Effects of holes in plastic film on the storage losses in total mixed ration silage in round bales

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ABSTRACT: The ensilage of total mixed ration (TMR) is a technology designed to help farmers with limitations to provide a balanced diet for their herds. Our aim was to evaluate the conservation of TMR ensiled in round bales with or without holes in the wrapping plastic film. Eight round bales of a corn silage-based TMR of 1,000 kg (370 kg DM/m^3) were prepared. Ten days (d) after ensiling, four bales were randomly punctured with two holes of 25 cm^2 each in opposite sides of the bale. The temperature in the center of the bales was recorded during the storage using dataloggers. After 60 d of storage, bales were weighted to assess dry matter (DM) recovery. Silages were sampled for measuring DM content, chemical composition, pH, lactic acid, and microbial counts. The temperature of the sliced bale face was assessed by infrared thermography. The holes in the plastic affected the DM content, DM recovery, and pH, whereas lactic acid, microbial counts, and temperature were not affected by treatments. The holes in the sealing plastic film should be avoided. However, holes of 25 cm^2 each were not capable of causing expressive losses in TMR silage stored in 1,000 kg bales.

Key words: dry matter loss, microbial count, plastic damage, storage losses, TMR silage

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INTRODUCTION

The total mixed ration (TMR) composed of forage, cereal grains, protein sources, minerals, vitamins, and additives is widely used in dairy farms (Sova et al., 2014). The mixture of these ingredients aims to provide a complete nutrition of cattle. However, some of the wet ration ingredients are sensible to deterioration, and if the farms did not have adequate feed management and/or trained workforce, it can be restrictive to the TMR use (Abdollahzadeh et al., 2010; Yuan et al., 2015; Liu et al., 2016).

The ensiling of TMR allows marketing to ready-to-use ruminant feed, in addition to better use of wet coproducts. This technology was conceived to aid farmers with limitations of specialized workforce, machinery, or without a production scale with the constant acquisition of ration constituents and even for farmers with limited knowledge of proper ration balancing (Miyaji and Nonaka 2018). It eliminates the need of daily preparation of TMR, improves ingredient preservation, and optimizes the transport

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and commercialization of the ready-to-use ration (Ishida et al., 2012; Chen et al., 2015), outsourcing feed production for ruminants.

In Brazil, TMR silage has been mostly commercialized in 1,000 kg round bales sealed with six to eight layers of plastic film. A disadvantage of this strategy is the poor handling of bales during at-farm transport and storage (mainly due to their weight) that can lead to the appearance of some damages in the plastic film which can facilitate the penetration of air into the ensiled mass. According to McDonald et al. (1991), the presence of oxygen in forage silages (maize, sugarcane, grasses, and sorghum) during the fermentative process triggers the proliferation of undesirable microorganisms like yeasts, fungi, and aerobic bacteria that grow using the energy sources available in the materials. This process causes losses in the nutritional value of the silages, increasing the temperature and decreasing the amount of silage consumed by the animals (Borreani et al., 2018).

There are no data in the literature on losses caused by the penetration of air due to damage in the covering plastic film in rich soluble nutrients feed such as TMR silage. Our hypothesis is that the entrance of oxygen during the fermentative process may cause losses in TMR silage. This study aimed to evaluate the DM recovery, chemical composition, temperature, and microbial counts of round-baled TMR silage with and without holes in the sealing.

MATERIALS AND METHODS

Experimental Design and Treatments

The TMR was formulated to meet the nutritional requirements of lactating dairy cows producing 25 kg/d of milk according to NRC (2001). Ingredients and composition of the TMR before ensiling are shown in Table 1.

