	7.60	17.0	1.49	<0.01
Kernel processing score (g kg^{-1})	620	494	10.1	<0.01

FM - fresh matter; SEM - standard error of the mean.
[1] LDM: low dry matter (300 g kg^{-1}); HDM: high dry matter (400 g kg^{-1}).

Table 2 - Statistical analysis (P-values) of the influence of maturity (M) and storage length (S) on the fermentation profile and nutritive value of corn silage

Item	M	S	M × S	Item	M	S	M × S
DM	<0.01	0.26	0.90	Propionic acid	<0.01	<0.01	0.50
DMR	<0.01	<0.01	<0.01	Butyric acid	0.19	0.11	0.63
DMD	<0.01	<0.01	<0.01	i-Butyric acid	0.82	<0.01	0.11
RDMD	<0.01	<0.01	<0.01	Valeric acid	0.38	0.34	0.45
Starch	<0.01	0.95	0.99	i-Valeric acid	0.14	<0.01	0.23
StarchD	<0.01	<0.01	<0.01	Methanol	<0.01	<0.01	0.18
CP	<0.01	0.80	0.99	i-Propyl alcohol	0.65	0.01	0.66
CPD	<0.01	<0.01	<0.01	Ethanol	<0.01	<0.01	0.91
NDF	<0.01	0.91	0.98	1-propanol	<0.01	<0.01	0.39
NDFD	<0.01	0.18	0.32	1,2-Propanediol	<0.01	0.01	0.02
NH_3-N	<0.01	<0.01	0.21	2,3-Butanediol	0.26	<0.01	0.99
pH	0.08	<0.01	<0.01	Ethyl lactate	<0.01	<0.01	0.40
Lactic acid	<0.01	<0.01	<0.01	Ethyl acetate	<0.01	<0.01	0.41
Acetic acid	<0.01	<0.01	0.94	Propyl acetate	<0.01	0.86	0.86

DM - dry matter; DMR - DM recovery; DMD - ruminal DM disappearance; RDMD - recovery of digestible DM; StarchD - ruminal starch disappearance; CP - crude protein; CPD - ruminal CP disappearance; NDF - neutral detergent fibre; NDFD - ruminal NDF disappearance.

The pH dropped during the onset of fermentation (first week of storage) and remained low (around 3.7), with slight variations thereafter. Lactic acid steeply increased during the first 14 d of fermentation, with higher values for the LDM silage (Figure 1).

The concentration of acetic acid increased with storage length and remained higher in LDM than in HDM silage up to 180 d. The content of 1,2-propanediol remained low across storage in HDM silage, while steeply increased from 60 d up to 120 d in LDM silage. The LDM silage also had a higher level of 1-propanol, which increased mainly during the first 30 d. In HDM silage, 1-propanol slowly increased up to 180 d of fermentation. Propionic acid was primarily synthetized in LDM silage up to 7 d of fermentation with minimal fluctuation thereafter. In HDM silage, propionic acid concentration was lower than in LDM and gradually increased up to 90 d of storage (Figure 2).

In LDM and HDM silages, ethanol increased over time, mainly during the first 30 d of fermentation, with higher levels in LDM than in HDM silage. Ethyl lactate and ethyl acetate concentrations were greater in LDM silage. In both silages, ethyl esters were formed mostly during the first 30 d of fermentation.

