University of São Paulo
Luiz de Queiroz College of Agriculture

Propionic acid-based additive with surfactant action in reconstituted corn grain silage on nutritive value and performance of ruminants

Álvaro Bernardo da Silva Neto

Dissertation presented to obtain the title of Master in Science. Area: Animal Science and Pastures

Piracicaba
2022

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **LUIZ GUSTAVO NUSSIO**

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1.Silagem de milho reconstituído 2. Surfactante 3. Ácido propiônico 4. Ruminantes I. Título

To my parents and friends

I DEDICATE

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CONTENTS

RESUMO

Aditivo com base em ácido propiônico e agente surfactante em silagem de grão de milho reconstituído no valor nutritivo e no desempenho de ruminantes

A ensilagem do grão de milho moído com alto teor de umidade é uma técnica que, além de permitir o armazenamento do grão, proporciona um alimento com melhor qualidade nutricional em relação ao milho seco, devido às transformações físico-químicas que resultam no aumento da disponibilidade de amido. Esse aumento na digestibilidade do amido ocorre devido ao processo de proteólise da matriz proteica que envolve os grânulos de amido, desencadeado principalmente por proteases bacterianas. O emprego de aditivos que auxiliam no processo de proteólise é uma alternativa para se obter um alimento de alta qualidade nutricional com tempo de fermentação reduzido. Em um estudo de Oliveira (2020) com silagem de milho reidratado, foram encontradas evidências de que a ensilagem com um aditivo à base de ácido propiônico e agente surfactante pode potencializar a proteólise em milho reconstituído e aumentar sua degradabilidade ruminal. Para avaliar essa hipótese, foram elaborados dois experimentos com milho reidratado ensilado por diferentes tempos sobre o desempenho de duas categorias de ruminantes. O primeiro experimento utilizou 18 vacas da raça Holandesa, em quadrado latino 3x3, com três tratamentos: milho reidratado ensilado por 30 dias (30 CON); ensilado por 60 dias (60 CON); e ensilado com aditivo por 30 dias (30 MYC), visando avaliar a ingestão de matéria seca e nutrientes, produção e composição do leite, comportamento ingestivo e seletivo e digestibilidade de nutrientes. O segundo experimento utilizou 36 borregas da raça Dorper, em delineamento de blocos casualizados com dois períodos subsequentes, com três tratamentos: milho reidratado ensilado por 50 dias (50 CON); ensilado por 90 dias (90 CON); e ensilado com aditivo por 50 dias (50 MYC), visando avaliar o consumo de matéria seca e nutrientes, ganho de peso e comportamento seletivo. Em ambos os experimentos, foram analisados os perfis bromatológico e fermentativo das silagens e realizado um ensaio de degradabilidade ruminal em vacas leiteiras. **Experimento 1**: as vacas foram alojadas em um sistema de confinamento *Free Stall*, e alocadas em cochos individuais equipados com sistema de restrição e monitoramento. O manejo alimentar consistiu em duas ofertas diárias, sendo as vacas ordenhadas duas vezes ao dia. O maior tempo de conservação da silagem 60 CON aumentou o teor de nitrogênio solúvel e a degradabilidade ruminal da matéria seca em relação às demais silagens com 30 dias. Embora a silagem tratada com aditivo tenha apresentado teor de proteína solúvel semelhante à silagem 30 CON, o teor de amônia e sua concentração no N solúvel foram significativamente maiores para a primeira. A silagem 30 MYC apresentou maior teor de ácido lático em relação às demais silagens. Houve redução significativa do tamanho médio geométrico das partículas e aumento da área superficial e proporção de partículas finas na silagem de milho reidratado com o uso do aditivo. Não foram observadas alterações na degradabilidade da matéria seca da silagem 30 MYC em relação à silagem controle, porém houve redução significativa da taxa de degradação ruminal (k_d) com o uso do aditivo. Não houve mudanças significativas no consumo de matéria seca, produção e composição do leite para as vacas alimentadas com a silagem tratada com aditivo. Porém, a presença do aditivo na silagem de milho reidratado promoveu maior seleção contra partículas longas da dieta, e maior seleção em favor de partículas finas, refletindo em menor consumo de FDN pelas vacas tratadas com a dieta contendo silagem 30 MYC, porém sem prejuízo às atividades de ruminação e mastigação. **Experimento 2**: o maior tempo de conservação da silagem 90 CON e entre os períodos promoveu aumento nos teores de proteína solúvel e amônia e na degradabilidade ruminal da matéria seca. Embora nenhum ganho na extensão da degradação tenha sido observado, o maior tempo de ensilagem aumentou a fração solúvel em detrimento da fração potencialmente degradável. Embora a silagem 50 MYC tenha apresentado teor de nitrogênio solúvel semelhante ao da silagem 50 CON, seu teor de amônia foi significativamente maior e sua concentração de NH_3-N no N solúvel foi maior do que a observada nos demais tratamentos. A silagem 50 MYC apresentou as menores concentrações de ácido lático entre os tratamentos, porém os teores de etanol e ácido butírico foram significativamente menores na silagem tratada com aditivo. Não houve mudanças na degradabilidade da matéria seca após 12 h de incubação ruminal e na taxa de degradação (k_d) da silagem 50 MYC contra a silagem 50 CON, porém o aditivo apresentou maior fração indigestível do milho em relação aos demais tratamentos, além de uma redução na fração solúvel no rúmen apenas no segundo período em relação à silagem 50 CON. A degradabilidade efetiva somente tendeu a ser menor para a silagem tratada com aditivo quando as taxas de passagem (k_p) foram consideradas a 5 e 8%/h, mas a 2%/h foi significativamente menor em relação à silagem 50 CON. Não houve efeito do tempo de ensilagem ou do aditivo sobre o consumo de matéria seca, ganho de peso e eficiência alimentar das borregas. A silagem tratada com o aditivo não alterou o comportamento seletivo das borregas.

Palavras-chave: Silagem de milho reconstituído, Surfactante, Ácido propiônico, Ruminantes

ABSTRACT

Propionic acid-based additive with surfactant action in reconstituted corn grain silage on nutritive value and performance of ruminants

The ensilage of ground corn grain with high moisture content is a technique that, in addition to allowing the storage of the grain, provides a food with better nutritional quality compared to dry corn, due to physicochemical transformations that result in an increase in availability of starch. This increase in starch digestibility occurs due to the proteolysis process of the protein matrix that surrounds the starch granules, mainly triggered by bacterial proteases. The use of additives that help in the proteolysis process is an alternative to obtain a high nutritional quality food with a reduced fermentation time. In a study by Oliveira (2020) with rehydrated corn silage, evidence was found that ensiling with a propionic acid-based additive with surfactant properties can enhance proteolysis in reconstituted corn and its ruminal degradability. To evaluate this hypothesis, two experiments were designed with rehydrated corn ensiled for different times on the performance of two categories of ruminants. The first experiment used 18 Holstein dairy cows, under a 3x3 Latin square design, with three treatments: reconstituted corn ensiled for 30 days (30 CON); ensiled for 60 days (60 CON); and ensiled with additive for 30 days (30 MYC), seeking to evaluate dry matter and nutrient intake, milk yield and composition, ingestive and selective behavior and digestibility of nutrients. The second experiment used 36 Dorper lambs, in a randomized complete block design with two subsequent periods, with three treatments: reconstituted corn ensiled for 50 days (50 CON); ensiled for 90 days (90 CON); and ensiled with additive for 50 days (50 MYC), seeking to evaluate dry matter and nutrient intake, weight gain and selective behavior. In both experiments, the bromatological and fermentative profile of the silages were analyzed, and a ruminal degradability assay was conducted using dairy cows. **Experiment 1**: the cows were housed in a free stall confinement system, and allocated to individual feed bunks equipped with individual access control and feed monitoring system. The feeding management consisted of two daily offers, and the cows were milked twice a day. The extended conservation time of the 60 CON silage increased the soluble nitrogen content and the ruminal degradability of dry matter in relation to the other silages with 30 days. Although the silage treated with additive had similar soluble protein content compared to 30 CON silage, the ammonia content and its concentration in soluble N were significantly higher for the former. The 30 MYC silage had higher lactic acid content compared to the other silages. There was a significant decrease in the geometric mean particle size and an increase in the surface area and proportion of fine particles in the rehydrated corn silage with the use of the additive. No changes were observed in the dry matter degradability of 30 MYC silage against the control silage, but there was a significant decrease of the ruminal degradation rate (k_d) with the use of the additive. There were no significant changes in dry matter intake, milk yield and composition for the cows fed the additive-treated silage. However, the presence of the additive in rehydrated corn silage promoted greater selection against long particles in the diet, and a greater selection in favor of fine particles, reflecting in lower NDF intake by cows when treated with the diet containing 30 MYC silage, but without harming rumination and chewing activity. **Experiment 2**: the longer conservation time of 90 CON silage and between periods promoted an increase in the soluble protein and ammonia contents and in the ruminal degradability of dry matter. Although no gain in the extent of degradation was observed, the longer ensiling time increased the soluble fraction at the expense of the potentially degradable fraction. Although the 50 MYC silage had similar soluble nitrogen content compared to 50 CON silage, its ammonia content was significantly higher, and its concentration of NH_3-N in soluble N was higher than that observed in the other treatments. The 50 MYC silage presented the lowest concentrations of lactic acid between the treatments, however ethanol and butyric acid contents were significantly lower in the additive-treated silage. There were no changes in dry matter degradability after 12 h of ruminal incubation and in degradation rate (k_d) of the 50 MYC silage against the 50 CON silage, but the additive promoted a higher indigestible fraction of corn compared to the other treatments, and there was a reduction in the ruminal soluble fraction only in the second period in relation to the 50 CON silage. The effective degradability only tended to be lower for the additive-treated silage when passage rates (k_p) were assumed at 5 and 8%/h, but for 2%/h it was significantly lower in relation to the 50 CON silage. There was no effect of ensilage time or additive on dry matter intake, weight gain and feed efficiency of the lambs. The silage treated with the additive did not change the selective behavior of the lambs.

Keywords: Reconstituted corn grain silage, Surfactant, Propionic acid, Ruminants

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1. INTRODUCTION

Corn is the main cereal used in animal feed, being the main energy source in the formulation of diets for ruminants. Although starch can represent up to 72% of the average composition of the corn grain (Paes, 2006), a large part of it is unavailable, preventing its complete digestion and use by the animal. This is due to the physicochemical barrier imposed by the protein matrix that surrounds the starch granules (Owens et al., 1986), mainly in the vitreous endosperm, where the interaction between the starch granules and hydrophobic prolamines (especially zeins) is particularly strong, inhibiting the penetration of both water and amylases and maltases responsible for breaking down starch into the rumen and intestine (McAllister, 2001).

Ensiling ground grain with a high moisture content is an alternative to the storage and use of dry grain in animal feed. The fermentation that takes place in ensiled grains causes chemical changes in the protein matrix, reducing its content of zeins and making starch granules available, increasing their digestibility (Hoffman et al., 2011), which results in a starch source with higher nutritional quality. Harvesting wet grain, however, reveals a series of operational risks, both because of the narrow harvest window that can be hampered by the occurrence of rain at the same time, and because of the difficulty in milling grains with a high moisture content, resulting in constant stuffing of the grinders and clogging of the sieves (Arcari, 2017).

As an alternative to wet grain ensilage, there is the corn rehydration technique. This strategy allows the grain to be harvested mature, even at a more advanced maturity stage, extending the harvest window, to then be ground and submitted to the rehydration or reconstitution process, which consists of adding water until it reaches a moisture content between 30 and 40 %, enough for lactic acid bacteria to multiply and ferment the ensiled substrate, preserving it through the ensiling process (Defoor et al., 2006). The degradation of the protein matrix containing prolamins in corn grain silages is mainly due to the action of bacterial proteases, followed by proteases from the grain itself and, to a lesser extent, by the action of fungi and solubilization by the acid products of fermentation (Junges et al., 2017).

In a study by Oliveira (2020), with rehydrated corn silage, there is evidence that a chemical additive based in propionic acid with surfactant properties can potentialize proteolysis in grains. The additive utilized consisted of a combination of organic acids, including propionic acid, and surfactants, whose purpose is to aid the penetration of water into the corn grains destined to the flocculation process and improve aerobic stability. Although the mechanism of action has not been reported, a hypothesis raised is that the additive should act to break the bonds between the starch granules and the prolamines, which consequently results in the exposure of the starch to the action of the proteases present in the silage (bacterial and from the grain, mainly) faster than by natural exclusive fermentation. The objective of this trial was to evaluate the effect of including an additive with surfactant properties in the silage of rehydrated corn kernels on proteolysis and performance of growing lambs and dairy cows.

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2. LITERATURE REVIEW

2.1. High moisture grain silage

The processing technique through ensiling of high moisture grain targets the protein matrix surrounding the starch granules, constituting a processing strategy based on biochemical intervention. Instead of physically disrupting the matrix, as in grinding or flocculation, ensiling of grains is associated with chemical changes in the protein matrix through the activity of proteases mainly from microorganisms and the grain itself, reducing the content of zein and making starch available, increasing its digestibility (Hoffman et al., 2011) both in the rumen and in the intestine (Owens & Soderlund, 2006).

The digestibility of ensiled corn grain has a high correlation with the soluble protein content of the silage (Fernandes et al., 2021), since, during fermentation, occurs degradation of the zeins surrounding the starch granules (Hoffman et al., 2011; Prigge, 1976; Rooney & Pflugfelder, 1986; Owens & Zinn, 2005). Zeins are hydrophobic proteins, components of the protein matrix that surrounds starch granules in grains such as corn and sorghum, making them inaccessible when ingested by the animal and therefore, not digested in the gastrointestinal tract. During ensiling under conditions of high humidity, zeins are degraded, at different scales, by bacterial proteases (60.4%) or from the grain itself (29.5%), by the action of fungi (5.3%) and by acid solubilization by fermentation products (4.8%), as reported by Junges et al. (2017) in corn silages rehydrated and ensiled for 90 days. Hoffman et al. (2011), evaluating the transformations in α, β, γ and δ-zein subunits in two corn hybrids ensiled for 240 days, reported a considerable decrease in γ-zeins, subunits that are located more superficially in the protein matrix, which changed the level of organization of starch granules, individualizing them and making them more accessible.

For the degradation of the protein matrix to occur, it is necessary that the ensiled grain should have a moisture content between 35 and 40%, ideal for microbial development and the kinetics of the enzymes, through the penetration of moisture into the endosperm of grains (Defoor et al., 2006). In the case of grains with vitreous endosperm, moisture penetration is hampered by the high degree of compaction of the granules and their strong adhesion to the matrix, but this is solved with sufficient storage time, so as the matrix is degraded, the deeper the water can penetrate the endosperm.

As a result of matrix degradation, grain silage is characterized by an increase in the levels of soluble nitrogen, particularly non-protein nitrogen (NPN), and ammonia, both being good indicators of proteolysis of the protein matrix and the evolution of the silage (Gervásio, 2021; Hoffman et al., 2011; Arcari, 2013; da Silva et al., 2019; Fernandes et al., 2021). Thus, in addition to the greater availability of starch, ensiling also contributes to increased grain protein digestibility (Hinders, 1976), by degrading fractions that would normally not be degraded while remaining in the rumen environment. The largest increases in dry matter digestibility and rumen degradable protein occur within the first 56 days of ensiling, according to Benton et al. (2005), as well as soluble protein (Hicks & Lake, 2012). Prigge (1976) observed an increase from 15.8 to 38.2% of soluble nitrogen in relation to the total nitrogen of corn silage with 28% moisture, stored for 56 days. Results from Gervásio (2021) reinforce the time of 60 days as the minimum ensiling time for satisfactory degradation of prolamines in corn, and as the minimum time for the prolamines content to reach a similar level regardless of the particle size of the grain milling. Fernandes et al. (2021) also reported similar results for two corn hybrids, both ensiled as high moisture grains or rehydrated grains, with greater decrease in prolamins on the first 60 days of ensiling, and continuous increase in the NH_3-N fraction, with the greater increases also occurring in the first weeks of ensiling.

There is some confusion of terminology when referring to high moisture grain silage processing in Brazil. This technique is commonly known as “wet grain silage”, a term used to define both the actual wet grain silage, harvested before physiological maturation, and the rehydrated grain silage, usually harvested after maturation and with a composition quite different from the first one. Technically, wet grain silage itself consists of harvesting the grain in a physiological stage such that the protein matrix is not yet fully developed, with moisture already at the ideal range (between 35 and 40%), ground and immediately unloaded into the silo without any intermediate storage steps. The silage of rehydrated or reconstituted grains, on the other hand, can even be a variation of the silage of moist grains, when they end up being harvested outside the cutting window with a moisture content below the ideal, requiring an additional step of adding water at the time of unloading in the silo. However, the term is more used for the silage of harvested mature grains, previously dried (11-12% moisture) and stored awaiting rehydration and ensiling.

For the ensiling of rehydrated corn, grains that are already dry are used, normally harvested after physiological maturation. Thus, they are subjected to grinding and rehydration until they reach an adequate moisture content for ensiling, from at least 35% to a maximum of 40%. When the harvest occurs after the physiological maturation, the protein matrix is already fully developed. However, the starch filling of the grain is also complete, which assures the maximum starch content in the grain. Although this strategy has a higher cost in relation to wet grain ensiling, it allows greater flexibility in planning and logistical improvements, as it is not limited to a restricted time of harvesting window.

Ensiling corn grains with high moisture content is an efficient storage strategy for this type of feed source, providing gains in nutritional value. Dry matter losses during fermentation are less than 4% (Kung Jr. et al., 2007; da Silva et al., 2015), which are close to the losses of 1-2% in dry grain storage (Santos et al., 1994), and considerably lower than the losses of 15% for the storage of dry grain still on the ear, by insects and rodents (Santos, 2006). Thus, rehydration and ensilage of corn grains can be used as an alternative solution for storing dry grains, which are very subject to attack by pests such as rodents and weevils, providing better conservation and greater nutritional quality to corn. Another advantage is the possibility of purchasing the corn grain on the market at a low price (off season) and storing it in the form of rehydrated silage, constituting a form of "energy saving" that generates "energy income" for cattle, so that in time of high prices (off-season) the producer can stop buying the grain corn and use his ensiled corn reserve to offer to the animals.

2.2. Proteolysis in corn silages

Proteolysis in silages is generally referred as a negative process of changing the nutritional quality of the feed, specifically the protein fraction, since part of the true protein is degraded to NPN by the plant's own proteases, and subsequently deaminated to ammonia and amines by microbial proteases (Ohshima and McDonald, 1978). This conversion can interfere with the efficiency of nitrogen utilization by ruminants (Waldo, 1985). Despite the possibility of the soluble protein fraction passing more quickly through the rumen and compose the rumen undegradable protein (Broderick et al., 2010; Huhtanen et al., 2014), NPN is the main source of nitrogen used by rumen microorganisms for microbial protein synthesis, therefore, excessive proteolysis of silage may interfere with the ruminal degradation synchrony and compromise the supply of amino acids to the ruminant. The proteolysis process is naturally triggered in plant cells by their death after the moment of cutting, being favored by conditions of high moisture (Van Soest, 1994). One of the objectives of management of the ensiling process is to reduce this proteolytic activity by providing an oxygen-free condition, favoring the development of anaerobic bacteria population which ferment part of the soluble carbohydrates and acidify the environment. Lowering pH delays the activity of proteases with linear decrease in activity between pH 6.0 and 4.0 (Muck, 1988; McKersie, 1985). This acidification begins right after a brief lag time when the residual oxygen is depleted by aerobic respiration. The following anaerobic fermentation within the first 3 to 5 days of ensiling yields organic acids, initially acetic, with low acidification capacity (pKa = 4.76), then lactic acid (pKa = 3.85), which usually decreases the silage pH during the next 15-20 days of ensiling until the range of 3.8-4.2, enough to stop bacterial activity and the synthesis of additional bacterial proteases (Harris Jr., 1984).

To evaluate the proteolytic process during storage of corn grain and whole-plant silages, a meta-analytic study was carried out from 40 studies with whole-plant corn silage (WPCS), being 8 full-length papers (Queiróz et al., 2012, 2013; Rabelo et al., 2017; Silva et al., 2017; Rosa et al., 2004; Auerbach and Nadeau, 2013; Salvati et al., 2020; Salvo et al., 2020), 31 published abstracts on International Silage Conference recent editions (Queiróz et al., 2015; Gerlach et al., 2015; Reis et al., 2015; Rabelo et al., 2015; Souza et al., 2015; Milora et al., 2015; Nadeau et al., 2015; Bereterbide et al., 2015; Sá Neto et al., 2015; Loučka et al., 2015, 2018; Smith et al., 2015; Daniel et al., 2015; Solórzano et al., 2015; Nooijen et al., 2015; Copani et al., 2018; Khan and Khan, 2018; Christou et al., 2018; Wang et al., 2018; Zopollatto et al., 2018; Monge et al., 2018; Borreani et al., 2018a, 2018b; Huenting et al., 2018a, 2018b; Jatkauskas et al., 2018; Jilg, 2018; Szucs et al., 2018; Milimonka et al., 2018; Agarussi et al., 2018; Arriola et al., 2018) and 1 thesis still not published as a paper (Reis, 2021), and from 33 studies with high moisture and rehydrated corn grain silages, being 26 full-length papers (Hoffman et al., 2011; Junges et al., 2017; da Silva et al., 2015, 2018, 2019; Fernandes et al., 2021; Silva et al., 2018; Bolson et al., 2020; Cruz et al., 2021; Bíro et al., 2006, 2009; Canibe et al., 2014; Doležal and Zeman., 2005; Ferraretto et al., 2015; Flores-Galarza et al., 1985; Gálik et al., 2008; Kung Jr. et al., 2004, 2007, 2014; Loučka, 2010; Morais et al., 2012; Prigge et al., 1976; Pyś et al., 2009; Reis et al., 2008; Taylor and Kung Jr., 2002; Wardynski et al., 1993), 2 published abstracts on the International Silage Conference (Auerbach et al., 2015; Gallo et al., 2015) and 5 theses still not published as papers (Oliveira, 2020; Gervásio, 2021; Arcari, 2013; Morais, 2016; Gritti, 2021). Of the set of studies with WPCS, only 22 reported proteolysis indicators as NH_3-N contents, and only the thesis of Reis (2021) also reported the soluble N content. Of the set of studies with corn grain silage, only 24 reported NH_3-N contents and 10 reported soluble N contents.