The corn silage and ryegrass haylage were already fermented separately (without any inoculant) in large-scale bunker silos for at least 90 d before preparing the TMR. These silages were taken using a silage defacer following good feedout practices. The ingredients were mixed in a stationary wagon (TRIOLIET model mat 16000L) for 7 min. After mixing, the TMR was packed in a cylindrical baler (ORKEL—MP 2000 Compactor) to produce 1,000 kg bales with a bulk density of 370 kg DM/m^3. Bales were immediately wrapped with seven layers of plastic film (SILOTITE, 750 mm width, 25 µm thickness) and weighted.

Table 1. Ingredients, chemical composition, pH, and microbial count of total mixed ration before ensiling

Ingredients	Total mixed ration, % of DM
Maize silage	44.9
Ryegrass haylage	19.5
Ground corn	13.6
Soybean meal (46%)	9.3
Corn gluten meal (*Promil*)[1]	9.3
Sodium bicarbonate	1.0
Mineral mixed (*Nucleo Melk*)[2]	1.0
Sodium chloride	0.5
Urea	0.4
Calcitic limestone	0.4
Chemical composition	Mean ± SEM[3]
pH	4.92 ± 0.02
Dry matter, %	41.9 ± 2.1
Crude protein, %	15.8 ± 0.9
Neutral detergent fiber, %	37.5 ± 6.3
Acid detergent fiber, %	19.1 ± 3.5
In vitro DM digestibility, %	83.4 ± 9.7
Starch, %	31.0 ± 2.4
Ether extract, %	2.8 ± 0.5
Ash, %	7.4 ± 0.9
NH_3-N[4], % of total N	15.5 ± 1.3
Microbial count, $\log_{10}$ cfu/g	Mean ± SEM[3]
Lactic acid bacteria	5.5 ± 0.03
Yeast	4.9 ± 0.08
Molds	3.9 ± 0.11

[1] Crude protein (24.4% DM), total fibber (9.4% DM), starch (8.9% DM), ash (7% DM).

[2] S (0.7% DM), Na (0.04% DM), Cl (0.35% DM), Fe (144 ppm), Mg (23 ppm), Zn (85 ppm), Cu (7.5 ppm), Mo (1.7 ppm), dietary cation-anion difference (5.8 meq 100 g), total digestible nutrients (77% MS), non-fibrous carbohydrates (19% MS).

[3]Standard error of the mean.

[4]Ammonia nitrogen.

The trial enrolled eight bales. Four bales were kept intact during the storage, whereas four bales had their plastic damaged with two holes of 25 cm^2. Holes were manually made on the 10th day of storage, in opposite sides of the bales. The size of the holes was based on our knowledge, in order to simulate bad management during transport and storage. The bales were stored outdoor for 60 d, from October 15 to December 15, 2015. Ambient temperature was recorded daily by a meteorological station (SMAABC 2015) close to the experimental site. Samples (1 kg, wet basis) were collected before (n = 4) and after (n = 8) the storage to evaluate TMR composition. At ensiling other two samples were taken to evaluate the TMR particle size using a particle separator (Kononoff et al., 2003). At ensiling, two dataloggers (USB-EL1—LASCAR Inc.) were placed in the center of each bale for

recording the temperature every 30 min during the whole trial. For placing it, a researcher climbed the baler and as a bale started being processed, two PVC-protected dataloggers were thrown at the middle of the rolling bar.

Sample Collection and Analyses

After 60 d of storage, the bales were weighed again and manually cut into two halves. The cutting plane was made longitudinally, passing through both holes in the plastic film. For each bale, one half (~500 kg) was homogenized and sampled for measuring DM content, chemical composition, microbial counts, lactic acid, NH_3-N, and pH. The DM recovery was determined by weight difference. The other bale half was kept intact for thermal image capturing using an infrared thermographic camera (FLUKE Ti25) in order to identify possible heat sites. Using the SMARTVIEW software, three areas of 600 cm^2 were isolated in the thermal image of each bale, being two at the edge (near the hole in that treatment) and one at the center (Figure 1). The difference between the mean temperature of the edges and the central temperature of the bales was calculated (Δt).

