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Corn silage aerobic stability, dry matter loss, and microbiology are affected by the use of organic acids, depending on the plant maturity stage

Estabilidade aeróbica da silagem de milho, perda de matéria seca e a microbiologia são afetadas pelo uso de ácidos orgânicos, dependendo da maturidade da planta

Thomer Durman[1]*; Antonio Vinicius Iank Bueno[1]; Clóves Cabreira Jobim[2]; André Martins de Souza[3]; Egon Henrique Horst[3]; Paulo Victor Pinheiro Cesar[4]; Livia Alessi Ienke[5]; Nicolli Soethe Mokochinski[5]; João Antonio de Arruda Giacomet[5]; Mikael Neumann[6]

Highlights

Organic acids improve the fermentation profile of corn silage
Organic acids reduce yeast count and dry matter losses in corn silage
Organic acids are effective in increasing the digestibility of corn silage

Abstract

This study aimed to evaluate the fermentation profile, nutritional quality, microbial count, fermentation losses, and aerobic stability of corn silages at different stages of maturity that were treated with organic acids (OA; Mold-Zap, Alltech, Nicholasville, KY). The OA (8 g kg^{-1} DM) was applied to corn silage harvested at the early, medium, or late stage, before compaction in the bunk. Lactic acid decreased with silage maturity ($P<0.01$). Compared to the control, the OA-treated silage had higher lactic acid content in the early-maturity silage (90.0 vs. 51.7 g kg^{-1} DM), but lower lactic acid in the late-maturity silage (33.7 vs.

[1] Drs., Animal Science, Department of Animal Science, Universidade Estadual de Maringá, UEM, Maringá, PR, Brazil. E-mail: thomerdurman@hotmail.com; vinicius_bueno602@hotmail.com
[2] Prof. Dr., Department of Animal Science, UEM, Maringá, PR, Brazil. E-mail: ccjobim@uem.br
[3] Drs., Animal Science, Department of Animal Science, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: andrems_92@hotmail.com; egonhh@yahoo.com.br
[4] M.e Veterinary Science, Department of Veterinary Medicine, Universidade Estadual do Centro-Oeste, Guarapuava, PR, Brazil. E-mail: paulo_victor147@hotmail.com
[5] Students of the graduation course in veterinary medicine, Department of Veterinary Medicine, UNICENTRO, Guarapuava, PR, Brazil. E-mail: liviaienke@gmail.com; nickmokochinski@gmail.com; joaogiacomet3@gmail.com
[6] Prof. Dr., Department of Veterinary Medicine, UNICENTRO, Guarapuava, PR, Brazil. E-mail: neumann.mikael@hotmail.com
* Author for correspondence

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51.6 g kg^{-1} DM). Both early- and late-maturity silages with OA had lower yeast counts and DM losses (P<0.01). Silage heating upon air exposure was also lower in late-maturity silage with OA (148 vs. 112 hours of aerobic stability). The use of OA increased the soluble fraction (a), the potentially degradable fraction (b), and the effective degradability (ED) (P<0.01), and reduced the undegradable fraction (U) in intermediate- (268 vs. 287 g kg^{-1} DM) and late-maturity (245 vs. 322 g kg^{-1} DM) silages. In general, organic acids effectively mitigated aerobic deterioration and promoted DM digestibility in corn silage with a DM content above 350 g kg^{-1}.
Key words: Aerobic stability. Ammonium hydroxide. Corn silage. Dry matter loss. Propionic acid. silage additive.

Resumo

Este estudo teve como objetivo avaliar o perfil de fermentação, qualidade nutricional, contagem de microrganismos, perdas de fermentação e estabilidade aeróbica de silagens de milho em diferentes estágios de maturidade que foram tratadas com ácidos orgânicos (AO; Mold-Zap, Alltech, Nicholasville, KY). O AO foi utilizado (8 g kg^{-1} MS) na silagem de milho colhida em estágios inicial, médio ou tardio antes da compactação nos silos. O ácido láctico diminuiu com a maturidade da silagem (P<0,01). Em comparação com a silagem controle, a silagem tratada com AO apresentou maior ácido láctico na silagem de maturidade inicial (90,0 vs. 51,7 g kg^{-1} MS), mas menor ácido láctico na silagem de estágio tardio (33,7 vs. 51,6 g kg^{-1} MS). A contagem de leveduras e a perda de MS foram menores tanto na silagem de maturidade inicial quanto na tardia com AO (P<0,01). O aquecimento da silagem após exposição ao ar também foi menor na silagem de maturidade tardia com AO (148 vs. 112 horas de estabilidade aeróbica). O uso de AO aumentou a fração solúvel (a), a fração potencialmente degradável (b) e a degradabilidade efetiva (DE) (P<0,01), e reduziu a fração indigerível (U) nas silagens em maturidade média (268 vs. 287 g kg^{-1} MS) e tardia (245 vs. 322 g kg^{-1} MS). Em geral, os ácidos orgânicos foram eficazes em mitigar a deterioração aeróbica e promover a digestibilidade da MS em silagens de milho colhidas com teor de MS acima de 350 g kg^{-1}.
Palavras-chave: Estabilidade aeróbica. Hidróxido de amônio. Silagem de milho. Perda de matéria seca. Ácido propiônico. Aditivo para silagem.

