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CHEMICAL COMPOSITION, DIGESTIBILITY AND AEROBIC STABILITY OF CORN SILAGES HARVESTED AT DIFFERENT MATURITY STAGES[1]

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ABSTRACT - The objective of this study was to evaluate the effect of corn plant harvested in diferente maturity stages on the chemical composition, digestibility and aerobic stability of silages. The corn used in the study was the hybrid BM3061 harvested after 114, 121, 126, 133 and 140 days of sowing at five maturity stages based on the advancement of the grain milk line (early dent (ED), 1/3 of milk line (ML), 1/2 ML, 2/3 ML and black layer (BL)). A variation from 242.7 to 377.4 g of dry matter (DM) kg^{-1}as fed was observed between the ED and BL stages; however, the measurements performed in this study did not exhibited major changes in the composition of these silages. The silages produced with plants that were harvested at the 1/2 ML, 2/3 ML and BL stages showed lower DM loss during the fermentative process. The silages produced with plants that were harvested at the ED and 1/3 ML stages showed higher *in vitro* organic matter digestibility (IVOMD) (0.584 and 0.631 g g^{-1} of OM, respectively). The corn silages produced at the maturity stage of 2/3 ML showed a higher aerobic stability (104 hours) during the aerobic exposure. The 1/3 and 2/3 ML maturity stages seem to be the best harvest stages for the production of corn silage in tropical climates.

Key words: Digestibility. Fermentation. Fiber.Tropical climate.

COMPOSIÇÃO QUÍMICA, DIGESTIBILIDADE E ESTABILIDADE AERÓBIA DE SILAGENS DE MILHO COLHIDAS EM DIFERENTES ESTÁDIOS DE MATURIDADE

RESUMO - O objetivo deste estudo foi avaliar o efeito do estádio de maturidade do milho sobre a composição química, digestibilidade e estabilidade aeróbia das silagens produzidas. O milho utilizado no estudo foi o híbrido BM3061 colhido em cinco estádios de maturidade baseado no avanço da linha de leite no grão (sem linha de leite (ED), com 1/3 de linha de leite (ML), 1/2 ML, 2/3 ML e camada negra (BL)), os quais corresponderam a 114, 121, 126, 133 e 140 dias após o plantio. Os valores de MS variaram de 242,7 a 377,4 g kg^{-1} de matéria natural entre os estádios ED e BL, contudo, não houve maiores alterações na composição química das silagens pelas análises realizadas neste estudo. As silagens produzidas com plantas colhidas nos estádios 1/2 ML, 2/3 ML e BL apresentaram menores perdas de MS durante o processo fermentativo. No entanto, as silagens produzidas nos estádios ED e 1/3 ML apresentaram maiores coeficientes de digestibilidade *in vitro* da matéria orgânica (0,584 e 0,631 g g^{-1} de MO, respectivamente). As silagens produzidas no estádio 2/3 ML apresentaram maior estabilidade aeróbia após abertura dos silos (104 horas). Os estádios de maturidade 1/3 ML e 2/3 ML mostraram ser os melhores estádios de maturidade para a produção de silagens em clima tropical.

Palavras-chave: Clima tropical. Digestibilidade. Fermentação. Fibra.

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INTRODUCTION

Ensiling process is based on the fermentation of sugars into lactic acid by lactic acid bacteria that causes a rapid pH decrease inhibiting the growth of undesirable microorganisms (MCDONALD et al., 1991). The most widely forage used as silage is corn, due to its high energy, low fiber content, and high dry matter (DM) yield per unit area. Thus, the stage of maturity when the plant is harvested can be considered the main determinant of the nutritive value of the silage (ATIS et al., 2013). With the advancing physiological maturity of whole-crop cereal, there is a reduction in the protein concentration (BAL, 2006), whereas the concentration of fiber in the whole-plant usually is reduced due to accumulate of starch in the grains (NADEAU, 2007). Usually, silages produced from plants with a higher moisture content present greater digestibility compared to plants with higher DM concentration (JENSEN et al., 2005; BAL, 2006; NADEAU, 2007), but in opposite, these forages can promote greater DM losses during fermentation due to high effluent production (RABELO et al., 2012), besides low aerobic stability in some cases (HU et al., 2009; RABELO et al., 2012).

After silos are open, silages rich in nutrients have lower aerobic stability due to spoilage microorganisms using lactic acid and residual soluble carbohydrates as substrates for their development (WILKINSON; DAVIES, 2012). These events are enhanced in warm climates and reduce silage quality (KIM; ADESOGAN, 2006). Although there are various studies that have evaluated the harvest time in corn for ensilage in tropical conditions, few studies have assessed the silage quality (VILELA et al., 2008; FERRARETTO; SHAVER, 2012). There is still no consensus as to ideal moment of harvest due to the influence of the hybrids, climate and fertilization. The climate interferes directly on the chemical composition of plants (ABEYSEKARA et al., 2013), and knowledge about the ideal moment of corn harvest for ensilage in tropical climates is relatively scarce.

Therefore, the objective of this study was to evaluate the effect of corn plant maturity on silage chemical composition, digestibility and aerobic stability.