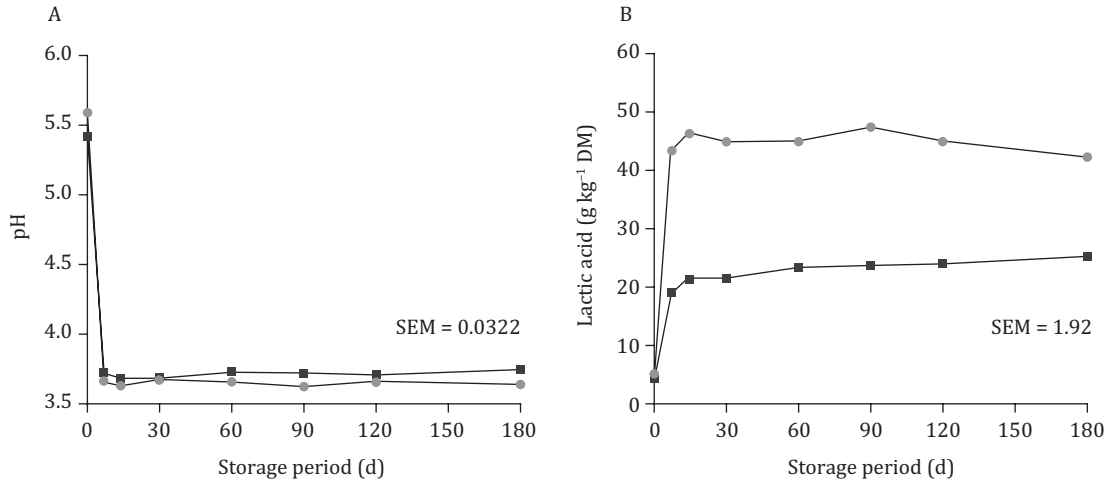


Figure 1 - Influence of maturity (low dry matter ●; high dry matter ■) and storage length on pH (A) and lactic acid (B) in flint corn silage.

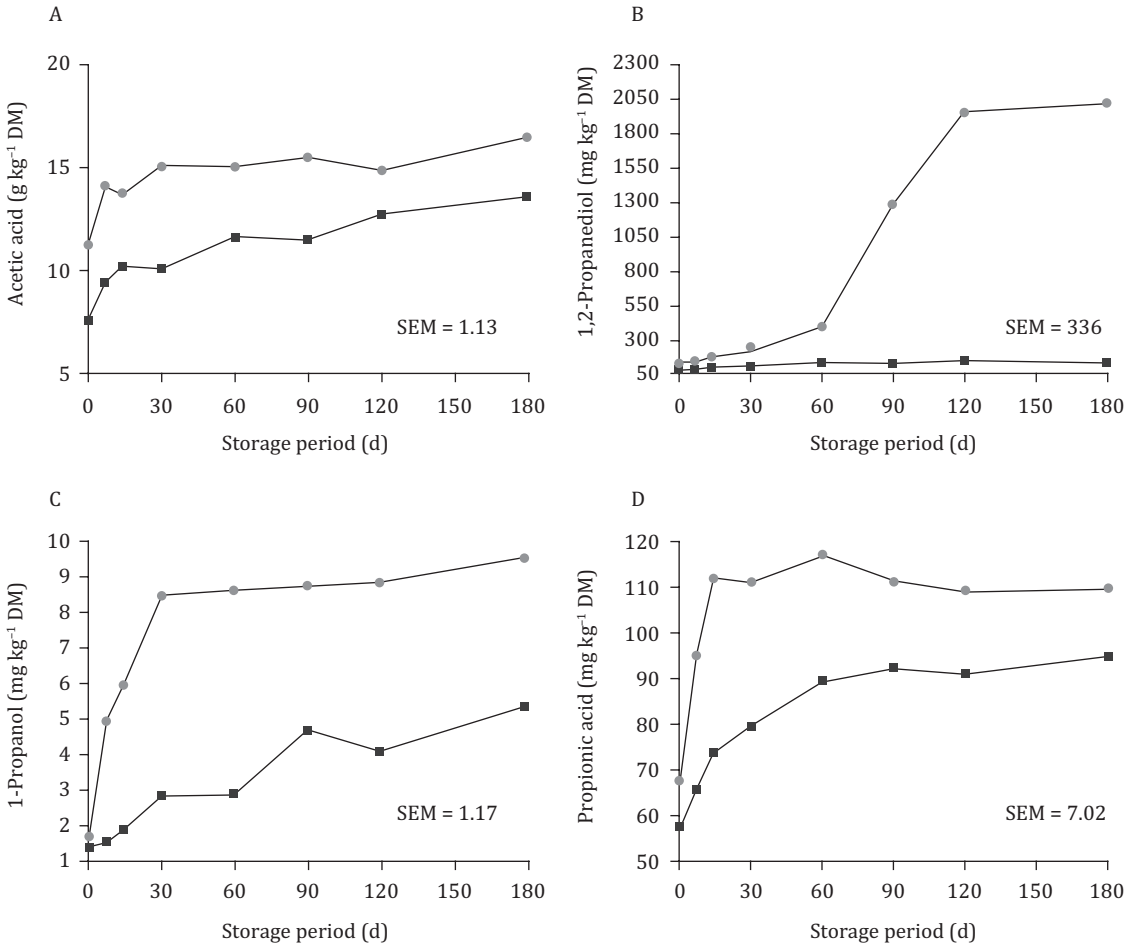


Figure 2 - Influence of maturity (low dry matter ●; high dry matter ■) and storage length on acetic acid (A), 1,2-propanediol (B), 1-propanol (C), and propionic acid (D) in flint corn silage.

