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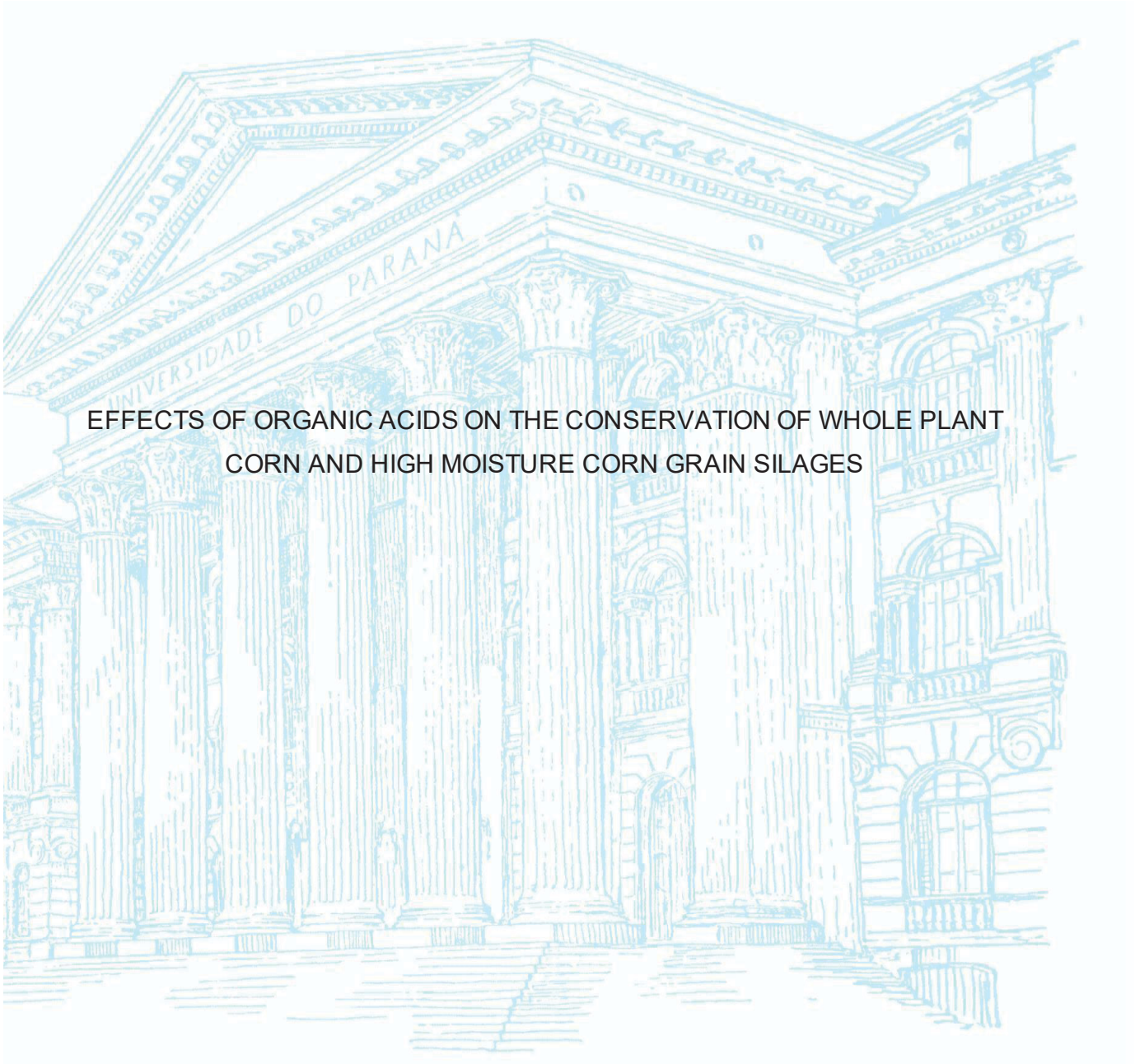

EFFECTS OF ORGANIC ACIDS ON THE CONSERVATION OF WHOLE PLANT CORN AND HIGH MOISTURE CORN GRAIN SILAGES

CURITIBA

2025

JULIANA APARECIDA DE ASSIS

EFFECTS OF ORGANIC ACIDS ON THE CONSERVATION OF WHOLE PLANT CORN AND HIGH MOISTURE CORN GRAIN SILAGES

Tese apresentada ao Programa de Pós-Graduação em Zootecnia, Setor De Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Zootecnia.

Orientador: Prof. Dr. Patrick Schmidt

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ZOOTECNIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **JULIANA APARECIDA DE ASSIS**, intitulada: **EFFECTS OF ORGANIC ACIDS ON THE CONSERVATION OF WHOLE PLANT CORN AND HIGH MOISTURE CORN GRAIN SILAGES**, sob orientação do Prof. Dr. PATRICK SCHMIDT, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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Aos meus pais, Maria Assis e José Sebastião de Assis.

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Gratidão à vida!

RESUMO

O objetivo deste estudo foi avaliar os efeitos de uma mistura de ácidos orgânicos na silagem de planta inteira de milho e na silagem de grão úmido de milho armazenadas por diferentes períodos, além de analisar o impacto do ácido propiônico na silagem de milho sob duas densidades de compactação. Para isso, a tese foi composta por três experimentos. Experimento I - Silagem de planta inteira de milho com os seguintes tratamentos: Controle (C) – sem aditivo; Anti Fun (AF) – adição de Anti Fun (ácidos acético, propiônico, fórmico e sórbico – Agrifirm® 0,5 L ton^{-1}); Inoculante (LB) – adição de *Lentilactobacillus buchneri* (1,0 x 10^5 ufc/g). Os silos foram armazenados por 15 e 60 dias. O maior tempo de armazenamento resultou em maiores perdas fermentativas e aumentou os teores de PB, PS, FDA e a digestibilidade da FDN e do amido. Por outro lado, silagens armazenadas por 15 dias apresentaram maior contagem de BAL e leveduras, além de maior teor de amido. O tratamento LB resultou na menor contagem de leveduras após 60 dias de armazenamento. O tratamento AF apresentou maior solubilidade proteica e digestibilidade do amido em comparação aos tratamentos C e LB. Experimento II: Silagem de grão úmido de milho com os tratamentos: Controle (C) – sem aditivo; Anti Fun (AF) – adição de Anti Fun (1,0 L ton^{-1}); Ácido propiônico (AP) – adição de ácido propiônico tamponado (1,0 L ton^{-1}). Os silos foram armazenados por 15 e
60 dias. Os ácidos orgânicos reduziram a produção total de gases. Silagens armazenadas por 60 dias tiveram estabilidade aeróbia 53% superior às armazenadas por 15 dias. O tratamento AP apresentou maior estabilidade aeróbia. O tempo de armazenamento reduziu a população de BAL. Os tratamentos AP e C apresentaram maiores teores de proteína solúvel do que as silagens tratadas com AF. Os teores de N-NH_3 e cinzas foram menores em silagens armazenadas por 60 dias. As silagens tratadas com PA apresentaram menores teores de N-NH_3 em comparação às silagens dos tratamentos C e AF. O aumento no tempo de armazenamento elevou a digestibilidade da fração B do amido e a digestibilidade da FDN em 36% e 20%, respectivamente. Experimento III: Silagem de planta inteira de milho com os seguintes tratamentos: Alta densidade (HD) – sem aditivo, controle positivo; Alta densidade com ácido propiônico (HDPA); Baixa densidade (LD) – sem aditivo, controle negativo, simulando uma condição de silo superfície; Baixa densidade com ácido propiônico (LDPA). Os silos foram armazenados por 60 dias. O ácido propiônico e a alta densidade reduziram a produção total de gases. Silagens tratadas com ácido propiônico apresentaram maior teor de MS do que as silagens controle, que, consequentemente, tiveram maiores perdas de MS durante a fermentação. O ácido propiônico e a compactação diminuíram as perdas de MS durante a exposição aeróbia pós abertura. Silagens tratadas com ácido propiônico apresentaram maior aeróbia em comparação às silagens controle. Com base nos dados obtidos nos dos três experimentos, observa- se que o blend de ácidos foi eficiente para algumas variáveis pontuais, mas os tratamentos controles, LB e PA foram mais eficazes na conservação da silagem. O tempo de armazenamento influenciou a composição química e a estabilidade aeróbia. Além disso, o ácido propiônico reduziu as perdas durante a fermentação e após a abertura do silo, independentemente da densidade de compactação.

Palavras-chaves: ácido propiônico; densidade; estabilidade aeróbia; inoculante

ABSTRACT

The objective of this study was to evaluate the effects of an organic acid blend on whole-plant corn silage and high-moisture corn silage stored for different periods, as well as to analyze the impact of propionic acid on corn silage under two compaction densities. To achieve this, the thesis comprised three experiments. Experiment I – Whole-plant corn silage with the following treatments: Control (C) – no additive; Anti Fun (AF) – addition of Anti Fun (acetic, propionic, formic, and sorbic acids – Agrifirm® 0.5 L ton^{-1}); Inoculant (LB) – addition of *Lentilactobacillus buchneri* (1.0 x 10^5 ufc/g forage). The silos were stored for 15 and 60 days. Longer storage time resulted in greater fermentation losses; however, it increased CP, SP, ADF content, and the digestibility of NDF and starch. On the other hand, silages stored for 15 days had higher counts of LAB and yeasts, as well as a higher starch content. The LB treatment resulted in the lowest yeast count after 60 days of storage. The AF treatment showed higher protein solubility and starch digestibility compared to the C and LB treatments. Experiment II – High-moisture corn silage with the following treatments: Control (C) – no additive; Anti Fun (AF) – addition of Anti Fun (1.0 L ton^{-1}); Propionic Acid (PA) – addition of buffered propionic acid (1.0 L ton^{-1}). The silos were stored for 15 and 60 days. Organic acids reduced total gas production. Silages stored for 60 days had 53% higher aerobic stability than those stored for 15 days. The PA treatment showed greater aerobic stability. Storage time reduced the LAB population. The PA and C treatments had higher soluble protein content than silages treated with AF. NH_3-N and ash levels were lower in silages stored for 60 days. Silages treated with PA had lower NH_3-N levels compared to the C and AF treatments. Increased storage time improved the digestibility of starch fraction B and NDF by 36% and 20%, respectively. Experiment III – Whole-plant corn silage with the following treatments: High density (HD) – no additive, positive control; High density with propionic acid (HDPA); Low density (LD) – no additive, negative control, simulating a surface silo condition; Low density with propionic acid (LDPA). The silos were stored for 60 days. Propionic acid and high density reduced total gas production. Silages treated with propionic acid had higher DM content than control silages, which consequently had greater DM losses during fermentation. Additionally, propionic acid and compaction reduced DM losses during aerobic stability. Silages treated with propionic acid had greater aerobic stability compared to control silages. Based on the data obtained from the three experiments, it is observed that the acid blend was effective for some specific variables; however, the control, INO, and PA treatments were more effective in silage preservation. Storage time influenced chemical composition and aerobic stability. Furthermore, propionic acid reduced losses during fermentation and after silo opening, regardless of compaction density.

Keywords: aerobic stability; density; inoculant; microbiology; propionic acid

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SUMMARY

1. INTRODUCTION

Corn silage and its grains are widely used in ruminant nutrition as a source of digestible fiber and readily fermentable energy, especially for high-performance animals (Hoffman et al., 2011; Gheller et al., 2021). Nutrient preservation and improved starch digestion efficiency during the ensiling process are effective strategies to enhance feed utilization by animals. However, losses can occur during fermentation due to undesirable fermentations and after silo opening, when oxygen exposure promotes the growth of aerobic microorganisms, leading to silage quality deterioration.

The preservation of forage quality requires a reduction in cellular respiration after plant cutting and silo sealing, as well as the control over aerobic microorganism activity, combined with predominant fermentation by lactic acid bacteria (McDonald et al., 1991). The use of organic acids at ensiling can help lowering the pH of the medium, improving silage fermentation and inhibiting the action of undesirable microorganisms such as yeasts and fungi (Kung et al., 2018). This can lead to reduced nutrient losses throughout the fermentation process. Additionally, organic acids are known for their antifungal properties, which are crucial for both fermentation and aerobic stability after silo opening (Randby and Bakken, 2021).

Studies in the literature report the efficiency of organic acids for increasing silage aerobic stability (Arthur, 2019), increasing water-soluble carbohydrate concentration (Muck et al., 2018), and inhibiting yeasts growth (Bernardes et al., 2014), and its effects under different dry matter conditions (Huhtanen et al., 2013). However, no studies have investigated their use in pile silos, where ideal bulk density is often not achieved, or their effects on different storage durations in whole-plant corn silage and its grains. Additionally, there is no conclusive information of their impact on starch digestibility or the chemical composition of the silage. Therefore, further studies are needed on the application of organic acids, recommended doses, and the associated costs to better understand their actual influence and effectiveness in silage preservation and animal nutrition.

In recent years, microbial inoculants have received considerable attention, as their function in the ensiling process are essentially the same as those of organic acids (Borreani et al., 2018; Muck et al., 2018; Liu et al., 2024). However, the effectiveness of inoculants can be influenced by various silage characteristics, such as the type of forage used, moisture content, epiphytic microbial community, and water-soluble carbohydrate concentration (Muck et al., 2018). From this perspective, organic acids, in addition to rapidly lowering the pH, are less dependent on forage characteristics, which may lead to lower losses and greater efficiency in the ensiling process. Thus, the aim of this study was to evaluate the effects of a blend of organic acids in whole-plant corn silage and high-moisture corn silage stored for different periods. The impact of propionic acid on corn silage at two compaction densities was also assessed.

2. CHAPTER I - LITERATURE REVIEW

2.1 Silage process

Silage is one of the main ingredients in the diets of domestic ruminants and has been widely studied to develop increasingly innovative and viable management practices. There is a great diversity of forage plants used for silage. However, intrinsic and extrinsic factors of the plants, such as DM content, soluble carbohydrates, anaerobiosis, buffering capacity, among others (Kung et al., 2018), are essential for achieving the desired fermentation of the ensiled material.

The fermentation process consists of the consumption of soluble carbohydrates present in the forage by enterobacteria and lactic acid bacteria under anaerobic conditions, resulting in the formation of organic acids (Jobim and Nussio, 2013). The fermentation process and the proliferation of lactic acid bacteria promote a reduction in the pH (Kung et al., 2018). This pH reduction contributes to controlling the development of undesirable microorganisms, such as clostridia, enterobacteria, fungi, and yeasts, which are responsible for the loss of nutritional value of the ensiled material during fermentation and after silo opening (Muck et al., 2018). Thus, the faster and more efficient the production of lactic and acetic acids, the lower the losses in the fermentation process (Rodrigues et al., 2014).

The biochemical pathways through some organisms metabolize plant soluble carbohydrates were described by Woolford (1984) and later adapted by McDonald et al. (1991), with an emphasis on fermentations carried out by lactic acid bacteria (homo and heterofermentative), clostridia, and yeasts (Table 2.1.1). Fermentation promoted by lactic acid bacteria is desirable, as they are capable of producing organic acids, such as lactic and acetic acids, without causing significant losses during the process. On the other hand, fermentation by bacteria of the genus *Clostridium* and by yeasts are considered undesirable, as they utilize soluble carbohydrates to produce mainly butyric acid (a weak acid), ethanol, and CO_2, and are also associated with greater losses of dry matter and energy in the ensiled material.

Table 2.1.1 – Biochemical pathways and losses of DM and energy during silage fermentation (Adapted from McDonald et al., 1991)

Organism	Pathway	Substrate	Products	Loss (% substrate) DM	Gross energy
LAB	Ho	Glucose	2 lactates	0	0.7
LAB	He	Glucose	1 lactate, 1 ethanol, 1 CO_2	24	1.7
LAB	He	3 Fructose	1 lactate, 1 acetate, 2 mannitol, 1 CO_2	4.8	1.0
LAB	Ho/He	2 Citrate	1 lactate, 3 acetate, 3 CO_2	29.7	-1.5
LAB	Ho/He	Malate	1 lactate, 1 CO_2	32.8	-1.8
Enterobacteria		2 Glucose	2 lactates, 1 acetate, 1 ethanol, 2 CO_2	17	11.1
Clostridia		2 Lactate	1 butyrate, 2 CO_2, 2 H_2	51.1	18.4
Yeasts		Glucose	2 ethanol, 2 CO_2	48.9	0.2

LAB = lactic acid bacteria; Ho = homofermentative; He = heterofermentative.

