Effect of the ensiling time of hydrated ground corn on silage composition and *in situ* starch degradability

Efeito do tempo de ensilagem do milho moído hidratado sobre a composição da silagem e degradabilidade in situ do amido

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Abstract

The aim of this study was to evaluate the effect of time of ensiling on chemical composition and *in situ* degradability of starch of hydrated ground corn (HGC) with medium grain vitreousness. Corn grains harvested at 83% of dry matter (DM) and vitreosity content of 67% ± 3, were dried to 87% DM. Grains were milled into a device with 2 mm sieve, reconstituted to reach 67% DM, and ensiled (density of 880 Kg/m³) for up to 330 days. One HGC sample was collected monthly for *in situ* determination of composition, fermentation end products and for corn starch degradability. Ensiling time did not affect the DM and crude protein (CP) content of the HGC. However, starch concentration was reduced by 2.4 percentage points at 330 days compared to 3 days of ensiling. Increased concentrations of NH₃-N (8.5 times), lactic acid (3.45 times), acetic acid (4.1 times), propionic acid (1.7 times), butyric acid (2.8 times) and alcohol (2.4 times) were observed during the ensiling period. The rapidly degradable fraction (fraction A) and the rate of degradation of the slowly degradable fraction (fraction C) of HGC starch were increased 3.51 and 2.21 times, respectively, during the ensiling period. Conversely, the slowly degradable fraction (fraction B) of the HGC starch was decreased 1.93 times during the ensiling period. The effective degradability of the starch of HGC increased for passage rates by 0.02/h (79.9% vs. 94.5%); 0.05/h (65.9% vs. 90.01%) and 0.08/h (56.98 vs. 86.52%) when it was evaluated at 3 vs 330 days of ensiling, respectively. In conclusion, ensiling time affected the chemical composition and increases rumen starch degradability of HGC with medium vitreousness of the grain endosperm.

Keywords: Corn. Starch degradability. Ensiling time. Chemical composition.

Resumo

O objetivo deste estudo foi avaliar o efeito do tempo de ensilagem sobre a composição química e a degradabilidade *in situ* do amido do milho moído hidratado (MMH) em grãos de média vitreosidade. Os grãos de milho foram colhidos com 83% de matéria seca (MS) e vitreosidade de 67% ± 3, e foram secos até atingirem 87% de MS. Os grãos foram moídos a dois milímetros, sendo posteriormente reconstituídos, 67% MS, e ensilados (densidade de 880 kg / m³) para até 330 dias. Uma amostra MMH foi coletado mensalmente para a determinação da composição, produtos finais da fermentação e para degradabilidade *in situ* do amido de milho. O tempo de ensilagem não afetou o teor de MS e proteína bruta (PB). No entanto, a concentração de amido foi reduzido em 2,4 pontos percentuais em comparação de 3 com 330 dias de ensilagem. Foram observados o aumento das concentrações de N-NH3 (8,5 vezes), ácidos láctico (3,45 vezes), acético (4,1 vezes), propionico (1,7 vezes), butírico (2,8 vezes) e álcool (2,4 vezes), durante o período de ensilagem. A fracção rapidamente degradável (fração A) e a taxa de degradação da fracção lentamente degradável (fração C) do amido do MMH foram aumentadas 3,51 e 2,21 vezes, respectivamente, durante o período de ensilagem. Por outro lado, a fração lentamente degradável (fração B) do amido do MMH foi diminuída em 1,93 vezes durante o período de ensilagem. A degradabilidade efetiva do amido do MMH foi aumentado para as taxas de passagem de 0,02 / h (79,9% vs. 94,5%); 0,05 / h (65,9% vs 90,01%) e de 0,08/h (56,98% vs. 86,52%) quando foi comparada o período de 3 vs 330 dias de ensilagem, respectivamente. Em conclusão, o tempo de ensilagem afetou a composição química e aumentou a degradabilidade ruminal do amido do MMH de grãos com média vitreosidade.

Palavras-chave: Milho. Degradabilidade do amido. Tempo de ensilagem. Composição química.

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Introduction

Corn grain is one of the main energy sources used in dairy cow diets, mainly due to the high starch content in its chemical composition. However, the starch digestibility of corn grain used for dairy cow feeding ranges from 70 to 100% (FIRKINS et al., 2001). Among the main factors affecting corn starch digestibility is the storage method, grain processing methods, particle size and type of endosperm (PHILIPPEAU et al., 2000; CORREA et al., 2002; NGONYAMO-MAJEE et al., 2008).

Grain at higher concentrations of vitreous endosperm has lower ruminal *in situ* degradability, as well as lower *in vitro* and *in vivo* digestibility (CORREA et al., 2002; ALLEN et al., 2008). The ruminal degradability and intestinal digestibility of starch in dairy cows was higher for corn grain derived from cultivars with chalky endosperm (3% of the vitreous starch) in relation to cultivars with larger endosperm vitreousness (67% of vitreous starch) (TAYLOR; ALLEN, 2005). After physiological maturity, corn grain reaches its point of maximum vitreousness (PEREIRA et al., 2004) and there is a significant decrease in the fermentable starch content in the rumen (BAL et al., 1997; JOHNSON et al., 1999).