Introduction

Several factors can influence dry matter and quality losses in silages, such as field and pre-ensiling conditions, respiration and temperature during ensiling, fermentation patterns, and the storage phase (Borreani et al., 2018). Good management practices, including the use of silage additives, can delay aerobic deterioration and improve silage quality by inhibiting lactate-utilizing yeasts. These yeasts are the primary microorganisms responsible for initiating aerobic deterioration and dry matter losses (Pahlow et al., 2003). Organic acids such as propionic, acetic, sorbic, and benzoic have a long history of research regarding the inhibition of yeasts and molds in silage, and their effectiveness depends upon the application rate (Kung et al., 2004). Several studies have shown that salt-based additives, such as those containing buffered

propionic acid, are effective in improving aerobic stability by inhibiting yeast and mold activity. However, the outcome may be affected by the maturity stage of the forage at the time of harvest (Muck, 2010). For example, forages that were not treated and had a low dry matter content showed a lower concentration of lactic acid than silages that were treated with salt-based additives, such as buffered propionic acid (Knický & Spörndly, 2009, 2011; Auerbach et al., 2016).

Buffered propionic acid products can promote better fermentation patterns in silages, improving the final product's quality. Several studies have shown that when nitrite-containing additives are used for forages ensiled at low dry matter content, a higher lactic acid concentration is observed (Knický & Spörndly, 2009, 2011; Auerbach et al., 2016). However, at later stages, bacteria (e.g., Bacillus spp.) and molds can contribute to deterioration by increasing temperature, which leads to reduced nutritive value and performance (Wilkinson & Davies, 2013; Auerbach & Nadeau, 2018; Borreani et al., 2018).

Additives such as salt-based propionic acid and ammonium hydroxide have been reported to prevent the formation of unwanted fermentation products and improve the chemical profile. Reported benefits include decreased ethanol concentration and improved aerobic stability (Kung et al., 2000), decreased butyric acid levels (Kung & Ranjit, 2001; Kung et al., 2004), reduced yeast count and dry matter loss (Nadeau, 2007; Knický & Spörndly, 2015), and decreased clostridial spore counts (König et al., 2017).

This study aimed to evaluate the fermentation profile, nutritional quality, microbial count, fermentation losses, and aerobic stability of corn harvested at different maturity stages and ensiled with an organic acid additive (OA).

Material and Methods

Materials and experimental design

The crop was planted on November 17, 2018, with the hybrid P2501 (Pioneer®), a conventional forage hybrid. It was planted in a no-till system with 0.55 m line spacing, a sowing depth of 4 cm, and a distribution of 5 seeds per linear meter. The goal was to achieve a final population of 90,000 plants ha^{-1}. The basic fertilizer, an NPK fertilizer formulated with 8% N, 30% P_2O_5, and 20% K_2O, was applied at a rate of 430 kg ha^{-1}. Urea was applied at a dose of 420 kg ha^{-1} (193 kg of N per ha). The fertilizer was applied during the vegetative stage, when there are 5 fully expanded leaves (V5 stage).

The experimental design used was a randomized block design with two factors (maturity stage and organic acids) in a 3 × 2 factorial arrangement. The three maturity stages were defined using the phenological scale described by Ritchie et al. (1993): early R4 (floury grain) at 122 days after emergence (DAE) and 33% DM; intermedia R4-R5 (transition) at 129 DAE and 40% DM; and late R5 (hard grain) at 135 DAE and 45% DM. The plant production and composition of corn silages harvested at these three stages are listed in Table 1. The use of organic acids (550 g kg^{-1} propionic acid and 120 g kg^{-1}

ammonium hydroxide, Mold-Zap, Alltech, Nicholasville, KY) at the time of ensiling is also presented. Each maturity x OA treatment was replicated 4 times, with each repetition represented by a PVC laboratory silo (50 cm high and 10 cm in diameter), aiming to obtain 700 kg m^{-3} as fed (AF). For each evaluation, ten whole plants in the useful area of each of the four plots were harvested at a height of 20 cm from the ground using the triple pairing method, where plants were marked with ribbons at harvest, allowing similar plants, based on their development at the previous harvest, to be harvested at a later stage. The harvested plants were minced in a Nogueira PN-Plus 2000 stationary ensiling machine, with a particle size range of 8 to 12 mm. Sequentially, organic acids were applied and homogenized in the chopped forage. The OA was applied with the aid of a manual sprinkler at a dose of 8 g kg^{-1} DM with organic acids. The control treatment underwent the same procedure, but it was sprayed with distilled water.

Table 1
Production and composition of corn silages harvested at three maturity stages

Item	Early maturity	Medium maturity	Late maturity
Yield of Dry matter, kg ha^{-1}	31822	32418	33616
Plant height, m	2.33	2.34	2.33
Ear height, m	1.20	1.19	1.20
Number of green leaves	13	10	8
Number of senescent leaves	1	4	6
Stalk %	19.24	18.34	16.60
Leaves %	20.40	18.97	16.60
Bracts + cob %	14.85	12.29	11.31
Grains %	45.51	50.43	55.5

Dry matter loss and aerobic stability

After 60 days of fermentation, the silos were opened and DM losses were determined by measuring the weight of the PVC silos before and after ensilaging, according to the methodology described by Neumann et al. (2007).

