UNIVERSIDADE FEDERAL DE LAVRAS

WESLEY DE REZENDE SILVA

PERFORMANCE OF LACTATING DAIRY COWS FED AN ALPHA-AMYLASE ENABLED CORN

LAVRAS-MG

2025

WESLEY DE REZENDE SILVA

PERFORMANCE OF LACTATING DAIRY COWS FED AN ALPHA-AMYLASE ENABLED CORN

Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para a obtenção do título de Doutor.

Prof. Marcos Neves Pereira
Orientador

LAVRAS-MG

2025

Ficha Catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Silva, Wesley de Rezende.
Performance of lactating dairy cows fed an alpha-amylase enabled corn / Wesley de Rezende Silva. - 2025.
141 p. : il.

Orientador: Marcos Neves Pereira

Tese (Doutorado) - Universidade Federal de Lavras, 2025.
Bibliografia.

1. Nutrição de vacas leiteiras. 2. Amilase em dietas de bovinos leiteiros. 3. Silagem de milho reidratado. I. Pereira, Marcos Neves. II. Universidade Federal de Lavras. III. Título.

WESLEY DE REZENDE SILVA

DESEMPENHO DE VACAS LEITEIRAS ALIMENTADAS COM MILHO DE ALTA CONCENTRAÇÃO DE ALFA AMILASE

PERFORMANCE OF LACTATING DAIRY COWS FED AN ALPHA-AMYLASE ENABLED CORN

Tese apresentada à Universidade Federal de Lavras como parte das exigências do Programa de Pós Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para a obtenção do título de Doutor.

Aprovada em 04 de Agosto de 2025.

Dr. Marcos Neves Pereira, DZO - UFLA.
Dra. Marina de Arruda Camargo Danés, DZO - UFLA.
Dra. Carla Luiza da Silva Ávila, DZO - UFLA.
Dra. Renata Apocalypse Nogueira Pereira, Epamig.
Dr. João Luiz Pratti Daniel, Departamento de Zootecnia - Universidade Estadual de Maringá.

Prof. Marcos Neves Pereira
Orientador

LAVRAS-MG

2025

AGRADECIMENTOS

Agradeço primeiramente a Deus por todas as bençãos colocadas sobre mim, por ter me iluminado e me dado forças para superar todas as adversidades que surgiram ao longo do caminho.

Aos meus pais, Marilza e Edmilson, que sempre me apoiaram e deram força, me educando e me direcionando para o caminho certo, através de todos os ensinamentos que transmitiram ao longo desses anos. Ao meu irmão Pierry por todo o apoio e suporte dado ao longo da minha pós graduação.

À minha noiva Letícia, por todos os conselhos, incentivos e diversas manifestações de carinho, que me fizeram seguir em frente, superando as dificuldades que apareceram ao longo desse caminho. Sem seu apoio nada seria possível.

Ao professor Marcos Neves pela orientação durante todos esses anos como bolsista, membro do Grupo do Leite e pós graduando, pela dedicação e tempo destinado a transferência de conhecimento. A sua esposa Renata, por toda a ajuda, paciência e compreensão dada a mim durante minha trajetória na UFLA.

Aos integrantes do Grupo do Leite por todo o aprendizado, pela ajuda dada durante a condução dos experimentos, pelo bom convívio e amizade formada durante esse tempo.

Aos pós graduandos do Grupo do Leite, pessoas que tive prazer de trabalhar, pela amizade, paciência e apoio durante todo meu doutorado. Em especial ao Josué e o João Pedro, pela parceria, amizade e por toda contribuição durante a realização do meu projeto de pesquisa.

Aos bolsista de iniciação científica por toda a ajuda durante o experimento e realização das análises, em especial a Ana Júlia e a Mariane que me ajudaram durante todo o período do doutorado e hoje só tenho gratidão por todas a horas de empenho dedicadas na realização das análises e pelo companheirismo.

Agradeço aos funcionários da Fazenda São Francisco (Tião, Geraldo, Carlinhos, Tonho e Josi), por todos os momentos de amizade, diversão e trabalho duro que tivemos ao longo desses anos. Por terem se dedicado e contribuído para a realização dos experimentos.

Aos membros do grupo Cow Crew da University of Guelph pelo suporte durante os 8 meses de doutorado sanduíche, em especial ao professor Trevor DeVries que me orientou durante esse tempo, pela oportunidade dada e todo o apoio e ensino durante minha estadia no Canadá.

À Universidade Federal de Lavras e ao Departamento de Zootecnia, pelo excelente ensino.

À Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo apoio durante a realização desse trabalho e disponibilização de recurso para a realização do doutorado sanduíche.

Muito obrigado a todos.