The database was created from information that allowed the extraction of exact values at different ensiling times, using only the control silages, which were not treated with any type of chemical additive or microbial inoculant. Corn grain silages included both high moisture harvested and rehydrated grains, processed or whole. The variables NH_3-N and soluble N had to be adapted in some studies so they could be expressed as a % of the total nitrogen. The analyzes were based on regression of the dependent variables as a function of storage time and adjustment for the random effect of study (St-Pierre, 2001). Preliminary analyzes of the variables over storage time were performed using the LOESS procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) to determine the most suitable regression models to be tested (Cohen, 1999; Ryan & Porth, 2007). The regressions were performed using the SAS MIXED procedure, considering continuous and piecewise models, and the occurrence of a lag-time at the beginning of ensiling. The covariance structure was defined based on the corrected Akaike's criterion, and degrees of freedom were calculated using the Kenward-Roger option. The final equations were obtained using the SAS NLIN procedure, and the best regression model for each variable was chosen based on the lowest root mean square of error (RMSE) value.

Whole-plant corn silages typically reach pH values between 3.5 and 4.0 (Figure 1). However, grain silages usually achieve stability under higher pH values (Figure 2), between 3.7 and 5.0, due to the low moisture content (30 - 40%), which inhibits the microbial development and organic acids production (Morais et al., 2017). Fernandes et al. (2021) observed that ensiled rehydrated corn grains had lower pH than high moisture grain silages, often reaching values close to or less than 4.0 (Morais, 2016; Arcari, 2013; Oliveira, 2020; Junges et al., 2017; Silva et al., 2018; Bolson et al., 2020; Gervásio, 2021), but similar values have also been reported for high moisture grain silages (Auerbach et al., 2015; da Silva et al., 2015; Gallo et al., 2015). The extent and rate of pH drop seem to be more related to the moisture content (Van Soest, 1994; Muck, 1988; Morais et al., 2017) than to the type of grain silage.

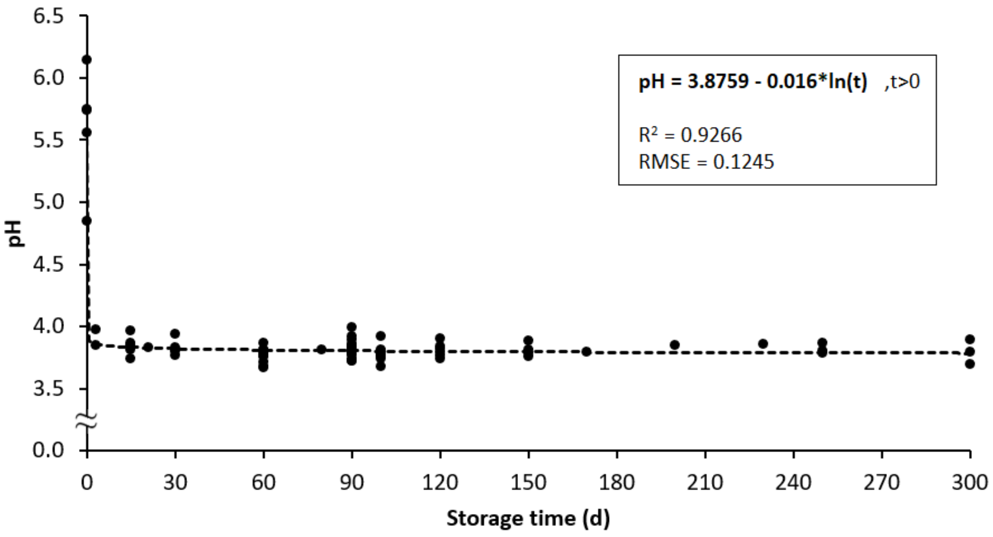

Figure 1. Adjusted pH values for whole-plant corn silage (n=81) compiled from 40 studies (Queiróz et al., 2012, 2013, 2015; Gerlach et al., 2015; Reis et al., 2015; Rabelo et al., 2015, 2017; Silva et al., 2017; Souza et al., 2015; Milora et al., 2015; Nadeau et al., 2015; Rosa et al., 2004; Bereterbide et al., 2015; Sá Neto et al., 2015; Loučka et al., 2015, 2018; Auerbach and Nadeau, 2013; Reis, 2021; Smith et al., 2015; Daniel et al., 2015; Solórzano et al., 2015; Nooijen et al., 2015; Salvati et al., 2020; Salvo et al., 2020; Copani et al., 2018; Khan and Khan, 2018; Christou et al., 2018; Wang et al., 2018; Zopollatto et al., 2018; Monge et al., 2018; Borreani et al., 2018a, 2018b; Huenting et al., 2018a, 2018b; Jatkauskas et al., 2018; Jilg, 2018; Szucs et al., 2018; Milimonka et al., 2018; Agarussi et al., 2018; Arriola et al., 2018). Values refer to silages without treatment (chemical additives or microbial inoculants).

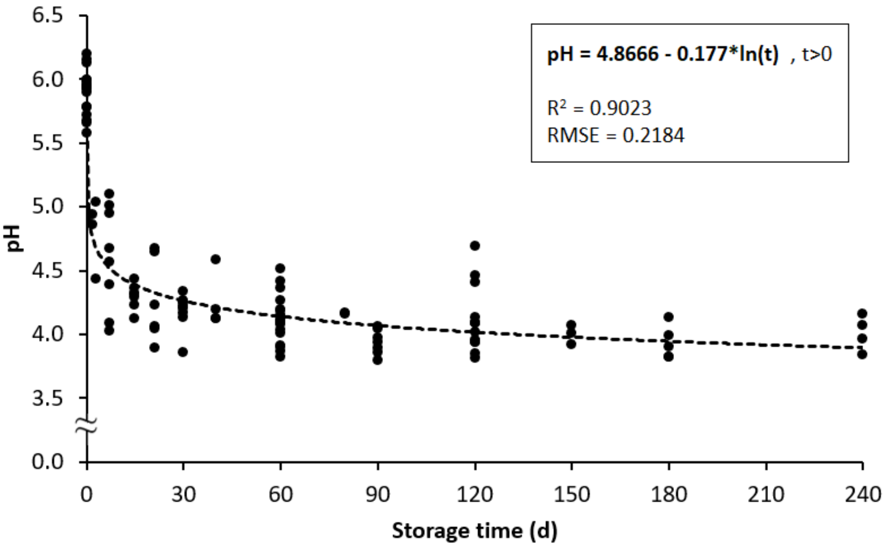

Figure 2. Adjusted pH values (n=106) compiled from 33 studies with high moisture and rehydrated corn grain silages (Oliveira, 2020; Gervásio, 2021; Hoffman et al., 2011; Arcari, 2013; Morais, 2016; Junges et al., 2017; da Silva et al., 2015, 2018, 2019; Fernandes et al., 2021; Auerbach et al., 2015; Gallo et al., 2015; Silva et al., 2018; Bolson et al., 2020; Cruz et al., 2021; Gritti, 2021; Bíro et al., 2006, 2009; Canibe et al., 2014; Doležal and Zeman., 2005; Ferraretto et al., 2015; Flores-Galarza et al., 1985; Gálik et al., 2008; Kung Jr. et al., 2004, 2007, 2014; Loučka, 2010; Morais et al., 2012; Prigge et al., 1976; Pyś et al., 2009; Reis et al., 2008; Taylor and Kung Jr., 2002; Wardynski et al., 1993). Values refer to silages without treatment (chemical additives or microbial inoculants).

A typical WPCS may be composed of about 45% DM in grains (Philippeau and Michalet-Doreau, 1998). The occurrence of a certain degree of proteolysis in this type of silage is desirable due to the need of degrading the protein matrix that surrounds the starch granules in the grains, especially in the case of high vitreous endosperms, such as in most of the tropical hybrids (Correa et al., 2002). Although the ensiling process is commonly associated with the absolute decrease of proteolysis in silages, what actually happens is a containment of the proteolytic process, since even at pH 4.0 proteases can still present from 15 to 35% of the observed activity at pH 6.0 (Muck, 1988). In WPCS, this lower activity of proteases allows a certain degree of proteolysis, beneficial to improve the nutritional value of the grain fraction, and at the same time prevents a generalized protein degradation of plant tissues in the rest of the silage, preserving the nutritional value of the vegetative fraction.

The NH_3-N content in silages is a good indicator of proteolytic activity and can be used as an index of nutritional quality. Suitable levels for WPCS are between 3-5% of total-N, but values observed in the literature are frequent between 3 and 8% of total-N over time, depending on the ensiling conditions (moisture, time between cutting and sealing, storage time) and the hybrid used (Figure 3).

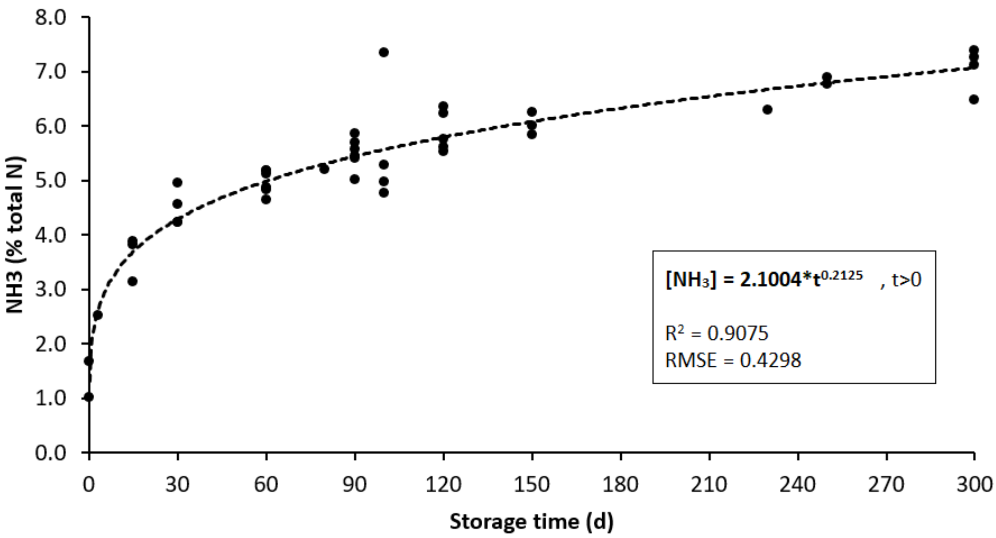

Figure 3. Adjusted NH_3-N values for whole-plant corn silage (n=43) compiled from 22 studies (Gerlach et al., 2015; Rabelo et al., 2015, 2017; Silva et al., 2017; Nadeau et al., 2015; Bereterbide et al., 2015; Sá Neto et al., 2015; Solórzano et al., 2015; Nooijen et al., 2015; Queiróz et al., 2012, 2013; Rosa et al., 2004; Auerbach and Nadeau, 2013; Reis, 2021; Salvati et al., 2020; Copani et al., 2018; Wang et al., 2018; Monge et al., 2018; Huenting et al., 2018a, 2018b; Jatkauskas et al., 2018; Szucs et al., 2018). Values refer to silages without treatment (chemical additives or microbial inoculants).

The occurrence of proteolysis in grain silages, such as corn and sorghum, is desirable due to the need of degrading the prolamins present in the protein matrix of the grains. However, grain silage has been commonly associated with increasing proteolytic activity over time (Hoffman et al., 2011; Gervásio et al., 2021; Fernandes et al., 2021; Arcari, 2013), in contrast to the general expectation of reduced proteolysis by traditional silage conservation concepts, to which forage silages, including whole-plant corn, are associated. However, the extent of this proteolytic activity, in absolute values, reaches levels similar or even lower than those observed in whole-plant corn silages, as reported by the levels of NH_3-N in the literature, ranging mainly between 2 and 6% of total-N for ensiled high moisture corn grains (Figure 4).

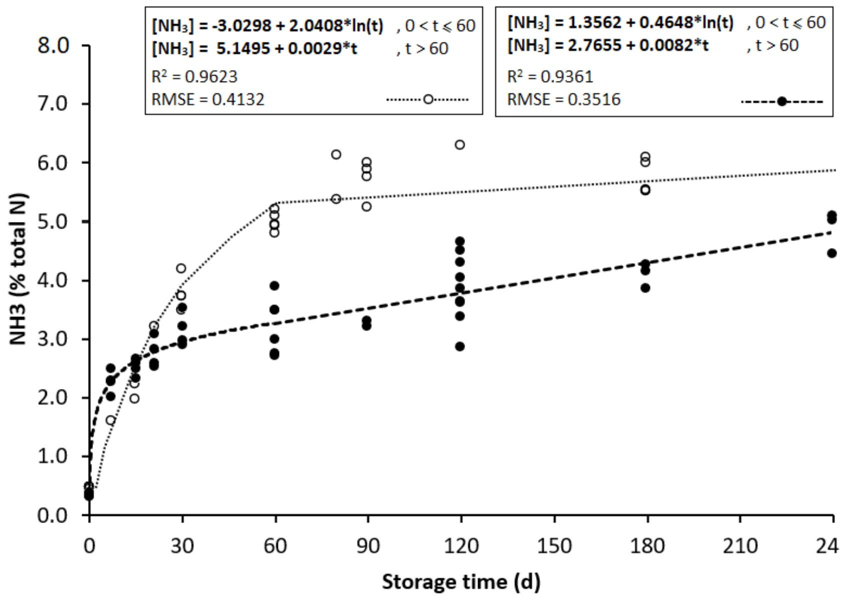

Figure 4. Adjusted NH_3-N values (n=76) compiled from 24 studies with high moisture and rehydrated corn grain silages (Oliveira, 2020; Gervásio, 2021; Hoffman et al., 2011; Arcari, 2013; Morais, 2016; Junges et al., 2017; da Silva et al., 2015, 2018, 2019; Fernandes et al., 2021; Bolson et al., 2020; Auerbach et al., 2015; Bíro et al., 2009; Doležal and Zeman., 2005; Ferraretto et al., 2015; Gálik et al., 2008; Kung Jr. et al., 2004, 2007, 2014; Morais et al., 2012; Pyś et al., 2009; Reis et al., 2008; Taylor and Kung Jr., 2002; Wardynski et al., 1993) when stabilized at pH < 4.0 (○) and pH ≥ 4.0 (●).Values refer to silages without treatment (chemical additives or microbial inoculants).

High levels of NH_3-N seem to be more frequent in corn grain silages with low pH values (Figure 4), which is an uncommon trend for traditional forage silages. Platikanow and Sandew (1962) reported reduced proteolysis with the addition of urea in corn silage, and Rayetskaya et al. (1964) reported an increase in true protein containing N^{15} from urea. Both effects were not observed by Lopez et al. (1970), but Lessard et al. (1978) reported that treatment with urea at the time of ensiling preserved the true protein content of corn silage after 20 days, while there was a decrease in the control silage. At the same time, these authors also observed a considerable increase in free amino acids for the urea-treated silage. The results of Rayetskaya et al. (1964) and Lessard et al. (1978) suggest the occurrence of microbial AA and protein synthesis from exogenous NPN, which may delay bacterial proteolysis of true protein. Also, the presence of an abundant source of NPN at the beginning of ensiling may favor the growth of microbial populations and consequently the production of organic acids during fermentation, especially lactic acid (Owens et al., 1969; Demirel et al., 2003; Lessard et al., 1978), enhancing silage acidification. Demirel et al. (2003) also observed that the variation in lactic acid content in urea-treated corn silages appears to be cultivar-dependent, probably related to different microbial populations, towards a greater presence of homolactic or heterolactic bacteria (Morais et al., 2017).

Thus, although lower pH values seem contradictory in silages with high ammonia content, this could make biological sense considering the occurrence of high populations of lactic acid bacteria (LAB) with a homolactic fermentation pattern in the silage, which would result in greater expression of proteolysis promoted by bacteria, producing ammonia, and in greater yield of lactic acid, decreasing the silage pH (Figure 5). Morais et al. (2017), in a review involving high moisture corn silages treated or not with microbial inoculants, observed an increase in lactic acid and a decrease in pH for silages inoculated with homolactic LAB, however, only a numerical increase in soluble protein (36.75 vs. 22.50 %CP, P=0.41) was observed, and no change in NH_3-N content. The crude protein, however, was greater for the inoculated silage, which probably suggests the occurrence of microbial protein synthesis. Although silages inoculated with heterolactic LAB did not show a decrease in pH, there was a significant increase in NH_3-N in relation to control silages. Thus, higher ammonia values may occur even without a decrease in pH promoted by lactic acid bacteria.

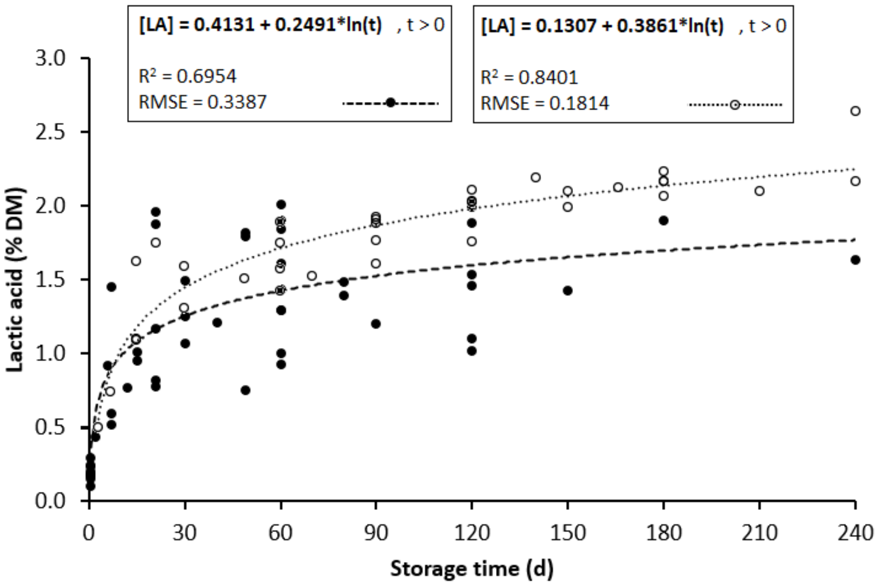

Figure 5. Adjusted lactic acid values (n=85) compiled from 28 studies with high moisture and rehydrated corn grain silages (Oliveira, 2020; Gervásio, 2021; Hoffman et al., 2011; Arcari, 2013; Morais, 2016; Junges et al., 2017; da Silva et al., 2015, 2018, 2019; Fernandes et al., 2021; Auerbach et al., 2015; Gallo et al., 2015; Silva et al., 2018; Gritti, 2020; Bíro et al., 2006, 2009; Canibe et al., 2014; Doležal and Zeman, 2005; Ferraretto et al., 2015; Gálik et al., 2008; Kung Jr. et al., 2004, 2007, 2014; Loučka, 2010; Prigge et al., 1976; Pyś et al., 2009; Taylor and Kung Jr., 2002; Wardynski et al., 1993) when stabilized at pH < 4.0 (○) and pH ≥ 4.0 (●).Values refer to silages without treatment (chemical additives or microbial inoculants).

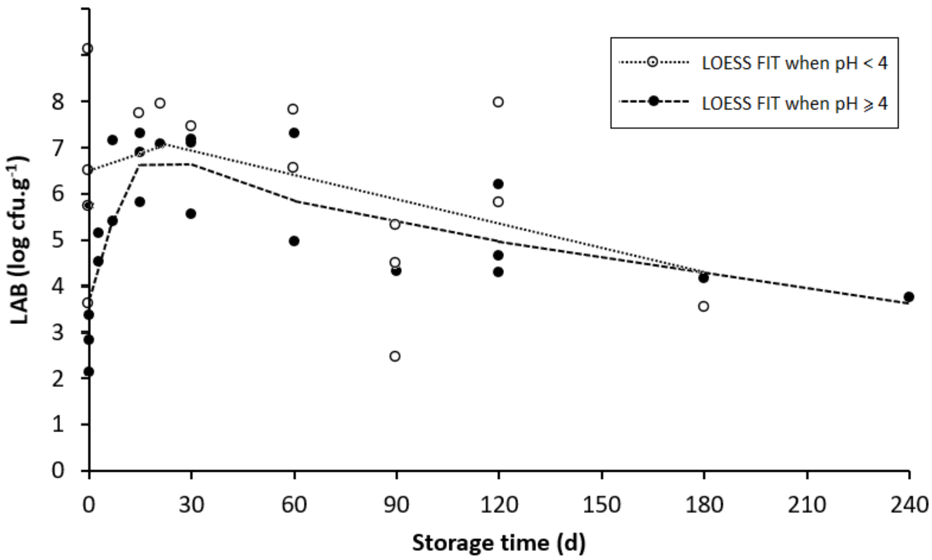

Figure 6. Lactic acid bacteria (LAB) counts (n=38) compiled from 10 studies with high moisture and rehydrated corn grain silages (Gervásio, 2021; Morais, 2016; Junges et al., 2017; da Silva et al., 2015, 2018, 2019; Dawson et al., 2018; Flores-Galárza et al., 1985; Kung Jr. et al., 2007, 2014) when pH < 4.0 (○) and pH ≥ 4.0 (●).Values refer to silages without treatment (chemical additives or microbial inoculants).

