UNIVERSIDADE FEDERAL DE VIÇOSA

Strategies to enhance corn utilization in dairy cows

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Doctor Scientiae

VIÇOSA - MINAS GERAIS
2025

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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2025

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T

M386s
2025

Martins, Bernardo Magalhães, 1983-
Strategies to enhance corn utilization in dairy cows / Bernardo Magalhães Martins. – Viçosa, MG, 2025.
1 tese eletrônica (141 f.): il.

Texto em inglês.
Orientador: Polyana Pizzi Rotta.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Zootecnia, 2025.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2025.297
Modo de acesso: World Wide Web.

1. Bovinos de leite - Alimentação e rações. 2. Milho - Silagem. 3. Digestibilidade. 4. Rações - Aditivos. I. Rotta, Polyana Pizzi, 1987-. II. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 22. ed. 636.2142

Bibliotecário(a) responsável: Bruna Silva CRB-6/2552

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APPROVED: February 17, 2025.

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À minha mãe, Romina Moreira de Magalhães Gomes, que sempre me demonstrou,
por meio de seu exemplo, que continuar os estudos é sempre o melhor investimento.

In memoriam.
Ao meu avô materno,
Vasco de Magalhães Gomes, que sempre me incentivou ao estudo e me ensinou a
apreciar a Zootecnia.

ACKNOWLEDGMENTS

À Universidade Federal de Viçosa pela oportunidade de cursar toda pós-graduação, do mestrado ao doutorado, de forma simultânea com meu trabalho como servidor.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES).

À minha orientadora Profa. Polyana Pizzi Rotta pela oportunidade, orientação e paciência.

Ao Prof. Alex Lopes da Silva, pela valiosa ajuda com a tese, pela compreensão e incentivo enquanto coordenador da UEPE-Gado de Leite, onde atuo e pude conciliar as atividades profissionais com o curso, o que possibilitou a conclusão do doutorado.

Ao Prof. Marcos Inácio Marcondes, por todo o apoio durante o curso de doutorado e por ter aberto as portas da pós-graduação para mim, uma vez que foi meu orientador no mestrado.

Ao Prof. Edenio Detmann, pela valiosa assistência e coorientação ao longo do curso, além de ser uma referência em ciência animal de excelência e rigor.

Ao Prof. Ricardo Augusto Mendonça Vieira pela valiosa contribuição para o artigo.

Ao Prof. Luiz Felipe Ferraretto por toda ajuda com os artigos.

À excepcional equipe que, por meio de sua valiosa colaboração nas atividades de campo e laboratório durante os experimentos, possibilitou a conclusão deste curso: Carina Bittencourt, Dayanne Lima, João Vítor Coelho, Thaís Soares, Luís Henrique Silva, Luciano Santos, Poliana Salgado, Rayce Ferreira, Sophia Cattleya Donde e Wellington Paulo.

Ao meu filho Leonardo e minha esposa Lisandra pelo companheirismo, incentivo e paciência.

À minha mãe por todo apoio e incentivo.

This work has been sponsored by the following Brazilian research agencies: Coordination for the Improvement of Higher Education Personnel (CAPES; Financing code 001), Minas Gerais State Foundation for Research Aid (FAPEMIG) and National Council of Scientific and Technological Development (CNPq).

“Every year is getting shorter
Never seem to find the time.
Plans that either come to naught
Or half a page of scribbled lines
Hanging on in quiet desperation is the English way
The time has gone, the song is over,
Thought I'd something more to say.”
(Time, Pink Floyd)

ABSTRACT

MARTINS, Bernardo Magalhães, D.Sc., Universidade Federal de Viçosa, February, 2025. **Strategies to enhance corn utilization in dairy cows**. Adviser: Polyana Pizzi Rotta. Co-advisers: Alex Lopes da Silva, Luciana Navajas Renno, Edenio Detmann and Marcos Inacio Marcondes.

Corn grain is the primary energy source in dairy cow diets, but its digestibility is influenced by processing and preservation methods. The main processing methods include grinding, rolling, and steam flaking, with the latter being a thermal treatment. Ensiling also affects starch digestibility, with the impact dependent on fermentation duration. Additionally, the maturity stage at harvest influences starch availability, which is higher in grains harvested at greater moisture due to reduced complexation with zein proteins. To ensure silage quality, ensiling high-moisture corn or rehydrating dry grains requires the use of additives and/or inoculants. Given this context, this study aimed to evaluate, in three stages, the effects of processing and ensiling duration on dry matter intake (DMI), ruminal fermentation, and productive performance in dairy cows. In the first stage, three treatments were compared: ensiled rolled high-moisture corn, finely ground dry corn, and ensiled rehydrated corn. Higher DMI was observed for high-moisture corn compared to dry ground corn, with rehydrated corn showing intermediate values. Ruminal digestibility was similar among treatments, as was total tract digestibility, except for protein digestibility, which was higher for ensiled treatments compared to dry ground corn. Ruminal volatile fatty acid (VFA) proportions did not differ among treatments. However, ruminal ammonia tended to be lower for dry ground corn. Regarding production parameters, a trend was observed for greater milk yield and energy-corrected milk (ECM) in cows fed high-moisture corn compared to dry ground corn. Fat yield was also higher for high-moisture corn than for dry corn. Urea excretion was greater for ensiled treatments compared to dry ground corn. Among blood metabolites, only non-esterified fatty acids (NEFA) differed, with higher values for ensiled treatments than for dry ground corn. The results indicated similarities among treatments for most response variables, with a slight advantage for ensiled rolled high-moisture corn in terms of productive performance. In the second stage, the effects of ensiling duration and the addition of a propionic acid-based chemical additive on silage quality and ruminal degradability were investigated. Ensiling periods of 180 days or longer improved digestibility and silage quality parameters. Furthermore, the propionic acid-based additive at 0.25% of dry matter (DM) did not provide additional benefits compared to the 0.5% DM dosage.

Conversely, increasing the additive dosage reduced ruminal degradability of high-moisture corn. Finally, in the third stage, a meta-regression was conducted using literature data on different corn processing methods and their effects on dairy cows. The results indicated that dry ground corn, compared to other processing methods, may impair ruminal fermentation and starch digestibility, leading to lower productive performance. However, particle size effects on these parameters should be considered before partially or completely replacing this type of corn with alternatives. In contrast, ensiled high-moisture corn reduced DMI and negatively affected ruminal pH but did not compromise milk yield or total solids synthesis, suggesting the need for dietary formulation adjustments to mitigate its impacts on ruminal health and potential production issues. The findings of this study enhance the understanding of the effects of corn processing and ensiling on dairy cow performance.

Keywords: Additive; Corn grain; Dairy cows; Degradability; Digestibility; Ensiling; Meta-regression; Production

RESUMO

MARTINS, Bernardo Magalhães, D.Sc., Universidade Federal de Viçosa, fevereiro de 2025. **Estratégias para melhorar a utilização do milho em vacas leiteiras**. Orientadora: Polyana Pizzi Rotta. Coorientadores: Alex Lopes da Silva, Luciana Navajas Renno, Edenio Detmann e Marcos Inacio Marcondes.

O milho grão é a principal fonte energética nas dietas de vacas leiteiras, mas sua digestibilidade é influenciada pelo processamento e método de conservação. Os principais processamentos incluem moagem, laminação e floculação, sendo esta última um método térmico. A ensilagem também afeta a digestibilidade do amido, com impacto dependente do tempo de fermentação. Além disso, o estádio de maturação na colheita influencia a disponibilidade de amido, sendo maior em grãos colhidos com maior umidade devido à menor complexação com as proteínas zeínas. Para garantir a qualidade da silagem, a ensilagem do milho úmido ou a reidratação de grãos secos requerem o uso de aditivos e/ou inoculantes. Diante disso, este estudo teve como objetivo avaliar, em três etapas, os efeitos do processamento e do tempo de ensilagem sobre o consumo de matéria seca, a fermentação ruminal e o desempenho produtivo de vacas leiteiras. Na primeira etapa, foram comparados três tratamentos: milho úmido laminado ensilado, milho seco moído finamente e milho reidratado ensilado. Foi observado maior consumo de matéria seca para milho úmido em relação a milho seco moído, com reidratado ficando com valores intermediários. As digestibilidades ruminais foram similares entre os tratamentos, assim como as digestibilidades do trato total, com exceção da digestibilidade da proteína, que foi maior para os tratamentos ensilados comparado ao milho seco e moído. As proporções dos ácidos graxos voláteis ruminais foram similares entre os tratamentos. Já para amônia ruminal houve uma tendência de menor concentração para o milho seco moído. Relativo aos quesitos de produção, tendência foi observada para maiores produções de leite e de leite corrigido para energia para o milho úmido em relação ao milho seco moído. Foi também observada maior produção de gordura para o tratamento de milho úmido em relação ao milho seco. A excreção de ureia foi maior para os tratamentos ensilados comparativamente ao milho seco moído. Com relação aos metabolitos sanguíneos, o único a apresentar diferença foi o NEFA, maiores valores para os tratamentos ensilados comparado ao milho seco moído. Os resultados indicaram semelhança entre os tratamentos para as principais variáveis de resposta, com uma leve vantagem para o milho úmido laminado ensilado em termos de desempenho produtivo. Na segunda etapa, investigou-se o efeito do tempo de ensilagem e da adição de um aditivo químico à base de ácido propiônico sobre a qualidade da silagem e a degradabilidade ruminal. Observou-se que períodos de ensilagem iguais ou superiores a 180 dias melhoraram a digestibilidade e os parâmetros de qualidade da silagem. Além disso, a dose do aditivo a base de ácido propiônico na dosagem de 0.25% da matéria seca não apresentou benefícios adicionais quando comparada à dose de 0.5% da matéria seca. Pelo contrário, o aumento da dose do aditivo reduziu a degradabilidade ruminal do milho úmido. Por fim, na terceira etapa, foi conduzida uma meta-regressão com dados da literatura sobre diferentes tipos de processamento do milho e seus efeitos sobre vacas leiteiras. Os resultados indicaram que o milho seco moído, em comparação a outros métodos de processamento, pode comprometer a fermentação ruminal e a digestibilidade do amido, resultando em menor desempenho produtivo, contudo é importante considerar o efeito de tamanho de partícula sobre esses parâmetros antes da substituição parcial ou completa desse tipo de milho por outro. Em contrapartida, o milho úmido ensilado reduziu o consumo de matéria seca e afetou negativamente o pH ruminal, mas não prejudicou a produção de leite e a síntese de sólidos totais, o que sugere a necessidade de ajustes na formulação da dieta para mitigar seus impactos sobre a saúde ruminal, e possíveis problemas na produção. Os achados deste estudo contribuem para a compreensão dos efeitos do processamento e da ensilagem do milho sobre o desempenho de vacas leiteiras.

Palavras-chave: Aditivo; Degradabilidade ; Digestibilidade ; Ensilagem; Meta-regressão; Milho grão; Produção; Vacas leiteiras

GENERAL INTRODUCTION

Corn Grain and Starch: Nutritional Implications for Dairy Cow Feeding

The corn kernel is a primary energy source in ruminant diets and is protected by a pericarp layer, composed primarily of hemicellulose (50-67%), cellulose (23-40%), lignin (0.1-1.3%), and protein (~2.4%) (Santiago-Ramos *et al.*, 2018). In its whole form, the kernel is highly resistant to enzymatic and microbial degradation (Kang *et al*., 2021). The endosperm comprises the bulk of the kernel, approximately 83%, and consists of two main types: vitreous and floury endosperm (Holmes *et al*., 2019). The relative proportions of these endosperm types vary according to plant genetics and maturity stage (Allen and Ying, 2021). Corn kernels are typically classified by the vitreousness of their endosperm. Hard kernels maintain a high proportion of vitreous endosperm throughout development, while floury kernels consist predominantly of floury endosperm. Dent corn kernels, derived from the floury class, exhibit a variable balance of vitreous and floury endosperm, representing an intermediate texture between these two classes. The endosperm texture is determined by the interaction between starch granules and the protein matrix, as well as the nature of this interaction (Kljak *et al*., 2018).

Starch granules vary in size and shape and consist primarily of two polymers: amylose and amylopectin. Amylose is a linear polymer of α-1,4-linked glucose units with occasional α-1,6 linkages, while amylopectin is branched, containing glucose units linked by both α-1,4 and α-1,6 bonds (Wang, 2021). Amylopectin is the predominant component in corn grain, comprising approximately 75% of the starch, with amylose making up the remaining 25% (Yu and Moon, 2022). Sucrose is the primary carbohydrate form produced by photosynthesis and is transported through the plant's phloem to reach the developing maize kernel. Upon arrival, sucrose must be broken down into fructose and glucose to enter the cytosol. Within the cytosol, these sugars are reassembled into sucrose, which is subsequently hydrolyzed by sucrose synthase into fructose and UDP-glucose. These products are then converted to ADP-glucose through the action of ADP-glucose pyrophosphorylase. In the amyloplast, ADP-glucose molecules are polymerized into either amylose or amylopectin. Amylose synthesis requires the action of a single enzyme (wx1), whereas amylopectin synthesis involves a coordinated action of multiple enzymes (ae1 and su1). Enzyme manipulation has enabled the development of hybrids with a predominance of either amylose or amylopectin for specific industrial applications (Yu and Moon, 2022). Waxy hybrids, which contain nearly 100% amylopectin, typically yield 5% less than non-modified hybrids; the reasons for this yield reduction are not yet fully understood (Gao et al., 2020). Studies on the use of these hybrids in dairy cattle have shown inconsistent results: some studies report advantages (Akay and Jackson, 2001), usually when compared with dent hybrids, while others find no differences compared to dent or BMR hybrids (Schroeder *et al.*, 1997; Barlow *et al.*, 2012).

The prolamins of corn kernels, known as zeins, are the primary storage proteins in these grains, comprising approximately 50 to 60% of total kernel proteins and characterized by a high proline content, which makes them highly hydrophobic (Hoffman and Shaver, 2011). Zeins are classified into four subclasses based on their molecular weight: α-zeins (19-22 kDa), β-zeins (14 kDa), γ-zeins (15-27 kDa), and δ-zein (10 kDa) (Holmes *et al.*, 2019). Initially, zeins are located on the outer layer of starch granules, but as the kernel matures, β-zeins and γ-zeins form covalent bonds, after which α-zeins and δ-zeins integrate into this network, encapsulating the starch within a hydrophobic protein matrix (Hoffman *et al.*, 2011). The vitreous endosperm contains higher concentrations of zeins and amylose than the floury endosperm, as well as smaller, less spherical starch granules (Kljak *et al.*, 2018). Additionally, the degree of starch granule encapsulation by zeins is greater in the vitreous endosperm (Hoffman and Shaver, 2011). Ensiling facilitates the breakdown of this protein matrix primarily through microbial activity, enhancing the action of ruminal microbial enzymes and potentially increasing digestibility (Hoffman *et al*., 2011).

Starch granules may also be associated with lipids, which can bind to the granule surface or internally, with a greater affinity for amylose due to its ability to form hydrogen bonds with hydrophobic compounds (Cervantes-Ramirez et al., 2020). Studies indicate that lipids can insert into the cavity of the amylose helix, either naturally or through heating and cooling processes commonly used in industrial feed production (Wang et al., 2020). The extent of amylose complexes with lipids varies widely, with reports indicating that 15 to over 55% of amylose may form such complexes (Giuberti et al., 2014). This type of binding increases the density of starch and may reduce its digestibility (Giuberti et al., 2014).

Starch can also undergo gelatinization, a process in which it becomes viscous under specific conditions involving water and a defined temperature, which varies by starch source. During gelatinization, the breakdown of intermolecular hydrogen bonds allows water molecules to penetrate between starch molecules, enhancing the accessibility of starch to digestive enzymes (Donmez *et al*., 2021). Upon cooling, starch molecules can reassociate and expel water, transforming the viscous gel into a solid—a process known as retrogradation (Donmez *et al*., 2021).

Processing of Corn Grains for Use in Dairy Cow Diets

Mechanical processing methods for corn grains are categorized into thermal and non-thermal techniques. Steam-flaked, which is the most common thermal method, involves crushing grains with rollers, raising the temperature, and adding moisture for a specified period to promote starch gelatinization, thereby enhancing digestibility by facilitating enzymatic and microbial action (Kang *et al*., 2021). Non-thermal methods, such as grinding and rolling, breaks the pericarp and can improve starch utilization by ruminants (Kang *et al*., 2021).

Most studies examining corn grain processing methods for dairy cows focus on using steam-flaked corn as a substitute for dry ground corn. This method requires specialized equipment and is more costly than other techniques, such as high-moisture corn silage or dry ground and rehydrated dry corn. Steam-flaked corn is more common in the United States and less frequently used in Brazil. Study results on parameters such as dry matter intake, starch intake, forage inclusion, milk yield, and milk solids production have varied significantly (Rafiee and Darabighane, 2021). To clarify these responses, a recent meta-analysis was conducted, in which Rafiee and Darabighane (2021) found no difference in dry matter intake when steam-flaked corn replaced ground dry corn, consistent with findings from a previous meta-analysis by Ferraretto *et al*. (2013). The meta-analysis also reported an increase in starch digestibility and a trend towards improved dry matter digestibility, although no effect was observed in fiber digestibility. Regarding milk production, a trend toward increased yield was identified for treatments that incorporated steam-flaked corn. Additionally, reductions in milk fat content and increases in milk protein content were noted. For ruminal fermentation parameters, no significant differences were found in volatile fatty acid concentrations between treatments using steam-flaked corn and dry ground corn, while values observed in the ruminal pH were similar. In comparison to ground corn, the average density of 350 g/L in steam-flaked corn is cited as a factor in maintaining its pH stability, along with its larger particle size and highly effective neutral detergent fiber content. The decrease in the ruminal ammonia concentration was attributed to improved nitrogen utilization efficiency facilitated by the use of steam-flaked corn.

Ahmadi *et al*. (2020) evaluated ground corn with various particle sizes (2, 3, and 4 mm) and steam-flaked corn on dairy cow performance, ruminal parameters, nutrient digestibility, and selection index. The authors reported no differences in the dry matter intake, milk yield, energy-corrected milk yield, or milk solids. The observed ruminal pH was however higher in the steam-flaked corn treatment, and volatile fatty acid concentrations in the rumen were lower, indicating a shift in the site of digestion from the rumen to the intestine. The flake density of 400 g/L used in this study may have notably contributed to this ruminal “protection” effect. Other similar studies involving flakes with densities lower than 400 g/L have reported improved digestibility, particularly for dry matter and starch, an optimum feed gain ratio, and enhanced milk production (Cooke *et al*., 2009; Savari *et al*., 2018; Martins *et al*., 2019).

The use of ensiled high-moisture corn, whether ground or cracked, presents a veritable alternative, as it allows for earlier clearing of the planting area, facilitating more efficient land availability and utilization for other uses. Additionally, the widely used method of harvesting at maturity stage enhances starch digestibility, yet studies on high-moisture corn are scarce in the literature compared to those on steam-flaked corn usage. In a meta-analysis by Ferraretto *et al*. (2013) comparing dry ground, cracked, and flaked corn categorized into five different particle sizes, high-moisture corn was classified into two particle size categories, and corn was also processed as steam-flaked or steam-rolled. No differences were observed among the three corn processing methods in terms of ruminal starch digestibility, neutral detergent fiber digestibility, or total tract digestibility of dry matter and neutral detergent fiber. The total tract starch digestibility, however, exhibited the highest digestibility in high-moisture corn, followed by steam-flaked and steam-rolled corn, with dry corn showing the lowest digestibility from the report. High-moisture corn was associated with lower dry matter intake, while the other two groups showed similar intakes; no differences in milk production were noted. Within each corn type, particle size influenced digestibility in dry ground corn, with smaller particle sizes enhancing dry matter and starch digestibility, although no effect was found on dry matter intake or milk production.

A meta-analysis by Torres *et al*. (2021) evaluated the performance of dairy cows fed ground dry corn or high-moisture corn silage. The authors found that dry matter intake was affected by the inclusion level of high-moisture corn silage. When more than 30% of dry ground corn was replaced by high-moisture corn silage, the dry matter intake decreased while milk production remained unaffected, indicating improved efficiency as cows consumed less feed while maintaining milk yield. This improvement in efficiency was attributed to enhanced digestibility and energy availability. Torres *et al.* (2021) also reported increased total tract digestibility of dry matter and crude protein, though not for starch. They attributed this to the floury endosperm profile of the corn used in the study, suggesting that vitreous corn might have amplified the benefits of high-moisture corn.

In an interesting study by Allen and Ying (2021a), corn endosperm types (floury and vitreous) and processing methods (dry ground or high-moisture corn) were compared by evaluating the performance and digestive parameters in dairy cows. It was observed that regardless of the preservation method (ground or high moisture), no differences were found in the dry matter intake, milk yield or milk solids. The total tract dry matter digestibility was, however, higher for high-moisture corn. Starch digestibility was influenced by both the endosperm type and preservation method, with high-moisture corn showing higher digestibility than dry ground corn and floury endosperm exhibiting greater digestibility than vitreous, as expected. In this study, the percentage of starch digested relative to total starch consumed was consistently higher for floury than vitreous endosperm in the rumen and higher for high-moisture corn than dry ground corn. Approximately 82% of starch in high-moisture corn was digested in the rumen, whereas dry ground corn showed a lower ruminal starch digestibility (~60.6%). For post-ruminal starch digestion, corn with vitreous endosperm exhibited greater digestion in the intestine than floury endosperm, regardless of preservation method, suggesting a compensatory mechanism for the lower ruminal digestion associated with vitreous endosperm. This study noted significant differences in total tract digestibility, with higher values in high-moisture corn compared to dry ground corn and for floury endosperm compared to vitreous within the dry ground corn treatment. However, for high-moisture corn, the two endosperm types showed similar total tract digestibility.