Most endosperm proteins are composed of prolamins, which in corn are classified as zeins (BUCHANAN et al., 2000). These proteins are hydrophobic and have low solubility in alcoholic solution. Because proteins of corn endosperm are composed of up to 79% zein, these proteins may limit

the degradability of the starch granules (HOFFMAN et al., 2011). Ensiling of ground corn grain increases ruminal degradability of starch, which may suggest that the process of silage fermentation can increase the access of rumen bacteria to starch granules by removing the protein barrier (PHILIPPEAU; MICHALET-DOREAU, 1998). The ensiling process of high-moisture corn grain increased the ruminal degradability of starch by 22.1% compared with dried corn grains (JURJANZ; MONTEILS, 2005). The effect of ensiling on the degradability of corn starch may occur by increasing the rapidly degradable fraction (fraction A) and the rate of starch degradation of corn grains in the silage.

Besides the fact that the ensiling process increases the degradability and total tract digestibility of starch, the longer the ensiling time, the higher the starch digestibility. Increases in corn grain starch and DM digestibility evaluated by *in vitro* and *in situ* assays, respectively, were reported after increasing the storage period of the entire crop, high-moisture corn grain and hydrated corn grain silages (BENTON et al., 2005; DER BEDROSIAN et al., 2012). The effect of ensiling time on silage degradability is probably due to the degradation and/or solubilization of zein subunits over the ensiling period (LAWTON, 2002; HOFFMAN et al., 2011).

The effect of the ensiling time on starch degradability and protein integrity of the endosperm matrix was previously described for silage of grains harvested with high-moisture corn (HOFFMAN et al., 2011). However, as far as we know, no studies reported the effect of longer ensiling periods on the composition and degradability of corn with medium vitreousness and high levels of DM. The aim of this study was to evaluate the effect of the length of ensiling period on the composition and *in situ* degradability of starch in ground hydrated corn grain with medium vitreousness harvested with high maturity.

Materials and Methods

Study design and ensiling procedures

The corn used in this study was harvested when the grains reached 83% DM. The maize crop was located in Pirassununga, São Paulo, Brazil. After harvesting, the grains were dried at a temperature of 60°C to reach 87% DM content, and then stored in a metal silo with forced ventilation and 1800-ton capacity. Dry matter (DM) was determined by forcedventilation oven drying for 72 h at 65°C and the vitreous endosperm content was determined according to the method described by Dombrink-Kurtzman and Bietz (1993). Corn grains were ground with a mill fitted with 2 mm screen. After milling, water and bacterial inoculant 600,00 (Lactobacillus buchneri strain CNCMI-4323, Katec Lellamand', Goiânia, Brazil) to reach 67% DM. The ground corn, water and inoculant was mixed in a mixer wagon for 10 min. The rehydrated corn was stored at room temperature (26 ± 10°C) in 20 PVC mini-silos (100 diameter x 600 mm length) in a density of $880 \pm 0.530 \text{ kg/m}^3$ (natural matter; NM) for a maximum of 330 days.

Sampling and Chemical Composition

Within the 330-day storage period and beginning at day three of ensiling, two experimental silos were collected monthly and stored at -20°C for interruption of the fermentation process. All hydrated ground corn (HGC) samples were frozen for at least 30 days in order to submit all samples to the freezing effect, independent of the time of fermentation interruption. After 360 days from the beginning of the study, all samples were thawed and submitted to pH evaluation according to Phillip and Fellner (1992).

HGC samples were compressed by a hydraulic press (15 tons/cm³; Marconi˚ São Paulo, Brazil) to obtain the liquid fraction, which was then used to measure the concentrations of NH₃-N and lactic acid, both analyzed by spectrophotometry using a commercial kit (Bioclin˚; Belo Horizonte, Brazil). The analysis of short-chain fatty acids (SCFA; butyrate,

propionate, acetate), and ethanol production were determined by gas chromatography according to Erwin et al. (1961). HGC samples were also analyzed for CP (AOAC, 1990; 988.05 method), starch (EHRMAN, 1996) and DM content (AOAC, 1990; 934.01 method).

In situ degradability

In situ degradability of HGC samples was performed according to the methodology of Mehrez et al. (1977). Briefly, nylon bags (10 x 19 cm) with porosity of 50 microns (Foraging Bag Ankon, Macedon, NY, USA) were filled with approximately 10 g of HGC, according to the ensiling period (3, 30, 60, 90, 120, 150, 180, 210, 240 and 330 days). Samples were weighed with a precision scale and after sealing were inserted via the ruminal cannula for 0; 1.5; 3; 6; 12; 24 and 48 h incubations. The bags were removed from the rumen cannulas, washed with running water and dried at 65°C for 72 h. The residue from all bags was analyzed for DM content (AOAC, 1990; 934.01 method) and starch content (EHRMAN, 1996) to determine the amount of starch remaining in bags for calculation of ruminal starch disappearance. Degradability at time zero was evaluated by immersing the bags into a container with water at 39°C for 15 min (CUMMINS et al., 1983). Degradability data was analyzed using the model of Ørskov and McDonald (1979). The effective degradability (ED) at 0.02; 0.05 and 0.08/h was calculated by the formula proposed by Ørskov et al. (1980).