In our review, 38 means for LAB counts were obtained from 10 studies with high moisture and rehydrated corn grain silages, being sorted by the silage stabilizing pH (≥ 4.0 and

< 4.0) (Figure 6). However, as an accurate regression model was not possible for the available data, figure 6 displays only a nonparametric smooth regression (Cohen, 1999), and it seems, indeed, that LAB counts alone do not explain the drop in pH, especially if a large portion of it corresponds to heterolactic bacteria (Morais et al., 2017).

Nonetheless, the corn grain silages that manage to reach stability at pH lower than 4.0 still seem to have some other factor that promotes higher levels of NH_3-N (Figure 4). Lowering the pH to values close to or lower than 4.0 also favors the activity of proteases from corn grains, which are the second largest contributor to proteolytic activity in high moisture corn silages (Junges et al., 2017). It happens because a series of proteases present in the corn grain endosperm are acid proteases, with greater or exclusive activity at acidic pH (Mitsuhashi and Oaks, 1994). Although some proteases show peak activity at pH 4.5 (Fujimaki et al., 1977) and 5.0 (Zhang et al., 2020), several proteases and endopeptidases with an optimum pH point of 3.8 were isolated in the works of Harvey and Oaks (1974), Barros and Larkins (1990) and Moureaux (1979), and a protease with peak activity at pH 3.0 was also identified by Abe et al. (1977). Fahmy et al. (2004) also isolated an acid protease with peak activity at pH 4.0 in wheat grain endosperm. This may explain the continuous proteolysis even after reaching pH stability in grain silage, and also the increase in ammonia contents, given the greater yield of peptides and amino acids from degradation by grain proteases, constituting a greater substrate for deamination by bacterial proteases.

The presence of acid proteases in the endosperm of corn grains may also explain the occurrence of similar or even higher levels of NH_3-N in whole-plant corn silage. WPCS easily achieves stability at pH less than 4.0, sufficient to reduce or even inhibit the activity of proteases from the vegetative fraction, characterized as neutral proteases (Davies et al., 1998), but at the same time favoring the activity of grain proteases. When the corn grain silage manages to naturally reach stability at pH below 4.0, its ammonia content reaches values similar to those observed in WPCS (Figures 3 and 4).

Rumen DM and starch digestibility are well correlated with the soluble protein content of the silage (Ferraretto et al., 2014; Philippeau et al., 1999). The soluble protein content is a better indicator of the full extent of degradation of the protein matrix (Heron et al., 1986), since NH_3-N is more related to the microbial deamination, although both of them can be used to assess the proteolytic activity in silages (Davies et al., 1998). Junges et al. (2017) demonstrated that proteases from the grain itself are the second largest contributor to proteolytic activity, accounting for around 30%, while bacterial proteases account for around 60%. As plant proteases generally degrade true protein to peptides and amino acids, while degradation to ammonia is mainly promoted by microbial proteases (Ohshima and McDonald, 1978), the overall increase in soluble protein may be more subtle than the increase in ammonia nitrogen. In our review, 51 means for soluble nitrogen were obtained from 10 studies with high moisture and rehydrated corn grain silages, however, it was not possible to sort them by the silage stabilizing pH as done for the other variables, since the available data for pH < 4.0 were insufficient to generate a regression model, so Figure 7 displays a regression equation for the overall values of soluble nitrogen over time.

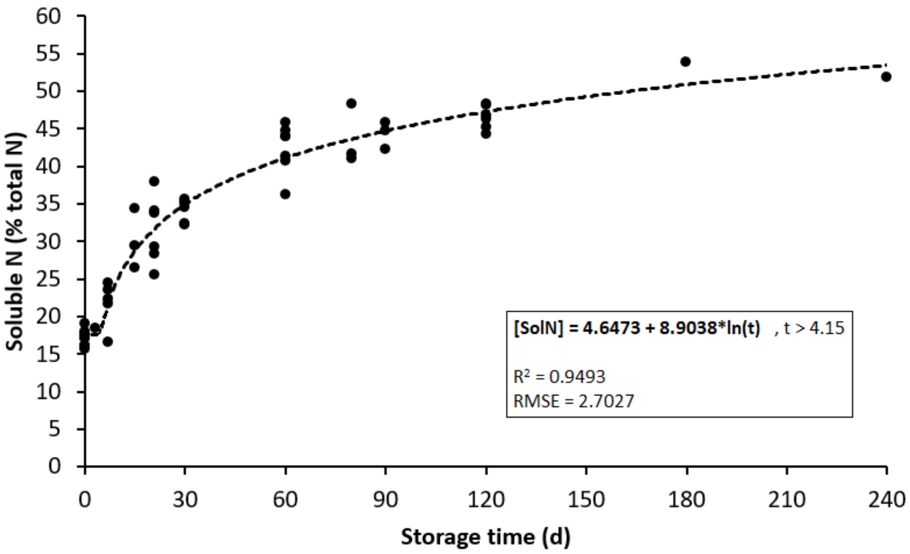

Figure 7. Soluble nitrogen values (n=51) compiled from 10 studies with high moisture and rehydrated corn grain silages (Oliveira, 2020; Hoffman et al., 2011; Morais, 2016; Junges et al., 2017; da Silva et al., 2015; Fernandes et al., 2021; Gritti, 2021; Ferraretto et al., 2018; Kung Jr. et al., 2014; Prigge et al., 1976). Values refer to silages without treatment (chemical additives or microbial inoculants).

2.3. Use of propionic acid-based additives in grain silages

The pH drop in rehydrated grain silages is remarkable, reaching values close to 4.0 quickly, but with a low final concentration of weak acids, such as lactic acid, insufficient to control yeast populations, which compromises the aerobic stability of the silage. In this context, the use of additives aiming to improve fermentation, providing good acidification of the ensiled grain, as well as the control of undesirable microorganisms, has become a practice of great interest (Morais, 2016). Organic acids, such as propionic, as well as combinations of these acids, have been the most successfully used.

The antifungal properties of propionic acid have been well known and explored in recent decades, being associated with improvements in the aerobic stability of forages and ensiled grains. This inhibitory property of yeasts and molds is linked to the non-dissociated form of the acid (COOH), which is more present the lower the pH of the medium (Lambert & Stratford, 1999). At pH 6.5, only 1% of the acid is in the undissociated form, while at pH 4.8 the dissociated form (COO-) is reduced to 50%. However, more evidence is needed to prove a better performance of animals that receive grain silages treated with propionic acid. Positive results such as greater weight gain and better feed efficiency have already been reported in studies with monogastrics (Jones et al., 1970; Livingstone et al., 1971), but for dairy cows the results are still inconclusive. High moisture corn silage usually promotes better feed efficiency through decrease in dry matter intake for cattle (Santos et al., 2016; Ferraretto et al., 2013), and the addition of propionic acid in the silage may enhance this effect due to the greater flow of propionate to the liver (Allen et al., 2009). However, there are reports of greater dry matter intake when cows were fed forage silages treated with propionic acid (Huber & Soejono, 1977; Stallings et al., 1979), and the increase was mainly due to greater intake of the treated forages. Other authors (Broderick et al., 1991; Bothast et al., 1978) did not observe changes in dry matter intake or milk yield. Clark et al. (1973) did not observe significative changes on dry matter intake when the cows were fed high moisture corn treated with propionic acid (1.3% fresh matter), and Kung Jr. et al. (1998) also did not observe changes on dry matter intake when a total mixed ration (TMR) was treated with a propionic acid-based additive. The uptake of propionic acid by the cows through a treated TMR or a diet containing treated ingredients is relatively low (1-2 mol/d) when compared to the natural production of propionate in the rumen even when submitted to a normal diet (13.3 mol/d) (Bauman et al., 1971). Consistent decreases on dry matter intake linked to propionic acid are usually reported in studies involving intraruminal infusion of this acid during the meal, when larger quantities of propionic acid are employed, close to the naturally produced by ruminal fermentation (Lemosquet et al., 2009 - 14.1 mol/d; Maldini and Allen, 2018 - 36.4 mol/d). Thus, the impact of propionic acid-based additives on dry matter intake is still inconclusive. It is possible that the reports of greater intake in the literature may be associated with a dose-dependent response based on palatability changes and/or moisture content of the feed. Further research is needed to better understand the effect of propionic acid treatment on silages and total diets for dairy cows.

Like other organic acids, propionic acid is difficult to handle due to its strong and aggressive odor and its volatile nature, and for being corrosive to metal. Thus, it is common to use buffering salts (calcium, sodium, and ammonium) in commercial products for safety reasons (Kung Jr. et al., 2003). Surfactants are substances with dual affinity, interacting with both polar (water) and non-polar (oils and organic compounds with large carbon chains) substances and reducing the surface tension between them. In the food and pharmaceutical industries, this property is used to improve the stability of emulsions and provide moisture retention (humectants), as well as other purposes (Mahungu & Artz, 2001). Polysorbates are nonionic synthetic surfactants formed by ethoxylated sorbitan fatty acid esters (ethylene oxide). Depending on the fatty acid of origin and the degree of ethoxylation, polysorbates with different HLB (hydrophilic-lipophilic balance) are obtained, ranging from zero (greater affinity for oil) to 20 (greater affinity for water). Synthetic surfactants used in foods can act on the physical structure by interacting with the amylose and amylopectin chains of starch (Vernon-Carter et al., 2018). However, there are few studies on the effect of synthetic surfactants on the digestibility of animal feeds such as silages, so their impact is still unknown (Oliveira, 2020).

Mycoflake™ is described as a liquid surfactant produced by Kemin Industries Inc. to aid the penetration of moisture into the corn kernel during the flocculation process. The additive is a water-diluted combination of organic acids (propionic, acetic), surfactants (including sarsaponin from yucca extract), buffers (ammonium hydroxide and chloride), an emulsifier (sodium phosphate tri-basic) and other components (n-propyl alcohol, propylene glycol). In a study conducted by Oliveira (2020) in the Forage Quality and Conservation research group at ESALQ/USP with rehydrated corn grain silage in laboratory mini-silos, evidence was found that the surfactant component of this additive may enhance the proteolysis of grains, accelerating the process. Combined with the preservative properties of propionic acid, Mycoflake™ appears to be a potential additive for high moisture grain silages. However, its mechanism of action was not reported and still lacks studies to be clarified.

2.4. Implications of increasing starch availability on ruminant performance

The objective of grain processing techniques is to increase the availability of starch to be digested by animals, preventing excessive excretion in feces. More intensive processing techniques, such as flocculation and high moisture grain silage, may also increase starch digestibility in the digestive tract of ruminants (Ferraretto et al., 2013; Owens et al., 1986; Harmon, 2009; Owens & Soderlund, 2006). In comparison to dry corn, there is also a shifting of the digestion site and kinetics mainly to the rumen. Owens and Soderlund (2006) reported starch degradation values in the rumen of around 84-86% for high moisture silage and flocculation, compared to 64% for dry rolled corn. The availability of the undegraded starch fraction would also be considerably high in the small intestine, with digestibility in this portion of the gastrointestinal tract reaching 92 to 95%, compared to 56% for dry rolled corn. In the total tract, both strategies can provide digestibilities as high as 99% of the ingested starch. As it usually happens by grinding grains, high moisture corn has a small average particle size, which, together with proteolysis occurring in the silo, provides a higher rate of ruminal degradation (Pereira & Pereira, 2013; Ferraretto et al., 2013), being this strategy the most concerning in terms of risk of ruminal acidosis.

The influence of this higher starch availability on the performance of ruminants is, however, quite varied in the literature. Alvarez et al. (2001) reported no changes in ruminal pH, volatile fatty acid production and acetate:propionate ratio when offering high moisture corn silage in substitution to cracked corn to dairy cows. However, they observed lower ammonia concentration in the rumen, indicating better synchronization between degradable carbohydrate and nitrogen fermentation, suggesting greater microbial protein synthesis. The total starch digestibility was higher for the wet grain diet, but without changes in milk yield, milk composition or body condition score.

Bradford & Allen (2004), however, reported differences in milk composition in cows fed a wet grain diet, with a decrease in the percentage of fat without changes in the levels of protein and lactose. Blood plasma glucose levels were also higher compared to the ground dried corn diet. The authors also reported lower dry matter intake (-2.0 kg/d) and increased total starch and dry matter digestibility with the wet grain diet, although the individual variation in food intake was exceptionally large (de +0.9 kg/d -6.0 kg/d). Oba & Allen (2003) attributed the lowest dry matter intake to the smaller meal size, due to the rapid fermentation generating satiety more quickly. The lowering on dry matter intake was also reported by Ferraretto et al. (2013) in cows fed ensiled high moisture grain in contrast to dry corn (-1.2 kg/d).

Bitencourt (2012), evaluating the performance of dairy cows on a dry ground corn diet (17.7% in inclusion DM) and the same rehydrated and ensiled hybrid (16.7% in inclusion DM), did not observe significant differences in dry matter intake, milk yield and composition, feed efficiency and body score. However, the concentration of milk urea nitrogen was lower in animals that received rehydrated corn silage. Cows supplemented with wet corn silage had higher milk yield (+2.4 kg/d) compared to cracked corn supplementation according to Wu et al. (2001). Ferraretto et al. (2013) reported a lower percentage of fat in milk, although they did not observe differences in milk production.

With beef cattle, the use of high moisture grain corn silage has resulted in greater feed efficiency compared to dry corn, mainly due to the decrease in dry matter intake (Santos et al., 2016). In studies with Brazilian flint corn, greater weight gain in addition to reduced consumption has also been reported (Santos et al., 2016). Reis et al. (2001), working with growing lambs, reported that animals fed wet corn grain silage instead of dry corn in the diet showed greater weight gain, shortening the time to slaughter. The authors attributed the greater efficiency of these animals to the greater starch digestibility with wet grain silage. Almeida Júnior et al. (2004) also reported greater weight gain for Suffolk lambs fed with high moisture grain silage instead of dry corn, on a 15% hay diet, reducing the finishing time required for slaughter with 28 kg.

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3. PROPIONIC ACID-BASED ADDITIVE WITH SURFACTANT ACTION ON THE NUTRITIVE VALUE OF RECONSTITUTED CORN GRAIN SILAGE FOR DAIRY COWS PERFORMANCE

ABSTRACT

Eighteen Holstein cows with 218 ± 106 days in milk (mean ± SD) were assigned to a 3x3 Latin square design to evaluate the effects of including a propionic-based additive with surfactant action during the ensiling of reconstituted corn on its nutritional value and animal performance. Dry ground corn grain was rehydrated to 40% moisture, with or without the additive, and ensiled in 200-L plastic drums. Treatments were prepared as follows: reconstituted corn grain silage (RCGS) stored for 30 days without additive (30 CON), RCGS stored for 60 days without additive (60 CON) and RCGS stored for 30 days with the additive at 2 L/t (30 MYC). Diets contained 20.7% of RCGS. Cows were housed in a free stall confinement system, and allocated to individual feed bunks equipped with individual access control and feed monitoring system. The feeding management consisted of two daily offers, and the cows were milked twice a day. The extended conservation time of the 60 CON silage increased the soluble nitrogen content and the ruminal degradability of dry matter in relation to the other silages with 30 days. Although the silage treated with additive had similar soluble protein content compared to 30 CON silage, the ammonia content and its concentration in soluble N were significantly higher for the former. The 30 MYC silage had higher lactic acid content compared to the other silages. There was a significant decrease in the geometric mean particle size and an increase in the surface area and proportion of fine particles for the rehydrated corn silage with the use of the additive. No changes were observed in the dry matter degradability of 30 MYC silage against the control silage, but there was a significant decrease of the ruminal degradation rate (k_d) with the use of the additive. There were no significant changes in dry matter intake, milk yield and composition for the cows fed the additive-treated silage. However, the presence of the additive in rehydrated corn silage promoted greater selection against long particles in the diet, and a greater selection in favor of fine particles, reflecting in lower NDF intake by cows when treated with the diet containing 30 MYC silage, but without harming rumination and chewing activity. The use of an additive based on propionic acid containing a surfactant agent in its composition did not enhance the proteolysis of corn grains during ensiling, but it had the potential to reduce the rate of ruminal degradation without harming starch availability, possibly resulting in a safer fermentation in the rumen and lower risk of acidosis.

Keywords: Reconstituted corn grain silage, Surfactant, Propionic acid, Dairy cows

3.1. Introduction

The ensiling of cereal grains with high moisture aims to promote the degradation of the protein matrix that surrounds the starch granules in the endosperm, facilitating the availability of starch in the rumen environment and its fermentation by microorganisms (Hoffman et al., 2011; Owens et al., 1986). Silage storage time is a determining factor in the extent of its degradation. Increases in the soluble fraction of nitrogen and ammonia are considered typical outcomes observed on the ensiling of corn and sorghum grains, indicating the occurrence of proteolysis (Ohshima & McDonald, 1978). The offering of ensiled high moisture corn to dairy cows in the literature has shown variable results in animal performance, but quite positive in relation to dry corn, such as increased feed efficiency and greater use of starch contained in grains by ruminants (Ferraretto et al., 2013; Santos et al., 2016).

The use of chemical additives in rehydrated corn, mainly those based on propionic acid, has been aimed at improving the conservation of silages, aerobic stability after during feed-out (Morais, 2016) and maintaining its nutritional quality while in the feed bunk, resulting in some cases even in higher dry matter intake. The impact of the presence of a surfactant in a feed additive is still poorly understood, especially in feed used in the animal industry (Oliveira, 2020). Mycoflake^{TM} is an additive that combines propionic acid (and other organic acids) and a surfactant component intended for the flocculation process, for moisture retention by the grains in the first stage, in a heated chamber under high pressure. To date, its use as a silage additive is unknown.

A recent trial conducted by Oliveira (2020) with this additive showed favorable results in ensiled rehydrated corn, such as greater ruminal degradability of dry matter after 12 hours of incubation, a decrease in the mean particle size and a possible enhancement of grain proteolysis. The aim of this trial was to evaluate the effect of this additive in rehydrated corn silage on the performance of lactating dairy cows, as well as to evaluate its impact on protein degradation in the silo and on the ruminal degradability of corn. The set of treatments were designed with the intention to promote a potential anticipation on the silo opening with enhanced starch digestibility being achieved earlier by using additive in substitution to extended time of storage.

3.2. Material and methods

All experimental procedures were approved by the ethics committee for animal use of University of São Paulo (protocols: 2018.5.1093.11.4 / nº 8340290120).

3.2.1. Preparing the reconstituted corn grain silages

The experimental silages were prepared and stored according to the fermentation times in 200-L plastic drums with internal plastic bags of 0.20 mm thick. The corn grain source used was an unknown hybrid, grounded with a 5 mm sieve and whose particle size distribution was determined using a sample of approximately 100 g in an electromagnetic shaker (Bertel® Ltda., Caieiras, Brazil) equipped with screens of 4.75, 3.35, 2.36, 1.70, 1.18 and 0.60 mm plus the bottom pan. The ground grain source showed a mean particle size of 1.00 mm, characterizing fine ground corn (Litherland, 2006).

The rehydration of the ground dry corn was carried out in a vertical mixing wagon with the addition of water (30 CON and 60 CON) or water + additive (30 MYC) to reach 40% moisture, mixed through 15 minutes before being conditioned and packed by feet in the drums, until reaching a density of 1,250 kg/m^3. Into the silages where the commercial additive was included, it was applied at a dosage of 2 L/t of rehydrated corn, mixed in the rehydration water. The plastic drums were lined inside with plastic bags, and after filling with the ground rehydrated grain and packing, bags were then closed and sealed with plastic clamps, without placing the covers on the drums, which were stored in a free stall barn in a dry, ventilated and shaded place.

3.2.2. Experimental diets

The basal diet of the experiment was formulated according to the NRC 2001 program (version 1.1.9, December 2012). The nutritional composition and the proportion of ingredients are shown in Table 1. The proposal of this trial was based on the total replacement of dry ground corn by the reconstituted corn silage sources tested. The inclusion of the grain silages was the same in the diets of all treatments.