MATERIALS AND METHODS

The trial was conducted at Universidade José do Rosário Vellano/UNIFENAS, Alfenas campus - MG, Brazil. Alfenas climate is characterized as humid subtropical (AWA) with two distinct seasons, a dry season from April to September and a rainy season from October to March. The climatic conditions during the trial are shown in Figure 1. The chemical characteristics of the soil used in the experiment were pH (H_2O) = 6.0; P Mehlich = 12 mg dm^{-3}; K = 61 mg dm^{-3}; Ca^{2+} = 3.2 $cmol_c$ dm^{-3}; Mg^{2+} = 1.2 $cmol_c$ dm^{-3}; Al^{3+} = 0.0 $cmol_c$ dm^{-3}; H + Al = 2.9 $cmol_c$ dm^{-3}; sum of bases (SB) = 4.5 $cmol_c$ dm^{-3}; CCHO effective (t) = 4.5 $cmol_c$ dm^{-3}; CCHO potential (T) = 7.4 $cmol_c$ dm^{-3}; V (%) = 61.0; m (%) = 0.0; organic matter (O.M.) = 5.0 dag kg^{-1}; and P remainder = 16.0 mg L^{-1}.

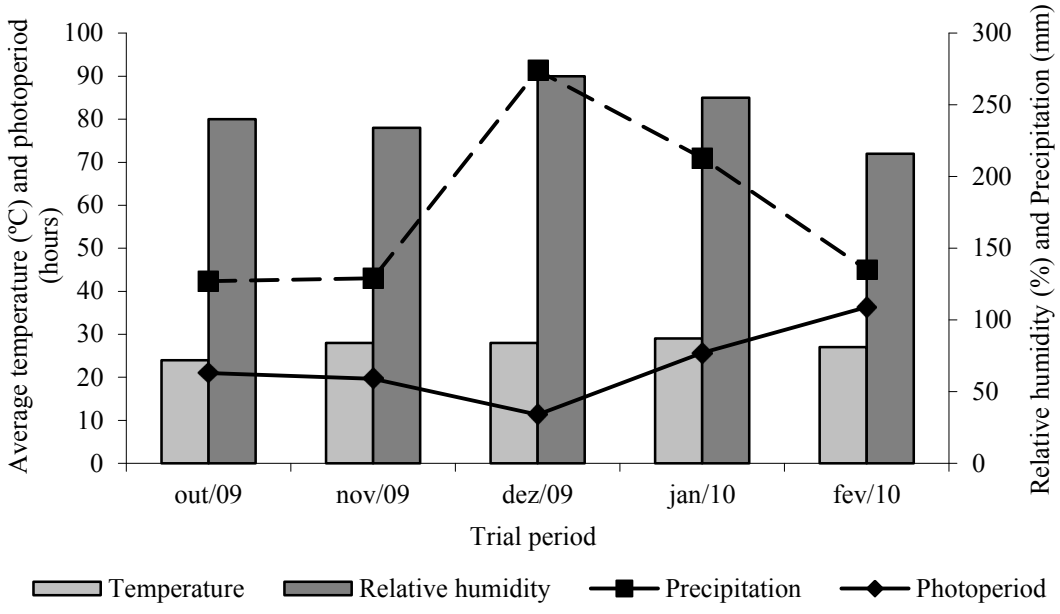

Figure 1. Maximum and minimum temperature and rainfall during the growth of the corn plants in Alfenas, Minas Gerais State, Brazil.

The BM3061 hybrid corn used in the study had texture of the grain dent (Biomatrix, Patos de Minas, MG, Brazil), and was sowed at an average 5 cm depth using 10 seeds per meter at a spacing of 80 cm. The NPK 10-20-10 was used in the soil fertilization at 400 kg ha^{-1}. Twenty days after planting, when the plants had 3 or 4 pairs of leaves, we thinned the field to 5 plants per meter (20 plants per row), obtaining an average standard of 62.500 plants ha^{-1}.

The crop was harvested, and evaluated at five maturity stages: early dent (ED), 1/3 of milk line (ML), 1/2 ML, 2/3 ML and black layer (BL). Weekly observations were performed at various places in the area to monitor the reduction of the milk line in the grain. When the pre-established moment to harvest was achieved, all of the corresponding plants in the sub-plot were harvested by hand at 10 cm from the soil level, and chopped in a stationary mill with adjustable knives for cutting particles close to 2.0 cm. The experimental silos were PVC tubes with a capacity for 4 L. Each tube had a cover adapted with a Bunsen valve for gas escape and 0.5 kg of sand at the bottom. A thin plastic screen was used to prevent contact between the sand and forage. Samples were taken for chemical characterization at ensiling for each stage of maturity (Table 1). After 55 days of ensiling, the silos were opened, and samples were taken for silage characterization. The total DM losses, gases and effluent losses during the silage fermentation phase were determined (JOBIM et al., 2007).

