RE-ENSILING AND MICROBIAL INOCULANT USE EFFECTS ON THE QUALITY OF MAIZE SILAGES EXPOSED TO AIR

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ABSTRACT – Re-ensiling has increased worldwide due to the increase in marketing of silage between farms. However, litlle is known about the aerobic deterioration and microbial inoculants effect in this process. The aim of this study was to determine the re-ensiling and microbial inoculant use effects on the maize silage quality. Experimental treatments included maize silage exposed to air for zero or 18 h, with or without microbial inoculant use containing a mixture of Lactobacillus plantarum and Propionibacterium acidipropionici. The forage was ensiled in 20 plastic buckets (20 liters capacity) with five repetitions per treatment. The silos were opened after 116 days and the chemical composition, in vitro dry matter digestibility (IVDMD), fermentative parameters, microorganism counts, total dry matter (DM) losses and aerobic stability of silage were evaluated. Re-ensiling reduced the non-fibrous carbohydrates content by 13.0%. The IVDMD was reduced by 6.1% in re-ensiled silages. In addition, re-ensiling increased the losses due to gases, effluents, and total DM. Lower lactic acid content and higher acetic acid content in re-ensiled maize silages were also observed. However, IVDMD increased by 4.96% in inoculated silages. Re-ensiling reduces the nutritive value and IVDMD of maize silages and should be avoided in production systems. The microbial inoculant use is not effective in improving the re-ensiled silage quality, which does not justify its use in these conditions. Farms should avoid purchasing maize silage due to reduction in nutritional value in the re-ensiling process. However, in some cases where farms need to purchase silage for feeding planning, the time between silo opening and re-ensiling should be as short as possible to reduce nutrient loss. Furthermore, in this situation the inoculant use does not improve silage conservation, which makes its use unnecessary.

Keywords: aerobic deterioration, *Lactobacillus plantarum*, *Propionibacterium acidipropionici,* silage relocation

EFEITOS DA UTILIZAÇÃO DE REENSILAGEM E INOCULANTE MICROBIANO SOBRE A QUALIDADE DE SILAGENS DE MILHO EXPOSTAS AO AR

RESUMO – A reensilagem tem aumentado em todo o mundo devido ao aumento da comercialização de silagem entre fazendas. Porém, pouco se sabe sobre os efeitos da deterioração aeróbia e de inoculantes microbianos nesse processo. O objetivo deste estudo foi determinar os efeitos do uso da reensilagem e de inoculante microbiano sobre a qualidade de silagens de milho. Os tratamentos experimentais incluíram silagem de milho exposta ao ar por zero ou 18 h, com ou sem a utilização de inoculante microbiano contendo uma mistura de *Lactobacillus plantarum* e *Propionibacterium acidipropionici*. A forragem foi ensilada em 20 baldes plásticos (capacidade de 20 litros) com cinco repetições por tratamento. Os silos foram abertos após 116 dias e avaliados a composição química, digestibilidade *in vitro* da matéria seca (DIVMS), parâmetros fermentativos, contagem de microrganismos, perdas de matéria seca (MS) total e estabilidade aeróbia da

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silagem. A reensilagem reduziu o teor de carboidratos não fibrosos em 14,8%, enquanto aumentou o teor de fibra em detergente neutro em 6%. A DIVMS foi reduzida em 6,1% nas silagens reensiladas. Além disso, a reensilagem aumentou as perdas por gases, efluentes e MS total. Menor teor de ácido lático e maior teor de ácido acético nas silagens de milho reensiladas também foram observados. No entanto, a DIVMS aumentou 4,6% nas silagens inoculadas. A reensilagem reduziu o valor nutritivo e a DIVMS das silagens de milho e deve ser evitada nos sistemas de produção. O uso de inoculante microbiano não foi eficaz na melhoria da qualidade da silagem reensilada, o que não justifica seu uso nessas condições. As fazendas devem evitar a compra de silagem de milho devido à redução do valor nutricional no processo de reensilagem. No entanto, em alguns casos em que as fazendas precisam adquirir silagem para o planejamento da alimentação, o tempo entre a abertura do silo e a reensilagem deve ser o menor possível para reduzir a perda de nutrientes. Além disso, nesta situação o uso de inoculante não melhora a conservação da silagem, o que torna seu uso desnecessário.