Table 1. Ingredient participation and nutritional composition of formulated diets

Ingredients	30 CON	30 MYC	60 CON
Corn silage, % DM	39.9	39.9	39.9
Tifton 85 haylage, % DM	9.8	9.8	9.8
RCGS (30 d), % DM	20.7	_	_
RCGS (60 d), % DM	_	_	20.7
RCGS (30 d + additive), % DM	_	20.7	_
Citrus pulp, % DM	9.8	9.8	9.8
Soybean meal, % DM	17.3	17.3	17.3
Mineral premix, % DM	2.5	2.5	2.5
Chemical composition			
DM, % as fed	44.7 ± 0.6	45.4 ± 0.4	45.2 ± 0.5
CP, % DM	13.6 ± 0.2	13.0 ± 0.3	13.7 ± 0.2
NDF, % DM	38.2 ± 0.1	38.7 ± 0.2	38.1 ± 0.3
Forage NDF, % DM	26.7 ± 0.2	26.7 ± 0.2	26.7 ± 0.2
Ash, % DM	9.0 ± 1.0	8.7 ± 0.5	8.6 ± 0.5
Starch origin, % of total			
Corn silage	36.6	36.6	36.6
Grain silage	63.4	63.4	63.4
Particle Size			
> 19 mm, % as fed	22.2 ± 2.3	20.6 ± 2.7	24.6 ± 3.0
< 19 mm and > 8 mm, % as fed	15.3 ± 0.9	15.8 ± 0.9	14.7 ± 0.9
< 8 mm and > 4 mm, % as fed	19.7 ± 1.0	19.5 ± 0.9	19.0 ± 0.6
< 4 mm, % as fed	42.8 ± 2.4	44.1 ± 2.1	41.8 ± 2.6

RCGS – reconstituted corn grain silage; DM – dry matter; CP – crude protein; NDF – neutral detergent fiber

Table 2. Chemical composition of the reconstituted corn grain silages utilized in the feeding trial

Items	30 CON	30 MYC	60 CON
DM, % as-fed	58.4 ± 0.9	59.5 ± 1.2	58.3 ± 0.8
CP, % DM	8.24 ± 0.12	8.53 ± 0.26	8.67 ± 0.24
NDF, % DM	12.5 ± 1.1	12.5 ± 1.0	10.3 ± 0.9
Ash, % DM	1.35 ± 0.05	1.35 ± 0.07	1.36 ± 0.02
Soluble Protein, % CP	26.26 ± 0.68	27.19 ± 1.32	46.59 ± 2.76

Table 3. Chemical composition of the ingredients utilized in the feeding trial

Items	Corn silage	Tifton haylage	Citrus pulp	Soybean meal
DM, % as-fed	27.0 ± 0.8	73.6 ± 1.9	83.7 ± 0.9	86.6 ± 1.6
CP, % DM	7.7 ± 0.1	11.4 ± 0.1	7.4 ± 0.3	47.0 ± 1.3
NDF, % DM	48.3 ± 0.7	75.4 ± 0.5	25.0 ± 0.7	18.7 ± 0.5
Ash, % DM	7.6 ± 1.2	8.8 ± 0.3	7.3 ± 0.2	7.3 ± 0.3

3.2.3. Feeding trial

The animal performance trial was conducted at the Experimental Facility for Confinement of Dairy Cattle “Prof. Vidal Pedroso de Faria" in the Animal Science Department of the Luiz de Queiroz College of Agriculture (ESALQ) – University of São Paulo (USP), located in Piracicaba-SP, using 18 Holstein cows with an average weight of 625 ± 113 kg, in advanced stage of lactation (mean DIM of 218 ± 106 days) and daily milk production of 22 ± 7.6 kg on average. The cows were ranked by milk production and live weight, then allocated in a 3x3 Latin square design with six contemporary squares, in periods of 20 days each (14 of adaptation and 6 of collections), with the following treatments: rehydrated corn grain silage stored for 30 days (30CON); rehydrated corn grain silage stored for 60 days (60CON); and rehydrated corn grain silage stored for 30 days + Mycoflake™ 2 L/t (30MYC) (Kemin South America, Indaiatuba, SP, Brazil).

The animals were housed in a free stall barn designed with a slotted concrete floor, sand beds and roof provided with an open ridge to assure satisfactory environmental conditions and animal welfare. Furthermore, the barn provided free access to water and was equipped with individual feed bunks of 60 cm of linear space each, capable of individual access control through identification by chip (tag) attached to the ear of the animal and individual feed monitoring system (Intergado Ltda., Contagem, Minas Gerais, Brazil). Animals were allotted to the facilities in three lots in the barn, resulting in a density of 1 cow/21.6 m^2 (15.8 m^2 of floor + 5.8 m^2 of bedding), which fulfilled the protocols for confined animals. The cows were grouped into the lots 15 days before the beginning of the trial to promote social adaptation of the animals to each other and to identify possible dominance relationships, in addition to allocating the cows to their respective feed bunks and promoting the habitual adaptation of feed intake.

Feed management was divided into two daily meals, one in the morning at 6 am and the other in the afternoon at 6 pm, with total collection of orts in the feed bunks the following morning before the first meal. All ingredients were mixed in a vertical mixer wagon for approximately 15 minutes and delivered to the feed bunks as a total mixed ration. The amount of ration offered per cow was calculated based on the weight back of the orts from the previous day, to assure a minimum of 10% orts in the feed bunk the next morning. Cows were milked twice daily, the first time at 5 am and the second at 5 pm, using a double 4 herringbone system. The waiting and milking rooms were located 100 m from the free-stall barn, distance covered 4 times/day, which was the only walking outside of the barn to which the animals were submitted during the experimental period. Some aside facilities such as the footbath alley and the body weighting scale were available next to the free-stall barn. The passage of the animals through the footbath occurred three times a week, on returning from the milking parlor.

3.2.3.1. Animal performance: nutrients intake, milk yield and composition

The feeding trial consisted of three periods of 20 days each, being 14 days of adaptation and 6 days of samplings and measurements, totalizing an experimental period of 60 days. In each period, each group of 6 animals was fed the ration from one of the treatments, so that all cows had gone through all treatments at the end of the experiment, following a Latin square design. The feed-out scheme of the silos utilized in the feeding trial was sequential according to use, so the silage matched the treatment fermentation time (30 or 60 days) right on the evening of the evaluation and collection period. The milk yield of each cow was measured through days 15 to 19 of each period, with samples being collected on d-16 and d-18 for analysis (Clínica do Leite, Piracicaba, São Paulo, Brazil) of fat, crude protein, lactose and urea nitrogen contents and somatic cell count (SCC). Energy corrected milk (ECM) was calculated according to Tyrrell & Reid (1965), as follows:

$$\text{ECM (kg/d)} = (0.327 \times \text{milk yield}) + (12.95 \times \text{fat yield}) + (7.2 \times \text{protein yield}) \quad \text{(Eq. 1)}$$

The energy content and excretion in milk were calculated from the equations present in the NRC (2001), as follows:

$$\text{Milk } NE_L \text{ (Mcal/kg)} = (0.0929 \times \%\ \text{fat}) + (0.0547 \times \%\ \text{protein}) + (0.0395 \times \%\ \text{lactose}) \quad \text{(Eq. 2)}$$

$$NE_L \text{ excretion (Mcal/d)} = \text{Milk yield (kg/d)} \times \text{Milk } NE_L \text{ (Mcal/kg)} \qquad \text{(Eq. 3)}$$

All ingredients used in the diets were sampled daily between d-15 and d-19 of each period, with the daily sub-samples being immediately frozen at -20 ºC. At the end of each period, the sub-samples were thawed and mixed to form the composite sample. The ingredients samples were then dried in a forced air circulation oven at 55 ºC for 72 hours and grounded in a Willey mill with a 1 mm sieve, then analyzed in the laboratory to determine the contents of dry matter (AOAC, 1990, method 934.01), ash (AOAC, 1990, method 924.05), crude protein (combustion by the Dumas method – AOAC, 1990, method 992.23) and neutral detergent fiber corrected for ash with thermostable amylase and sodium sulfite (Mertens, 2002). The offered diet was weighed and sampled for each animal every day between d-15 and d-19 of each period, and the daily sub-samples were immediately frozen at -20 ºC. At the end of each period, the sub-samples were thawed and mixed to form the composite sample. The orts from each animal were collected every day between d-16 and d-20 before the first morning meal, weighed and sampled, and the daily sub-samples were immediately frozen at -20 ºC, being thawed and mixed to form the composite sample at the end of each period. A fraction of 400 g of each composite sample of offered diet and orts was separated to determine the particle size distribution using the Penn State Particle Separator (Lammers et al., 1996; Heinrichs & Jones, 2013), using 19 mm, 8 mm and 4 mm sieves, plus the bottom box. Another fraction of 500 g of each composite sample of offered diet and orts was separated for chemical analysis, being dried in a forced air circulation oven at 55 ºC for 72 hours and grounded in a Willey mill with a 1 mm sieve, to determine the contents of dry matter (AOAC, 1990, method 934.01), ash (AOAC, 1990, method 924.05), crude protein (combustion by the Dumas method – AOAC, 1990, method 992.23) and neutral detergent fiber corrected for ash with thermostable amylase and sodium sulfite (Mertens, 2002). Individual intake of as-fed matter by each cow was measured daily between d-15 and d-19 of each period using the feed monitoring system installed at the feed bunks (Intergado Ltda., Contagem, Minas Gerais, Brazil).

3.2.3.2. Ingestive and selective behavior

The evaluation of ingestive behavior was carried out for 24 hours, with observations every 10 minutes, considering the activities of feed intake, water intake, rumination and idleness. The recording started right after the morning feeding on d-17 and ended at the same time on d-18 of each period. During milking and while in the waiting room, the animals did not have access to food, only water, so the assessment of ingestive behavior included two hours of diet restriction (5 am-6 am and 5 pm-6 pm) in each period, recording only the other activities. Average time and size of meal and meal frequency were calculated using the feed monitoring system installed in the feed bunks (Intergado Ltda., Contagem, Minas Gerais, Brazil). The first feed intake activity after the morning feed was considered as the first meal.

For selective behavior, the particle size distribution of the offered diets and orts of each animal was determined using the Penn State Particle Separator (Lammers et al., 1996; Heinrichs & Jones, 2013), as described in section 3.2.3.1. The intake for each particle size retained in the *i*-mm sieve is calculated as follows:

$$[\text{Offered x } (\%\text{sieve\_offer}_{(i)}/100)] - [\text{Orts x } (\%\text{sieve\_orts}_{(i)}/100)] = \text{Intake}_{(i)} \qquad \text{(Eq. 4)}$$

With the actual intake for each sieve, a particle size distribution for intake ($\%\text{sieve\_intake}_{(i)}$) can be estimated. The particle sorting indexes for each sieve is then calculated by dividing [($\%\text{sieve\_intake}_{(i)}$ / $\%\text{sieve\_offer}_{(i)}$) x 100], indicating refusal (<100%), preferential intake (>100%) or no selection (=100%) (Leonardi & Armentano, 2003).

3.2.3.3. Digestibility of nutrients

Fecal samples were collected every 8 hours for three days, between d-18 and d-20, and immediately frozen at -20 ºC. Subsequently, the samples from each cow were thawed and mixed to form a composite sample for laboratory analysis, being dried in a forced air circulation oven at 55 ºC for 72 hours and grounded in a Willey mill with a 1 mm sieve, to determine the contents of dry matter (AOAC, 1990, method 934.01), ash (AOAC, 1990, method 924.05), crude protein (combustion by the Dumas method – AOAC, 1990, method 992.23) and neutral detergent fiber corrected for ash with thermostable amylase and sodium sulfite (Mertens, 2002).

To determine the total tract digestibility of nutrients, the indigestible fraction of NDF (iNDF) was used as a marker. Dry and ground 7 g subsamples of the offered diets, orts and feces were incubated into the rumen through a ruminal cannulated cow for 288 h (Huhtanen et al., 1994), adapted to a diet with 50% corn silage, 21% dry ground corn, 9.5% citrus pulp, 17% soybean meal and 2.5% mineral premix.

3.2.3.4. Fermentative profile of the reconstituted corn grain silages

Samples of reconstituted corn silage were collected at the moment of silos opening for each treatment when they reached the respective fermentation times (30 and 60 days) and daily between d-15 and d-19 of each period, being immediately frozen at -20°C. Subsequently, the daily subsamples were thawed and mixed to form a composite sample. A fraction of 100 g was separated and sent to prepare aqueous extracts, by using 25 g of sample and 225 g of deionized water. The extracts were homogenized in a stomacher for 4 minutes, then filtered through 3 folder cheesecloths. The pH was measured immediately, and samples were placed in 2 mL micro tubes and frozen at -40 ºC awaiting analyzes for the determination of ammonia nitrogen (Chaney & Marbach, 1962, adapted by Weatherburn, 1967), lactic acid (Pryce, 1969), volatile fatty acids, esters and ethanol by gas chromatography (GCMS QP2010 Plus; Shimadzu®, Kyoto, Japan) using a capillary column (Stabilwax; Restek®, Bellefonte, PA; 60 m long, 0.25 mm outer diameter, 0.25 µm film thickness) and correction of dry matter content for volatile compounds according to the equation of Weissbach (2009).

Another fraction of 400 g was kept for chemical analysis destined to the ingredients (described in section 3.2.3.1), in addition to the determination of the soluble protein content by mixing in a borate-phosphate buffer solution at 39 ºC for one hour, followed by filtration (Krishnamoorthy et al., 1982) and measurement of nitrogen by the Dumas method (AOAC, 1990, method 992.23). To determine the kernel particle size distribution, a fraction of 100g was separated and dried in a forced air circulation oven at 55 ºC for 72 hours, then submitted to an electromagnetic shaker (Bertel® Ltda., Caieiras, Brazil) equipped with screens of 4.75, 3.35, 2.36, 1.70, 1.18 and 0.60 mm plus the bottom pan. Geometric mean particle size (GMPS; µm) and surface area (cm^2/g) were calculated using a log normal distribution (Baker & Herrman, 2002) as described in Dias Junior et al. (2016).

3.2.3.5. Ruminal *in situ* degradability of the reconstituted corn grain silages

For the ruminal *in situ* degradability of dry matter, 5.5 g dry and ground subsamples of the silages were placed in 20 cm x 10 cm nylon bags with a porosity of 30 µm, and subsequently incubated in triplicate in the rumen of two Holstein cows, adapted to a diet similar to the experimental one, with only the replacement of rehydrated corn by dry ground corn. The incubation times used were 0, 12, 24 and 48 hours, with the bags being incubated in reverse order, so that they could be removed simultaneously. After removal, the bags were immediately immersed in iced water to stop fermentation. Then, the bags were washed until the washing water became clear and dried in a forced air circulation oven at 55 ºC for 72 hours, being then weighed to determine the percentage of dry matter degradation for each incubation time.

The degradability parameters were determined through the PROC NLIN of SAS 9.4 (SAS Institute Inc., Cary, NC) according to the model proposed by Orskov & McDonald (1979), whose equation is given by:

$$Y_{(t)} = A + B^*(1 - e^{-k^*t}) \qquad \text{(Eq. 5)}$$

Where A = fraction that immediately escapes from the bag (soluble fraction + particles smaller than the porosity of the bag); B = potentially degradable fraction; k = degradation rate (%/h); and t = incubation time (h). The following fractions were obtained from the degradation data: soluble (A), obtained by the difference between the incubated weight and the zero-time residue; indigestible (C) = residue at time 48h and the potentially degradable fraction (B), calculated by the difference 100 – (A + C). Potential degradability was calculated as potDEG = (100 – C) and the effective degradabilities (Eq. 6) were calculated according to Ogden et al. (2005) considering the passage rates of 2%/h, 5%/h and 8%/h.

$$eDEG\ (\%) = A + [B^*k_d / (k_d + k_p)] \qquad \text{(Eq. 6)}$$

3.2.4. Statistical analysis

The parameters of nutrient intake and digestibility, milk production and composition, and ingestive and selective behavior obtained in the feeding trial were analyzed using the SAS MIXED procedure (SAS Institute Inc., Cary, NC), as a Latin square design, including the fixed effect of Latin square (1 to 6), fixed effect of treatment (30 CON, 60 CON and 30 MYC), fixed effect of period (1 to 3) and the random effect of cow within square (1 to 18).

The parameters regarding the chemical composition and fermentative profile of the reconstituted corn silages were analyzed as a randomized block design, including fixed effect of treatment (30 CON, 60 CON and 30 MYC) and random effect of block (periods 1 to 3).

The ruminal degradability assay was analyzed as a randomized block design using the SAS MIXED procedure (SAS Institute Inc., Cary, NC), including fixed effect of treatment (30 CON, 60 CON and 30 MYC) and random effect of block (cow A and B). Two contrasts were used to compare treatments: 1) 30 CON vs. 30 MYC and 2) 30 MYC vs. 60 CON. Significant differences were declared when $P \leq 0.05$ and trends when $0.05 < P \leq 0.10$.

3.3. Results and discussion

3.3.1. Silages

The additive did not promote changes on chemical composition of the grain silage (Table 4) regarding the contents of crude protein, ash and NDF, as could have happened according to Morais et al. (2017), but at the same time in agreement with Kung Jr. et al. (1998, 2000, 2001), who consistently reported no changes in these nutrients (including ADF and starch) with buffered propionic-based additives. A minor decrease in NDF content was observed as a result of the extending storage time to 60 days, probably due to physical changes in the grains with the ensiling, as also reported by Morais (2016), however this decrease was not significant.

The soluble protein content is a good indicator of the occurrence of proteolysis in silage, as is the ammonia nitrogen content (Ohshima & McDonald, 1978). The rehydrated corn silage at 60 days promoted an increase of almost 20 percentage units in the soluble fraction of the protein, and an increase in the concentration of ammonia nitrogen compared to ensiling with only 30 days, evidencing the greatest degradation of the protein matrix with the longer storage time. The observed soluble protein content, 46.6%, was higher than that reported by Prigge (1976) of 38.2% for similar storage time (56 days), however the author studied corn grain silage at 28% moisture, which may have limited the activity of proteolytic enzymes for matrix degradation. Hicks & Lake (2012) suggest that the largest increases in soluble protein occur within the first 56 days of ensiling. Morais (2016) reported 48.5% of soluble protein for rehydrated corn grain silage at 90 days, so the content of 46.6% observed in the present trial at 60 days seems to fit into the range of the reported values available in the literature.

Table 4. Chemical composition of the reconstituted corn grain silages

Item	Treatment			SEM	*P*-value[1]	
	30CON	30MYC	60CON		C_1	C_2
DM, % as-fed	58.4	59.5	58.3	0.53	0.44	0.41
CP, % DM	8.24	8.53	8.67	0.13	0.25	0.53
NDF, % DM	12.5	12.5	10.3	0.62	0.99	0.20
Ash, % DM	1.35	1.35	1.36	0.03	0.99	0.90
Soluble Protein, % CP	26.26	27.19	46.59	3.44	0.66	<0.01
NH_3-N, % DM	0.05	0.08	0.08	0.01	0.02	0.44
NH_3-N, % Total N	3.97	5.44	5.82	0.30	0.02	0.50
NH_3-N, % Soluble N	15.13	20.42	12.68	1.03	0.02	<0.01
Particles <1,18mm, %	50.3	56.3	46.6	1.28	<0.01	<0.01
GMPS, µm	1105.0	995.7	1127.5	20.73	0.01	<0.01
Surface area, cm^2/g	29.5	31.3	29.5	0.29	<0.01	<0.01

[1]Contrasts include C_1: 30 CON x 30 MYC, C_2: 30 MYC x 60 CON

The use of the additive did not promote significant changes on the levels of soluble protein after 30 days of ensiling, but its concentration of NH_3-N was significantly higher from that observed for 30 CON at both DM and total N basis, reaching similar levels to the observed in the corn ensiled for 60 days. Oliveira (2020), analyzing the effects of the same additive used in the present trial, reported inconsistent data regarding soluble protein contents. However, the results from that study suggest a possible increase on the soluble protein fraction after 30 days of ensiling with the additive, which could indicate a favoring of matrix proteolysis. In the present trial, however, the additive failed to increase the soluble protein content after 30 days of ensiling, which was similar to that observed for the control silage (27.19 vs. 26.26 % CP, respectively). The 30 MYC silage had a higher concentration of NH_3-N at 30 days compared to the control (0.08 vs. 0.05 % DM, respectively), in contrast with the reported by Oliveira (2020), who observed no effect of the additive on the concentration of ammonia nitrogen. Kung Jr. et al. (2004), working with a propionic acid-based additive from the same company, also reported higher ammonia concentrations in the treated silage compared to the control. However, the authors suggest that this was due to the composition of the additive itself, which includes ammonium propionate as buffering salt. Since the additive used in the present trial contains ammonium hydroxide and ammonium chloride in its composition, the higher ammonia concentrations observed in the 30 MYC silage are probably due to the presence of these compounds, but their concentrations are not known. Manufacturers of this type of product do not usually describe the composition or concentrations of the ingredients used. Thus, it was not possible to state with certainty whether the results for N-NH_3 were due to the action or composition of the additive.

As for the contribution of ammonia in the soluble nitrogen content, the additive-treated silage presented a significantly higher NH_3-N concentration in the soluble N in relation to the other treatments. A possible explanation for the greater contribution of ammonia in the soluble nitrogen content of the 30 MYC silage would be if the additive somehow promoted the deamination process during the fermentation of the protein matrix. However, to the moment, there is no data in the literature that support this hypothesis or suggest such an action mode from a chemical additive. The values observed for ammonia nitrogen were also higher than that reported by Kung Jr. et al. (2004) of 0.03% DM for high moisture corn ensiled for 120 days, but close to the values reported by Oliveira (2020) and Arcari (2013) for the same ensiling times of the present trial. The differences in ammonia and soluble protein contents in the literature may be related to the type of corn hybrid used in the trials, with possible variations in arrangement of the protein matrix and starch granules, which may interfere with the degradation pattern during ensiling (Hoffman et al., 2011).

The silage that received the additive showed a high concentration of lactic acid at 30 days (1.18 % DMcorr), which was significantly higher than that observed for both the control silage (0.92 % DMcorr) and the silage at 60 days (0.80 % DMcorr). However, the pH values of the extracts were similar for the three rehydrated corn silages. Oliveira (2020) did not report effect of the additive on the concentration of lactic acid in rehydrated corn silage with 15, 30 and 60 days, which contrasts with the results of the present study. The fermentation pattern exhibited by the control silages was a heterolactic one, with considerable production of acetic acid and ethanol besides lactic acid. The high concentration of 1,2-propanediol in the 60 CON silage in relation to 30 CON also suggests the occurrence of lactic acid degradation, usually carried out by bacteria like *L. diolivorans* and *L. buchneri* (Daniel, 2019). 1,2-propanediol can also be further degraded to 1-propanol and propionic acid (Morais et al., 2017). Mycoflake™ contains in its composition "end products" of lactic acid degradation, such 1,2-propanediol, 1-propanol and mainly propionic acid. The presence of these compounds with the use of the additive may promote an effect of chemical balance, inhibiting the degradation of lactic acid, which could explain the greater content of this acid in the 30 MYC silage through its preservation. Since all silages stabilized under a similar pH, the difference in lactic acid content could be related to posterior degradation of this acid in the control silages, while the additive managed to inhibit this process in the 30 MYC silage. One may also consider that the presence of ammonium salts in Mycoflake™ could lead to a greater yield of lactic acid in order to compensate the buffering effect, therefore reaching stability under similar pH as in the control silages.