Aerobic stability was determined by placing 2.5 kg of silage in piles and maintained in a closed space at ambient temperature (barn). The ambient and silage temperatures were measured at 8 hours intervals for 7 days using thermometers placed 10 cm from the center of the masses. In the same pile, approximately 15 g were taken to determine the pH values (same evaluation times as for the temperature). The average room temperature when the silages were exposed to oxygen was 22.41ºC, with average temperatures of 19.93, 25.63 and 21.21ºC at 06:00 a.m, 02:00 p.m and 10:00 p.m (time for data sampling), respectively. The aerobic stability was defined as the length of time required for the temperature of corn silages to increase to 2°C above the baseline after exposure to air. Moreover, we evaluated the maximum temperature, the number of hours to reach the maximum temperature, and the rate of temperature rise (°C/hour) using the higher observed temperature value divided by the time (in hours) needed to reach that value (RUPPEL et al., 1995). The average pH values and maximum pH also were measured during the aerobic exposure, as well as the time needed to reach these values. As known, the evaluation of the aerobic stability silages in ambient temperature is more accurate to estimate the spoilage of silages in the field when compared to studies in room with controlled temperature (JOBIM et al., 2007).

Silage and fresh plant samples were dried in a ventilated oven at 55°C for 72 hours, ground in a knife mill *Willey* to pass through 1 mm screen sieve, and analyzed for dry matter - DM (105°C for 12 h) and ash (500°C for 5 h). The total nitrogen (TN) and ether extract (EE) were determined following the recommendations of the AOAC (1996, methods no. 930.15 and 920.39, respectively). The crude protein (CP) content was obtained by the product between the total nitrogen (TN) and the factor 6.25. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured using the techniques described by Van Soest et al. (1991). The NDF was assayed with a heat stable amylase and expressed inclusive of residual ash (aNDF). The ADF also was expressed inclusive of residual ash. The lignin content was determined after solubilization of the cellulose in 72% sulfuric acid (VAN SOEST; ROBERTSON, 1985). The total carbohydrate (CHO) and non-fiber carbohydrate (NFC) contents were calculated according to Sniffen et al. (1992). Fresh silage samples were used to determine the pH values in a Beckman pH meter Expandomatic SS-2.

Table 1. Agronomic and chemical characteristics (g kg^{-1} of DM) of corn plants harvested at different stages of maturity.

Item	Maturity stages[1]				
	ED	1/3 ML	1/2 ML	2/3 ML	BL
Days after sowing	114	121	126	133	140
Ear, g kg^{-1}	-	321.0	310.2	342.9	334.3
Grain, g kg^{-1}	-	182.1	215.4	220.5	235.3
Density, kg m^{-3}	570.15	606.12	577.91	594.95	541.77
Dry matter, g kg^{-1} of fresh matter	261.3	290.9	321.1	340.2	385.8
Ash	41.2	39.3	40.5	50.3	43.6
Crude protein	72.4	58.8	67.2	55.3	62.3
Ether extract	24.7	21.6	28.6	34.4	22.4
Total carbohydrates	861.7	880.3	863.8	860.0	871.6
Non-fiber carbohydrates	197.7	295.2	229.8	311.2	268.8
Neutral detergent fiber	663.9	585.2	634.0	548.9	602.9
Acid detergent fiber	336.2	266.1	290.7	278.9	318.3
Lignin	66.9	75.9	57.8	64.8	53.8

[1]ED = early dent; ML = milk line; BL = black layer.

The *in vitro* gas assay was conducted for 72 hours following the procedures described by Mauricio et al. (1999). The ruminal fluid was collected from two Nellore cattle cannulated fed with a diet based on corn silage (60% of the diet on a DM basis). The ruminal fluid was filtered in two layers of cotton gauze under the continuous injection of CO_2 and kept in a water bath at 39°C. After, the ruminal fluid was mixed with a buffer solution in order to artificially reproduce the effect of salivation under *in vivo* conditions. The fluid containing liquid rumen + artificial buffer was placed in bottles (30 mL fluid in bottles with a capacity of 110 mL) with 0.2 g dried silage. The bottles were immediately closed with rubber stoppers and sealed with aluminum foil.

The pressure accumulated in the bottles was measured via a pressure transducer connected at its end to a needle. The readings were taken at 2, 4, 6, 8, 10, 12, 24, 26, 28, 30, 32, 36, 48, 52, 56, 60 and 72 hours after incubation. To deduct the gas volume from the ruminal fluid over the buffer solution, two flasks were incubated without samples (blank), which can be used to correct the changes in the atmospheric pressure (ARAUJO et al., 2011). Thus, each reading of the gas volume in bottles with the sample was subtracted from the volume of the blanks. From the *in vitro* test for gas production, the *in vitro* organic matter digestibility (IVOMD) was calculated according to the equation described by Menke and Steingass (1988):

Equation 1 → IVOMD (g g^{-1} of OM) = 14.88 + (0.889 * PG24h) + (0.045 * CP) + (0.065 * MM)

Where: PG24h = gas production obtained after 24 h of fermentation; CP and MM are expressed in g kg^{-1} DM.

The potential of gas production was calculated according to the following equation (dual pool logistic model) described by Schofield et al. (1994):

Equation 2 → Y_t (mL $g^{-1}$of OM) = V1 (1 + exp (2 - 4*D1*(t - L)))$^{-1}$ + V2 (1 + exp (2 - 4*D2*(t - L)))$^{-1}$

Where: Y_t = gas volume produced in the t time; V1 and V2 = volume obtained by NFC and fiber carbohydrate (FC) fermentation, respectively; D1 and D2 = fractional degradation rate of NFC and FC, respectively; t = time of fermentation; and L = lag time (h).