Within context, silage can exhibit different profiles and concentrations of fermentative products, depending on the type of microorganism acting on the ensiled material during the fermentation process. The presence of organic acids in the ensiling process can help to inhibit undesirable fermentations and preserve nutrients that could otherwise be used as substrates by fermentative microorganisms (Pordeus et al., 2022). Although the potential of these acids as additives in silage has been known for decades (McDonald et al., 1991), their use is still incipient in Brazil.

2.2 Factors affecting the fermentation process of silage

The most desirable group of microorganisms during the fermentation process is LAB, as they efficiently convert soluble carbohydrates into lactic acid, leading to lower DM losses (Table 2.1.1). The concentration of water-soluble carbohydrates in the ensiled plant is a critical factor for the success of fermentation process (Borreani et al., 2018). Various forage species can be used for silage production, however, corn stands out for naturally meeting the basic requirements for efficient fermentation, as well as being a crop with high energy content compared to other forages (Jobim and Nussio, 2013). For effective fermentation, it is estimated that the plant should have a minimum water-soluble carbohydrates concentration of >2.5% on a DM basis (Piltz and Kaiser, 2004). Despite variations in carbohydrate concentration due to the stage of maturity at harvest, the levels

typically found in corn generally exceed this minimum threshold (Jobim and Nussio, 2013), ensuring adequate conditions for fermentation.

The fermentation process in silage occurs efficiently after all residual oxygen in the silo has been consumed (McDonald et al., 1991). This oxygen consumption is driven by the plant's cellular respiration and the activity of aerobic microorganisms, which utilize nutrients and energy from the ensiled forage through chemical and biological reactions, resulting in the production of CO_2, H_2O, free ammonia, and heat (Queiroz et al., 2018). Because of these activities, silage temperature increases. According to Adesogan and Newman (2010), an increase of up to 12°C above the initial forage temperature is considered normal. However, heat production is associated with nutrient and dry matter losses. Borreani et al. (2018) indicate that although respiration and the activity of aerobic microorganisms contribute to residual oxygen consumption and heat generation, cellular respiration is the primary driver of these processes. The authors also report that high temperatures can cause detrimental effects, such as protein denaturation (Ashbell et al., 2002) and an increase in NH3-N levels in the silage (Hoffman et al., 2011). Additionally, overheating may lead to reduced feed intake and digestibility, as well as darkening of the ensiled material (Borreani et al., 2018).

Residual oxygen in silage is directly related to silo density. Thus, the greater the efficiency in compacting the ensiled material, the lower the residual oxygen levels in the silo, resulting in reduced nutrient and DM losses caused by cellular respiration and activity of undesirable microorganisms (Liu et al., 2024). According to Borreani et al. (2018), porosity is defined as the volume of air spaces in the silage as a fraction of the volume of the silo. These authors demonstrated that reducing porosity is associated with increased density, particularly within a DM content range of 30-40% (Figure 2.1.1). DM levels above this range compromise the ensiling process, as they negatively impact particle size and compaction efficiency. Plants with higher DM levels exhibit higher fiber content and reduced adhesion between particles during compaction, increasing porosity and, consequently, the residual oxygen levels within the silo (Kung et al., 2018). Conversely, DM levels below this range increase water content in the silo, facilitating undesirable fermentations, such as those caused by *Clostridium* species (Muck et al., 2018). These

fermentations lead to greater fermentative losses, reduced DM, and excessive effluent production (Queiroz et al., 2018).

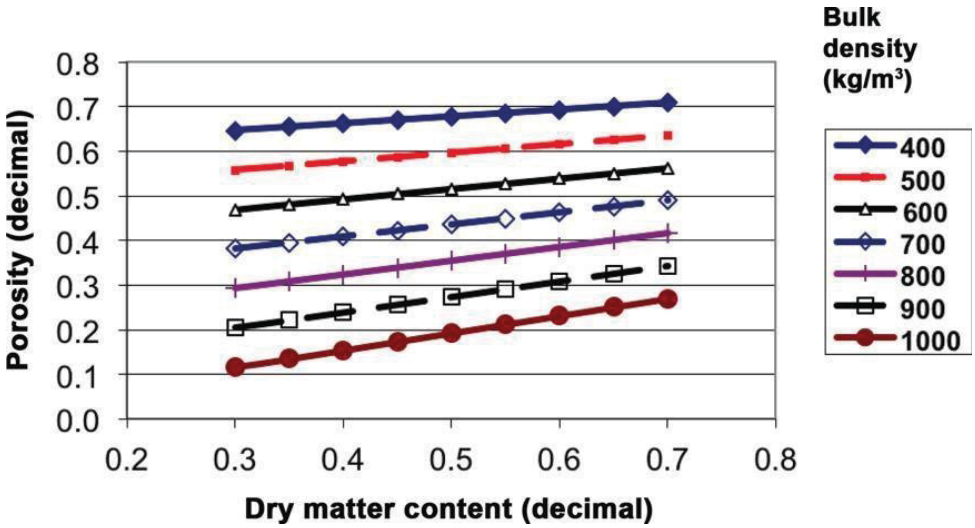

Figure 2.1.1 – Porosity as a function of DM content and bulk density of silage (Borreani et al., 2018).

Part of the DM of ensiled forage can be lost due to gas production during the fermentation process (Table 2.1.1). According to Schmidt et al. (2011), gas production can exceed 90% of total DM losses. In this context, reducing residual oxygen in the silo, achieved through higher silage density, contributes to mitigating DM losses during the ensiling process. The highest gas production occurs within the first hours after sealing the silo in well-processed silages (Schmidt et al., 2012). Besides residual oxygen, differences in the soluble carbohydrate content of the ensiled plant can also be a significant factor influencing the volume of gases generated during fermentation (Assis et al., 2024). Souza (2015) evaluated the gas volume produced by different forages under similar methodological conditions and reported gas productions of 4.9 and 29.1 L kg^{-1} DM for corn and sugarcane silages, respectively, under similar bulk densities of 614.7 and 622.7 kg FM m^{-3}. Consequently, sugarcane silages showed greater DM losses compared to corn silages.

The study conducted by Koehler et al. (2013) demonstrated an inverse and significant relationship between DM losses and silage density, as well as feeding rate, indicating that higher densities result in lower DM losses and increased intake. Sun et al. (2021) reported higher that corn silages with greater compaction exhibit higher stability

after air exposure, reduced fermentative losses, lower levels of acetate and NH3-N, as well as higher DM digestibility and energy content. Thus, increased densities not only reduces residual oxygen in the silo, limiting cellular respiration and the activity of undesirable microorganisms, but also mitigates fermentative losses and extends silage stability after exposure to air.

The structure of silo used is another factor that significantly influences DM losses during the ensiling process. Under certain field conditions, achieving the desired density may not be feasible, even with proper management practices, as observed in pile silos and bunker (vertical silos) (Hutnik and Kobielak, 2012). According to Johnson and Harrison (2001), structural challenges in these silos often lead to difficulties in achieving uniform compaction throughout the silo. This results in increased levels of residual oxygen inside the silo, leading to higher DM losses, particularly in the upper and lateral layers. The authors also reported that despite these limitations, pile silos are widely used globally, especially in large-scale production systems, due to their lower capital investment compared to other types of silos. Bolsen et al. (1993) reported DM losses of 9.5 percentage points in corn silage stored in vertical silos compared to laboratory silos stored horizontally. In such cases, management interventions, including the use of additives such as organic acids, can help preserve the ensiled material by reducing the pH and limiting the activity of undesirable microorganisms.

As previously mentioned, cellular activities, such as respiration, continue to occur after cutting plants. Thus, the time between cutting and silo sealing can directly influence the onset of the fermentation process, as well as the availability of substrates in the plants that will be utilized during fermentation (McDonald et al., 1991). Bruning et al. (2018) reported that a four-day delay in silo sealing resulted in an increase of up to 11% in DM losses, higher yeast counts, and greater formation of ethyl esters. This delay also reduced water-soluble carbohydrate levels by up to 65% and decreased the aerobic stability of corn silage after exposure. In a lab-scale study, Melo et al. (2023) evaluated different sealing times (30, 90, 150, and 210 minutes after chopping), and observed that longer delays resulted in higher gas production, lower starch and lactic acid levels, and significantly greater total DM losses, both during the ensiling process and after exposure to air. Additionally, silages with longer sealing delays contained higher levels of ADF, NDF,

and lignin. These findings emphasize that delayed silo sealing compromises both the quantity and quality of the ensiled material, negatively impacting its subsequent use and utilization by animals, even considering short harvesting-ensiling intervals.

The storage time of silage also is a determining factor for the fermentation process, especially in corn silages. As demonstrated earlier (Table 2.1.1), different microorganisms act at distinct stages of the fermentation process. Most active metabolites in the silo tend to cease their activity between 2 and 6 weeks after the start of ensiling (Pahlow et al., 2003). However, this duration is variable and can be influenced by factors such as density, epiphytic microbial community, environmental conditions at the time of ensiling, plant chemical microbial composition, among others (Kung et al., 2018). It has been observed that corn silage (Der Bedrosian et al., 2012) and high-moisture corn silage (Hoffman et al., 2011) exhibit a prolonged duration of the fermentative phase. In silages with shorter storage times, the fermentation process may not be fully completed, which compromises the goal of ensiling, the efficient preservation of feed.

The primary objective of corn ensiling is to provide effective fiber while increasing starch availability for animals (Der Bedrosian et al., 2012). During the ensiling process, some primary proteins of the starch-protein matrix, such as zeins, are degraded in the acidic environment generated by fermentation, enhancing starch availability in silage (Hoffman et al., 2011). A review by Kung et al. (2018) presented several studies indicating an increase in starch digestibility with prolonged storage times (Figure 2.1.2). Der Bedrosian et al. (2012) observed a significant increase on in vitro starch digestibility over extended storage periods (0 to 360 days). The authors emphasized that, although the active fermentation phase occurs within a limited timeframe, secondary metabolic processes remain active during prolonged storage, leading to changes in the silages nutritional value.

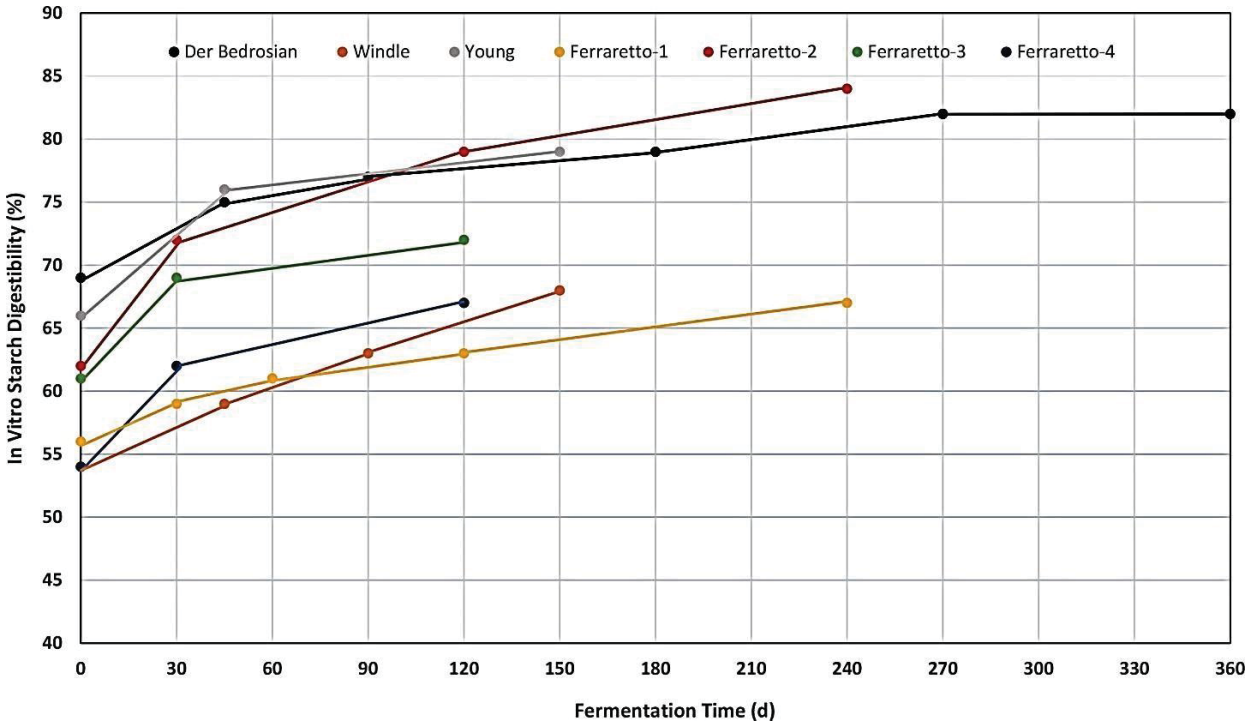

Figure 2.1.2 – Effect of ensiling duration on *in vitro* starch digestibility (Kung et al., 2018).

The efficiency of the fermentation process and the compounds resulting from it influence the stability of silage after exposure to air (Kung et al., 2018). The data presented so far have demonstrated the negative effects of oxygen presence throughout the forage preservation process. After silo opening, aerobic microorganisms, such as fungi and yeasts, resume their activity, consuming soluble carbohydrates and silage nutrients, leading to gas and heat production, which causes material deterioration (Borreani et al., 2018). Schmidt et al. (2010) reported a strong correlation between gas production and increased silage temperature under aerobic exposure when evaluating CO_2 release in silages exposed to air under continuous oxygen flow. It is important to highlight that all gas production is associated with nutrient and DM losses in silages (Table 2.1.1; Assis et al., 2024). Several studies have shown that the use of inoculants and additives, such as organic acids, can help reduce DM losses during the fermentative process and after silo opening (Der Bedrosian et al., 2012; Morais et al., 2017; Borreani et al., 2018, Muck et al., 2018; Lui et al., 2024). Thus, although corn has favorable characteristics for efficient fermentation, several factors from the field to silo opening can influence the fermentation

process and aerobic stability of silage. These factors should be carefully monitored to ensure greater efficiency in preserving the ensiled material.

2.3 Effect of organic acids on the silage

Brönsted defines acids as chemical compounds capable of donating protons (H+ ions), with organic acids being characterized by the presence of carboxyl group (-COOH) in their structure (Marzzoco and Torres, 2015). These organic acids occur naturally in various biological sources and play essential roles in metabolism and fermentation (McDonald et al., 1991). In general, the structure of many cellular molecules and most biochemical processes are highly sensitive to variations in medium pH, which are influenced by the presence of organic acids (Marzzoco and Torres, 2015). In silage, the most found and studied organic acids include lactic, acetic, and propionic acids (Kung et al., 2018). Each of these acids is produced by the action of specific microorganisms and plays a distinct role in the preservation process of ensiled material, aspects that will be further discussed below.

In silage production, pH can be considered a measure of the acidity of the forage (Kung et., 2018). In biochemistry, within the classification of acids, lactic acid is considered a weak acid. However, among the most common organic acids in silage, it has the lowest dissociation constant (pK_a = 3.86), making it stronger than acetic acid (pK_a = 4.75) and propionic acid (pK_a = 4.87) (Marzzoco and Torres, 2015). Thus, lactic acid plays a fundamental role in the fermentation phase and silage preservation, as its higher concentration contributes to a rapid decrease in pH within the silo, inhibiting the activity and growth of undesirable microorganisms (Kung et al., 2018). The lactic acid present in silage is the primary end-product of fermentation by LAB and is generally found in higher concentrations in high-quality silages (Table 2.1.2; Kung et al., 2018). In corn silages that undergo efficient fermentation, lactic acid typically accounts for more than 60% of the total organic acids, with concentrations ranging from 3 to 6% of the DM (Table 2.1.2; Jobim and Nussio, 2013).