Research on rehydrated ground corn is scarce compared to other corn processing forms, likely because it is more commonly used in Brazil. Arcari *et al*. (2016) observed an increased starch digestibility with higher dietary inclusion levels of rehydrated corn, as well as increased dry matter intake and milk production when 100% of dry ground corn was replaced by rehydrated and ensiled corn (90 days), in their study. Another study by Castro *et al.* (2019) evaluated rehydrated ground corn ensiled for 247 days, testing two starch levels (29.1 vs. 23.5%) and two particle sizes (1.58 vs. 2.18 mm) on dairy cow performance and digestive parameters. Ensiling coarsely ground vitreous corn (2.18 mm) for over 200 days nullified any effects of particle size on cow performance, digestion, or feeding behavior. The authors emphasized that coarser grinding requires less electrical energy and labor without affecting cow performance; however, the prolonged storage period necessary to achieve these effects may limit practical feasibility.

Gomide *et al*. (2023) assessed finely ground corn (1.26 mm) and coarsely ground corn (1.65 mm) that were rehydrated and ensiled for 40 days and also compared these treatments to dry ground corn (0.36 mm) in a grazing system. Coarse rehydrated corn reduced total tract dry matter and starch digestibility but did not affect milk yield or milk solids like in other treatments. Finely ground rehydrated corn demonstrated greater feed efficiency than coarse rehydrated or dry ground corn. Gomes *et al*. (2020) explored various silo opening durations (30 to 180 days) with different particle sizes and moisture levels of rehydrated corn. They reported increased ruminal degradability over time, with higher moisture and smaller particle sizes enhancing degradability relative to dry corn with lower moisture content. Fernandes *et al*. (2021) evaluated ensiling durations (7, 21, 60, and 120 days) in silos with rehydrated corn, using both vitreous and floury hybrids. They concluded that proteolysis positively affected digestibility, recommending at least 60 days of ensiling to enhance digestibility, especially for vitreous corn. In a rare comparative study on high-moisture and rehydrated corn, Silva *et al*. (2019) examined in situ ruminal degradability across silo opening durations (15 to 300 days) with incubation times of 12 or 24 hours. They recommended a minimum of 52 days for rehydrated corn and 71 days for high-moisture corn to maximize endosperm protein matrix degradation and improve digestibility, considering particle size differences (1.68 mm for rehydrated and 4.27 mm for high moisture corn).

Rehydrated, ensiled ground corn may enhance starch digestibility through prolamin degradation during the ensiling process, potentially improving animal performance. However, further research is needed, as current findings do not consistently demonstrate an advantage over dry ground corn for dairy cow performance. Dry ground corn remains more commonly used, as it does not require silo structures or associated labor costs.

Ensiled rehydrated corn offers potential advantages over high-moisture corn, particularly when high-moisture harvests face logistical challenges, such as rain. This approach also allows for strategic grain procurement and subsequent rehydration and ensiling. However, there is a lack of research comparing these processing methods side-by-side to determine whether one method offers superior performance, digestibility, or health benefits in dairy cows.

The literature on these processing methods presents limited and sometimes contradictory findings. Furthermore, optimal ensiling duration for improved digestibility and performance outcomes remains unclear. Evidence suggests that very short ensiling durations offer no significant benefits, yet studies investigating opening duration effects are scarce.

Use of Inoculants and additives in Corn Grain Silage

Ensiling corn grains is a particularly significant practice, especially in Brazilian conditions where flint corn predominates. This process promotes proteolysis, breaking down the protein matrix of the endosperm and enhancing starch digestibility (Diogénes *et al*., 2023). Ensiling is an anaerobic process that relies on lactic acid bacteria to metabolize water-soluble carbohydrates, producing organic acids, primarily lactic acid, which lowers the pH and inhibit undesirable microorganisms (Torres *et al*., 2021).

Adhering to fundamental principles is crucial for successful ensiling. These include ensuring appropriate moisture content, compacting the silage to remove oxygen, and properly sealing the silo. These steps aim to achieve a rapid pH drop, minimizing the proliferation of spoilage organisms (Gervásio *et al*., 2023). However, challenges such as reduced aerobic stability and high microbial activity during the ensiling process can result in dry matter losses. In this context, additives play a critical role in maintaining silage quality (Torres *et al*., 2021).

Additives are categorized into different types such as microbial (Inoculants), chemical, enzymatic, or combined (Muck *et al*., 2018). For corn grain silage, microbial and chemical additives, as well as their combinations, are most used. Microbial inoculants can be classified as homolactic or heterolactic. Homolactic bacteria produce high levels of lactic acid, effectively lowering pH but with limited antifungal activity. Conversely, heterolactic bacteria produce weaker acids, such as acetic and propionic acids, which exhibit strong antifungal properties, thereby improving aerobic stability. Combining these bacteria within a single product leverages their complementary effects, thereby enhancing silage quality (Morais *et al*., 2017; Muck *et al*., 2018).

A meta-analysis by Torres *et al*. (2021) evaluating additives for high-moisture corn (HMC) generally found no significant positive effects on silage quality. However, Lactobacillus buchneri stood out, producing the lowest pH and highest concentrations of acetic and propionic acids among the treatments. This inoculant reduced fungal counts, likely due to the antifungal properties of acetic and propionic acids.

A review by Diogénes *et al.* (2023) emphasized the benefits of fermentation-promoting additives, particularly Lactobacillus buchneri, in rehydrated corn grain silage. Among 15 studies conducted, 13 done in Brazil underscored the importance of ensiling processes, driven by the predominance of Flint-type corn and the pursuit of higher-quality nutritional materials.

In another study, microbial additives (*Lactobacillus plantarum, Lactobacillus brucei, and Lactococcus lactis*) improved the fermentation quality of high-moisture ear corn silage at moisture levels of 42.5% and 47.5%. These moisture levels yielded higher concentrations of lactic and acetic acids, resulting in lower pH values and improved silage quality (Li *et al.*, 2023).

Chemical additives, classified as acids or salts, also play an essential role in silage making. Common acids such as formic, propionic, sorbic, benzoic, and acetic acids, which act by either lowering the pH or inhibiting fungal growth in the silage, results in enhanced aerobic stability (Muck *et al.*, 2018). Salts, such as sodium benzoate and ammonium propionate, also release their respective acids in the silage, further improving its stability. The efficacy of these additives, however, depends on proper application rates (Muck *et al*., 2018).

Oliveira *et al*. (2023) evaluated the effects of propionic acid and polysorbate 80 in rehydrated corn grain silage. Dry matter losses were reduced in all treatments observed, the combination of additives also improved nutrient availability but failed to enhance aerobic stability, likely due to dosage issues. A related study by Silva *et al*. (2023) reported similar findings, noting the additive's influence on silage particle size and feed selectivity without affecting dairy cow performance.

Chemical additives containing sodium benzoate, potassium sorbate, and sodium nitrite significantly reduced yeast counts in high-moisture corn silage, improving dry matter recovery and aerobic stability (Da Silva *et al*., 2015). A review by Morais *et al*. (2017) highlighted the positive effects of chemical additives in reducing dry matter losses and enhancing silage stability, though variations in dosage across studies contributed to inconsistent results.

Daniel *et al*. (2022) highlighted the susceptibility of high-moisture and rehydrated corn grain silage to aerobic deterioration due to low antifungal fermentation products. They emphasized that appropriate dosages of sorbate, benzoate, and propionate salts enhance silage aerobic stability. Future studies on dose-response relationships of inoculants and their effects on silage quality, dry matter losses, and animal performance are needed to optimize silage management.

This comprehensive understanding of microbial inoculants and chemical additives underscores their critical role in improving the nutritional quality, stability, and efficiency of corn grain silage in dairy production systems.

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CHAPTER 1

This chapter is written according to the standards of the Journal of Dairy Science

Interpretative summary: **Impacts of reconstituted corn grain silage or high-moisture corn grain silage as replacers for ground grain when using a flint corn hybrid for lactating cows.** By Martins et al. This study compared dry ground corn, reconstituted corn silage, and high moisture corn silage in diets for dairy cows. High moisture corn increased milk yield and feed intake compared with dry ground corn. Both ensiled treatments improved milk fat percentage, while digestibility and feed efficiency remained similar across treatments. Despite additional preparation and storage costs, ensiled corn offered slight performance benefits. High moisture corn showed the most promising results, also contributing to improved land-use efficiency. These findings highlight the importance of evaluating processing methods when formulating diets to optimize milk production and resource utilization in dairy systems.

Running Head: Corn processing for dairy cows

Impacts of reconstituted corn silage or high-moisture corn silage as replacers for ground grain when using a flint corn hybrid for lactating cows

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ABSTRACT

This study aimed to assess the impact of replacing dried ground corn (**DGC**) by reconstituted corn silage (**RCC**) or high moisture corn silage (**HMC**) on nutrient intake and digestibility, milk yield and composition, rumen fermentation profile, and blood parameters of dairy cows. A total of nine Holstein dairy cows, including six rumen-cannulated and three non-cannulated, with an average BW of 639.1 ± 15.07 kg, milk production of 30.4 ± 1.58, parity of 2 ± 0.5, and DIM of 99.7 ± 10.70 were blocked by milk yield and DIM. Subsequently, they were randomly assigned to a treatment sequence following a replicated 3 × 3 Latin squares design. Three following treatments were evaluated in this study: 1) a control diet with concentrate based on DGC; 2) a diet with replacement of DGC with RCC; and 3) a diet with replacement of DGC for HMC. All treatments utilized the same flint corn hybrid. Dried ground corn and RCC were ground through a 3 mm sieve; RCC and HMC were ensiled for 258 d. The starch in diet was 22%, approximately. Results showed that DM, CP, NDF, starch, and ether extract intakes were greater for HMC compared to DGC. The total digestibility of DM, NDF, and starch did not differ among treatments. However, the total digestibility of CP was greater for RCC and HMC than DGC. The ruminal flow of DM was greater for both RCC and HMC. No effect was observed on the ruminal digestibility of DM, NDF, and starch among treatments. No differences were observed in milk yield, whether corrected for energy or not, among treatments. Regarding milk solids, a higher fat content was observed for RCC and HMC compared to DGC. The feed efficiency (milk/DM intake) was similar among treatments. Similarly, the percentage values of the main volatile fatty acids were similar among treatments. The concentration of non-esterified fatty acids was greater for both RCC and HMC than for DGC. The results of this study suggest caution when selecting the type of corn processing, as the outcomes for DGC and the ensiled treatments, which involve additional costs for preparation and storage, were very similar. A slight advantage was observed for HMC in some productive parameters.

Key Words: corn processing, flint, performance, starch

INTRODUCTION

Corn grain serves as a primary energy source in dairy cow diets, largely due to its high energy content. The endosperm, which comprises about 83% of the grain, is rich in starch and can be either vitreous (hard) or floury (soft). The proportion of each type of endosperm varies depending of factors like hybrid genetics and the maturity stage of the grain (Singh et al., 2014). Corn starch is undoubtedly a vital energy source for milk production, as ruminal degradation and intestinal digestion of starch provide glucose precursors for lactose synthesis in the mammary gland (Allen and Piantoni, 2014).

Processing methods like grinding, rolling, and ensiling may significantly impact on the rate and site at digestion, thereby affecting the efficiency of energy utilization from starch (Nunes et al., 2020). Ensiling triggers proteolytic activity of bacteria and kernel enzymes that help break down the protein matrix within the corn grain endosperm, potentially enhancing starch digestibility (Junges et al., 2017; Torres et al., 2021). This effect may be achieved by ensiling either high-moisture corn (**HMC**) grain or dry reconstituted corn grain (**RCC**), both of which may be viable options.

In certain agricultural regions, particularly in specific tropical areas, the window for harvesting HMC is narrow and often coincides with periods of intense rainfall (Ferraretto et al., 2018). In such cases, RCC silage is an intriguing alternative (Castro et al., 2019). However, it is important to note that RCC silage may exhibit lower ruminal and total-tract starch digestibility compared to HMC silage due to the vitreousness of the corn, which increases with the advancement of the physiological maturity of the grains, causing an increased prolamin content in endosperm (Ferraretto et al., 2013). Ensiling of HMC involves harvesting the grain at an earlier stage, when it is more immature and has lower prolamin content.

Few studies in the literature have addressed factors such as processing type, corn grain ensiling, dairy cow performance, and ruminal dynamics in a single study. Allen et al. (2021) evaluated all these parameters by comparing dry ground corn (**DGC**) to HMC. Some studies have compared HMC to steam-flaked corn, assessing animal performance and some digestive parameters (Eun et al., 2014; Tye et al., 2017). More commonly, several other studies have evaluated HMC versus DGC (Knowlton et al., 1998; Krause et al., 2002a, b; Oba and Allen, 2003a, b). However, no study has assessed digestive and performance characteristics using the same flint corn hybrid while simultaneously comparing DGC, RCC, and HMC.

Thus, we aimed to evaluate the effects of replacing DGC with either HMC or RCC silage on nutrient intake and digestibility, milk yield and composition, microbial protein synthesis, ruminal fermentation profile, and blood characteristics of lactating dairy cows. We hypothesized that HMC will have higher digestibility compared to DGC and RCC, resulting in improved dairy cow performance.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of Animal Use in Brazil (protocol number 60/2020) and was carried out at the Teaching, Research, and Extension Unit in Dairy Cattle of the Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

Animals and Management

Nine Holstein dairy cows, comprising six rumen-cannulated and three non-cannulated individuals, with an initial average BW of 639.1 ± 15.07 kg, milk production of 30.4. ± 1.58, parity of 2 ± 0.5, and DIM of 99.7 ± 10.70 were blocked by milk yield and DIM. The animals were organized in a replicated 3 × 3 Latin square design, with two squares formed by rumen-canulated animals. The cows were housed in individual free stalls with 12.7 m² per cow, with free access to fresh water, and were fed experimental TMR three times a day at 0700, 1500, and 2100 h, allowing 100 g/kg in refusals (as fed). Daily records of the weight of feed offered and refusals were maintained for all cows. Milking occurred three times a day at 0630, 1430, and 2030 h, with the cows brought to the sprinkler room 30 min before milking for a cooling process.

Treatments

Three treatments were evaluated: 1) control diet with concentrate based on DGC; 2) replacement of DGC for RCC, and 3) replacement of DGC for HMC. The remaining components of the diet were corn silage, Tifton hay, soybean meal, cottonseed whole, sodium bicarbonate, magnesium oxide, urea, and mineral and vitamin premix (Table 1). Diets were formulated to meet specifications of 17.0% CP, 22.0% starch, and 4.6% ether extract, targeting a milk yield of 32 kg/d according to NASEM (2021) guidelines. The particle distribution of the experimental diets, analyzed using the Penn State Particle Separator, is presented in Table 1. The experiment consisted of three experimental periods, each lasting 27 d, with 14 d for the animals to adapt to the treatments (Machado et al., 2016). The animals were weighed at the end of each experimental period to calculate the average BW and relative voluntary intake.

Corn Grain Processing

The same corn hybrid was used for all treatments (hybrid LG 36799; Limagrain, Goianesia, Goias, Brazil), which presented a vitreousness of 80%, measured using the method described by Dombrink-Kurtzman and Bietz (1993). Corn for the experiment was planted during off-season, targeting a DM content of approximately 60% for high-moisture silage. However, for the RCC treatment, the corn was harvested dry (89% DM) and then the reconstitution process to restore moisture was carried out. The grains utilized for HMC were processed using a 350 S2 roller mill (Murska®, Ylivieska, Finland) powered by a tractor and subsequently ensiled in 500 L capacity polyethylene drums, capable of storing approximately 500 kg of HMC. The roller mill had a capacity of 5 ton/h for wet grains, requiring 30-40 HP of energy. To prevent the development of bacteria and fungi, a propionic acid-based additive (90.5%; Lupro-Grain®, BASF S.A., São Paulo, Brazil), was utilized at a dose of 5 L/ton. The average density of the silos was 1,100 kg/m^3.

Dry corn grain of the same hybrid (hybrid LG 36799; Limagrain, Goianesia, Goias, Brazil) was utilized for RCC and DGC treatments, with the detailed nutrient composition presented in Table 2. The grain with a moisture of 11% was ground with a hammer mill (3-mm screen) for DGC and RCC. For the treatment of RCC, water was added to corn ground to 3 mm to achieve a moisture level of approximately 40% before ensiling. The same propionic acid-based additive (90.5%; Lupro- Grain®, BASF S.A., São Paulo, Brazil) was used to prevent the development of bacteria and fungi, applied at a dose of 5 L/ton. The silos for RCC also had a density of 1,100 kg/m^3. Both HMC and RCC were stored for 258 d before opening. The geometric mean particle size (**GMPS**) analysis (Table 2) was performed for corn samples prior to ensiling, both for corn ground to 3 mm and for rolled corn, following the methodology described by Baker and Herman (2002).

Sampling

Between day 15 and day 17 of each experimental period, milk yield was recorded at all milking sessions. During these days, milk samples (350 mL) were also collected from each cow at each of the three daily milkings, using an electronic milking flow meter (GEA Westfalia Surge, GEA Farm Technologies of Brazil, Jaguariuna, São Paulo, Brazil). These samples were subsequently analyzed for fat, protein, and lactose content using a Lactoscan S LP ultrasonic milk analyzer (Milkotronic LTD, Nova Zagora, Bulgaria). Parameters were analyzed separately for each milking session, and data were then aggregated according to milk yield.

Voluntary intake by cow was measured from day 15 to day 17 of each experimental period, with both feeds offered and refusals weighed to quantify intake. Each day, representative samples of forages, concentrate ingredients, RCC, HMC, and TMR were collected and stored at -20°C until analysis. Throughout the experiment, three batches of concentrate mixes were prepared for each treatment, and individual concentrate ingredient samples were collected with each batch. Concentrate samples were taken at the feed mill and stored at -20°C until analysis. All feed samples were oven-dried at 55°C, then ground through a 2-mm sieve, a particle size required for uNDF determination. Half of this 2-mm ground sample was subsequently ground through a 1-mm sieve using a Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA) and this sample was used for all other analyses.

Fecal samples were collected from the rectum of each cow for three consecutive days, from day 18 to day 20 of each period, directly from the animal's rectum, following these different times throughout the 3 collection days: 0000, 0300, 0600, 0900, 1200, 1500, 1800, and 2100 h. Fecal pH was measured immediately using a pHmeter (Tecnal Tec-3MP, Piracicaba, São Paulo, Brazil) as described by Sulzberger et al. (2016). Subsequently, samples were oven-dried (55°C) and ground, as previously described.

Urine spot samples were obtained from three consecutive days, on day 18 to day 20 of each experimental period, following different times throughout the 3 collection days: 0000, 0300, 0600, 0900, 1200, 1500, 1800, and 2100 h. Urine samples were collected from the animals by stimulating excretion through vulva massage. These samples were then stored in a freezer at -20°C until analysis. At the end of each collection day, spot samples were pooled per animal (equal volumes for each collection time) and analyzed for urea, creatinine, uric acid, and allantoin concentrations.

Ruminal digesta outflow was assessed through omasal digesta sampling. Rumen-canulated cows received 6 g/d of Co-EDTA divided into four ruminal infusions at 0000, 0600, 1200, and 1800 h from day 17 to day 23 of each period. The schedule used sampling at 9-h intervals (Allen and Linton, 2007) to represent every 3 h of a 24-h period to account for diurnal variation. Sampling was on day 21 at 0600, 1500, and 0000 h; day 22 at 0900 and 1800 h; and day 23 at 0300, 1200, and 2100 h. Approximately 800 mL of omasal digesta was filtered through a 100-μm nylon filter (Sefar Nitex 100/44; Sefar, Thal, Switzerland) to separate the particle and fluid phases. Samples from each phase were freeze-dried, ground as previously described, pooled by animal, and analyzed independently to estimate omasal digesta flow. Omasal digesta flow was estimated using the digestion technique developed by Faichney (1975), employing a dual-marker system with Co-EDTA as the liquid phase marker and uNDF as the particle phase marker, measured at distinct stages of digestion. Sample collection and compositing per animal followed the methodology described by Rotta et al. (2014).

Prior to the omasal digesta sampling, samples of rumen fluid were taken to assess rumen fermentation pattern. Samples were taken in the liquid-solid interface of the rumen mat and filtered through a nylon filter (100 µm, Sefar Nitex 100/44; Sefar, Thal, Switzerland) and evaluated for pH using a digital potentiometer. Then, a 20-mL aliquot of ruminal fluid was combined with 5 mL of a metaphosphoric acid solution (250 g/L) and frozen (-20◦C) for subsequent analysis of VFA. Another 40-mL aliquot was combined with 1 mL of a H_2SO_4 solution (9 M) and frozen (-20◦C) for later analysis of rumen ammonia nitrogen concentration. A composite sample was made for each cow in each experimental period, for both VFA's and ammonia analysis.

