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SCIENTIFIC NOTE

Overtime losses of nutritional and fermentation traits in re-ensiled maize silage

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ABSTRACT

Re-ensiled maize (*Zea mays* L.) silage is an important ready-to-use product for feeding ruminants in urgent circumstances at dairy or beef farms. However, in recent decades, there has been little investigation into the possible re-ensiled silage losses caused by the storage time. The objective of this study was to evaluate losses in chemical, fermentation and digestibility parameters of whole-plant maize silage re-ensiled in proper plastic bags over time. Treatments consisted of six silage storage periods in the reallocation silos (30, 60, 90, 120, 150 and 180 d). Contents of DM, starch, non-fiber carbohydrates, and total digestible nutrients decreased linearly over time ($P < 0.001$). In contrast, consistent gains in crude protein, ether extract, neutral detergent fiber (NDF), lignin, ash, and Ca concentrations were observed as the storage time increased ($P < 0.001$). Concentrations of digestible NDF after 240 h and digestible *in situ* starch decreased as the storage time advanced ($P < 0.001$). The lactic/acetic acid ratio decreased from day 73rd onwards ($P = 0.002$) while fermentation losses increased from day 54th onwards ($P = 0.001$). The nutritional value and fermentation quality of re-ensiled maize silage decrease over time. However, it is possible to open reallocation silos from 30 to 150 d after the re-ensiling process to obtain a proper-parameter maize silage.

Key words: Ensilage, lactic acid, ruminant feeding, silage reallocation, *Zea mays*.

INTRODUCTION

Commercializing re-ensiled maize (*Zea mays* L.) silage is of great economic importance in developing countries like Brazil, with several companies engaged in producing and developing specific machinery for this purpose (compaction and packing). There is no official economic data on re-ensiled silages but there is a large supply of this ready-to-use product for feeding ruminants. The silage transfer among farms has occurred due to reduced crop yields and climatic variations during the rainy season (Anjos et al., 2018), limited forage allowance and excessive forage losses, low machinery availability, and inadequate topography (Marafon et al., 2015).

Air exposure during the re-ensiling process has been focused in scientific investigations (Faria et al., 2020; Tian et al., 2022) since it leads to silage deterioration (Kung Jr. et al., 2021). In these researches, the storage time in reallocation silos varied such as in livestock farms. However, possible losses in the re-ensiled silage over time has been little investigated.

Silage reallocation is often performed using plastic bags 200 µm thick with 30 to 40-kg capacity (Santos et al., 2023). However, plastic film traits affected corn silage's pH, lactic and acetic acid concentrations, and lactic/acetic acid ratio (Borreani et al., 2014). Good-quality silages are rich in sugars, free of butyric acid, and have moderate-to-low acetic acid concentrations. Conversely, various nutrients, mainly the soluble carbohydrates, deteriorate as soon as oxygen meets the material (Wilkinson and Davies, 2013).

In countries reallocating silages, the silages must be unpacked and transported to other locations, where it will be compacted and sealed again (Anjos et al., 2018). We hypothesized that the nutritional value and fermentation traits of whole-plant maize silage can be preserved even with unavoidable losses caused by the reallocation process. This study aimed to evaluate possible changes in chemical, fermentation, and digestibility parameters of whole-plant maize silage re-ensiled in plastic bags over time to simulate what happens in the field.

MATERIALS AND METHODS

Study site and maize cropping

The trial was conducted in Januária (15°29'17" S, 44°21'43" W, 554 m a.s.l.), Minas Gerais, Brazil. The site's climate is Aw according to the Köppen classification (Alvares et al., 2013), with an annual average temperature of 24.5 °C. The hybrid corn (*Zea mays* L.) was sown in a 2.5 ha irrigated area, with a density of 66 000 plants ha^{-1}. The planting fertilization was performed using 55 and 50 kg ha^{-1} P_2O_5 and K_2O, respectively, while the topdressing fertilization was performed using 165, 70 and 8 kg ha^{-1} N, K_2O, and $MgSO_4$, respectively.