Propyl acetate was only found in LDM silage (Figure 3). The formation of methanol occurred during the first 14 d of fermentation and was higher in LDM silage (Figure 4).

The content of NH_3-N increased during fermentation and was higher in LDM silage. Although the DM recovery markedly decreased during the first 60 d of fermentation, it continued until 180 d of storage in both silages. In comparison with HDM, ensiling corn with LDM reduced DM recovery during silage fermentation (Figure 5).

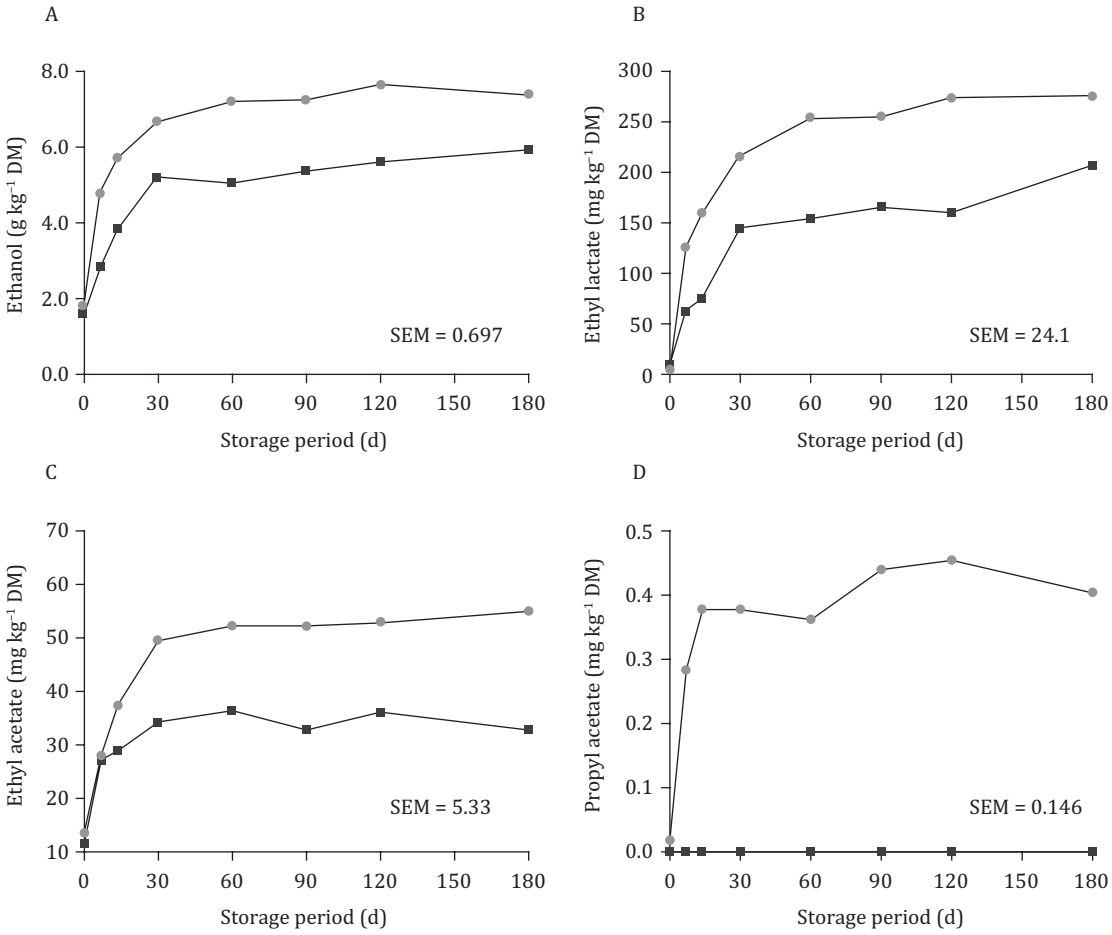


Figure 3 - Influence of maturity (low dry matter ●; high dry matter ■) and storage length on ethanol (A), ethyl lactate (B), ethyl acetate (C), and propyl acetate (D) in flint corn silage.