The total rumen evacuation procedure was conducted on day 24 and day 26 to estimate rates of passage and digestion (Allen and Linton, 2007). On day 24, 4 h after feeding and on day 26, before feeding, to represent maximum and minimum ruminal content, all rumen contents were removed and filtered through a double layer of cheesecloth for separation into solid and liquid fractions. These fractions were collected in 60 L plastic barrels weighed and sampled (approximately 2.5 kg). Afterward, the remaining content was immediately remixed and replaced in the rumen. The samples were then oven-dried and ground as previously described.

Blood samples were collected from all cows at day 27 through tail vessels approximately 4 h after feeding. Coagulation activator tubes with serum separating gel (BD Vacutainer®, Becton, Dickinson and Company, Franklin Lakes, NJ) were used for Cholesterol, IGF-1, non-esterified fatty acids (**NEFA**), and urea analysis. Tubes with clot activator and sodium fluoride (BD Vacutainer® Fluorinated/EDTA, São Paulo, Brazil) were employed to quantify plasma glucose concentration.

Laboratory Analysis

Samples of feed, ruminal digesta, omasal digesta, and feces underwent analysis for DM (method G-001/2 and method G-003/2), ash (method M-001/3), CP (method N-001/3, N × 6.25, Kjeldahl method), NDF (method F-002/3), and ether extract (method G-005/2) following the procedures described by Detmann et al. (2025). Starch analysis was conducted using the acetate buffer method as described in Hall (2009). Samples of feed, feces, ruminal, and omasal digesta, processed to pass through a 2-mm screen sieve, were analyzed for uNDF content using non-woven textile (100 g/m^2) and a 288-h *in situ* incubation procedure (Valente et al., 2011). Omasal digesta samples (liquid and solid phases) were evaluated for cobalt concentration by inductively coupled plasma optical emission spectroscopy (ICP-OES; PerkinElmer, Shelton, CT) after nitric-perchloric digestion of the samples (Palma et al., 2015).

Concentration of uric acid (automatic biochemical analyzer; autoanalyzer, model BS200E, Mark Mindray; Model BS200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China) and allantoin in urine was determined using the methods described by Chen and Gomes (1992). Volatile fatty analysis was performed using HPLC chromatography (Shimadzu LC-20AT, Kyoto, Japan) following the techniques outlined by Siegfried et al. (1984). Ammonia was analyzed through Chaney and Marbach (1962) method. Urea, cholesterol, and glucose were analyzed using the BS-380 Mindray equipment (Shenzhen, Guangdong, China), employing the urease UV method for urea, the enzymatic method for glucose, and the enzymatic colorimetric method for cholesterol. For NEFA analysis, the AU680 (Beckman Coulter Analyzer, Brea, CA) was used. The IGF-1 analysis was performed on the Immulite Analyzer (Siemens, Erlangen, Germany) using the chemiluminescence method.

Calculations

Ruminal rates were calculated according to Oba and Allen (2003a), as follows:

- Turnover rate in the rumen, %/h = [(intake of component, kg/d) / (ruminal pool of component, kg)] / 24 × 100
- Passage rate from the rumen, %/h = [(omasal flow of component, kg/d) / (ruminal pool of component, kg)] / 24 × 100
- Digestion rate in the rumen, %/h = (turnover rate in the rumen, %/h) – (passage rate from the rumen, %/h)

Where, the nutrient intake was calculated as the difference between the amount offered and the refusals. The rumen pool was first estimated by averaging the rumen volumes measured over 2 days (d24 and d26) of rumen evacuation. Rates for potentially digestible NDF (pdNDF) were calculated based on the difference between NDF intake and undegradable NDF (uNDF) intake, as well as the difference between the NDF pool and the uNDF pool. Omasal flow was calculated as described by Rotta et al. (2014) using the double marker system, with an adapted method for determining starch concentration in the liquid phase. Due to insufficient sample volume for analysis in the liquid phase, total starch concentration from the single-phase sample was used instead. The calculation procedure for microbial synthesis estimation followed the method described by Chen et al. (2004).

The milk production was correct for energy through the following equation: 0.25 × kg milk + 12.2 × kg fat + 7.7 × kg crude protein (Sjaunja et al., 1990).

Particle size distribution of the diet was conducted through the Penn State Particle Separator (Lammers et al., 1996) utilizing 500 g of samples of the diets and refusals taken at d 25 of each experimental period. The GMPS was analyzed prior to the ensiling process, for the HMC and RCC treatments. The number of particles and surface area of DGC, RCC, and HMC were calculated as described in Baker and Herman (2002), using a Ro-Tap shaker with sieves of the following meshes: 4.76, 3.36, 2.38, 1.68, 1.19, 0.71, and 0.297 mm with approximately 50 g of dry sample stirred for 10 min.

Statistical Analysis

Data was submitted to analysis of variance using the function lmer of lme4 package of R (R Core Team, 2023), according to the following model:

$$Y_{ijklm} \quad = \mu + T_i + LS_j + (T \times LS)_{ij} + A_{(j)k} + P_{(j)l} + \varepsilon_{ijklm}$$

where: Y_{ijklm} = dependent variable; μ = overall constant; T_i = fixed effect of treatment; LS_j = random effect of Latin square; $T \times LS_{ij}$ = random effect of interaction between treatment and Latin square (this effect was not significant for all variables and was removed from the model); $A_{(j)k}$ = random effect of animal within Latin square; $P_{(j)l}$ = random effect of period within Latin square and ε_{ijklm} = random error.

Variables measured over time were incorporated as repeated measures in the model, and the most appropriate covariance matrix was selected based on the lowest Akaike Information Criterion with correction. The sequentially rejective Bonferroni *t*-test adjustment was employed to compare treatment means after an overall significant *F*-test with differences considered significant at $P < 0.05$, and trends at $P \leq 0.10$

RESULTS

Intake and Digestibility

The DM, OM, CP, and starch intakes differed among treatments, with greater values for HMC compared to DGC ($P < 0.05$; Table 3). The RCC presented intermediate positions between DGC and HMC in general.

Neutral detergent fiber intake was greater ($P = 0.04$) for HMC compared to the other treatments. Ether extract intake was greater ($P = 0.04$) for RCC and HMC than for DGC.

Ruminal digestibility of DM, NDF, and starch were similar ($P > 0.10$) among treatments (Table 4). This pattern was also observed for total tract apparent digestibility. However, total tract apparent digestibility of CP was greater ($P < 0.01$) for RCC and HMC than for DGC.

In terms of ruminal rates (Table 4), in general there was no difference ($P > 0.05$) among treatments for ruminal turnover rate, ruminal passage rate, or ruminal digestion rate. Except for the pdNDF digestion rate, which tended to be greater in RCC than in DGC ($P = 0.07$).

Rumen pH and VFA

An interaction between time and treatment was observed for rumen pH ($P < 0.01$; Figure 1). Rumen pH reached its lowest levels in the early morning hours, followed by an increase that peaked shortly before the morning feeding. Thereafter, ensiled treatments exhibited greater pH fluctuations throughout the day compared with DGC, which maintained higher pH values between daytime feedings but converged to similar levels by the end of the day, approximately 3 h after the afternoon feeding. Notably, pH values around feeding times were higher for the ensiled treatments compared with DGC. A treatment × time interaction was observed for fecal pH ($P < 0.01$; Figure 2), with the DGC treatment showing consistently lower values than RCC and HMC throughout the day. Fecal pH followed a pattern like ruminal pH, reaching a minimum value a few hours after feeding, followed by a gradual increase until the next feeding time.

The levels of NH3-N present a trend ($P < 0.10$) among treatments, in which the DGC presented a lower value in relation to the ensiled treatments, which presented similar values (Table 5). In general, corn processing methods did not affect rumen VFA concentration ($P > 0.05$, Table 5), except for isovalerate ($P = 0.07$), which tended to be higher for HMC compared to DGC; however, RCC presented intermediate values.

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Milk Yield and Composition

A trend for greater milk yield in HMC compared with DGC was observed ($P < 0.10$, Table 6), as well as a trend for higher ECM production in HMC than in DGC ($P < 0.05$). For milk yield and ECM, RCC presented intermediate values. Milk fat and total solids concentrations, and fat yield were greater for HMC than for DGC ($P < 0.05$) with RCC not differing among them. The percentage of total solids in milk tended to be higher for HMC compared to DGC. Protein and lactose contents did not differ among treatments ($P > 0.05$). The treatments did not differ in terms of feed efficiency ($P = 0.60$).

Protein Metabolism and Blood Metabolites

The RCC and HMC treatments showed higher urinary urea excretion values compared with DGC ($P < 0.01$; Table 7). Microbial protein production tended to be greater in RCC than in DGC ($P = 0.07$). Similarly, microbial efficiency tended to be higher in RCC compared to DGC ($P = 0.05$), with HMC exhibiting no difference among them in both cases. For blood metabolites, only NEFA differed among treatments ($P = 0.03$), with higher values for both RCC and HMC compared with DGC (Table 8).

DISCUSSION

This study evaluated the effects of DGC, RCC, and HMC treatments on intake, digestive processes, performance, and blood metabolites in dairy cows. The objective was to assess how the same flint corn hybrid, processed using three different methods influences these response variables. A trend toward increased milk and ECM yield was observed in cows fed HMC compared to those fed DGC. This suggests that the higher intake associated with HMC may have contributed to greater energy availability compared to the DGC treatment, thus allowing this trend towards greater production.

The ruminal digestibilities of DM, NDF, and starch did not differ, contrary to what might be expected given the prolonged ensiling process to which the RCC and HMC treatments were subjected. The DGC and RCC were finely ground, whereas HMC had larger particle sizes. Castro et al. (2019) reported no differences in total tract DM, NDF, or starch digestibilities in diets with 23.5% starch when comparing finely and coarsely ground RCC ensiled for 247 d, suggesting that prolonged ensiling neutralized particle size effects. Conversely, Gomide et al. (2023) found that coarsely ground RCC ensiled for 40 d reduced total tract starch digestibility, indicating that ensiling duration influences digestibility in relation to particle size. Thus, the observed similarity in digestibility between RCC and HMC in the present study may be attributed to the extended ensiling duration (258 d), which likely mitigated the potential effects of particle size differences. Notably, the reduced GMPS in DGC contributed to similar digestibility compared with HMC. A meta-analysis organized by Ferraretto et al. (2013) found similar ruminal digestibility among DGC, steam-flaked corn, and HMC, despite expectations for greater digestibility in corn ensiled for extended periods with lower DM. The very fine particle size in DGC partially contributed to the comparable digestibility responses observed in this study.

Given the similar digestibility values between treatments, as previously highlighted the higher DMI observed for HMC may have been insufficient to produce significant differences in milk production among treatments, although a trend to higher milk yield and ECM for HMC was observed. Among the milk solids, milk fat yield and milk fat percentage were the only parameters that differed among treatments, being higher for HMC compared with DGC. Considering the same NEL intake among treatments, the higher blood NEFA observed for HMC compared with DGC may help explain this difference. The elevated NEFA concentration in HMC suggests greater mobilization of body reserves compared with DGC. In HMC, it is possible that an insulin peak was reached more quickly, followed by a rapid decrease, allowing NEFA concentration to rise sooner (Allen, 2023). Pulsatile insulin secretion clears NEFA from the blood, increasing feed consumption (Allen et al., 2009). Freitas et al. (2020), studying feedlot cattle fed either whole shelled corn or cracked corn, observed a similar effect, with NEFA being the only blood metabolite altered, showing an increase with cracked corn. The concentrations of the main ruminal VFA did not differ among treatments, supporting the hypothesis that elevated plasma NEFA was the primary factor contributing to the increased milk fat observed in HMC. This finding is particularly relevant, as acetate—a primary precursor for milk fat—showed no difference among treatments. In this case, the production pathway responsible for the difference in milk fat production possibly did not involve *de novo* synthesis but rather the pathway of long-chain fatty acids (C16–C22) derived from circulating NEFA (Tian et al., 2022).

Cows fed all three treatments were able to maintain pH values above 5.8 for most of the measured time points in this study, on one occasion among the eight time points of pH measurement throughout the day, the pH in DGC dropped below 5.8, whereas in RCC, it fell below this threshold twice, and in HMC, once likely to indicate no occurrence of subacute ruminal acidosis. This finding aligns with expected outcomes based on the diets' low-moderate starch levels, adequate forage fiber inclusion, and use of buffers (sodium bicarbonate and magnesium oxide), as confirmed by the milk fat concentration. The DGC treatment exhibited less pH fluctuation throughout the day compared with RCC and HMC, possibly due to the higher fermentability of RCC and HMC, which caused more rapid and pronounced pH declines, showing well-defined patterns of pH drop and recovery (Oba and Allen, 2003b). This reduced fluctuation in DGC may benefit ruminal health, emphasizing that pH stability, alongside average pH values, is a key indicator of ruminal health (Krause et al., 2002).

Given the lower pH fluctuation in DGC relative to RCC and HMC, this processing method may be more advantageous for ruminal health in diets with higher starch concentration or limited in forage fiber and buffering capacity. Reflecting dietary starch, fiber, and buffering concentrations, fecal pH values ranged from 6.5 to 7.5 among treatments, suggesting no hindgut acidosis (Palladino et al., 2022).

Microbial protein production and efficiency showed a trend to be higher in HMC compared with DGC. The RCC showed the trend in greater microbial protein and microbial efficiency compared to DGC, despite not showing significance, possibly due to the experimental sample size. All these findings are consistent with the higher CP digestibility observed in HMC compared with DGC. Additionally, urea excretion was significantly higher in the ensiled treatments, aligning with the previously presented results. This suggests that although protein availability was higher in this treatment, the increased excretion indicates that the animals' requirements were met under the study conditions, with surplus protein being excreted.

CONCLUSIONS

The results of this study suggest that caution is required when selecting the method of corn processing, as the evaluated parameters were generally similar among treatments when using a diet with 22% starch and high-vitreous corn. Although factors such as prolonged storage time and high moisture content could potentially benefit the ensiled treatments, they did not result in significant differences in parameters such as ruminal starch digestibility. High moisture corn silage led to greater DMI, a trend for higher milk yield, and an increased milk fat content. In addition, the possibility of earlier harvest and, consequently, improved land use makes high moisture corn silage an interesting alternative. However, given the similarity observed in several digestive and productive parameters, dry ground corn, which does not require ensiling or the associated costs of this process and storage, should be considered in feasibility assessments when selecting decision.

NOTES

We acknowledge Brazilian foundations for help: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, DF, Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, DF, Brasília) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, MG, Brazil). The study was approved by the Ethics Committee for Animal Welfare and Use of Universidade Federal de Viçosa under the number 60/2020. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: DGC = dried ground corn; GMPS = geometric mean particle size; HMC = high moisture corn silage; NEFA = non-esterified fatty acids; RCC = reconstituted corn silage

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Table 1. Ingredients and composition of diets with different corn processing methods and particle size analysis.

	DGC[1]	RCC[2]	HMC[3]
Ingredients (% DM basis)			
Corn silage	51.2	51.2	51.2
Dry ground corn	16.0	0.0	0.0
Rehydrated ground corn	0.0	16.0	0.0
High moisture corn	0.0	0.0	16.0
Soybean meal	14.0	14.0	14.0
Whole cottonseeds	8.2	8.2	8.2
Tifton hay	5.7	5.7	5.7
Minerals and vitamins premix[4]	3.09	3.09	3.09
Sodium bicarbonate	0.90	0.90	0.90
Magnesium oxide	0.45	0.45	0.45
Urea	0.43	0.43	0.43
Diet composition (% DM)			
Dry matter	47.1	45.0	44.0
Crude protein	17.5	17.8	17.7
Rumen degraded protein	11.2	11.4	11.4
Rumen undegraded protein	6.3	6.4	6.3
Starch	22.2	22.2	21.7
Neutral detergent fiber	32.5	31.8	31.5
Neutral detergent $\text{fiber}_{\text{forage}}$	25.5	25.5	25.5
Ether extract	4.3	4.6	4.4
NEL (Mcal/kg)	1.82	1.84	1.84
Particle distribution (%)[5]			
19 mm	6.53	5.32	5.26
8 mm	42.7	41.0	47.0
Pan	50.7	53.7	48.2

[1] DGC = dry ground corn.

[2] RCC = reconstituted corn silage.

[3] HMC = high moisture corn silage.

[4] calcium: 180 (g/kg), phosphorus: 40 (g/kg), magnesium: 25 (g/kg), sodium: 75 (g/kg), sulfur: 20 (g/kg), copper: 594 (mg/kg), zinc: 3.000 (mg/kg), manganese: 2.475 (mg/kg), selenium: 16.5 (mg/kg), cobalt: 45 (mg/kg), iodine: 34.10 (mg/kg), Vitamin A: 300.000 (UI), Vitamin D3: 90.000 (UI), Vitamin E: 1.440 (mg/kg), Biotin: 50 (mg/kg), Monensin: 600 (mg/kg).

[5] Feed basis

Table 2. Composition of corn treatments used in the experiment, particle size distribution, geometric mean particle size, particle per gram, and surface area.

Items (% DM basis)	DGC[1]	RCC[2]	HMC[3]
Dry matter	89.0	58.4	49.5
Organic matter	98.1	98.7	99.0
Crude protein	8.42	9.20	8.61
Starch	70.5	70.6	68.9
Neutral detergent fiber	8.32	6.81	6.66
Ether extract	5.02	4.41	4.69
% retained on screen[4]			
4760 µm	0.92	0.92	23.5
3360 µm	0.92	0.92	16.0
2380 µm	0.98	0.98	19.6
1680 µm	2.58	2.58	22.5
1190 µm	5.01	5.01	7.90
710 µm	29.9	29.9	7.59
297 µm	48.5	48.5	2.34
Pan	11.2	11.2	0.52
GMPS[5] (µm)	615	615	2,665
Surface area[5] (cm^2/g)	37	37	19

[1] DGC = dry ground corn.

[2] RCC = reconstituted corn silage.

[3] HMC = high moisture corn silage.

[4] The analysis was conducted prior to the ensiling process.

[5] GMPS = geometric mean particle size. Kansas State University: MF-2051 (Baker and Herrman, 2002).

Table 3. Dry matter and nutrient intake (kg/d on DM basis) in dairy cows fed with different corn processing methods.

Items (kg/d)	DGC[1]	RCC[2]	HMC[3]	SEM	*P*-value
Dry matter	19.1^{b}	19.8^{ab}	21.1^{a}	1.43	0.03
Organic matter	17.3^{b}	17.8^{ab}	18.9^{a}	1.29	0.04
Crude protein	3.34^{b}	3.42^{ab}	3.63^{a}	0.229	<0.01
Neutral detergent fiber	6.23^{b}	6.24^{b}	6.73^{a}	0.482	0.04
Starch	5.37^{b}	4.96^{ab}	5.52^{a}	0.260	<0.01
Ether extract	0.76^{b}	0.91^{a}	0.93^{a}	0.074	0.04

[ab] Means in a row with differing superscripts differ at $P < 0.05$ by the Bonferroni test.

[1] DGC = dry ground corn.

[2] RCC = reconstituted corn silage.

[3] HMC = high moisture corn silage.

Table 4. Ruminal digestibility, total tract apparent digestibility, ruminal flow, and ruminal rates in dairy cows fed different corn processing methods.

	DGC[1]	RCC[2]	HMC[3]	SEM	*P*-value
Ruminal digestibility (% of total)					
Dry matter	53.4	51.0	48.6	3.72	0.21
Neutral detergent fiber	48.5	48.4	48.6	2.30	0.18
Starch	81.1	77.9	78.5	2.32	0.35
Total tract apparent digestibility (%)					
Dry matter	59.3	58.1	59.1	1.63	0.48
Crude protein	68.1^{b}	71.7^{a}	73.1^{a}	1.90	<0.01
Neutral detergent fiber	48.8	49.2	49.5	0.98	0.87
Starch	97.3	97.6	97.7	0.57	0.73
Ruminal turnover rate (%/h)					
Undigestible neutral detergent fiber	2.68	3.15	2.55	0.305	0.29
pdNDF[4]	4.01	4.86	4.17	0.368	0.18
Starch	27.5	31.7	28.6	2.99	0.38
Ruminal passage rate (%/h)					
Undigestible neutral detergent fiber	4.24	5.17	3.77	0.608	0.15
pdNDF	1.82	1.72	2.18	0.247	0.42
Starch	14.9	19.8	15.6	2.42	0.24
Ruminal digestion rate (%/h)					
pdNDF	2.10^{B}	2.86^{A}	2.36^{AB}	0.249	0.07
Starch	12.6	11.9	13.0	2.84	0.48

^{ab}Means in a row with differing superscripts differ at $P < 0.05$ by the Bonferroni test.

^{AB}Means in a row with differing superscripts representing statistical tendency at $P \leq 0.10$ by the Bonferroni test.

[1] DGC = dry ground corn.

[2] RCC = reconstituted corn silage.

[3] HMC = high moisture corn silage.

[4] pdNDF = potential degradable neutral detergent fiber.

Table 5. Ruminal ammonia and volatile fatty acids concentration in ruminal samples of dairy cows fed different corn processing methods.