Aerobic stability assessments of the silages were obtained by measuring temperature, pH, DM, and ash content immediately after opening the silos on their respective dates. In each silo, the silage was decompressed to expose the ensiled material to air, as described by Kung et al. (2000). A 500-g sample of the material was placed in 1-kg buckets. The buckets were stored in a room with constant temperature at 25 ± 1 °C during the evaluation period. To determine aerobic stability, we measured

the temperature of the silages directly in the buckets using a long-stem digital thermometer inserted into the center of the mass. Temperature and pH measurements were taken during the first 48 hours at 4-hour intervals (06:00h; 10:00h; 14:00h; 18:00h; 22:00h; 02:00h) and thereafter every 8 hours (06:00h; 14:00h; 22:00h). The pH measurements were taken using a digital potentiometer according to the methodology established by Cherney and Cherney (2003). The aerobic stability breakpoint was defined as the point at which the silage temperature exceeded the ambient temperature by 2 °C, as reported by Taylor and Kung (2002).

Fermentative profile

A sample of 15 g was collected when the silos were opened and homogenized with 250 mL of distilled water in a blender. After blending for one minute, the sample was filtered through a four-layer cloth. The liquid fraction was then centrifuged at 2,000 rpm for 15 minutes. A 2-mL aliquot of the supernatant was then removed and stored in an Eppendorf tube. The lactic acid concentration was determined according to the methodology described by Pryce (1969).

From the same sample material, the ethanol, acetic, propanoic, butyric, valeric, and isovaleric acids concentrations were determined by gas chromatography using a Shimadzu© GC-2010 Plus chromatograph equipped with an AOC-20i automatic injector, a Stabilwax-DA™ capillary column (30 m, 0.25 mm ID, 0.25 µm df, Restek©), and a flame ionization detector (FID). Afterwards, it was acidified with 1 M phosphoric acid P.A. (Ref. 100573, Merck©), fortified with the WSFA-2 standard (Ref. 47056, Supelco©), and injected at a 40:1 split ratio using helium as the carrier gas at a linear speed of 42 $cm.s^{-1}$, achieving separation of the analytes in an 11.5-minute chromatographic run. The injector and detector temperatures were 250 ºC and 300 ºC, respectively, and the initial column temperature was 40 ºC. The column temperature ramp began with a gradient of 40 to 120 °C at a rate of 40 °C min^{-1}, followed by gradients of 120 to 180 °C and 180 to 240 °C at rates of 10 and 120 °C min^{-1}, respectively. The temperature was maintained at 240 °C for an additional 3 minutes. To quantify the analytes, the method was calibrated using dilutions of the WSFA-2 standard (Ref. 47056, Supelco©), glacial acetic acid (Ref. 33209, Sigma-Aldrich©), and HPLC-grade ethanol (Ref. 459828, Sigma-Aldrich©), which were analyzed under the aforementioned conditions. Peak detection and integration were performed using GCsolution v. 2.42.00 (Shimadzu©).

The $N\text{-}NH_3$ concentration was measured through colorimetry following the indophenol blue technique (Weatherburn, 1967). Samples were first thawed at 4°C, then centrifuged at 13,000 × g for 15 minutes at the same temperature. Next, 1 mL of each sample was combined with 5 mL of a phenol and sodium nitroprusside solution and 4 mL of a NaClO and NaOH solution. This mixture was incubated in a water bath at 39°C for 15 minutes. Subsequently, absorbance was recorded at 625 nm using a spectrophotometer. Calculations were calibrated against a curve prepared from a 50 mM ammonium sulfate solution.

Microbiological counts

Immediately after opening the silos, a sample was collected for microbiological analysis. For this analysis, 25 g of fresh silage was collected and diluted in 225 mL of sterile saline solution (49.67 g of NaCl in 1,000 mL of H_2O). Serial dilutions were made (10^{-1} to 10^{-6}) to count fungi (yeasts and molds). Glass plates containing dichloran rose bengal chloramphenicol agar (DRBC agar) were incubated at 30 °C for 72 h before counting colony-forming units (CFUs). The CFU values were converted to a logarithmic scale (log10) and expressed as log CFU g^{-1} silage.

Nutritional quality

After opening each silo, a sample of 500 g was collected, weighed, and dried in a forced air oven at 55°C until constant weight. Then, the samples were ground in a Wiley mill with a 1 mm mesh sieve. The pre-dried, ground samples were analyzed for total dry matter in an oven at 105 °C and for ash by incineration at 550°C for 4 h. The crude protein (CP) content was determined using the micro-Kjeldahl method (Association of Official Analytical Chemists, 1995). The content of neutral detergent fiber (aNDF) was obtained using thermostable amylase (Van Soest et al., 1991). The acid detergent fiber (ADF) content was determined according to the method of Goering and Van Soest (1970). Both fiber fractions were expressed inclusive of residual ash. Lignin (sa) was subsequently determined using the methods of Goering and Van Soest (1970). Hemicellulose was estimated by the difference between the NDF and ADF, and cellulose was estimated by the difference between the ADF and lignin (sa). Total digestible nutrients (TDN) were obtained using the equation suggested by Sniffen et al. (1992). Water-soluble carbohydrate (WSC) content was determined by colorimetry after reacting with anthrone reagent (Thomas, 1977).