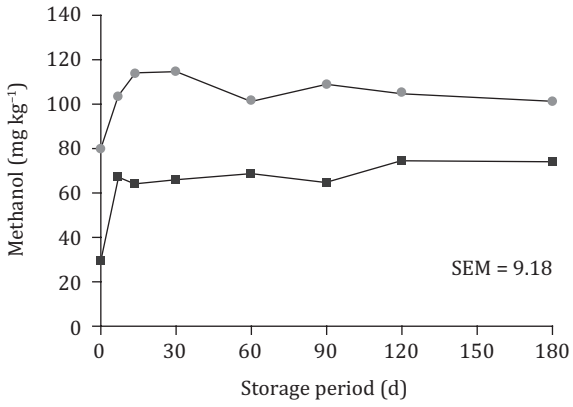


Figure 4 - Influence of maturity (low dry matter ●; high dry matter ■) and storage length on methanol in flint corn silage.

The concentrations of i-butyric acid, i-valeric acid, i-propyl alcohol, and 2,3-butanediol were only affected by storage length and increased from 6.30 to 9.37 mg kg^{-1} DM, 14.7 to 12.5 mg kg^{-1} DM, 0.49 to 1.98 mg kg^{-1} DM, and 8.24 to 10.7 g kg^{-1} DM, respectively, from ensiling to 180 d of storage. Butyric (average 10.0 mg kg^{-1}) and valeric (average 2.06 mg kg^{-1} DM) acids did not change among maturities and storage lengths (data not shown in figures).

The contents of DM, starch, NDF, and CP were only affected by maturity. The HDM silage had higher levels of DM and starch, whereas LDM silage had greater concentrations of NDF and CP (Figure 6). The

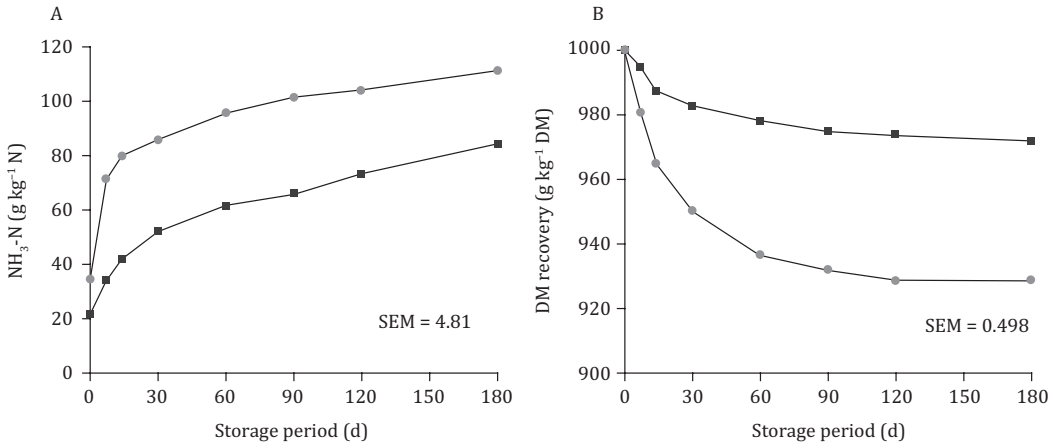


Figure 5 - Influence of maturity (low dry matter ●; high dry matter ■) and storage length on NH_3-N (A) and DM recovery (B) in flint corn silage.