Item	DGC[1]	RCC[2]	HMC[3]	SEM	*P*-value
Ammonia (mg/dL)	7.59^{B}	8.85^{A}	8.84^{A}	0.630	0.09
Acetic (%)	66.9	66.5	66.1	1.22	0.61
Propionic (%)	20.8	21.1	20.7	0.74	0.74
Butyric (%)	8.51	9.40	9.55	0.730	0.22
Isovalerate (%)	2.02^{B}	2.41^{AB}	2.61^{A}	0.306	0.07
Isobutyric (%)	1.85	1.36	0.25	1.254	0.15
Valerate (%)	0.87	0.96	0.83	0.135	0.61
Acetic/propionic	3.25	3.17	3.22	0.163	0.72

^{AB}Means in a row with differing superscripts representing statistical tendency at $P \leq 0.10$ by the Bonferroni test.

[1] DGC = dry ground corn.

[2] RCC = reconstituted corn silage.

[3] HMC = high moisture corn silage.

Table 6. Milk yield and milk composition in cows fed different corn processing methods.

Items	DGC[1]	RCC[2]	HMC[3]	SEM	*P*-value
Yield (kg/d)					
Milk	28.6^{B}	29.3^{AB}	31.0^{A}	2.84	0.09
Energy corrected milk	26.5^{B}	27.9^{AB}	29.6^{A}	2.37	0.05
Fat	1.03^{b}	1.12^{ab}	1.19^{a}	0.723	0.03
Protein	0.88	0.90	0.95	0.727	0.14
Lactose	1.32	1.35	1.43	1.081	0.13
Total solids	3.23^{b}	3.37^{ab}	3.57^{a}	0.273	0.04
Milk composition (%)					
Fat	3.59^{b}	3.81^{ab}	3.86^{a}	0.232	0.04
Protein	3.06	3.08	3.07	0.044	0.75
Lactose	4.60	4.59	4.61	0.065	0.87
Total solids	11.7^{B}	12.2^{AB}	12.3^{A}	0.28	0.08
FE[4] (kg milk/kg DMI)	1.50	1.48	1.47	0.068	0.60
FE (kg ECM[5]/kg DMI)	1.35	1.47	1.43	0.068	0.45

^{ab}Means in a row with differing superscripts differ at $P < 0.05$ by the Bonferroni test.

^{AB}Means in a row with differing superscripts representing statistical tendency at $P \leq 0.10$ by the Bonferroni test.

[1] DGC = dry ground corn.

[2] RCC = reconstituted corn silage.

[3] HMC = high moisture corn silage.

[4] FE = feed efficiency.

[5] ECM = energy corrected milk.

Table 7. Urea excretion, microbial protein, microbial efficiency, RDP/RUP and MP of dairy cows fed different corn processing methods.

Item	DGC[1]	RCC[2]	HMC[3]	SEM	*P*-value
Urea excretion (g/d)	158^{b}	220^{a}	223^{a}	8.1	<0.01
Microbial protein (g/d)	$1,333^{B}$	$1,549^{A}$	$1,514^{AB}$	114	0.07
Microbial efficiency (g N/kg of DOM[4])	13.0^{B}	14.9^{A}	13.6^{AB}	0.87	0.05

^{ab}Means in a row with differing superscripts differ at $P < 0.05$ by the Bonferroni test.

^{AB}Means in a row with differing superscripts representing statistical tendency at $P \leq 0.10$ by the Bonferroni test.

[1] DGC = dry ground corn.

[2] RCC = reconstituted corn silage.

[3] HMC = high moisture corn silage.

[4] DOM = digestible organic matter.

Table 8. Blood metabolites of dairy cows fed different corn processing methods.

Item	DGC[1]	RCC[2]	HMC[3]	SEM	*P*-value
Cholesterol (mg/dL)	154	178	169	17.0	0.28
BUN[4] (mg/dL)	17.0	19.2	18.5	5.52	0.43
IGF-1 (ng/mL)	128	130	135	26.0	0.87
NEFA (mmol/L)	0.0700^{b}	0.0878^{a}	0.1056^{a}	0.01020	0.03
Glucose (mg/dL)	53.9	53.0	55.3	1.61	0.30

^{ab}Means in a row with differing superscripts differ at $P < 0.05$ by the Bonferroni test.

[1] DGC = dry ground corn.

[2] RCC = reconstituted corn silage.

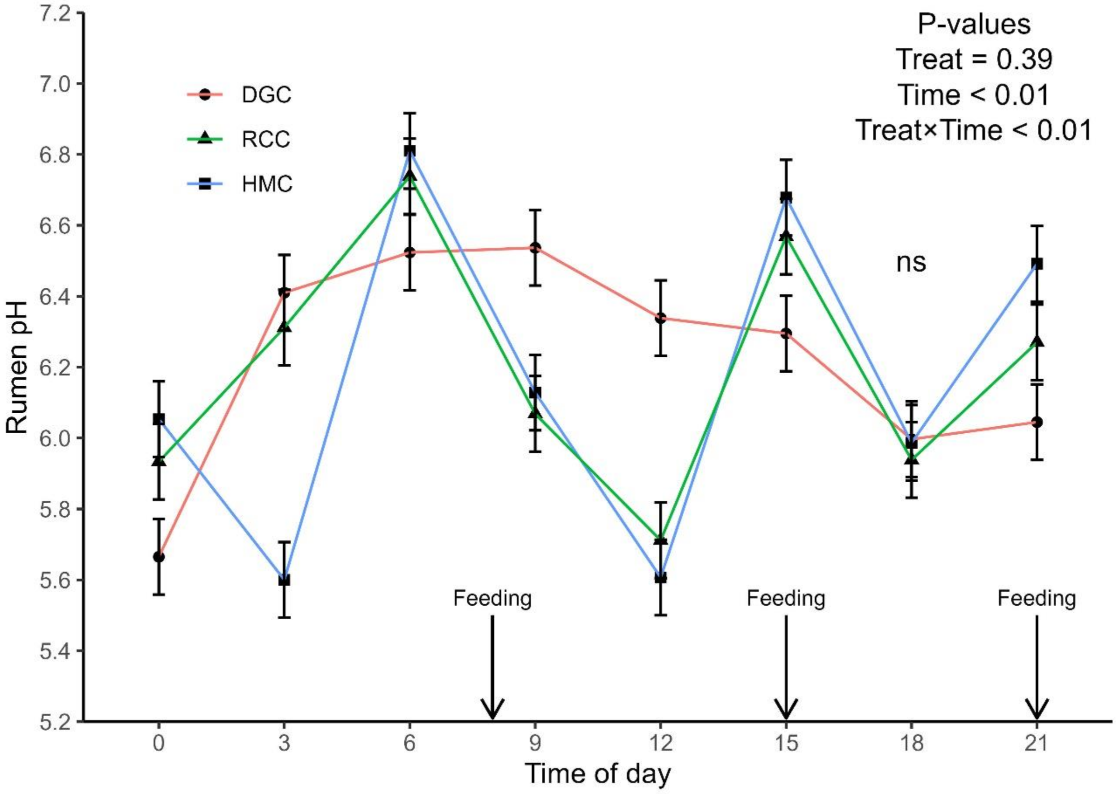
[3] HMC = high moisture corn silage.

[4] BUN = blood urea nitrogen.

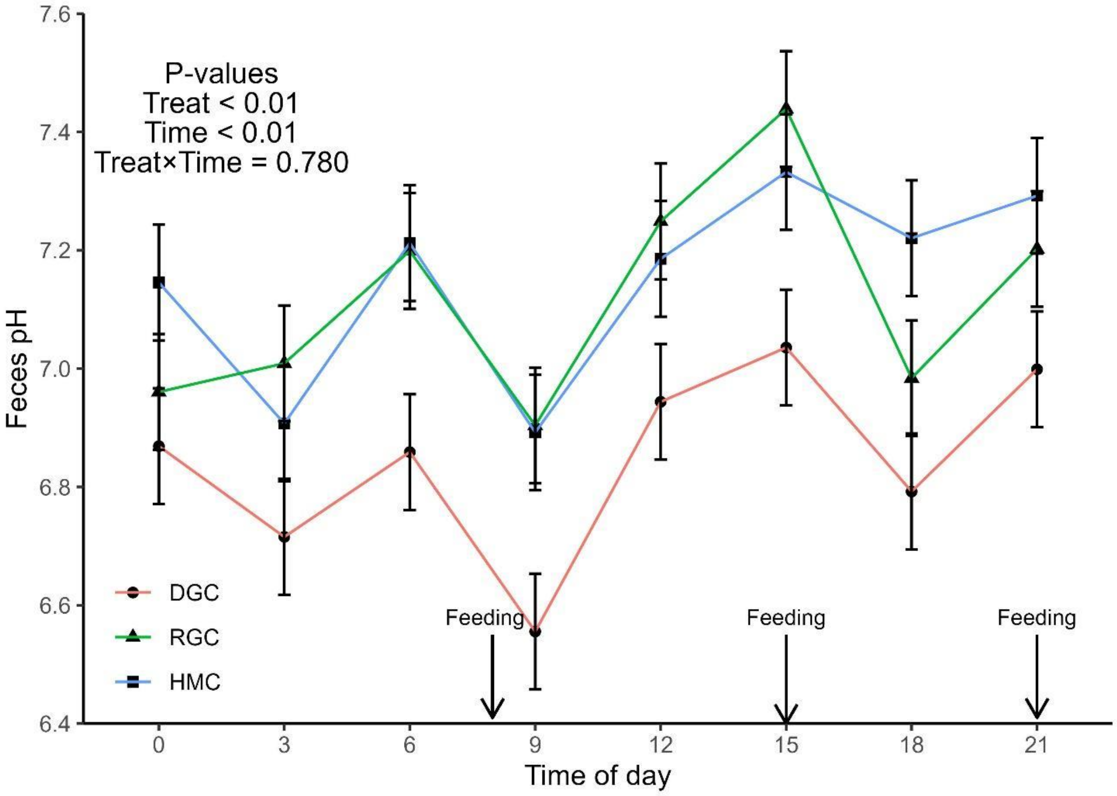
Figures

Figure 1. Ruminal pH in cows fed different corn processing methods. ns = non significative.

Figure 2. Fecal pH in cows fed different corn processing methods. ns = non significative.



Martins, Figure 1.



Martins, Figure 2.

CHAPTER 2

This chapter is written according to the standards of the Journal of Dairy Science

Interpretative summary: **Effects of propionic acid inclusion and ensiling duration on fermentation characteristics, dry matter recovery, and in situ ruminal starch degradability of high-moisture corn.** By Martins et al. This research investigated the use of propionic acid, a common silage preservative, at varying doses and storage periods on the quality and nutritional value of high-moisture corn silage used in dairy cow diets. The results indicated that adding propionic acid did not prevent nutrient losses during storage and tended to decrease how well cows could digest the starch. However, extending the fermentation time to 180 d improved the digestibility of starch, suggesting longer storage periods can enhance the nutritional quality of high-moisture corn silage.

Running Head: Storage length and propionic acid in corn

Effects of propionic acid inclusion and ensiling duration on fermentation characteristics, dry matter recovery, and in situ ruminal starch degradability of high-moisture corn

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ABSTRACT

This study aimed to evaluate the effects of propionic acid inclusion (**PAI**) at two levels (0.25 and 0.50% of fresh matter) or no inclusion (0% - control) on the fermentation characteristics, chemical composition, and in situ ruminal starch degradability of high-moisture corn (**HMC**) ensiled for 30, 60, 90, 120, 150, and 180 d. High-moisture corn was ensiled in mini silos made from 10-L polypropylene buckets, sprayed with propionic acid prior to ensiling, and compacted to achieve an average density of 1,300 kg/m^3. A completely randomized experimental design was used in a 3 × 6 factorial arrangement (three levels of propionic acid × six storage lengths). Ruminal starch degradability was assessed using nylon bags incubated in three pluriparous Holstein cows. Data analysis employed the NLMIXED procedure of SAS, with the Newton-Raphson optimization algorithm and adaptive Gaussian quadrature for likelihood integration. Contrasts were conducted to compare PAI versus control treatment and between the PAI levels. An interaction between PAI and ensiling duration was observed for DM losses. During the initial 60 d of ensiling, DM losses ranged between 5 and 6%; beyond this period, the 0.50% PAI treatment exhibited the highest DM losses, exceeding 6%. Treatments with PAI had slightly lower pH values compared to the control. Crude protein concentrations decreased, and ammonia-N levels increased progressively up to 180 d of storage. Ruminal starch degradation rates increased with extended ensiling duration, reaching the highest values in control treatment. The PAI did not effectively mitigate DM losses and negatively affected ruminal starch degradability. Extending the fermentation period of HMC to 180 d enhanced starch degradability and altered chemical composition favorably. Under the conditions of this study, the use of PAI as a silage additive for HMC did not improve fermentative characteristics or starch degradability compared with untreated control silage.

Key Words: high-moisture corn, in situ degradability, propionic acid, starch, storage length

INTRODUCTION

Corn grain-based concentrates play a critical role in dairy cattle nutrition by providing an essential source of energy, primarily through starch content, which supports increased milk production and ruminal microbial protein synthesis (Batistel et al., 2017). In tropical countries, the predominant use of flint corn, mainly characterized by a high vitreousness, presents challenges for ruminal starch digestibility, even when finely ground (Correa et al., 2002; Allen et al., 2021; Jacovaci et al., 2021), which can impair animal performance.

To improve ruminal starch degradation, a variety of processing techniques, including milling and rolling are used to increase starch exposure to rumen microbes. Additionally, ensiling ground corn grain may disrupt the protein matrix surrounding the starch granules, further enhancing its ruminal availability (Ferraretto et al., 2015). The use of HMC, whether ground or cracked, presents an interesting alternative since harvesting the high moisture material at its optimal point allows for the earlier release of the planting area for other uses, compared to harvesting at the traditional stage to produce dry ground corn (Torres et al., 2021).

Extended ensiling periods may facilitate the disruption of the hydrophobic protein matrix—particularly prolamins such as zein—thereby enhancing starch availability (Kung et al., 2018). Previous studies have shown that this effect becomes more pronounced after 240 d of ensiling (Hoffman et al., 2011; Ferraretto et al., 2014). However, maintaining a sealed silo for such extended durations is often impractical for many dairy producers. Silva Neto et al. (2023) reported that opening silos at 30 d was insufficient, as no improvements in cow performance were observed. For rehydrated corn silage, a minimum storage period of 60 d is generally recommended (Fernandes et al., 2021). Nevertheless, further research is needed to determine the optimal storage duration for HMC silage treated with PAI, as no studies to date have specifically addressed this.

Propionic acid is widely recognized as an antimicrobial agent that effectively reduces losses during ensiling (Morais et al., 2017; Gheller et al., 2021). Despite its benefits, the high cost and corrosiveness of propionic acid limit its widespread use (Kung et al., 1998). Buffered propionic acid-based additives at inclusion rates of 0.1 to 0.2% of fresh matter may present a practical approach for minimizing ensiling losses (Kung et al., 1998; Kung et al., 2004). The concentrations of additives based on or exclusively with propionic acid vary greatly, as do the application rates. As described in the review by Morais et al. (2017), the concentrations of propionic acid in different products ranged from 5 to 90%, and the application rates ranged from 1.5 to 6.0 L.ton^{-1}. Research outcomes regarding the use of additives in high moisture corn demonstrate considerable heterogeneity, as evidenced by a recent meta-analysis (Torres et al., 2021) However, optimal dosages for commercial additives for HMC remain unclear, underscoring the need for further research.

This study aims to evaluate the effects of PAI on HMC ensiled up to 180 d on chemical characteristics, DM losses, starch degradation and perform a characterization of the ensiled mass regarding the presence of mycotoxins. We hypothesize that a minimum of 60 d of ensiling is required to optimize fermentation patterns and improve ruminal starch degradability. Furthermore, we hypothesize that a low dose of PAI is sufficient to achieve adequate fermentation profile while minimizing losses.

MATERIAL AND METHODS

The study was conducted at the Dairy Unit of the Teaching and Research Farm affiliated with the Department of Animal Science at Universidade Federal de Viçosa, located in Viçosa, Minas Gerais, Brazil. The animal care and handling procedures were approved by the Ethics Committee on the Use of Production Animals of the Universidade Federal de Viçosa (protocol number 60/2020).

Corn Processing and Ensiling Procedure

A corn hybrid (variety LG 76799; Limagrain, Goiás, Brazil) was harvested at approximately 45% moisture content using a 350 S2 harvester (Murska®, Subotica, Serbia), which required 30-40 HP from a tractor, and with a processing capacity of 5 ton/h.

The vitreousness of the corn grains of 80% was determined according to protocols by Dombrink-Kurtzman and Bietz (1993). Experimental treatments involved the application of a propionic acid-based additive (Lupro-Grain®; BASF S.A., São Paulo, Brazil) containing 91% propionic acid, 3.5% ammonia, 3.5% 1,2-propanediol, and 2% water. The HMC was ensiled in laboratory silos (polypropylene buckets), measuring 29.5 cm height, 24 and 22 cm on top and base diameters, respectively, and with 10 L of capacity each. The buckets were sealed with adhesive tape to reduce contact between the silage mass and the air.

For ensiling, corn was sprayed with the propionic acid-based additive, manually mixed, and placed into buckets, then compacted using wooden stakes to achieve an average density of 1,300 kg/m^3. An average of 1,389.4 ± 16.84 kg/m^3 was obtained. The experiment followed a completely randomized design in a 3 × 6 factorial arrangement (addition of 0, 0.25, or 0.50% PAI in a fresh matter basis, and 30, 60, 90, 120, 150, and 180 d of ensiling). Therefore, nine silos were opened at each storage length, three being for each PAI addition level, totaling 63 experimental silos (i.e., experimental units).

Sampling and Laboratory Analysis

Upon opening, the top 10 cm of HMC silos was discarded. The buckets were then weighed, and their tare weights were subtracted to determine the net weight of the contents of the mini silos. The remaining material was homogenized, and a laboratory sample of approximately 2 kg was placed in plastic bags and frozen at -20°C for subsequent analysis. Another aliquot was taken, and an aqueous extract was prepared by blending 25 g of the homogenized material with 225 mL of distilled water for 1 min, and the pH was measured using a digital potentiometer (Tec-3MP; Tecnal, Piracicaba, São Paulo, Brazil). Ammonia-N was analyzed through Chaney and Marbach (1962) method, and organic acids (lactic, acetic, propionic, and butyric) were quantified following the methods described by Siegfried et al. (1984). Organic acids analysis was performed using HLPC chromatography (Shimadzu LC-20AT, Kyoto, Japan).

Posteriorly, a portion of the frozen samples from each experimental silo were thawed at room temperature, oven-dried (55°C/72 h), and ground in a knife mill to pass through a 1-mm screen sieve (model 3; Arthur H. Thomas Co., Philadelphia, PA). Analyses of DM (dried over-night at 105°C, method G-001/2), ash (combustion in a muffle furnace at 550°C, method M-001/3), CP (Kjeldahl procedure, method N-001/3), NDF (using a thermostable α-amylase without sodium sulfite, method F-002/3), and ether extract (**EE**, Randall extraction, method G-005/2) were conducted according to the protocols outlined by Detmann et al. (2025). Starch content was determined using the enzymatic method as described by Silva et al. (2019).

Additionally, study materials were also analyzed for mycotoxins using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The mycotoxins quantified included Aflatoxin, Deoxynivalenol, Fumonisins, Ochratoxin A, T2 Toxin, and Zearalenone.

Calculations

Total DM losses were quantified by calculating the difference between the initial and final gross DM weights in the silos relative to the ensiled forage mass, using the equation provided by Schmidt (2006):

$$TLDM = [(ADMi - ADMf)] \,/\, ADMi \times 100$$

in which TLDM represents the total DM loss (% DM), ADMi is the initial DM amount (calculated as the weight of the silo post-filling minus the weight of the empty set pre-filling, adjusted by the DM content of the forage in the silage), and ADMf is the final DM amount (calculated as the weight of the filled silo pre-opening minus the weight of the empty set post-opening, adjusted by the DM content of the forage at opening).

Ruminal Degradation Procedures

The in situ starch ruminal degradation was assessed using nylon bags (Sefar Nitex, Switzerland; 50 μm porosity, 4 × 7 cm size). A reserved part of the samples was ground through a Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA) using a 6-mm screen (Fernandes et al., 2018), for each treatment within each opening time of the mini silos, a composite sample was prepared based on DM and proportionally derived from the three replicates. Three multiparous Holstein cows were utilized for this study. These cows had an average BW of 668.7 ± 49.90 kg, an average milk yield of 37.2 ± 4.51 kg/d and were at 73.2 ± 21.10 DIM. The cows were fed a diet comprising 75% forage (90 corn silage + 10% Tifton hay) and 25% concentrate (43% soybean meal + 20% dry ground corn + 30% whole cottonseed + 2% urea + 5% minerals-vitamins premix).

Material for each treatment was incubated in three different cows. A 6-g sample (on a DM basis) was used per nylon bag, with the total number of bags adjusted to reach the desired sample amount per incubation time: 6 g for the 0 h time point (not incubated in the rumen), 18 g for 3 h, 24 g for 7 h, and 102 g for 48 and 120 h (Fernandes et al., 2018). These quantities were replicated in two incubations per cow, with a 15-d interval between them, to ensure sufficient residual material remained for the analyses planned.