Statistical analyses

The times of ensiling were analyzed as repeated measures by MIXED procedure of SAS (2002). Covariation within mini-silo was specified by the REPEATED statement, where several error structures were investigated, and the chosen structure for each variable was evaluated according to Bayesian information criteria (BIC) according to the following model: $Y_{ij} = \mu_i + e_{ij}$, where $Y_{ij} = 0$ b s e r v e d

= overall mean; T_i = effect of time i; and, E_{ij} = random error associated with each observation. Statistical significance was defined at P < 0.05.

Results

Vitreousness

The corn grains used to produce the HCG in our study had medium vitreousness (67%). Correa et al. (2002) reported an endosperm vitreosity averaging 73% (64.2 to 80%) for the corn harvested in Brazil. Corn kernels with medium vitreousness has the potential to reduce starch availability in the rumen to less than 60% when harvested mature and milled without hydration (CORREA et al., 2002). Thus, the corn grains used in this study were considered to have hard endosperm, with the potential to have a reduced ruminal starch degradability when used for feeding dairy cows.

Chemical composition and fermentation end-products

Ensiling time did not affect the DM and crude protein (CP) content of the HGC. However, ensiling time reduced the starch content (P < 0.0001) by 2.4 percentage points at 330 days compared to 3 days of ensiling, pH (P < 0.0001) decreased below 4.0 after 30 days of ensiling. NH₃-N (P < 0.0001) increased concentrations (8.5 times) content as percentage of total nitrogen, the same manner as the ensiling time increased the HGC concentrations of the lactic (P < 0.0001) (3.45 times), acetic (P < 0.0001) (4.1 times), propionic (P < 0.0001) (1.7 times), and butyric (P < 0.0001) (2.8 times) acids, as well as the HGC alcohol concentration (P < 0.0001) (2.4 times).

In situ degradability

The results of *in situ* degradability were used to calculate the percentage of fractional degradation (A and B), the degradation rate of fraction B (fraction C) and effective degradability (ED) at the following passage rates (%/h): 2 (ED2), 5 (ED5) and 8 (ED8).

The increase of the storage period affected all variables of starch degradability of HGC. The ED of the starch increased in all simulations (ED2, ED5, and ED8) according to the increase in ensiling time. The rapidly degradable fraction (fraction A) (P < 0.0001) and the rate of degradation of the slowly degradable fraction (fraction C) (P < 0.0001) of HGC starch were increased 3.51 and 2.21 times, respectively, during the ensiling period. Conversely, the slowly degradable fraction (fraction B) (P < 0.0001) of the HGC starch was decreased 1.93 times during the ensiling period. The effective degradability of the starch of HGC increased for passage rates by 0.02/h (79.9% vs. 94.5%); 0.05/h (65.9% vs. 90.01%) and 0.08/h (56.98 vs. 86.52%) when it was evaluated at 3 vs 330 days of ensiling, respectively.

Discussion

Corn grain starch is the main source of nonstructural carbohydrate fed to high-producing dairy cows. Corn harvested mature has high levels of vitreous endosperm and lower digestibility of starch compared to corn grains containing lower levels of vitreous endosperm. Better understanding of the effect of rehydration of vitreous corn over the ensiling time on ruminal starch degradability can improve dietary formulations of dairy cows. Furthermore, the quality of the corn can benefit from this method, as the ensiling process uses no heat, which could affect the digestibility of starch, or chemicals to prevent insect damage.

Chemical composition and fermentation end-products

The dry matter and crude protein content of the silage did not show significant variations during the ensiling time. These results can be explained by the absence of silage mass deterioration, which could be responsible for the protein degradation and its volatilization to the NH₃-N form, as well as degradation of other components with consequent dry matter losses (Figure 1).

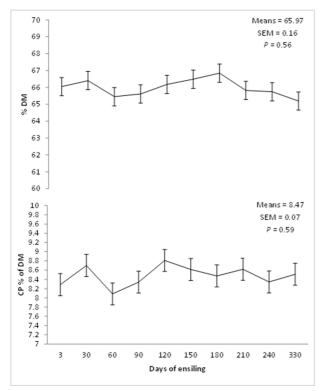


Figure 1 – Effect of ensiling time in the hydrated ground corn (HGC) on the concentration of dry matter (DM) and crude protein (CP) in the mass ensiled

Starch content of the HGC after 330 days of ensiling was reduced by 2.4% when compared to the third day of ensiling period (Figure 2). The disappearance of HGC starch was relatively constant across the ensiling time. However, after 240 days of ensiling, the curve of starch disappearance reached an apparent stabilization. A starch reduction during the ensiling process was also reported by other studies using corn silage (PETTERSON; LINDGREN, 1990) and sorghum (OJEDA; DÍAZ, 1992).