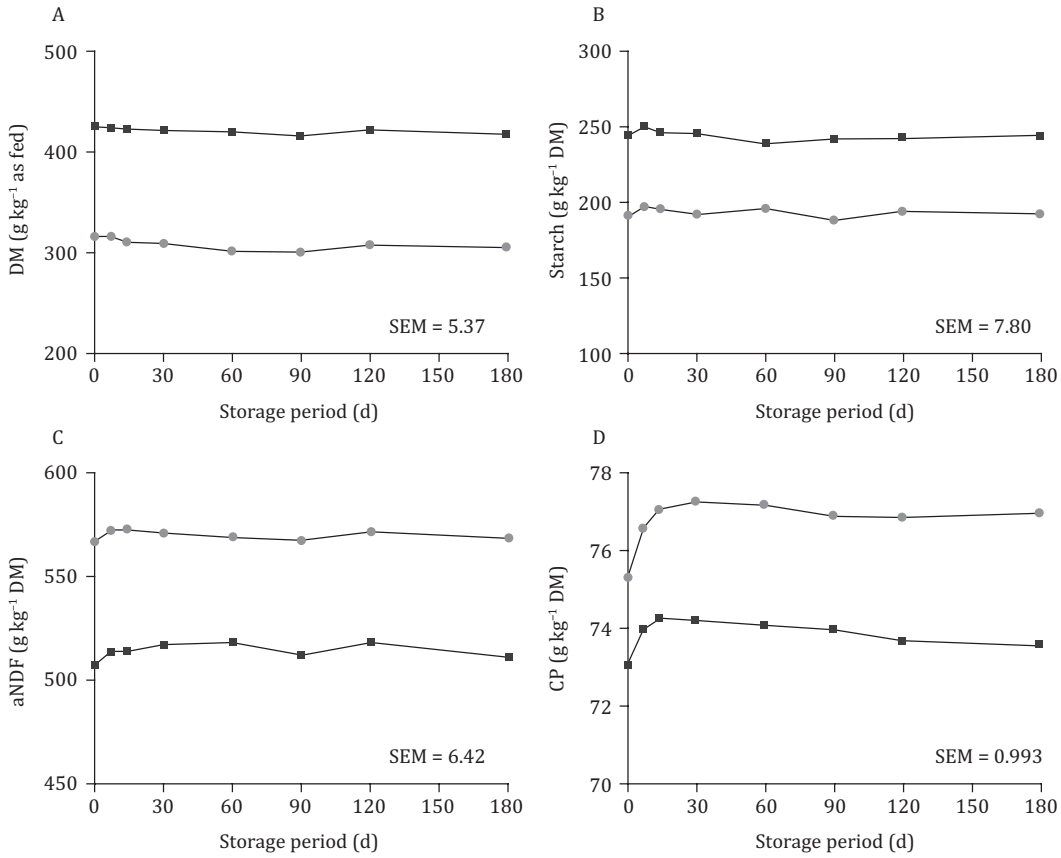
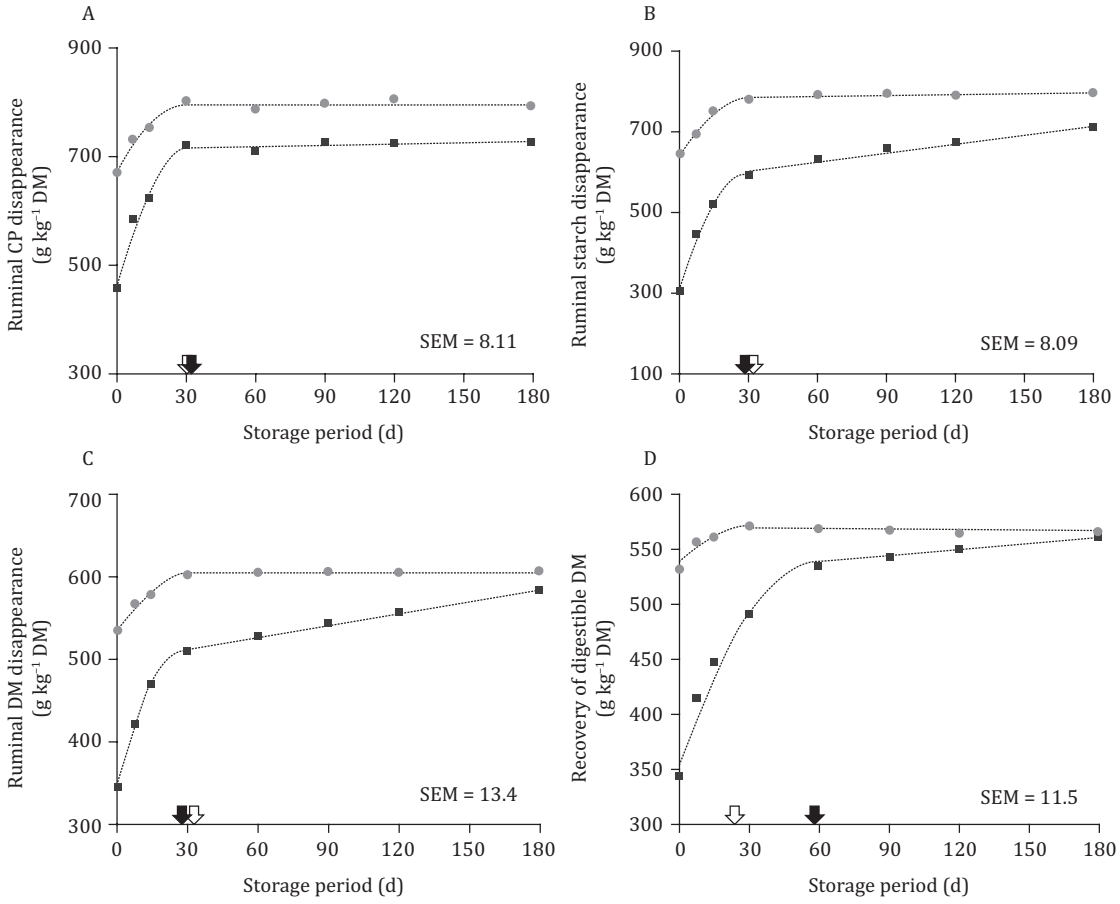