Palavras-chave: deterioração aeróbia, manejo da fazenda, *Lactobacillus plantarum*, *Propionibacterium acidipropionici*, realocação de silagem.

INTRODUCTION

Silage is the main forage source used in dairy cattle diets (Weinberg et al., 2004). In Brazil, maize silage is the primary source of energy and fiber in dairy cattle diets (Bernardes and Rêgo, 2014). Thus, losses from fermentation and aerobic silage exposure may compromise the dietary balance and animal performance.

The conservation of fresh forage as silage occurs under anaerobic conditions via a lactic-acid dominated fermentation. Lactic acid bacteria convert soluble carbohydrates into organic acids, mainly lactic acid, thus reducing the silage pH. As a result, the material is protected from the activity of aerobic microorganisms such as molds and yeasts, which are the primary cause of aerobic deterioration that results in reduced fermentation quality and nutritional value of silage (Pahlow, 2003; Michel et al., 2017).

Some factors including silage commercialization between farms have contributed to increase in aerobic exposure of silage prior to the feed-out phase. This practice has increased in Brazil and other countries of the world (Chen and Weinberg, 2014) due to the climatic variability and the low availability of machinery and specialized workforce for silage production (Lima et al., 2017). Because of increasing demand, some farmers have started specializing in silage production for marketing purposes or resale. Buying and selling silage involves re-ensiling, which is a process in which the material is opened, transported and re-ensiled at the destination. During this operation, the silage is inevitably exposed to air allowing the proliferation of deteriorating microorganisms (Chen and Weinberg, 2014). In Brazil, these re-ensiling stages have in most cases required one day to be carried out. Maize silage is the most commercialized forage between farms because it is the most widely-grown crop for whole-plant silage.

Re-ensiling may allow the growth of molds and yeasts that reduce silage quality. Some microbial inoculants have been used to reduce possible nutritional and dry matter (DM) losses due to re-ensiling. Studies on the effect of re-ensiling and microbial inoculant use are recent (Chen and Weinberg, 2014; Michel et al., 2017; Dos Anjos et al., 2018; Santos et al., 2021). More information is needed regarding the re-ensiling process and microbial inoculants use containing *Lactobacillus plantarum* and *Propionibacterium acidipropionici* on the maize silage quality. The current study aimed to determine the effects of re-ensiling and the use of a mixture of facultative heterofermentative lactic acid bacteria *Lactobacillus plantarum* and the propionic bacteria *Propionibacterium acidipropionici* on the maize silage quality.

MATERIALS AND METHODS

Planting, harvesting, and ensiling

The BRS 1055 maize crop was planted in November 2013 in the experimental area of Embrapa Maize and Sorghum, located in Sete Lagoas, Minas Gerais, Brazil (19°28′S, 44°15′W, altitude 732 m). Maize was planted in one hectare (10000 m^2), subdivided into five blocks to provide samples representing a range of moisture and soil fertility. The space between rows was 70 cm and there was 20 cm between plants in the same row. At planting, fertilizer was applied at 400 kg/ha of 8-28-16 (N:P:K formulation) + 0.5% Zinc, according to soil analysis and crop need. Subsequently, top-dressed nitrogen (N) application (100 kg N/ha) was made 30 d after planting. The crop was harvested at one-third milk line maturity stage (298 g/kg DM) and chopped into lengths of between 1 and 2 cm, using conventional forage harvester (JF C120 AT; JF Agricultural Machines, Itapira, Brazil).

The chopped forage was sampled (800 g/block) and analyzed before being inoculated. The material harvested in each block was separated into four parts and randomly allocated to each treatment. Two parts was weighed and inoculated with the inoculum composed of the facultative heterofermentative lactic acid bacteria *Lactobacillus plantarum* MA18/5U and the propionic bacteria *Propionibacterium acidipropionici* MA26/4U at 2.5 x 10 Colony Forming Units per gram of product (CFU/g) for each microorganism (Biomax Maize, Lallemand, Saint-Simon, France). Inoculation was performed at ensiling, and the inoculum was diluted in distilled water at room temperature in the ratio of 1 g/L. The mixture of distilled water and inoculant was uniformly sprayed onto the forage with a backpack sprayer in the ratio of 200 mL per 100 kg of fresh matter (FM), according to the manufacturer's recommendation. The other two parts of the FM was weighed, and distilled water was added at a rate identical to that of the inoculated silage (200 mL of distilled water for 100 kg of FM).