Table 5. Fermentative profile of the reconstituted corn grain silages

Item	Treatment			SEM	*P*-value[1]	
	30CON	30MYC	60CON		C_1	C_2
DMcorr, % as fed[2]	58.7	59.9	58.7	0.36	0.13	0.13
pH	3.77	3.75	3.75	0.03	0.48	0.90
Lactic acid, %	0.92	1.18	0.80	0.06	0.05	0.01
Acetic acid, %	0.26	0.25	0.30	0.01	0.52	0.02
Propionic acid, mg/kg	39.0	540.3	64.4	61.59	<0.01	<0.01
Butyric acid, mg/kg	4.39	4.19	4.70	0.22	0.74	0.36
Ethanol, %	0.13	0.10	0.33	0.04	0.73	0.01
1,2-Propanediol, mg/kg	39.7	38.6	73.0	5.02	0.86	<0.01
1-Propanol, mg/kg	5.71	13.20	9.05	1.45	0.04	0.22
Ethyl acetate, mg/kg	1.61	2.03	2.01	0.22	0.43	0.97
Ethyl lactate, mg/kg	79.7	49.5	136.9	11.61	0.15	<0.01
2,3-Butanediol, mg/kg	48.2	485.9	72.4	51.30	<0.01	<0.01

[1]Contrasts includes C_1: 30 CON x 30 MYC, C_2: 30 MYC x 60 CON
[2]Dry matter corrected for volatile compounds, according to the equation of Weissbach (2009)

Propionic acid-based additives are commonly associated to better conservation of silages due to the antimicrobial properties of the undissociated form of this acid (Morais et al., 2017), which is favored by the low pH environment of silages due to its high pKa. This effect has been reported by several authors, resulting in both yeast and mold control during storage and improved aerobic stability during feed-out (Woolford, 1975; Kung Jr. et al., 2000, 2004; Oliveira, 2020), been achieved with application rates close to 0.1-0.3% (fresh weight) of propionic acid-based additives. Mycoflake^{TM} presents a considerably lower concentration of propionic acid (about 28% wt,wt) in relation to additives commonly used in forage and grain silages. This is because Mycoflake^{TM} was developed to treat grains destined to flocculation, which present lower moisture content (~20%) than rehydrated grains. Higher moisture content of feeds requires higher rates of propionic acid to control microbial growth (Collins, 1995). However, Oliveira (2020) found evidence of yeast control in RCGS treated with Mycoflake^{TM}. In the present trial, although no microbiological profile analysis was performed and the decrease in ethanol content for the 30 MYC silage was only numerical, the silos that received the additive were the only ones that did not show mold or color change at the top layer at the time of opening. The application rate adopted for the additive was 0.2% (silage fresh matter), as in the study of Oliveira (2020), which corresponded to 0.06% of propionic acid, considerably lower in comparison to the usually employed in silages for conservation purposes (Kung Jr. et al., 1998, 2000, 2004; Morais et al., 2017). However, the surfactant components of Mycoflake^{TM} can also promote antimicrobial activity, since its chemical nature allows the interaction with the hydrophobic molecules (lipidic portions) of the cellular membranes, leading to its rupture and consequent microbial death (Zhang et al., 2009). Thus, despite the low content of propionic acid, the combination with surfactant agents seems to give Mycoflake^{TM} the potential to preserve rehydrated corn grain silages, preventing mold and yeasts growth during ensiling. As for aerobic stability, the only data available for Mycoflake^{TM} to the moment and to our knowledge are those reported in the work of Oliveira (2020), showing no improvement with its use in RCGS.

2,3-butanediol is a fermentation product usually associated with the presence of enterobacteria (Nishino & Shinde, 2007) and can also be produced by clostridia (Nishino et al., 2007). However, the high levels presented by the 30 MYC silage seem to be in discrepancy with its overall fermentative profile, since ethanol and butyric acid, also linked to enterobacteria and clostridia activity, presented the lowest levels in the additive-treated silage. Also, one can notice that 2,3-butanediol seems to “follow” the pattern for propionic acid between the treatments. The determination of 2,3-butanediol through gas or liquid chromatography may present a problem of co-eluting peaks during passage through the column, as the first peak can co-elute with propionic acid and the second peak, with iso-butyric acid (Daniel, 2019). Since all fermentative compounds were determined through gas chromatography in the present study, the observed high value for 2,3-butanediol is probably an issue of co-eluting peak with propionic acid.

The silage treated with additive did not differ from the 30 CON silage in terms of ruminal degradation fractions or degradability, although a significant decrease in the degradation rate was observed (P = 0.03). The 60 CON silage was superior in all ruminal degradability parameters in comparison to the 30 MYC silage.

Table 6. Ruminal *in situ* degradability parameters of the reconstituted corn grain silages

Item	Treatment			SEM	*P*-value[1]	
	30CON	30MYC	60CON		C_1	C_2
A (%)	34.9	35.8	49.9	1.18	0.61	<0.01
B (%)	63.5	63.8	47.3	2.33	0.95	<0.01
C (%)	1.21	1.15	0.67	0.09	0.72	0.02
k_d (%/h)	12.28	10.63	19.48	1.50	0.03	<0.01
potDEG (%)	98.8	98.9	99.3	0.09	0.72	0.02
eDEG, 2%/h	89.4	88.2	94.9	0.98	0.28	<0.01
eDEG, 5%/h	79.9	77.8	89.7	1.70	0.23	<0.01
eDEG, 8%/h	73.4	70.9	85.5	2.06	0.22	<0.01
DEG_{12h} (%)	80.0	79.0	93.0	2.70	0.76	<0.01

[1]Contrasts includes C_1: 30 CON x 30 MYC, C_2: 30 MYC x 60 CON

The results for soluble fraction (A) of the silages were consistent with the observed soluble protein contents, suggesting that the greater soluble fraction of the 60 CON silage was due to greater degradation during ensiling, mainly of the protein matrix (Hoffman et al., 2011). The value of 49.9% observed in the present trial seems to be higher, as Morais (2016) reported 48.7% for the soluble fraction of rehydrated corn silage at 90 days. However, fraction A considers, in addition to the soluble fraction itself, particles fine enough to pass through the pores of the bag and disappear at the zero-time incubation. As the corn was submitted to grinding before incubation, this may have generated a greater amount of particles in the form of powder, which would explain the high value of fraction A observed in the present trial, collaborating to the experimental error of methodology.

The longer storage time considerably favored the dry matter degradability of rehydrated corn since the indigestible fraction within 48 hours was significantly lower than in the additive-treated silage with only 30 days of storage. Oliveira (2020) reported greater degradability of dry matter and starch within 12 hours with an increase in the storage time of rehydrated corn from 30 to 60 days. In the case of corn, proteolysis indicators, such as soluble protein, have a good correlation with the in vitro degradability of starch (Ferraretto et al., 2014). The ruminal in situ degradability of dry matter is also highly correlated with the ruminal degradability of starch in corn (Philippeau et al., 1999), since 65 to 70% of mature corn grain is composed of starch (Paes, 2006; Huntington, 1997; Rooney & Pflugfelder, 1986). Thus, the results obtained in the present study suggest and consolidate that extending the ensiling of rehydrated corn from 30 to 60 days offers considerable gains in starch availability in the ruminal environment.

The additive-treated silage did not change the soluble fraction (A) or the indigestible fraction at 48 hours (C), so its potential degradability was similar to the 30 CON silage, which is in agreement with the absence of additive effect on the soluble protein content. These results suggest that the additive had no effect on protein matrix degradation in the same way that the increase in ensiling time to 60 days did. Oliveira (2020) reported greater dry matter degradability after 12 hours of ruminal incubation for rehydrated corn silage treated with the additive, although this increase was not significant in relation to the control treatment. In the present trial, the 30 MYC silage did not show any change in DM degradability at 12 hours of incubation compared to the 30 CON silage. However, Oliveira (2020) suggested that the greater DM degradability would have been due to changes in the particle size of the silage treated with the additive, which had a smaller geometric mean particle size (GMPS), greater surface area and higher percentage of fine particles compared to control silage, which was also observed in the present study (Table 4). However, as all silages had their particle size standardized by grinding before incubation, any possible effect on ruminal degradation due to changes in surface area and GMPS by the additive was nullified. All these results suggest that improvements in ruminal degradability of dry matter and starch of reconstituted corn silages treated with this additive are not due to a greater extent of proteolysis of the protein matrix, but to changes in particle size distribution, with increased surface area available for microbial attack in the rumen.

One may notice that the mean particle size presented by the silage treated with Mycoflake^{TM} after 30 days of ensiling is actually similar to the original particle size of the dry ground corn used to prepare the silages (995.7 µm vs. 998.0 µm, respectively) , while the control silages presented increases in GMPS over time (1105.0 and 1127.5 µm for 30 CON and 60

CON, respectively). This increase was also reported by Oliveira (2020) for rehydrated corn grain with extending ensiling time from 15 to 60 days. The reason for this phenomenon is still poorly understood, and according to Gomes et al. (2020), two possible mechanisms may explain this behavior for rehydrated corn grain silages. The first and more likely is an aggregation of small particles favored by the degradation process and the high moisture environment. The cohesion of corn starch particles is increased when submitted to moisture levels around 32-55% (Shotton & Harb, 1966), and the degradation of hydrophobic structures of the protein matrix surrounding the starch granules favors the exposure of starch to water, leading to the formation of aggregates that may not be completely separated during dry sieving (Gomes et al., 2020). Other possible explanation would be the disappearance of small particles with the solubilization and degradation during ensiling, which would decrease the recovery of particles retained in the lower sieves. Gomes et al. (2020) indeed observed recovery loss of small particles, suggesting they may be degraded or solubilized. However, for rehydrated corn grain, the recovery in the upper sieves were greater than 100%, suggesting that particle aggregation may also have occurred. Thus, it seems the lower mean particle size observed for the additive treated silage was not due to actual breakage of the grain, but to an effect of weakening the particle aggregation by the surfactant molecules, along with a possible delay in the solubilization of the protein matrix, as will be discussed further, favoring the relative maintenance of the grain silage GMPS.

The inclusion of the additive in the rehydrated corn silage reduced the ruminal fractional degradation rate (k_d) compared to the control silage, but without significantly changing the effective degradability of corn considering the passage rates of 2%/h, 5%/h and 8%/h. Vernon-Carter et al. (2018), studying the in vitro digestibility of gelatinized corn starch, reported that increasing additions of a surfactant, polysorbate 80, increased the rapidly digestible fractions of starch and also the resistant (indigestible) fraction. By using optical microscopy analysis, the authors observed that the surfactant was adsorbed on the surface of the starch granules and formed complexes through cross-linking. As the effect was more intense for normal corn than for waxy corn, the authors suggested that these complexes are formed preferentially with the amylose chains. However, as the addition of the surfactant was prior to the gelatinization process, it would be possible for the complex to be formed due to thermal treatment at 90 ºC. Ghiasi et al. (1982), using X-ray diffraction patterns, also reported the formation of complexes between non-ionic surfactants and amylose in gelatinized wheat starch at 75°C, but reported the disappearance of the surfactant-amylose complex when gelatinization occurred at 95°C. Schoch (1965) postulated that the surfactant, when making contact with the starch granule, immediately forms a complex with amylose, a theory reinforced by Longley & Miller (1971) and Miller et al. (1973). Ghiasi et al. (1982) observed the formation of the surfactant-amylose complex even at temperatures as low as 60°C. According to these authors, the immediate formation of the complex, as suggested by Schoch (1965), would be the only possible explanation for the observed results.

The formation of this surfactant-amylose complex seems to delay the release of solubles and the destruction of starch granules (Ghiasi et al., 1982), which may explain the slower ruminal degradation of 30 MYC silage in the present trial, due to the presence of the surfactant component of the additive. Oliveira (2020) also observed decrease in soluble nitrogen and ammonia contents in rehydrated corn silage treated with polysorbate 80 until 30 days of ensiling, suggesting a delay in the protein matrix degradation by the surfactant. Studies on the use of surfactants in bakery products (Stampfli & Nersten, 1995; Mahungu & Artz, 2001) reported that polysorbate was adsorbed on the surface of the starch granules, forming complexes and reducing the rate of water migration to the crust in bread. It is possible that, as the degradation of the protein matrix progresses during ensiling, the surfactant component of the additive will increasingly migrate to the starch granules, forming complexes and hindering the advancement of proteolysis itself through the effect of moisture retention, instead of allowing greater mobilization of enzymes. This interpretation seems to be in agreement with the results obtained for the soluble protein content of the 30 CON and 30 MYC silages, which despite being similar for both, the higher concentration of NH_3-N in the 30 MYC silage seems to have compensated for its lower protein degradation, “deceiving” the soluble nitrogen content as an indicator of proteolysis. This hypothesis is reinforced by the greater concentration of NH_3-N in the soluble nitrogen content observed for the 30 MYC silage, so if this excess ammonia comes from the additive composition, then this would be evidence of less protein degradation. Also, this exogenous ammonia could be used by silage bacteria as a source of NPN for microbial protein synthesis and growth (Rayetskaya et al., 1964; Lessard et al., 1978), decreasing the need for proteolysis of the silage true protein and consequently delaying the protein matrix degradation. However, as already mentioned, it is not known whether the ammonia present in 30 MYC silage is due to the action or composition of the additive.

The decrease in the rate of degradation of dry matter and, by correlation, of starch from rehydrated corn silage, without changing the potentially degradable fraction in the rumen, the 12-hour degradable fraction and the effective degradability, suggest that the use of this additive can assure the ruminal starch fermentation safer, reducing the risk of acidosis that is commonly associated with high-moisture corn silage (Hicks & Lake, 2006; Benton, 2005; Owens et al., 1997). Some beef feedlots in the USA (Hicks & Lake, 2006) have adopted the technique of mixing steam-flaked corn (relative slower degradation rate due to the largest particle size) with high moisture corn as a strategy to control ruminal acidosis without harming starch availability (Stock & Erickson, 2006). The possibility of obtaining this result with exclusively ensiled high moisture corn by using an additive is an interesting perspective, especially for dairy cattle, and deserves further investigation.

3.3.2. Animal performance

There were no significant differences among treatments for dry matter intake (DMI), however the treatment with reconstituted corn at 60 days (60 CON) presented a noticeable trend to decrease in DMI compared to the 30 CON silage in almost 1.6 kg/day (Table 7). Ferraretto et al. (2013), in a meta-analysis on the replacement of dry corn by high moisture corn in dairy cow diets, reported a decrease in dry matter intake with this practice. The decrease in DMI in this scenario seems to be linked to greater availability of fermentable starch in the rumen, as reported by Allen (2000), which would trigger a negative feedback on feed intake through hepatic oxidation of propionate, as described according to the most recent theory of Allen et al. (2009). Cows that received the 60 CON diet do not seem to have experienced limited intake by physical filling, as NDF intake was similar to that of cows fed the 30 CON diet. However, the 60 CON diet offered corn with greater degradability and faster fermentation, which may explain the decrease in DMI as reported by Allen (2000).

Although it did not result in a significant change in the DMI, the additive caused a significant decrease in NDF intake compared to the control silage ($P = 0.01$) and decreased crude protein intake compared to 60 CON ($P = 0.03$). The lower NDF intake seems to be related to the selective behavior of cows when they received the additive-treated corn grain silage (Table 8) – as will be discussed further, since the chemical analysis of the offered diets showed no difference in NDF content among treatments. However, the 30 MYC diet had slightly lower crude protein content in the analyses, although the silages did not differ in this parameter, which may explain the lower crude protein intake when cows received the 30 MYC diet.

Table 7. Performance of dairy cows: nutrient intake, milk yield and composition

Item	Treatment			SEM	*P*-value[1]	
	30CON	30MYC	60CON		C_1	C_2
DMI, kg/d	20.1	19.6	18.5	0.49	0.46	0.16
OMI, kg/d	17.6	17.3	18.0	0.49	0.82	0.53
NDFI, kg/d[2]	8.65	7.28	8.06	0.35	0.01	0.14
CPI, kg/d[3]	2.62	2.44	2.77	0.08	0.18	0.03
Milk, kg/d	22.8	21.9	21.9	0.71	0.06	0.93
FCM 3,5%, kg/d	19.7	19.3	19.2	0.81	0.33	0.90
ECM, kg/d	22.5	22.1	22.0	0.55	0.35	0.90
Milk/DMI	1.12	1.10	1.19	0.04	0.80	0.18
Fat, %	3.39	3.35	3.41	0.08	0.70	0.58
Fat, kg/d	0.67	0.66	0.66	0.03	0.68	0.98
Protein, %	3.51	3.50	3.49	0.05	0.86	0.85
Protein, kg/d	0.71	0.68	0.68	0.03	0.10	0.73
Lactose, %	4.12	4.15	4.13	0.05	0.72	0.83
Lactose, kg/d	0.86	0.84	0.82	0.04	0.40	0.28
Milk NE_L, Mcal/kg	0.67	0.67	0.67	0.01	0.81	0.76
NE_L, Mcal/d	14.9	14.6	14.5	0.37	0.45	0.73
Milk NE_L/DMI	0.74	0.75	0.80	0.02	0.83	0.20
MUN, mg/dL	12.2	12.6	12.9	0.23	0.26	0.25
SCC, mil/ml	169	145	178	26.6	0.48	0.33

[1]Contrasts includes C_1: 30 CON x 30 MYC, C_2: 30 MYC x 60 CON
[2]NDF intake
[3]Crude Protein intake

There were no significant changes in milk yield, yield corrected for 3.5% fat and milk composition (Table 7), except for a trend in lower milk yield by the cows when fed the additive-treated silage in comparison to the 30 CON silage (P = 0.06). However, the energy excretion in milk (NE_L) and the milk yield corrected for energy (ECM) did not differ across treatments. Several authors (Alvarez et al., 2001; Voelker et al., 1985, 1989; Morais, 2016; Bittencourt, 2012) have reported no effect of high moisture corn grain silage on milk yield, replacing dry corn in the diet, which is in agreement with the results of the meta-analysis by Ferraretto et al. (2013), containing published articles between 2000 and 2011. Knowlton et al. (1999), working with 30% moisture corn (ensiling time not reported), observed a trend towards an increase in milk yield in the first trial, but this effect disappeared in the second trial. Wilkerson et al. (1997) also observed a trend towards an increase in milk yield studying cows in early lactation between 3 and 9 weeks. The studies that did not report changes in milk yield utilized cows at mid or late lactation stages (100-300 DIM). The present trial used late lactation cows, with an average DIM of 218 days at the beginning of the feeding trial, which may explain the observed milk yield values and the absence of treatment effects on this variable. Feed efficiency also did not present significant differences between treatments.

The observed milk fat content of around 3.4% was close to that reported for the Holstein breed consuming diets with high moisture corn (Gritti, 2021; Voelker et al., 1989; Ferraretto et al., 2013), although higher values were reported by Arcari (2013), of 4.0%, and Morais (2016), of 3.6%, working with cows in advanced stage of lactation. Ferraretto et al. (2013) reported, in their meta-analysis, a decrease in milk fat content with increased starch availability in the rumen through high moisture corn silage. In the present trial, the increase in available starch with ensiling for 60 days did not cause milk fat depression. According to the currently most accepted theory (Bauman & Griinari, 2001), high moisture corn can contribute to the depression of milk fat by reducing the ruminal pH to such a level that the biohydrogenation process is compromised, altering the biochemical pathway, and producing CLA trans-10, cis-12, the isomer associated with inhibition of *de novo* synthesis in the mammary gland (Griinari et al., 1998). The lack of effect on fat content suggests that cows did not experience prolonged periods of low ruminal pH or acidosis. Despite the higher starch degradability in the rumen with the 60 CON silage, the total starch content of the diets was moderate, not exceeding 25% of DM, and both rumination and total chewing activities were not affected by the treatments (Table 8). Other authors (Morais, 2016; Arcari, 2013; Voelker et al., 1985; Knowlton et al., 1999) also reported no changes in milk fat with the use of more processed corn.

According to the theory of Bauman & Griinari (2001), another factor that can lead to milk fat depression is the increase in the ruminal rate of passage (k_P), which can prevent the complete biohydrogenation of unsaturated fatty acids due to the low retention time in the rumen. The silage treated with additive (30 MYC) reduced the intake of NDF and long particles retained in the 19-mm sieve (Table 8), in addition to a higher intake of fine particles (<4 mm), which could result in a higher passage rate in cows when they received this treatment. However, the decrease in the milk fat content with the additive (30 MYC) was not significant in relation to the other treatments without the additive.

There were no changes in the protein content of milk with the extended storage time, which is in agreement with the results from other studies (Morais, 2016; Arcari, 2013; Voelker et al., 1985, 1989; Knowlton et al., 1999) which reported no differences in milk protein with the use of more digestible corn starch in the diet. Alvarez et al. (2001) observed an increasing trend in milk protein content with high moisture corn and attributed this result to a likely greater flow of microbial protein into the duodenum. However, the meta-analysis by Ferraretto et al. (2013), reported no difference in protein content between dry and high moisture corn, only for flocculated corn, which has a higher fraction of undegradable protein in the rumen (RUP) due to the heat treatment during its processing. Microbial protein synthesis may indeed be higher with high moisture corn, due to the higher fraction of rumen degradable protein (RDP), but it is possible that its contribution to metabolizable protein (MP) and, consequently, to the excretion of protein in milk, is conditioned by the supply of RUP in the total diet or by the level of meeting with the cow's MP requirement (Alvarez et al., 2001). The silage treated with the additive did not alter the excretion of protein in milk, which seems to be in agreement with the lack of effect of the additive on the protein fractions of corn.