In situ ruminal degradation kinetics of DM

The ruminal digestibility of neutral detergent fiber (NDF-D) was estimated using the *in situ* technique. Two adult bulls with permanent rumen cannulas were kept in individual stalls with *ad libitum* access to water and corn silage. Approximately 5 g of each sample, dried and ground to 1 mm, was weighed and placed in 12 × 8 cm nylon bags with 50 µm pores for subsequent rumen incubation for 168 h (Nocek, 1988). After removing the bags from the rumen, the samples were washed with iced water and dried in a forced air oven at 55 °C for 48 h. The aNDF was determined from the residue according to the Van Soest et al. (1991) assay, which used a heat-stable amylase, and was expressed inclusive of residual ash. All procedures involving animals were approved by the ethics committee on the use of animals in experiments under approval number 002/2019 - CEUA / UNICENTRO.

The ruminal degradability of dry matter was estimated using the *in situ* technique. Five grams of the sample were dried, ground to 1 mm, weighed, and placed in 12 × 8 cm nylon bags with 50 µm pores for subsequent rumen incubation (Nocek, 1988). The evaluated incubation times were 0, 2, 4, 6, 12, 24, 36, 48, 72, and 96 h. After removal, the samples were washed with iced water

and dried in a forced air oven at 55 °C for 48 h. The weight was recorded, and the dry matter disappearance was calculated. Dry matter degradation parameters were estimated using the exponential equation proposed by Ørskov and McDonald (1979):

$$DMD = a + b(1 - e^{-ct})$$

where: DMD is the *in situ* DM disappearance (as % of DM incubated) at incubation time t (h), a is the intercept representing the fraction (%) that disappears instantly at t = 0, b is the potentially degradable fraction (%), and c is the fractional degradation rate (per h).

The effective degradability (ED) of dry matter in the rumen was calculated as suggested by Ørskov and McDonald (1979):

$$ED = a + b \times [c/(c + k)]$$

Where: k is the fractional passage rate of digesta through the rumen, and is assumed to be 4% or 6% per h for two levels of medium feed intake.

Statistical analysis

For the data analysis, the following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \delta_k + \varepsilon_{ijk}$$

Where: Y_{ijk} is the observation for each variable related to maturity stage *i* from additive (OA) *j* in plot *k*; μ is the overall mean; α_i is the effect of maturity stage *i* (*i* = early, medium or late); β_j is the effect of additive (OA) *j* (*j* = with or without OA); γ_{ij} is the interaction of maturity stage *i* with additive (OA) *j*; δ_k is the blocking effect of plot *k* (*k* = 1, 2, 3, 4) and ε_{ijk} is the random error associated with each observation of Y_{ijk}. General linear models (PROC GLM) in SAS were used for analysis of variance (ANOVA), and Tukey's test was applied for multiple comparisons of means at a 5% significance level.

Results

Fermentation profile, microbial counts, and dry matter loss

The pH levels observed at silo opening were affected (P<0.01) only by maturity stage (Table 2), with more mature forage resulting in silage with a higher pH. Neither the use of OA nor the maturity stage influenced the concentration of acetic acid, ethanol, or mold counts.

Table 2
Dry matter, fermentation profile, microbial counts, and aerobic stability of corn silages harvested at three maturity stages and treated with organic acids (OA)

Item	Early maturity		Medium maturity		Late Maturity		SEM	*P*-value		
	OA	Control	OA	Control	OA	Control		A	M	A × M
Dry matter, g kg^{-1} AF	356^{c}	410^{b}	425^{b}	461^{a}	465^{a}	466^{a}	3.440	<0.01	<0.01	<0.01
pH	3.83^{c}	3.83^{c}	3.84^{c}	3.86^{bc}	3.94^{a}	3.92^{ab}	0.207	0.89	<0.01	0.32
Lactic acid, g kg^{-1} DM	90.0^{a}	51.7^{c}	72.8^{b}	69.7^{b}	33.7^{d}	51.6^{c}	2.894	<0.01	<0.01	<0.01
Acetic acid, g kg^{-1} DM	5.24	4.87	4.60	4.94	4.34	3.74	0.653	0.59	0.10	0.58
Propionic acid, g kg^{-1} DM	2.79^{a}	0.28^{c}	1.65^{b}	0.21^{c}	0.81^{bc}	0.12^{c}	0.598	<0.01	<0.01	<0.01
Butyric acid, g kg^{-1} DM	0.14	0.29	0.06	0.25	0.07	ND	0.041	<0.01	0.13	0.46
Ethanol, g kg^{-1} DM	4.34	3.58	2.36	3.90	2.99	2.50	0.739	0.81	0.08	0.07
NH_3-N, g kg^{-1} N	105^{a}	81.8^{c}	90.7^{bc}	64.4^{d}	97.3^{bc}	51.8^{e}	3.318	<0.01	<0.01	<0.01
Yeast, $\log_{10}$ CFU g^{-1}	6.03^{b}	7.29^{a}	5.77^{b}	6.12^{b}	3.50^{c}	6.13^{b}	0.250	<0.01	<0.01	<0.01
Mold, $\log_{10}$ CFU g^{-1}	< 2	< 2	< 2	< 2	< 2	< 2	-	-	-	-
DM loss, g kg^{-1} DM	44.8^{b}	94.0^{a}	60.7^{b}	61.0^{b}	48.6^{b}	100^{a}	9.412	<0.01	0.04	<0.01
Aerobic stability, h	32.0^{c}	32.0^{c}	36.0^{c}	36.0^{c}	148^{a}	112^{b}	9.520	0.04	<0.01	0.02