Nylon bags were attached to a chain and placed inside the rumen via a cannula, then the bags were removed simultaneously. Post-ruminal incubation, including the 0 h bags, were washed in cold water using a washing machine and rinsed 5 times for 1 min each (Detmann et al., 2025). The samples were oven-dried (55°C/72 h) and ground in a knife mill to pass through a 1-mm screen sieve (model 3; Arthur H. Thomas Co., Philadelphia, PA), subsequently analyzed for DM and starch as previously described.

Quantitative Approach for Starch Degradation Kinetics

The *in-situ* degradation kinetics of starch (D_t) was obtained with rumen cannulated cows, in which cows were considered random effects and grouping factors. The PAI levels and silo opening times (30, 60, 90, 120, 150, and 180 d) were considered fixed, categorical effects for the first fitting procedure. The first modeling approach was to estimate parameters of cumulative starch degradation in the rumen, as follows:

$$dD_t/dt = \kappa\left(D_f - D_t\right), t \geq 0. \qquad \text{Eq. (1)}$$

By integrating Eq. (1), one can obtain a stochastic version as follows:

$$D_t = D_f - B\exp(-\kappa t) + e \qquad \text{Eq. (2)}$$

in which D_f is the dimension less (dmls) asymptotic fraction of starch degraded, $B = D_f - D_0$ is a scale correction (dmls) because of initial solubility of starch (D_0), which was assumed instantaneously degraded, κ (1/h) is the fractional rate of starch degradation in the rumen, and e is the error term.

The gnls function of nlme package (Pinheiro et al., 2017) was used to fit several versions of Eq. (2) to the data. The model versions were created iteratively, so that the first model version was Eq. (2) with no fixed treatment effects, no random effects, and with independent and identical normal errors, i.e., $e \sim N.i.i.d.(0, \sigma^2)$. The model complexity increased by attributing treatment effects, silo opening time effects, or both to each parameter of Eq. (2). The random effects were attributed to each parameter by fitting the model with the nlme function. Variance functions (power-of-the-mean, and heterogeneous variances per fixed effect level) and continuous auto-regressive correlations were fitted in addition to the traditional homoscedastic variance. The information-theoretic (I-T) approach (Burnham et al., 2011a; b) was used to evaluate the quality of fit of the different model versions (Table 1).

The best solution of Eq. (2) to in situ starch degradation was Eq. (2)-$B\kappa$~ot×trt, κ~c, σ^2_{ot}, which means that the interacting effects PAI by silo opening times were attributed to parameters B and κ of Eq. (2), whereas parameter D_f functioned as an intercept constant that was affected neither by PAI nor by silo opening time. In addition, the random effect of cow was attributed to parameter κ, and heterogeneous variances by silo opening times

(t_o) were necessary to achieve the most suitable solution, given the data (Table 1). Because κ was affected by the random cow effect, a two-step process was done to model κ by generating a random sample of κ's and modeling this new random variable as follows:

$$d\kappa(t_o)/dt_o = \begin{cases} 0, & t_o \leq \mu \\ (\kappa_f - \kappa_0)f(t_o), & t_o > \mu \end{cases} \qquad \text{Eq. (3)}$$

$$f(t_o) = (\eta/\beta)((t_o - \mu)/\beta)^{\eta-1}\exp(-((t_o - \mu)/\beta)^{\eta}), t_o > \mu \qquad \text{Eq. (4)}$$

In Eq. (3), κ_0 (1/h) is the fractional rate constant of starch degradation in the corn forage mass at the time of ensiling, i.e., at $t_o = 0$. A Weibull probability density function (Weibull, 1951; Carlton and Devore, 2014), namely $f(t_o)$, was assumed for describing the process of increasing the fractional rate constant of in situ starch degradation in the corn silage mass as the time for silo opening (t_o, d) advances. In that case, $\kappa(t_o)$ reaches an asymptote as $\lim_{t_o \to \infty} \kappa(t_o) = \kappa_f$ (1/h). Because κ_f and κ_0 are rate constants, there is a closed-form solution for $\kappa(t_o)$, which was obtained by integrating Eq. (3) from zero to t_o:

$$\kappa(t_o) = \begin{cases} \kappa_0, & t_o \leq \mu \\ \kappa_0 + (\kappa_f - \kappa_0)(1 - \exp(-((t_o - \mu)/\beta)^{\eta})), & t_o > \mu \end{cases} \qquad \text{Eq. (5)}$$

The increase in the fractional degradation rate of starch is given by $\kappa_b = \kappa_f - \kappa_0$. Parameter μ is a location parameter expressed in days (d), β is a scale parameter (d), and η is a dmls shape parameter of the Weibull cumulative distribution function, namely $F(t_o) = (1 - \exp(-((t_o - \mu)/\beta)^{\eta}))$. The fixed treatment effects (PAI) were attributed to the parameters κ_0, κ_b, μ, β, and η. The random effect of cow was attributed to the intercept κ_0, and the errors were assumed $N.i.i.d.(0, \sigma^2)$. The model described by Eq. (5) was fitted to the random fractional rates of starch degradation with the NLMIXED procedure of SAS (SAS® OnDemand for Academics, Copyright © 2021 SAS Institute Inc. | Release 3.1.0), using the Newton-Raphson optimization algorithm with ridging, and the adaptive gaussian quadrature as the likelihood integration method. One of the advantages of the Weibull model fitted by NLMIXED is the possibility of obtaining an inverse prediction of t_o for a given probability $p = \kappa_{t_o}/(\kappa_0 + \kappa_b)$. If one divides both sides of Eq. (5) by $(\kappa_0 + \kappa_b)$ and solves for t_o it yields Eq. (6), as follows:

$$t_o = \mu + \beta\left(-\log\left(1 - \left(p\kappa_f - \kappa_0\right)/\kappa_b\right)\right)^{1/\eta}. \qquad \text{Eq. (6)}$$

In sequence, the silo opening time was estimated for $p = 0.25$, 0.50, and 0.75 with the estimate sentence of the NLMIXED procedure. Besides, contrasts between inoculants and control, and contrasts between inoculants were created and tested with the contrast sentence of NLMIXED. The concordance correlation coefficient (Lin, 1989) was computed either as point and interval estimate to evaluate the adequacy of Eq. (5) to describe the random fractional rate constants.

Statistical Analysis for Fermentative Profile and Chemical Characteristics

Statistical analysis of the fermentative profile and chemical composition were performed using the GLIMMIX procedure in SAS Studio. A completely randomized design was employed, with fixed effects for treatment and opening time, as well as their interaction. When the interaction between time and treatment was significant, a partitioned analysis was conducted using the SLICE statement. Significant differences among means were evaluated using Tukey's test, with the Type I error rate set at 0.05.

RESULTS

Mycotoxins

Among the six types of mycotoxins analyzed (Table 2), Aflatoxin was the most prevalent in the samples, followed by Ochratoxin, and T2 toxin. However, it is worth noting that the levels of all mycotoxins were within the limits established by European Union regulation (1881/2006).

Chemical Composition

No interaction was observed between PAI and storage time for any characteristic of silage chemical composition ($P > 0.05$, Table 3). Neither DM, nor OM contents were affected by PAI or storage length ($P > 0.05$, Table 3).

Crude protein content was influenced by storage length ($P < 0.01$). Despite some overlapping, CP content tends to decrease as storage length extended, reaching the lowest values at 180 d. However, PAI did not affect CP content ($P = 0.07$).

Starch content was affected by PAI ($P < 0.01$). Starch content decreased when PAI was added regardless of the amount. Starch concentration was also affected by storage length ($P < 0.01$), where concentrations remained stable up to 150 d of storage, but increased at 180 d.

Ammonia concentration (% total N) increased as storage length extended ($P < 0.01$). On the other hand, its concentration decreased ($P < 0.01$) with PAI (Table 3).

Fermentation Profile

An interaction between PAI and storage length was observed ($P < 0.01$) in concern to DM losses. Dry matter losses increased when 2.5% PAI were added to the ensiled material and were similar for 0 and 5.0% PAI. Effects of PAI in DM losses ($P < 0.01$) showed a similar pattern, except for 60 d, which showed no difference ($P = 0.76$) between the PAI levels (Table 4).

Final pH showed an interaction between PAI and storage length ($P < 0.01$, Table 4). The effect of PAI was detected across storage length ($P < 0.01$), except for 90 d ($P = 0.54$) where values were similar. In general, PAI led to a decrease in final pH, and the differences between 2.5 and 5.0% became more prominent as the storage length extended, with lower values obtained with the highest PAI level.

No interaction effect ($P = 0.54$; Table 4) was detected for lactic acid concentration. However, lactic acid concentrations remained similar until 150 d and increased at 180 d of the storage length ($P < 0.01$). The PAI did not affect ($P = 0.48$) lactic acid concentrations in the silages.

The acetic acid concentration exhibited an interaction between PAI and storage time ($P = 0.01$, Table 4). The cutoff of this effect did not show any PAI effect at 90 d ($P = 0.33$) and 120 d ($P = 0.77$). For the other storage periods, the acetic acid concentrations were lower ($P < 0.05$) when 5.0% PAI was added to the material compared to the control (0% PAI), except for 180 d where the concentration became higher for 5.0 than 2.5% PAI. The 2.5% PAI showed no difference compared to 0 or 5.0% PAI.

No interaction ($P = 0.26$, Table 4) was observed for propionic acid concentration. However, both PAI ($P < 0.01$) and storage length ($P = 0.01$) affected propionic acid concentration. As expected, the propionic acid concentration increased as PAI increased. On the other hand, propionic acid concentration was similar until 150 d, being increased when storage length extended to 180 d.

Regarding butyric acid, no effect was observed for PAI (*P* = 0.15) or storage length (*P* = 0.59), and there was no significant interaction between them (*P* = 0.85).

Ruminal in situ Degradability

The storage length was affected by the concentrations of additives, as evidenced by the contrasts between treatments control and PAI in the time required to reach half (*P* = 0.04) and three-quarters (*P* = 0.04) of the maximum starch degradation rate (Figure 1). The other contrasts were not significant (*P* > 0.05). Treatments with higher levels of PAI required more days to reach the maximum degradation rate (Table 5), and treatments with higher PAI exhibited lower degradation rates over time (Figure 1).

DISCUSSION

This study evaluated the effects of PAI (0, 0.25, and 0.50% fresh matter) on fermentation, chemical composition, and ruminal starch degradability of HMC ensiled for periods up to 180 d. High-moisture corn was ensiled in mini silos with a density of 1,300 kg/m^3. Treatments with PAI showed slightly reduced pH levels but experienced increased DM losses after 60 d, especially at 0.50% PAI. Crude protein content decreased while ammonia-N levels rose with longer ensiling periods. Ruminal starch degradation rates increased over time, achieving highest values in untreated silage. Overall, PAI did not enhance fermentative characteristics or ruminal starch degradability compared to the control, and extending ensiling duration to 180 d improved starch degradability and influenced chemical composition positively.

Dry matter losses in corn grain silage typically range between 1 and 3%, although some studies have reported values as high as 5%. In the present study, observed DM losses ranged from 4.84 to 6.75%, without a consistent trend over time. These losses were notably influenced by PAI, with the 0.25% PAI treatment showing increased losses compared to other treatments. Therefore, the inclusion of PAI did not effectively reduce DM losses in HMC silage. Nevertheless, the actual differences among treatments were minimal (less than 1 percentage point), suggesting a limited practical impact on overall HMC production.

The low pH values indicate stable silage fermentation, an essential factor that suppresses undesirable microbial activity. The remarkably low pH observed could partially be attributed to interactions between water and nitrogen oxides present in HMC, potentially forming nitric acid, as described by Kung et al. (2018). Typically, pH reduction during fermentation primarily results from lactic acid production. However, despite similar lactic acid concentrations across treatments, lower pH values were especially evident in PAI-treated silages at extended storage durations (150 and 180 d). This observation suggests an additional acidifying effect of propionic acid, despite its relatively low acidogenic potential compared to lactic acid. Nevertheless, given the similarity in DM losses between 0.50% PAI and control treatments, pH alone did not appear to strongly influence silage quality.

Butyric acid, an undesirable fermentation byproduct, was detected at concentrations between 0.3 to 0.4% of DM, slightly above the average 0.24% reported in a comprehensive review by Morais et al. (2017). The slightly elevated butyric acid concentrations could indicate some degree of clostridial fermentation, despite the low pH and adequate lactic acid levels observed. Lactic acid concentrations ranged between 0.6 and 1.1% of DM, consistent with typical HMC silages. Acetic acid concentrations (0.2–

0.3% of DM) were within normal ranges and aligned with expected fermentation patterns. Together, these acid concentrations confirm effective fermentation in the evaluated silages, irrespective of treatment.

Propionic acid, typically absent in significant concentrations unless deliberately added or produced during severe clostridial fermentations, reached expected levels (0.15–0.30%) due to its intentional addition. Similar concentrations were previously reported in studies using ammonium propionate-based additives (Kung et al., 2004; da Silva Neto et al., 2023). Interestingly, ammonia concentrations increased with higher PAI levels, reaching 1.85% of total N at 0.50% PAI compared to 1.69% for control. Although elevated ammonia typically indicates enhanced protein degradation, this increase likely originated from ammonia present in the additive formulation itself rather than from microbial protein breakdown. Supporting this interpretation, the increased ammonia levels did not correlate with improvements in ruminal starch degradability.

Consistent with previous studies, CP content decreased while ammonia levels increased progressively, indicating ongoing proteolysis. This shift in chemical composition aligns with enhanced starch availability for ruminal degradation at extended storage durations, a pattern also noted by Oliveira et al. (2023). Importantly, starch content remained stable throughout storage, supporting findings by Saylor et al. (2022), who also observed no starch losses over time.

Ruminal starch degradability increased continuously with storage duration, consistent with literature suggesting increased enzymatic and bacterial proteolytic activity (Junges et al., 2017; Hoffman et al., 2011). Silva et al. (2019) recommended at least 71 d of storage for optimal starch degradability in rolled HMC, while Fernandes et al. (2021) suggested at least 60 d for flint-type hybrids. Contrarily, in the present study, PAI negatively impacted ruminal starch degradability. The control treatment showed the highest degradation rates, whereas increased PAI levels progressively reduced starch degradability. These results differ from studies using lower propionic acid doses, such as Oliveira et al. (2023), who found similar degradability between treatments containing lower propionic acid concentrations. Furthermore, Silva Neto et al. (2023) reported no differences between control and low-dose propionic acid treatments, emphasizing the importance of additive dosage.

The inhibitory effect of propionic acid on microbial activity is well-known, extensively documented in food preservation studies (Ammar and Philippidis, 2021). This antimicrobial effect likely hindered microbial activity within the rumen environment, particularly within nylon bag incubations, reducing starch and protein matrix degradation. Consequently, treatments with higher PAI required longer periods to achieve similar starch degradation levels compared to control. The control achieved 50% of maximum starch degradability after approximately 90 d, whereas treatments with higher PAI levels required over 132 d.

In summary, the results suggest that although propionic acid inclusion slightly reduced silage pH, it did not effectively decrease DM losses nor improve starch degradability. Optimal starch utilization required extended storage periods exceeding 90 d, highlighting the limited practicality of short-term fermentation durations in maximizing the nutritional value of HMC silages.

CONCLUSION

Under the conditions evaluated in this study, adding propionic acid to high-moisture corn did not enhance fermentative characteristics and negatively impacted ruminal starch degradability. Since no fermentation issues were observed in untreated control silages, the inclusion of propionic acid is not recommended for improving silage quality or ruminal starch utilization. Extending the ensiling period proved beneficial, improving starch degradability. Therefore, a storage duration of at least 180 d is advised, depending on the practical and operational conditions of individual dairy operations, to maximize the nutritional value of high-moisture corn silage.

NOTES

We acknowledge Brazilian foundations for the support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, Distrito Federal, Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, Distrito Federal, Brasília), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, Minas Gerais, Brazil). The study was approved by the Ethics Committee for Animal Welfare and Use of Universidade Federal de Viçosa under the number 60/2020. The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: CON = control treatment; HMC = high moisture corn silage; PAI = propionic acid inclusion

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Table 1. Evaluation of model versions (Eq. 2) according to the information-theoretical approach.

Model[1]	d.f.[2]	$-\hat{\ell}$[3]	AICc[4]	Δ[5]	w[6]	ER[7]
Eq.(2)-$B\kappa$~ot×trt, κ~c, σ^2_{ot}	51	817.0	1,756	0	$\cong 1.0$	1
Eq.(2)-$B\kappa$~ot×trt, $\sigma^2_{ot \times trt}$	64	810.7	1,783	26.6	10^{-6}	597,196
Eq.(2)-$B\kappa$~ot, σ^2_{ot}	28	875.9	1,814	57.4	10^{-13}	10^{12}
Eq.(2)~ot×trt, $N.i.i.d.$[8]	64	919.7	2,001	244.6	10^{-54}	10^{53}
Eq.(2), general, PM[9], CAR[10]	6	1,218	2,448	692.1	10^{-151}	10^{150}
Eq.(2)~trt, PM, CAR	12	1,213	2,452	695.7	10^{-152}	10^{151}
Eq.(2), general, PM	5	1,235	2,481	724.7	10^{-158}	10^{157}
Eq.(2), general, $N.i.i.d.$	4	1,243	2,493	737.2	10^{-161}	10^{160}

[1] General means with no fixed effect attributed to the parameters of Eq. (2).
[2] Degrees of freedom of the model.
[3] Negative logarithm of the likelihood function.
[4] Akaike information criterion corrected for small samples.
[5] Akaike differences.
[6] Model probability.
[7] Evidence ratio.
[8] $N.i.i.d.$ is independent and identically distributed normal errors.
[9] PM is power-of-the-mean variance function.
[10] CAR is a continuous autoregressive correlation.

~trt means that fixed treatment effects were attributed to the model, ~ot×trt means that the interacting effects of treatment and silo storage length were attributed to parameters of Eq. (2), $B\kappa$~ot means that fixed effects of silo opening times were attributed only to parameters B and κ, κ~c means that the random effect of cow was attributed to parameter κ, and σ_{ot}^{2} and $\sigma_{ot\times trt}^{2}$ are heterogeneous variances per ot or ot×trt stratum, respectively.

Table 2. Mycotoxins (ppb) analyzed in the experimental silages.

SL[1]	Treatment	Aflatoxin	Deoxynivalenol	Fumonisins	Ochratoxin	Toxin-T2	Zearalenone
		ppb					
0	Control	1.52	ND[2]	ND	ND	40.4	ND
	0.25% PAI[3]	NA	ND	ND	3.94	ND	ND
	0.50% PAI	2.16	ND	ND	ND	33.9	ND
30	Control	1.63	ND	ND	ND	ND	ND
	0.25% PAI	1.16	ND	ND	2.5	51.2	ND
	0.50% PAI	2.27	ND	ND	5.9	60.4	ND
60	Control	1.80	ND	ND	4.78	55.2	ND
	0.25% PAI	2.05	ND	ND	ND	ND	ND
	0.50% PAI	1.55	ND	ND	2.48	29.5	ND
90	Control	3.39	ND	ND	11.3	22.6	ND
	0.25% PAI	3.02	ND	ND	8.31	ND	ND
	0.50% PAI	3.54	11.2	ND	27.2	ND	ND
120	Control	3.70	ND	ND	15.0	ND	ND
	0.25% PAI	3.59	ND	ND	15.6	36.0	ND
	0.50% PAI	1.30	ND	ND	3.64	ND	ND
150	Control	3.42	ND	ND	8.48	ND	ND
	0.25% PAI	3.80	ND	ND	16.8	31.9	ND
	0.50% PAI	4.25	ND	ND	33.2	ND	ND
180	Control	2.50	ND	ND	6.09	20.8	ND
	0.25% PAI	2.75	ND	ND	5.03	ND	ND
	0.50% PAI	4.50	ND	ND	21.7	ND	ND
EU[4]		5.00	1,000	2,000	1,000	100	500

[1] SL = storage length in days.

[2] ND = not detect.

[3] PAI = propionic acid inclusion on a fresh matter basis.

[4] Europe Union legislation for animal feeding limits (1881/2006).

Table 3. Chemical composition of high-moisture corn silage with different doses of propionic acid inclusion and at different silo storage length.