Whole-plant maize ensilage

Whole-plant maize was harvested for ensiling when the corn kernel milk line had between 1/3 and 2/3 already filled with starch (from dough to dent stage). Whole plant was chopped into 1 or 2 cm particles using a forage machine, transported, compacted and stored in a surface silo. During ensiling, biological inoculant composed of homofermentative bacteria *Lactobacillus plantarum* and *Propionibacterium acidipropionici* was applied according to the manufacturer's recommendation (SILOBAC, Ourofino PLC, São Paulo, Brazil).

The surface silo was opened 60 d after sealing, from which random samples were taken and stored in plastic bags. The silage fits fermentative and chemical traits of a high-quality product according to Kung Jr. et al. (2018). Laboratory analyses were conducted at the Food Analysis and Animal Nutrition Laboratory of the Department of Agricultural Sciences at Universidade Estadual de Montes Claros (Unimontes). Silages were analyzed for concentrations of DM (analysis method 934.01), crude protein (CP; analysis method 954.01), ether extract (EE; analysis method 920.39), ash (analysis method 942.05), and lignin (analysis method 973.18) following the recommendations of AOAC (Horwitz, 2005). Concentrations of NDF and ADF were determined using methods of van Soest et al. (1991). The NDF and ADF were analyzed without a heat-stable amylase and expressed including residual ash. Non-fiber carbohydrate (NFC) content was calculated using the formula proposed by Sniffen et al. (1992):

$$NFC = 1000 - (NDF + CP + ash + EE)$$

where NFC, CP, ash, and EE were the concentrations of non-fiber carbohydrates, crude protein, ash and ether extract, respectively.

Total digestible nutrient (TDN) concentration was determined according to NRC (2001). Concentrations of Ca, P, starch, digestible NDF after 48 h (dNDF 48h), digestible NDF after 240 h (dNDF 240h), digestible starch (dStarch), besides pH, lactic acid, acetic acid and fermentation losses were analyzed using a near-infrared spectroscopy (NIRS DS2500 Analyzer, FOSS Analytical, Hillerød, Denmark).

The average chemical composition was DM = 338 g kg^{-1}; CP = 80 g kg^{-1}; EE = 27.1 g kg^{-1}; ash = 41.4 g kg^{-1}; Ca = 2.1 g kg^{-1}; P =1.7 g kg^{-1}; starch = 291.5 g kg^{-1}; NDF = 424.8 g kg^{-1}; ADF = 261.4 g kg^{-1}; lignin = 45.0 g kg^{-1}; NFC = 436.8 g kg^{-1}; TDN = 719.2 g kg^{-1}; dNDF 48h = 0.50 g g^{-1}; dNDF 240h = 0.66 g g^{-1}; dStarch = 0.91 g g^{-1}; pH = 3.87; lactic acid = 4.65 mol 100 mol^{-1}; acetic acid = 1.17 mol 100 mol^{-1}; fermentation losses = 2.30 g 100 g^{-1}.

Re-ensiling process and experimental design

After opening the silo, 1 m layer of silage was discarded on each side. Then, the silage was uncompressed and re-ensiled. Silage was re-ensiled in recyclable polyethylene plastic bags, black color, without anti-UV additive, no side weld, measuring 1.10 × 0.51 m, with 200 µm thickness and 40 kg capacity. A silage compaction-and-packing machine was used to standard 30 kg silage bags (experimental units) sealed with nylon plastic cable ties; thus, the silage density was 50 kg m^{-3}. Experimental treatments consisted of six storage periods of re-ensiled silos (30, 60, 90, 120, 150, and 180 d) with 10 replicates (plastic bags) in a completely randomized design.

Chemical analyses, fermentation profile and digestibility

After each storage period, silos (plastic bags) were opened and the silage at the ends was discarded and the rest of the material was homogenized and sampled for chemical analysis, fermentation profile and digestibility. The material was frozen at -20 °C. The samples were analyzed for concentrations of DM (analysis method 934.01), CP (analysis method 954.01), EE (analysis method 920.39), ash (analysis method 942.05), and lignin (analysis method 973.18) following the recommendations of AOAC (Horwitz, 2005). Concentrations of NDF and ADF were determined using methods of van Soest et al. (1991). The NDF and ADF were analyzed without a heat-stable amylase and expressed including residual ash.