The corn used in the experiment was sowed in the field under randomized blocks design to control possible effects of soil fertility. However, after harvest, the whole-crop corn from each parcel (blocks) was chopped and mixed at various times to ensure a good homogenization and eliminate possible effects of soil fertility. Moreover, the environmental conditions were not controlled because maturity stages were our treatments, and so, when the corn was harvested, there was not link with time. Therefore, because these reasons described earlier, the experiment was conducted in a completely randomized design, as well as the *in vitro* gas production assay, evaluating five treatments (maturity stages ED, 1/3 ML, 1/2 ML, 2/3 and BL) with four replicates. Prediction regressions of DM accumulated during the corn plant growth were generated using the MIXED procedure of SAS (v. 9.2, SAS Inst. Inc., Cary); the equations' intercepts and slopes were estimated using the ESTIMATE statement. The tests of aerobic stability and *in vitro* gas production were conducted as split plot, where the factor of plots was the treatments, and the factor attributed to the sub-plots was the time. The chemical composition and digestibility were analyzed as a completely randomized design using the MIXED procedure of SAS (v. 9.2, SAS Inst. Inc., Cary). Differences between the means were determined using the PDIFF at $P \leq 0.05$, which differentiates the means based on Fisher´s *F*-protected least significant difference test.

The gas production functions were jointly fitted for the five stages of maturity using the NLIN procedure of SAS (v. 9.2, SAS Inst. Inc., Cary) according to the following model:

$$Y_{ij} = (V1+dV1_2+dV1_3+dV1_2+dV1_5)\,(1+\exp\,(2\text{-}4*(D1+dD1_2+dD1_3+dD1_4+dD1_5)*(t-(L+dL_2+dL_3+dL_4+dL_5))))^{-1}+(V2+dV2_2+dV2_3+dV2_4+dV2_4)\,(1+\exp(2\text{-}4*(D2+dD2_2+dD2_3+dD2_4+dD2_5)*(t-(L+dL_2+dL_3+dL_4+dL_5))))^{-1}+e_{ij}$$

where: Y_{ij} = the observed value for the j^{th} bottle of the i^{th} maturity stage of corn, i = 1, …,5; V1 and V2 = the volume obtained by NFC and FC fermentation, respectively, for the subclass (early dent) that serves as the class of reference; $dV1_i$ and $dV2_i$ = the deviations of the volume obtained by NFC and FC fermentation for the remaining 4 subclasses from the volume obtained by the NFC and FC fermentation of the reference class, respectively; D1 and D2 = fractional degradation rate of NFC and FC, respectively, for the subclass (early dent) that serves as the class of reference; $dD1_i$ and $dD2_i$ = the deviations of the fractional degradation rate of NFC and FC for the remaining 4 subclasses from the fractional degradation rate of NFC and FC of the reference class, respectively; t = time of fermentation; L = the lag time (h) for the subclass (early dent) that serves as the class of reference; dL_i = the deviations of lag time for the remaining 4 subclasses from the lag time of the reference class; and e_{ij} = the residual errors $\sim N(0, \sigma_e^2)$. This model allows for testing the effect of the maturity stage on the functional parameters using a straightforward t-test and whether the confidence intervals of the $dV1_i$, $dV2_i$, $dD1_i$, dD2 and dL_i overlap zero. Standard residual plots were used to assess the homogeneity of the residual variance (SNEDECOR;COCHRAN, 1989). The residual plots were obtained using the PLOT statement of the GPLOT procedure plotting Studentized residual against predicted values of the dependent variable obtained from the STUDENT statement, and NLIN procedure.

The pH and temperature means they were analyzed over time in a completely randomized de-

sign with repeated measures. The data were analyzed using the MIXED procedure of SAS (v. 9.2, SAS Inst. Inc., Cary). Various error covariance structures (ANTE(1), AR(1), ARH(1), ARMA(1,1), CS, CSH, FA0(1), FA(1), HF, SIMPLE, TOEP, TOEPH, UN and VC) were investigated, and the one that best fit the data according to the Bayesian information criterion (BIC) was selected. The significant level was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

The DM concentration of the corn plants increased 4.7 g kg^{-1} daily, whereas the DM concentration of the silage increased 5.0 g kg^{-1} (Figure 2), which could be a good indicative to management the harvest time. Nevertheless, some care is needed as the climate, such as an absence of rain, can interfere markedly with the DM deposition, affecting mainly the grain filling (ABEYSEKARA et al., 2013). Thus, in our study, the greatest value of DM was observed in the silage that was produced during the BL stage. However, the DM concentration of silages obtained from plants that were harvested at the stages ED and 1/3 ML was below the minimum value recommended (FERRARETTO; SHAVER, 2012), which is approximately 280 g kg^{-1} DM (Table 2).

All pH values of corn silages were within the recommended range (3.8 to 4.2), indicating adequate fermentation (MCDONALD et al., 1991). Silages produced with wetter plants (ED and 1/3 ML) showed higher fermentative losses due to the great effluent production (Table 2). Silages made with wetter plants endure more pressure during compaction, causing the disruption of the cell wall and thereby losing soluble constituents present in the cells, which can greatly reduce the nutritional value of the silage (KHORVASH et al., 2006). The results of this study are in accordance with the data reported by Vilela et al. (2008), which studied the effect of maturity stage at harvest in different corn hybrids (range of DM concentration from 242 to 464 g kg^{-1}) and found increases on effluent production in corn silages with high-moisture.