Item	PAI[1]	Storage length, d								*P*-value		
		30	60	90	120	150	180	Mean[2]	SEM	PAI	SL[3]	PAI×SL
Dry matter, %	0	51.2	49.7	51.3	50.2	50.6	50.3	50.7	0.20	0.81	0.06	0.86
	2.5	51.2	51.1	50.7	50.6	50.6	49.9	50.8				
	5.0	50.9	50.8	50.4	50.6	50.7	49.7	50.6				
	Mean[4]	51.1	50.5	50.8	50.5	50.7	50.0					
Organic matter, %	0	50.2	48.6	50.2	49.2	49.4	49.0	49.4	0.21	0.82	0.16	0.87
	2.5	50.3	50.0	49.5	49.6	49.4	48.8	49.6				
	5.0	49.8	49.7	49.3	49.3	49.4	48.8	49.4				
	Mean	50.1	49.4	49.7	49.3	49.4	48.8					
Crude protein, %	0	8.52	8.33	8.21	8.30	8.29	8.17	8.29	0.033	0.07	<0.01	0.31
	2.5	8.42	8.40	8.26	8.28	8.42	8.11	8.33				
	5.0	8.43	8.38	8.47	8.34	8.51	8.09	8.40				
	Mean	8.46A	8.37A	8.31AB	8.31AB	8.41A	8.13B					
Starch, %	0	69.0	68.4	68.4	69.1	68.9	71.5	69.0A	0.16	<0.01	<0.01	0.19
	2.5	67.8	68.7	66.6	67.7	68.3	70.6	68.1B				
	5.0	68.0	67.7	66.8	67.4	67.6	71.8	68.0B				
	Mean	68.3B	68.3B	67.3B	68.0B	68.3B	71.3A					
NH3-Total N, %	0	1.36	1.48	1.67	1.86	1.84	1.95	1.69C	0.020	<0.01	<0.01	0.51
	2.5	1.51	1.55	1.74	1.84	1.96	1.97	1.76B				
	5.0	1.61	1.70	1.75	1.93	2.01	2.11	1.85A				
	Mean	1.49D	1.57D	1.72C	1.88B	1.94AB	2.01A					

[1] PAI = propionic acid inclusion (L/ton) on a fresh matter basis.

[2] Means in the line, within the different propionic acid inclusion levels, followed by lowercase letters differ ($P < 0.05$).

[3] SL = storage length.

[4] Means in rows or columns followed by capital letters differ ($P < 0.05$).

Table 4. Fermentative profile of high-moisture corn silage with different doses of propionic acid inclusion and at different silo storage length.

Item	PAI[1]	Storage length, d								*P*-value		
		30	60	90	120	150	180	Mean[2]	SEM	PAI	SL[3]	PAI×SL
Dry matter losses, %	0	5.59ab	5.65	5.37b	5.35b	5.23b	5.15b	-	0.151	<0.01	0.72	<0.01
	2.5	5.88a	5.76	5.92a	6.36a	6.52a	6.75a	-				
	5.0	5.28b	5.60	5.18b	5.24b	4.88b	4.84b	-				
	Mean[4]	-	-	-	-	-	-					
	P-value[5]	0.03	0.76	<0.01	<0.01	<0.01	<0.01					
pH	0	3.61a	3.56a	3.61	3.60a	3.57a	3.54a	-	0.073	<0.01	<0.01	<0.01
	2.5	3.60ab	3.60b	3.60	3.51b	3.50b	3.49b	-				
	5.0	3.58b	3.61b	3.61	3.48c	3.44c	3.45c	-				
	Mean	-	-	-	-	-	-					
	P-value	<0.05	<0.01	0.54	<0.01	<0.01	<0.01					
Lactic acid, %	0	0.92	1.12	0.79	0.87	1.01	1.21	0.98	0.039	0.48	<0.01	0.54
	2.5	1.03	0.85	0.91	1.12	1.07	1.22	1.04				
	5.0	0.87	0.85	0.83	1.21	1.03	1.48	1.05				
	Mean	0.94B	0.94B	0.85B	1.07B	1.04B	1.30A					
Acetic acid, %	0	0.27a	0.29a	0.13	0.21	0.25a	0.25ab	-	0.029	0.03	<0.01	0.01
	2.5	0.25ab	0.22ab	0.19	0.22	0.20ab	0.21b	-				
	5.0	0.17b	0.16b	0.15	0.19	0.13b	0.32a	-				
	Mean	-	-	-	-	-	-					
	P-value	0.04	0.01	0.33	0.77	0.03	0.03					
Propionic acid, %	0	0.01	0.01	0.01	0.06	0.04	0.08	0.03C	0.017	<0.01	0.01	0.26
	2.5	0.01	0.17	0.15	0.10	0.19	0.23	0.16B				
	5.0	0.24	0.29	0.20	0.32	0.18	0.41	0.27A				
	Mean	0.11B	0.16B	0.12B	0.16B	0.14B	0.24A					
Butyric acid, %	0	0.43	0.56	0.31	0.41	0.36	0.39	0.41	0.034	0.15	0.59	0.85
	2.5	0.27	0.35	0.27	0.32	0.38	0.31	0.32				
	5.0	0.37	0.37	0.34	0.35	0.28	0.46	0.36				
	Mean	0.36	0.43	0.30	0.36	0.34	0.39					

[1] PAI = propionic acid inclusion (L/ton) on a fresh matter basis.

[2] Means in the line, within the different propionic acid inclusion levels, followed by lowercase letters differ ($P < 0.05$).

[3] SL = storage length.

[4] Means in rows or columns followed by capital letters differ ($P < 0.05$).

[5] *P*-values referring to the comparison between PAI levels within different opening times.

Table 5. Parameter estimates related to the fractional rate parameter of in situ starch degradation as a function of time of storage length, as described by Eq. (5), and additional estimates regarding the storage length (t_o, d) for a 0.25, 0.5, and 0.75 proportion (p) to achieve the asymptotic starch degradation rate ($\kappa_0 + \kappa_b$).

Parameter[1]	Estimate	$\pm$SE	Lower limit	Upper limit
β~t1	100.5	9.69	78.2	122.9
β~t2	175.0	37.21	89.2	260.8
β~t3	275.0	86.90	74.6	475.4
η~t1	0.9	0.08	0.7	1.1
η~t2	0.9	0.10	0.7	1.1
η~t3	0.7	0.04	0.6	0.8
μ~t1	30.0	4.08	20.6	39.4
μ~t2	30.0	6.19	15.7	44.3
μ~t3	29.9	0.31	29.2	30.7
$100 \times \kappa_0$~t1	9.47	0.753	7.73	11.2
$100 \times \kappa_0$~t2	8.48	0.751	6.75	10.2
$100 \times \kappa_0$~t3	10.08	0.978	7.82	12.3
$100 \times \kappa_b$~t1	119.0	6,170	104.8	133.2
$100 \times \kappa_b$~t2	120.2	14,390	87.0	153.4
$100 \times \kappa_b$~t3	122.7	18,560	79.9	165.5
$100 \times \sigma_{cow}$	0.97	0.316	0.24	1.70
$100 \times \sigma$	3.42	0.151	3.07	3.76
t_o~t1, $p = 0.25$	48.6	2.0	43.9	53.3
t_o~t2, $p = 0.25$	61.4	6.1	47.3	75.4
t_o~t3, $p = 0.25$	60.0	9.6	38.0	82.0
t_o~t1, $p = 0.5$	89.5	5.2	77.5	101.4
t_o~t2, $p = 0.5$	132.7	21.2	83.9	181.6
t_o~t3, $p = 0.5$	168.0	43.7	67.3	268.8
t_o~t1, $p = 0.75$	164.7	15.6	128.8	200.6
t_o~t2, $p = 0.75$	269.3	58.2	135.1	403.6
t_o~t3, $p = 0.75$	431.6	138.2	112.9	750.2

[1] t1, t2, and t3 correspond to control, 0.25% PAI, and 0.50% PAI, respectively, which were ascribed to parameters of Eq. (5). σ_{cow} and σ are cow and residual standard deviations, respectively. $100 \times$ means that the original value was multiplied by 100 to be presented.

Supplementary Table. Fixed parameter estimates and respective standard errors (± SE), and point and 0.95 confidence limits (lower limit, upper limit) for variance components obtained by fitting Eq. (2) to in situ data on starch degradation as affected by inoculant and silo storage length.

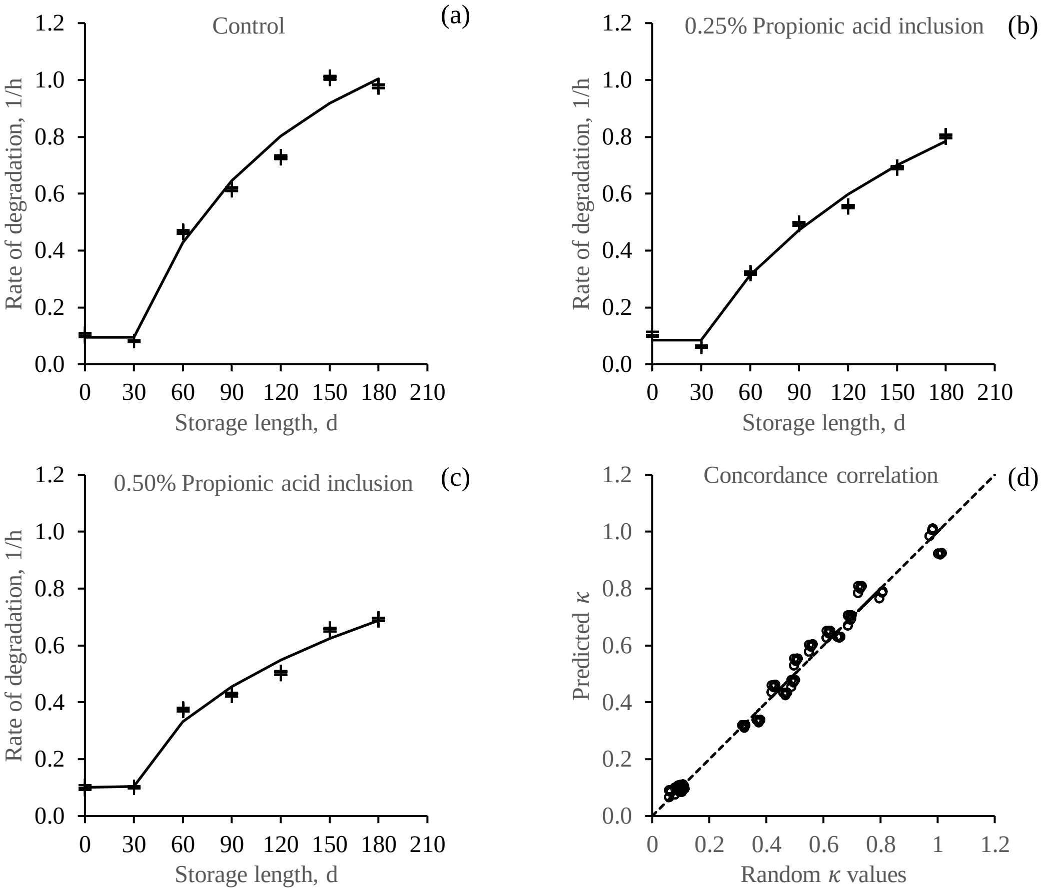
Parameter[1]	Estimate ± $\widehat{\text{SE}}$	Parameter	$100 \times \left(\hat{\kappa} \pm \widehat{\text{SE}}\right)^2$
D_f	99.0 ± 0.23	–	–
B ~ t1×ot0	78.3 ± 1.93	κ ~ t1×ot0	10.3 ± 0.96
B ~ t2×ot0	65.3 ± 1.94	κ ~ t2×ot0	10.6 ± 1.11
B ~ t3×ot0	73.6 ± 1.93	κ ~ t3×ot0	9.9 ± 0.99
B ~ t1×ot30	64.9 ± 5.63	κ ~ t1×ot30	8.7 ± 2.61
B ~ t2×ot30	71.2 ± 5.30	κ ~ t2×ot30	6.7 ± 1.93
B ~ t3×ot30	72.2 ± 5.74	κ ~ t3×ot30	10.5 ± 2.61
B ~ t1×ot60	78.6 ± 2.21	κ ~ t1×ot60	46.7 ± 3.73
B ~ t2×ot60	71.8 ± 2.20	κ ~ t2×ot60	32.2 ± 2.47
B ~ t3×ot60	72.7 ± 2.21	κ ~ t3×ot60	37.4 ± 2.95
B ~ t1×ot90	67.7 ± 1.71	κ ~ t1×ot90	61.8 ± 5.37
B ~ t2×ot90	71.4 ± 1.71	κ ~ t2×ot90	49.5 ± 3.48
B ~ t3×ot90	66.3 ± 1.71	κ ~ t3×ot90	42.7 ± 3.00
B ~ t1×ot120	68.0 ± 1.51	κ ~ t1×ot120	72.9 ± 6.61
B ~ t2×ot120	63.5 ± 1.51	κ ~ t2×ot120	55.7 ± 4.19
B ~ t3×ot120	63.7 ± 1.51	κ ~ t3×ot120	50.5 ± 3.56
B ~ t1×ot150	77.1 ± 1.17	κ ~ t1×ot150	101.0 ± 10.48
B ~ t2×ot150	77.2 ± 1.17	κ ~ t2×ot150	69.3 ± 4.06
B ~ t3×ot150	74.4 ± 1.17	κ ~ t3×ot150	65.5 ± 3.76
B ~ t1×ot180	72.4 ± 1.60	κ ~ t1×ot180	97.9 ± 13.90
B ~ t2×ot180	66.2 ± 1.60	κ ~ t2×ot180	80.3 ± 8.97
B ~ t3×ot180	75.7 ± 1.60	κ ~ t3×ot180	69.3 ± 5.64
Variance components			
$100 \times \sigma_{cow}$[b]	0.879	$\sigma_{t_o=90}$	2.22
	(0.218, 3.546)		(1.67, 2.97)
$\sigma_{t_o=0}$	2.68	$\sigma_{t_o=120}$	1.96
	(1.98, 3.63)		(1.47, 2.62)
$\sigma_{t_o=30}$	8.00	$\sigma_{t_o=150}$	1.50
	(5.98, 10.70)		(1.12, 2.02)
$\sigma_{t_o=60}$	3.55	$\sigma_{t_o=180}$	2.07
	(2.89, 4.36)		(1.55, 2.78)

[1] Parameter D_f is the asymptotic in situ potential degradation of starch (g/100 g). B is a scale parameter (g/100 g). Acronyms t1, t2, and t3 mean control, 0.25% PAI, and 0.50% PAI; and ot0, ot30, …, ot180 mean storage length (t_o), and the × means an interaction between treatment and storage length. The standard deviations related to t_o are $\sigma_{t_o=0}$, …, $\sigma_{t_o=180}$. [2] κ is the fractional rate of in situ starch degradation,

expressed as 1/h, whereas σ_{cow} is the standard deviation of the random cow effect. Both parameters were presented multiplied by 100.

Figure

Figure 1. On panels (a), (b), and (c) are displayed the fractional rate of starch degradation (ordinates, 1/h) as a function of storage length (days), t_o (abscissas, d) of control, 0.25% propionic acid inclusion and 0.50% propionic acid inclusion, respectively. Crosses are random κ values, and solid lines are estimated ones. On panel (d), circles correspond to pairs of random κ values on ordinates and predicted κ on abscissas, and the dashed line is the unity line (y = x). The concordance correlation coefficient for the observed versus predicted κ estimates (panel d) was 0.993, with 0.95 confidence lower and upper limits as (0.992, 0.995). The dashed line is the unity line.



Martins, Figure 1.

CHAPTER 3

This chapter is written according to the standards of the Journal of Dairy Science

Interpretative summary: **Meta-regression analysis of corn processing effects on intake, digestibility, ruminal fermentation, and performance of dairy cows.** By Martins et al. This study quantifies how different corn grain processing methods affect feed intake, nutrient digestibility, ruminal fermentation, and milk production in dairy cows. Results indicate that dry ground corn, commonly used on many dairy farms, was associated with lower milk production and less favorable ruminal fermentation outcomes compared with other processing types. In contrast, high-moisture corn enhanced digestibility but could negatively influence intake and some fermentation parameters when dietary starch levels are high, highlighting the need for careful diet formulation.

Running Head: Corn processing effects on dairy cows

Meta-regression analysis of corn processing effects on intake, digestibility, ruminal fermentation, and performance of dairy cows.

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ABSTRACT

This meta-regression study provides a comprehensive evaluation of five corn grain processing methods—dry ground (DGC), dry rolled (DRR), steam-flaked (FLA), high-moisture (HMC), and rehydrated (RCC)—on the performance and digestive parameters of dairy cows. The analysis encompassed 42 peer-reviewed studies published between 1997 and 2023, resulting in 178 treatment means that varied in corn dietary inclusion levels, forage types, and other nutritional factors. All studies reported outcomes on feed intake, ruminal fermentation (e.g., pH, volatile fatty acid concentrations), nutrient digestibility (dry matter, starch, and neutral detergent fiber), and production parameters (milk yield (MY), milk composition, and ECM. Data was analyzed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC), employing a random coefficients model to account for study-level variability and to allow covariance between intercept and slope. A backward elimination procedure was used to remove non-significant ($P > 0.05$) variables sequentially, retaining only those that significantly contributed to the final model. Results highlighted that DGC consistently exerted the most negative effects among the processing categories, reducing propionate production, nutrient digestibility, and milk yield. In contrast, HMC diets—while beneficial for starch digestibility—reduced dry matter intake and lowered ruminal pH. These shifts adversely affected neutral detergent fiber digestibility and ECM, although MY and milk solids were not negatively impacted. Other processing methods (DRR, FLA, and RCC) showed intermediate effects on both digestive traits and production outcomes. Overall, the findings underscore the importance of strategically selecting and including specific corn processing methods in dairy rations to optimize ruminal fermentation, nutrient utilization, and MY. Dairy nutritionists and producers should carefully balance dietary starch levels—particularly when using HMC—to avoid compromising ruminal health and overall performance.

Key words: corn, dairy cows, meta-regression, processing.

INTRODUCTION

Modern dairy cows have exceptionally high energy demands, necessitating strategic dietary formulations to maximize nutrient availability and optimize milk production. Corn (*Zea mays* L.) is a principal energy source in dairy rations due to its high starch content, but whole, unprocessed corn kernels are relatively resistant to digestive enzymes and ruminal microbial degradation (Kang et al., 2021). To overcome these limitations and improve starch digestibility, a variety of processing methods have been developed, each with distinct effects on ruminal fermentation, nutrient utilization, and MY.

Dry ground corn (**DGC**) is the most common form of corn processing for supply to dairy cows (Malekkhahi et al., 2021), and is therefore commonly used as a control treatment in studies comparing forms of corn processing. Steam-flaking (**FLA**) is one of the most extensively researched thermal processing methods, demonstrating improvements in starch digestibility and feed efficiency (Ferraretto et al., 2013). High-moisture corn (**HMC**), widely adopted in several countries, is a form of mechanical processing often noted for its favorable impacts on ruminal fermentation and total-tract digestibility (Kang et al., 2021). However, excessive starch availability from HMC can decrease ruminal pH and negatively affect fiber digestibility and DMI (Rafiee and Darabighane, 2021). In tropical regions, rehydrated or reconstituted ground corn (**RCC**) is also employed to improve starch utilization, but studies on this method have been more limited, restricting our understanding of its broader applications (Arcari et al., 2016; Gomide et al., 2023).

Although existing literature provides valuable insights into the effects of individual corn processing methods, findings are often conflicting or difficult to compare. For instance, Ferraretto et al. (2013) compared the influence of particle size across DGC, HMC, and FLA corn on starch digestibility and milk production, whereas Rafiee and Darabighane (2021) compared DGC with FLA corn inclusion levels. Furthermore, Torres et al. (2021) examined the influence of silage additives on HMC corn, highlighting possible interactive effects of storage techniques and processing on performance parameters. Taken together, these studies underscore the complexity of corn processing outcomes and the need for an integrative approach to reconcile apparent inconsistencies. In general, these meta-analysis and meta-regression studies compared DGC with one or two categories of corn processing. However, a multiple comparison of the most commonly used processing categories for dairy cows—namely, DGC, dry rolled corn (**DRR**), FLA, HMC, and RCC—along with an evaluation of inclusion levels, is lacking in the literature.

A comprehensive meta-regression analysis can synthesize data across multiple processing methods while accounting for variations in diet composition, forage type, and inclusion level of processed corn. By quantifying and comparing the effects of DGC, DRR, FLA, HMC, and RCC corn on intake, ruminal fermentation, nutrient digestibility, and MY, a more nuanced understanding of optimal corn processing strategies can be achieved. Therefore, the objective of this study was to conduct a meta-regression of published studies to provide a comparison of the main categories of corn processing simultaneously through a quantitative analysis of their impact on fermentative, digestive and performance characteristics of dairy cows.

MATERIALS AND METHODS

Data Set

Relevant peer-reviewed articles were identified using Google Scholar (scholar.google.com) and ScienceDirect (sciencedirect.com). The advanced search feature in each database was applied to ensure that specific keywords appeared in the title, abstract, or keywords. The search terms included “steam-flaked,” “high-moisture,” “rehydrated/reconstituted,” or “dry ground,” each followed by “corn,” in combination with “dairy” and “cows.”

Studies were eligible for inclusion if they met the following criteria: (1) provided quantitative data on dairy cow performance (milk production and/or milk solids yield), and (2) reported gastrointestinal digestibility or ruminal fermentation parameters. Articles that did not satisfy both criteria or failed to include relevant data were excluded. The full texts of all potentially eligible studies were then retrieved, and only those meeting the inclusion criteria in their entirety were retained for subsequent data extraction and analysis.

Data Extraction

For the search term: “steam-flaked corn dairy cows,” ScienceDirect returned 56 articles; of these, 37 did not meet the inclusion criteria and 4 were review articles, and leaving 15 articles for inclusion. The same term in Google Scholar yielded 18 articles; 9 were duplicates, 1 was a meta-analysis, and 8 did not meet the inclusion criteria, leaving no articles retained. Among these, 1 focused exclusively on FLA, while the other 14 compared FLA to DGC.