A total of 10.0 ± 0.1 kg of chopped forage was placed in each silo, and the forage was then manually compacted into experimental mini-silos at a density of 500 kg/m^3. For each block, one silo was made for each of the four treatments, giving a total of 20 experimental silos. Each silo consisted a 20 liters plastic bucket equipped with a fitted lid Bunsen valve to allow only the gas to exit. A cotton bag was placed inside each bucket with approximately 2 kg of dry sand to allow the measurement of the effluents produced.

Aerobic exposure

Re-ensiling was performed 56 d after ensiling. The silos previously defined randomly were opened, and the material was removed and then re-ensiled after 18 h of air exposure. The non-exposed silo remained closed. This procedure was carried out in a shed, starting at 2 p.m. on 7 November 2014. During the aerobic exposure period, the temperature ranged from 23.5 to 29.6 °C, whereas relative humidity varied from 39 to 67%, according to data obtained from the automatic weather station of the Brazilian National Institute of Meteorology (116 - INMET), located 2.4 km from the area.

After 60 d of re-ensiling, all silos were opened. Samples were taken for analysis to determine chemical composition, *in vitro* digestibility, fermentative parameters (pH, ammonia nitrogen (NH_3-N) and lactic, acetic, propionic, and butyric acids), silage losses (gases, effluents, and total DM losses), aerobic stability, and total count of molds, yeasts, and bacteria.

Experimental design

The re-ensiling process after 18 h of air exposure and the use of a microbial inoculant during ensiling were evaluated. The treatments were arranged in a 2 × 2 factorial scheme, with 5 repetitions each (blocks). The first variable examined was the re-ensiling process (with or without), and the second variable investigated was the microbial inoculant use (used or not used). A randomized block design with five repetitions was used.

Chemical analysis and in vitro dry matter digestibility

Fresh forage samples were dried in a forced-ventilation oven at 55 °C for 72 h and then processed in a knife mill (sieve size = 1 mm; Thomas Wiley model 4, Thomas Scientific, Swedesboro, NJ). Dry matter at 105 ºC, ash, crude protein (CP) and ether extract (EE) contents were determined according to Cunniff (1995). Cell-wall components [neutral detergent-insoluble fiber (NDF), acid detergent-insoluble fiber (ADF) and acid detergent lignin (ADL)] were determined by the sequential method according to Van Soest et al. (1991). Heat-stable amylase was used in NDF analysis. The NDF and ADF residues were subjected to ash and CP analyses to determine the amount of neutral detergent fiber insoluble protein (NDIP) and acid detergent fiber insoluble protein (ADIP). These values were used to correct NDF and ADF for ash and protein (NDFap and ADFap, respectively). The non-fibrous carbohydrates (NFC) concentration was calculated using the equation proposed by Hall (2003): NFC = 100 – (%NDFap + %CP + %EE + %Ash), where NDFap is the concentration of NDFap, CP is the concentration of CP, EE is the concentration of EE, and Ash is the concentration of ash.

The *in vitro* dry matter digestibility (IVDMD) was determined according to the procedure described by Tilley and Terry (1963) and modified by Holden (1999) using the DaisyII Incubation system (Ankom Technology, Fairport, New York, USA). The ruminal fluid was collected from a ruminally cannulated cow whose diet was composed of 10 kg (DM) of maize silage and 3 kg (DM) of commercial feed with 24% CP. In the IVDMD procedure, the samples were incubated for 72 h. After the initial 48 h of incubation, which simulates ruminal digestion, 40 ml of 6N HCl and 8 g of pepsin were added to simulate digestion.