However, the decrease in the HGC starch concentration observed in our study was different from the results reported by Kung Jr. et al. (2014) who described relative starch stability over 140 days of ensiling for high-moisture corn. The maintenance in the concentration of starch during the ensilage period can be attributed to the unavailability of starch for fermentation by rumen bacteria, which first ferment soluble sugars present in the diet (MCDONALD et al.,

1991). According to Van Soest (1994), the contents of starch, pectin and hemicellulose are not degraded by microorganisms that produce lactate during the ensiling process, but it can be degraded by other bacteria that do not produce lactate, proportionally to the fermenting activity of the silo. The results regarding starch concentration observed in our study suggest an activity of lactate-producing microorganisms, which may have reduced the concentration of starch with advancing ensiling time.

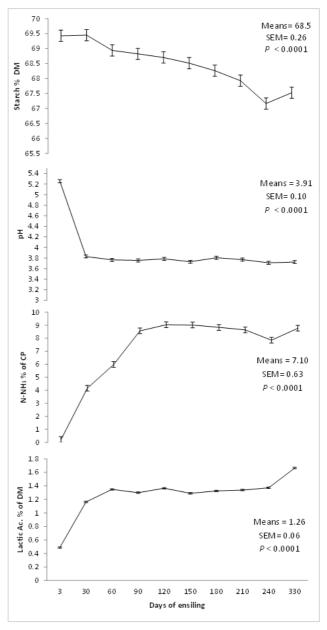


Figure 2 – Effect ensiling time of hydrated ground corn on the concentration of starch, pH, NH_3 -N and lactate in the mass ensiled

The pH of the HGC decreased (P < 0.0001) according to ensiling time and a pH < 4.0 was observed after 30 days of the ensiling process (Figure 2). Hoffman et al. (2011) also reported a marked decrease in the pH of high-moisture corn silage during the first 15 days of ensiling, but a slight variation was observed up to 240 days of ensiling. The CP content of the HGC did not alter (P = 0.59) across the ensiling time (Figure 1). Our results are similar to those described by Der Bedrosian et al. (2012), which evaluated corn silage of whole-plant stored during 360 days. However, an increase of ammoniacal nitrogen (NH₃-N) (P < 0.0001) was observed in this study in comparison to total N across the ensiling period (Figure 2). The NH₃-N content of the ensiled HGC increased by 8.5 times at 330 days compared to three days of ensiling. The concentration of NH₃-N had a more expressive increase up to 90 days of the ensiling process and presented less NH3-N variation up to 330 days of storage. The increase in ammonia concentration suggests the degradation of proteins by bacterial action during the ensiling process. The results of our study are similar to those reported by Hoffman et al. (2011), which also reported an increase in NH₃-N of high-moisture corn silage in comparison to the total nitrogen over the ensiling period. Similar results were also reported for whole-plant corn silage with the advancing ensiling time (DER BEDROSIAN et al., 2012). According to Ohshima and McDonald (1978), NH₃-N was described as an exclusive product of proteins deamination. Thus, it could be suggested that the increase in NH3-N observed in our study originates from the bacterial activity on corn grain proteins. The continuous bacterial activity on the corn proteins may alter the integrity of the protein matrix surrounding the starch granule, facilitating the exposure of starch to bacterial fermentation.

In this study, the concentration of HGC lactic acid (Figure 2) increased (P < 0.0001) 2.93-fold during the ensiling period (330 days). HGC lactic acid production increased more extensively up to 60 days of ensiling time, and subsequently, a slight increase of

lactic acid concentration was observed. The increase in the concentration of lactic acid in our study was similar to the results described by Hoffman et al. (2011), which evaluated the storage of high-moisture corn silage for a period of 240 days. In the study described by Der Bedrosian et al. (2012) the most extensive increase of lactic acid concentration was observed only for corn harvested with high DM content (41% DM) and ensiled for 360 days, which was compared with corn harvested with 32% DM content. In our study, increased concentration of lactate occurred even when the ensiled corn had 33% of DM.

The results of our study are different from those reported by other authors (KLEINSCHMIT; KUNG JUNIOR, 2006; HERRMANN al., 2011; WEINBERG; CHEN, 2013), which described lower lactic acid concentration and higher acetic acid concentration of corn and wheat (whole-plant) silages stored for long periods in comparison to silages stored for a shorter period (few months). The reduction of lactic acid concentration in silages stored for long periods may be attributed to the ability of certain bacteria strains to use lactic acid in anaerobic conditions in the lack of glucose (LINDGREN et al., 1990).

Acetic acid concentration increased (P < 0.0001) 12.4-fold during the entire ensiling period (Figure 3). The increase in the concentration of acetic acid was constant from beginning to end of the experimental period. In our study, the concentration of acetic acid during the ensiling time corroborate with those described by Hoffman et al. (2011), which evaluated high-moisture corn silage. Similar results were also reported by other studies evaluating whole-plant corn silage (DER BEDROSIAN et al., 2012; WEINBERG; CHEN, 2013). The increase in acetic acid concentration may be attributed to the characteristic of the inoculant used in the silage (Lactobacillus buchneri), which is hetero-fermentative and maintain its activity for long periods even at low pH, converting lactic to acetic acid and 1.2-propanediol

(PAHLOW et al., 2003; KLEINSCHMIT; KUNG JUNIOR, 2006; WANG; NISHINO, 2009). The constant increase of lactate and acetate concentration in our study suggests that both populations of bacteria producing acetate and lactate were active during the ensiling time.