For the search term ‘high-moisture corn dairy cows,’ ScienceDirect returned 129 articles. Of these, 113 were excluded for not meeting the inclusion criteria, while 14 met the criteria and were retained. Additionally, one study on rehydrated corn and one on steam-flaked and dry ground corn were also included. Google Scholar returned 34 articles for the same search; 28 did not meet the criteria, 1 was a book, and 4 were duplicates, leaving 1 retained. Among these search term, 2 had treatments with only HMC, 11 compared HMC corn with DGC, and 2 compared HMC with FLA.

The search term 'rehydrated/reconstituted corn dairy cows' retrieved 5 articles from ScienceDirect, of which 2 were excluded and 3 met the inclusion criteria. A parallel search in Google Scholar identified 4 articles; 1 did not meet the criteria, and 3 were duplicates, resulting in no additional retained articles. Among the eligible publications, 2 studies focused solely on RCC, and 1 compared RCC with DGC.

Finally, “dry ground corn dairy cows” returned 213 articles in ScienceDirect; 199 did not meet the criteria, and 14 that met the criteria were retained. The same term in Google Scholar yielded 4 results; 3 were duplicates and 1 was excluded, leaving no articles retained. Among these 14 selected articles, 1 compared DGC to RCC, 3 compared DRR with FLA, 7 were exclusively about DGC, 2 compared DGC with DRR, and 1 article was exclusively about DRR.

In total, 42 articles published between 1997 and 2023 met the selection criteria (Table 1). From these 42 articles, 178 treatment means were extracted. Studies were required to report individual treatment least squares means and either the standard error of the mean (SEM) or the standard deviation (SD). For studies reporting only SD, the SEM was calculated using the method described by Steel et al. (1997). A descriptive statistical analysis was performed to characterize the studies used (Table 2).

Data Compilation and Statistical Analysis

A total of 42 studies were classified into five corn categories: DGC, DRR, FLA, HMC, and RCC. The inclusion level (%) of each category was recorded along with additional variables, including flake density, corn type (flint, semi-flint, or dent), vitreous endosperm percentage, type of inoculant, DM percentage of the corn category, starch percentage in the grain, grinding particle size, storage duration if HMC or RCC, dietary inclusion percentages of concentrated and bulky ingredients, and the use of buffers. Information on diet starch content, NDF, forage inclusion, and total corn inclusion (sum of all corn categories in the diet) was also gathered.

Performance data included DMI, MY, and ECM (Tyrrell et al., 1965). Measurements of fat, protein, lactose, total milk solids, and MUN in milk were recorded, along with FE, BW, and BCS. Digestibility parameters included total tract digestibility of DM, OM, NDF, and starch. Ruminal VFA, expressed as a percentage of total VFA concentration, and ruminal pH were also documented.

Prior to statistical modeling, a graphical examination was performed to assess relationships among dependent and independent variables and to identify potential outliers (Sauvant et al., 2008). Data were weighted by the inverse of the SEM to account for varying precision among studies (Roman-Garcia et al., 2016; Brandão et al., 2019).

Analyses were conducted using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). A random coefficients model was specified, with individual studies as random effects. Covariance between the intercept and slope was allowed, and the significance of the covariance parameter was tested at $P < 0.05$. Fifteen variance-covariance structures were evaluated, and the structure yielding the lowest Akaike's information criterion was chosen. Region was included as a secondary random effect to capture geographical variability.

A comprehensive initial model contained the following variables: DGC, DRR, FLA, HMC, RCC, starch level, NDF and forage inclusion in diet. A backward elimination procedure was employed to refine this model: non-significant variables ($P > 0.05$) were sequentially removed, starting with the variable with the highest P-value. At each step, only one variable was eliminated, ensuring that strongly explanatory variables remained in the final model. Although some variables were correlated, the backward elimination inherently prioritized those with the most robust associations, as supported by subsequent analyses.

Final model fit was assessed via R^2 and mean squared error, adjusted for study-level effects (Tables 3–5). Regression estimates are presented as mean ± SEM. Further details on mixed-model methodologies for integrating quantitative results are provided by St-Pierre (2001).

Because this study was not designed to develop predictive models, the focus was on critically evaluating the effects of differing corn grain sources and inclusion levels on key performance and digestive responses in dairy cows. The significance threshold for all fixed and random effects was set at $P < 0.05$.

RESULTS

Dry matter intake decreased with increasing inclusion of HIM and elevated dietary NDF (Table 3, figure 1). Although HIM can enhance ruminal starch availability, high levels may suppress overall feed intake. Dry matter digestibility was negatively affected by DRG, FLA, and REH, corn, dietary starch, NDF, and forage. Similarly, OM digestibility was reduced when DRG and REH were used, indicating that these two corn processing methods may lessen OM matter utilization. Starch digestibility was negatively associated with DRG and DRR, suggesting these forms of mechanical processing may provide less ruminally available starch than other methods. In contrast, NDF digestibility was negatively influenced by FLA, HIM, and dietary starch.

Acetate concentrations increased in diets containing DRG, DRR, and higher forage levels, but decreased with greater dietary starch (Table 4, figure 2). Propionate showed an opposite trend to acetate, exhibiting a positive association with dietary starch and a negative association with DRG and DRR. Ruminal pH decreased significantly when HIM was included in the diet.

Milk production was negatively correlated with DRG, dietary NDF, and forage (Table 5, figure 3). Energy-corrected milk differed from milk yield in its associated variables: HIM and dietary NDF which both showed negative effects on ECM. In this context, while HIM may provide readily fermentable starch, it can also reduce DMI, potentially mitigating any positive effect on overall milk solids production.

Milk fat yield was positively influenced by DRG, DRR, REH, and dietary NDF but decreased with increasing starch (Table 5, figure 3). This result is consistent with a greater proportion of lipogenic precursors (e.g., acetate) arising from fiber fermentation. By contrast, milk protein levels were positively correlated with FLA and dietary starch.

DISCUSSION

Dry matter intake declined with increasing HIM and with greater dietary NDF. One possible explanation for the negative impact of HIM on DMI is that enhanced ruminal starch digestibility can raise energy supply, thereby reducing intake (Torres et al., 2021), Another possibility would be a drop in ruminal pH due to greater starch digestibility and possible subclinical acid, which would result in a drop in consumption (Golder and Lean, 2024). Specifically, in Torres et al. (2021) meta-analysis, when HIM replaced DRG at inclusion rates exceeding 30%, DMI was reduced. In contrast, Allen and Ying (2021), whose study was included in the present meta-analysis database, utilized a diet containing 38.9% corn and 27% starch but did not observe differences in DMI between DRG and HIM, even with this high inclusion rate. They did report, however, that starch intake and gastrointestinal tract digestibility were higher with HIM. Similarly, Eun et al. (2014) and Tye et al. (2017), who used FLA compare to HIM corn, with inclusion rates ranging from 12 to 17% of DM and starch levels below 20%, found that DMI was greater with FLA than with HIM corn, although starch intake and total tract starch digestibility did not differ between treatments. While ruminal starch digestibility was not reported in the current analysis, DMI has been shown to be inversely correlated with ruminal starch digestibility (Firkins et al., 2001). Additional evidence of this effect was described by Oba and Allen (2003), who compared DRG and HIM at two dietary starch levels (21 *vs*. 31%); although HIM lowered DMI at the 31% starch level, no significant differences were observed at 21% starch.

Regarding the negative impact of NDF on the DMI equation, as the NDF content of the diet reflects the fiber concentration of the diet, and forages often provide the primary source of NDF, a negative relationship between DMI and dietary NDF is expected (Shi et al., 2023). Therefore, this observation aligns with the role of dietary NDF in the DMI equation, where it exerted a stronger negative effect on intake than HIM. Organic matter digestibility was negatively affected by DRG, FLA, and REH. However, Oba and Allen (2003) reported that total tract OM digestibility depended more on the dietary starch level than on the corn processing type (DRG *vs.* HIM). In their study, ensiled corn sources increased ruminal starch digestibility, raising ruminal OM digestibility by 20%. Although total tract OM digestibility improved by only 6%, the net effect still favored greater OM digestibility with ensiled corn. In our meta-analysis, diets involving FLA and REH corn typically contained lower levels of starch (25.4%and 24.9, respectively) compared with diets containing DRG (26.8%). High moisture diets exhibited the highest starch concentration (28.6%). Hence, it seems plausible that the starch content in these diets, combined with the ensiling or thermal processes, played a role in the negative inclusion of DRG, FLA, and REH in the OM digestibility equation.

Dry ground corn was also negatively associated with starch digestibility, along with DRR corn. It is important to emphasize that particle size during grinding will influence this. In this study, the works used reported an average particle size of 1.5 mm for DRG, with variation between studies ranging from 0.3 to 4 mm. For DRR, the average particle size was 1.8 mm, varying between 1.1 and 3.2 mm. This variability in particle size affects starch digestibility, with finer particles being more digestible than coarser ones (Ferraretto et al., 2013). Given this, it would be interesting to analyze the impact of particle size on digestibility within this corn category. The negative effect of these categories on starch digestibility, without considering the particle size effect, is likely due to the lack of silage or thermal processing in DRG and DRR compared to the other methods. Silage processing promotes the proteolysis of the prolamins in the endosperm, thereby improving starch accessibility (Hoffmann et al., 2011; Castro et al., 2019). Castro et al. (2019) demonstrated that storage periods greater than 200 days in REH corn can effectively negate the effect of particle size on starch digestibility, likely due to prolonged proteolytic activity. Thermal processing, specifically steam-flaking, increased starch digestibility in the total tract compared to DRG, according to the meta-regression by Rafiee and Darabighane (2021).

Likewise, FLA corn disrupts the protein matrix and gelatinizes starch, thus enhancing starch digestibility (Rafiee and Darabighane, 2021). Because neither DRG nor DRR benefits from extended ensiling or heat treatment, it is logical that both would appear as negative factors in the starch digestibility equation. It is important to emphasize that the coefficient of determination (R^2) for this equation was the lowest (R^2=0.45) among all the variables analyzed. Starch digestibility is influenced by a variety of factors, such as particle size of grinding, type of corn endosperm, silage fermentation time, and the quality and quantity of fiber in the diet (Allen and Ying, 2021). Therefore, it may be necessary to develop a model that includes additional variables to achieve a more reliable outcome. It is important to emphasize once again that further studies are needed to address the issue of particle size for DRG or DRR in dairy cow diets, as the particle size used can often lead to misinterpretations depending on the chosen size.

High moisture corn, FLA, and dietary starch had negative effects on NDF digestibility. Studies focusing on HIM often employed diets with higher starch levels (28.6%, as noted) and the lowest overall NDF (27.1%). Higher starch availability can lead to rapid ruminal acid production, potentially inhibiting fibrolytic bacteria, and reducing fiber degradation. Although the FLA corn category did not generally include such high dietary starch concentrations (25.4%), it nonetheless negatively influenced NDF digestibility. This could be attributed to the large, flattened particles that enhance starch availability (Rafiee and Darabighane, 2021), shifting ruminal fermentation toward more propionate production, which can reduce the pH, and impair fiber digestion. Thus, despite the potential for higher starch digestibility with flaking, there may be trade-offs regarding fiber degradation. The NDF digestibility also exhibited a low R^2 (0.67) when compared to the other equations. This may be due to the high variability of fiber sources used in different regions of the world, as well as the interaction between starch and fiber under various conditions.

With respect to ruminal VFA, both DRG and DRR appeared in the acetate and propionate equations. In the acetate equation, DRG and DRR were positively correlated, whereas in the propionate equation they were negatively correlated. This outcome is consistent with the premise that DRG and DRR have no fermentable processes compared with FLA or HIM, producing less lactate and less propionate, and possibly mitigating the risk of subacute ruminal acidosis. Forage also positively influenced acetate, which aligns with well-established knowledge that fiber digestion supports acetate production (Agle et al., 2010). Conversely, starch level strongly promoted propionate, as diets rich in highly fermentable carbohydrates favor propionogenesis (Wang et al., 2020). Butyrate was not extensively discussed here, but the data indicated a reduction in butyrate concentration with higher starch and NDF, likely reflecting altered ruminal microbial populations and fermentation pathways.

Milk production was negatively associated with DRG, as well as with dietary NDF, and forage inclusion. This finding corroborates with previous equations showing that DRG adversely affected OM digestibility and starch digestibility, thereby reducing the net energy available for lactation. Excessive NDF or forage in the ration likewise can increase rumen fill and diminish the diet’s energy density, reducing milk yield (Shi et al., 2023). Energy-corrected milk followed a slightly different pattern, in which HIM and dietary NDF displayed negative associations, replacing the negative effect of DRG noted for milk yield. These results may reflect the partial suppression of milk fat production in diets high in rapidly fermentable starch, a phenomenon also reported by Torres et al. (2021).

Milk fat was positively correlated with DRG, DRR, and REH corn. In many of these diets, the lower ruminal starch fermentability may have increased the production of acetate and other lipogenic precursors, promoting higher milk fat percentages.

Rehydrated corn diets were also characterized by lower average dietary starch levels in this study, which may explain its positive impact on milk fat. Additionally, dietary NDF positively contributed to milk fat, presumably via enhanced acetate production (Shi et al., 2023), whereas starch was negatively linked to milk fat—a well-documented effect of more rapidly fermentable carbohydrates shifting VFA profiles away from lipogenic precursors (Koch et al., 2019; Liu et al., 2023).

Milk protein displayed a positive correlation with FLA corn and dietary starch, mirroring the importance of readily available energy and, by extension, enhanced microbial protein synthesis (Kim and Lee, 2021). Indeed, Eun et al. (2014) reported that FLA corn promoted higher starch digestibility without depressing ruminal pH, a likely explanation for improved protein output. Steam-flaking's greater extent of starch gelatinization and disruption of the protein matrix presumably helps optimize both microbial fermentation and nutrient availability for milk protein synthesis (Rafiee and Darabighane, 2021).

Overall, these findings reinforce the complexity of balancing starch and fiber sources in dairy diets. Although FLA, HIM and REH processing strategies can improve starch digestibility, each also carries potential drawbacks for feed intake, fiber digestion, or milk component yield. Conversely, DRG and DRR may be advantageous for supporting milk fat, but they can negatively affect total milk yield and nutrient digestibility. Future studies should therefore explore the interplay among corn processing method, dietary starch level, and forage quality to identify optimal feeding strategies that maximize lactation performance while maintaining rumen health.

CONCLUSION

Results from this meta-regression analysis indicate that dry ground corn (particle size average of 1.5mm)—although commonly used—was consistently associated with the greatest reductions in nutrient digestibility, propionate production, and milk yield among the processing methods evaluated. High-moisture corn, in contrast, depressed DMI, lowered ruminal pH, and negatively affected fiber digestibility and ECM, yet did not compromise milk or milk solids production. These findings suggest that caution is warranted when formulating diets with high level of starch using high-moisture corn. Rehydrated corn, analyzed largely in diets with comparatively low starch inclusion, did not emerge as uniformly superior or inferior, underscoring the need for further research on varying corn types across diverse feeding scenarios. Overall, optimizing corn processing methods and dietary composition is critical for achieving desirable production responses while maintaining ruminal health in modern dairy systems.

NOTES

We acknowledge Brazilian foundations for the support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, DF, Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, DF, Brasília), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, MG, Brazil).

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Table 1. Characterization of studies included in the meta-regression analysis

Study	Year	DOI	Journal	Region
Ahmadi et al.	2020	https://doi.org/10.3168/jds.2019-17344	JDS	Middle east
Allen and Ying	2021 a	https://doi.org/10.3168/jds.2020-18881	JDS	North America
Allen and Ying	2021 b	https://doi.org/10.3168/jds.2020-18882	JDS	North America
Arcari et al.	2016	https://doi.org/10.1016/j.anifeedsci.2016.08.005	J. Anim. Sci. Technol.	South America
Castro et al.	2019	https://doi.org/10.3168/jds.2019-16559	JDS	South America
Cooke et al.	2008	doi:10.3168/jds.2007-0715	JDS	North America
Cooke et al.	2009	doi:10.3168/jds.2008-1481	JDS	North America
Crocker et al.	1998	https://doi.org/10.3168/jds.S0022-0302(98)70131-6	JDS	North America
Dhiman et al.	2002	https://doi.org/10.3168/jds.S0022-0302(02)74070-8	JDS	North America
Eastridge et al.	2011	https://doi.org/10.3168/jds.2010-3908	JDS	North America
Ekinci and Broderick	1997	https://doi.org/10.3168/jds.S0022-0302(97)76305-7	JDS	North America
Eun et al.	2014	http://dx.doi.org/ 10.3168/jds.2014-8425	JDS	North America
Ferraretto et al.	2012	http://dx.doi.org/ 10.3168/jds.2011-5190	JDS	North America
Fredin et al.	2015	http://dx.doi.org/ 10.3168/jds.2014-8502	JDS	North America
Gencoglu et al.	2010	doi: 10.3168/jds.2009-2673	JDS	North America
Gomide et al.	2023	https://doi.org/10.3390/ani13121932	Animals	South America
Guyton et al.	2003	https://doi.org/10.3168/jds.S0022-0302(03)74008-9	JDS	North America
Ipharraguerre et al.	2005	https://doi.org/10.3168/jds.S0022-0302(05)72931-3	JDS	North America
Joy te al.	1997	10.3168/jds.S0022-0302(97)76154-X	JDS	North America
Knowlton te al.	1998	https://doi.org/10.3168/jds.S0022-0302(98)75771-6	JDS	North America
Krause and Combs	2003	https://doi.org/10.3168/jds.S0022-0302(03)73722-9	JDS	North America
Krause et al	2003	https://doi.org/10.3168/jds.S0022-0302(03)73719-9	JDS	North America
Krause et al. a	2002	https://doi.org/10.3168/jds.S0022-0302(02)74270-7	JDS	North America
Krause et al. b	2002	https://doi.org/10.3168/jds.S0022-0302(02)74271-9	JDS	North America
Lopes et al.	2009	doi: 10.3168/jds.2009-2090	JDS	North America
Malekkhahi et al.	2021	https://doi.org/10.3168/jds.2020-19202	JDS	Middle east
Martins et al.	2019	https://doi.org/10.3168/jds.2018-15553	JDS	South America
Mathew et al.	2011	https://doi.org/10.3168/jds.2010-3580	JDS	North America

Oba and Allen a	2003	https://doi.org/10.3168/jds.S0022-0302(03)73598-X	JDS	North America
Oba and Allen b	2003	https://doi.org/10.3168/jds.S0022-0302(03)73599-1	JDS	North America
Rafiee-Yarandi et al. a	2019	https://doi.org/10.1016/j.livsci.2019.01.001	Livest. Sci.	Middle east
Rafiee-Yarandi et al. b	2019	https://doi.org/10.1016/j.livsci.2019.01.019	Livest. Sci.	Middle east
Rémond et al.	2004	https://doi.org/10.3168/jds.S0022-0302(04)73288-9	JDS	Europe
San Emeterio et al.	2000	https://doi.org/10.3168/jds.S0022-0302(00)75184-8	JDS	North America
Santos et al.	1999	https://doi.org/10.3168/jds.S0022-0302(99)75290-2	JDS	North America
Savari et al.	2018	https://doi.org/10.3168/jds.2017-12776	JDS	Middle east
Shen et al.	2015	http://dx.doi.org/10.5713/ajas.14.0504	AAAP	Asia
Silva Neto et al.	2023	https://doi.org/10.1016/j.livsci.2023.105292	J. Anim. Sci. Technol.	South America
Taylor and Allen a	2005	https://doi.org/10.3168/jds.S0022-0302(05)72809-5	JDS	North America
Taylor and Allen b	2005	https://doi.org/10.3168/jds.S0022-0302(05)72810-1	JDS	North America
Tye et al.	2017	https://doi.org/10.1139/cjas-2016-0220	CJAS	North America
Uchida et al.	2001	https://doi.org/10.3168/jds.S0022-0302(01)74495-5	JDS	North America
Volker and Allen a	2003	https://doi.org/10.3168/jds.S0022-0302(03)73959-9	JDS	North America
Volker and Allen b	2003	https://doi.org/10.3168/jds.S0022-0302(03)73960-5	JDS	North America
Volker and Allen c	2003	https://doi.org/10.3168/jds.S0022-0302(03)73961-7	JDS	North America
Weiss et al.	2011	https://doi.org/10.3168/jds.2010-3766	JDS	North America
Yu et al.	1998	https://doi.org/10.3168/jds.S0022-0302(98)75634-6	JDS	North America
Zhong et al.	2008	doi:10.3168/jds.2007-0957	JDS	Asia
Zhou et al.	2015	http://dx.doi.org/10.3168/jds.2015-9312	JDS	Asia

Table 2. Descriptive statistics of key variables from the studies included in the meta-regression