RESUMO GERAL

O uso do milho com alta concentração de alfa amilase (AAC; Enogen, Syngenta) pode ser uma alternativa para melhorar a eficiência alimentar de vacas leiteiras. Nesse projeto, 2 experimentos foram realizados para avaliar os efeitos do fornecimento do milho AAC sobre o desempenho lactacional, consumo, eficiência alimentar, digestibilidade dos nutrientes, perfil fermentativo no rúmen, partição de N e comportamento ingestivo e de ruminação de vacas em lactação. As vacas da raça Holandês (20 vacas no experimento 1 e 24 vacas no experimento 2) formaram grupos de acordo com a paridade e a produção de leite, e foram alocadas em uma das 4 dietas experimentais em delineamento quadrado Latino 4 × 4 com períodos de 21 d (14 d de adaptação). Os tratamentos foram fornecidos em arranjo fatorial 2 × 2. No experimento 1 os fatores foram: concentração de milho [Alta (28,7% de amido, 24,6% de milho e 2,5% de polpa cítrica) vs. Baixa (21,5% de amido, 14,1% de milho e 13,8% de polpa cítrica)] e tipo de milho (AAC vs. controle isogênico - CTL fornecidos na forma de silagem de milho reidratado). No experimento 2 os fatores foram: processamento do milho (milho reidratado e ensilado vs. milho moído) e tipo de milho (AAC vs. CTL). As dietas do experimento 2 tinham 22,4% de amido (% da MS). A vitreosidade dos híbridos utilizados foi 48,8% em AAC e 51,1% em CTL nos experimentos 1 e 2. O milho reidratado e ensilado dos experimentos 1 e 2 foram armazenados por 28 ± 3 d antes do fornecimento aos animais. O uso do AAC na forma de concentrado melhorou a digestibilidade da fibra em detergente neutro (experimento 1) e do amido (experimento 2) e aumentou a eficiência alimentar quando fornecido em dieta com baixa concentração de milho, sem afetar a produção de leite e o consumo de matéria seca. A composição do leite e o comportamento ingestivo dos animais foram pouco afetados pelo fornecimento do AAC em ambos os experimentos. O milho AAC não afetou a proporção dos principais ácidos graxos voláteis e a síntese microbiana no rúmen, mas afetou a proporção molar dos ácidos graxos de cadeia ramificada no experimento 1. A partição de N não foi afetada pela suplementação de AAC no experimento 2, mas quando fornecido em combinação com a dieta de alta inclusão de milho (experimento 1) reduziu o N-ureico no leite e no sangue, e a excreção de N pela excreta (fezes + urina). O fornecimento do AAC pode melhorar a eficiência alimentar de vacas leiteiras, mas esse resultado é aparentemente influenciado pelo perfil de carboidratos que compõe a dieta.

Palavras-chave: enzima; Enogen; processamento; amido.

GENERAL ABSTRACT

The alpha-amylase enabled corn (AAC; Enogen, Syngenta) may be an alternative to improve feed efficiency of lactating dairy cows. Three experiments were conducted to evaluate the effects of the AAC corn on lactation performance, intake, feed efficiency, total tract digestibility, ruminal fermentation profile, N partition, and eating and rumination behaviors of lactating dairy cows. Holstein cows (20 cows in experiment 1; and 24 cows in experiment 2) formed groups based on parity and milk yield and were allocated to one of 4 treatments using a 4 × 4 Latin Square design with 21-d periods (14 d for diet adaptation). Treatments were in a 2 × 2 factorial arrangement. The factors evaluated in experiment 1 were: corn concentration [High (28.7% starch, 24.6% corn, 2.5% citrus pulp) vs. Low (21.5% starch, 14.1% corn, 13.8% citrus pulp)] and corn type (AAC vs. Isogenic control - CTL fed as a rehydrate and ensiled corn). The factors evaluated in experiment 2 were: corn processing (rehydrated and ensiled corn vs. ground corn) and Factor 2) corn type (AAC vs. CTL). Diets in experiment 2 had 22.4% of starch on DM basis. Vitreousness of corn hybrids was 48.8% for AAC and 51.1% for CTL in experiments 1 and 2. Rehydrated and ensiled corn used in experiments 1 and 2 were stored for 28 ± 3 d before fed to the animals. The utilization of AAC fed as a concentrate source improved neutral detergent fiber (experiment 1) and starch digestibility (experiment 2) and improved feed efficiency in diets with low corn concentration, without affecting milk yield and dry matter intake. No major effects of AAC on milk composition and chewing behavior were observed in both experiments. The AAC corn did not affect the molar proportion of the major volatile fatty acids in the rumen and the ruminal microbial synthesis but affected the proportion of ruminal branched chain volatile fatty acids in experiment 1. The N partition was not affected by AAC in experiment 2, but when fed in a diet with high corn concentration (experiment 1) AAC decreased blood and plasma urea-N, and the excretion of N in total excreta (feces + urine). There is a possibility of using AAC as rehydrated and ensiled corn to improve lactating dairy cows efficiency, but it seems to be affected by the carbohydrate profile of the diet.