The NFC was calculated according to the method of Sniffen et al. (1992). The TDN concentration was determined according to NRC (2001). Concentrations of Ca, P, starch, dNDF 48h, dNDF 240h, dStarch, besides pH, lactic acid, acetic acid and fermentation losses were analyzed using a near-infrared spectroscopy (NIRS DS2500 Analyzer, FOSS Analytical, Hillerød, Denmark).

Statistical analysis

Data were subjected to a normality residual test (Shapiro-wilk, $P \geq 0.05$), ANOVA and orthogonal polynomial contrasts (linear and quadratic) using a general linear model procedure of the R-Studio software (RStudio, Boston, Massachusetts, USA) to analyze the storage time effect on dependent variables of the re-ensiled maize silages. The probability of error was 5% for both tests ($P < 0.05$). The statistical model was:

$$y = \beta_0 + \beta_1 x + \beta_2 x^2$$

where y is the dependent variable, β_0 is the intercept value when the independent variable is zero (day 0). The x is the independent variable (storage time) while β_1 and β_2, are the linear and quadratic effects.

RESULTS

The storage time affected almost all the chemical composition variables of re-ensiled maize silage, except for the P content, which was 1.8 g kg^{-1} (Table 1). The DM content decreased linearly from 269.1 to 241.4 g kg^{-1} as the storage period increased, and the starch content diminished from 193.5 to 111.5 g kg^{-1}. The NFC and TDN concentrations also reduced linearly as the storage period increased.

Table 1. Chemical composition and digestibility of re-ensiled corn silage subjected to different storage times. DM: Dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non-fiber carbohydrates; TDN: total digestible nutrients; dNDF 48h: concentration of digestible neutral detergent fiber after 48 h rumen incubation; dNDF 240h: concentration of digestible neutral detergent fiber after 240 h rumen incubation; dStarch: concentration of digestible starch; SEM: standard error of the mean. Orthogonal contrasts were significant at 5% of probability error ($P < 0.05$).

Variable	Storage time (d)						SEM	Contrast, *P*-values	
(g kg^{-1})	30	60	90	120	150	180		Linear	Quadratic
DM	269.1	260.5	248.2	262.7	256.4	241.4	2.04	< 0.001	0.850
CP	89.5	91.8	93.6	92.5	96.0	100.9	0.68	< 0.001	0.112
EE	30.1	31.2	32.4	31.6	33.2	36.4	0.41	< 0.001	0.127
Ash	52.7	55.1	56.6	58.6	57.4	62.4	0.62	< 0.001	0.835
Ca	2.4	2.5	2.6	2.6	2.7	2.7	0.02	< 0.001	0.602
P	1.8	1.8	1.8	1.8	1.9	1.8	0.01	0.195	0.194
Starch	193.5	155.9	123.7	148.5	119.9	111.5	6.08	< 0.001	0.186
NDF	507.3	530.3	542.0	534.5	556.2	557.4	4.37	< 0.001	0.449
ADF	329.5	345.1	353.2	351.1	367.5	371.0	3.16	< 0.001	0.658
Lignin	61.0	64.1	62.7	66.9	68.4	69.9	0.76	< 0.001	0.787
NFC	331.7	303.6	287.5	295.0	269.9	256.5	5.22	< 0.001	0.712
TDN	659.4	642.7	653.6	653.0	621.4	627.8	3.02	< 0.001	0.382
dNDF 48h	490.0	500.3	489.8	490.1	490.2	489.8	20.07	0.380	0.796
dNDF 240h	600.1	600.4	610.0	580.2	600.1	579.8	30.10	< 0.001	0.268
dStarch	890.3	879.8	880.3	899.7	859.8	850.1	40.03	< 0.001	0.280

Conversely, the CP concentration increased linearly from 89.5 to 100.9 g kg^{-1} over reallocation time, and the lignin content increased from 61.0 to 69.0 g kg^{-1}. Concentrations of EE, NDF, ADF, ash, and Ca also increased as the storage time advanced.