Processing corn by rehydration and ensiling may promote better synchronization between carbohydrates and protein ruminal degradation, which is evidenced by reports of lower ruminal ammonia concentration (Voelker et al., 1989; Arcari, 2013) and decrease in milk urea nitrogen (MUN) content (Pereira & Pereira, 2013; Morais, 2016; Bittencourt, 2012). In the present study, the MUN was not significantly affected by the intake of rehydrated corn grain silage treated with the additive. The levels of MUN observed in this trial are within the range considered adequate for the health of dairy cows, between 11 and 16 mg/dl (Campos, 2002), and close to the values reported by Morais (2016) and Gritti (2021) for diets with rehydrated corn silage.

Table 8. Ingestive and selective behavior of dairy cows

Item	Treatment			SEM	*P*-value[1]	
	30CON	30MYC	60CON		C_1	C_2
DMI, kg/d	20.1	19.6	18.5	0.49	0.46	0.16
NDFI, kg/d[2]	8.65	7.28	8.06	0.35	0.01	0.14
CPI, kg/d[3]	2.62	2.44	2.77	0.08	0.18	0.03
Eating, min/d	207	217	212	9.5	0.24	0.60
Rumination, min/d	487	471	468	9.0	0.40	0.89
Chewing, min/d	694	687	679	15.0	0.74	0.74
Meal frequency, meal/d	7.3	7.4	7.2	0.29	0.91	0.75
Meal length, min/meal	32.4	29.5	30.2	2.16	0.29	0.81
Meal size, kg/meal	3.0	2.8	2.8	0.15	0.51	0.95
First meal length, min	53.8	39.8	47.6	4.99	0.24	0.52
First meal size, kg	5.6	4.8	4.6	0.44	0.38	0.84
Particle Sorting, % as fed						
> 19 mm	89.2	82.1	90.5	1.66	0.01	0.01
8-19 mm	97.7	99.2	99.4	0.69	0.11	0.82
4-8 mm	102.3	103.2	102.1	0.44	0.28	0.16
< 4 mm	104.2	106.8	104.4	0.52	0.03	0.04
$peNDFI_{>4}$, kg/d[4]	4.82	4.02	4.66	0.18	0.01	0.04
$peNDFI_{>8}$, kg/d[5]	3.06	2.47	3.07	0.11	0.01	0.01
Rumination/$peNDFI_{>4}$	111.2	121.7	110.0	4.98	0.41	0.38
Rumination/$peNDFI_{>8}$	174.9	202.7	163.0	8.50	0.19	0.08

[1]Contrasts includes C_1: 30 CON x 30 MYC, C_2: 30 MYC x 60 CON
[2]NDF intake
[3]Crude Protein intake
[4]physically effective NDF above the 4 mm PSPS sieve intake
[5]physically effective NDF above the 8 mm PSPS sieve intake

No significant differences were observed for the ingestive behavior of animals across treatments, and there were also no differences in the parameters of stimulation of chewing activity, despite the lower intake of NDF for 30 MYC compared to 30 CON (Table 8). The silage with additive resulted in greater selection against long particles (retained in the 19-mm sieve) and selection in favor of the bottom fraction (< 4 mm) in relation to the other treatments.

The overall results suggest a preferential selection for the concentrate present in the smaller fractions of the diet, regardless of treatment, with sorting indexes above 100% for particles smaller than 8 mm, with the use of rehydrated corn. This behavior has been consistent in dairy cows fed diets containing high moisture corn, with reports of lower forage intake in favor of concentrate (Macleod et al., 1973; Palmquist & Conrad, 1970; Clark & Harshbarger, 1972). It is well stablished in the literature that the intake of feed and diets by dairy cattle is favored when they have an adequate moisture content (Arcari, 2013), which can also reduce the selection of ingredients (Lahr et al., 1983; Leonardi & Armentano, 2003) during the meal. Morais (2016), offering a diet of 17% rehydrated corn, 37% corn silage and 10% coast-cross hay (based on total diet DM) for Holstein cows, reported greater selection in favor of particles smaller than 8 mm, behavior also reported by Gritti (2021), offering a similar diet with high moisture corn, corn silage and oat haylage at similar proportions in diet DM, suggesting that the higher moisture content of the concentrate due to the presence of ensiled corn may have been more attractive to the animals and, consequently, increased the selection in their favor. Voelker et al. (1985), however, offering a diet of 33.8% high moisture corn, 41.4% corn silage and 16.2% alfalfa haylage, reported that cows tried to select high moisture corn in favor of roughage. However, the corn used in that study had less than 30% moisture (between 71.6 and 74.8% DM), considerably drier than that reported in the trials of Morais (2016) and Gritti (2021), which may not have been enough to promote the same sorting effect. In the present trial, a diet containing corn grain silage with 40% moisture was offered, so that the results of selective behavior seem to agree with the stated in the literature.

The results suggest that the presence of the additive in the rehydrated corn silage may have intensified the selection by the animals for the concentrate present in the smaller fractions of the diet. It is known that high moisture feed, such as silages, are the most perishable fraction of the diet in the feed bunk characterized by lower aerobic stability, becoming less attractive to animals throughout the day due to the deterioration effect by exposure to oxygen (Dawson et al., 1998). In this context, propionic acid-based additives have resulted in greater aerobic stability, besides the better control of yeasts and molds in the silo (Woolford, 1975; Kung Jr. et al., 2004; Oliveira, 2020; Morais, 2016), which may help to preserve the nutritional quality of the feed in the feed bunk for a longer time. According to the results of Oliveira (2020), although the concentration of propionic acid in Mycoflake^{TM} did not seem to be high enough to improve aerobic stability of rehydrated corn grain silages, this additive still presented a preservative potential during ensiling, and in the present trial, the silos treated with Mycoflake^{TM} were the only ones that did not show signs of mold or a spoiled top layer. It is possible that, by controlling yeast and mold growth during ensiling, the sensory quality of the feed may be also preserved, enhancing its palatability in comparison to untreated silages, stimulating its preferential intake by the animal. However, further research is needed to assess this possibility.

Besides the preservation purpose, propionic acid and its salts are also used as flavoring agents in food industry (Samel et al., 2018). Macleod et al. (1973) reported no difference in palatability with the treatment of high moisture shelled corn at 24% moisture with propionic acid, and Clark et al. (1973) also did not observe significant changes in concentrate intake when high moisture corn (~25% moisture) was treated with 1.3% (wt/wt) propionic acid. However, a recent trial conducted in the Forage Quality and Conservation group at ESALQ/USP (Ribeiro, unpublished) evaluated the use of a propionic acid-based additive on the total diet of dairy cattle, similar in composition to the present trial (44,6% corn silage, 6,4% Tifton haylage, 21,1% rehydrated corn), at time of feeding. Preliminary results suggest that the additive applied to the total diet inhibited selection, so that the animals consumed both concentrate and forage at the same proportion as they were offered, contrary to the trend observed in most studies with high moisture corn in the diet. Similar behaviors were also observed for forage silages treated with propionic acid in the works of Huber & Soejono (1977) and Stallings et al. (1979), with increased intake of the treated forages by dairy cows. Since in the present trial the grain corn was rehydrated to a higher moisture level (40%) than in the works of Clark et al. (1973) and Macleod et al. (1973), and a distinct selection in favor to concentrate was observed for the diet containing the additive-treated grain silage, it seems that moisture level of the feed may be a critical parameter in this issue.

The intake of physically effective NDF (peNDF) showed significant differences, so animals receiving grain silage with additive consumed less peNDF compared to treatments without additive. Considering that the peNDF is calculated from the NDF content and the percentage of material retained in the sieves associated with physical effectiveness, the lower intake of peNDF by the cows when fed the 30 MYC diet can be explained by the greater selection against long particles (>19 mm) and, consequently, to the lower intake of particles with physically effective size, since the NDF content of the diets was similar according to laboratory analyses. This methodology, however, assumes that NDF is evenly distributed across all particle sizes (Mertens, 1997). If true, NDF intake by cows fed the 30 MYC diet should not be different from the other treatments, as dry matter intake did not show any significant difference. In fact, the larger particles retained in the Penn State Particle Separator top sieves were composed primarily of straw, leaves and stalk from corn and Tifton plants, structures whose fiber content is considerably higher than the grain fractions often more present in the bottom sieves (Van Soest, 1994). Thus, although the diets offered in all treatments were similar in NDF content, the diet consumed by cows receiving 30 MYC silage would be less concentrated in NDF due to the selection against larger particles in this treatment, which explains the lower intake of NDF by cows when they received the 30 MYC diet.

The concept of peNDF is linked to the stimulus of rumination and total chewing activity (Mertens, 1997), through the formation of ruminal mat, whose presence stimulates rumen muscle contractions and favors regurgitation for rumination (Beauchemin, 2019). Particles retained in the 19-mm and 8-mm sieves actively participate in the formation of the ruminal mat, thus having a direct relationship in the stimulus of rumination (Beauchemin, 2019; Heinrichs & Jones, 2013), while particles retained in the 4-mm sieve would have a more passive participation in the stimulus, as they still make up the ruminal mat, although they seem to depend on the previous formation of raft by the larger particles. Beauchemin (2019) suggests that particles larger than 19 mm in the diet are less effective in maintaining ruminal pH due to the potential of being sorted by the cows in the feed bunk, so the greater retention of fiber in the 8-mm sieve is more desirable (Heinrichs & Kononoff, 2002). Adequate supply of particles retained in the 8-mm sieve also reduces the need for particles larger than 19 mm and for forage-NDF (Beauchemin, 2019; White et al., 2017). This consideration seems to explain the lack of effect of the lower intake of long particles on rumination time in cows receiving the 30 MYC silage, in addition to the fact that the intake of particles between 4 and 19 mm seems to have been sufficient for the formation of ruminal mat and stimulate rumination in all treatments.

Table 9. Total tract apparent digestibility of nutrients

Item	Treatment			SEM	*P*-value[1]	
	30CON	30MYC	60CON		C_1	C_2
DMDig, %	61.9	61.4	66.0	1.37	0.88	0.22
OMDig, %	66.0	65.3	70.3	0.96	0.79	0.07
CPDig, %	57.0	54.0	62.5	1.65	0.46	0.07
NDFDig, %	51.3	48.6	50.9	1.51	0.45	0.54

[1]Contrasts includes C_1: 30 CON x 30 MYC, C_2: 30 MYC x 60 CON

There were no significant differences for total-tract apparent digestibility (TTAD) of nutrients between treatments (Table 9). The digestibility values of dry matter, organic matter and NDF were close to those reported by Gritti (2021) and Arcari (2013) for diets containing rehydrated corn ensiled for 80 and 90 days, respectively. By producing high moisture grain silage it can provide increases in starch availability in the rumen and the rest of the digestive tract, which results in higher dry matter digestibility coefficients in the total tract. The degradation of the protein matrix reflects in an increase in the soluble fraction of crude protein, also resulting in higher values of protein TTAD. Morais (2016) and Arcari (2013) reported significant increases in total-tract digestibility of these nutrients with the replacement of dry corn by ensiled high moisture corn in dairy cattle diets. In the present study, the increase in conservation time from 30 to 60 days provided a slight increase in the digestibility coefficients of DM, OM and CP. The use of the additive did not change the digestibility of these nutrients in relation to the diet containing 30 CON silage, which seems to be in agreement with the results observed for ruminal degradability of the silages (Table 6).

The NDF total-tract apparent digestibility values observed in the present trial were similar to the values reported by Gritti (2021) and Morais (2016). The increase in storage time of the corn silages from 30 to 60 days does not seem to have altered NDF digestibility of the respective diets. Owens & Soderlund (2006) suggested that the increase in ruminal starch degradability may result in a shift of the fiber digestion site. This occurs when the ruminal environment is acidified by high starch fermentation to the point of inhibiting cellulolytic bacteria. Rumen undigested fiber has a new opportunity for fermentation in the large intestine, specifically in the cecum, but the contribution of cecal fermentation to total-tract digestion is still low compared to ruminal digestion. Thus, high moisture corn silage may result in lower ruminal degradability and lower TTAD of dietary fiber in cattle (Owens & Soderlund, 2006).

However, the similarity of NDF TTAD values between the 60 CON and 30 CON silages in the present trial suggests that there was not enough ruminal acidification to compromise fiber degradation, which must be related to the low starch content of the diets. Morais (2016) and Arcari (2013), working with diets containing 21% and 25% starch, respectively, also did not report decrease in the dietary NDF digestibility in dairy cattle with higher ruminal starch availability.

3.4. Conclusions

The increase in ensiling time from 30 to 60 days improved the nutritional value of reconstituted corn and its ruminal dry matter degradability. Mycoflake^{TM} promoted a decrease in particle size and an increase in surface area, but did not enhance the proteolysis of the reconstituted corn grain silage. Despite not improving the nutritional value in relation to the increase in ensiling time to 60 days, the additive had the potential to reduce the ruminal fermentation rate of corn grain silage without compromising its ruminal degradability. Rehydrated corn ensiled with Mycoflake^{TM} influenced the selection pattern of dairy cows against the intake of long particles and in favor of the concentrate, but without compromising chewing activity. The additive did not promote significant changes on the performance of dairy cows when used in rehydrated corn grain silage.

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4. PROPIONIC ACID-BASED ADDITIVE WITH SURFACTANT ACTION ON THE NUTRITIVE VALUE OF RECONSTITUTED CORN GRAIN SILAGE FOR GROWING LAMBS PERFORMANCE

ABSTRACT

Thirty-six Dorper lambs with an initial age of 5 months and initial weight of 27.9 ± 4.3 kg were allocated in a randomized complete block design to evaluate the effects of including an additive with surfactant action and propionic acid during the ensiling of reconstituted corn on its nutritional value and animal performance. Ground dry corn was rehydrated to 40% moisture, with or without additive, and ensiled in 200-L plastic drums. The treatments were prepared as follows: reconstituted grain corn silage (RCGS) stored for 50 days without additive (50 CON), RCGS stored for 90 days without additive (90 CON) and RCGS stored for 50 days with additive at 2 L/t (50 MYC). Diets contained 59.99% of RCGS. The extended conservation time of 90 CON silage and during the trial promoted an increase in soluble protein and ammonia contents and in the ruminal degradability of dry matter. Although no gain in the extent of degradation was observed, the longer ensiling time increased the soluble fraction at the expense of the potentially degradable fraction. Although the 50 MYC silage had similar soluble nitrogen content compared to 50 CON silage, its ammonia content was significantly higher, and its concentration of NH_3-N in soluble N was higher than that observed in the other treatments. The 50 MYC silage presented the lowest concentrations of lactic acid between the treatments, however ethanol and butyric acid contents were significantly lower in the additive-treated silage. There were no changes in dry matter degradability after 12 h of ruminal incubation and in degradation rate (k_d) of the 50 MYC silage against the 50 CON silage, but the additive promoted a higher indigestible fraction of corn compared to the other treatments, and there was a decrease in the ruminal soluble fraction only in the second period in relation to the 50 CON silage. The effective degradability only tended to be lower for the additive-treated silage when passage rates (k_p) were assumed at 5 and 8%/h, but for 2%/h it was significantly lower in relation to the 50 CON silage. There was no effect of ensiling time or additive on dry matter intake, weight gain and feed efficiency of the lambs. The silage treated with the additive did not change the selective behavior of the lambs. The results suggest that the additive seems to promote a delay in the reconstituted corn grain protein matrix degradation while in the silo even with extended storage times.

Keywords: Reconstituted corn grain silage, Surfactant, Propionic acid, Growing lambs

4.1. Introduction

One of the main limitations of corn digestibility in ruminants is the natural retention time in the rumen, which does not allow a satisfactory degradation of the protein matrix of the grain endosperm by proteolytic microorganisms due to the kinetics, preventing complete access to starch. By producing high moisture grains silages, the protein matrix of the grain can be modified, further degrading it in the silo and delivering to the animals a corn with fewer barriers to access to starch granules by ruminal microorganisms and digestive enzymes (Hoffman et al., 2011). Storage time is a determining factor in both the extent of degradation and solubilization of the protein matrix, so increases in soluble protein and ammonia content in the silage normally signal the evolution of the proteolytic process (Ohshima & McDonald, 1978). The use of high moisture ensiled corn in the literature has shown variable results, but positive in relation to dry corn, such as improved feed efficiency and decreased dry matter intake in cattle (Santos et al., 2016; Ferraretto et al., 2013) and increased daily weight gain in sheep (Reis et al., 2001; Almeida Júnior et al., 2004).

Chemical additives based on organic acids, such as propionic, have been used in both forage and grain silages with the purpose of microbial control and improvement of fermentation conditions in the silo, helping to maintain aerobic stability after opening the silos (Morais, 2016) and in the preservation of the nutritional and sensory quality of the silages while in the feed bunk. The impact of using an additive containing a surfactant agent in its composition in the food industry is still poorly understood, especially for animal nutrition (Oliveira, 2020). Several authors have reported the formation of cross-linked complexes involving surfactant and amylose chains in cereals (Ghiasi et al., 1982; Vernon-Carter et al., 2018).

Mycoflake™ is an additive that combines organic acids, including propionic, and a surfactant component designed to increase the retention and penetration of moisture into the grain during the flocculation process. So far, its use as an additive for silage is unknown. Oliveira (2020) evaluated the use of this additive in rehydrated corn silage and reported favorable results in ruminal dry matter degradability, decrease in average particle size and a possible enhancement of grain proteolysis. The aim of the present trial was to evaluate the impact of this additive in reconstituted corn silage conservation and its effects on the performance of growing lambs.

4.2. Material and methods

All experimental procedures were approved by the ethics committee for animal use of University of São Paulo (CEUA protocol nº 4233220520).

4.2.1. Preparing the reconstituted corn grain silages

The experimental silages were prepared and stored according to the fermentation times in 200-L plastic drums with internal plastic bags of 0.20 mm thick. The corn grain source was an unknown hybrid, grounded with a 5 mm sieve and whose particle size distribution was determined using a sample of approximately 100 g in an electromagnetic shaker (Bertel® Ltda., Caieiras, Brazil) equipped with screens of 4.75, 3.35, 2.36, 1.70, 1.18 and 0.60 mm plus the bottom pan. The ground grain had an average particle size of 1.00 mm, matching the traditional patterns for fine ground corn (Litherland, 2006).

The rehydration of the ground dry corn was carried out in a vertical mixing wagon with the addition of water (50 CON and 90 CON) or water + additive (50 MYC) to reach 40% moisture, mixed through 15 minutes before being conditioned and packed by feet into the 200-L plastic drums, until reaching a density of 1,250 kg/m^3. In the silages where the commercial additive was included, it was applied at a dosage of 2 L/t of rehydrated corn, mixed in the rehydration water. The plastic drums were lined inside with plastic bags, and after filling with the ground rehydrated grain and packing, bags were then closed and sealed with plastic clamps, without placing the covers on the drums, which were stored in a dry, ventilated and shaded place just aside the barn facilities were the animals were allocated.

4.2.2. Experimental diets

The basal diet of the experiment was formulated according to the SRNS program (version 1.11.7154.28131), in order to reach a predicted weight gain of 300 g/d. The nutritional composition and the proportion of ingredients are shown in Table 10. The proposal was to proceed with a typical high concentrate feedlot diet for sheep, with 60% inclusion of corn in the total dry matter, but totally replacing the dry corn by the rehydrated corn silages. The inclusion of the grain silages was the same for the diets of all treatments. The high inclusion of corn grain silages also aimed to evaluate possible effects of the additive on the palatability of the silage and from the diets that received it, since lambs are animals with high selective capacity (Van Soest, 1994).

Table 10. Ingredient participation and nutritional composition of formulated diets

Ingredients	50 CON	50 MYC	90 CON
Sugarcane bagasse, % DM	21.50	21.50	21.50
RCGS (50 d), % DM	59.99	_	_
RCGS (90 d), % DM	_	_	59.99
RCGS (50 d + additive), % DM	_	59.99	_
Soybean meal, % DM	16.00	16.00	16.00
Urea, % DM	0.50	0.50	0.50
Limestone, % DM	2.00	2.00	2.00
Mineral premix, % DM	0.01	0.01	0.01
Chemical composition			
DM, % as fed	60.3 ± 0.1	58.9 ± 0.3	59.3 ± 0.6
CP, % DM	10.4 ± 0.0	11.1 ± 0.5	10.9 ± 0.7
NDF, % DM	21.0 ± 2.0	18.5 ± 0.8	20.1 ± 1.7
Forage NDF, % DM	15.3 ± 0.1	15.3 ± 0.1	15.3 ± 0.1
Ash, % DM	6.0 ± 0.5	5.5 ± 0.6	5.6 ± 0.3
Particle Size			
> 19 mm, % DM	11.4 ± 0.8	11.4 ± 0.8	11.4 ± 0.8
< 19 mm and > 8 mm, % DM	6.9 ± 0.8	6.9 ± 0.8	6.7 ± 0.8
< 8 mm and > 4 mm, % DM	11.9 ± 1.3	11.4 ± 1.4	9.2 ± 1.4
< 4 mm, % DM	69.8 ± 0.1	70.4 ± 0.2	72.7 ± 0.2

RCGS – reconstituted corn grain silage; DM – dry matter; CP – crude protein; NDF – neutral detergent fiber.