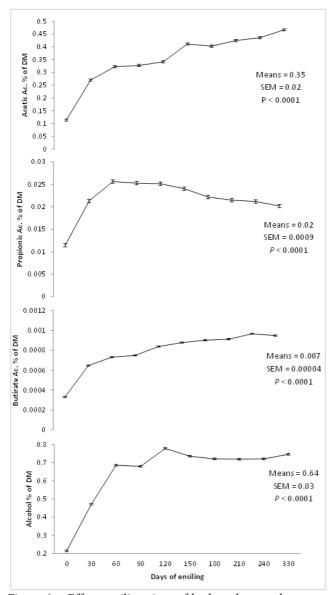


Figure 3 – Effect ensiling time of hydrated ground corn on the concentration of short-chain fatty acid (acetate, propionate and butirate) and alcohol in the mass ensiled

The concentration of propionic acid also increased (P < 0.0001) over the ensiling time, with a maximum concentration obtained at 60 days of storage (Figure 3). This increase of propionic acid was about twice

higher than the lowest concentration observed on the curve, which remained stable for 30 days, and subsequently, presented a progressive decrease up to the end of the storage time. The increase in the concentration of propionic acid may be due to the degradation of lactic acid by butyric acid bacteria (ROTH; UNDERSANDER, 1995). The concentration of butyric acid also increased (2.84-fold; P < 0.0001) during 330 days of ensiling when compared to the level observed in the initial storage period. Although the presence of high levels of butyric acid may indicate silage deterioration, concentrations < 0.10% DM do not compromise the nutritional quality of corn silage (ROTH; UNDERSANDER, 1995). In this study, butyrate concentrations were lower than 0.1% DM, indicating that the HGC did not deteriorate during the ensiling process.

As for butyric acid, high alcohol concentrations are also harmful for silage quality. The concentration of alcohol in the present study increased (P < 0.0001) throughout the ensiling period (Figure 3). The greatest concentration of alcohol in the present study occurred at 120 days of ensiling, when alcohol concentration was 3.63-fold higher than at three days of ensiling. After 120 days, the alcohol concentration decreased and remained relatively stable until day 330 of the ensiling period. Similar results on alcohol concentration have been described by Hu et al. (2009) and Der Bedrosian et al. (2012) using whole-plant corn silage with high DM content and stored for long Although the increase periods. concentration observed in this study had similar profile to the whole-plant corn silage with high DM content (41%), the concentrations were lower than those reported for silages with lower DM content (32%) (DER BEDROSIAN et al., 2012). Lower concentrations of alcohol observed in our study may be the result of the bacterial inoculant (Lactobacillus buchneri), which does not have the acetaldehyde dehydrogenase enzyme responsible for reducing the acetaldehyde into ethanol (OUDE ELFERINK et al., 2001). Thus, the decrease in ethanol production may

have favored the production of acetic acid (MCDONALD et al., 1991).

In situ degradability

Other studies have also reported that increased ensiling time resulted in higher starch digestibility *in vitro* (HALLADA, 2009; DER BEDROSIAN et al., 2012). However, Weinberg and Chen (2013) reported a reduction of digestibility of whole-plant corn or wheat silage stored for 12 months compared with the whole plant before ensiling.

According to Benton et al. (2005), when only the corn grains were ensiled, there was an increase of *in situ* DM degradability of high-moisture corn grain and HGC with the advance of the ensiling period. However, they described a more pronounced increase in degradability of corn DM during the first 28 days of storage, which differs from the present study, because the ED increased constantly throughout the ensiling period. The difference in the degradability profile of the latter study in comparison to ours could possibly be explained by the difference among corn grain vitreousness, however, this variable was not described by Benton et al. (2005) (Figure 4).

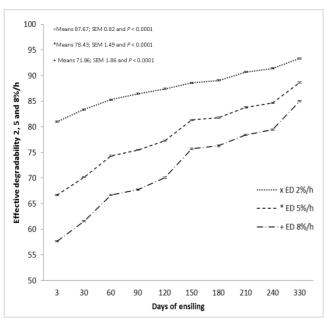


Figure 4 – Efectt of ensiling time in the hydrated ground corn on *in situ* starch effective degradation (ED) in rates simulated passage 2 (\times), 5 (*) and 8 (+)%/h

The increase of effective degradability of HGC starch over the ensiling period may have occurred by the increase of starch rapidly degradable fraction in the rumen in this study. The fraction of starch with degradation rate corresponding to the fraction A increased linearly (P < 0.0001) during the storage period. The amount of starch in HGC, which had a behavior of rapidly degradable fraction at 330 days, was approximately 250% higher than at three days of ensiling. Ensiled corn grains had higher amount of fraction A, which may explain the highest degradability of ensiled corn grains in comparison to non-ensiled corn grains (JURJANZ; MONTEILS, 2005). One consequence of the increased rapidly degradable fraction is the reduction of potentially degradable fraction (fraction B). The increase in the storage time of the HGC decreased linearly (P < 0.0001) the fraction B of the starch. The fraction B of the starch was reduced by approximately two times at 330 days of ensiling compared to the same fraction after three days of ensiling. Fraction C of HGC increased linearly (P < 0.0001) from 2.95%/h on day three to 28.7%/h on day 330 of the ensiling period. The increase of degradation rate (fraction C) may have made the fraction B of corn starch more rapidly degradable over the ensiling time. This result may also have contributed to the starch ED increase during the ensiling period.