Concentration of dNDF after 240 h decreased linearly from 600.1 to 579.8 g kg^{-1} with the storage time, and the digestible starch content depressed from 890.3 to 850.1 g kg^{-1} overtime (Table 1). The dNDF after 48 h was not affected by the silage reallocation management. Values of pH increased from the 90th day (3.95) of re-ensiling and reached its highest point on the 180th day (4.08) (Figure 1). Acid lactic displayed the top concentration on the 92nd day (4.6 mol 100 mol^{-1}), while the acetic one increased linearly, from 3.2 to 4.3 mol 100 mol^{-1}. Lactic/acetic acid ratio decreased from the 73rd day onwards (from 1.4 to 0.8), while the fermentation losses increased from the 54th day onwards (from 5.9 to 8.5 g 100 g^{-1}).

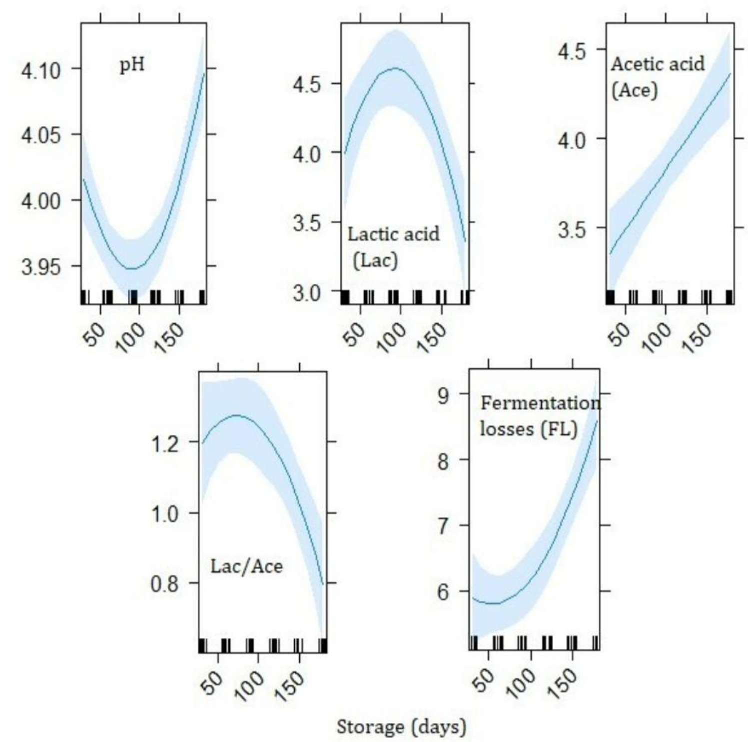

Figure 1. Fermentation parameters of re-ensiled corn silage subjected to different storage times. Orthogonal contrasts were significant at 5% of probability error ($P < 0.05$). Lactic (Lac) and acetic (Ace) acids were measured in mol 100 mol^{-1}. Fermentation losses were measured in g 100 g^{-1}.

DISCUSSION

Maintaining quality characteristics during storage, reducing the loss of DM and energy to a minimum are the key objectives of a forage conservation system (Borreani et al., 2018). The DM content losses, observed in this study, is indicative of biological and chemical processes by microorganisms that consume nutrients and energy for produce water, carbon dioxide, heat and free ammonia (Costa et al., 2022). The growth of fungi, yeast, and bacteria during the aerobic deterioration is one of the main factors influencing silage quality. The main substrates used by these microorganisms are lactic acid, ethanol, and soluble sugars, resulting in decreased digestibility and energy content of silages (Kung Jr. et al., 2018).