Table 2.1.2 – Suggested typical concentrations of common fermentation end-products in corn silage and high-moisture corn (Kung et al., 2018)

Item	Corn Silage (30-40% DM)	High-moisture corn (70-75% DM)
pH	3.7 - 4.0	4.0 - 4.5
Lactic acid, % DM	3 - 6	0.5 - 2.0
Acetic acid, % DM	1 - 3	<0.5
Propionic acid, % DM	<0.1	<0.1
Butyric acid, % DM	0	0
Ethanol, % DM	1 - 3	0.2 - 2.0
NH3-N, % of total N	5 - 7	<10

Given the efficiency of lactic acid in the fermentation process, the scientific community began investing the addition of homofermentative LAB in the ensiling process, aiming to improve the preservation of some forages (Woolford and Wilkins, 1975). However, subsequent studies demonstrated that inoculation with homofermentative LAB did not always enhance silage fermentation, as factors such as the availability of soluble carbohydrates, dry matter content, residual oxygen, among others, directly influence this process (Woolford et al., 1984; McDonald et al., 1991; Buxton et al., 2003).

The isolated use of heterofermentative LAB was also studied, primarily to enhance the aerobic stability of silage after silo opening and extend its utilization window (Kung et al., 2003). Studies have demonstrated that inoculation with *Lentilactobacillus buchneri* does not always reduce fermentation losses but increases aerobic stability in corn silage (Ferrero et al., 2020. Liu et al., 2024). *L. buchneri* possesses an anaerobic metabolic pathway that converts lactic acid into acetic acid, 1,2-propanediol, and ethanol (Kung et al., 2003). Additionally, in some cases, inoculation with *L. buchneri* has resulted in higher concentrations of propionic acid (Taylor et al., 2002). It is believed that the accumulation of acetic and propionic acids is the main factor responsible for the increased aerobic stability of silage. Subsequent research has shown that the combination of homofermentative and heterofermentative LAB can yield better results on both the fermentation and the silage stability after air exposure (Kung et al., 2003).

The acetic acid present in silage can originate from different metabolic pathways, including the activity of heterofermentative LAB and enterobacteria (McDonald et al.,

1991). As previously demonstrated, heterofermentative LAB can convert soluble carbohydrates into high concentrations of acetic acid, which are associated with minimal DM losses in ensiled material (Table 2.1.1). Additionally, acetic acid exhibits antifungal properties, inhibiting the growth of filamentous fungi and yeasts (Kung et al., 2018). Thus, this acid plays a role both during the fermentation phase and in the aerobic phase after silo opening, contributing to the aerobic stability of silage. On the other hand, acetic acid derived from the fermentative activity of enterobacteria is less desirable, as these microorganisms compete directly with LAB for soluble carbohydrates. In addition to acetic acid, their fermentation also produces ethanol and CO_2, which can lead to undesirable losses (McDonald et al., 1991).

A study published by Muck et al. (2018) evaluated more than 24 peer-reviewed articles on the use of combined inoculants in silages from various crops. The authors reported that *L. buchneri* dominated the later stages of storage, reducing lactic acid levels, increasing acetic acid concentration, and prolonging the aerobic stability of silages compared to untreated silages in most of the analyzed studies. Although acetic acid has antifungal properties and consequently improves silage stability after air exposure, its concentration should be carefully monitored, since high levels (>4-6% of DM) may compromise voluntary feed intake in animals (Kung., 2003). On the other hand, when present at adequate levels (Table 2.1.2), acetic acid from silage can be absorbed in the rumen of ruminants, being used as an energy source or incorporated into milk and body fat (Kung et al., 2018).

Propionic acid in silage can be produced by the action of propionic bacteria, which are capable of converting glucose and lactic acid into propionic and acetic acids (Borreani et al., 2018). Among the organic acids present in silage, propionic acid is found in the lowest proportion (<0.10%) (Table 2.1.2). However, it exhibits greater antifungal activity compared to acetic and lactic acids. Kung et al. (2003) stated that propionic acid can exist in either the free form ($COO\text{-}H^+$) or the dissociated form (COO^-), with pH influencing both its form and antifungal activity. According to the authors, as pH decreases, the concentration of free-form propionic acid increases, leading to greater antifungal activity. Thus, the lower the pH of the environment, the more active propionic acid becomes. The mechanism of action of this acid involves penetrating fungal and yeast

cells, promoting the release of H^+ ions, oxidation of the medium, and consequently, the death of microorganisms (Borreani., 2018). It can act both during the fermentation process and after silo opening. However, due to its low concentration in silage, the addition of this acid during ensiling could be an effective strategy to help control undesirable microorganisms.

The review published by Kung et al. (2003) reported that the addition of 12.5 g kg^{-1} of propionic acid completely preserved the DM of corn silages with 34% DM and reduced DM losses from 175 to 95 g kg^{-1} in corn silages with 20% DM after 19 days of air exposure. Although the isolated application of propionic acid has a positive effect on silage stability and preservation, several commercially available additives on the market use propionic acid or its salts (buffered acid) as a base. Due to its strong odor, high corrosive on solid steel, and relatively short residual time resulting from its volatile nature, in addition to its reduced cost, propionic acid has been more commonly used in its buffered form (Kung et al., 2018). Morais et al. (2017), when evaluating a set of studies, reported that among 29 additives tested in grain silages, 17 contained propionic acid in their composition . Among the additives whose composition was detailed, the proportion of propionic acid ranged from 5 to 90%. Thus, propionic acid can be used in different proportions and combinations, adapting to the specific needs of silage preservation.

Formic acid, unlike lactic, acetic, and propionic acids, is considered a strong acid and can promote a rapid reduction in pH (Marzzoco & Torres, 2015). Kung et al. (2003) reported that the ranking of organic acids based on their ability to lower pH is as follows: formic acid ~ lactic acid ~ acetic acid ~ propionic acid. The rapid pH drop can inhibit the activity of microorganisms sensitive to acidic environments, such as aerobic bacteria and enterobacteria, creating ideal conditions for the activity of LAB. This process results in lower dry matter losses due to reduced gas production and decreased activity of undesirable microorganisms (Gheller et al., 2021). According to Kung et al. (2003), formic acid partially inhibits fermentation, leading to high-quality silage but with low stability after air exposure. For this reason, the authors suggest combining it with another organic acid that provides benefits after silo opening, such as propionic acid. Another factor that may influence the isolated use of formic acid is its cost, which is higher compared to other acids

and additives. Thus, combining formic acid with other acids could help reduce the final product cost.

Several commercial products are used as silage additives, containing propionic acid and formic acid in their composition, either alone or in combination with other compounds, and have shown promising results. Pordeus et al. (2022) observed that snaplage treated with organic acids exhibited higher DM content, better preservation of nutrients (starch, ether extract, and non-fibrous carbohydrates), a higher count of LAB compared to other treatments, and a decrease in fungal and mold counts, when compared to the control group. Gheller et al. (2021) reported that the incorporation of organic acids into whole-plant corn silages reduced dry matter losses and increased aerobic stability. Furthermore, a meta-analysis conducted by Goeser et al. (2015) on various studies demonstrated that the inclusion of chemical additives containing organic acids inhibited the growth of undesirable microorganisms and reduced dry matter losses in silage. Therefore, the use of these organic acids, either in combination or individually, can help preserve nutrients in whole-plant and corn grain silages, both during the fermentation process and after silo opening, depending on the intended objective.

Sorbic acid is widely used in food preservation, particularly for controlling the growth of fungi and yeasts (McDonald et al., 1991). Similar to the propionic acid, the free form of sorbic acid has the ability to penetrate the cell membrane of undesirable microorganisms, releasing H^+ ions and acidifying the cytoplasm, ultimately leading to microbial death (Kung et al., 2003). Additionally, its effectiveness is influenced by the pH of the environment, the lower the pH, the greater its activation and efficiency. Woolford et al. (1975) demonstrated that, in forage with pH 6, a concentration of 225 mmol L^{-1} inhibited yeast endospores, and fungi. However, when the authors used fresh forage with pH 5, the concentration required to inhibit those microorganisms was reduced by half (112.5 mmol L^{-1}). Like the other acids discussed, sorbic acid is a component of various chemical additives used for silage preservation (Morais et al., 2017).

The presence of organic acids in silage, whether produced during the fermentation process or added to the forage at ensiling, helps reduce fermentative losses and losses after silo opening. Scientific literature indicates that the use of organic acids as silage additives dates back to the 1960s. Most early studies were conducted in Europe, where

adverse climatic conditions make it challenging to achieve optimal dry matter levels for silage production. Since then, research in other regions has also demonstrated the effectiveness of organic acids in silage preservation, with predominantly positive results. Although the addition of organic acids represents an additional cost to the ensiling process, their application might minimize losses during storage, even when good production practices are adopted. Thus, this strategy can contribute to optimizing silage production and improving the economic efficiency of the activity.

2.4 Whole plant corn silage

Corn silage is widely studied in forage conservation due to its importance in ruminant nutrition worldwide. The study conducted by Bernardes and Do Rêgo (2014) found that among 260 dairy farms in Brazil, 82.7% use corn silage exclusively or in combination with another forage source, highlighting its relevance to the brazilian livestock production. Although corn has favorable characteristics for natural fermentation, some losses during the ensiling process are inevitable and cannot be entirely controlled under natural conditions.

Corn silage offers several advantages, and it is important to highlight that the natural fermentation profile of well-processed corn silage (without additives) is predominantly homolactic, characterized by high lactic acid levels and a rapid reduction in pH (Arthur, 2019). However, after silo opening and oxygen exposure, aerobic microorganisms proliferate, primarily oxidizing lactic acid, increasing pH, and promoting aerobic deterioration (Jobim & Nussio, 2013). In Selwet (2008) study, whole-plant corn silage showed a significant increase in fungal cell counts between day 0 and day 7, with yeast growth rising by 68.7% (from 8.5 to 27.2 CFU g^{-1}) and mold growth by 51.3% (from 15.2 to 31.2 CFU g^{-1}). In this context, microbial proliferation under aerobic conditions after silo opening occurs rapidly and naturally, leading to nutrient degradation. The addition of organic acids can help extend the aerobic stability of silage and reduce nutrient losses after silo opening.

The aerobic stability of silage treated with a chemical additive (5% propionic acid, 21% sodium formate, 20% formic acid, 4% sodium propionate, and 20% water) was significantly higher than the control silage (Arthur, 2019). The time to aerobic stability

breakdown was approximately twice as long in the treated silage compared to the control (78 h vs. 144 h). Thus, the use of chemical additives, such as organic acids, may serve as an alternative for preserving silage nutrients, particularly during the aerobic phase after silo opening. However, several questions regarding their efficacy and mode of action remains unclear. For instance, Pordeus et al. (2022) observed that snaplage treated with organic acids showed an increase in lactic acid bacteria counts but also a rise in aerobic bacteria populations compared to other treatments.

The effects of silages treated with organic acids on animal performance remain inconclusive. Additionally to providing greater temperature and pH stability to corn silage, propionic acid increased milk production (25.7 vs. 21.6 kg/day, PA vs. C) and dry matter intake (1.86 vs. 1.56% BW, PA vs. C) in lactating cows (Huber and Soejono, 1976). However, Arthur (2019) reported that, despite greater aerobic stability and a fivefold reduction in ethanol content (0.43 vs. 0.09% corrected DM, C vs. Additive) in silages treated with organic acid-based additives, no effects were observed on intake, performance, or production. From an economic perspective, the use of these additives may still be advantageous, even if they do not provide clear benefits for animal production. The reduction in fermentative and post-opening losses, along with an increased shelf life of silage on the farm, may make it a financially viable option compared to untreated silage.

In this context, studies that explore the advantages of adding organic acids, their mode of action, benefits, appropriate dosage, proper management, and the selection of the most effective acids for different conditions can provide data for their recommendation. Such research is essential to address uncertainties and validate the use of organic acids as a viable alternative in the management of whole-plant corn silage.

2.5 High moisture grain silage

Corn grain is one of the most commonly ingredients in ruminant diets, serving as a primary energy source for these animals. In recent years, there has been a significant increase in the processing and utilization of grain silages (Gervásio et al., 2021) to enhance starch utilization by ruminants. Many methods can improve starch digestion efficiency in animal nutrition, and grain ensiling stands out as a viable alternative within this context.

During the fermentation process of ensiled grains, proteolysis of the protein matrix occurs due to proteolytic enzymes from both microorganisms and the grains itself (Junges et al., 2017). As a result, starch digestibility in the grains increases (Morais et al., 2017). However, despite this significant advantage, as previously mentioned, factors in the process of ensilage may lead to potential losses throughout the fermentation process, storage, and silo opening.

In the ensiling of wet corn grain, the kernels must reach a specific stage of physiological maturity, typically when they have around 35% moisture content. The moisture content is crucial for the fermentation process, making it essential to harvest at the proper time. Low moisture alters fermentation and compromise the digestibility of the grains (Gervásio et al., 2021). Additionally, the degradability of starch is positively correlated with the degradability of dry matter in grain silage, influencing its nutritional quality.

The most predominant type of corn in Brazil is flint corn, which has a high vitreous endosperm (Correia et al., 2002). This characteristic makes starch release more difficult, and therefore, processing and better utilization of the grains are necessary. Flint corn has a significant amount of prolamin (Gervásio et al., 2021), which further hinders starch accessibility, and the storage time of this material can influence this aspect. Da Silva et al. (2019) tested different storage periods (15, 30, 60, 90, 120, 180, 240, and 300 days) in high-moisture corn and rehydrated corn silages and observed a gradual decrease in prolamin content, accompanied by an increase in ammonia nitrogen over time, likely due to the proteolytic action of bacteria present in the silage (Junges et al., 2017).

Storage time can influence the aerobic stability of grain silages due to the gradual accumulation of fermentation products with antifungal properties, such as acetic and propionic acids (Gervásio et al., 2021). Proteolysis during storage slowly breaks down the protein matrix, increasing starch digestibility over time (Hoffman et al., 2011). The study by Jurjanz and Monteils (2005) demonstrated that acidic proteases applied during ensiling can enhance proteolytic activity and improve ruminal starch digestion. However, significant challenges remain in developing an additive that enhances starch digestion in corn silage or high-moisture corn stored for only a few months (Muck et al., 2018).

According to McDonald et al. (1991), organic acids can be effective in controlling aerobic deterioration in both whole-plant and wet grain corn silages, as it inhibits yeasts, which are major consumers of lactic acid when silage is exposed to air. However, studies on the inclusion of organic acids in the wet grain corn silage process are limited, and further research is needed to assess the effectiveness of these compounds in preserving silage nutrients. Thus, evaluating wet grain silage with addition of organic acids at different opening times may offer benefits for nutrient preservation and material conservation, as well as improve starch digestibility by enhancing the protein matrix.

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3. CHAPTER II – ORGANIC ACIDS ON THE CONSERVATION OF WHOLE PLANT CORN SILAGE AND HIGH MOISTURE CORN GRAIN SILAGE AT DIFFERENT STORAGE TIMES

3.1 ABSTRACT

The objective of this study was to evaluate the effects of an organic acid blend on whole-plant corn silages and high-moisture corn silages stored at different times, focusing on gas production, DM content, fermentative losses, aerobic stability, pH, chemical composition, and microbiological characteristics. Two experiments were conducted. Experiment 1 – Whole-plant corn silage, with the treatments: Control (C) – no additive; Anfi Fun (AF) – addition of Anti Fun (acetic, propionic, formic, and sorbic acids – Agrifirm® - 0.5 L ton^{-1}); Inoculant (LB) – addition of *Lentilactobacillus buchneri* (1.0 x 10^{11} cfu/g – Lallemand Animal Nutrition). Experiment 2 – High-moisture corn silage, with the treatments: Control (C) – no additive; Anfi Fun (AF) – addition of Anti Fun (acetic, propionic, formic, and sorbic acids – Agrifirm® - 1.0 L ton^{-1}); Propionic Acid (PA) – addition of buffered propionic acid (1.0 L ton^{-1}). The silos were stored for 15 or 60 days. In Experiment 1, silages stored for 60 days showed higher fermentative losses of 0.97 and 2.05% DM for 15 and 60 days, respectively. Silages stored for 15 days had higher counts of LAB and yeasts. The LB treatment exhibited the highest LAB count and the lowest yeast count after 60 days of storage compared to the C and AF treatments, respectively. Storage time reduced the NDF content and increased CP, ADF, and NDF digestibility levels. Silages treated with AF and LB had higher crude protein content compared to the C treatment. Silages stored for 15 days had higher starch content, with values of 23.57 and 22.18% DM. Soluble protein and starch digestibility were higher in silages stored for 60 days. The AF treatment had higher soluble protein and starch digestibility compared to the C and LB treatments. In Experiment 2, organic acids reduced total gas production, with gas volumes of 1.61, 1.27, and 1.24 L kg^{-1} DM for C, AF, and PA, respectively. Silages stored for 15 days had lower fermentative losses, with values of 1.34, 1.90, and 0.49% DM for C, PA, and AF, respectively. Silages stored for 60 days had aerobic stability 53% higher than those stored for 15 days. The PA treatment demonstrated 32% and 25% greater aerobic stability compared to the C and AF

treatments, respectively. Storage time reduced LAB counts, with values of 8.17 and 5.83 log cfu/g for 15 and 60 days, respectively. Soluble protein was 10% higher in silages stored for 60 days. The PA and C treatments had higher soluble protein levels than silages treated with AF. The NH3-N and ash contents were lower in silages stored for 60 days. Silages treated with PA had NH3-N levels 7% and 10% lower than silages treated with C and AF, respectively. Prolonged storage time increased starch digestibility (fraction B) and NDF digestibility by 36% and 20%, respectively. The organic acid blend was effective for some specific variables, but the positive controls, BL and PA, were more efficient in silage preservation. Storage time influenced the chemical composition and aerobic stability of the silages.