Keywords: Enzyme; Enogen; processing; starch.

INDICADORES DE IMPACTO

Devido ao avanço no melhoramento genético e na intensificação da produção de leite, as vacas leiteiras têm se tornado cada vez mais produtivas, o que resultou em aumento das exigências nutricionais. Por isso, a adoção de estratégias que aumentam a degradação ruminal do amido e a digestibilidade do amido no trato digestivo total podem auxiliar no atendimento dos requerimentos nutricionais e favorecer o desempenho produtivo e a eficiência alimentar dos animais. No Brasil, a digestibilidade do amido dos híbridos de milho cultivados é comprometida devido a alta vitreosidade dos mesmos, fazendo com que alternativas capazes de favorecer a digestão do amido sejam consideradas por nutricionistas e produtores. O ganho em eficiência alimentar através da utilização de fontes de amido mais degradáveis pode reduzir a emissão de metano e favorecer a assimilação de nitrogênio pelos microorganismos ruminais, reduzindo a excreção de nitrogênio pelas fezes e urina. Além disso, o aumento da eficiência alimentar pode reduzir o custo com alimentação dos animais e aumentar a lucratividade da propriedade. Portanto, estratégias nutricionais, como a utilização do milho com alta concentração de amilase, capazes de aumentar a eficiência alimentar e de utilização de nitrogênio em bovinos leiteiros pode resultar em aumento da lucratividade e em redução do impacto ambiental gerado pela produção de leite.

IMPACT INDICATORS

Throughout the years, dairy cows have been selected based on their genetic merit for milk yield, resulting in increased nutritional requirements. Therefore, nutritional strategies that can enhance ruminal starch degradation and total tract starch digestibility may help nutritionists meet the increasing nutritional demands of high-producing dairy cows, thereby improving productive performance and feed efficiency. In Brazil, the digestibility of starch in conventional corn hybrids is frequently reduced because of their high vitreousness. This has led nutritionists and dairy producers to adopt nutritional strategies with the objective of improving starch digestion in lactating cows. Improving feed efficiency with the utilization of more digestible starch sources can reduce methane emissions and improve nitrogen utilization by ruminal microorganisms, decreasing nitrogen in feces and urine. Furthermore, it can decrease feed cost and improve profitability. Therefore, nutritional strategies such as the utilization of an alpha-amylase enabled corn, that can increase feed efficiency and nitrogen usage efficiency in dairy cows, have the potential to improve profitability and reduce the environmental impact of milk production.

Alpha-amylase enabled corn on lactating dairy cow diets

Elaborated by **Wesley R. Silva** and advised by **Dr. Marcos N. Pereira**

Corn is the main energy source used for dairy cows due to its high starch content. Brazilian corn hybrids are known to have high concentrations of prolamin, a protein that impair starch digestibility in the digestive tract of animals. Therefore, strategies that improve starch digestibility can be an alternative to increase milk yield and feed efficiency of dairy cows. The alpha-amylase enabled corn (AAC. Enogen, Syngenta) may be an alternative to improve nutrient digestibility, feed efficiency and lactation performance of lactating dairy cows. Two experiments were conducted to evaluate the effects of the alpha-amylase enabled corn on lactation performance, intake, feed efficiency, total tract digestibility, ruminal microbial synthesis and fermentation profile, and eating and rumination behaviors of lactating dairy cows. Cows formed groups based on parity and milk yield and allocated to one of 4 treatments using a 4 × 4 Latin Square design with 21-day periods (14 days for diet adaptation). Treatments were a 2 × 2 factorial arrangement of corn concentration (low vs. high) and corn type [AAC vs. Isogenic control (CTL) fed as a rehydrate and ensiled corn] in experiment 1; and processing (rehydrated and ensiled corn vs. ground corn) and corn type (AAC vs. CTL) in experiment 2. Holstein cows were used in all experiments (20 and 24 cows in each experiment, respectively). In general, AAC can improve nutrient digestibility (NDF and starch) when fed as rehydrated and ensiled corn and improve feed efficiency in diets with low corn concentration, without affecting milk yield and dry matter intake. There is a possibility of using AAC as rehydrated and ensiled corn to improve lactating dairy cows efficiency, but it seems to be affected by the carbohydrate profile of the diet.