Analysis of fermentative parameters

Silage juice was extracted by a hydraulic press (2.5 kilogram-force/cm^2) to determine the pH, NH_3-N

and organic acids. The pH was measured with a digital potentiometer (HI 221, Hanna Instruments, USA). Distillation to determine NH_3-N was performed in Kjeldahl equipment using magnesium oxide and calcium chloride as a neutralizing medium for the evaporation of ammonia, with boric acid as the receptor solution and *0.1 M* hydrochloric acid as the titrant. The concentration of organic acids were determined by gas chromatography (GC-17 Shimadzu gas chromatograph; Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector and fitted with a Nukol capillary column according to the methodology described by Playne (1985). The gas chromatograph was operated isothermally with a column temperature at the temperature slope ranging from 90 to 200 °C and with an inlet and detector temperature of 225 °C.

The pH values of samples subjected to the aerobic stability test were determined as follows: fresh silage (9 g) was added to 60 mL of distilled water, and pH values were measured after 30 min (Silva and Queiroz, 2002).

Analysis of loss

The weight of the empty silos plus lid and dry sandbags was recorded before the ensiling process. The silos were then filled with forage, compacted, covered, sealed with adhesive tape, and weighed again. Silos subjected to the re-ensiling procedure were opened at 56 d and weighed before and after forage removal to determine the production of gases and effluents. The DM content in the forage was also determined. The sand deposited at the bottom of each silo was replaced, after which the empty set (bucket, lid and dry sandbags) was weighed again. The forage was re-ensiled after 18 h of aerobic exposure. After filling and sealing, the experimental silos were weighed again to determine the total weight.

After 60 d of re-ensiling, all experimental silos were weighed to determine the loss of gases, according to the formula: G = {[(Wen – SWen) × DMen] – [(Wop – SWen) × DMop] × 100} / [(Wen – SWen) × DMen], in which: G represents the gas losses (% of DM); Wen is the weight of the bucket full (kg) at ensiling; SWen is the set weight (empty bucket + lid + dry sand + bag) (kg) at ensiling; DMen is the forage DM content (%) at ensiling; Wop is the weight of the bucket full (kg) at opening; DMop is the forage DM content (%) at opening.

The buckets were then opened and silages were then removed, and the silos were weighed to quantify the effluents produced, according to the formula EL = (Wef × 1,000) / FMi, in which: EL represents the effluent losses (g/kg FM); Wef is the weight of effluent (weight of empty set after opening - weight of empty set before filling); FMi is the amount of organic matter (kg) ensiled.

Total DM loss was estimated as the difference between the gross weight of the initial and final dry mass of the experimental silos in relation to the dry silage weight minus the weight of the set of bucket at ensiling and opening of the silos (Jobim et al., 2007), according to the formula: TDM = {[(Wen – SWen) × DMen] – [(Wop – SWop) × DMop] × 100} / [(Wen – SWen) × DMen], in which: TDM is the total DM loss (%); Wen is the weight of the full bucket (kg) at ensiling; SWen is the weight (kg) of the set (empty bucket + lid + dry sand + bag) at ensiling; DMen is the forage DM content (%), at ensiling; Wop is the weight of the full bucket (kg) at opening; SWop is the weight (kg) of the set (empty bucket + lid + dry sand + bag) at opening; DMop is the forage DM content (%) at opening.

Aerobic stability test

Plastic buckets (28-cm diameter) containing 1.5 kg of silage per repetition were placed in a room at 25 ± 1°C to evaluate aerobic stability. Silage temperature was monitored every 10 min with the aid of a temperature data logger inserted 15 cm into the center of mass. In addition, another set of 1.5 kg of silage was placed in another set of buckets to track changes in microbial and pH counts. Aerobic deterioration was considered to have occurred if the temperature difference between the materials and ambience reached 2 °C (Ranjit and Kung, 2000). Finally, at aerobic stability loss the microorganisms were also counted in silage samples.