Silage samples were dried in a forced air oven at 55°C for 72 h. DM data were not corrected for volatiles. Dried samples were ground in a Willey mill using a 1-mm mesh sieve for measuring: DM (method number 934.01; AOAC 1990), crude protein (CP) by the Dumas method (FP-528, LECO, combustion N analyzer) according to Wiles et al. (1998), ash (method number 924.05; AOAC, 2012), ether extract (EE) (method number 920.39; AOAC 2012), neutral detergent fiber (aNDF) with thermostable alpha-amylase and sodium sulphite (Mertens 2002), and acid detergent fiber (ADF) (Van Soest et al., 1991), determined sequentially, using an ANKOM A^{200} Fiber Analyzer. The in vitro DM digestibility (IVDMD) was determined according to Tilley and Terry (1963) using an ANKOM DAISY^{II} incubator (Holden 1999).

Microbial counts were performed in aqueous extracts. The extracts were prepared in sterile plastic bags containing 25 g of sample and 225 mL of Ringer solution homogenized for 4 min in a stomacher blender (MARCONI-MA 440/CF) and filtered with four layers of cheesecloth. Ten-fold dilutions in MRS broth (for lactic acid bacteria—LAB) or in Ringer solution (for yeasts and molds) were prepared and plated in 3M PETRIFILM. The count of LAB was made in PETRIFILM AC, after incubation for 48 h at 32°C in anaerobic jars. Yeasts and molds were enumerated in PETRIFILM YM after incubation at 25°C for 72 and 120 h, respectively. Microbial counts were expressed as $\log_{10}$ cfu/g of silage. The concentrations of NH_3-N (Chaney and Marbach 1962) and lactic acid (Pryce 1969) were determined in the aqueous extract using colorimetric methods. The pH was

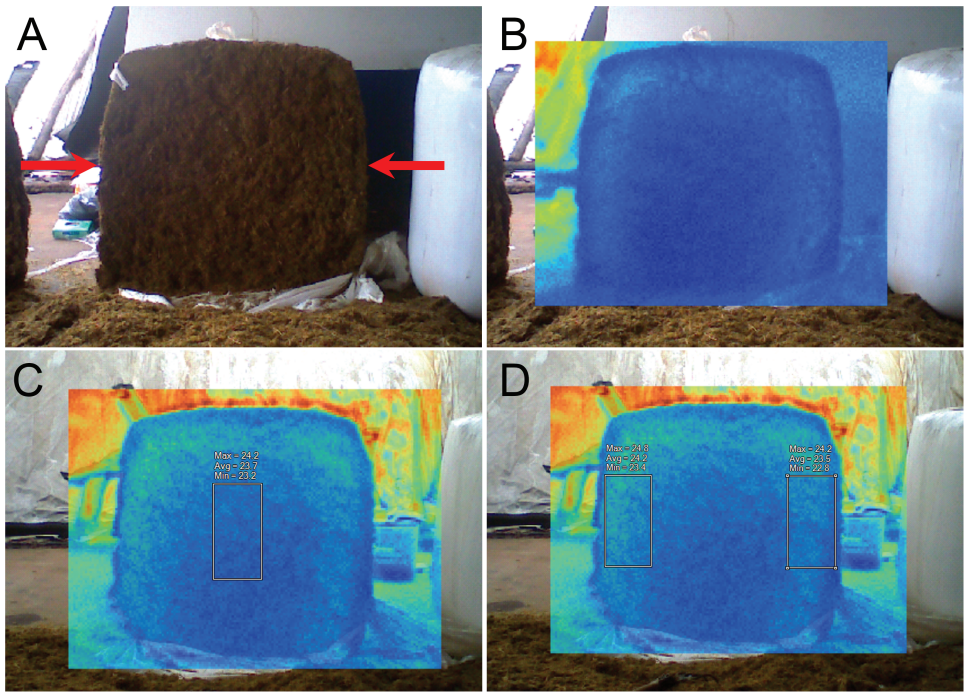

Figure 1. Thermal images of longitudinally cut bales after 60 d of storage. A bale with holes, place indicated by red arrows (A); a thermal image of the same half-cut bale (B); delimited areas and temperature (maximum, mean, and minimum) of the center (C); delimited area and temperature (maximum, mean, and minimum) of the edges (D). Squares delimitate the areas for calculation of temperature difference (Δt = edge − center).

measured using a digital pH meter (PG 1400, GEHAKA—Brazil).