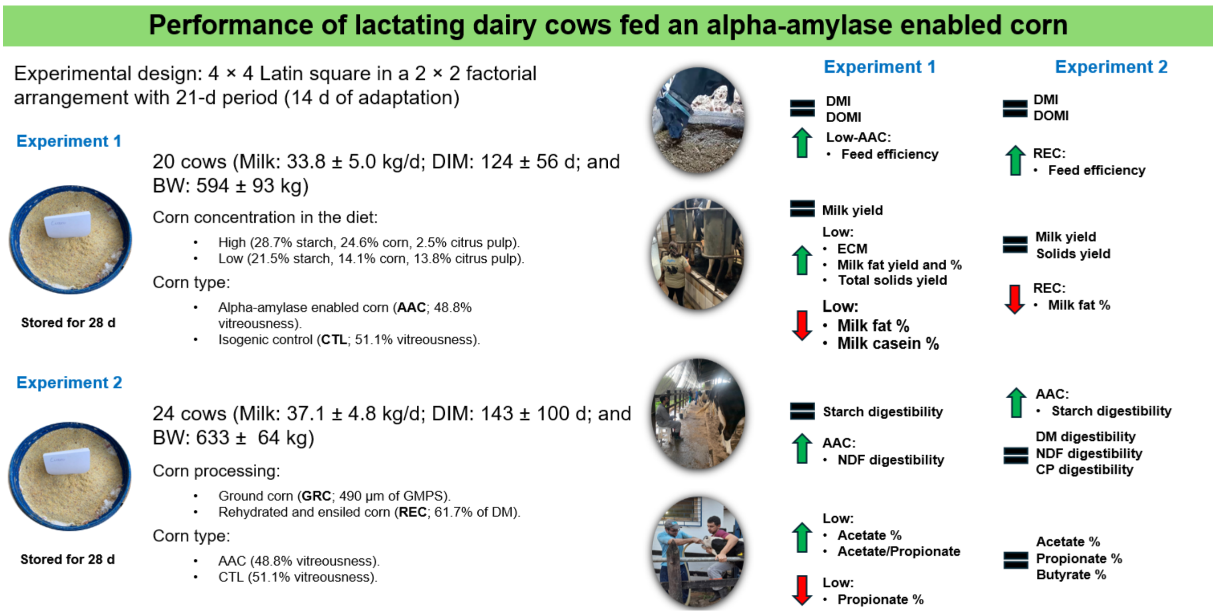

Figure 1. Lactation performance of dairy fed an alpha-amylase enabled corn.

PhD Thesis in Animal Science at UFLA, defended on August 04, 2025.

SUMÁRIO

GENERAL INTRODUCTION

Corn is frequently included in diets for lactating dairy cows as a key energy ingredient, given its elevated starch concentration (*National Academies of Sciences Engineering Medicine* - NASEM, 2021). Although other non-fibrous carbohydrates can be used as energy source, starch seems to maximize ruminal microbial synthesis (Hall and Herejk, 2001) and animal performance (Leiva, Hall, and Van Horn, 2000; Broderick, Mertens, and Simons, 2002; Salvador *et al.*, 2008). Recommended starch levels in dairy cow diets typically range from 22% to 30% of dietary dry matter (DM) (NASEM, 2021). The inclusion of starch in the diet should consider the variability in ruminal starch degradation among different starch sources, as well as the physical effectiveness of fiber, particularly the proportion of long particles in the ration (Zebeli et al., 2012; NASEM, 2021).

Total tract starch digestibility can vary from 80 to 90% of starch intake based on the different starch sources, processing of corn kernels, and diet composition (Nocek and Tamminga, 1991; Firkins, 2001; Ferraretto, Crump, Shaver, 2013). However, the variability in ruminal starch degradation is typically greater than that observed for total tract starch digestibility (ranging from 40% to 90% of starch intake) (Nocek and Tamminga, 1991; Firkins *et al.*, 2001; Ferraretto, Crump, and Shaver, 2013), suggesting that intestinal starch digestion may partially compensate for impaired ruminal degradation. This variation in starch digestibility and degradation influences both the site of starch digestion and the efficiency of nutrient utilization, ultimately affecting animal performance.

Increasing ruminal starch degradation can be associated with a shift in ruminal fermentation pattern towards an increase in propionate production (Oba and Allen, 2003a, b; Bannink *et al.*, 2006). Propionate is the major gluconeogenic precursor in ruminants (Amaral *et al.*, 1990; Nocek and Tamminga, 1991; Huntington, 1997). Therefore, an increase in starch degradation can improve the supply of propionate to the liver (Nocek e Tamminga, 1991), which can reduce DM intake (DMI) (Allen, Bradford, and Oba, 2009) and affect eating (Allen, 2000; Oba and Allen, 2003a) and sorting behavior (DeVries, Dohme, and Beauchemin, 2008; Maulfair, McIntyre, and Heinrichs, 2013) of dairy cows. Furthermore, glucose produced from propionate can be used in the mammary gland as a substrate for lactose production and increase milk yield (Osorio, Lohakare e Bionaz, 2016; Costa *et al.*, 2019). Increasing ruminal starch availability can stimulate microbial synthesis (Sniffen and Robinson, 1986; Firkins *et al.*, 2001; Hall and Herejk, 2001; NASEM, 2021) and increase the supply of metabolizable protein, which may positively affect milk production and milk solids concentration and secretion (Lapierre *et al.*, 2012; Lean *et al.*, 2018; Yoder *et al.*, 2020). Those responses can result in an improvement in feed efficiency by a decrease in DMI or an increase in milk yield when ruminal starch degradation is increased (Allen, 2000; Firkins *et al.*, 2001; Andreazzi *et al.*, 2018; Castro *et al.*, 2019).