Microbiological analyses

Samples were collected for total aerobic microbial counts (yeasts, molds, and bacteria). Analysis of the microbiota was performed using a standard dispersion plate method (Pitt and Hocking, 2009). Initially, 25 g of silage were diluted in 225 mL of 0.1% peptone water. Appropriate serial dilution (10_{-2} to 10_{-6}) for each sample was performed in 0.1% peptone solution and then homogenized for 2 min. From each dilution step, 0.1 mL was transferred to a Petri dish, and the inoculum was spread onto the surface of the agar with a Drigalski spatula. Total bacterial counts were determined aerobically on plate count agar (PCA-BD ™ Difco, Sparks, MD, USA) following an incubation period of 1 to 3 d at 36 ± 1 °C. Total yeast counts were determined on tryptone glucose yeast extract agar according to Pitt and

Hocking (2009). Next, aerobic samples were incubated for 1 to 3 d at 30 ± 1 °C. Total mold count was determined on dichloran rose-bengal chloramphenicol agar according to Pitt and Hocking (2009) after anaerobic incubation period of 5 to 7 d at 25 ± 1 °C. The plates were examined daily for typical colony and morphological characteristics associated with each growth medium. Total microbial counts were expressed as CFU/g.

Statistical analyses

The results of chemical composition, *in vitro* digestibility, fermentative parameters, losses, aerobic stability, and total counts of yeasts, molds, and bacteria were analyzed in randomized blocks in 2 × 2 factorial scheme (re-ensiling or not and inoculant use or not) with five repetitions. All microbial counts were $\log_{10}$ transformed to obtain lognormal distribution. Data were submitted to analysis of variance. When significant interactions were identified, further analyses of simple effects were conducted (ANOVA). When interactions were found to be insignificant, the effects were analyzed separately by *F*-tests at 5% significance. The analysis was performed using the PROC GLM software from SAS (SAS Institute Inc., Cary, NC, USA).

RESULTS

The fresh maize before ensiling and inoculation had 298 g/kg DM and chemical composition of 40 g/kg ash, 76 g/kg CP, 565 g/kg NDFap, 297 g/kg ADFap and 37 g/kg EE. The re-ensiling process and microbial inoculation did not influence the DM, CP, NDFap, ADFap, ADIP, NDIP and ADL contents of maize silage (Table 1; P>0.05). However, re-ensiling reduced the NFC content and IVDMD by 13.0% (P<0.01) and 5.99% (P<0.01). The microbial inoculant use increased IVDMD 4.96% (P<0.05).

Table 1 - Chemical composition (g/kg of DM unless noted) and *in vitro* dry matter digestibility of maize silage treated with inoculants and after re-ensiling

Variables	Treatments				S.E.M	P-value		
	Control		Inoculant					
	SIL	RE	SIL	RE		I	R	I × R
DM (g/kg FM)	247	252	251	248	0.317	NS	NS	NS
Ash	45.2	49.5	45.3	49.1	0.076	NS	**	NS
CP	85	88	87	86	0.055	NS	NS	NS
EE	33	44	48	52	0.232	**	NS	NS
NDFap	502	518	509	551	0.727	NS	NS	NS
ADFap	252	259	235	267	0.624	NS	NS	NS
ADIP (g/100 g CP)	0.159	0.144	0.124	0.148	0.006	NS	NS	NS
NDIP	0.384	0.413	0.384	0.500	0.021	NS	NS	NS
ADL	39.9	42.6	36.6	41.1	0.116	NS	NS	NS
NFC	335	301	311	262	0.953	NS	**	NS
IVDMD	614	595	664	605	0.808	*	**	NS

SIL, silage; RE, re-ensiled; S.E.M, standard error of mean; I, inoculant effect; R, re-ensiling effect; I x R, interaction effect; * P<0.05; ** P<0.01; NS, not significant; FM, fresh matter; DM, dry matter; CP, crude protein; EE, ether extract; NDFap, neutral detergent insoluble fiber corrected for ash and protein; ADFap, acid detergent insoluble fiber corrected for ash and protein; ADIP, acid detergent insoluble protein; NDIP, neutral detergent insoluble protein; ADL, acid detergent lignin; NFC, Non-fibrous carbohydrate; IVDMD, *in vitro* dry matter digestibility.

Re-ensiling and inoculation did not influence the pH, NH_3-N/total nitrogen (TN), and the propionic and butyric acid concentrations of silages (Table 2; P>0.05). However, re-ensiling reduced the lactic acid concentration by 29.4% (P<0.05), whereas it increased the acetic acid concentration by 101% (P<0.05) compared to control

silage. Re-ensiled silages had greater losses due to gases (P<0.01), effluents (P<0.05) and total losses (P<0.01) (Table 2). The inoculation did not influence these variables (P>0.05).