Statistical Analysis

The experimental design was completely randomized with two treatments (bales with holes or without holes) and four replicates. Data were analyzed using the GLIMMIX procedure of SAS (v. 9.2) (SAS, 2001). The statistical model used for the analysis was the following:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij},$$

which Y_{ij} is the response variable, μ is the overall mean; T_i is the effect of treatment (holes), and ε_{ij} is the error. *F*-test was used to compare treatment means, and significance declared at $P \leq 0.05$.

RESULTS

The particle size of the TMR before the ensilage was distributed in the following proportions: 1) 30.0% of particles higher than 19 mm; 2) 29.0% of particles between 8 and 19 mm; 3) 28.5% of particles between 1.18 and 8 mm; and 4) 12.5% of particles lower than 1.18 mm.

The differences in DM content and DM recovery between the treatments are shown in Table 2. Intact (undamaged) bales showed the highest DM content ($P < 0.01$) and DM recovery ($P = 0.02$). However, the magnitude of differences was very small. Even the bales with holes showed a high DM recovery (98.5%).

The chemical composition (CP, aNDF, ash, and EE) and IVDMD were not affected by treatments ($P > 0.05$). It was similar to the composition of TMR before ensiling, likely as a consequence of low DM loss.

Ensiling the TMR led to a small increase in IVDMD (1.4%) and a slightly greater increase of NH_3-N (12.8%), probably due to the proteolysis during the fermentation.

The pH value presented a slight increase when the plastic film was damaged ($P < 0.01$). However, the lactic acid content was high and similar between treatments (8.07%).

The holes in the plastic film did not affect the microbial counts (LAB, yeasts, and molds; $P > 0.05$). The TMR right exposed from the holes was dark, dry, and showing a few visible molds. However, this spoiled surface was thin of no more than 5 cm depth (data not taken). When comparing the counts of LAB, yeast, and molds before and after the ensilage, an increase in LAB (6.9 $\log_{10}$ cfu/g) population and a decrease of yeasts (2.5 $\log_{10}$ cfu/g) and molds (2.4 $\log_{10}$ cfu/g) seem to be observed.

Contrary to the expected, the thermography of the inner surface of the bales (Figure 1) has shown no heating spots, even in the areas near the holes. This observation is in accordance with the values of internal temperature (Figure 2) collected by data

Table 2. Dry matter recovery, chemical composition, pH, microbial count, and temperature of the total mixed ration ensiled for 60 d, with and without holes in the sealing plastic film

Item	Holes	Intact	SEM[1]	*P*
Dry matter recovery, %	98.5	99.5	2.20	0.02
Dry matter, %	41.4	41.7	0.32	<0.01
Crude protein, % of DM	15.7	15.8	0.38	0.17
Neutral detergent fiber, % of DM	36.6	37.0	2.29	0.23
Acid detergent fiber, % of DM	19.3	19.1	0.69	0.07
Ash, % of DM	7.3	7.2	0.67	0.94
Ether extract, % of DM	2.8	2.8	0.44	0.88
In vitro DM digestibility, %	84.6	84.5	2.84	0.94
NH_3-N[1], % of total N	17.6	17.5	0.98	0.31
pH	4.38	4.24	0.03	<0.01
Latic acid, % of DM	8.0	8.1	0.45	0.60
Lactic acid bacteria, $\log_{10}$ cfu/g	6.90	6.95	0.06	0.58
Yeast, $\log_{10}$ cfu/g	2.52	2.51	0.08	0.92
Molds, $\log_{10}$ cfu/g	2.45	2.32	0.06	0.18
Δt[2], °C	0.51	0.57	0.13	0.75
Edges temperature, °C	23.9	22.5	0.49	0.08
Center temperature, °C	23.4	21.9	0.47	0.07

[1]Standard error of the mean.