The silage produced with plants harvested during the 1/2 ML stage had the highest value of CP, although it was not significantly different during the 1/3 ML and BL stages. The aNDF decreased with the advancement of the maturity stage (except the ED stage), but this result did not imply in increase of the coefficients of IVOMD (Table 2).

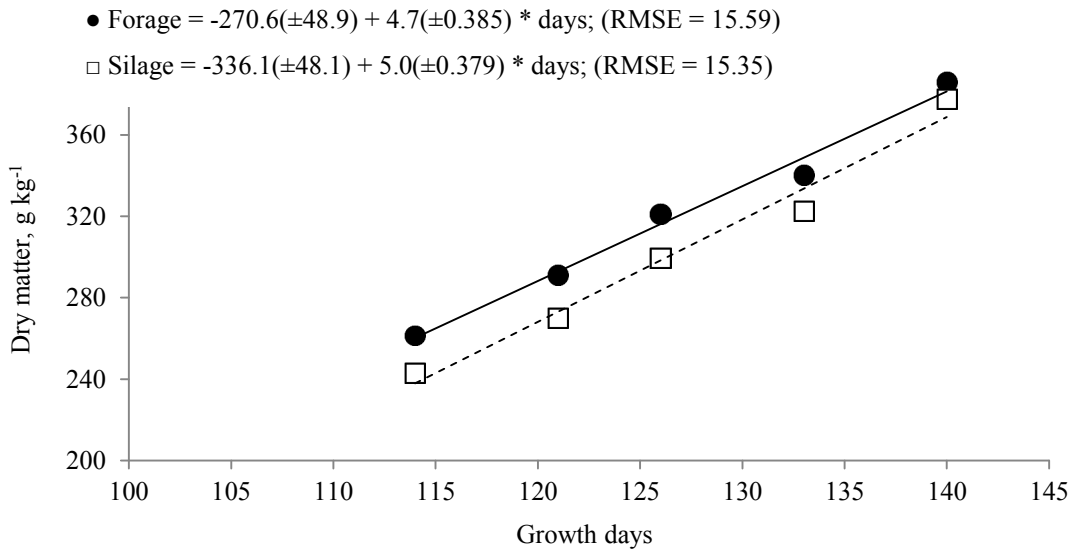

Figure 2. Dry matter content of corn plants and silages according to the maturity.

Usually, with the advancing physiological maturity of the plant, there is a reduction in the protein concentration (VILELA et al., 2008) and fiber due to accumulate of starch in the grains (NADEAU, 2007). However, in our study, these changes did not occur, possibly related to the fact that during the vegetative cycle, the DM accumulation in plants occurs mainly in the stems and leaves, whereas this accumulation during the reproductive phase is translocated to grains, maintaining the constancy throughout the production cycle with respect to the fiber fraction (ZOPOLLATTO et al., 2009). This result explains the absence of a tendency among the maturity stages in this research.

Table 2. Fermentative losses and chemical composition (± standard error of mean) of corn silages produced in different maturity stages.

Item[1]	Maturity stages[2]					P-value
	ED	1/3 ML	1/2 ML	2/3 ML	BL	
pH	3.81^{b}(±0.030)	3.92^{b}(±0.035)	3.92^{b}(±0.030)	4.10^{a}(±0.035)	3.95^{b}(±0.035)	0.0008
Dry matter losses, %	10.80^{a}(±0.77)	7.80^{ab}(±0.77)	6.19^{b}(±0.77)	5.62^{b}(±0.77)	4.81^{b}(±0.77)	0.0005
Gas losses, % DM	0.016^{b}(±0.009)	0.072^{a}(±0.007)	0.037^{b}(±0.008)	0.023^{b}(±0.004)	0.025^{b}(±0.002)	0.0003
Effluent, kg t^{-1}	28.7^{a}(±3.8)	19.8^{ab}(±4.3)	10.0^{bc}(±3.6)	6.4^{bc}(±4.3)	1.7^{c}(±0.5)	<0.0001
Dry matter	242.7^{d}(±5.8)	269.9^{cd}(±5.8)	310.0^{bc}(±6.7)	322.3^{b}(±5.8)	377.4^{a}(±5.8)	<0.0001
Ash	43.6^{ab}(±1.4)	41.4^{ab}(±1.6)	44.0^{ab}(±1.4)	37.2^{b}(±1.4)	45.3^{a}(±1.4)	0.0083
Crude protein	55.5^{b}(±3.1)	61.6^{ab}(±3.1)	69.7^{a}(±3.1)	52.9^{b}(±3.1)	59.2^{ab}(±3.1)	0.0160
Ether extract	28.6(±2.4)	33.9(±2.4)	29.9(±2.4)	29.0(±2.4)	29.0(±2.4)	0.5210
Total carbohydrates	872.2^{ab}(±4.5)	865.1^{ab}(±4.5)	856.2^{b}(±4.5)	880.8^{a}(±4.5)	866.3^{ab}(±4.5)	0.0221
Neutral detergent fiber	526.5^{b}(±5.3)	567.2^{a}(±6.1)	543.0^{ab}(±5.3)	518.8^{b}(±5.3)	539.8^{b}(±5.3)	0.0004
Acid detergent fiber	299.7^{ab}(±6.8)	283.0^{bc}(±6.8)	308.3^{a}(±6.8)	268.3^{c}(±6.8)	309.7^{a}(±6.8)	0.0027
Lignin	56.3^{a}(±1.9)	47.7^{b}(±1.9)	57.7^{a}(±1.9)	52.8^{ab}(±1.9)	47.8^{b}(±2.2)	0.0071
NFC	345.6^{ab}(±7.9)	306.1^{c}(±7.9)	313.2^{bc}(±7.9)	361.9^{a}(±7.9)	326.5^{bc}(±7.9)	0.0010
IVOMD, g g^{-1} of OM	0.584^{ab}(±0.13)	0.631^{a}(±0.13)	0.563^{b}(±0.13)	0.532^{bc}(±0.13)	0.491^{c}(±0.13)	<0.0001