Keywords: aerobic stability; inoculant; microbiology; propionic acid

3.2 INTRODUCTION

Ensilage is a widely used feed conservation technique based on the fermentation of plant-soluble sugars by fermentative bacteria in an aerobic environment. This process generates organic acids that preserve the feed by lowering the pH of the medium. During ensiling, different microorganisms can influence the fermentation, lactic acid bacteria, for instance, produce lactic acid and are desirable due to their fermentative efficiency. In contrast, enterobacteria and fungi can impair the process by metabolizing nutrients into compounds that do not benefit forage conservation, thus being undesirable (Kung et al., 2018).

Several factors can influence silage quality, but it is the fermentation process that ensures nutrient preservation and quality in the ensiled material. The presence of organic acids, such as lactic, acetic, and propionic acids, enhances fermentation efficiency and inhibits undesirable fermentation (Gheller et al., 2021). Lactic acid, being the strongest acid present in silage, quickly reduces the pH, preserving silage quality (Borreani et al., 2018). On the other hand, acetic and propionic acids, especially propionic acids, have antifungal properties, acting against fungi both during the fermentation phase and in maintaining aerobic stability after silo opening (Morais et al., 2021). Thus, the use of organic acids in the ensiling process can help preserve essential nutrients, reducing the losses associated with silage production.

Various plants can be used for silage production, however, corn stands out due to its characteristics that support good natural fermentation, such as an appropriate dry matter content, high levels of soluble carbohydrates, and low buffering capacity. Thus, ensiling the whole corn plant or just its grains, with the addition of organic acids, can result in better dry matter and nutrient preservation, while also reducing fermentation losses during the ensiling process and after silo opening, ultimately leading to higher-quality silage.

In this context, the present study aimed to evaluate and describe the effects of an organic acid blend on the fermentation and conservation of whole plants or high-moisture corn grain silages. This study is based on the hypothesis that adding organic acids to whole plants or high moisture corn grain silages can reduce fermentation losses, increase

lactic acid bacteria populations, and preserve the dry matter and starch content of the ensiled material, thereby serving as a management strategy to ensure better quality in these feeds.

3.3 MATERIAL AND METHODS

Experiment 1

3.3.1 Ensiling process

The research was performed in the Forage Research Center (CPFOR) in Curitiba (25°25'42" S and 49°16'24" W), located in the state of Paraná, Brazil. The corn hybrid P4285VYHR (Pioneer®) was harvested on March 25, 2023, at 32,4 % dry matter (DM, Table 3.3.1.1) and processed using a stationary chopper, adjusted to 10 mm particle size.

The chopped forage was homogenized and divided into three piles for treatment characterization. The treatments were: Control (C) – no additive; Anti Fun (AF) – addition of Anti Fun (acetic, propionic, formic, and sorbic acids – Agrifirm® 0.5 L ton^{-1}); Inoculant (LB) – addition of *Lentilactobacillus buchneri* (1.0 x 10^5 ufc/g forage – Lallemand Animal Nutrition). After spraying and manually homogenizing the additives in their respective treatments, the chopped forage was ensiled in 8.8 L PVC laboratory silos with 4.44 kg of forage per silo. After filing and compacting, the silos were sealed with a liquid sealant (Selabond®) and stored for 15 or 60 days.

The measurement of produced gas volume (GV) was daily performed during the fermentation period according to the methodology described by Restellato et al. (2019). The measurements were taken several times a day and, after each evaluation, the gas was released. The total GV was the sum of all measurements recorded for each silo during the evaluation period.

Table 3.3.1.1 – Chemical composition of whole-plant corn with and without organic acids and inoculant before ensiling

Variable	C	AF	LB	Mean
Dry matter	32.58	32.45	32.33	32.45
Crude protein (% DM)	6.83	7.24	7.18	7.08
Soluble protein (% crude protein)	41.34	43.94	41.01	42.09
Neutral detergent fiber (% DM)	49.05	49.30	49.40	49.25
Acid detergent fiber (% DM)	25.20	24.84	25.56	25.20
48h digestibility NDF (% NDF)	56.97	58.35	58.47	57.93
Starch (% DM)	24.71	23.70	23.56	23.99
7h starch digestibility (% starch)	67.37	67.50	68.83	67.90
Ether extract (% DM)	3.56	3.28	3.39	3.41
Lignin (% DM)	4.49	4.85	4.89	4.74
Non-fibrous carbohydrate (% DM)	40.89	40.30	40.32	40.50

3.3.2 Sample preparation and laboratory analysis

After characterizing the treatments, two samples of each treatment of fresh forage (~500 g) were collected to determine the DM content and the chemical composition (Table 3.3.1.1). The samples were dried in a forced-air oven at 60°C for 72 hours. Subsequently, the samples were processed in Wiley mill (Marconi®, model M 680, Brazil) using a 1 mm mesh sieve. Next, approximately 70 g of the processed samples were sent to a commercial laboratory (3RLAB - Chapecó – SC) for chemical composition analysis by Near Infrared Spectroscopy (NIRS) applying a calibration curve from the Rock River Laboratory, USA.

Another set of samples (25 g) from each treatment was collected for pH evaluation. The samples were diluted in 225 mL of distilled water and homogenized for one minute using a rod. The pH reading was taken with a digital pH meter (Gehaka®, model PG2000) as described by Kung et al. (1984).

Upon sealing and before opening, the silos were weighed, and the gravimetric losses of gases and DM were calculated by the difference between the initial and final weight of each silo (Jobim et al. 2007). At silo opening, all silage was removed, placed in plastic bags, and homogenized. Then, two other samples (~500 g) from each silo were collected to determine the DM content and silage composition, as previously described.

3.3.3 Aerobic stability

After opening the silos, 3.0 kg of silage was homogenized and placed in 20L buckets for the evaluation of aerobic stability. The buckets remained in a temperature-controlled room (25±1ºC) for 240 hours. In each bucket, a datalogger was placed in the central region of the forage, programmed to take the temperature every 30 minutes. Additionally, another datalogger was placed near to the buckets to measure the room temperature. Aerobic stability was defined as the time (h) required for the silage to reach 2°C above the ambient temperature, as described by Kung et al. (2000). Another set of buckets with silage was used for taking samples for pH measurement every 2 days from

the opening day (day 0), for 10 days. Samples were taken from the central region of each bucket and processed, as previously described.

3.3.4 Microbiological analyses

At the time of closing and opening the silos, 25 g samples of fresh forage and silage from each replicate were collected for microbial analysis. Each sample was diluted in 225 mL of saline solution composed of distilled water, sodium chloride, calcium chloride hexahydrate, and potassium carbonate. The solution was prepared and autoclaved the day before the analysis, as described by Kung et al. (2000). Subsequently, the samples were homogenized for four minutes using a Stomacher-type homogenizer (MA 440/CF, Marconi®) at 150 rpm, filtered through three layers of gauze, and subjected to serial dilutions.

The dilution for counting lactic acid bacteria (LAB) was performed in MRS broth (ACC – Rogosa and Sharpe, Merck®), where 1 mL of the dilution was plated on Petrifilm™ plates (AC, 3M®). The plates were incubated in an anaerobic jar at 30°C for 48 ± 3 h, after which colonies were counted. For yeast and filamentous fungi (molds) evaluation, the dilution was performed in saline solution, where 1mL of the dilution was plated on Petrifilm™ plates (YM, 3M®) and incubated at 25°C. Yeast colonies were counted after 72 ± 3 h, and mold colonies were counted after 120 ± 3 h, using the same plate. Counting was conducted with the aid of a laboratory stereoscope.

3.3.5 Experimental design and statistical analysis

The experimental design was completely randomized in a 3 x 2 factorial scheme, with five replicates, totaling 30 experimental units. The statistical model used was

$$Y_{ijk} = \mu + A_i + T_j + (AT)_{ij} + e_{ijk}$$

In which μ = general mean; A_i = effect of the i-th level of additive factor; T_j = effect of the j-th level of the time factor, $(AT)_{ij}$ = effect of interaction between in the i-th level of the additive factor and j-th level of the time factor; e_{ijk} = experimental error. Additive and storage time were considered fixed effects. The data were analyzed using analysis of variance (ANOVA). Means were compared using Tukey's test with a significance of 0.05 to verify significant differences between treatments. All the computations were conducted

using the GLM procedure of SAS® (Statistical Analysis System) OnDemand for Academics.

Experiment 2

3.3.6 Ensiling process

The research was conducted in partnership with the Colégio Instituto Cristão in Castro (24° 47' 32'' S and 50° 0' 42'' W) and the Forage Research Center (CPFOR) in Curitiba (25º 25' 42'' S and 49° 16' 24'' W), both located in the state od Paraná, Brazil. The corn grains were harvested using a self-propelled harvester on March 27, 2023, at 33.16% moisture and processed using two cracker rolls with a 0.5 mm gap. The chemical composition the grain fresh this reported in Table 3.3.6.1.

The processed grains were homogenized and divided into three piles for the treatment characterization. The treatments were: Control (C) – no additive; Anti Fun (AF) – addition of Anti Fun (acetic, propionic, formic, and sorbic acids – Agrifirm® 1.0 L ton^{-1}); Propionic Acid (PA) – addition of buffered propionic acid (1.0 L ton^{-1}). After the manual spraying and homogenization of the additives for each respective treatment, the grains were ensiled in 8.8 L PVC laboratory silos with 8.02 kg of corn grains per silo. Following filling and compaction, the silos were sealed with a liquid sealant (Selabond®) and stored for 15 or 60 days. Gas volume measurement, sample preparation for laboratory analysis, aerobic stability assessment, microbiological analysis, experimental design, and statistical analysis were conducted similarly to those described in Experiment 1.

Table 3.3.6.1 – Chemical composition of high moisture grain with and without organic acids before ensiling

Variable	C	AF	PA	Mean
Dry matter (%)	66.78	66.86	66.86	66.83
Crude protein (% DM)	8.37	8.40	8.44	8.40
Soluble protein (% crude protein)	26.74	27.87	24.17	26.26
Neutral detergent fiber (% DM)	8.36	8.04	8.02	8.14
Acid detergent fiber (% DM)	3.10	2.84	2.85	2.93
Starch (% DM)	67.50	68.46	69.00	68.32
7h starch digestibility (% starch)	44.48	45.76	44.38	44.87
Ammoniacal nitrogen (% NTotal)	8.21	9.70	7.41	8.44
Ether extract (% DM)	4.28	4.28	3.88	4.14
Lignin (% DM)	0.51	0.48	0.49	0.49
Ashes (% DM)	2.13	2.07	2.03	2.08
Non-fibrous carbohydrate (% DM)	76.95	77.30	77.71	77.32
Lactic acid bacteria (log CFU/g)	7.70	7.63	7.67	7.67
Yeasts (log CFU/g)	6.67	6.75	6.32	6.58
Filamentous fungi (log cfu/g)	6.95	6.59	6.71	6.75

3.4 RESULTS

Experiment 1

The treatments did not affect gas production (P = 0.1898), with gas volumes of 5.7, 5.1, and 4.9 L kg^{-1} DM for LB, AF, and C, respectively (Figure 3.4.1). Silage stored for 60 days exhibited lower DM content at silo opening, with fermentative losses 53% higher than those observed in silages stored for 15 days (Table 3.4.1). Consequently, the longer storage period resulted in an approximately 13% increase in DM losses after silage exposure to air (Table 3.4.1). On the other hand, silages stored for 60 days showed greater aerobic stability compared to those stored for 15 days (Table 3.4.2). Regarding the treatments, silages treated with inoculant exhibited highest aerobic stability than the C and AF treatments (Table 3.4.1). The maximum temperature of the silages was not affected by the treatments after aerobic exposure (P = 0.1071), with average values of 41, 39, and 38°C for AF, C, and LB, respectively. Accumulated

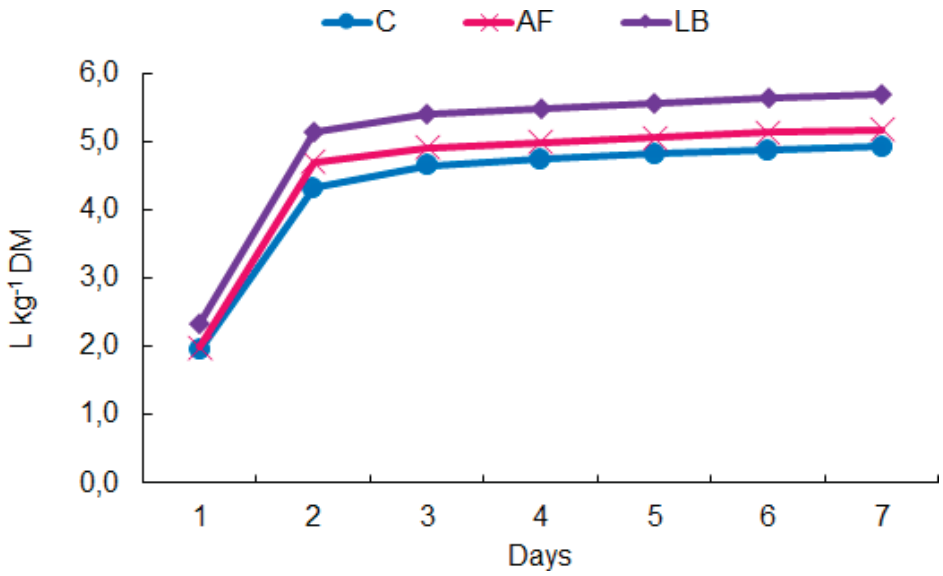

Figure 3.4.1 – Accumulated gas produced during fermentation of whole-plant corn silage treated with organic acids or inoculant.

Table 3.4.1 – Fermentation losses, losses after exposure to air and aerobic stability of whole plant corn silage at different storage times

Variable	Treatment[1]			Mean	SEM[2]	Contrast *P*-value		
	C	AF	LB			Treatment	Time	Interaction
Dry matter (%)								
15	32.48^{A}	32.28^{A}	32.26^{A}	32.33	0.113	0.0527	0.0261	0.5413
60	32.37^{B}	31.82^{B}	31.89^{B}	32.02				
DM losses (% DM)								
15	0.95^{A}	0.96^{A}	1.00^{A}	0.97	0.349	0.4768	0.0139	0.4986
60	1.40^{B}	2.52^{B}	2.25^{B}	2.05				
DM losses at AS (% DM)								
15	14.04^{A}	14.73^{A}	13.83^{A}	14.24	0.812	0.5186	0.0333	0.1677
60	17.43^{B}	14.30^{B}	17.27^{B}	16.33				
Aerobic stability (h)								
15	28.4^{Bb}	30.25^{Bb}	35.80^{Ba}	31.57	2.687	0.0021	<0.0001	0.0914
60	45.20^{Ab}	42.00^{Ab}	66.00^{Aa}	50.00				
Maximum temperature (ºC)								
15	36.90	39.50	39.00	38.40	1.089	0.1349	0.4052	0.1071
60	40.30	42.10	36.20	39.53				

[1]C – Control, no additive; AF – Anti Fun (0.5 L ton^{-1}); LB – *Lentilactobacillus buchneri* (1.0 x 10^5 ufc/g).
[2]SEM – Standard error of the mean.
Lowercase letters compare rows, and uppercase letters compare columns using Tukey test (P≤0.05).