The extension and rate of corn starch degradability may be associated with the integrity of grain protein matrix. The ruminal degradation of starch was higher in grains harvested and ensiled with high moisture in comparison to dried corn starch (FIRKINS et al., 2001). Studies evaluating different varieties of corn during the advance of plant maturity (30 to 35% DM) reported decreased starch degradability from 62.2 to 47.8% for vitreous corn and from 87.0 to 69.0% for chalky corn. The reduction of starch degradability does not depend on the genotype of corn and is associated mainly with the decrease of rapidly degradable fraction of the starch (fraction A) (PHILIPPEAU et al., 1996). The alteration in ratio of

degradability of corn grains starch fractions with high levels of vitreous endosperm suggest an increase of effective rumen degradability of the starch with the advance of ensiling period (Figure 5).

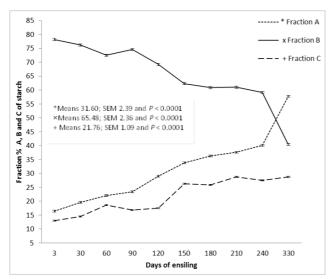


Figure 5 – Effect of ensiling time of hydrated ground corn on the *in situ* starch fractions concentrations A (*), B (×) and C (rate of degradation %/h of fraction B) (+), in the mass ensiled

The results of our study are consistent with the hypothesis that the protein matrix of corn grain is degraded during the ensiling process, which facilitates the access of rumen microorganisms to the starch granule (PHILIPPEAU; MICHALET-DOREAU, 1998; JURJANZ; MONTEILS, 2005). The progressive degradation and solubilization of the corn protein matrix by rumen microbial activity and by the end products of fermentation may possibly explain the increase in starch degradability throughout storage time.

Conclusions

Time of ensiling of HCG alters the composition and proportion of starch fractions A, B and C, and the effective degradability of DM and starch. The advance of ensiling time decreases starch concentration and increases concentrations of NH₃-N, lactate, acetate, propionate, butyrate and alcohol, which suggest a constant activity of microbial populations in the silo. In addition, there is an increase of fractions A and C

of the HCG starch and the effective degradability of DM, and a decrease in starch fraction B over the ensiling time. The results of this study indicate that the composition and characteristics of degradability of HGC changes according to time of ensiling.

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References

ALLEN, M. S.; LONGUSKI, R. A.; YING, Y. Endosperm type of dry ground corn grain affects ruminal and total tract digestion of starch in lactating dairy cows. **Journal of Dairy Science**, v. 91, p. 529, 2008. Supplement 1.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY (AOAC). Official methods of analysis of the Association of Official Analytical Chemists. Arlington, VA: AOAC, 1990.

BAL, M. A.; COORS, J. G.; SHAVER, R. D. Impact of maturity of corn for use as silage in the diets of dairy cows on intake, digestion, and milk production. **Journal of Dairy Science**, v. 80, n. 10, p. 2497-2503, 1997. doi: http://dx.doi.org/10.3168/jds.S0022-0302(97)76202-7.

BENTON, J. R.; KLOPFENSTEIN, T. J.; ERICKSON, G. E. Effects of corn moisture and length of ensiling on dry matter digestibility and rumen degradable protein. **Nebraska Beef Cattle Report**, n. 151, p. 31-33, 2005.

BUCHANAN, B. B.; GRUISSEM, W.; JONES, R. L. (Ed.). **Biochemistry and molecular biology of plants**. Rockville, Md.: American Society of Plant Physiologists, 2000. 1367 p.

CORREA, C. E. S.; SHAVER, R. D.; PEREIRA, M. N.; LAUER, J. G.; KOHN, K. Relationship between corn vitreousness and ruminal in situ starch degradability. **Journal of Dairy Science**, v. 85, n. 11, p. 3008-3012, 2002. doi: http://dx.doi.org/10.3168/jds.S0022-0302(02)74386-5.

CUMMINS, K. W.; SEDELL, J. R.; SWANSON, F. J.; MINSHALL, G. W.; FISHER, S. G.; CUSHING, C. E.; PETERSEN, R. C.; VANNOTE, R. L. Organic matter budgets for stream ecosystems: problems in their evaluation. ln: BARNES, J. R.; MINSHALL, G. W. (Ed.). **Stream ecology:** application and testing of general ecological theory. New York: Plenum, 1983. p. 299-353.

DER BEDROSIAN, M. C.; NESTOR JUNIOR, K. E.; KUNG KUNIOR, L. The effects of hybrid, maturity, and length of storage on the nutritive value of corn silage. **Journal of Dairy Science**, v. 95, n. 9, p. 5115-5126, 2012. doi: http://dx.doi.org/10.3168/jds.2011-4833.

DOMBRINK-KURTZMAN, M. A.; BIETZ, J. A. Zein composition in hard and soft endosperm of maize. **Cereal Chermistry**, v. 70, n. 1, p. 105-108, 1993.