OA: with organic acids; Control: without organic acids.
SEM: standard error of the mean; A: additive (OA) effect; M: maturity effect; A × M: interaction between additive (OA) and maturity.

The silage DM content, the concentrations of lactic acid, ammonia, and propionic acid, as well as DM loss, yeast counts, and aerobic stability were influenced (P<0.01) by the interaction between the OA and maturity stage (P<0.05; Table 2). The DM content increased by maturity stage, but a difference related to OA use was only observed (P<0.01) in early-harvested silage. Lactic acid concentration decreased (P<0.01) according to maturity stage in the treated group, but it was highest at medium maturity in the control group. Compared to control silage at the same maturity stage, the lactic acid concentration was higher in early-maturity silage with OA use, similar in medium-maturity silage, and lower in late-maturity silage. NH_3-N was lower in the control silage than in the OA silage at all three maturity stages and decreased as harvest was delayed. Propionic acid concentration decreased from early to late maturity and was significantly higher in OA silage at early- and medium-maturity stages, and higher at the late-maturity stage. Butyric acid concentration was lower in OA-treated silages harvested at the early- and medium-maturity stages compared to control silage. At the late stage, butyric acid was only detected in OA silage; however, values were lower than those reported for silage harvested at earlier stages.

Aerobic stability

The microbial count was affected by the OA. Yeast counts and DM losses were lower in OA silage harvested at early and late maturity stages, but not different between control and OA silage at the medium-maturity stage. Heating upon air exposure was lower in late maturity OA silage; however, silages harvested at earlier stages heated faster than late-harvested silage. The pH upon aerobic exposure did not change over time in OA silage harvested at medium- and late-maturity stages (Figure 1). In early harvested OA silage, the pH actively increased after 104 h. The pH of control silages started to increase (P<0.01) at 56, 88, and 104 h after aerobic exposure in early, medium, and late-harvested silages, respectively.