Figure 6 - Influence of maturity (low dry matter ●; high dry matter ■) and storage length on DM (A), starch (B), aNDF (C), and CP (D) in flint corn silage.

ruminal NDF disappearance was not affected by storage length and was higher in LDM than in HDM silage (387 vs. 338 g kg^{-1} DM; P<0.01; data not shown in figures). There was an interaction between maturity and storage length for the ruminal disappearance of starch, CP, and DM and recovery of digestible DM. Ruminal CP disappearance increased primarily during the first month of storage and was higher in LDM silage. Ruminal starch disappearance was greater in LDM but increased in both LDM and HDM silages, especially during the first month of storage. After 30 d, ruminal starch disappearance continued improving in HDM silage. The ruminal DM disappearance increased along the storage and was higher in LDM than in HDM silage. In both silages, the breakpoint for ruminal disappearance of DM, CP, and starch modelled with segmented regressions occurred around 30 d of storage. After the first month of fermentation, ruminal DM disappearance reached a plateau in LDM, while linearly increased in HDM up to 180 d. The recovery of digestible DM increased along the storage in both LDM and HDM silages. In LDM, the recovery of digestible DM reached a plateau at 26 d of storage, whereas it increased during 180 d in HDM silage, but the main increase occurred during the first 60 d of storage (breakpoint) (Figure 7).



Dashed lines indicate curve trends modelled with segmented regression (quadratic-linear); arrows indicate curve brake-point.

Figure 7 - Influence of maturity (low dry matter ●; high dry matter ■) and storage length on ruminal CP disappearance (A), ruminal starch disappearance (B), ruminal DM disappearance (C) and recovery of digestible DM (D) in flint corn silage harvested with a pull-type machine without a kernel processor.

Discussion

In tropical areas, many farmers are harvesting corn crop earlier than desirable (<300 g kg^{-1} of DM), mainly due to the use of forage harvesters without a kernel processor (Bernardes and Do Rêgo, 2014;

Daniel et al., 2019) and high vitreousness of the corn hybrids (Correa et al., 2002), which has been called harder to processing.

Harvesting corn plants with high DM content has been associated with poorer processing of both stover and kernel fractions (Ferraretto et al., 2018). In this study, harvesting at HDM with a pull-type machine without a kernel processor affected forage processing by increasing the proportion of very long (>19 mm) and fine particles (<8 mm) in comparison with LDM. Bernardes et al. (2012) also reported a higher proportion of particles >19 mm in corn harvested at higher DM content, whereas a positive correlation between DM content and particles >19 mm was higher in corn silages harvested with pull-type rather than self-propelled machines. In our study, harvesting at HDM also tended to increase the proportion of fine particles (<8 mm), besides the greater proportion of particles >19 mm. Therefore, the mean particle length was similar between LDM and HDM, but the geometric standard deviation was higher in HDM corn. Higher proportion of very long particles increases the resistance to packing at ensiling, resulting in higher silage porosity (Muck et al., 2003; Pries et al., 2018). Long particles also increase the risk of sorting in the feedbunk (Leonardi and Armentano, 2003). Excessive sorting increases the risk of ruminal acidosis and reduces the nutritive value of the ration remaining in the feedbunk, particularly in the later hours past the time of feed delivery (DeVries et al., 2008), resulting in lower milk yield (Sova et al., 2013). On the other hand, a larger proportion of fine particles (<8 mm) decrease the content of physically effective NDF and increase the risk of ruminal acidosis (Zebeli et al., 2012).