Category	Variable	N	Mean	Min.	Max.	Mode	Std Deviation
DRG[1]	Starch, %	76	26.84	16.20	37.50	26.80	4.628
DRR[2]	Starch, %	14	27.20	13.70	35.20	35.20	8.096
FLA[3]	Starch, %	37	25.42	14.10	39.20	26.10	5.556
HIM[4]	Starch, %	30	28.59	14.70	38.70	35.20	7.534
REH[5]	Starch, %	12	24.87	23.13	29.20	23.50	2.117
DRG	Forage, %	78	45.39	30.00	66.10	50.00	7.007
DRR	Forage, %	14	45.77	35.00	55.00	45.30	6.488
FLA	Forage, %	39	44.08	30.00	61.50	35.00	8.526
HIM	Forage, %	30	46.96	39.00	65.80	45.30	7.494
REH	Forage, %	12	48.79	46.03	55.86	46.03	3.119
DRG	NDF, %	78	31.67	23.40	43.80	37.00	4.523
DRR	NDF, %	14	30.76	26.40	37.00	26.40	3.897
FLA	NDF, %	39	32.50	24.90	43.00	30.00	3.504
HIM	NDF, %	30	27.12	23.10	35.70	24.30	3.271
REH	NDF, %	12	35.91	32.53	38.20	36.10	1.728
DRG	DMI, kg/d	77	23.71	15.90	30.10	22.90	2.997

DRR	DMI, kg/d	14	23.63	17.70	27.50	NA	3.217
FLA	DMI, kg/d	38	23.75	17.50	27.80	24.90	2.796
HIM	DMI, kg/d	30	23.12	19.30	25.80	23.40	1.888
REH	DMI, kg/d	12	19.30	16.70	21.00	18.70	1.314
DRG	StarchD, %	73	93.38	69.50	98.40	95.60	4.528
DRR	StarchD, %	13	86.56	76.40	96.60	NA	6.918
FLA	StarchD, %	32	96.08	85.13	99.20	95.10	3.081
HIM	StarchD, %	28	92.05	83.20	98.80	98.20	4.898
REH	StarchD, %	12	95.45	88.30	99.00	96.20	3.343
DRG	Ruminal pH	60	6.15	5.78	6.76	6.04	0.245
DRR	Ruminal pH	11	6.31	5.97	6.6500	NA	0.243
FLA	Ruminal pH	26	6.25	5.91	6.7600	5.9100	0.248
HIM	Ruminal pH	30	6.02	5.61	6.4700	5.9400	0.195
REH	Ruminal pH	6	6.99	6.06	7.4500	7.4400	0.694
DRG	Milk, kg/d	78	36.95	18.20	52.9000	33.8000	7.822
DRR	Milk, kg/d	14	36.1285	21.0000	46.4000	37.2000	6.651
FLA	Milk, kg/d	39	35.9676	25.8000	46.9300	33.1000	5.582
HIM	Milk, kg/d	30	37.4700	28.1000	48.2000	35.9000	4.793

REH	Milk, kg/d	12	23.8658	19.4000	31.1000	19.4000	4.672
DRG	Fat, %	78	3.4626	2.4800	4.3100	3.2900	0.387
DRR	Fat, %	14	3.5216	2.8732	4.0700	NA	0.338
FLA	Fat, %	39	3.2731	2.6200	4.2300	2.9300	0.404
HIM	Fat, %	30	3.4183	2.4300	3.9500	3.4900	0.356
REH	Fat, %	12	3.4675	2.9400	4.1700	3.3900	0.403
DRG	Prot, %	78	2.9743	0.9600	3.5294	2.9500	0.363
DRR	Prot, %	14	2.9394	2.6300	3.1000	NA	0.149
FLA	Prot, %	39	2.9283	0.9700	3.5223	3.0800	0.485
HIM	Prot, %	30	3.0267	2.7957	3.2200	3.0300	0.121
REH	Prot, %	12	3.2683	3.0000	3.5500	3.1300	0.174
DRG	Particle size, mm	57	1.5100	0.3200	4.00000	3.00000	0.913
DRR	Particle size, mm	9	1.8900	1.1100	3.2800	1.1100	0.888
FLA	Particle size, mm	4	4.4100	3.8000	5.3000	NA	0.724
HIM	Particle size, mm	18	2.8006	0.3900	9.5000	0.3900	2.749
REH	Particle size, mm	12	3.6661	0.998	9.0000	0.998	3.114

[1] DRG=dry ground corn

[2] DRR=Dry rolled corn

[3] FLA=Steam-flaked corn

[4] HIM=High moisture corn

[5] REH=Rehydrated corn

Table 3. Equations related to digestibility parameters for different corn processing methods

Variable	Equation	MSE[1]	AIC[2]	R^2
DMI, kg/d	$Y = 28.1026_{\pm 2.0589} - 0.0324_{\pm 0.0119} \times$ HIM[3] $- 0.1790_{\pm 0.0500} \times$ NDF	1.5736	593.7	0.868
OMD[4], kg/d	$Y = 70.7590_{\pm 0.9190} - 0.0646_{\pm 0.0283} \times$ DRG[5] $- 0.1532_{\pm 0.0715} \times$ REH[6] $- 0.0764_{\pm 0.0347} \times$ FLA[7]	6.4893	708.8	0.709
StarchD[8], %	$Y = 95.5763_{\pm 0.6980} - 0.1078_{\pm 0.0103} \times$ DRG $- 0.1577_{\pm 0.0483} \times$ DRR[9]	18.5233	824.0	0.450
NDFD[10], %	$Y = 62.5541_{\pm 4.8552} - 0.1101_{\pm 0.0310} \times$ HIM $- 0.0827_{\pm 0.0261} \times$ FLA $- 0.5276_{\pm 0.1780} \times$ Starch	30.4691	903.4	0.678

MSE[1] = Mean squared error

AIC[2] = Akaike information criterion

HIM[3] = High moisture corn

OMD[4] = Organic matter digestibility

DRG[5] = Dry ground corn

REH[6] = Rehydrated corn

FLA[7] = Steam-flaked corn

StarchD[8] = Starch digestibility

DRR[9] = Dry rolled corn

NDFD[10] = NDF digestibility

Table 4. Equations related to ruminal fermentation parameters for different processing methods.

Variable	Equation	MSE[1]	AIC[2]	R[2]
ACET[3], %	$Y = 58.8708_{\pm 2.9821} + 0.0493 \times$ DRG[4] $+ 0.1063_{\pm 0.0230} \times$ DRR[5] $- 0.1916_{\pm 0.0449} \times$ Starch $+ 0.1343_{\pm 0.0463} \times$ Forage	1.4279	419.4	0.917
PROP[6], %	$Y = 16.3220_{\pm 1.2371} - 0.0405_{\pm 0.0110} \times$ DRG $- 0.0733_{\pm 0.0249} \times$ DRR $+ 0.2677_{\pm 0.0453} \times$ Starch	2.0492	503.9	0.757
BUT[7], %	$Y = 18.1666_{\pm 2.3574} - 0.1113_{\pm 0.0286} \times$ Starch $- 0.1102_{\pm 0.0462} \times$ NDF	0.6235	288.2	0.910
Ruminal pH	$Y = 6.1227_{\pm 0.1751} - 0.0029_{\pm 0.0007} \times$ HIM[8] $+ 0.0059_{\pm 0.0026} \times$ Forage	0.0848	-170.7	0.942

MSE[1] = Mean squared error

AIC[2] = Akaike criterion information

ACET[3] = Acetate

DRG[4] = Dry ground corn

DRR[5] = Dry rolled corn

PROP[6] = Propionate

BUT[7] = Butyrate

HIM[8] = High moisture corn

Table 5. Equations related to milk production and composition parameters or different processing methods.

Variable	Equation	MSE[1]	AIC[2]	R^2
Milk, kg/d	$Y = 45.8010_{\pm 4.6761} - 0.0288_{\pm 0.0073} \times \text{DRG}^{3} - 0.2312_{\pm 0.0630} \times \text{NDF} - 0.1359_{\pm 0.0518} \times \text{Forage}$	1.7062	707.7	0.983
ECM, kg/d	$Y = 33.7697_{\pm 3.8420} - 0.0318_{\pm 0.0154} \times \text{HIM}^{4} - 0.1594_{\pm 0.0644} \times \text{NDF}$	1.6305	808.8	0.968
Fat, kg/d	$Y = 3.0445_{\pm 0.3955} + 0.0041_{\pm 0.0008} \times \text{DRG} + 0.0049_{\pm 0.0018} \times \text{DRR}^{5} + 0.0058_{\pm 0.0028} \times \text{REH}^{6} - 0.0139_{\pm 0.0055} \times \text{Starch} + 0.0208_{\pm 0.0071} \times \text{NDF}$	0.1268	-16.7	0.882
Protein, kg/d	$Y = 2.8985_{\pm 0.0679} + 0.0001_{\pm 0.0003} \times \text{FLA}^{7} + 0.0058_{\pm 0.0015} \times \text{Starch}$	0.0496	-356.1	0.877

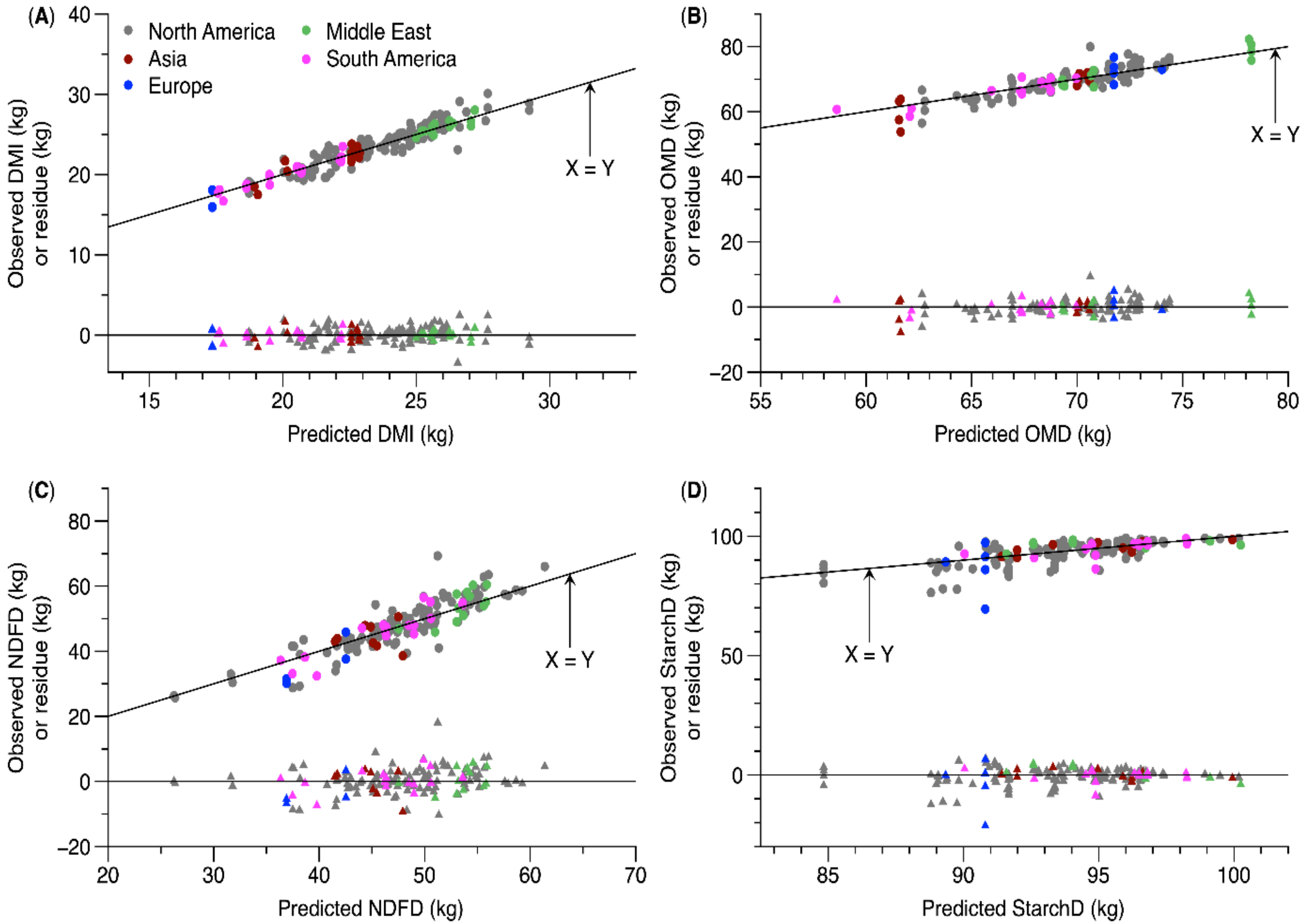
MSE[1] = Mean squared error
AIC[2] = Akaike criterion infomation
DRG[3] = Dry ground corn
HIM[4] = High moisture corn
DRR[5] = Dry rolled corn
REH[6] = Rehydrated corn
FLA[7] = Steam-flaked corn

Figure list.

Figure 1. Observed and predicted values by meta-regression equations for intake and digestibility parameters, the colors of the studies represent the regions to which they belong.

Figure 2. Observed and predicted values by meta-regression equations for volatile fatty acids and ruminal pH, the colors of the studies represent the regions to which they belong.

Figure 3. Observed and predicted values by meta-regression equations for milk production and composition parameters, the colors of the studies represent the regions to which they belong.



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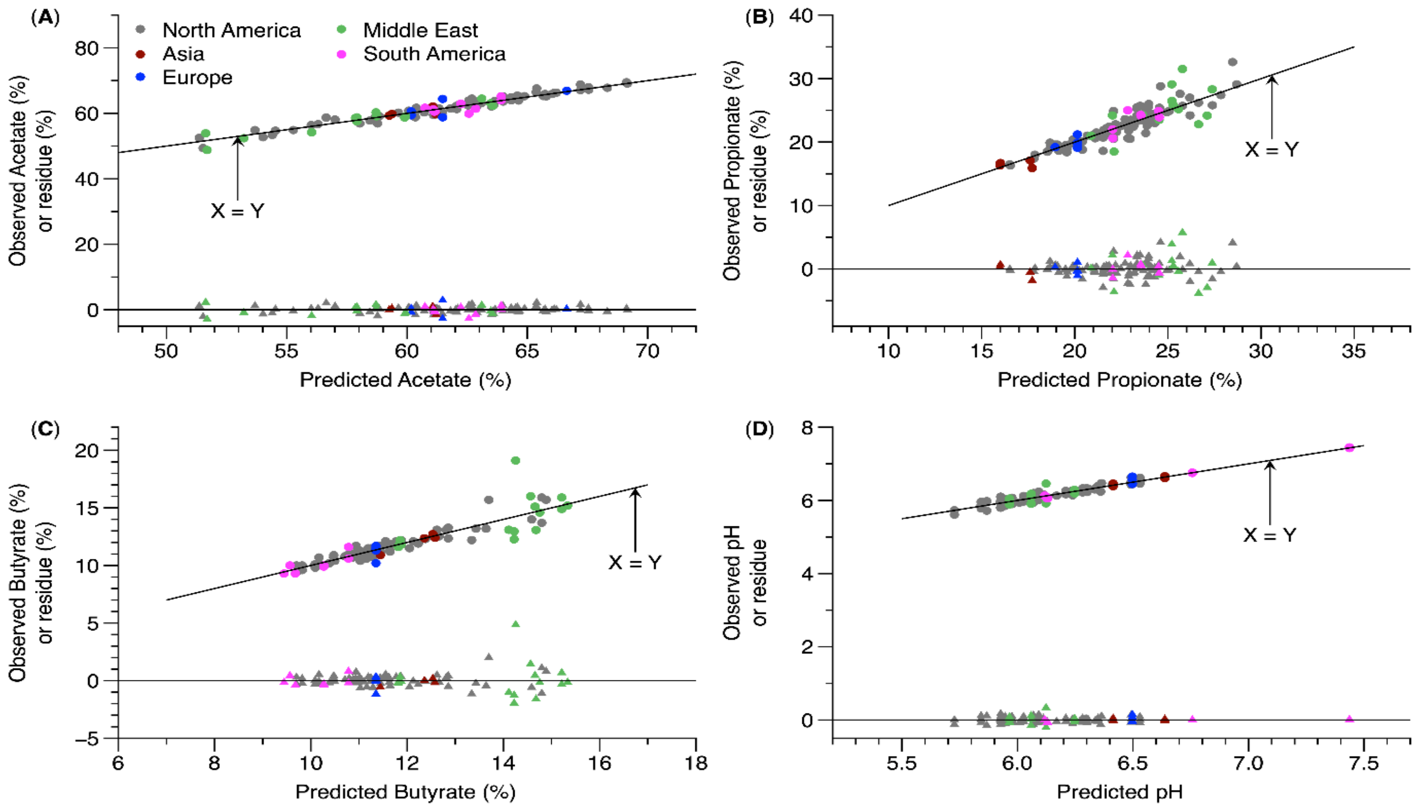
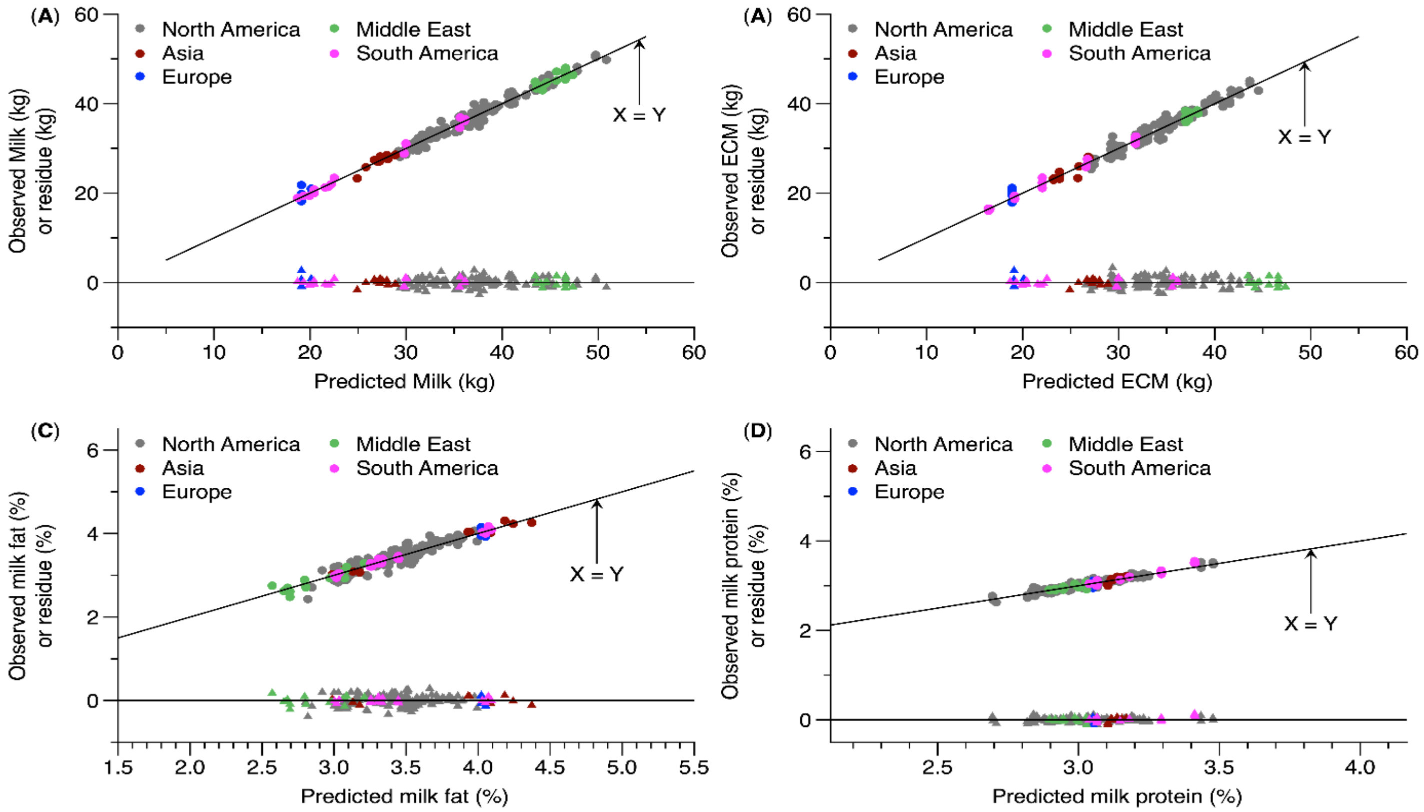
771 Martins, Figure 1.



Martins, Figure 2.



Martins, Figure 3.

GENERAL CONCLUSION

Given the results obtained, the importance of considering the specific conditions in which the work is conducted becomes evident, namely the granulometry of the corn, the possible ensiling duration for storing the corn, and the maturation stage of the corn crop at harvest. Since these factors will influence the digestive and performance parameters of the animals, finely ground dry ground corn (DGC) demonstrated good performance compared to reconstituted corn (RCC) and high-moisture corn (HMC). However, the meta-regression performed revealed negative impacts on digestive and productive parameters when compared to other categories, although particle size variability must be considered. HMC showed a slight advantage in some productive parameters relative to DGC in diets with low starch content and should, when possible, always be ensiled for periods exceeding 180 days to improve starch digestibility. Caution should be exercised when using an additive based on propionic acid, as it had a negative impact on starch digestibility. Additionally, HMC in diets with higher starch levels should be used with care due to its potential negative effect on ruminal pH.