Brazilian corn hybrids have been reported to exhibit lower starch digestibility and degradation than those cultivated in other countries, mainly attributed to their greater kernel vitreousness (Correa *et al.*, 2002). Vitreousness is associated with the concentration of prolamin proteins in the corn kernel endosperm (Philippeau, Landry, Michalet-Doreau, 2000; Holding, 2014). In corn kernels, the main prolamin proteins are referred to as zeins, which can represent 40 to 60% of total protein in corn kernels (Hamaker *et al.*, 1995). Zeins are synthesized on the rough endoplasmic reticulum of the amyloplast (Lending *et al.*, 1988) and can be classified into α, β, γ, and δ subclasses based on their solubility and ability to form disulfide bonds (Esen, 1987). Zeins are embedded within the protein matrix that envelops the starch granules in the corn kernel endosperm (Mu-Foster and Wasserman, 1998), forming the characteristic starch–protein matrix. The amount of zeins in this matrix can influence the morphology of starch granules and the strength of their interactions (Coleman and Larkins, 1999). These proteins encapsulate starch granules within a hydrophobic starch-protein matrix (Mu-Foster and Wasserman, 1998), reducing the accessibility of ruminal microorganisms (McAllister *et al.*, 1993) and limiting ruminal starch degradation, which ultimately impairs starch digestibility in ruminants (Philippeau and Michalet-Doreau, 1998; Correa *et al.*, 2022). Therefore, there is an inverse relationship between vitreousness and ruminal starch degradation (Philippeau, Landry, Michalet-Doreau, 2000; Correa *et al.*, 2002; Varela *et al.*, 2023).

Ruminal starch degradation (81.6 vs 71.1% of starch) and the rapidly degradable starch fraction (51.9 vs 35.1% of starch), evaluated *in situ*, were reduced in vitreous corn (55.4% vitreousness) compared to floury corn (40.3% vitreousness), regardless of the conservation method (ensiled or unensiled) or preparation form (chopped corn plant or ground corn kernels) used (Philippeau and Michalet-Doreau, 1998). Similarly, Philippeau, De Moredon, and Michalet-Doreau (1999) evaluated the *in situ* kinetics of starch degradation in vitreous (71.8% vitreousness) and floury (51.4%

vitreousness) corn and observed reduced starch degradation (61.9 vs 46.2% of starch), a lower rapid degradable starch fraction (26.6 vs 19.4% of starch), and a slower fractional degradation rate (5.9 vs 3.9%/hour) in the vitreous corn. Additionally, these variables were positively and significantly correlated with DM degradation (Philippeau, De Moredon, and Michalet-Doreau, 1999), which can impair energy supply and lactation performance of lactating dairy cows.

In a sequence of published papers, Taylor and Allen (2005a, b, c) evaluated the effects of the supplementation of dairy cows with vitreous (67.2% of vitreousness) or floury (3.0% of vitreousness) corn fed as ground corn kernels in diets with 38.7% of forage, 22.7% of ground corn, and 29.6% of starch, on DM basis. The utilization of the vitreous corn decreased ruminal starch degradation (46.3 vs 62.1% of starch intake), ruminal digestion rate (12.9 vs 21.9%/hour), and total tract starch digestibility (91.7 vs 96.3% of starch intake), and increased the passage of starch from the rumen to the duodenum (56.8 vs 39.3% of starch intake) compared to the floury corn (Taylor and Allen, 2005c). Furthermore, the improvement in ruminal starch degradation with floury corn supplementation decreased the mean ruminal pH, acetate proportion in ruminal fluid, and the acetate to propionate ratio, with an increase in the propionate proportion (Taylor and Allen, 2005b). Although corn kernel vitreousness affected the fermentation process in the rumen, N metabolism in the rumen and N supply to the animal was not influenced by treatments (Taylor and Allen, 2005b). There were no effects of corn kernel vitreousness in lactation performance and DMI, but floury corn tended to improve feed efficiency (1.68 vs 1.64; 3.5% fat corrected milk/DMI) in the study of Taylor and Allen (2005a). The authors observed that the utilization of vitreous corn decreased total chewing time (713 vs 742 min/day) and eating time (250 vs 263 min/day) of the cows (Taylor and Allen, 2005a).