Table 2 - Fermentation quality variables (g/kg of DM unless noted) of maize silages after re-ensiling and treatment with inoculants

Variables	Treatments				S.E.M	P-value		
	Control		Inoculant					
	SIL	RE	SIL	RE		I	R	I x R
pH	3.87	3.98	3.94	3.82	0.036	NS	NS	NS
NH_3-N/NT	74.7	72.0	76.6	76.3	0.172	NS	NS	NS
Lactic acid	65.7	49.8	61.6	40.3	0.357	NS	*	NS
Acetic acid	14.7	29.3	12.3	25.2	0.259	NS	*	NS
Propionic acid	0.500	1.40	1.90	1.80	0.036	NS	NS	NS
Butyric acid	2.80	1.30	0.00	0.200	0.053	NS	NS	NS
Gas loss	181	261	188	292	1.792	NS	**	NS
Effluent loss (g/kg FM)	14.4	18.1	11.2	22.4	2.057	NS	*	NS
Total loss	197	269	201	301	1.761	NS	**	NS

SIL, silage; RE, re-ensiled silage; DM, dry matter; NH_3-N, ammonia nitrogen; TN, total nitrogen; FM, fresh matter; S.E.M, standard error of mean; I, inoculant effect; R, re-ensiling effect; I × R, interaction 1 effect; *P<0.05; **P<0.01; NS, not significant.

Re-ensiling and the microbial inoculant did not influence the aerobic stability of maize silages (Table 3; P>0.05). The same response was observed for the bacteria, yeast, and mold counts after opening the experimental silos. However, after the loss of aerobic stability, a lower mold count was observed in inoculated silages.

Table 3 - Aerobic stability (h) and microbial total count (log10 CUF/g) in various conditions

Variables	Treatments				S.E.M	P-value		
	Control		Inoculant					
	SIL	RE	SIL	RE		I	R	I x R
Aerobic stability	208	211	220	192	11.5	NS	NS	NS
Microbial counts at silo opening								
Bacteria	6.67	6.63	6.75	6.64	0.211	NS	NS	NS
Yeasts	3.81	3.99	2.00	3.67	0.224	NS	NS	NS
Molds	3.13	3.63	2.30	3.21	0.151	NS	NS	NS
Counts at loss of stability								
Bacteria	9.44	10.1	9.51	10.0	0.183	NS	NS	NS
Yeasts	9.57	10.1	9.54	9.88	0.472	NS	NS	NS
Molds	8.18	8.34	8.35	7.78	0.645	*	NS	NS

SIL, silage; RE, re-ensiled silage; S.E.M, standard error of mean; I, inoculant effect; R, re-ensiling effect; I × R, interaction effect; *P<0.05; NS, not significant.

DISCUSSION

During air exposure, the ensiled mass is susceptible to the action and growth of aerobic microorganisms (Woolford, 1990) and facultative anaerobes. Consequently, aerobic deterioration of silages exposed to air may occur, with loss of nutritive value (Filya et al., 2006). The degree of nutrient and DM losses in the silage depends on the quality of the ensiled material and the length of aerobic exposure (Chen and Weinberg, 2014; Lima et al., 2017). It also depends on the efficiency of the silage-making processes, including silo compaction, filling time and sealing.

In the current study, 18 h of aerobic exposure changed the chemical composition and IVDMD of silages. The re-ensiling process reduced the NFC content by 13.0% (323 vs. 282 g/kg of DM), whereas it increased the ash content by 8.2%. According to Michel et al. (2017) the action of molds and yeasts reduces soluble carbohydrates, which can reduce NFC and proportionally increase fibrous fractions. However, we did not observe any change in the NDFap. Furthermore, according to Muck (1999, 2010), under aerobic conditions, acetic acid bacteria can also consume soluble carbohydrates, which justifies the NFC reduction.