[2]Δt = Edge mean temperature − Center temperature.

loggers throughout the storage, as well as the chemical variables.

No difference was observed in bale temperature variation (Δt = edge − central temperature) between treatments (P = 0.75). No heating was detected throughout the ensiling process. The average temperature at the edges and center of the bales showed trends of treatment effect (P = 0.08 and P = 0.07, respectively), and the intact round bales had the lowest temperature. However, the average temperature of both sites was small and biologically similar.

The temperature dynamic inside the TMR silage bales was also similar between treatments (Figure 2). From days 5 to 25, a progressive decrease in temperature was observed. After this, the temperature reached a plateau about 20°C to 22°C.

DISCUSSION

The lack of a relevant negative effect of the holes in the TMR silage was probably related to the high bulk density (370 kg DM/m³), to the high DM content (41.8%), and to the adequate particle size (41.0% < 8 mm). These factors are interrelated and hinder the air flow into the silage, thus inhibiting the proliferation of aerobic microorganisms (McDonald et al., 1991).

Other authors have also reported a high DM recovery in TMR silages. Hu et al. (2015) found a DM recovery of 97.4% after 56 d of storage. Miyaji et al. (2013) observed DM recovery of 99.3%. Hence, properly prepared TMR silages seem to be less susceptible to DM losses even under inadequate management when compared with plant silage data. The same pattern was found in our previous trial using lab scale silos, testing the same TMR, treated or not with microbial inoculants (Restelatto et al., 2019).

Lee et al. (2011) verified a small but no significative increase in NH_3-N content (2.98% on the first storage d and 4.25% after 21 d of storage) for fermented TMR. The greater values observed in the present study (17.5% of total N) are likely related to the presence of urea in the TMR (Table 1).

Apparently, the homolactic LAB was predominant during the fermentation process. The great value of LAB count in the TMR silage observed in this work and in the literature (Wang and Nishino 2008; Yuan et al., 2015) is a result of three main factors: 1) LAB are predominant microorganisms in the anaerobic fermentation; 2) there are available substrates for microbial growth and; 3) the moisture content of the silages is adequate. Additionally, corn and wilted ryegrass silages carried a high population of LAB to the TMR.

Opposed to the current study, Wang and Nishino (2008) assessed the effects of air infiltration (200 mL of air in 300 g of TMR silage) and found a significative increase of pH value due to this. On the other hand, Nishino et al. (2007) verified pH value between 4.07 and 4.23 for inoculated TMR silages after 60 d of storage. These pH values are closer to the values found here (4.38 and 4.24, with and without holes, respectively), and they are capable of inhibiting the growth of undesirable microorganisms during the storage of TMR silages with high DM content (Yuan at al., 2015). The lower pH of intact bales could be related to other acids than the lactic acid, from heterofermentative BAL. However, those fermentation products were not assessed in this trial.

The magnitude of temperature differences (Δt) between the center and the edges of bales was small,