Harvesting corn plants at HDM usually increases starch concentration, while decreases starch digestibility (Allen et al., 2003). Kernel maturation is associated with development of the protein matrix that surrounds the starch granules and increases the proportion of vitreous endosperm, especially in flint genotypes (Philippeau and Michalet-Doreau, 1997; Pereira et al., 2004; Ngonyamo-Majee et al., 2009). Endosperm vitreousness is negatively correlated with starch digestibility in non-fermented corn (Correa et al., 2002; Pereira et al., 2004). Moreover, more mature kernels are more vitreous and harder to damage upon forage chopping. Philippeau and Michalet-Doreau (1998) reported larger particle size for flint than dent corn ground through the same screen, and Ngonyamo-Majee et al. (2009) mentioned a larger particle size for ground corn kernels harvested at black layer than at half-milk line stage. Pericarp integrity and larger pieces of endosperm, with lower surface area available for enzymatic hydrolysis and microbial attachment, are physical constrains to starch digestibility (McAllister et al., 1994). In our study, harvesting at HDM increased the proportion of intact kernels and decreased the KPS compared with LDM. Lower KPS has been associated with lower starch digestibility and milk yield (Ferraretto and Shaver, 2012; Braman and Kurtz, 2015; Salvati et al., 2017).

Harvesting corn at HDM increased starch concentration by 28%, while reduced the ruminal disappearance of nutrients (starch, CP, and NDF) at ensiling, compared with LDM. Therefore, ruminal DM disappearance decreased by 36% at ensiling in HDM compared with LDM. However, the ruminal DM disappearance increased during storage for both LDM and HDM silages by 14 and 70%, respectively. The large difference in ruminal DM disappearance at ensiling markedly decreased during storage.

Ensiling corn causes degradation of endosperm hydrophobic proteins (Hoffman et al., 2011) mainly due to the proteolytic activity of bacteria and plant enzymes (Junges et al., 2017). In corn silage, the occurrence of proteolysis is positively correlated with starch digestibility (Ferraretto et al., 2015; Kung Jr. et al., 2018). Therefore, harvesting corn at LDM and storing silages for longer periods increased NH_3-N concentration and ruminal starch disappearance, and in turn, ruminal DM disappearance. Plant enzymes (proteases and deaminases) and bacteria (e.g. enterobacteria, clostridia, and bacilli) are involved in protein breakdown (McDonald et al., 1991; Pahlow et al., 2003; Rooke and Hatfield, 2003). Although low pH is recognized in reducing proteolysis (McDonald et al., 1991), it should be noted that LDM silage had higher NH_3-N concentration despite a tendency to lower pH. Hence, the higher moisture content in LDM must have prevailed in determining the extent of protein breakdown. Such increase in proteolysis was in line with the higher ruminal CP disappearance.

In both silages, the increase in ruminal DM disappearance occurred mainly during the first month of storage, due to an increase in both CP and starch disappearance in the same period. Despite the lower KPS in HDM silage, longer storage periods decreased the negative impact of maturity on nutrient availability. Although a minimum storage period of one month has been generally recommended for corn silage (Daniel et al., 2015), flint hybrids harvested at high DM contents (e.g. 400 g kg^{-1}) using pull-type forage harvesters without kernel processors requires longer storage periods (e.g. at least two months) to attenuate the negative impact of maturity on digestibility.

As expected, LDM silage showed a more intense fermentation. Most volatile organic compounds were detected in higher concentrations in LDM silage. Typically, wetter forages favour microbial development due to the higher water activity (McDonald et al., 1991; Pahlow et al., 2003). In this study, both LDM and HDM silages were well preserved, as noticed by low concentrations of butyric acid, whereas a more heterofermentative pattern was stimulated in LDM silage.