The NDFap concentration of silages was numerically reduced when compared with the NDFap concentration of fresh forage. In the silage fermentation process, not only the soluble carbohydrates are fermented. Some sources of structural carbohydrates, such as hemicelluloses, may also be substrates for volatile fatty acid (VFA) production (McDonald et al., 1991). Thus, in the current study the authors believe that part of the hemicelluloses was probably used for the synthesis of VFA, which may justify the silage NDFap reduction in relation to the FM. It is difficult to determine the exact losses of soluble carbohydrates due to the enzymatic activity of the plant, since the sugars lost by respiration can be partially replaced by the sugar liberated by the hydrolysis of the plant structural carbohydrates (McDonald et al., 1991). It is important to note that these losses of hemicelluloses, together with the aerobic losses and, to a lesser extent, losses due to undesirable products of the fermentation, were the factors responsible for the reduction of the DM concentration values of the silages in relation to the FM.

Since NFC is comprised of highly digestible organic compounds, its loss in silage may result in reduced DM digestibility (McDonald et al., 1991). In this sense, the IVDMD was reduced by 6.1% in re-ensiled silages when compared with control silages (600 *v.* 639 g/kg of DM). The lower IVDMD of re-ensiled maize silages is related to the reduction of NFC concentration in these silages. High losses due to effluents (20.2 g/ton of FM) and DM in re-ensiled silages (285 g/kg of DM) also contribute to the reduction of IVDMD. It leads to effluent losses due to nutrient leaching and reduced availability of digestible nutrients in DM. The greater effluent loss of re-ensiled maize silages possibly occurred because the material was compacted twice, one compaction during ensiling and another during re-ensiling, it resulted in a greater loss of water (Michel et al., 2017) and nutrients in re-ensiled silages. Therefore, the aerobic exposure of silages during the opening, transport, and re-ensiling may compromise the nutritive value of silages due to the lower availability of digestible nutrients.

The IVDMD was 4.96% greater in inoculated silages in relation to silages without inoculant. According to Weinberg et al. (2003, 2007), the inoculation of silages with *Lactobacillus plantarum* can optimize animal performance, regardless of whether there is an improvement in the silage fermentation process. This is possible because *Lactobacillus plantarum* survives under rumen conditions (Weinberg et al., 2003). Lactic acid bacteria are associated with better animal performance because of greater fiber digestibility and changes in ruminal fermentation (Weinberg et al., 2007); the mechanism for this response has been linked with greater IVDMD and fiber digestibility in maize silages inoculated with lactic acid bacteria. However, lactic acid bacteria cannot degrade fiber (Rooke and Hatfield, 2003). In this context, Weinberg et al. (2003) verified the ability of lactic bacteria to buffer rumen pH, thus avoiding pH drop. Since cellulolytic bacteria that degrade fiber exhibit activities in a medium where pH is low, the buffering promoted by *Lactobacillus plantarum* maintains a less-acidic pH and favors its greater activity (Muck, 2013). However, non-measurable effects were also indicated as justification for the improvement in the digestibility of inoculated silages (Silva et al., 2006). The results related to the higher IVDMD in silages inoculated with *Lactobacillus plantarum* are variable. However, none of these results include the mixture of *Lactobacillus plantarum* and *Propionibacterium acidipropionici*. In experiments using a mixture of *Lactobacillus plantarum* and *Propionibacterium acidipropionici*, there was no improvement in the IVDMD of silages (Filya et al., 2006; Chen and Weinberg, 2014; Michel et al., 2017). In the current study, the highest IVDMD in inoculated silages may be associated with higher fiber digestibility since there was no difference in the content of NFC and NDFap between control and inoculated silages.

Among the chemical composition variables, only the EE was influenced by inoculation, which was higher

for inoculated silages. However, this change does not have practical relevance since the EE did not compromise the fermentation process of silages. The increase of ash content were consistent with the greater DM loss observed in re-ensiled maize silage. This increase was probably due to silage contamination during air exposure.

The parameters of fermentative quality are in accordance with the values proposed by McDonald et al. (1991) for good-quality maize silages. However, re-ensiling reduced the lactic acid concentration, whereas it increased acetic acid production by 101% (13.5 v. 27.3 g/kg of DM) over the control silage. The higher concentration of acetic acid for re-ensiled silages may be related to the growth of acetic acid bacteria and facultative heterofermentative lactic acid bacteria. The aerobic exposure of silages favors the growth of microorganisms that produce acetic acid (Pahlow, 1991). According to Muck (2010), the observed pH values and aerobiosis allow the growth of acetic acid bacteria in silages. In addition, facultative heterofermentative lactic acid bacteria produce acetic acid (Pahlow, 2003).