Table 11. Chemical composition of the reconstituted corn grain silages at the beginning of the feeding trial

Items	50 CON	50 MYC	90 CON
DM, % as-fed	57.7 ± 0.1	59.5 ± 0.3	57.9 ± 0.1
CP, % DM	8.78 ± 0.30	8.73 ± 0.24	8.96 ± 0.06
NDF, % DM	7.0 ± 0.5	6.8 ± 0.3	5.0 ± 0.4
Ash, % DM	1.15 ± 0.02	1.09 ± 0.01	1.09 ± 0.03
Soluble Protein, % CP	49.6 ± 2.3	48.0 ± 3.3	58.4 ± 2.0

Table 12. Chemical composition of the ingredients utilized in the feeding trial

Items	Sugarcane bagasse	Mineral-protein mixture
DM, % as-fed	50.2 ± 4.6	95.3 ± 0.3
CP, % DM	6.6 ± 1.5	46.4 ± 0.0
NDF, % DM	71.0 ± 0.6	10.1 ± 0.3
Ash, % DM	15.1 ± 5.1	23.3 ± 1.8

4.2.3. Feeding trial

The animal performance trial was carried out at the feedlot facilities of the Intensive Production System of Sheep and Goats (SIPOC), on the Department of Animal Science of the Luiz de Queiroz College of Agriculture from University of São Paulo (ESALQ/USP), located in Piracicaba-SP. Thirty-six weaned Dorper lambs were used, with a mean initial age of 5 months and initial weight of 27.9 ± 4.3 kg. The animals were ranked and blocked by weight after fasting for 16 hours, and allocated to a randomized complete block design with two consecutive periods of 28 days each, totalizing 56 days of experimental period. The experimental unit consisted of a pen with two animals, so that each treatment had six pens. The treatments were: rehydrated corn grain silage (RCGS) at 50 days (50 CON); RCGS at 90 days (90 CON); and RCGS at 50 days + Mycoflake^{TM} 2 L/t (50 MYC) (Kemin South America, Indaiatuba, SP, Brazil).

Animals were dewormed and confined in covered pens with concreted floors and free water access. Eighteen pens were used with a capacity of two animals/pen, resulting in a density of 1 lamb/3 m^2. Feeding management consisted of only one daily feeding at 5 pm, with total collection of orts the following day before the next feeding. The concentrated ingredients, except for the silages, were previously mixed and stored in raffia bags as concentrated ration, so that ration, sugarcane bagasse and corn grain silage were mixed immediately before feeding to the animals. Given the small amount of feed to be offered, the ingredients were mixed manually for 10 minutes. The amount offered per pen was calculated based on the orts from the previous day, to ensure a minimum of 10% orts at the feed bunk.

4.2.3.1. Animal performance: nutrients intake and weight gain

The feeding trial consisted of two subsequent periods of 28 days each, being the first 14 days of adaptation and the following 14 days of evaluation and collections. There was no rotation of treatments between pens, so the animals remained in the same treatment until the end of the trial. The feed-out scheme of the silos utilized in the feeding trial was sequential according to use, with the opening of the first silos at the respective fermentation times of each treatment (50 and 90 days) at the beginning of the trial. In the second period of the feeding trial, a second set of silos were opened, following the ensiling time already elapsed (+28 days).

The concentrate premix, sugarcane bagasse and grain silages were sampled every other days, with the sub-samples immediately frozen at -10 ºC in walkin freezer, to later be thawed and mixed to form the composite sample for each period. The ingredient samples were dried in a forced air circulation oven at 55 ºC for 72 hours and grounded in a Willey type mill with a 1 mm sieve, then analyzed in the laboratory to determine the contents of dry matter (AOAC, 1990, method 934.01), ash (AOAC, 1990, method 924.05), crude protein (combustion by the Dumas method – AOAC, 1990, method 992.23) and neutral detergent fiber corrected for ash with thermostable amylase and sodium sulfite (Mertens, 2002).

The offered diet was weighed and sampled for each pen every day, and the daily sub-samples were immediately frozen at -10 ºC in walkin freezer, to later be thawed and mixed to form the composite sample for each period. The orts from each pen were collected daily before the next feeding, weighed and sampled, and the daily sub-samples were immediately frozen at -10 ºC in walkin freezer, to later be thawed and mixed to form the composite sample for each period. A fraction of 400 g of each composite sample of the offered diet and orts was kept to determine the average particle size through the Penn State Particle Separator (Lammers et al., 1996; Heinrichs and Jones, 2013), using 19 mm, 8 mm and 4 mm sieves, plus the bottom box. Another fraction of 500 g of each composite sample of the offered diet and orts was separated for chemical analysis, being dried in a forced air circulation oven at 55 ºC for 72 hours and grounded in a Willey type mill with a 1 mm sieve, to determine the contents of dry matter (AOAC, 1990, method 934.01), ash (AOAC, 1990, method 924.05), crude protein (combustion by the Dumas method – AOAC, 1990, method 992.23) and neutral detergent fiber corrected for ash with thermostable amylase and sodium sulfite (Mertens, 2002). The intake of as-fed matter in each pen was measured throughout d-15 and d-27 of each period, by weighing offers and orts. The animals weight gain was obtained by weighing them after fasting for 16 hours, on day zero and on the 28th day of each period. The net energy for maintenance (NEm) and gain (NEg) of the diets were calculated using mean values of observed body weight (BW), average daily gain (ADG) and dry matter intake (DMI) of the lambs in each pen, according to the equations of Zinn & Shen (1998).

4.2.3.2. Ingestive behavior

For ingestive behavior evaluation, the particle size distribution of the offered diets and orts of each pen was determined using the Penn State Particle Separator (Lammers et al., 1996; Heinrichs and Jones, 2013), as described in section 4.2.3.1. The intake for each particle size retained in the *i*-mm sieve is calculated as follows:

$$[\text{Offer x } (\%\text{sieve\_offer}_{(i)}/100)] – [\text{Orts x } (\%\text{sieve\_orts}_{(i)}/100)] = \text{Intake}_{(i)} \qquad \text{(Eq. 7)}$$

With the actual intake for each sieve, a particle size distribution for intake ($\%\text{sieve\_intake}_{(i)}$) can be estimated. The particle sorting indexes for each sieve is then calculated by dividing [($\%\text{sieve\_intake}_{(i)}$ / $\%\text{sieve\_offer}_{(i)}$) x 100], indicating refusal (<100%), preferential intake (>100%) or no selection (=100%) (Leonardi & Armentano, 2003).

4.2.3.3. Fermentative profile of the reconstituted corn grain silages

Samples of reconstituted corn silage were collected at the time of silo opening of each treatment (50 and 90 days of fermentation) and weekly to represent the silages throughout the time until the end of the feeding trial, with the samples being immediately frozen at -20°C. Subsequently, the subsamples collected every week were thawed and mixed to form a composite sample for each period. A fraction of 100 g was kept and sent to prepare aqueous extracts, by using 25 g of sample and 225 g of deionized water. The extracts were homogenized in a stomacher for 4 minutes, then filtered through 3 folder cheesecloths. Samples were placed in 2 mL micro tubes and frozen at -40 ºC awaiting analyzes for the determination of ammonia nitrogen (Chaney & Marbach, 1962, adapted by Weatherburn, 1967), lactic acid (Pryce, 1969), content of volatile fatty acids, esters and ethanol by gas chromatography (GCMS QP2010 Plus; Shimadzu®, Kyoto, Japan) using a capillary column (Stabilwax; Restek®, Bellefonte, PA; 60 m long, 0.25 mm outer diameter, 0.25 µm film thickness) and correction of dry matter content for volatile compounds according to the equation of Weissbach (2009).

Another fraction of 400 g was separated for chemical analysis destined to the ingredients (described in section 4.2.3.1), in addition to the determination of the soluble protein content by mixing in a borate-phosphate buffer solution at 39 ºC for one hour, followed by filtration (Krishnamoorthy et al., 1982) and measurement of nitrogen by the Dumas method (AOAC, 1990, method 992.23).

4.2.3.4. Ruminal *in situ* degradability of the reconstituted corn grain silages

For the ruminal *in situ* degradability of dry matter, 5.5 g dry and ground subsamples of the silages were placed in 20 cm x 10 cm nylon bags with a porosity of 30 µm, and subsequently incubated in duplicate in the rumen of two Holstein cows, adapted to a diet with 50% corn silage, 20.7% dry ground corn, 9.8% citrus pulp, 17% soybean meal and 2.5% mineral premix. The incubation times used were 0, 12, 24 and 48 hours, with the bags being incubated in reverse order, so they could be removed together. After removal, the bags were immediately immersed in iced water to stop fermentation. Then, the bags were washed until the washing water became clear and dried in a forced air circulation oven at 55 ºC for 72 hours, being then weighed to determine the percentage of dry matter degradation for each incubation time.

The degradability parameters were determined through the PROC NLIN of SAS 9.4 (SAS Institute Inc., Cary, NC) according to the model proposed by Orskov & McDonald (1979), whose equation is given by:

$$Y_{(t)} = A + B*(1 - e^{-k*t}) \qquad \text{(Eq. 8)}$$

Where A = fraction that immediately escapes from the bag (soluble fraction + particles smaller than the porosity of the bag); B = potentially degradable fraction; k = degradation rate (%/h); and t = incubation time (h). The following fractions were obtained from the degradation data: soluble (A), obtained by the difference between the incubated weight and the zero-time residue; indigestible (C) = residue at time 48h and the potentially degradable fraction (B), calculated by the difference 100 – (A + C). Potential degradability was calculated as potDEG = (100 – C) and the effective degradabilities (Eq. 9) were calculated according to Ogden et al. (2005) considering the passage rates of 2%/h, 5%/h and 8%/h.

$$eDEG\ (\%) = A + [B*k_d / (k_d + k_p)] \qquad \text{(Eq. 9)}$$

4.2.4. Statistical analysis

The parameters of animal performance and ingestive behavior obtained in the feeding trial were analyzed as a randomized block design using the SAS MIXED procedure (SAS Institute Inc., Cary, NC), including fixed effect of treatment (50 CON, 90 CON and 50 MYC), and random effect of block (1 to 6). Two contrasts were used to compare treatments: 1) 50 CON vs. 50 MYC and 2) 50 MYC vs. 90 CON.

The parameters regarding the chemical and fermentative profile of the reconstituted corn grain silages were analyzed as a completely randomized design with split-plot arrangement using the SAS MIXED procedure (SAS Institute Inc., Cary, NC), including fixed effect of silage (50 CON, 90 CON and 50 MYC), fixed effect of period (1 and 2) and interaction between silage and period. Least square means for interaction effect were adjusted and compared by Tukey ($\alpha = 0.05$). Two contrasts were used to compare silages: 1) 50 CON vs. 50 MYC and 2) 50 MYC vs. 90 CON.

The ruminal degradability assay was analyzed as a randomized block design with split-plot arrangement using the SAS MIXED procedure (SAS Institute Inc., Cary, NC), including fixed effect of silage (50 CON, 90 CON and 50 MYC), fixed effect of period (1 e 2), interaction between silage and period and random effect of block (cow A and B), with least square means for interaction effect adjusted and compared by Tukey ($\alpha = 0.05$). Two contrasts were used to compare silages: 1) 50 CON vs. 50 MYC and 2) 50 MYC vs. 90 CON. Significant differences were declared when $P \leq 0.05$ and trends when $0.05 < P \leq 0.10$.

4.3. Results and discussion

4.3.1. Silages

Table 13. Chemical composition of the reconstituted corn grain silages

Item	50 CON		50 MYC		90 CON		SEM	*P*-value[1]			Contrast *P*-value[2]	
	0-30	30-60	0-30	30-60	0-30	30-60		S	P	S × P	C_1	C_2
DM, % as fed	57.9	57.5	59.8	59.2	57.7	58.1	0.19	<0.01	0.28	0.08	<0.01	<0.01
CP, % DM	8.7	8.8	8.5	9.0	8.9	9.0	0.12	0.82	0.53	0.87	0.89	0.56
NDF, % DM	7.8	6.2	7.2	6.4	5.7	4.4	0.34	<0.01	<0.01	0.22	0.46	<0.01
Ash, % DM	1.17	1.13	1.09	1.09	1.11	1.06	0.02	0.17	0.31	0.73	0.11	0.99
Soluble Protein, % CP	45.64	53.51	43.51	52.48	55.34	61.51	1.94	0.01	0.01	0.83	0.51	<0.01
NH_3-N, % DM	0.05	0.06	0.07	0.08	0.07	0.08	0.00	0.03	0.04	0.80	0.02	0.82
NH_3-N, % total N	3.83	4.54	5.17	5.42	4.79	5.72	0.22	0.03	0.07	0.64	0.02	0.91
NH_3-N, % Soluble N	8.37	8.48	11.92	10.42	8.63	9.24	0.40	<0.01	0.41	0.06	<0.01	<0.01

[1]Probabilities for effect of silage (S), period (P) and interaction between silage and period (S × P)
[2]Contrasts include C_1: 50 CON x 50 MYC, C_2: 50 MYC x 90 CON

Table 14. Fermentative profile of the reconstituted corn grain silages

Item	50 CON		50 MYC		90 CON		SEM	*P*-value[1]			Contrast *P*-value[2]	
	0-30	30-60	0-30	30-60	0-30	30-60		S	P	S × P	C_1	C_2
DMcorr, % as fed[3]	58.6	58.2	60.6	60.1	58.6	58.6	0.19	<0.01	0.01	0.13	<0.01	<0.01
Lactic acid, %	2.40	2.56	2.11	2.46	2.57	2.76	0.05	<0.01	<0.01	0.20	<0.01	<0.01
Acetic acid, %	0.22	0.24	0.21	0.24	0.21	0.20	0.01	0.22	0.21	0.26	0.86	0.16
Propionic acid, mg/kg	43.2	35.9	433.5	428.1	29.2	21.0	56.69	<0.01	0.07	0.94	<0.01	<0.01
Butyric acid, mg/kg	4.87	5.41	2.67	2.78	6.30	5.78	0.50	0.02	0.95	0.83	0.03	0.01
Ethanol, %	0.54[b]	0.65[a]	0.23[c]	0.27[c]	0.67[a]	0.69[a]	0.06	<0.01	<0.01	0.02	<0.01	<0.01
1,2-Propanediol, mg/kg	74.1	73.8	60.1	59.7	66.4	57.4	7.57	0.83	0.88	0.98	0.58	0.94
1-Propanol, mg/kg	2.16	4.28	4.31	4.53	3.14	3.58	0.27	0.03	0.02	0.07	0.02	0.02
Ethyl acetate, mg/kg	7.75	11.80	5.15	7.81	12.15	12.67	0.88	<0.01	0.01	0.15	0.01	<0.01
Ethyl lactate, mg/kg	183.0	221.1	64.8	86.5	283.5	327.0	28.9	<0.01	<0.01	0.23	<0.01	<0.01
2,3-Butanediol, mg/kg	55.5	39.9	543.0	543.8	36.7	30.9	71.7	<0.01	0.64	0.89	<0.01	<0.01

[1]Probabilities for effect of silage (S), period (P) and interaction between silage and period (S × P)
[2]Contrasts include C_1: 50 CON x 50 MYC, C_2: 50 MYC x 90 CON
[3]Dry matter corrected for volatiles compounds, according to the equation of Weissbach (2009)

The use of additives in grain silages may promote changes in the concentration of some nutrients, such as crude protein and ash, as pointed out by Morais et al. (2017). However, no significant changes in crude protein, ash and NDF contents were observed with the use of the additive, which is in agreement with Kung Jr. et al. (1998; 2000; 2001), who also did not report changes in these nutrients with the use of an additive based on buffered propionic acid. However, the NDF content of the silages decreased with the extending storage time ($P < 0.01$). This may be due to fiber fermentation with enzymatic activity on hemicellulose (Morais et al., 2017), decreasing the NDF content over time. The DM content was greater for the silage treated with the additive, possibly due to lower losses of dry matter, suggesting a better conservation of the silage, which is in agreement with the lower concentrations of butyric acid and ethanol in relation to the other silages (Table 14). Propionic acid-based additives are commonly associated to the better conservation of silages, since the low pH environment favors the prevalence of the undissociated form of this acid, which can diffuse into microbial cells, leading to their death (Morais et al., 2017). Microbial growth control and improvements in aerobic stability have been reported with application rates in the order of 0.1-0.3% (fresh weight) (Kung Jr. et al., 1998, 2000, 2004) of propionic acid-based additives. Mycoflake™ presents a considerably lower concentration of this acid (~28% wt,wt) in relation to other additives commonly used in forage and grain silages for conservation purposes. This is because Mycoflake™ was developed for treatment of grains destined to the flocculation process, where a much lower moisture content is needed (~20%) than for rehydration of grains to be ensiled (~35%). Higher moisture content in feeds requires higher rates of propionic acid to control microbial growth (Collins, 1995). However, using this additive at 0.2% (fresh weight), Oliveira (2020) found evidence of yeast control in RCGS over the ensiling time, and in the present trial, there were also evidence of better silage conservation and control of undesirable microbial activity. Despite the low content of propionic acid that was applied (0.06% fresh weight), the surfactant components of Mycoflake™ may also promote antimicrobial activity, since its chemical nature allows the interaction with the hydrophobic structures (lipidic portions) of the cellular membranes, leading to its rupture and consequent microbial death (Zhang et al., 2009). Thus, the combination of propionic acid with surfactant agents seems to give Mycoflake™ the potential to preserve rehydrated corn grain silages, preventing mold and yeasts growth during ensiling. As for aerobic stability, the only data available for Mycoflake™ to the moment and to our knowledge are those reported in the work of Oliveira (2020), showing no improvement with its use in RCGS.

2,3-butanediol is a fermentation product usually associated with the presence of enterobacteria (Nishino & Shinde, 2007) and clostridia (Nishino et al., 2007). However, the high levels presented by the 50 MYC silage do not agree with its overall fermentation end products profile, since butyric acid and ethanol, also indicators of undesirable microbial activity, were significantly lower in this treatment. Also, one can notice that 2,3-butanediol seems to "follow" the pattern for propionic acid between the treatments. The determination of 2,3-butanediol by gas or liquid chromatography may present a problem of co-eluting peaks during passage through the column, as the first peak can co-elute with propionic acid and the second peak, with iso-butyric acid (Daniel, 2019). Since all fermentation compounds were determined through gas chromatography in the present study, the observed high value for 2,3-butanediol is probably an issue of co-eluting peak with propionic acid.

The lactic acid content of the 50 MYC silage was the lowest in comparison to the other silages without the additive ($P < 0.01$). Oliveira (2020), working with the same additive on rehydrated corn silage, did not report differences regarding lactic acid concentration. In our first trial (discussed in chapter 3), the additive-treated silage presented greater lactic acid concentration than the control silage at 30 days of storage. It is possible that the surfactant agents of Mycoflake™ may have inhibited part of lactic acid bacteria as well, which could explain a lower yield of lactic acid over a longer ensiling time.

The extending storage time promoted a significant increase on the soluble protein content ($P = 0.01$) over time, regardless of the treatments. There was also a significant increase in the concentration of ammonia nitrogen at DM basis ($P = 0.04$) and a trend for ammonia nitrogen at total-N basis ($P = 0.07$) in the silages with longer storage times, regardless of the treatment, which seems to agree with the reported by Hoffman et al. (2011). Soluble protein and ammonia contents are good indicators of the occurrence of proteolysis in silages (Ohshima & McDonald, 1978). The longer storage time between the two evaluation periods promoted an average increase of about 16 percentage units in the soluble protein content, and 17 percentage units in the ammonia nitrogen concentration, indicating greater degradation of the protein matrix. Although the largest increases in the soluble protein fraction may occur within the first 56 days of ensiling (Hicks & Lake, 2012), matrix degradation remain active for much longer, as reported by Hoffman et al. (2011), who observed a continuous decrease in the γ-zeins up to 240 days of ensiling. The soluble protein values observed in the present study were higher than those reported by Prigge (1976) and Oliveira (2020), of 38.2% and about 30%, respectively, for corn ensiled for 60 days. Although the first author has studied grain silage with a moisture content of only 28%, which may have limited the activity of enzymes in the ensiled grains, the second one worked with a 35% moisture, which is considered adequate for the rehydration of corn grains. Both in the present trial as well as in the trial of Oliveira (2020) the corn grain source provided had unknown origin, then it is possible that the differences in soluble protein may be linked to the hybrid germplasm origin, as also reported by Hoffman et al. (2011). However, the soluble protein results are close to those observed by Morais (2016) of 48.5% with 90 days and 61.0% with 180 days of conservation, and by Gritti (2021), of 52.7% with 80 days. The values observed in the present study for ammonia nitrogen were higher than that reported by Kung Jr. et al. (2004) of 0.03% DM for high moisture corn ensiled for 120 days, but were similar to that reported by Oliveira (2020) of 4% in total N with 60 days, and lower than the values reported in the trials of Morais (2016) and Arcari (2013). These differences are probably linked to the type of hybrid used in the respective trials, with different arrangements of the protein matrix and starch granules, which may interfere with the degradation pattern during ensiling (Hoffman et al., 2011).

The silage treated with the additive (50 MYC) showed no difference in soluble protein content compared to the 50 CON silage (47.99 vs. 49.58 %CP, respectively; P = 0.51). Oliveira (2020), analyzing the same additive used in the present study, reported inconsistent results regarding the soluble protein content, although they still suggested a possible increase in the soluble fraction after 30 days of ensiling with the additive. However, in the present trial, the additive failed to promote any significant change in the soluble protein content of the silage compared to the control silage in both experimental periods, between 50 and 110 days of storage. Although in the trial of Oliveira (2020) the additive had no effect on the concentration of ammonia up to 60 days of ensiling, the 50 MYC silage presented a higher concentration of NH_3-N compared to the 50 CON silage both at DM and total-N basis (P = 0.02), and similar to the observed in the 90 CON silage. Kung Jr. et al. (2004) also reported higher concentrations of ammonia nitrogen in silages treated with a propionic acid-based additive from the same company, but the authors suggested that this effect was due to the composition of the additive itself, which includes an ammonium salt (ammonium propionate) to buffer the acid. Since the additive used in the present trial contains ammonium hydroxide and ammonium chloride in its composition, the higher ammonia concentrations observed in the 50 MYC silage are probably due to the presence of these ingredients, but their concentrations are not known. Manufacturers of this type of product do not usually declare neither the ingredient composition nor their concentrations, which was not different for the additive used in this study. Thus, it is not possible to determine whether the results for NH_3-N were due to the action of the additive or its composition.