The pH of the silages at silo opening was similar regardless of the storage and treatments, with an average value of 3.6 (Figure 3.4.2). Silages stored for 15 days exhibited similar behavior throughout the evaluation period after silo opening. The pH of silages from all treatments remained stable until 48h after aerobic exposure, followed by a significant increase (Figure 3.4.2). In silages stored for 60 days, the C and AF treatments showed a pH between 48h. In contrast, the LB treatment kept stable pH until 96h after aerobic exposure.

Silages stored for 15 days exhibited higher counts of lactic acid bacteria (LAB, $P<0.0001$) and yeasts ($P = 0.0011$, Table 3.4.2) compared to silages stored for 60 days. Notably, the LB treatment showed the highest LAB count and the lowest yeast count after 60 days of storage when compared to C and AF treatments (Table 3.4.2). The presence of filamentous fungi (molds) was higher in treatments C and LB stored for 15 days compared to the same treatments stored for 60 days ($P = 0.0165$). However, no significant difference was observed for treatment AF between the two storage periods (Table 3.4.2). Similarly to the yeast results, the LB treatment showed 34 and 48% lower counts of molds compared to treatments C and AF, respectively.

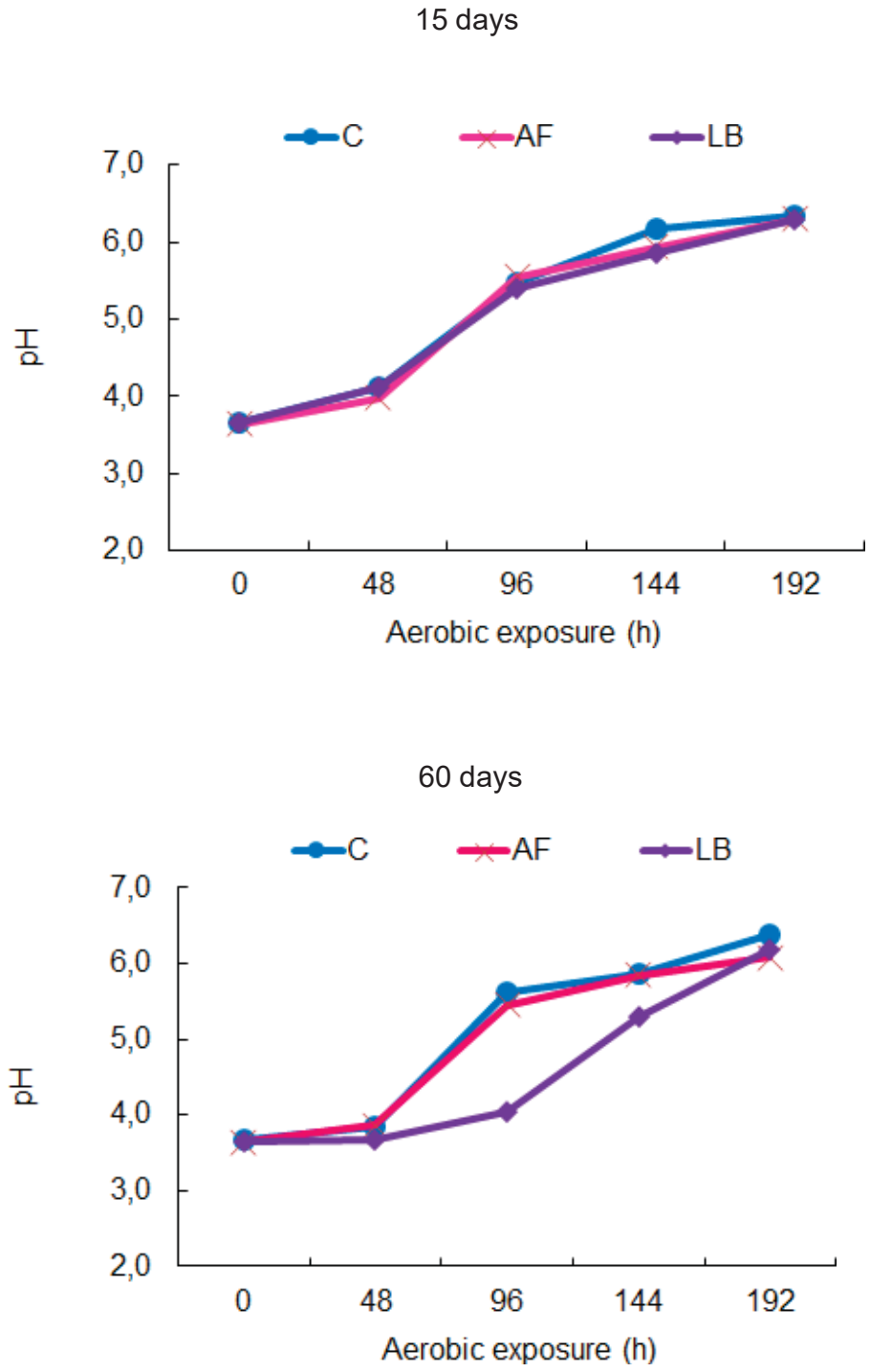

Figure 3.4.2 – pH values during aerobic exposure of whole plant corn silages treated with organic acids or inoculant at different storage times.

Table 3.4.2 – Microbial analysis of whole plant corn silages treated with organic acids and inoculant at different storage times

Variable	Treatment[1]			Mean	SEM[2]	Contrast *P*-value		
	C	AF	LB			Treatment	Time	Interaction
Lactic acid bacteria (log cfu/g)								
15	8.08^{Ab}	8.02^{Ab}	8.37^{Aa}	8.14	0.049	<.0001	<.0001	<.0001
60	5.88^{Bb}	6.01^{Bb}	8.33^{Aa}	6.83				
Yeasts (log cfu/g)								
15	4.66^{Ab}	4.58^{Aa}	4.64^{Ab}	4.63	0.105	0.0017	0.0002	0.0011
60	4.47^{Ba}	4.47^{Ba}	3.30^{Bb}	4.02				
Filamentous fungi log cfu/g)								
15	4.25^{Ba}	4.45^{Ba}	4.15^{Ab}	4.30	0.165	0.0039	0.8209	0.0165
60	4.94^{Aa}	4.52^{Aa}	3.26^{Bb}	4.39				

[1]C – Control, no additive; AF – Anti Fun (0.5 L ton^{-1}); LB – *Lentilactobacillus buchneri* (1.0 x 10^5 ufc/g).
[2]SEM – Standard error of the mean.
Lowercase letters compare rows, and uppercase letters compare columns using Tukey test (P≤0.05).

The longer storage time decreased the neutral detergent fiber (NDF) content and increased the crude protein (CP), acid detergent fiber (ADF), and NDF digestibility of the silages (Table 3.4.3). Silages treated with AF and LB exhibited higher CP content compared to the control (Table 3.4.3). Conversely, silages stored for 15 days showed a higher ether extract (EE) content (Table 3.4.3), with the C and LB treatments having the highest EE levels compared to the AF treatment. Additionally, storage time reduced the starch content and, interestingly, treatment C showed a higher starch content compared to the LB and AF treatments.

The storage increased the soluble protein content (SP, P = 0.0052) and reduced starch digestibility (P = 0.0046) of the silages. The AF treatments showed higher SP content and starch digestibility compared to the C and LB treatments (Table 3.4.3). Storage time and treatments had no significant effect on lignin and nom-fibrous carbohydrates (NFC), which presented average values of 4.6 and 41.2% DM for lignin and NFC, respectively.

.

Table 3.4.3 – Chemical composition and digestibility of whole plant corn silages treated with organic acids and inoculant at different storage times

Variable	Treatment[1]			Mean	SEM[2]	Contrast *P*-value		
	C	AF	LB			Treatment	Time	Interaction
Crude protein (% DM)								
15	6.53 ^{Bb}	6.74 ^{Ba}	6.70 ^{Ba}	6.65	0.056	0.0077	<0.0001	0.7851
60	7.12 ^{Ab}	7.39 ^{Aa}	7.40 ^{Aa}	7.30				
Soluble protein (% crude protein)								
15	47.68 ^{Bc}	55.71 ^{Ba}	51.31 ^{Bb}	52.55	0.642	0.0003	<0.0001	0.0052
60	61.96 ^{Ab}	63.09 ^{Aa}	62.11 ^{Aa}	62.42				
Acid detergent fiber (% DM)								
15	25.17 ^{B}	26.64 ^{B}	26.41 ^{B}	26.03	0.340	0.1817	<0.0001	0.3504
60	27.90 ^{a}	28.26 ^{a}	27.84 ^{A}	28.00				
Neutral detergent fiber (% DM)								
15	46.69 ^{A}	48.62 ^{A}	47.73 ^{A}	47.61	0.529	0.3933	0.0356	0.3764
60	46.57 ^{B}	46.59 ^{B}	47.77 ^{A}	46.29				
Digestibility NDF – 48h (% NDF)								
15	57.33 ^{B}	56.99 ^{B}	57.17 ^{B}	57.16	0.294	0.3271	<0.0001	0.6803
60	60.44 ^{A}	59.61 ^{A}	59.56 ^{A}	59.87				
Lignin (% DM)								
15	4.44	4.64	4.58	4.55	0.177	0.2851	0.2623	0.7153
60	4.60	4.67	4.63	4.64				
Ether extract (% DM)								
15	3.20 ^{Aa}	2.84 ^{Ab}	3.11 ^{Aa}	3.05	0.076	0.0130	0.0003	0.6534
60	2.72 ^{Ba}	2.48 ^{Bb}	2.82 ^{Ba}	2.67				
Starch (% DM)								
15	24.00 ^{a}	21.16 ^{b}	21.39 ^{b}	23.59	0.555	0.0178	0.0473	0.2392

60	24.17^{a}	22.11^{b}	24.18^{a}	22.18				
Starch digestibility (% starch)								
15	67.41^{Bb}	72.48^{Ba}	67.84^{Bb}	22.19	0.544	<0.0001	<0.0001	<0.0001
60	81.86^{Aa}	80.28^{Ab}	80.71^{Ab}	23.59				
Nom- fibrous carbohydrates (% DM)								
15	36.90	39.50	39.00	38.40	0.437	0.3001	0.0785	0.6648
60	40.30	42.10	36.20	39.53				

[1]C – Control, no additive; AF – Anti Fun (0.5 L ton^{-1}); LB – *Lentilactobacillus buchneri* (1.0 x 10^{5} ufc/g).
[2]SEM – Standard error of the mean.
Lowercase letters compare rows, and uppercase letters compare columns using Tukey test (P≤0.05).

Experiment 2

Organic acids reduced total gas production (P = 0.0074, Figure 3.4.3), with gas volumes of 1.61, 1.27, and 1.24 L kg^{-1} DM for treatments C, AF, and PA, respectively. Silages stored for 15 days had higher dry matter (DM) content at silo opening (P = 0.0429), resulting in lower fermentation losses (P = 0.0438) compared to silages stored for 60 days (Table 3.4.4). Among the evaluated treatments, silages with AF showed higher DM content than those treated with PA and C at both storage times. Notably, the AF treatment stored for 15 days exhibited DM losses 63 and 17% lower than treatments C and PA, respectively. However, the shorter storage duration increased DM losses after silage exposure to air and resulted in higher maximum temperatures (Table 3.4.4). It is noteworthy that silages treated with PA not only exhibited lower heating but also had lower DM losses after air exposure compared to those treated with AF or C (Table 3.4.4). These results align with the AS analysis showing that silages stored for 60 days had AS 53% higher than treatments C and AF, respectively (Table 3.4.8).

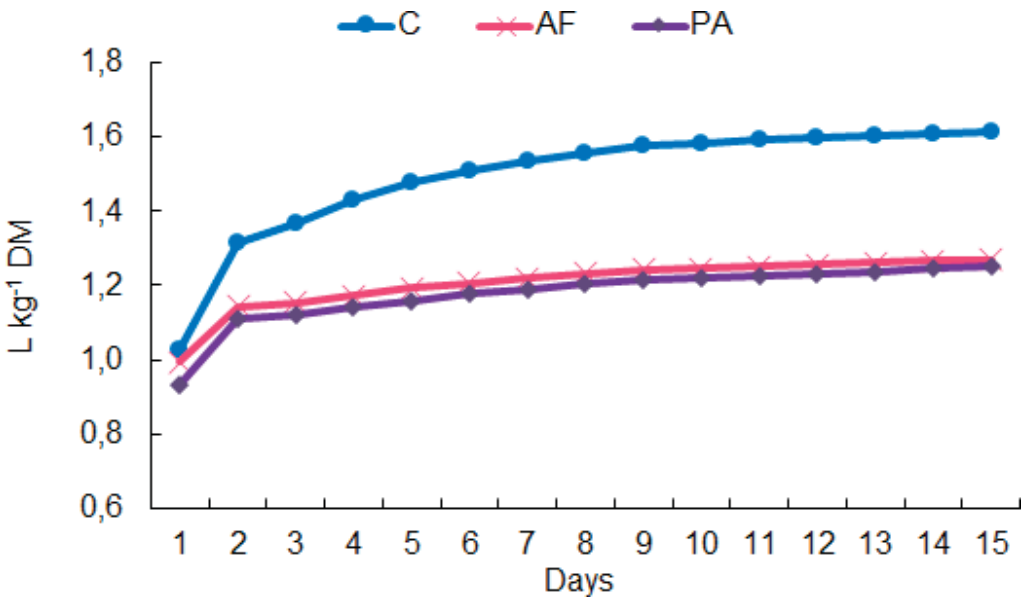

Figure 3.4.3 – Gas volume produced in high moisture corn grain silage treated with organic acids.

Table 3.4.4 – Fermentation losses, losses after exposure to air and aerobic stability of high moisture grain silage treated with organic acids at different storage times

Variable	Treatment[1]			Mean	SEM[2]	Contrast *P*-value		
	C	AF	PA			Treatment	Time	Interaction
Dry matter (%)								
15	66.27^{Ab}	66.72^{Aa}	66.12^{Ab}	66.37	0.071	0.0052	<0.0001	0.0429
60	65.71^{Bb}	65.86^{Ba}	65.80^{Ba}	65.79				
DM losses (% DM)								
15	1.34^{Ba}	0.49^{Bc}	1.09^{Bb}	0.97	0.115	0.0223	<0.0001	0.0438
60	2.13^{Aa}	1.94^{Ab}	1.94^{Ab}	2.00				
DM losses at AS (% DM)								
15	11.46^{Ab}	12.80^{Aa}	8.30^{Ac}	10.95	0.263	<0.0001	<0.0001	<0.0001
60	8.93^{Ba}	7.87^{Bb}	7.40^{Bc}	8.07				
Aerobic stability (h)								
15	56.00^{Bc}	74.10^{Bb}	115.56^{Ba}	81.89	2.687	0.0044	<0.0001	0.7647
60	160.12^{Ab}	162.50^{Ab}	199.60^{Aa}	175.07				
Maximum temperature (ºC)								
15	41.82^{Aa}	42.74^{Aa}	26.92^{Ab}	37.89	1.147	<0.0001	<0.0001	0.0006
60	29.14^{Ba}	26.82^{Bb}	25.46^{Bb}	27.14				

[1]C – Control, no additive; AF – Anti Fun (1.0 L ton^{-1}); PA – buffered propionic acid (1.0 L ton^{-1}).
[2]SEM – Standard error of the mean
Lowercase letters compare rows, and uppercase letters compare columns using Tukey test (P≤0.05).

The pH of the silages at silo opening was similar among treatments, regardless of storage time and treatments, with an average value of 3.8 (Figure 3.4.4). Silages from the C treatment maintained a stable pH for up to 48h of air exposure, followed by an increase in both the storages. Silages treated with AF stored for 15 days exhibited a pH change after 48h of air exposure, maintaining this trend until the end of the analysis, although presenting lower pH values compared to the C treatment. Conversely, silages treated with AF stored for 60 days showed a change in pH after 144h and 196 of air exposure, stabilizing thereafter at 196h. Silages treated with PA stored for 15 days exhibited a pH increase at 144h, but by the end of the evaluation period, the pH values were similar to those of the AF treatment. In contrast, silages treated with PA stored for 60 days showed virtually no changes in pH, reaching a value of 4.02 at the end of the evaluation period.

The storage time reduced LAB counts in the silages, with values of 8.17 and 5.83 log cfu/g for 15 and 60 days of storage, respectively (Table 3.4.5). Silages stored for 15 days showed lower yeast counts compared to those stored for 60 days (Table 3.4.5). However, the AF treatment showed similar counts of filamentous fungi across both storage times, while PA treatment showed similar counts of filamentous fungi across both the storage times, while PA treatment stood out for having the lowest counts compared to AF and C treatments.