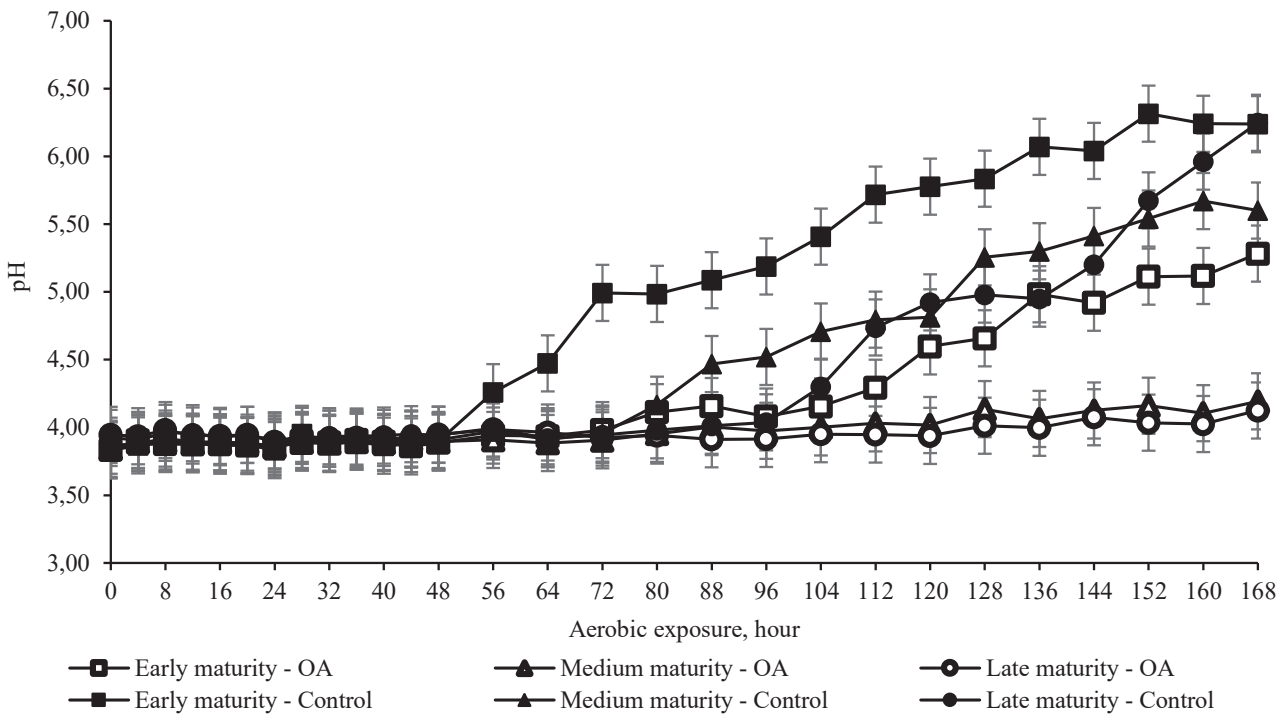

Figure 1. pH values of corn silages harvested at three maturity stages and treated with organic acids (OA) during aerobic exposure.
OA: with organic acids; Control: without organic acids.
SEM: 0.207; P < 0.01 for additive (OA) effect; P < 0.05 for maturity effect; P < 0.01 aerobic exposure length; P = 0.74 for interaction additive (OA) × maturity; P < 0.01 for interaction additive (OA) × aerobic exposure length; P < 0.01 for interaction maturity × aerobic exposure length; P < 0.01 for interaction additive (OA) × maturity × aerobic exposure length.

Nutritional quality

The CP and NDF contents were affected (P<0.01) only by maturity stage, not by their interaction (Table 3). Ash content was similar among maturity stages.

Table 3
Nutritional composition of corn silages harvested at three maturity stages and treated with organic acids (OA).

Item	Early maturity		Medium maturity		Late Maturity		SEM	*P*-value		
	OA	Control	OA	Control	OA	Control		A	M	A × M
Ash, g kg^{-1} DM	24.7	21.7	23.6	23.3	22.3	21.4	0.846	0.06	0.15	0.26
aNDF, g kg^{-1} DM	545^{a}	548^{a}	525^{b}	524^{b}	498^{c}	498^{c}	2.734	0.11	<0.01	0.18
ADF, g kg^{-1} DM	250^{a}	250^{a}	224^{b}	240^{a}	188^{c}	182^{c}	2.920	<0.01	<0.01	<0.01
HEM, g kg^{-1} DM	295^{b}	298^{b}	309^{ab}	285^{b}	310^{ab}	316^{a}	3.621	0.51	<0.01	0.02
CEL, g kg^{-1} DM	209^{a}	207^{a}	182^{b}	200^{a}	153^{c}	136^{c}	3.019	0.04	<0.01	<0.01
Lignin (sa), g kg^{-1} DM	41^{bc}	44^{ab}	42,5^{b}	40^{bc}	35^{c}	46^{a}	1.244	<0.01	0.31	<0.01
NDF-D, g kg^{-1} NDF	815^{a}	806^{ab}	807^{ab}	821^{a}	829^{a}	786^{b}	5.108	<0.01	0.44	<0.01
WSC, g kg^{-1} DM	34.3^{b}	28.8^{bc}	28.2^{c}	26.3^{c}	47.2^{a}	30.7^{c}	2.866	<0.01	<0.01	<0.01
CP, g kg^{-1} DM	61.2^{a}	60.5^{a}	58.5^{ab}	55.7^{b}	59.5^{ab}	57.7^{b}	1.118	0.07	<0.01	0.67
TDN, g kg^{-1} DM	696^{b}	693^{b}	710^{a}	696^{b}	713^{a}	712^{a}	2.043	<0.01	<0.01	<0.01
In situ ruminal degradation kinetics of DM										
a, g kg^{-1} DM	435^{c}	427^{c}	475^{a}	430^{c}	462^{ab}	443^{bc}	6.159	<0.01	<0.01	<0.01
b, g kg^{-1} DM	251^{bc}	255^{b}	275^{b}	283^{a}	293^{a}	234^{c}	6.386	<0.01	<0.01	<0.01
U, g kg^{-1} DM	315^{a}	318^{a}	268^{bc}	287^{b}	245^{c}	322^{a}	7.418	<0.01	<0.01	<0.01
c, % per h	3.81	4.25	3.29	4.12	3.29	3.45	0.361	0.06	0.03	0.43
ED 4% per h, g kg^{-1} DM	557^{b}	558^{b}	590^{a}	574^{ab}	593^{a}	551^{b}	9.276	<0.01	<0.01	0.01
ED 6% per h, g kg^{-1} DM	532^{b}	533^{b}	565^{a}	546^{ab}	565^{a}	529^{b}	8.962	<0.01	<0.01	0.03

OA: with organic acids; Control: without organic acids.
DM: dry matter; aNDF: neutral detergent fiber; ADF: acid detergent fiber; HEM: hemicellulose; CEL: cellulose; Lignin (sa): lignin determined by solubilization of cellulose with sulfuric acid; NDF-D: neutral detergent fiber digestibility; WSC: water-soluble carbohydrates; CP: crude protein; TDN: total digestible nutrients; a: soluble fraction; b: potentially degradable fraction; U: undegradable fraction; c: fractional degradation rate; ED: effective degradability
SEM: standard error of the mean; A: additive (OA) effect; M: maturity effect; A × M: interaction between additive (OA) and maturity.