[1]NFC = non-structural carbohydrates; IVOMD = *in vitro* organic matter digestibility (calculated at 24 h). Data expresses in g kg^{-1} of DM.
[2]ED = early dent; ML = milk line; BL = black layer.[a,b,c] Means in the same row with different superscripts differed by Fisher's test.

Although the silage produced during the 1/3 ML stage had a higher aNDF concentration, this same treatment exhibited lower lignin concentration together, at BL stage, besides lower NFC concentration, which resulted in high digestibility (Table 2). Probably, the fact of the 1/3 ML silage has higher aNDF and lower lignin may be related to partial acid hydrolysis of hemicellulose occurred in different intensities between silages, which might have resulted in higher indigestible NDF concentration (mainly lignin) in the silage since hemicellulose is the potentially digestible fraction of forage NDF (MCDONALD et al., 1991). Consequently, the high digestibility for this silage can be explained by the low lignin content of this silage, which interferes markedly with the digestibility of forages (GRABBER, 2005). Furthermore, the grain's starch of high-moisture plants exhibits higher digestibility (JENSEN et al., 2005), although starch digestibility was not measured in this study, and the leaf and stem of corn with high DM concentration exhibit lower digestibility due to enhance in the fiber concentration during growth. This fact could also have contributed to the lower coefficients of the IVOMD reported in the silages produced from plants that were harvested with higher DM concentrations in this study.

The silages produced during the 1/3 ML, 1/2 ML, and BL stages showed higher values of *in vitro* gas production due to the sum of NFC and FC fermentation. Evaluating the results of gas production separately, higher *in vitro* gas production from the NFC was found in the silages produced during the ED, 1/2 ML, and 2/3 ML stages. Likewise, these silages showed the necessity for short-term colonization by microorganisms. In contrast, the silages produced from plants that were harvested during the 2/3 ML, and BL stages showed lower degradability rates (Table 3).

The changes in the potential of gas production possibly can be associated with the ruminal degradation of starch. The differential association between starch, and proteins within each fraction of the endosperm is likely responsible for the variation of starch degradation during the maturity of the corn grain (JENSEN et al., 2005). In the vitreous endosperm (flint), starch granules are surrounded by protein bodies and are embedded in a dense matrix, which limits the action of hydrolytic enzymes. In contrast, in the floury endosperm (dent), starch granules are more accessible to ruminal bacteria due to their inclusion in a discontinuous protein matrix

(CORONA et al., 2006). In our study, the percentage of grains in the ensiled mass ranged from 182 to 235 g kg^{-1}. Thus, the difference in the starch degradation due to maturity stages is an important source contributing to the higher degradability, and gas production of these silages.

These results regarding the degradability rate, and lag time of our study suggest that silages produced with higher moisture content, present better nutritive value compared to silages produced with higher DM content because water soluble carbohydrates are more intensively degraded than starch (LANZAS et al., 2007). Moreover, corn silages produced from plants harvested with higher DM content required more time for the bacterial population colonize the feed.

After opening the silos, we observed higher aerobic stability in the corn silage produced with plants that were harvested during the 2/3 ML stage, whereas the 1/3 ML stage promoted lower aerobic stability (Table 4). Moreover, during aerobic exposure, the ED, and 2/3 ML stages showed the lowest average temperature during the 7 days of evaluation, and they took more time to reach the maximum temperature. The highest temperatures were found during the 1/3 ML and BL stages, whereas the highest rate of warming was in the silage produced during the 1/3 ML stage. The lower aerobic stability of silages produced during the 1/3 ML stage might be associated with a high concentration of nutrient. This result infers that silages with high nutrient concentrations show more rapid deterioration in the silos post-opening due to the greater activity of yeasts, and mold, which develop through the use of residual carbohydrates and lactic acid as substrates, causing the spoilage of mass and decreasing the nutritive value (WILKINSON; DAVIES, 2012).

Due to the advancement in the maturity stage of corn plants, the reduction in the soluble carbohydrates levels can decrease organic acid production. But, although the lactic acid also decreases, the relationship between the concentration of this acid, and other organic acids is increased (WARD, 2000), and the lactic acid does not control the yeast, and mold development after the silo opening (MOON, 1983).