The SP content was 10% higher in silages stored for 50 days, compared to those stored for 15 days (Table 3.4.6). Treatments PA and C showed higher SP levels than silages treated with AF. Ammoniacal nitrogen (NH_3-N) and ashes content decreased with increasing storage time (Table 3.4.6). Silages treated with PA had NH_3-N levels 8 and 20% lower than those treated with AF and C, respectively. Increased storage time enhanced the digestibility of starch fraction B and NDF digestibility by 36% and 20%, respectively (Table 3.4.6). The variables CP, NDF, ADF, starch, lignin, EE, and NFC were not influenced by time or treatments (Table 3.4.6).

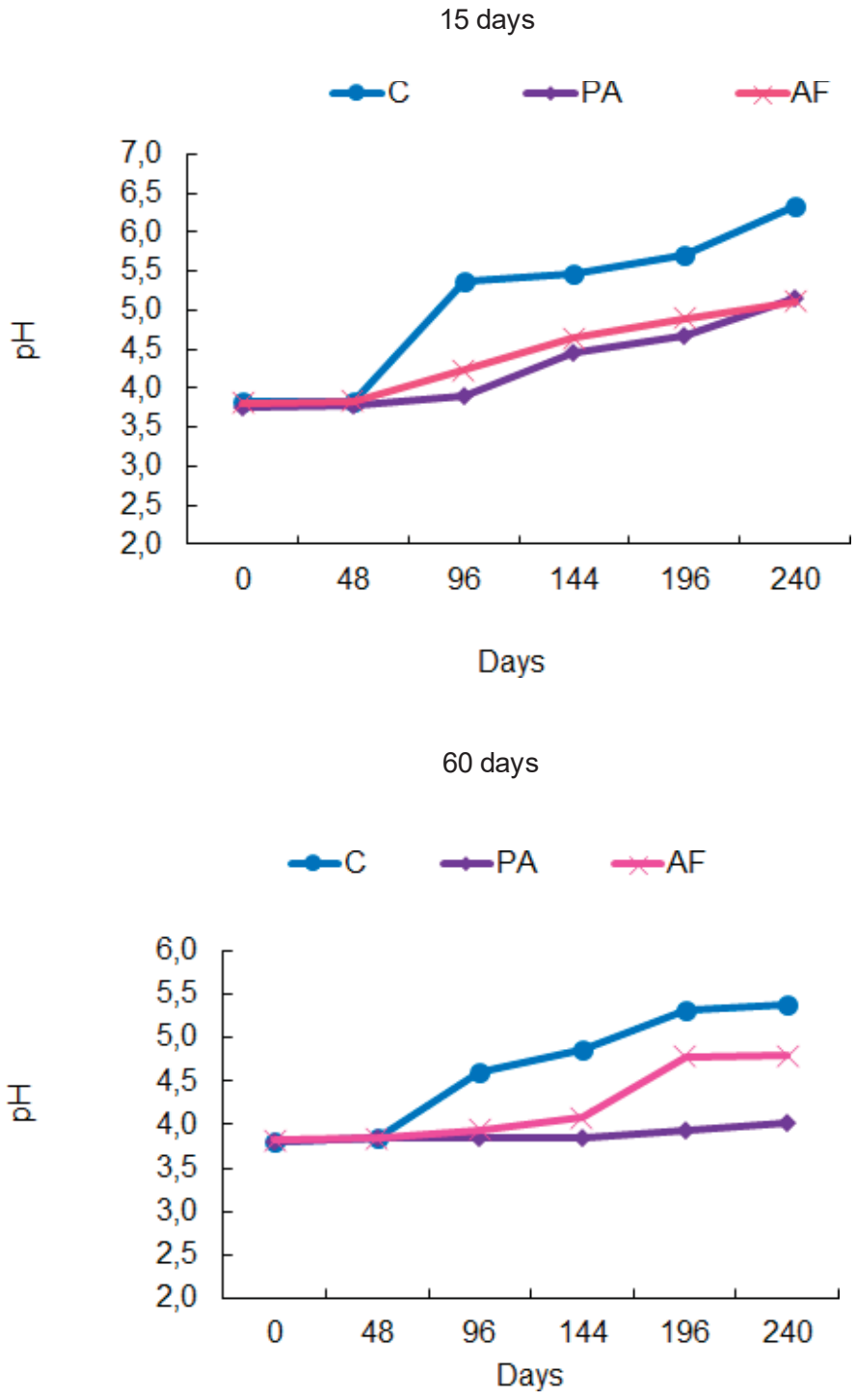

Figure 3.4.4 – pH values during aerobic exposure of high moisture corn grain silage treated with organic acids and inoculant at different storage times.

Table 3.4.5 Microbial analysis of high moisture corn grain silage treated with organic acids and inoculant at different storage times

Variable	Treatment[1]			Mean	SEM[2]	Contrast *P*-value		
	C	AF	PA			Treatment	Time	Interaction
Lactic acid bacteria (log cfu/g)								
15	8.24^{A}	8.24^{A}	8.02^{A}	8.18	0.071	0.2579	<0.0001	0.0858
60	5.94^{B}	5.63^{B}	5.89^{B}	5.83				
Yeasts (log cfu/g)								
15	4.53^{Ba}	4.15^{Bb}	4.34^{Ab}	4.35	0.642	0.0179	0.0009	0.0103
60	4.87^{Aa}	4.94^{Aa}	4.32^{Ab}	4.71				
Filamentous fungi log cfu/g)								
15	4.76^{Aa}	4.16^{Ac}	4.46^{Ab}	4.30	0.654	0.1993	<0.0001	0.0093
60	3.54^{Bb}	4.17^{Aa}	3.17^{Bc}	4.39				

[1]C – Control, no additive; AF – Anti Fun (1.0 L ton^{-1}); PA – buffered propionic acid (1.0 L ton^{-1}).
[2]SEM – Standard error of the mean
Lowercase letters compare rows, and uppercase letters compare columns using Tukey test (P≤0.05).

Table 3.4.6 Chemical composition of high moisture corn grain silage treated with organic acids and inoculant at different storage times

Variable	Treatment[1]			Mean	SEM[2]	Contrast *P*-value		
	C	AF	PA			Treatment	Time	Interaction
Crude protein (% DM)								
15	8.48	8.45	8.44	8.46	0.037	0.8675	0.1283	0.7883
60	8.51	8.55	8.51	8.52				
Soluble protein (% crude protein)								
15	41.67^{Ba}	37.03^{Bb}	41.84^{Ba}	40.31	0.603	0.0028	<0.0001	0.0751
60	45.41^{Aa}	44.15^{Ab}	45.19^{Ab}	44.91				
Acid detergent fiber (% DM)								
15	2.44	2.06	2.30	2.31	0.068	0.4057	0.4811	0.5147
60	2.36	2.33	2.41	2.37				
Neutral detergent fiber (% DM)								
15	7.51	7.26	7.18	7.32	0.121	0.6419	0.3752	0.5807
60	7.18	7.20	7.19	7.19				
Starch (% DM)								
15	68.12	68.92	68.40	68.48	0.172	0.3271	<0.0001	0.6803
60	69.05	68.78	68.67	68.93				
Starch digestibility – 7h (% DM)								
15	64.40^{B}	63.92^{B}	62.73^{B}	63.68^{B}	0.663	0.4451	<0.0001	0.6696
60	79.89^{A}	78.68^{A}	79.18^{A}	79.24				
Digestibility B fraction of the starch (% DM)								

15	14.50 ^{B}	14.07 ^{B}	13.54^{B}	14.00	0.076	0.2483	<0.0001	0.5828
60	22.20 ^{A}	21.39 ^{A}	21.76 ^{A}	21.78				
Ammoniacal nitrogen (% NTotal)								
15	7.86^{Ab}	7.98^{Aa}	6.98^{ac}	7.61	0.037	0.0001	<0.0001	0.2697
60	6.24 ^{Bb}	7.05 ^{Ba}	5.88 ^{Bc}	6.38				
Lignin (% DM)								
15	0.45	0.42	0.43	0.43	0.007	0.2541	0.5032	0.3970
60	0.44	0.43	0.44	044				
Ether extract (% DM)								
15	4.40	4.26	4.29	4.32	0.028	0.6716	0.1420	0.3037
60	4.19	4.36	4.25	4.27				
Nom- fibrous carbohydrates (% DM)								
15	77.92	78.39	78.25	78.18	0.437	0.6973	0.8740	0.2623
60	78.29	78.11	78.23	79.21				

[1]C – Control, no additive; AF – Anti Fun (1.0 L ton^{-1}); PA – buffered propionic acid (1.0 L ton^{-1}).
[2]SEM – Standard error of the mean
Lowercase letters compare rows, and uppercase letters compare columns using Tukey test (P≤0.05).

3.5 DISCUSSION

Experiment 1

Although gas production was not influenced by the treatments, the observed gas volume was relatively low (mean GV = 5.2), combined with the silage pH at silo opening (average pH = 3.6), which was similar across treatments which indicates that all treatments exhibited efficient fermentation. The decrease in DM content across all treatments after the ensiling process, regardless of storage duration, reflects the effects of the fermentation process, which include DM losses and carbon losses through microbial activity, cellular respiration, and the metabolism of anaerobic microorganisms (Junges et al., 2013). In silages stored for 60 days, greater microbial activity may have led to a reduction in the DM content of the silages, which also generated greater fermentation losses and DM losses after opening the silo. However, microbial activities during the ensiling process are associated with the production of metabolites such as organic acids (lactic, acetic, propionic), which play an important role both in the efficiency of the fermentation process and in the preservation of the silage after exposure to air (Gheller et al. 2021). This fact may explain the greater aerobic stability in silages stored for 60 days.

The purpose of adding *L. buchneri* and organic acids to silage is that both have the common function of reducing the pH of the medium and, consequently, controlling microbial activities, especially undesirable microorganisms such as yeasts (Muck et al., 2018; Gheller et al., 2021). However, the result of AS and microbial counts found in this study demonstrates the major effect of the INO treatment, which showed higher AS and BAL counts, with lower values of yeast and filamentous fungi compared to the AF and C treatments. In this way, the action of *L. buchneri* stands out as a causal agent of AS, associated with the reduction of yeast populations (Ferrero et al., 2020), particularly in silages stored for 60 days. Although the INO treatment showed better results, the recorded AS value (51h) is still below the values commonly reported in the literature for corn silages treated with LB-based inoculants (Ferrero et al., 2020; Melo et al., 2023; Tavares et al., 2024).

However, the study published by Agarussi et al. (2022), evaluating 11 strains of *L. buchneri* in corn silage, found that all strains used increased the AS of the inoculated

silage compared to the control silage, but there was variation in AS duration (approximately 38-70h), which may be related to the epiphytic community present in the forage at the time of ensiling (Silva et al., 2018). When comparing yeast and filamentous fungi counts in fresh forage and silage, it was observed that the inoculation performed in this study was effective but not sufficient to fully inhibit the growth of spoilage microorganisms. This may be attributed to the high counts of these microorganisms in the fresh forage, which resulted in lower AS compared to other studies.

The absence of significant differences in maximum temperature between the treatments indicates that, although variations in aerobic stability (AS) occurred, none of the additives were effective in mitigating the initial heating of the silages during exposure to air. The same effect was observed in the treatments' behavior concerning pH after the silo was opened. Ashbell et al. (2002) reported that temperatures between 20 and 30°C promote the growth of deteriorative microorganisms, accelerating the silage degradation process and increasing pH. Thus, the low AS, along with the high maximum temperatures observed in the treatments (41°C, 39°C, and 38°C for AF, C, and INO, respectively), suggests that the material may have started degrading within a short period. This aspect is undesirable from both an economic and animal health standpoint, as it is associated with the loss of feed quality, reduced animal production, and risks of negative health effects (Borreani et al., 2018).

Storage time is a critical factor for silage quality (Ferrero et al., 2020). In the present study, an increase in storage time resulted in a reduction of 1.3 percentage points in the NDF content and increases of 1.97 and 2.71 percentage points in the ADF content and NDF digestibility, respectively, indicating greater fiber degradation. Morrison (1979) reported that acid hydrolysis during hemicellulose degradation in the ensiling process may contribute to increased NDF digestibility, particularly with prolonged storage time. On the other hand, Kung et al. (2018), in a literature review, mentioned studies that reported no effect or even a reduction in NDF digestibility in corn silages with extended storage time. These authors suggest that the initial solubilization of fiber during ensiling could lead to a reduction in NDF digestibility over time. Thus, the relationship between storage time and increased NDF digestibility appears to be inconclusive.

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The small increase in CP with extended storage time may indicate an improvement in the nutritional value of silages stored for 60 days. During the fermentation process, proteolysis of the protein matrix and protein degradation occur, which tend to intensify with prolonged storage time (Der Bedrosian et al., 2012). The SP is a product of protein degradation and, although undesirable in excess, it is inevitable since the ensiling process involves microbial activity on the protein matrix. The INO and AF treatments exhibited higher SP levels, which can be explained by the fact that some proteins, such as zeins, are hydrophobic but soluble in organic acids (Hoffman et al., 2011). Therefore, the greater presence of organic acids generated by both the treatments may have favored the proteolysis process, resulting in an increase in the SP content of the silage.

Hydrophobic zein proteins are the primary proteins in the starch-protein matrix, mainly located on the outer surface of starch granules. As the granules develop, these proteins promote the encapsulation of starch within their hydrophobic matrix, making it progressively less available (Hoffman et al., 2011). However, as previously mentioned, these proteins are soluble in organic acids, suggesting that the ensiling process can promote their degradation, thereby increasing starch availability in silage. This effect was observed by Jurjanz and Monteils (2005), who reported higher ruminal starch digestibility in ensiled grains compared to non-ensiled grains, with values of 92.3 and 70.2%, respectively. Hoffman et al. (2011), evaluating the effect of storage time on hydrophobic zein proteins, found that in silages stored for prolonged periods (240 days), there was greater degradation of these proteins in the starch-protein matrix, resulting in increased starch digestibility. This fact explains the higher starch concentration in silages stored for 15 days, likely with lower availability, and the greater starch digestibility in silages stored for 60 days.

Following this line of reasoning, the fact that treatment C presented a higher starch content can be explained by the greater initial microbial activity in INO and AF silages for producing organic acids from carbohydrates (Muck et al., 2018; Kung et al., 2018), which may reduce the residual starch in the ensiled material. On the other hand, in treatment C, this activity was likely less intense, preserving a greater amount of starch. This dynamic may also have influenced starch digestibility, which was higher in treatment C with storage of 60 days compared to treatments INO and AF. This suggests that, under field conditions,

when the producer's main objective is to increase starch availability without considering other factors, such AS for instance, the whole-plant corn silage process with longer storage time may be sufficient to achieve good results.

The main hypothesis of this study was that the addition of blend of organic acids could reduce losses during the fermentation process, promoting better preservation of nutrients in whole-plant corn silage. However, the AF treatment was not efficient in most of the evaluated variables, such as DM, DM losses, AS, and microbiological analysis. Nevertheless, it is important to highlight that, although starch digestibility and SP levels were higher in silages stored for 60 days, regardless of the treatment, the AF treatment showed higher starch digestibility and SP levels after 15 days of storage compared to the other treatments. Thus, the blend of organic acids could be an alternative in situations requiring short storage periods. However, as mentioned earlier, storage time is a critical factor for silage quality (Ferrero et al., 2020), especially regarding starch availability. In this study, silages stored for 60 days showed starch digestibility 13.05 percentage points higher than fresh plants ensiled, while silages stored for 15 days had only a 1.34 percentage point increase. This highlights that the main impact of silages with shorter storage times is the lower starch availability, along with lower aerobic stability of the material, leading to double economic losses for the production system.

The results of this study demonstrate that, despite greater fermentative and DM losses after air exposure, prolonged storage of whole-plant corn silage is more effective as it promotes greater gains in the digestibility of NDF, CP, and especially starch, which is the primary objective of ensiling whole-plant corn. Additionally, it provides greater AS, which directly influences the usage window of the material on the farm. Regarding the treatments used, the AF treatment was not effective for the evaluated parameters. On the other hand, the C and INO treatments showed significant differences. While the C treatment exhibited higher starch digestibility, the INO treatment presented additional advantages, such as greater AS and LAB counts, with reduced levels of yeasts and filamentous fungi. These characteristics extend the viability of silage use, making it an interesting option from the perspective of long-term management and conservation.