The ADF, HEM, CEL, lignin, WSC, and NDF-D were affected (P<0.05) by the interaction between the additive and the maturity stage. In general, the ADF and CEL contents decreased due to delayed harvest. The ADF and CEL were higher with the use of additives in silage harvested at medium maturity. The HEM increased as DM increased, and the highest value was observed in the control silage harvested at the late stage. Differences in lignin content due to OA use were only observed in silages ensiled at the late maturity stage; silages with OA had lower lignin content than the control silage. Using the additive resulted in higher residual WSC content in silage harvested at late maturity stages with OA. The TDN content increased in late-maturity silage compared to early-maturity silage. In medium-maturity control silage, the TDN content was comparable to the values in the early stage but lower than the silage with OA at the same maturity stage. NDF-D did not differ in delayed harvest silage with OA, but it was lower in control silage harvested at late stages than in silage harvested at earlier stages.

We observed a significant interaction between the additive and the maturity stage for most variables related to the in situ rumen degradation kinetics of DM. The a and b fractions, as well as the ED, were higher in OA silages, regardless of maturity stage. Conversely, the U fraction was lower in medium and late-maturity silages. Conversely, the fractional degradation rate (c) was only affected by the maturity stage (P < 0.05).

Discussion

Fermentation profile, microbial counts, and dry matter loss

In general, the fermentation end-products were similar to those reported by other authors (Bernardi et al., 2019; Bueno et al., 2020). As expected, because the additive presented propionic acid and ammonium hydroxide in its formulation, silages with OA had higher amounts of these antifungal substances (Halverson & Emerick, 1982; Li et al., 1992; Ranjit & Kung, 2000; Kung et al., 2000). However, ethanol production was not significantly affected by the use of the ammonium hydroxide-based additive or by the corn maturity stage, and remained at low levels. The lack of ethanol reduction cannot be attributed solely to high yeast counts because other microorganisms also produce ethanol. Despite lower lactic acid concentrations at more advanced maturity stages, ethanol formation did not follow this trend, indicating that multiple factors, such as microbial diversity, substrate availability, and physicochemical conditions, influence its production. Therefore, the additive's effect on ethanol production is limited and depends on the complexity of the microbiota and the fermentation environment (Bernardi et al., 2019; Pinto et al., 2020). Nonetheless, all silages exhibited satisfactory lactic acid accumulation, resulting in low pH values (< 4.00). Compared to the control silage, the OA silages showed reduced DM loss in both early- and late-harvested silages. Additionally, they exhibited a delayed pH rise when exposed to aerobic conditions (< 168 hours) and increased in situ rumen degradability in medium and late-harvested silages.

Silage pH was influenced only by maturity stage, while lactic acid concentration was affected by the additive-maturity stage interaction. As expected, lower lactic acid content was associated with higher pH values since lactic acid is the main acid responsible for pH reduction during fermentation (Kung et al., 2018). OA silage harvested at late maturity showed lower lactic acid content than the control despite having lower yeast counts, indicating reduced lactic acid bacteria (LAB) development. This can be explained by the lower availability of water-soluble carbohydrates and reduced moisture content, which is essential for LAB growth (Rooke & Hatfield, 2003; Pahlow et al., 2003). The addition of ammonium hydroxide may have also limited LAB counts, as reported by Kung et al. (2000). Therefore, the combination of low moisture and ammonium hydroxide in OA silage likely restricted LAB growth at late maturity. In earlier stages of maturity, however, moisture was sufficient for effective LAB development despite the presence of ammonium hydroxide. Moreover, the additive containing ammonium hydroxide increased NH_3-N levels within the acceptable range for corn silage (100 g kg^{-1} N), which helps control mold growth after the silo is opened (Kung et al., 2018).

Butyric acid was detected in nearly all of the silages evaluated here, with concentrations similar to those reported by other researchers (Filya, 2004; Selwet, 2009; Bernardi et al., 2019). A butyric acid content greater than 5 g kg^{-1} DM may indicate high clostridial activity. However, bacilli and yeasts can also produce butyric acid in silages (McDonald et al., 1991; Muck, 2010; Kung et al., 2018). In general, the additive reduced butyric acid in our silages. Similar results were found by Kung et al. (2004) and Selwet (2009) when propionic acid was added to silage.

Under anaerobic conditions, yeasts consume nutrients, which increases DM losses in silages, as observed in our trial for the control silages (Rooke & Hatfield, 2003). Therefore, the OA silages had higher lactic acid content, which provided better conservation conditions, resulting in higher residual soluble sugar contents and lower DM losses in the early and late stages. Indeed, the residual soluble sugar content in OA silages was 33% higher, and the DM losses were 41% lower. Unexpectedly, ethanol production was not influenced by OA use in our trial. Although yeasts are considered the primary producers of ethanol in silages, an ethanol concentration above 40 g kg^{-1} DM may indicate an extensive yeast activity in silage (Kung et al., 2018). The average ethanol concentration was 3.3 g kg^{-1} DM, regardless of OA use. However, ethanol is not only formed by yeasts. In silages, enterobacteria, heterofermentative LAB, bacilli, and even clostridia may contribute to ethanol accumulation during fermentation (McDonald et al., 1991).

The variations observed in the DM content of the silage, relative to the DM content of the plant at harvest, resulted from the fermentation profile and DM losses occurring during storage, influenced by the maturity stage, treatment with OA, and other factors. According to Rabelo et al. (2012), the occurrence of losses and the use of the acid causes changes in DM content and nutritional composition, as well as significant differences in organic acid production.