Table 3. Potential gas production, degradability rate, and time of colonization (*lag time*) (± standard error of the mean) of corn silages produced during different maturity stages.

Item[1]	Maturity stages[2]					P-value
	ED	1/3 ML	1/2 ML		2/3 ML	BL
Gas production, mL g^{-1} of OM						
NFC	113.6^{b}(±13.3)	717.4^{a}(±58.9)	175.0^{a}(±13.1)	119.3^{b}(±5.8)	179.3^{a}(±20.3)	<0.0001
FC	162.5^{a}(±13.2)	121.7^{b}(±9.3)	127.0^{ab}(±16.2)	157.1^{ab}(±7.2)	117.9^{b}(±10.3)	<0.0001
Degradability rate, % $hour^{-1}$						
NFC	1.5^{a}(±0.3)	0.4^{c}(±0.02)	1.6^{a}(±0.2)	1.5^{b}(±0.6)	1.3^{b}(±0.2)	<0.0001
FC	5.0^{a}(±0.6)	7.6^{a}(±1.9)	5.6^{a}(±0.9)	6.3^{a}(±0.1)	5.6^{a}(±0.9)	<0.0001
Lag time, hour						
Feed	1.59^{b}(±0.74)	8.64^{a}(±1.40)	2.93^{b}(±0.81)	3.84^{b}(±0.44)	4.24^{b}(±0.89)	<0.0001

[1]NFC = non-fiber carbohydrates; FC = fibrous carbohydrates.[2]ED = early dent; ML = milk line; BL = black layer.[a,b,c]Means in the same row with different superscripts differed by the t-test, and the confidence intervals of the $dV1_i$, $dV2_i$, $dD1_i$, $dD2$ and dL_idid not overlap with zero.

Moreover, drier silages typically present higher pH because, the lactic: acetic ratio is even higher in drier silages compared with moisture silages (WARD, 2000). Thus, although our study has no data about the organic acid profile, the highest pH occurring in the silage produced during the 2/3 ML stage could indicate that the fermentation during the ensiling process has a higher acetic acid production because the pK_a values of acetic and propionic acids are similar (4.73 and 4.87), whereas the pK_a of lactate is 3.86 (MOON, 1983), since the BL stage showed lower pH, indicating probably low production of acetic acid. As known, acetic and propionic acids have antifungal properties (MOON, 1983), which could be associated with the highest aerobic stability being found in our study.

Temperature peaks were observed in all silages during the aerobic exposure, mainly in the silage produced from plants harvested during the 1/3 ML stage (Figure 3). However, all silages (except for

those produced during the 1/3 ML stage) showed temperature peaks after 45 hour of aerobic exposure, and the maximum temperatures were lower compared to those produced during the 1/3 ML stage (Table 4).

Table 4. Aerobic stability and characteristics of corn silages produced during different stages of maturity during aerobic exposure (± standard error of the mean).

Item[1]	Maturity stages[2]					P-value
	ED	1/3 ML	1/2 ML	2/3 ML	BL	
Aerobic stability, h	80^{ab}(±6.45)	32^{c}(±6.45)	56^{bc}(±6.45)	104^{a}(±6.45)	52^{bc}(±6.45)	<0.0001
Temperature, ºC						
Maximum	25.75^{b}(±0.64)	30.25^{a}(±0.64)	26.75^{b}(±0.64)	26.00^{b}(±0.64)	27.50^{ab}(±0.64)	0.0013
H. to max. value	138^{a}(±17.10)	42^{b}(±17.10)	120^{a}(±17.10)	138^{a}(±17.10)	72^{ab}(±17.10)	0.0034
Heating rate	0.046^{c}(±0.002)	0.248^{a}(±0.033)	0.064^{bc}(±0.014)	0.048^{bc}(±0.004)	0.132^{b}(±0.021)	<0.0001
pH						
Maximum	7.22^{c}(±0.17)	8.44^{ab}(±0.17)	7.45^{c}(±0.17)	7.68^{bc}(±0.17)	8.72^{a}(±0.17)	<0.0001
H. to max. value	160^{a}(±7.28)	108^{b}(±7.28)	160^{a}(±7.28)	166^{a}(±7.28)	128^{b}(±7.28)	<0.0001

[1] H. to max.value= hours necessary to achieve the maximum temperature and pH value; Heating rate = ºC hour^{-1}. [2]ED = early dent; ML = milk line; BL = black layer. ^{a,b,c} Means in the same row with different superscripts differed by the Fisher's test.