Experiment 2

Gas production during the ensiling process can occur from the residual air respiration, and due to the activity of both desirable microorganisms, such as heterolactic bacteria, and undesirable microorganisms, such as yeasts and clostridia (Borreani et al., 2018). Many of these microorganisms have their activity inhibited at lower pH levels (Kung et al., 2018). The higher presence of organic acids in the PA and AF treatments may explain the lower gas volumes observed in these treatments compared to the control (C) treatment. It is worth noting that, despite the differences in gas production among the treatments, the similarity in average pH values (3.8) indicates that all silages underwent an efficient fermentation process.

Similar to the first experiment, the increase in storage time reduced the DM content of the silages, suggesting prolonged microbial activity in silages stored for 60 days. The literature review published by Kung et al. (2018) discusses the effects of storage time on silage fermentation and raises questions about the duration of the fermentation phase, referencing various studies. One of the works mentioned was by Der Bedrosian et al. (2012), which reported a decline in the pH of whole-plant corn silage after 180 days, accompanied by a gradual increase in lactate and acetate concentrations with extended storage times (45, 90, 180, 270, and 365 days). These findings demonstrate that the duration of the fermentation phase is variable and does not always represent a negative aspect, particularly when it results in the formation of compounds such as lactate and acetate, which can benefit the fermentation process.

The fact that the AF treatment stored for 15 days showed fermentative losses 63% and 17% lower compared to the C and PA treatments, respectively, suggests that the acid blend could be recommended for preserving the DM of ensiled grain over short storage periods. However, the data on DM losses and maximum silage temperatures after air exposure demonstrated limited efficiency of the blend, particularly in silages stored for 15 days. As previously mentioned, the AF blend consists of acetic, propionic, formic, and sorbic acids. Acetic and propionic acids are well-known for their antifungal properties (Morais et al., 2018), acting both during the fermentation process and after silage exposure to air. Formic acid helps preserve nutrients during fermentation (Gheller et al., 2021), while sorbic acid reduces ethanol production (Hafner et al., 2016). Thus, the acids

used in the blend formulation have potential for preserving ensiled feed. Nevertheless, it is believed that the concentrations employed may have been insufficient to ensure greater efficacy. Due to confidentiality reasons, the manufacturer did not disclose the concentrations used.

In contrast to fermentative losses, the prolonged storage time increased the aerobic stability AS of the silages, reaching 175 hours. This result suggests that the microbial activities responsible for DM losses metabolized compounds that promoted greater AS in the silages, regardless of the treatment. Notably, the treatment with PA achieved 158 hours of AS. The pH behavior of the silages after exposure reflected the AS results. pH is an important indicator of silage acidity (Kung et al., 2018). As mentioned earlier, the initial pH of 3.8, similar across treatments, indicates a higher concentration of lactic acid and efficient fermentation (Muck et al., 2018). The aerobic deterioration of silage caused by yeast activity raises the pH (McDonald et al., 1991), which may explain the rapid pH change in the silages from the C treatment, regardless of storage time. On the other hand, the smaller pH alteration in the PA and AF treatments, particularly in silages treated with PA stored for 60 days, demonstrates greater resistance to the breakdown of aerobic stability. From a production system perspective, this result is highly relevant, as it extends the feed usage window without causing economic losses or reducing the animal's utilization efficiency. Thus, the use of propionic acid in high-moisture corn silage proves to be an alternative management strategy to improve silage aerobic stability.

Similarly to the experiment with whole-plant corn silage, an increase in storage time reduced the LAB count. However, conversely, yeast counts increased with prolonged storage time. Storm et al. (2010), when evaluating the microbiological dynamics in corn silage over 11 months, observed similar results, with a reduction in LAB counts over time and higher fungal growth between 5 and 7 months, followed by a decrease at 11 months. The authors identified and isolated filamentous fungi, highlighting *Aspergillus fumigatus*, *Penicillium roqueforti*, and *Penicillium paneum* as the most frequent species. According to the authors, these fungi are known for their resistance to low pH levels. In the present study, the count of filamentous fungi decreased with longer storage times, which is a positive result, as these microorganisms, in addition to impairing silage quality, can produce mycotoxins and compromise animal performance (Kung et al., 2018). Among the

evaluated treatments, PA demonstrated greater effectiveness in controlling filamentous fungi with prolonged storage time, confirming its antifungal action.

Storage time is an extensively studied variable in whole-plant corn silage (Kung et al., 2018), high-moisture corn silage (Hoffman et al., 2011), and rehydrated grain silage (Fernandes et al., 2021), aiming to improve nutrient availability, especially starch. Similarly to the experiment with whole-plant corn silage, wet corn grain stored for 60 days showed starch digestibility 34.37 percentage points higher than the grain prior to the ensiling process, while silages stored for 15 days exhibited an increase of 18.81 percentage points. These data demonstrate that ensiling process was effective for both storage durations, although the results were more pronounced with 60 days of storage. The higher SP content in silages stored for 60 days, observed in this and previous studies, can be explained by increased microbial enzyme activity acting on protein degradation during storage. Similar results were reported by Hoffman et al. (2011), who evaluated high-moisture corn silage over extended storage periods (0, 15, 30, 60, 120, and 240 days) and observed an increase in SP content from 1.5% to 2.0% of DM during the ensiling process, reaching levels above 4% DM in silages stored for 240 days.

The process of protein deamination involves the break of amino acids into ammonia, resulting in an increase in NH3-N (Kung et al., 2018). This process occurs due to the action of plant and microorganisms enzymes (Fernandes et al., 2021). Elevated NH3-N levels in silage are undesirable as they indicate protein losses (Hoffman et al., 2011). In this context, the higher NH3-N content associated with the lower SP levels in the AF treatment is unfavorable both for food preservation and for silage utilization by animals. On the other hand, in the case of high-moisture corn silage, the increase in ammonia indicates the breakdown of zeins and, consequently, better starch degradation. Despite variations in NH3-N levels among treatments, the values found of 6.29%, 6.85%, and 7.12% DM for PA, C, and AF, respectively, are within the recommended range for corn wet grain silages (<10% DM; Kung et al., 2018).

The prolongation of storage time increased the digestibility of starch fraction B and NDF, factors of great importance from a nutritional standpoint and for the utilization of silage by the animals, aligning with the objectives of various previous studies (Hoffmann et al., 2011; Fernandes et al., 2021). Although the variables CP, NDF, ADF, starch, lignin,

EE, and NFC were not significantly influenced by the treatments or storage time, the comparison of the chemical composition of grains and silage showed little to no variation in these parameters. These results indicate that the ensiling process was effective in preserving the nutrients of the high-moisture grains.

The results of this study demonstrate that, despite greater fermentative losses, prolonged storage of high-moisture corn silage is more effective, yielding significant improvements in starch digestibility and AS. It also reduces DM losses after air exposure, the presence of filamentous fungi, and NH3-N levels. These factors are critical for ensuring silage quality and usability. Although the AF treatment exhibited higher DM content and lower DM losses, the PA treatment stood out. It showed lower gas volumes, reduced maximum temperature, fewer DM losses after air exposure, and lower NH3-N levels, along with higher AS compared to the other treatments. These results make the PA treatment a promising strategy for managing the ensiling process of high-moisture corn.

3.6 CONCLUSIONS

Silages stored for 60 days demonstrated greater starch digestibility and aerobic stability in both experiments. While the blend of organic acids showed specific benefits in silages stored for 15 days, such as increased starch digestibility in whole-plant corn silage and higher DM content in high-moisture corn silage, the positive controls used (inoculant and propionic acid) were more effective in the fermentation process and silage preservation, especially after exposure to air.

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4. CHAPTER III – PROPIONIC ACID IN THE FERMENTATION AND CONSERVATION OF WHOLE PLANT CORN SILAGE AT TWO PACKING DENSITIES

4.1 ABSTRACT

The objective of this study was to evaluate the effects of propionic acid on whole-plant maize silage under two packing densities (608 or 428 kg m^{-3} for the high and low density, respectively) in terms of gas production, DM content, fermentative losses, aerobic stability, pH, and chemical composition. The treatments were: High density (HD) – no additive, positive control; High density with propionic acid (HDPA); Low density (LD) – no additive, negative control, simulating a pile silo condition; Low density with propionic acid (LDPA). Silos were stored for 60 days. Propionic acid and high density decreased total gas production (4.9, 5.5, 5.6, and 5.8 L kg^{-1} DM, for HDPA, LDPA, HD, and LD, respectively). Propionic acid silages presented higher DM than control silages, being 362.5 and 356.5 g kg^{-1} DM for PA and Control, respectively. Consequently, the control silages showed higher DM losses during fermentation than the propionic acid silages (31.1, 26.3, 13.1, and 7.1 g kg^{-1} initial DM, for LD, HD, LDPA, and HDPA, respectively). Additionally, propionic acid and the densities decreased DM losses during 10 days of aerobic exposure (132.4, 123.0, 96.3, and 85.3 g kg^{-1} silage DM for LD, HD, LDPA, and HDPA, respectively). Propionic acid silages showed longer aerobic stability compared to control silages (137, 134, 65, and 32 hours, for LDPA, HDPA, HD, and LD, respectively). The LD silages showed the highest maximum temperature during aerobic exposure (44, 39, 38.5, and 36 °C, for LD, HD, LDPA, and HDPA, respectively). Propionic acid decreases losses during both the fermentation and after opening the silo, for both the density levels.

Keywords: aerobic stability; bulk density; fermentative losses; forage conservation; organic acids

4.2 INTRODUCTION

Pile silos are commonly used in Brazil and worldwide due to their lower cost and large-scale production capacity. However, achieving adequate and uniform packing density of the ensiled mass is challenging, increasing residual oxygen (Kung et al., 2018). The presence of oxygen promotes greater respiration by aerobic microorganisms, which consume soluble carbohydrates and reduce the intensity of fermentation, compromising the quality of the silage (Hutnik and Kobielak, 2012).

Losses associated with the fermentation process and time of silage exposure to air in pile silos are generally higher compared to other types of silos (Johnson and Harrison, 2001). These losses are related to poor or uneven packing of the ensiled material, which can delay establishing the anaerobic condition necessary for proper fermentation. Consequently, respiration by undesirable aerobe microorganisms may occur, consuming soluble carbohydrates and other nutrients, and producing compounds that do not aid in the forage preservation process, such as CO_2, ethanol, H_2O, among others (Queiroz et al., 2018).

There are various chemical additives on the market designed to aid the fermentation process and aerobic stability of silages, many of which contain propionic acid in their composition (Morais et al., 2017). This can be explained by the fact that propionic acid is a short-chain fatty acid capable of penetrating microbial cells, such as yeasts and fungi, lowering internal pH and disrupting enzymatic activity, which interferes with enzymatic function and cellular metabolism, which ultimately leads to cell death (Lambert and Stratford, 1999).

When added to the forage, propionic acid also helps reduce its pH (Chen et al., 2017). Since most microorganisms that deteriorate silage struggle to survive in acidic conditions, the propionic acid inhibits undesirable microorganisms in silage by creating acidic conditions that hinder their survival (Zhao et al., 2022). In this way, the action of propionic acid can be effective both during the fermentation process and after the silage is exposed to air upon opening the silo. This results in more efficient fermentation with lower nutrient losses.

Propionic acid has been studied as a silage additive since the 1950s. However, no research associates its efficiency and benefits in preserving silage in pile silos or low-density silages. This experiment aimed to evaluate the effect of propionic acid on whole-plant corn silage at two packing densities.

4.3 MATERIAL AND METHODS

4.3.1 Ensiling process

The research was performed in the Forage Research Center (CPFOR) in Curitiba (25°25'42" S and 49°16'24" W), located in the state of Paraná, Brazil. The corn hybrid P4285VYHR (Pioneer®) was harvested on February 2, 2024, at 365 g kg^{-1} dry matter (DM) and processed using a stationary chopper, adjusted to 10 mm particle size.

The chopped forage was homogenized and divided into four piles for treatment characterization. The treatments were: High density (HD) – no additive, positive control; High density with propionic acid (HDPA) – addition of buffered propionic acid (1.0 L ton^{-1}); Low density (LD) – no additive, negative control, simulating pile silo condition; Low density with propionic acid (LDPA) – addition of buffered propionic acid (1.0 L ton^{-1}). After spraying and manually homogenizing the propionic acid in the respective treatments, the chopped forage was ensiled in 8.8 L PVC laboratory silos averaging 608 and 428 kg of forage per cubic meter for the high- and low-density treatments, respectively. After filing and compacting, the silos were sealed with a liquid sealant (Selabond®) and stored for 60 days.

The measurement of the produced gas volume (GV) was daily performed during the fermentation period according to the methodology described by Restellato et al. (2019). The measurements were taken several times a day and, after each evaluation, the gas was released. The total GV was the sum of all measurements recorded for each silo during the evaluation period.

4.3.2 Sample preparation and laboratory analysis

After characterizing the treatments, two samples of each treatment of fresh forage (~ 500 g) were collected to determine the DM content and the chemical composition (Table 4.3.2.1). The samples were dried in a forced-air oven at 60°C for 72 hours. Subsequently, the samples were processed in Wiley mill (Marconi®, model M 680, Brazil) using a 1 mm mesh sieve. Next, approximately 70 g of the processed samples were sent to the MS.DC Lab (Ponta Grossa - PR) for chemical composition analysis by Near Infrared Spectroscopy (NIRS) applying a calibration curve from the Dairyand Laboratories, EUA.

Table 4.3.2.1 – Chemical composition of fresh whole plant corn with and without propionic acid

Variable	Fresh Forage	Fresh Forage + PA
Crude protein (g kg^{-1} MD)	76.1	75.1
Neutral detergent fiber (g kg^{-1} DM)	506.9	444.6
Acid detergent fiber (g kg^{-1} DM)	281.8	243.1
Digestibility NDF – 48h (% NDF)	64.15	64.13
Starch (g kg^{-1} MD)	223.1	280.6
Total digestible nutrients (g kg^{-1} MD)	681.1	708.2

Another set of samples (25 g) from each treatment was collected for pH evaluation. The samples were diluted in 225 ml of distilled water and homogenized for one minute using a rod. The pH reading was taken with a digital pH meter (Gehaka®, model PG2000), as described by Kung et al. (1984).

Upon sealing and before opening, the silos were weighed, and the gravimetric losses of gases and DM were calculated as the difference between the initial and final weight of each silo (Jobim et al. 2007). At the silos opening, all the silage was removed, placed in plastic bags, and homogenized. Then, two other samples (~ 500 g) from each silo were collected to determine the DM content and chemical composition of the silage, as previously described.

4.3.3 Aerobic stability

After opening the silos, 3.0 kg of silage for high density and 2.5 kg of silage for low density were homogenized and placed in 20L buckets for the evaluation of aerobic stability. The buckets remained in a temperature-controlled room (25±2°C) for 240 hours. In each bucket, a datalogger was placed in the central region of the forage, programmed for taking the temperature every 30 minutes. Additionally, another datalogger was placed near buckets to measure the room temperature. Aerobic stability was defined as the time (h) required for the silage to reach 2°C above the ambient temperature, as described by Kung et al. (2000).

Another set of buckets with silage was used for taking samples for pH measurement every 2 days from the opening day (day 0), for 10 days. Samples were taken from the central region of each bucket and processed, as previously described.

4.3.4 Experimental design and statistical analysis

The experimental design was completely randomized in a 2 x 2 factorial scheme, with five replicates, totaling 20 experimental units. To evaluate the specific effects of the treatments, three orthogonal contrasts were defined. The contrasts were formulated so that the sum of the products of the coefficients was equal to zero (Table 4.3.4.1), thus ensuring orthogonality. The established contrasts were:

Table 4.3.4.1 – Contrast coefficients for each treatment.

Contrast	Treatment			
	HD	HDPA	LD	LDPA
1	-1	1	-1	1
2	1	0	-1	0
3	-1	0	0	1

Contrast 1: comparison between the control group {HD, LD} and treatments with propionic acid {HDPA x LDPA}

Contrast 2: Comparison of the density effect (HD x LD)

Contrast 3: Comparison between the ideal condition and the simulated field condition with propionic acid (HD x LDPA).