The additive-treated silage showed a significantly higher concentration of NH_3-N in the soluble N (11.17 %) compared to the other treatments (50 CON: 8.43 %; 90 CON: 8.94 %). A possible explanation for the greater contribution of ammonia in the soluble nitrogen content of the silage would be if the additive somehow promoted the deamination process during the degradation of the protein matrix. However, to the moment there is no data in the literature that support this hypothesis or suggest such an action mode from a chemical additive. Furthermore, this effect was not maintained throughout the trial, tending to disappear with the advance of the storage time between periods (P = 0.06). So, the NH_3-N concentration in the soluble N decreased and tended to approach the values observed in the silages without the additive in the second evaluation period. These results suggest that the contribution of ammonia in the soluble N content of the silages was not affected by the ensiling time, suggesting a possible synchrony between matrix proteolysis and the deamination process during ensiling. However, such behavior did not occur for the ammonia in the additive-treated silage, so this anomaly suggests that part of the NH_3-N from the 50 MYC silage does not follow the degradation dynamics observed in the other silages, possibly because this fraction corresponds to the ammonia “imported” by the additive in its composition. In theory, this fraction would remain relatively constant and would gradually have its participation in soluble N reduced as more NH_3-N from protein degradation would be produced by deamination with the advancing proteolysis over time.

Table 15. Ruminal *in situ* degradability parameters of the reconstituted corn grain silages

Item	50 CON		50 MYC		90 CON		SEM	*P*-value[1]			Contrast *P*-value[2]	
	0-30	30-60	0-30	30-60	0-30	30-60		S	P	S × P	C_1	C_2
A (%)	59.5[d]	69.9[b]	59.9[d]	65.3[c]	72.0[b]	76.7[a]	1.91	<0.01	<0.01	0.01	0.01	<0.01
B (%)	40.1[a]	29.9[c]	39.4[a]	33.9[b]	27.8[c]	23.0[d]	1.88	<0.01	<0.01	0.01	0.03	<0.01
C (%)	0.32	0.23	0.77	0.77	0.23	0.30	0.09	0.02	0.94	0.86	0.01	0.01
k_d (%/h)	16.98	19.73	15.98	19.13	20.53	22.88	1.19	0.03	0.03	0.90	0.46	0.02
potDEG (%)	99.7	99.8	99.2	99.2	99.8	99.7	0.09	0.02	0.94	0.86	0.01	0.04
eDEG, 2%/h	95.2	96.9	94.6	95.7	97.2	97.8	0.42	0.01	0.01	0.18	0.04	<0.01
eDEG, 5%/h	90.2	93.6	89.2	91.6	94.2	95.5	0.82	0.01	0.01	0.20	0.08	<0.01
eDEG, 8%/h	86.4	91.0	85.3	88.5	91.8	93.7	1.09	0.01	0.01	0.18	0.08	<0.01
DEG_{12h} (%)	93.6	96.4	93.9	95.6	96.9	98.0	0.68	0.04	0.04	0.59	0.77	0.02

[1]Probabilities for effect of silage (S), period (P) and interaction between silage and period (S × P)
[2]Contrasts include C_1: 50 CON x 50 MYC, C_2: 50 MYC x 90 CON

There was a significant increase in the ruminal soluble fraction (A), ruminal degradation rate (P = 0.03), effective dry matter degradability considering passage rates (k_p) at 2, 5 and 8%/h (P = 0.01) and in DM degradability after 12 hours of incubation (P = 0.04) with the extending conservation time between the evaluation periods, regardless of the treatments (Table 15). The results for soluble fraction (A) of the silages were consistent with the observed soluble protein contents (Table 13), suggesting that the greater soluble fraction of both the 90 CON silage and the silages over time was due to greater degradation of the protein matrix during ensiling (Hoffman et al., 2011). The values for fraction A were considerably high considering the storage times. Morais (2016) reported 48.7% of soluble fraction in rehydrated corn ensiled for 90 days, while Arcari (2013) observed values between 22.4 and 32.4% for corn ensiled at 60 and 150 days, respectively, only obtaining values greater than 50% after more than 300 days of ensiling. However, fraction A considers, besides the soluble fraction in the rumen itself, particles fine enough to pass through the pores of the bag and immediately disappear during the incubation. As the silages were ground before being incubated, this may have promoted a greater proportion of particles in the form of powder, which could explain the high fraction A values observed in the present study.

The absence of effect of storage time on the indigestible fraction (C) indicates that there were no gains in ruminal availability of starch with storage time greater than 50 days, only gains in the soluble fraction at the expense of the potentially degradable fraction, since the proteolysis in the silages remained active, as indicated by the soluble protein and ammonia results. Thus, the longer conservation time promoted significant improvements in ruminal degradability of dry matter and, by correlation, of starch contained in corn grains (Philippeau et al., 1999).

The 50 MYC silage presented lower soluble fraction and a higher potentially degradable fraction (B) compared with the 50 CON silage only in the second period, when they were already over 80 days of storage. These results suggest that the additive may have promoted a delay in the degradation of the ensiled corn, decreasing the soluble fraction of the silage and making its ruminal digestion more dependent on the degradation kinetics over the potential fraction (B) with extended storage times. Although no significant changes were observed in the soluble protein content, it is possible that the corn grain ensiled with additive has shown less degradation of its protein matrix, since the higher ammonia concentration of the 50 MYC silage seems to compensate for the soluble nitrogen content, “deceiving” its function as an indicator of proteolysis. The exogenous ammonia from the additive could also be used by silage bacteria as a source of NPN for microbial protein synthesis and growth (Rayetskaya et al., 1964; Lessard et al., 1978), decreasing the need for proteolysis of the silage true protein and consequently delaying the protein matrix degradation. As already mentioned, it is not known whether the ammonia present in the treated silage is due to the action or composition of the additive, however, the results of ruminal degradation and the behavior of NH_3-N concentration in soluble N (Table 13) over time seem to reinforce the hypothesis of less protein degradation in the silage treated with the additive.

Vernon-Carter et al. (2018), through optical microscopy analysis, reported the occurrence of cross-linked complexes between a non-ionic surfactant, polysorbate 80, and starch granules in gelatinized corn. These complexes would be formed as soon as the surfactant meets the starch amylose chains, as postulated by Schoch (1965) and further reinforced by Longley & Miller (1971) and Miller et al. (1973). The presence of the surfactant component of the additive used in this trial may have led to the formation of these surfactant-amylose complexes, which seem to delay the release of solubles and the destruction of starch granules (Ghiasi et al., 1982). Oliveira (2020) also observed decrease in soluble nitrogen and ammonia contents for rehydrated corn silage treated with a surfactant until 30 days of ensiling, suggesting a delay in the protein matrix degradation. It is possible that, as the degradation of the protein matrix progresses during ensiling, the surfactant component of the additive will increasingly gain access to the starch granules, forming complexes and hindering the advancement of proteolysis itself through the effect of moisture retention, reported by Stampfli & Nersten (1995) and Mahungu & Artz (2001), instead of allowing greater mobilization of enzymes. This interpretation seems to agree with the possible lower degradation of the protein matrix and the lower soluble fraction in the rumen, although it has not promoted a significant decrease in the fractional rate of degradation (k_d) in relation to the control silage ($P = 0.46$).

The fermentation of fraction B is totally conditioned by ruminal kinetics, with the degradation and passage rates determining how much of this fraction is actually digested. High passage rates, favored by high concentrate diets with a large amount of fine particles, decrease ruminal retention time and may suppress the contribution of fraction B to the feed ruminal digestion, resulting in lower effective degradability (eDEG). Although this dependence was observed for the 50 MYC silage subjected to an extended storage time in the second evaluation period (80 to 110 days), there were no significant losses in dry matter and, consequently, starch degradability after 12 hours of incubation compared to the control silage ($P = 0.77$), possibly due to the proximity between the degradation rates of both silages. Also, there was only a tendency in lower degradability for high passage rates at 5%/h and 8%/h ($P = 0.08$), which are more likely to have occurred in lambs fed a low roughage diet with high proportion of fine particles. Despite the proximity in k_d, the less effective degradability with k_p = 2%/h of the additive-treated silage compared to the 50 CON silage (P = 0.04) suggests that, with sufficiently high retention time, there may be a slight loss in the extension of the ruminal degradation of corn.

The larger indigestible fraction (C) of the 50 MYC silage in relation to the other treatments (P = 0.02) seems to be in agreement with that reported by Vernon-Carter et al. (2018), who observed an increase in the digestion resistant starch fraction with the addition of a surfactant in the gelatinization process at 90 ºC. Although the silage in the present study did not likely experience too high temperatures, it is possible that the unavailability of starch may be linked to the formation of the aforementioned surfactant-amylose complex, which could occur under the natural temperature conditions of the silo (Ghiasi et al., 1982). However, comparing the values observed for effective degradability and potential degradability, it seems that even with a very high ruminal retention time, promoted by low passage rates, the higher indigestible fraction would not be enough to result in some loss in the extension of corn degradability of the additive-treated silage.

The treatment of rehydrated corn silage with the additive promoted greater dry matter degradability after 12 hours of incubation in the trial of Oliveira (2020), although this increase was not significant in relation to the control. In the present trial, the 50 MYC silage did not show any change in DM degradability after 12 hours of incubation compared to 50 CON silage. Oliveira (2020) suggested that the increase in DM degradability was due to the smaller particle size, larger surface area and larger percentage of fine particles of the additive-treated silage. The present study did not assess the physical score of the silages. However, as all samples were ground before being incubated, a particle size standardization may have occurred, so that any possible effect on ruminal degradation due to changes in particle size was nullified. These results suggest that improvements in ruminal dry matter and starch degradability with the addition of the additive are related to changes in particle size distribution, with greater surface area available for microbial approach.

4.3.2. Animal performance

Table 16. Performance of lambs: nutrients intake and weight gain

Item	Treatment			SEM	*P*-value[1]	
	50CON	50MYC	90CON		C_1	C_2
DMI, kg/d	1.08	1.07	1.04	0.03	0.82	0.63
ADG, kg/d	0.217	0.206	0.206	0.011	0.68	0.99
ADG/DMI	0.201	0.192	0.194	0.008	0.66	0.94
OMI, kg/d	1.02	1.01	0.99	0.03	0.94	0.61
NDFI, kg/d[2]	0.169	0.166	0.156	0.005	0.78	0.35
CPI, kg/d[3]	0.112	0.120	0.115	0.003	0.21	0.37
Dietary NEm, Mcal/kg[4]	1.31	1.31	1.31	0.03	0.93	0.94
Dietary NEg, Mcal/kg[4]	0.74	0.74	0.74	0.02	0.93	0.94

[1]Contrasts include C_1: 50 CON x 50 MYC, C_2: 50 MYC x 90 CON
[2]NDF intake
[3]Crude Protein intake
[4]Estimated using the equations of Zinn & Shen (1998)

There was no effect of the treatments on the voluntary dry matter intake (DMI) of the animals. Owens et al. (1997), in a review of the effect of grain processing on the performance of beef cattle, reported a decrease in DMI with the use of more processed corn in the diet, and several more recent works compiled by Santos et al. (2016) have reported a similar effect by substituting dry corn for high moisture ensiled corn (Ladely et al., 1995; Huck et al., 1998; Scott et al., 2003; Henrique et al., 2007; Corrigan et al., 2009; Carareto et al., 2010). This decrease may be associated with daily variations on dry matter intake due to ruminal pH fluctuations, caused by the rapid and extensive production of volatile fatty acids from the fermentation of highly available starch (Stock et al., 1995). This high availability of fermentable starch can also cause a negative feedback effect on DMI (Allen, 2000) through hepatic propionate oxidation, signaling satiety and interruption of feed intake (Allen et al., 2009). However, the greater ruminal digestibility of starch with the extended storage time of the 90 CON silage did not significantly decrease the DMI of animals compared to the other treatments. Zietsman (2008), studying different grinding size patterns of corn grain in the diet of finishing lambs, reported higher dry matter intake by animals that received the finer ground corn, suggesting that the combination of high degradability and decreased particle size may have increased the rate of passage from the rumen and thus allowing for additional feed intake. It is possible that the use of a finely ground corn, with 1 mm GMPS, on the processing of the rehydrated silages for the present trial may have influenced the ruminal passage rate in order to offset the impact of the high starch fermentability over the DMI. Ruminal escape of starch to be digested in the small intestine yields glucose instead of volatile fat acids (Huntington, 1997; Harmon, 2009), and glucose uptake by the liver is negligible, so satiety signaling from intestinal digestion of starch and glucose oxidation is unlikely (Allen et al., 2009).

Our data revealed no effect of the treatments on the daily weight gain and feed efficiency of the animals. Owens et al. (1997) reported a decrease in the weight gain of confined beef cattle with the use of high moisture corn as a substitute for dry corn, suggesting that this was due to the lower dry matter intake with the greater availability of starch in the rumen. Santos et al. (2016), compiling more recent publications, reported variable results, but with scenarios of increased feed efficiency based on the relative decrease on the DMI. In the present study, the greater starch availability from extended storage time of the 90 CON silage does not seem to have changed the rate of weight gain of lambs in comparison to the shorter storage times, which is in agreement with other studies (Huck et al., 1998; Corrigan et al., 2009; Harrelson et al., 2009) that did not observe improvements in weight gain with substituting dry ground corn for high moisture corn grain silage.

According to Vaz (2007), sheep begin a period of less muscle growth after five months of age, when the optimum carcass point is reached in lambs for slaughter. Considering that the lambs in the present study started the trial at age of five months and remained in the diet for 56 days, the animal response to the diets was probably limited (Almeida Júnior et al., 2004). The NEm and NEg values calculated for the diets were also quite similar across the treatments. Although no carcass evaluation was performed for the lambs at the end of the trial, animals at advanced age and closer to maturity are more prone to increase body fat content instead of muscle growth, so it is possible that the more available energy from the corn grain silages with extended storage times was diverted to fat deposition on the carcass of the lambs. High moisture corn grain silage also may promote greater fat deposition on the carcass of feedlot animals, since the starch is more digested in the rumen and its escape to the intestine is decreased (Almeida Júnior et al., 2004; Harrelson et al., 2009; Owens et al., 1986).

The results for ingestive behavior (Table 17) suggest preferential intake for fine particles and high refusal for particles larger than 8 mm with the offer of a high concentrate diet based on rehydrated corn silage. Feedlot animals usually ingest a large amount of concentrate to meet their requirements (Carvalho et al., 2008), and sheep have an improved and a quite particular capacity for feed selection (Van Soest, 1994), based on sensory characteristics and on a long time of visual assessment before ingestion (Aguiar et al., 2015). It is known that a diet with adequate and uniform moisture reduces selection of ingredients (Leonardi & Armentano, 2003; Leonardi et al., 2005) and that dairy cattle show preference for wetter feed in the diet (Arcari, 2013), as also demonstrated by Kargar & Kanani (2019) in calves. The pattern observed in the present trial suggests that the lambs had preference for the fraction with greater presence of rehydrated corn, a moist ingredient with high palatability, while showing greater refusal for medium and long particles mainly composed of sugarcane bagasse.

Table 17. Ingestive behavior of lambs

Item	Treatment			SEM	*P*-value[1]	
	50CON	50MYC	90CON		C_1	C_2
DMI, kg/d	1.08	1.07	1.04	0.03	0.82	0.63
NDFI, kg/d[2]	0.169	0.166	0.156	0.005	0.78	0.35
Particle sorting, % as fed						
> 19 mm	81.4	85.8	84.7	2.69	0.48	0.86
8-19 mm	73.8	69.9	68.1	1.59	0.29	0.62
4-8 mm	99.2	99.3	94.6	0.51	0.90	<0.01
< 4 mm	106.3	105.6	106.4	0.43	0.47	0.40

[1]Contrasts includes C_1: 50 CON x 50 MYC, C_2: 50 MYC x 90 CON
[2]NDF intake

There was a significant treatment effect on the selection of particles retained in the 4-mm sieve, so the animals that received the diet containing 90 CON silage consumed less particles of this fraction in comparison to the animals fed the 50 MYC diet. However, there is no reasonable explanation for this behavior. Besides, these selection differences did not cause changes in nutrients intake, especially NDF, between treatments.

The use of the additive in rehydrated corn silage did not change the selective behavior of the lambs. It is known that rehydrated corn silages, as well as silages in general, starts a process of aerobic deterioration from the moment it is exposed to oxygen (Dawson et al., 1998), being highly perishable in the feed bunk, which makes animals lose interest for the food throughout the day. The use of propionic acid-based additives helps to control yeast and mold populations and to improve the aerobic stability of high-moisture feed, such as silages

(Woolford, 1975; Kung Jr. et al., 2004; Oliveira, 2020; Morais, 2016), which may help to maintain the nutritional quality of the food in the feed bunk for longer time. It is possible that, by controlling yeast and mold growth during ensiling, the sensory quality of the feed may be also preserved, enhancing its palatability in comparison to untreated silages, stimulating its preferential intake by the animal. However, the lambs in the present trial did not show any sign of preferential intake when fed the treated RCGS. Stallings et al. (1979), evaluating a forage silage treated with propionic acid fed to ruminants, observed greater intake of treated forage by the cows, while sheep also did not change ingestive behavior.

4.4. Conclusions

The increase in storage time between 50 and 150 days improved the nutritional value and ruminal degradability of reconstituted corn grain silage. The additive did not enhance the proteolysis of the grains in the silo, and seems to have caused a gradual delay in the solubilization of the protein matrix, with a significant decrease in the ruminal soluble fraction after 80 days of storage, but without harming dry matter ruminal degradability. Mycoflake™ showed potential as a preservative additive for rehydrated corn grain silage, possibly due to the presence of surfactant agents. The additive did not change the performance or selection pattern of the lambs.

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5. GENERAL CONCLUSIONS AND CONSIDERATIONS

The increase in digestibility of corn grains when submitted to rehydration and ensiling is well known in the literature, being the processing strategy that yields the greater ruminal degradable fraction of starch and protein (Ferraretto et al., 2013; Owens et al., 1986; Harmon, 2009; Owens & Soderlund, 2006). Although the silages manage to reach stability for conservation 20-25 days after ensiling, chemical changes continue to occur throughout the storage period, and a minimum ensiling time of two months has been reported and recommended in the literature in order to achieve the major gains in protein matrix degradation, starch availability and dry matter degradability for rehydrated corn grain silages (Gervásio, 2021; Fernandes et al., 2021; Da Silva et al., 2019). Our objective in this study was to verify the possibility of an early opening of the silos with only 30 days, through an additive with surfactant property. For this to be justified, the state of protein degradation and starch availability commonly reached after 60 days of storage must be reproduced in this shorter time.

In our study, Mycoflake™ did not show any potential to enhance proteolysis when applied to rehydrated corn grain silage. The presence of ammonium salts in its composition, a common strategy adopted by companies for buffering additives based on propionic acid, "deceives" the indicators of silage proteolysis, especially NH_3-N. This may even mask a probable scenario of partial inhibition of protein matrix degradation, since, although the surfactant favors the interaction of water with the highly hydrophobic structure of the prolamins, the moisture retention effect (Mahungu & Artz, 2001) could hinder the exhaust flow of solubilization products, creating a stagnant microenvironment over the protein matrix, delaying its proteolysis. Characterizing silages treated with additives containing nitrogen compounds before ensiling is a necessary practice to obtain true indicators of proteolysis.

Nonionic surfactants are known in the food industry literature as forming indigestible complexes with starch, forming a film over the granule surface, which can difficult the adhesion of ruminal bacteria and its degradation. This effect appeared in corn ensiled with Mycoflake™ after 30 days in our study, decreasing the ruminal degradation rate, but disappeared when ensiling was extended beyond 60 days. The indigestible portion of starch (<0.8% in our study) due to complexation also appears to be relatively negligible compared to the extent of total starch digestibility of high moisture ensiled grains in the ruminant tract (96.0 and 99.3% for dairy and beef cattle, respectively, according to Owens & Soderlund, 2006).

Since it was developed for use in flocculation, the propionic acid concentration of Mycoflake™ is much lower than the normally found in additives intended for conservation purposes in silages. However, both Oliveira (2020) and our study revealed the potential of Mycoflake™ as a preservative additive for rehydrated corn grain silage, possibly due to the presence of surfactant agents, so its application at a rate of 0.2% in silage fresh matter resulted in lower dry matter losses, decreased unwanted fermentation products such as ethanol and butyric acid, and a silage with better visual aspect without a spoiled top layer. Microbiological trials with this additive are still necessary to determine the real extent of control of fungi, yeasts and bacteria, including lactic acid bacteria.

Mycoflake™ has also shown a consistent and positive effect in yielding rehydrated grain silages with greater exposed area due to the smaller mean particle size, which seems to favor ruminal dry matter degradability (Oliveira, 2020). So far, the most likely hypothesis would be the action of the surfactant in preventing the aggregation of particles, a recurrent phenomenon in silage of rehydrated grains over the storage time (Gomes et al., 2020; Oliveira, 2020). Trials involving recovery of particles in sieves are necessary to investigate this effect in grain silage treated with the additive.

Thus, the use of Mycoflake™ did not justify the early opening of rehydrated corn grain silos after only 30 days of ensiling. However, Mycoflake™ has generated promising results in maintaining the physical and nutritional quality of these silages, so despite being developed for use in flocculation, it also has potential as an additive for rehydrated grain silage.

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