Re-ensiling also reduced the lactic acid content by 29.1% when compared with conventional silages (45.1 *v.* 63.6 g/kg of DM). The reduction of lactic acid can be explained by the growth of deteriorating aerobic microorganisms, such as molds, yeasts and some bacteria, although the microbiological count was not enough to detect statistical difference. These microorganisms use VFA as a substrate. Thus, one of the main products consumed is lactic acid, which can be used by yeasts to produce CO_2 and water (Tabacco et al., 2011). Despite the increased acetic acid and reduced lactic acid concentrations in re-ensiled silages, the pH values were not altered.

Re-ensiled silages had greater losses due to gases (185 v. 276 g/kg of DM), effluents (14.8 v. 20.2 g/kg FM) and total losses (199 v. 285 g/kg of DM). The stage of maturity at harvesting, particle size, filling time and silo sealing during the silage making process are determinant for the occurrence and severity of DM losses. These losses can vary from 3 to 25%, and the main factors involved are the presence of oxygen (aerobic deterioration), CO_2 production by anaerobic microorganisms, and effluent losses (Pitt, 1986), as well as gas losses. The high DM losses in the current study (285 g/kg of DM) may be a result of the greater amount of acetic acid produced. It agrees with the DM content of maize plant at ensiling and with the lower DM content observed after storage.

Re-ensiling also led to high losses of gas, contributing to the highest total DM loss. Aerobic exposure is one of the factors that may result in increased gas production in silages. Carbon dioxide (CO_2) is the main gas formed during the fermentation process, and it can be derived from the residual oxygen of the ensiled plant, or the penetration of O_2 into the ensiled material (Pahlow, 2003), as occurs during re-ensiling. In addition, anaerobic bacteria produce higher amounts of CO_2 during the fermentation of soluble carbohydrates and organic acids (Pahlow, 2003). The observed DM losses can aid in the decision-making process for buying silages and planning forage production on farms.

In the current study, a lower mold count in inoculated silages was observed after the loss of aerobic stability, but without benefit to aerobic stability. Possibly, the aerobic stability of silages was not increased because the yeast count was similar between silages with and without inoculant after the loss of aerobic stability. Yeasts are the first aerobic microorganisms to grow under aerobic conditions. According to the literature, mold counts increase significantly eight days after aerobic exposure (Gerlach et al., 2013). As a result, the lower mold count in inoculated silages did not influence aerobic stability.

Re-ensiling resulted in higher production of acetic acid and could contribute to an increase of aerobic stability of silages. According to Muck (2010), acetic acid can increase the aerobic stability of silages by inhibiting the growth of molds and yeasts. However, the higher production of acetic acid in re-ensiled silages did not benefit aerobic stability.

The re-ensiling process and application of microbial inoculant did not influence the microbiological count. Yeasts and molds are the main microorganisms involved in the aerobic deterioration of maize silages (Muck, 1988; Pahlow et al., 2003). Yeast and mold counts should exceed 9 and 8 $\log_{10}$ CFU/g of silage, respectively, at the onset of aerobic deterioration (Pitt, 1991). The values found at silage opening were below the values reported by Pitt (1991). Thus, silage quality was not compromised. After the loss of aerobic stability, yeast count was above 9 $\log_{10}$ CFU/g and mold count was below 8 $\log_{10}$ CFU/g, which are the reference values for the onset of aerobic deterioration (Pitt, 1991).

The higher production of acetic acid may have contributed to the lack of effect between treatments on aerobic stability in silages exposed to air since there was also no influence of re-ensiling on mold and yeast counts. Re-ensiling reduced the nutritive value and IVDMD of silages and could result in a greater need for concentrate in the diet.

CONCLUSION

Re-ensiling after 18 h reduces the nutritive value and IVDMD and increases losses of maize silages and should not be used in production systems. The use of microbial inoculant containing a mixture of *Lactobacillus plantarum* and *Propionibacterium acidipropionici* is not effective in avoiding nutritional value losses or losses due to gases, effluents and total dry matter of re-ensiled maize silages and should not be used in these conditions.

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