In the study of Lopes *et al.* (2009), lactating dairy cows were fed rolled shelled corn from conventional (64% vitreousness), floury, or opaque corn hybrids (both with 0% vitreousness) in diets containing alfalfa silage as the sole forage source and 19.4% starch on DM basis. Dry matter intake was similar across treatments (26.4, 25.3, and 25.2 kg/day, respectively), as was milk yield (44.0, 45.1, and 46.5 kg/day, respectively). However, milk protein concentration was lower in cows fed conventional corn compared to those fed floury or opaque corn (2.63, 2.77, and 2.68%, respectively) (Lopes et al., 2009). In the same study, conventional corn resulted in reduced total tract starch digestibility (89.6, 95.1, and 96.6% of starch intake, respectively) and lower *in situ* starch disappearance at 0 (9.5, 24.9, and 11.8%), 8 (34.7, 69.6, and 63.3%), and 16 hours (57.3, 85.7, and 85.2%) compared to floury and opaque hybrids. These reductions in starch degradation were associated with decreased ruminal concentrations of propionate and butyrate, and increased ruminal pH, acetate concentration, and acetate:propionate ratio in cows fed conventional corn (Lopes et al., 2009).

Allen and Ying (2021a, b) observed that the replacement of a vitreous corn (81% of vitreousness) by a floury corn (8.6% of vitreousness) fed as a high moisture (35.5% of diet DM) or ground corn (35.7% of diet DM) in diets with 41.3% (on DM basis) of alfalfa silage as the sole forage source and 28.2% (on DM basis) of starch did not affect milk yield (47.4 vs 47.5 kg/day), and DM (23.4 vs 23.8 kg/day) and starch (6.97 vs 6.87 g/day) intake. However, floury corn increased ruminal degradation of starch (83.7 vs 67.7% of starch intake), ruminal degradation rate (31.5 vs 25.4%/hour), and total tract starch digestibility (97.9 vs 96.9% of starch intake) (Allen and Ying, 2021b). The effects of corn vitreousness on starch degradation greatly affected ruminal fermentation pattern, with floury corn increasing propionate concentration in ruminal fluid, and decreasing acetate and the acetate to propionate ratio (Allen and Ying, 2021a). Furthermore, feeding floury corn improved microbial N flow to the duodenum (71.8 vs 60.2% of duodenal non ammonia N) and ruminal true N digestion (78.5 vs 70.9% of N intake) (Allen and Ying, 2021a). Although vitreousness affected ruminal fermentation profile, meal patterns and feeding behavior were not affected by treatments (Allen and Ying, 2021a).

In another study, floury corn (24.5% of vitreousness) tended to decrease DMI (23.8 vs 25.1 kg/day) relative to vitreous corn (66.4% of vitreousness) fed as a ground corn (38.9% of diet DM) in diets with 40.9% (on DM basis) of alfalfa silage and 29.2% (on DM basis) of starch. However, no difference in milk yield and milk solids yield between treatments was observed (Allen, Longuski, and Ying, 2021a). Allen, Longuski, and Ying (2021b) observed an increase in starch intake (8.15 vs 6.94 kg/day) and a decrease in ruminal degradation of starch (27.3 vs 57.5% of starch intake) and total tract starch digestibility (85.1 vs 92.2% of starch intake) on cows fed the vitreous corn. The replacement of vitreous corn by floury corn increased ruminal propionate concentration and decreased the ruminal acetate concentration and acetate to propionate ratio, but did not affect ruminal N metabolism (Allen, Longuski, and Ying, 2021a, b). Furthermore, floury corn tended to increase meal frequency (9.68 vs 9.58

meals/day) and decreased eating time (256 vs 270 min/day) compared to vitreous corn (Allen, Longuski, and Ying, 2021a).

In general, the results demonstrate that corn kernel vitreousness plays a critical role in modulating starch digestibility and ruminal fermentation in lactating dairy cows. Diets containing floury corn hybrids, can improve ruminal starch degradation, increase propionate concentration, and reduce ruminal pH and acetate:propionate ratio. Although increased ruminal starch degradation can reduce DMI (Allen et al., 2009), changes in DMI or milk yield are not consistently observed when vitreous corn are replaced by floury corn hybrids. However, minor improvements in feed efficiency and milk protein concentration have been reported in some cases. Despite the pronounced effects on ruminal fermentation patterns and starch digestion kinetics, responses in N metabolism and feeding behavior were generally limited or inconsistent. Overall, the replacement of vitreous corn with floury corn appears to enhance starch utilization in the rumen, potentially improving nutrient availability, which may lead to an improvement in animal performance.