EHRMAN, T. LAP-016: determination of starch in biomass samples by chemical solubilization and enzymatic digestion. In: UNITED TATES. Department of Energy. Midwest Research Institute (MRI). National Renewable Energy Laboratory (NREL). Chemical analysis and testing laboratory analytical procedures. Washington, D.C.: National Bioenergy Center, 1996.

ERWIN, E. S.; MARCO, G. J.; EMERY, E. M. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. **Journal of Dairy Science**, v. 44, n. 9, p. 1768-1771, 1961. doi: http://dx.doi.org/10.3168/jds.S0022-0302(61)89956-6.

FIRKINS, J. L.; EASTRIDGE, M. L.; ST-PIERRE, N. R.; NOFTSGER, S. M. Effects of grain variability and processing on starch utilization by lactating dairy cattle. **Journal of Animal Science**, v. 79, p. E218-E238, 2001. Supplement. doi: http://dx.doi.org/10.2134/jas2001.79E-SupplE218x.

HALLADA, C. Why new corn silage doesn't feed as well. [S.l.]: Hoard's Dairyman, 2009.

HERRMANN, C.; HEIERMANN, M.; IDLER, C. Effects of ensiling, silage additives and storage period on methane formation of biogas crops. **Bioresource Technology**, v. 102, n. 8, p. 5153-5161, 2011. doi: http://dx.doi.org/10.1016/j.biortech.2011.01.012.

HOFFMAN, P. C.; ESSER, N. M.; SHAVER, R. D.; COBLENTZ, W. K.; SCOTT, M. P.; BODNAR, A. L.; SCHMIDT, R. J.; CHARLEY, R. C. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. **Journal of Dairy Science**, v. 94, n. 5, p. 2465-2474, 2011. doi: http://dx.doi.org/10.3168/jds.2010-3562.

HU, W.; SCHMIDT, R. J.; MCDONELL, E. E.; KLINGERMAN, C. M.; KUNG JUNIOR, L. The effect of *Lactobacillus buchneri* 40788 or *Lactobacillus plantarum* MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents. **Journal Dairy Science**, v. 92, n. 8, p. 3907-3914, 2009. doi: http://dx.doi.org/10.3168/jds.2008-1788.

JOHNSON, L.; HARRISON, J. H.; HUNT, C.; SHINNERS, K.; DOGGETT, C. G.; SAPIENZA, D. Nutritive value of corn silage as affected by maturity and mechanical processing: a contemporary review. **Journal of Dairy**

Science, v. 82, n. 12, p. 2813-2825, 1999. doi: http://dx.doi.org/10.3168/jds.S0022-0302(99)75540-2.

JURJANZ, S.; MONTEILS, V. Ruminal degradability of corn forages depending on the processing method employed. **Animal Research**, v. 54, n. 1, p. 3-15, 2005. doi: http://dx.doi.org/10.1051/animres:2004041.

KLEINSCHMIT, D. H.; KUNG JUNIOR, L. The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn silage. **Journal of Dairy Science**, v. 89, n. 10, p. 3999-4004, 2006. doi: http://dx.doi.org/10.3168/jds.S0022-0302(06)72443-2.

KUNG JUNIOR, L.; WINDLE, M. C.; WALKER, N. The effect of an exogenous protease on the fermentation and nutritive value of high-moisture corn. **Journal of Dairy Science**, v. 97, n. 3, p. 1707-1712, 2014. doi: http://dx.doi.org/10.3168/jds.2013-7469.

LAWTON, J. W. Zein: a history of processing and use. **Cereal Chemistry**, v. 79, n. 1, p. 1-18, 2002. doi: http://dx.doi.org/10.1094/CCHEM.2002.79.1.1.

LINDGREN, S. E.; AXELSSON, L. T.; MCFEETERS, R. F. Anaerobic L-lactate degradation by *Lactobacillus plantarum*. **FEMS Microbiology Letters**, v. 66, n.1-3, p. 209-213, 1990. doi: http://dx.doi.org/10.1016/0378-1097(90)90284-W.

MCDONALD, P.; HENDERSON, N.; HERON, S. **The biochemistry of silage**. 2nd ed. Heighwoods Drive: Chalcomb Publications, 1991. 340 p.

MEHREZ, A. Z.; ØRSKOV, E. R.; MCDONALD, I. Rates of rumen fermentation in relation to ammonia concentration. **British Journal of Nutrition**, v. 38, n. 3, p. 437-443, 1977. doi: http://dx.doi.org/10.1079/BJN19770108.

NGONYAMO-MAJEE, D.; SHAVER, R. D.; COORS, J. G.; SAPIENZA, D.; LAUER, J. G. Relationship between kernel vitreousness and dry matter degradability for diverse corn germplasm: II. Ruminal and post-ruminal degradabilities. **Animal Feed Science and Technology**, v. 142, n. 3-4, p. 259-274, 2008. doi: http://dx.doi.org/10.1016/j.anifeedsci.2007.09.001.