Aerobic stability

Yeast counts in the silages were higher than 5 log CFU g-1, indicating a high susceptibility to aerobic deterioration (Kung et al., 1998). After aerobic exposure, yeasts consume residual sugars and lactic acid through respiration, increasing silage pH and temperature, enabling the development of a wide range of opportunistic aerobic microorganisms, and reducing the nutritional quality of the silage (Wilkinson & Davies, 2013; Wang et al., 2016; Bernardi et al., 2019).

The pH measurement during aerobic exposure is considered an effective indicator of aerobic stability because pH values are primarily influenced by microbial activity, specifically lactic acid consumption. However, silage temperature may be affected by environmental temperature (Oliveira et al., 2018; Bernardi et al., 2019). Volatile fatty acids (VFAs), such as acetic and propionic acids, as well as ammonia, are considered antifungal substances that protect silage against yeast spoilage during aerobic exposure (Danner et al., 2003; Kung et al., 2000).

Propionic acid is a useful additive for preserving silage due to its bacteriostatic and antifungal properties. It works by directly inhibiting the growth of various microorganisms, including lactic acid bacteria and yeasts commonly found in silage. Propionic acid disrupts cellular functions and lowers the pH, reducing the growth of spoilage organisms and improving aerobic stability (Zhang et al., 2015). Compared to untreated silage, silages treated with propionic acid had better pH control during aerobic exposure, especially those harvested at medium and late stages. However, the temperature rise was only delayed in silages harvested at the late maturity stage.

Nutritional quality

The decrease in fiber components with advancing plant maturity was more significant for NDF and ADF. As plants mature, there is an increased accumulation of starch in the grains (Table 1), thereby reducing the proportion of fibrous components and increasing the DM content (Neumann et al., 2007). However, the cell wall compounds are not used as fuel during silage fermentation, as silage bacteria lack the enzymes necessary to ferment these compounds for energy metabolism (Rooke & Hatfield, 2003). Additionally, protein loss in silage is minimal due to the proteolytic activity of bacterial and plant enzymes, which can diminish protein fractions (e.g., prolamins) (Rooke & Hatfield, 2003; Hoffman et al., 2011; Silva et al., 2019). Delaying harvest typically increases starch accumulation in kernels and reduces fiber and crude protein content (Hunt et al., 1989; Ferraretto & Shaver, 2012; Bueno et al., 2020). Higher WSC and cell wall constituents in OA silage indicate lower yeast activity. Hemicellulose may degrade in acidic conditions (Dewar et al., 1963); thus, the lower pH observed in early-harvested silages is probably the reason for their lower HEM content. Lignin accumulation normally increases in plants over time for structural purposes (Jung et al., 1993; Taiz & Zeiger, 2006).

Regardless of OA use, silages harvested at an early stage had a similar chemical composition and analogous *in situ* rumen degradation kinetics of DM.

Conversely, delaying harvest worsened silage digestibility. Still, the greater DM values in OA silage indicate some ability to recover digestibility. Yeast counts in control silage remained unchanged, whereas yeast counts in OA silage were lower than in control silage. Further, we observed higher residual sugar content and lower lignin content, as well as DM loss, in OA silage harvested at late stages. At the medium maturity stage, ADF was lower in OA silage, despite having a similar residual sugar content and DM loss. Taken together, these results likely explain the higher ED values in mature silages compared to those harvested early.

The greater contribution of fractions a and b, as well as the consequent increase in ED observed in silages treated with the OA additive regardless of maturity stage, indicates that OA promotes higher nutrient availability for rumen microorganisms. This improvement in degradability can be attributed to OA's ability to preserve residual sugars and reduce less digestible fibrous compounds, particularly in silages harvested at the medium and late stages when these fibrous compounds typically limit digestion. The decrease in the undegradable fraction (U) at these stages further supports the recovery of silage quality. The increase in ED of mature silages implies greater feed utilization efficiency by animals, which can lead to improved dry matter intake and enhanced productive performance. Additionally, reduced yeast counts and DM losses point to better fermentation conditions and silage preservation with the OA additive (Senger et al., 2005; Neumann et al., 2021).

The antifungal additive based on buffered propionic acid and ammonium hydroxide is more effective in corn silages that are harvested at the medium and late maturity stages (DM >350 g kg^{-1}). At these stages, the additive controls yeast, reduces DM losses, delays aerobic deterioration, and improves rumen degradability. At the early maturity stage, the additive's effect on aerobic stability is inconsistent, possibly due to higher moisture content and microbial activity. It is preferably recommended for intermediate to advanced maturities, with application adjusted according to management for optimal results.

Conclusions

This study highlights the effectiveness of an antifungal additive containing organic acids derived from propionic acid and ammonium hydroxide in controlling yeast and reducing dry matter losses, which depends on the maturity of the corn plant. The additive improved the fermentation profile and rumen degradability in silages with dry matter content exceeding 350 g kg^{-1}, demonstrating a consistent impact on aerobic stability during the medium and late maturity stages. Therefore, using the additive is recommended during these stages, with application tailored to specific management practices.

Conflicts of Interest

The authors declare no conflicts of interest.

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