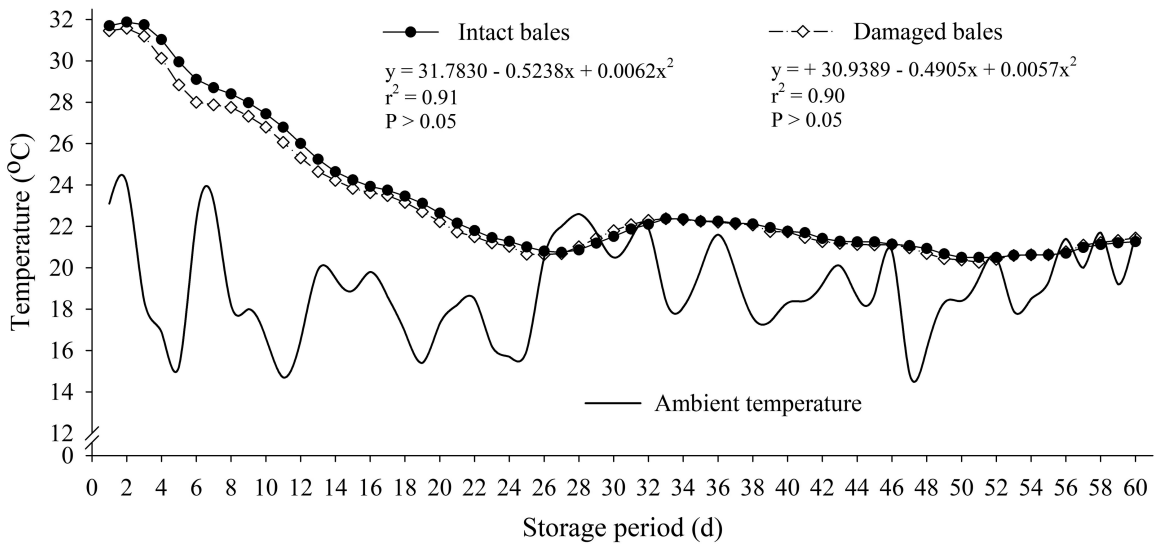

Figure 2. Ambient temperature and the average temperature inside the bales containing total mixed ration silage.

which means that the opportunistic aerobic microorganisms have not been able to develop significantly to cause quantitative or qualitative losses in the TMR silage, even when plastic film was submitted to holes. In the first 5 d of storage, the internal temperature of the bales remained around 30°C, probably related to the temperature of the ingredients at ensiling and some heating produced by respiration. Silage gradually lost temperature to the environment and no heat was produced in this phase, even in bales submitted to holes.

Lee et al. (2011) verified an increase in temperature (23°C–27°C) during the first 21 d of ensilage period. During the aerobic phase of silage production, the temperature increases as a result of the respiratory activity, which plays an important role over the losses of the ensiled material. Most of the heat is retained in the silage mass resulting in a progressive increase of the temperature (above 35°C; McDonald et al., 1991). An excessive heat production, compared with ambient temperature, may result in Maillard reaction or dimming, which reduces protein and fiber digestibility (Bolsen et al., 1996). This process was inhibited in the TMR silages, likely due to their high bulk density.

The small silage particle size improves the fermentation, optimizes the packing density, minimizes silage porosity, prevents aerobic spoilage (Bernardes et al., 2018), and influences animal intake (Allen 1997). The particle size of the TMR used in this study was close to the values recommended by Lammers et al. (1996) for TMR. The small particle size and the presence of concentrates in the TMR silages lead to a bulk density of 370 kg of DM/m^3. In traditional forage silages (maize, sugarcane, and sorghum), the bulk density remains below 250 kg of DM/m^3 (Holmes 2009). This difference helps us to understand why holes in the wrapping plastic film did not cause great impact in the chemical variables evaluated here as well as the dynamic of temperature inside the TMR silage bales.

Considering the experimental conditions of our study, the maize silage-based TMR stored for 60 d maintains its chemical composition close to the original values. The high bulk density, the great DM content, and the predominance of LAB fermentation led to very small DM disappearance. Thus, TMR can be stored as silage with inexpressive losses or changes in chemical composition.

Although the presence of holes in the process of sealing and storage of silages should be avoided, under the experimental conditions two holes of 25 cm^2 in the plastic film did not influence the quality of TMR ensiled in round bales of 1,000 kg.

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Conflict of interest statement. None declared.

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