The adoption of strategies that can improve starch digestion in ruminants are important, especially in Brazil. Corn kernel processing is a well-known method to increase starch digestibility in ruminants, by increasing surface area to ruminal microbial attachment and enzymatic degradation (Huntington, 1997). Therefore, reduction in particle size of ground corn kernels can be positively associated with starch digestibility (Dias Junior *et al.*, 2016; Ahmadi *et al.*, 2020). Evidence indicates that corn kernel vitreousness is not correlated with particle size after grinding, implying limited impact of vitreousness on grinding resistance (Philippeau, De Monredon, Michalet-Doreau, 1999). Although grinding can enhance corn starch digestibility, it does not overcome the negative impact of vitreousness on starch degradation.

Throughout the silage fermentation process, microbial enzymes, endogenous plant enzymes (Simpson, 2001; Junges *et al.*, 2017), and fermentation acids produced by bacterial growth (Lawton, 2002) can contribute to the degradation of the protein matrix in the corn kernel endosperm. Moreover, a positive relationship has been reported between storage duration and starch digestibility in whole plant corn silage (Kung Jr *et al.*, 2018; Varela *et al.*, 2023), high moisture corn (Hoffman *et al.*, 2011; Kung Jr, Windle, and Walker, 2014), and rehydrated and ensiled corn (Fernandes *et al.*, 2022). This association is likely driven by the cumulative activity of enzymes over time, which promotes the breakdown of prolamins during storage (Hoffman *et al.*, 2011; Varela *et al.*, 2023).

Hoffman et al. (2001) reported that ensiling high moisture corn for 240 days reduced the α-, β-, γ-, and δ-prolamin subunits compared to unensiled corn, which was associated with an increase in ammonia concentration throughout the storage period. Varela *et al.* (2023) ensiled whole plant corn silages from different genotypes for 0, 30, 60, 120, and 240 days to evaluate those effects on fermentation profile, prolamin, and starch degradation. Regardless of genotype, increasing storage length improved *in vitro* starch degradation in 7 hours incubation, with higher values at 120 (65.8% of starch) and 240 (68.4% of starch) days compared to 30 (62.6% of starch) and 60 (62.4% of starch) days of ensiling (Varela *et al.*, 2023). In the same study, total zeins and α-zeins subunits decreased, and both ammonia N and soluble protein increased over time. However, an interaction between corn genotype and storage length was observed, and the genotype with higher vitreousness (74.5 vs 38.8% of vitreousness) had higher α-zeins subunits concentration in all storage times evaluated. Furthermore, *in vitro* starch degradation in 7 hours incubation was negatively associated with total zeins ($r = -0.69$) and α-zeins subunits ($r = -0.72$), but positively correlated with soluble protein ($r = 0.76$), and ammonia N ($r = 0.67$) (Varela *et al.* 2023). These results demonstrate that extending the storage period of silages mitigates the negative effects of kernel vitreousness on starch degradation, primarily through the breakdown of prolamins. Moreover, the concentrations of soluble protein and ammonia N in silages may serve as useful indicators of prolamin degradation and starch digestibility.

Although corn kernel processing and conservation methods are commonly used to improve starch digestibility, the use of exogenous amylases may be an alternative to enhance starch utilization in dairy cows (Pech-Cervantes, Ferraretto, and Ogunade, 2022). However, the results of amylase supplementation do not improve starch digestibility in all situations (Gencoglu *et al.*, 2010; Weiss, Steinberg e Engstrom, 2011; Nozière *et al.*, 2014). Andreazzi *et al.* (2018) provided exogenous alpha amylase to lactating dairy cows for 63 days (± 10 g/day) in diets containing 32.1% of starch (% of DM) and whole plant corn silage, rehydrated and ensiled corn, and ground corn as starch sources. The authors did not observe difference between control and amylase treatments in the total tract digestibilities of DM (69.4 vs. 70.3% of DMI), organic matter (OM) (70.9 vs. 71.5% of OM intake), neutral detergent fiber (NDF) (44.3 vs 44.4% of NDF intake), and starch (96.1 vs 96.4% of starch intake), and in ruminal fermentation profile (Andreazzi *et al.*, 2018). Although nutrients digestibility was not affected by treatments, milk yield (32.3 vs. 33.0 kg/day) increased and DMI (20.7 vs. 19.7 kg/day) decreased in cows fed the enzyme, which led to an improvement in feed efficiency (milk yield/DMI) (1.58 vs. 1.70) (Andreazzi *et al.*, 2018). Furthermore, enzyme supplementation decreased chewing time and meal frequency while increasing the selection for long particles throughout the day (Andreazzi *et al.*, 2018).