Critical DM losses in the re-ensiled maize silage quality occurred after 150 d. The DM contents inferior to 250 g kg^{-1} indicate high *Clostridium* sp. activity in performing proteolysis (Marte-Pereira et al., 2025). In this sense, the maize silages were subjected to consecutive DM losses until achieving 241 g kg^{-1}, an undesirable value for a proper anaerobic fermentation.

The results suggest that the predominant bacterial group changed over time, from homofermentative to heterofermentative, as the acetic acid production increased significantly (Obinwanne et al., 2023). Even with the greater acidification power of lactic acid compared to acetic one (pKa = 3.86 *vs*. 4.74) (Narendranath et al., 2001), pH-values for all storage times did not show patterns outside what is recommended for silages (3.8-4.2) (Kung Jr. et al., 2018), but fermentation losses increased significantly.

The increase in crude protein (CP) content occurred because of the reduction in non-fiber carbohydrates (NFC) and growth of microorganisms resulted in a higher concentration of N compounds (Pinheiro et al., 2020). Fiber components such as neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin increased because the NFC diminished over time, so we could observe increments in the plant cell wall structural components that reduces the re-ensiled material digestibility. Coelho et al. (2018) also observed increase in cellular wall (NDF) and CP in reallocated corn silages.

Fiber components are negatively correlated with the roughage nutritional value. The NDF content is related to reduced intake, while ADF and lignin are related to reduced digestibility (Daniel et al., 2019). As NFC are highly digestible organic compounds, their concentration reduction may result in poor silage digestibility (McDonald et al., 1991). The total digestible nutrients (TDN) contents in the re-ensiled material decreased because of DM losses, high intake of starch and NFC by bacteria, and increments in NDF, ADF, and lignin contents.

Concentrations of ether extract (EE), ash and Ca in the re-ensiled material increased over storage time, a pattern not observed for P. Such increases likely suggest reductions in the organic matter because of the soluble carbohydrate consumption by microorganisms during the storage (Borreani et al., 2018).

Re-ensiled material lost quality more intensely from 54^{th} day onwards likely because of four main reasons: 1) Low packing density, which resulted in greater air flow in the re-ensiled material (Kung Jr. et al., 2021); 2) air permeability of the plastic bag, even with the same technical specification as the tarpaulins used to make the conventional silos (Borreani et al., 2018; Neumann et al., 2021); 3) greater contact surface of the reagent substances in the silos with the air, due to their smaller volumes, which results in a higher speed of chemical reactions and, consequently, more losses over time; and 4) the high environmental temperature of the region where the study was carried out.

A significant decline in starch digestibility was observed from 150 to 180 d, as we could observe for DM contents during this period. Such reduction can be explained by the dense protein matrix surrounding starch granules formed after so long storage (Silva et al., 2025). In this sense, it is possible to open reallocation silos from 30 to 150 d after re-ensiling maize silage, while still maintaining proper parameters for fermentation and digestibility.

CONCLUSIONS

The nutritional value and fermentation quality of re-ensiled maize silage decrease over time. However, it is possible to open reallocation silos from 30 to 150 d after re-ensiling maize silage, while still maintaining proper parameters for fermentation and digestibility.

Author contributions

Conceptualization: R.M.M. Methodology: R.M.M., T.L.M., D.S.G. Formal analysis: R.M.M., T.L.M., F.V.S., D.S.G., C.M.O., J.P.S.R., P.H.F.S., V.L.M. Investigation: R.M.M., T.L.M., D.S.G., C.M.O., V.L.M. Resources: R.M.M., T.L.M., D.S.G. Writing-original draft: R.M.M., T.L.M., F.V.S., D.S.G. Writing-review & editing: R.M.M., T.L.M., F.V.S., D.S.G., C.M.O., J.P.S.R., P.H.F.S., V.L.M. Visualization: R.M.M., T.L.M., F.V.S., D.S.G., C.M.O., J.P.S.R., P.H.F.S., V.L.M. Supervision: R.M.M., T.L.M., D.S.G. Project administration: R.M.M., T.L.M., D.S.G. Funding acquisition: R.M.M., T.L.M., D.S.G. All co-authors reviewed the final version and approved the manuscript before submission.

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