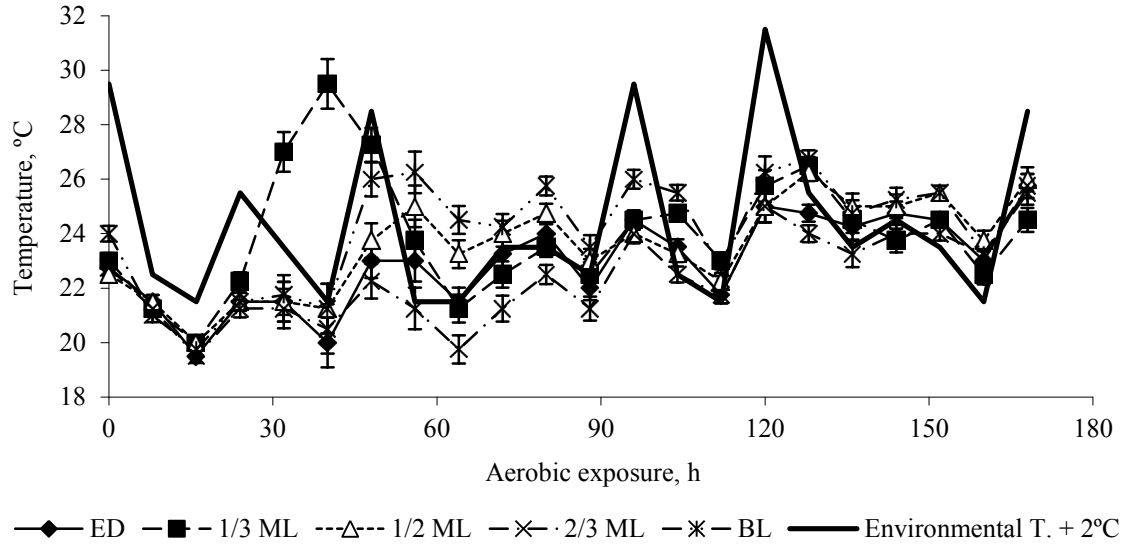

Figure 3. Temperature change of corn silages produced during different stages of maturity during the aerobic exposure (effect of maturity = P<0.01; aerobic exposure days = P<0.01; interaction between the factors = P<0.01).

As Known, the action of spoiling microorganisms after silo opening is potentiated under tropical conditions, and it is impossible to control the environmental temperature (KIM; ADESOGAN, 2006). Moreover, the increase in the temperature during the period of aerobic exposure occurs as a function of the increase in the amount of oxidative yeast, causing an increase in the balance between the rate of heat produced by microbial activity and heat loss, which is directly related to the oxidation of DM and causes losses in the form of carbon dioxide (CO_2), resulting in a decrease in the nutritive value of the feed (WILKINSON; DAVIES, 2012). As result, the pH increase (Figure 4) favoring the growth of spoilage microorganisms besides yeast (WILKINSON; DAVIES, 2012). Therefore, under high temperatures, the silage produced with plants harvested in the 2/3 ML stage contributed to an increase in the

silage quality persistence after exposure to air.

The pH values remained stable for 30 hour after the silos opened (Figure 4). However, after this time, the silages showed pH peaks, mainly in the silage produced from plants that were harvested during the 1/3 ML stage.

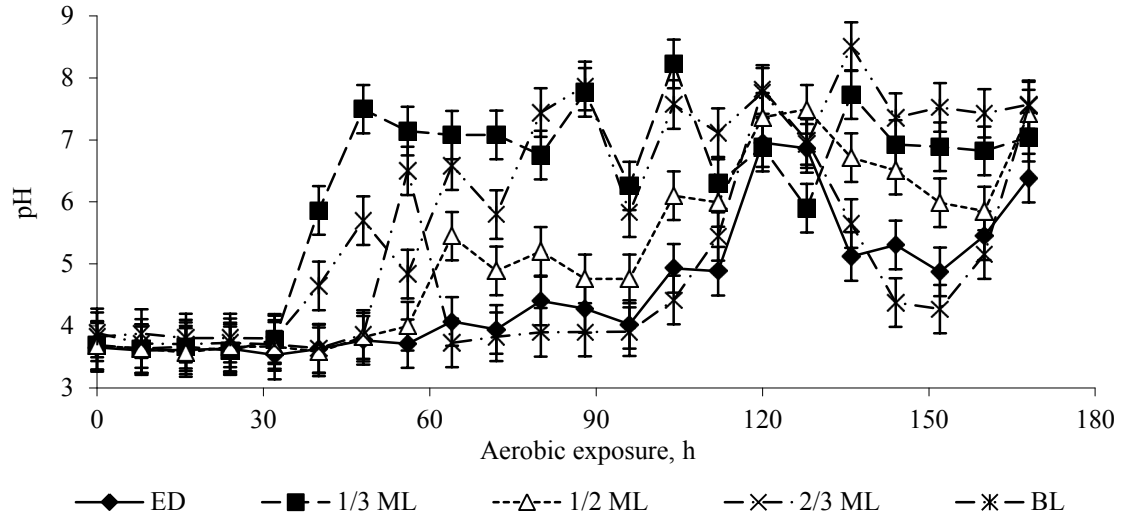

Figure 4. Change in the pH values of corn silages produced during different stages of maturity during exposure aerobic (effect of maturity = P<0.01; aerobic exposure days = P<0.01; interaction between the factors = P<0.01).

It is important to say that even with greater stability in the silage produced during the 2/3 ML stage, the management of silos on farms should be adequate for maximum efficiency. In this case, the silage must be rapidly removed from the silos due the great reduction in the nutritive value during aerobic exposure under tropical conditions. Therefore, the silage produced during the 1/3 ML stage can also be used on farms, since quickly removed.

CONCLUSIONS

The 1/3 and 2/3 ML stages of maturity seem to be the best maturity stages for the production of corn silage from the hybrid corn BM3061 in tropical climates.

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