OHSHIMA, M.; MCDONALD, P. A review of the changes in nitrogenous compounds in herbage during ensilage. **Journal of the Science of Food and Agriculture**, v. 29, n. 6, p. 497-505, 1978. doi: http://dx.doi.org/10.1002/jsfa.2740290602.

OJEDA, F.; DÍAZ, D. Estudio de diferentes proporciones de sorgo y dolichos ensilados con y sin preservantes. **Pastos y Forrajes**, v. 15, n. 1, p. 77-87, 1992.

ØRSKOV, E. R.; MCDONALD, I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. **The Journal of Agricultural Science**, v. 92, n. 2, p. 499-503, 1979. doi: http://dx.doi.org/10.1017/S0021859600063048.

ØRSKOV, E. R.; HOVELL, F. D. B.; MOULD, F. The use of the nylon bag technique for evaluation of feedstuffs. **Tropical Animal Production**, v. 5, n. 3, p. 195-213, 1980.

OUDE ELFERINK, S. J. W. H.; KROONEMAN, J.; GOTTSCHAL, J. C.; SPOELSTRA, S. F.; FABER, F.; DRIEHUIS, F. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. **Applied and Environmental Microbiology**, v. 67, n. 1, p. 125-132, 2001. doi: http://dx.doi.org/10.1128/AEM.67.1.125-132.2001.

PAHLOW, G.; MUCK, R. E.; DRIEHUIS, F.; OUDE ELFRINK, S. J. W. H.; SPOELSTRA, S. F. Microbiology of ensiling. **Agronomy Monograph, Silage Science and Technology**, v. 42, p. 31-93, 2003. doi: http://dx.doi.org/10.2134/agronmonogr42.c2.

PEREIRA, M. N.; PINHO, R. G. Von; BRUNO, R. G. S.; CALESTINE, G. A. Ruminal degradability of hard or soft texture corn grain at three maturity stage. **Scientia Agricola**, v. 61, n. 4, p. 358-363, 2004. doi: http://dx.doi.org/10.1590/S0103-90162004000400002.

PETTERSON, K. L.; LINDGREN, S. The influence of the carbohydrate fraction and additives on silage quality. **Grass and Forage Science**, v. 45, n. 2, p. 223-233, 1990. doi: http://dx.doi.org/10.1111/j.1365-2494.1990.tb02202.x.

PHILIPPEAU, C.; CHAMPION, M.; MICHALET-DOREAU, B. Influence du génotype et du stade de maturité sur la digestion ruminale de l'amidon de maïs récolté au stade ensilage. In: SYMPOSIUM ON SILAGE MAIZE, 1996, Nantes. **Annales...** Nantes, 1996. p. 379-380.

PHILIPPEAU, C.; LANDRY, J.; MICHALET-DOREAU, B. Influence of the protein distribution of maize endosperm on ruminal starch degradability. **Journal of the Science of Food and Agriculture**, v. 80, n. 3, p. 404-408, 2000. doi: http://dx.doi.org/10.1002/1097-0010(200002)80:3<404::AID-JSFA541>3.0CO;2-Z.

PHILIPPEAU, C.; MICHALET-DOREAU, B. Influence of genotype and ensiling of corn grain on in situ degradation of starch in the rumen. **Journal of Dairy Science**, v. 81, n. 8, p. 2178-2184, 1998. doi: http://dx.doi.org/10.3168/jdsS0022-0302(98)75796-0.

PHILLIP, L. E.; FELLNER, V. Effects of bacterial inoculation of high moisture ear corn on its aerobic

stability, digestion and utilization for growth by beef steers. **Journal of Animal Science**, v. 70, n. 10, p. 3178-3187, 1992. doi: http://dx.doi.org/1992.70103178x.

ROTH, G.; UNDERSENDER, D. Silage additives. In: ALLEN, M.; ROTH, G.; UNDERSANDER, D. (Ed.). **Corn silage production, management, and feeding**. Madison, WI: American Society of Agronomy-Crop Science Society of America-Soil Scien Society of America, 1995. p. 27-29.

STATISTICAL ANALYSIS SYSTEM (SAS). **System for microsoft windows**: release 8.2. Cary: SAS, 2002.

TAYLOR, C. C.; ALLEN, M. S. Corn grain endosperm type and brown midrib 3 corn silage: feeding behavior and milk yield of lactating cows. **Journal of Dairy Science**, v. 88, n. 4, p. 1425-1433, 2005. doi: http://dx.doi.org/10.3168/jdsS0022-0302(98)75796-0.

VAN SOEST, P. J. **Nutritional ecology of the ruminant**. 2nd ed. Ithaca: Cornell University Press, 1994. 476 p.

WANG, F.; NISHINO, N. Association of *Lactobacillus buchneri* with aerobic stability of total mixed ration containing wet brewers grains preserved as a silage. **Animal Feed Science and Technology**, v. 149, n. 3-4, p. 265-274, 2009. doi: http://dx.doi.org/10.1016/j.anifeedsci.2008.06.012.

WEINBERG, Z. G.; CHEN, Y. Effects of storage period on the composition of whole crop wheat and corn silages. **Animal Feed Science and Technology**, v. 185, n. 3-4, p. 196-200, 2013. doi: http://dx.doi.org/10.1016/j.anifeedsci.2013.08.009.