The statistical model used was

$$Y_{ijk} = \mu + D_i + P_j + (DP)_{ij} + e_{ijk}$$

In which μ = general mean; D_i = effect of the i-th level of the density factor; P_j = effect of the j-th level of the propionic acid factor, $(DP)_{ij}$ = effect of interaction between in the i-th level of the density factor and the j-th level of the propionic acid factor, e_{ijk} = experimental error. Propionic acid and packing density were considered fixed effects. The data were

analyzed using analysis of variance (ANOVA). Means were compared using Tukey's test with a significance level of 0.05 to verify significant differences between treatments. Subsequently, orthogonal contrasts were applied to decompose the total variability and evaluate the specific effects of the treatments. All the computations were conducted using the GLM procedure of SAS® (Statistical Analysis System) OnDemand for Academics.

4.4 RESULT

Propionic acid decreased total gas production (P<0.0001), with no difference between the HD and LDPA treatments (P = 0.2603) and density (P=0.1282), with gas volume of 4.9, 5.5, 5.6 and 5.8 L kg^{-1} DM for HDPA, LDPA, HD and LD, respectively (Figure 4.4. and Table 4.4.1). Propionic acid silages presented higher DM content than control silages (P = 0.0025, Table 4.4.1). Consequently, the control silages showed higher DM losses during fermentation than the propionic acid silages (P<0.0001, Table 4.4.1). High density reduces DM losses, however, low density combined with propionic acid results in lower DM losses compared to high density (Table 4.4.1).

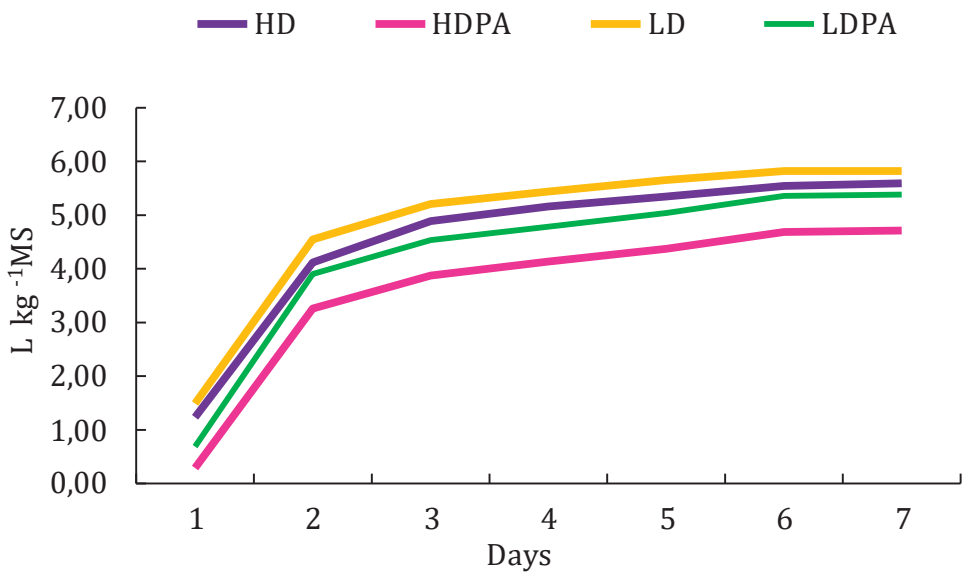

Figure 4.4.1 – Gas volume produced in whole plant corn silage treated with propionic acid and two packing densities.

Additionally, propionic acid decreased DM losses during 10 days of aerobic exposure (P<0.0001, Table 4.4.1). As expected, higher-density silages have lowered DM losses compared to those with lower density (P = 0.0207). However, LDPA treatment still shows lower DM losses than HD during aerobic exposure (P<0.0001). Propionic acid silages showed longer aerobic stability compared to control silages. Notably, LDPA silages had 52% greater aerobic stability than HD. Lower-density silages exhibited higher maximum temperature during aerobic exposure compared to higher-density silages (Table 4.4.1).

Table 4.4.1 – Fermentation losses and aerobic stability of whole plant corn silages treated with propionic acid at two packing densities

Variable	Treatment[1]				SEM[2]	Contrast[3] *P*-value		
	HD	HDPA	LD	LDPA		1	2	3
	Fermentative losses							
Gas volume produced (L kg^{-1} DM)	5.6	4.9	5.8	5.5	0.138	<.0001	0.1282	0.2603
Dry matter (g kg^{-1})	357.1	363.2	355.5	361.8	0.172	0.0025	0.5162	0.0742
DM losses (g kg^{-1} DM)	26.3	7.1	31.1	13.1	0.116	<.0001	0.0092	<.0001
	Losses after exposure to air							
DM losses in at AS (g kg^{-1} DM)	123.0	85.3	132.4	96.3	0.259	<.0001	0.0207	<.0001
Aerobic stability (h)	65	134	32	137	5.187	<.0001	<.0001	<.0001
Maximum temperature (ºC)	39	38.5	44	36	1.594	0.4344	<.0001	0.1039

[1]HD – High density – no additive; HDPA – High density with propionic acid (1.0 L ton^{-1}); LD – Low density – no additive; LDPA – Low density with propionic acid (1.0 L ton^{-1}).
[2]SEM – Standard error of the mean; DM – dry matter; AS – aerobic stability.
[3]C1 = comparison {HD, LD} and {HDPA x LDPA}; C2 = Comparison HD x LD; C3 = HD x LDPA.

The pH values were similar among treatments at the opening, with an average value of 3.74 (Figure 4.4.2). The LD silage showed an increase in pH before the first 48h, remaining high until the end of the evaluation. In contrast, the pH values of the HD, HDPA, and LDPA silages remained similar until 48h of aerobic exposure. However, in the HD silages, the pH increased after this period, reaching similar values to those of the LD silages up to 96h, and remained high until the end of the aerobic exposure. The LDPA silages kept lower pH values up to 192h after aerobic exposure. The HDPA silages kept reduced pH values up to 96h, increasing linearly until the end of the evaluation period. After 240h, all treatments reached similar pH values.

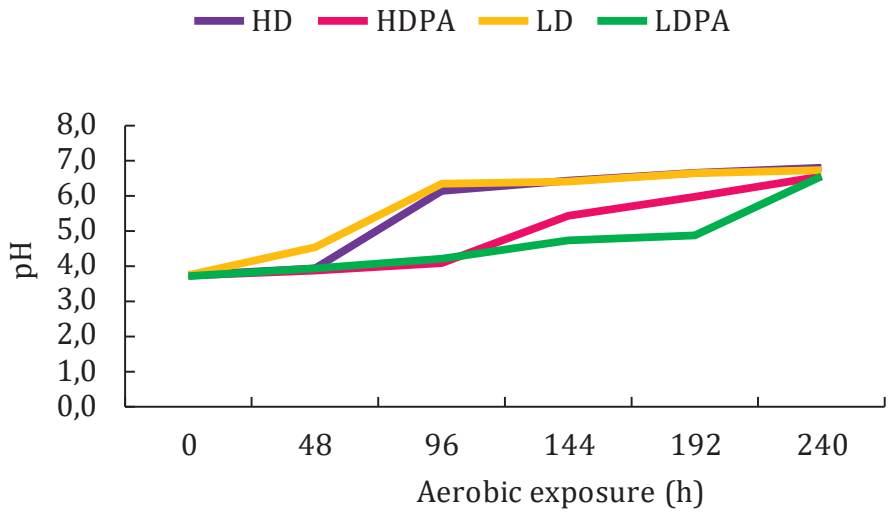

Figure 4.4.2 – pH values during aerobic exposure of whole plant corn silages treat with propionic acid at two packing densities.

Regarding the chemical composition, HD and HDPA silages showed higher crude protein content compared to the LD and LDPA ones (Table 4.4.2). The values of NDF, ADF, NDF digestibility (48h), starch, and TDN were similar among the treatments (Table 4.4.2).

Table 4.4.2 – Chemical composition of whole plant corn silages treated with propionic acid at two packing densities

Variable	Treatment[1]				Mean	SEM[2]	Contrast[3] *P*-value		
	HD	HDPA	LD	LDPA			1	2	3
Crude protein (g kg^{-1} DM)	80.0	80.0	77.0	77.0	78.0	0.046	0.9487	0.0004	0.0004
Neutral detergent fiber (g kg^{-1} DM)	415.3	427.0	418.1	420.5	420.2	1.032	0.5165	0.8514	0.7264
Acid detergent fiber (g kg^{-1} DM)	233.4	237.6	233.5	235.0	234.8	0.584	0.6360	0.9924	0.8545
Digestibility NDF – 48h (% NDF)	60.7	61.9	63.2	63.2	62.2	0.881	0.5231	0.0644	0.0652
Starch (g kg^{-1} DM)	325.2	313.6	331.2	314.4	321.1	1.074	0.2050	0.6980	0.4873
Total digestible nutrients (g kg^{-1} DM)	714.9	712.0	714.9	713.8	713.9	0.409	0.6366	0.9891	0.8516

[1]HD – High density – no additive; HDPA – High density with propionic acid (1.0 L ton^{-1}); LD – Low density – no additive; LDPA – Low density with propionic acid (1.0 L ton^{-1}).
[2]SEM – Standard error of the mean; DM – dry matter.
[3]C1 = comparison {HD, LD} and {HDPA x LDPA}; C2 = Comparison HD x LD; C3 = HD x LDPA.

4.5 DISCUSSION

Our study hypothesized that the inclusion of propionic acid in whole plant corn silage could reduce fermentative losses and preserve the DM of the ensiled material, serving as a strategy to ensure better feed quality, particularly in low-density silos. The addition of propionic acid to silage promotes a rapid drop in pH, inhibiting the activity of undesirable microorganisms and consequently reducing fermentative losses (Chen et al., 2017). In this study, we observed that controlling the microbial community through propionic acid, combined with higher density, reduces the degradation of carbohydrates into gases, minimizing DM losses. On the other hand, low density combined with propionic acid resulted in similar gas production to high-density silages, suggesting that on field conditions where ideal bulk density is not achievable, propionic acid can help reduce DM losses from gases. Due to higher residual respiration, especially in the early days of fermentation, low-density silos (LD) showed greater gas production compared to high-density silos, although this difference was not statistically significant. It is important to note that gas production is usually the main source of DM losses in silages (Schmidt et al., 2012).

As a result of the lower gas production, silages treated with propionic acid showed higher DM content at silo opening (362 g kg^{-1}) and lower DM losses during the fermentation process (7.1 and 13.1 g kg^{-1} initial DM for HDPA and LDPA, respectively), regardless of density. However, due to higher residual respiration, especially in the early days, low-density silos (LD) experienced greater DM losses compared to higher-density silos (Schmidt et al., 2012). Carvalho (2013) found that pile silos with lower densities had greater silage losses due to increased porosity, the presence of oxygen, and aerobic microorganisms. These results indicate that density has a direct influence on DM losses during fermentation, nevertheless, propionic acid helps reduce losses during forage preservation. Similar results were found by Chadwade et al. (2024), who observed higher DM content in grass silages treated with propionic acid compared to the control (270 and 250 g kg^{-1} initial DM for PA and Control, respectively).

After aerobic exposure, microorganisms such as yeasts and molds can proliferate rapidly (Liu et al., 2024). Many factors such as silage density, epiphytic microbial

community, removal process, and duration of use affect the rate of feed degradation (Carvalho, 2013). The greater aerobic stability (AS) observed in silages treated with propionic acid (137 and 134h for LDPA and HDPA, respectively) highlights its significant role over post-silo opening, particularly in low-density silos. Low-density silos treated with propionic acid showed 52% greater AS than high-density silos, indicating that while density improves AS, propionic acid further supports stability. Similar results were observed by Arthur (2019), who reported greater aerobic stability in corn silage treated with a commercial propionic acid-based additive compared to the control (144 vs 78h for Additive and Control, respectively).

Although the present study did not include microbial analyses of silage, previous research has indicated that higher concentrations of propionic acid reduce microbial populations such as *Clostridium* (0.6 vs 5.3%) and *Enterobacteria* (3.0 vs 11.1%), while increasing *Lactobacillus* (45.0 vs 32.7%) and *Bacillus* (0.35 vs 0.15%), acting as an inhibitor of undesirable fermentation (Zhao et al., 2022; Chadwade et al., 2024). This inhibition occurs because propionic acid, at a pH lower than its pKa (4.87) remains in its undissociated form during the ensiling process, penetrating yeast and fungal cells. Once inside the cells, if forces the microorganism to expend energy to expel H^+ ions out, which can reduce microbial growth and lead to cell death (Lambert and Stratford, 1999). This explains the crucial role of propionic acid both during the fermentation process and after silo opening.

Aerobic stability is crucial for forage preservation, ensuring that silage supplies sufficient nutrients for animal feed (Wilkinson and Davies, 2013). The reduced AS of low-density silage (32h) may reflect the activity of aerobic microorganisms metabolizing carbohydrates and organic acids, producing CO_2 and H_2O, generating heat, and raising pH levels (Figure 2, Wilkinson and Davies, 2013), leading to rapid aerobic deterioration. This outcome is undesirable due to economic losses from unutilized silage and decreased animal performance. Therefore, adequate pressing density or the use of additives like propionic acid is important under field conditions where ideal density is unattainable.

Silages treated with propionic acid exhibited the lowest DM losses after exposure to air (85.3, 96.3, 123.0, 132.4 g kg^{-1} silage DM for HDPA, LDPA, HD and LD, respectively), likely due to the reduced growth of fungi, yeasts, and other microorganisms responsible

for silage degradation (Pordeus et al., 2022). The fermentation process involves a complex microbial symbiosis (Zhao et al., 2024), where undesirable microorganisms such as yeasts and enterobacteria may not be fully eliminated and can remain inactive in the absence of oxygen. After re-exposure to air, these yeasts can metabolize lactic acid, increasing the silage pH and creating favorable conditions for aerobic microorganisms, such as bacteria and fungi, to develop, initiating material degradation (Kung et al., 2018). Therefore, the antifungal properties of acetic and propionic acids at the silo opening slow the degradation process, minimizing DM losses after exposure to air (Morais et al., 2017, Pordeus et al., 2022).

The bulk density of silage is a key factor for both anaerobic fermentation and aerobic stability (Hutnik and Kobielak ,2012). In this study, LD silages exhibited lower AS reaching a maximum temperature of 44°C after 42h of air exposure, along with a rapid pH rise and higher DM losses during the fermentation process and after silo opening. In contrast, the LDPA silages showed lower maximum temperatures, higher aerobic stability, and reduced losses throughout the ensiling process, indicating that propionic acid plays an important role in inhibiting undesirable microorganisms that compromise forage preservation.

The chemical composition of the fresh forage samples showed variation in NDF, ADF, and starch content. Given the inconsistency of these results, we believe that a potential sampling error may have occurred. Despite all attention to the sampling protocol, chopped corn forage segregate the kernel from forage, possibly leading to this issue. Although propionic acid significantly contributed to the fermentation process and aerobic stability of whole-plant corn silage, it did not affect the chemical composition of the silages in this study. Only crude protein showed a significant difference, with higher-density silages (HD and HDPA) having 3 g kg^{-1} DM more CP than lower-density silage ones. Senger et al. (2005) reported no changes in DM, CP, NDF, and ADF in corn silages with densities ranging from 350 to 700 kg m^{-3}. However, an increase in crude protein in corn silage (Gheller et al., 2021) and grass silage (Chadwade et al., 2024; Zhao et al., 2024) has been reported. Additionally, snaplage silages treated with propionic acid additives had higher starch content compared to control (593 vs 549 g/kg DM; Pordeus et al., 2022). Despite its unclear role in nutrient preservation, propionic acid improves aerobic stability and reduces DM losses, as shown in previous in studies.

4.6 CONCLUSIONS

Propionic acid decreases losses during the fermentation and after opening the silo, at both packing density evaluated. This indicates propionic acid may be used to decrease losses in piles or bunker silos.

5 FINAL CONSIDERATIONS

The blend used consists of organic acids known to have beneficial effects on the ensiling process. However, it is believed that the inclusion rates may not have been sufficient to produce significant results in terms of nutrient preservation and aerobic stability in whole-plant corn silage and high-moisture corn silage.

Propionic acid may serve as a management alternative under field conditions where optimal silage density cannot be achieved. However, future studies should be conducted to assess whether silage treated with propionic acid has any impact on nutrient utilization and animal performance.

4.7 REFERENCES

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