A wide range of bacteria produces acetic acid (McDonald et al., 1991). During earlier stages of fermentation, enterobacteria play an important role in acetic acid synthesis; however, other microorganisms such as heterolactic bacteria can also produce acetic acid in silage (Pahlow et al., 2003). Nevertheless, the activity of *Lactobacillus buchneri*-like strains probably had a secondary contribution to acetic acid formation, once acetic acid was mostly produced during the first month of storage in both silages. Moreover, a significant accumulation of 1,2-propanediol (a marker of *L. buchneri* activity; Oude-Elferink et al., 2001) was noticed after 60 d of storage, almost exclusively in LDM silage. Interestingly, this accumulation in 1,2-propanediol in LDM silage was not followed by an increase in 1-propanol and propionic acid, as often reported in the literature (Raun and Kristensen, 2010; Kleinshmitt et al., 2013; Gomes et al., 2019). Krooneman et al. (2002) demonstrated that *Lactobacillus diolivorans*, an indigenous strain in corn silage, is capable of converting 1,2-propanediol to approximately equimolar amounts of 1-propanol and propionic acid. In our study, the concentration of propionic acid was much greater than 1-propanol in both silages (on average 18-fold), suggesting that propionic acid might have been synthetized in small amounts by a variety of microorganisms, such as clostridia, yeasts, and, perhaps, propionibacteria (McDonald et al., 1991; Rooke and Hatfield, 2003).

Ethanol is the alcohol most commonly found in silages (Pahlow et al., 2003). Higher concentrations (>30-40 g kg^{-1} DM) of ethanol are often associated with yeast development in silage (Kung Jr. et al., 2018). However, low concentrations of ethanol were found in this trial (<7 g kg^{-1} DM), which can be considered within normal range for whole-plant corn silage (Kung Jr. et al., 2018). Such levels indicate that ethanol was probably produced in small amounts by a variety of microbes, such as yeasts, enterobacteria, heterolactic bacteria, bacilli, and clostridia (McDonald et al., 1991; Rooke and Hatfield, 2003).

Low molecular weight esters are likely formed in silages by abiotic esterification of carboxylic acids and alcohols under low pH conditions (Hangx et al., 2001). Therefore, ethyl acetate and ethyl lactate are positively correlated with ethanol concentrations, whereas propyl acetate is correlated with 1-propanol concentration (Weiss, 2017). In this study, LDM silage had higher concentrations of lactic and acetic acids, ethanol, and 1-propanol, resulting in higher concentrations of ethyl lactate, ethyl acetate, and propyl acetate. Interestingly, propyl acetate was only formed in LDM silage, suggesting that a minimal concentration of alcohol is required for ester formation. The concentration of 1-propanol was lower than 5 mg kg^{-1} DM in HDM silage. Although the possible impacts of esters on feed intake have been conjectured (Weiss, 2017), Gerlach et al. (2019) reported that ethyl esters, as single substance or in combination with ethanol, did not affect the feeding behaviour of goats, then suggesting that esters are unlikely to impair the feed intake of ruminants.

The mechanisms of methanol production in silages are not clear. Although methanol may be produced by clostridia (Hippe et al., 1992), in the current study, the low concentrations of butyric acid suggest that methanol was probably a product of pectin demethylation by plant and bacterial enzymes (Fall and Benson, 1996; Steidlová and Kalac, 2002). The higher concentrations of methanol suggest that

pectin demethylation must have occurred under greater intensity in LDM silage, perhaps due to the less mature cell wall and higher moisture content.

These large alterations in fermentation patterns led to a marked difference in DM recovery among silages. The greater formation of fermentation end-products from pathways associated with CO_2 production resulted in lower DM recovery in LDM than in HDM silage (McDonald et al., 1991; Rooke and Hatfield, 2003).

Lastly, the recovery of digestible DM (= DM recovery × ruminal DM disappearance) followed a similar pattern as observed for ruminal DM disappearance. In this way, the delay in harvest reduced the recovery of digestible DM, but this effect was counterbalanced by prolonged storage, despite the occurrence of DM loss during silage fermentation. In LDM silage, the recovery of digestible DM slightly changed over time, but it markedly increased in HDM silage, mainly up to 60 d of fermentation.

Conclusions

Corn silage harvested at low dry matter (300 g kg^{-1}) had less starch and a more intense fermentation with higher dry matter loss during storage. On the other hand, harvesting corn at a more mature stage (400 g kg^{-1} of DM) with a pull-type forage harvester without a kernel processor worsened forage processing and decreased the ruminal disappearance of nutrients. However, in both silages, there was an increase in ruminal dry matter disappearance during fermentation, mainly in the first month of storage. Afterwards, the ruminal dry matter disappearance reached a plateau in low dry matter silage, whereas it continued increasing in high dry matter silage. Therefore, longer storage of high dry matter silage is a plausible strategy to attenuate the negative effect of maturity on digestibility of flint corn silage harvested with a pull-type forager without a kernel processor.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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