Dairy cows fed ± 13 g/day of exogenous amylase for 12 weeks and diets with 21.2% of starch (on DM basis) from ground corn and whole plant corn silage had lower DMI (25.9 vs. 29.1 kg/day) and similar milk yield (50.4 vs. 50.9 kg/day) in comparison to control treatment, those responses increased feed efficiency from 1.77 to 1.98 in amylase treatment (Gencoglu *et al.*, 2010). In the same study, exogenous amylase improved total tract DM (71.0 vs. 74.7% of DMI), OM (73.1 vs. 76.6% of OM intake), crude protein (CP) (68.8 vs. 73.0% of CP intake), and NDF (54.1 vs. 57.8% of NDF intake) digestibility, but no difference in total tract starch digestibility was observed (97.2 vs. 97.6% of starch intake) (Gencoglu *et al.*, 2010).

The supplementation of exogenous amylase (30.8 g/day) in diets with high (31.1% of starch on DM basis) or low (25.9% of starch on DM basis) starch content, and coarse ground corn and whole plant corn silage as starch sources for dairy cows in a Latin square (24-days period) experiment did not affect lactation performance (Weiss, Steinberg e Engstrom, 2011). However, the enzyme treatment tended to increase NDF digestibility (49.7 vs. 51.0% of NDF intake) without affecting starch digestibility (87.7 vs. 88.0% of starch intake) regardless of starch concentration of the diet. Similarly, Nozière *et al.* (2014) did not observe differences in total tract starch digestibility (98.0 vs. 98.4% of starch intake) when lactating dairy cows were fed exogenous amylase (10 g/day) in diets containing 30 or 20% of starch (% of diet DM) when ground corn was the major starch source. Besides, in the same study, the amylase treatment increased ruminal starch degradation by 8.7% (75.1 vs. 81.6% of starch intake) (Nozière *et al.,* 2014). The *in vivo* experiments conducted with exogenous amylase normally evaluated the total tract starch digestibility. However, Nozière *et al.* (2014) findings suggests that post rumen starch digestibility can compensate for a decrease in ruminal starch degradation in cows fed control diets relative to exogenous amylase fed cows. Therefore, the utilization of amylase may change the site and extent of starch degradation without affecting total tract starch digestibility. There is evidence that exogenous amylase can improve NDF digestibility due to a possible increase in ruminal availability of starch hydrolysis products to fiber digesting bacteria (Cotta, 1988).

In a recent meta-analysis, Pech-Cervantes, Ferraretto, and Ogunade (2022) synthesized data from 18 studies evaluating the effects of exogenous amylase supplementation in diets for lactating dairy cows. Although amylase supplementation improved total tract digestibilities of DM and starch, it did not significantly affect DMI, milk yield, feed efficiency, or total tract NDF digestibility. The authors suggested that variations in dietary starch and NDF concentrations, experimental design, and enzyme dosage may partially explain differences in animal responses. However, days in milk appeared to exert a greater influence on the response of lactating dairy cows to amylase supplementation than the factors previously mentioned. Under these conditions, dairy cows fed an alpha-amylase enabled (AAC; Enogen, Syngenta) corn may have similar response to the utilization of exogenous amylase.

The AAC is a transgenic corn that contains a microbial gene able to increase the expression alpha-amylase in the endosperm of corn kernels (APHIS, 2008). The genus *Thermococcus* has been utilized in the production of AAC due to its ability to produce a thermostable alpha-amylase (AMY797E) (APHIS, 2008). This technology was initially developed as an alternative to improve the efficiency of ethanol production in the industry (APHIS, 2008). However, the utilization of AAC as a feed ingredient for dairy (Cueva *et al.*, 2021; Krogstad and Bradford, 2023; Silva *et al.*, 2025) and beef cattle (Jolly-Breithaupt *et al.*, 2018; Johnson *et al.*, 2019; Volk *et al.*, 2021) has been evaluated either as a forage (Cueva *et al.*, 2021, 2025; Krogstad and Bradford, 2023) or a concentrate source (Horton *et al.*, 2020; Rebelo *et al.*, 2023; Silva *et al.*, 2025).

The alpha-amylase trait was not expected to affect nutrient composition of the plant, except for increasing amylase concentration and activity in the corn kernels (APHIS, 2008). Silva et al. (2025) did not observe differences between rehydrated and ensiled AAC and isogenic control corn in nutrient composition. However, Heinzen Jr. et al. (2024) found higher starch concentration and lower NDF and acid detergent fiber concentrations in whole plant corn silage (harvested at 2/3 to 3/4 kernel milk line for both silages) and earlage silage produced from AAC relative to control, which may be related to maturity at harvesting, as AAC (37.4 and 59.4% on an as-fed basis, respectively) had higher DM concentration than control (34.2 and 57.8% on an as-fed basis, respectively) in both silages. The evidence suggests that the nutritional composition of AAC and its isogenic counterpart is expected to be similar when both are harvested at the same stage of maturity. Therefore, differences in animal response to AAC supplementation are likely attributable to the presence of alpha-amylase and its interaction with other dietary factors.

Silva *et al.* (2025) fed lactating dairy cows with AAC as a rehydrated and ensiled corn (28 ± 3 days of storage) in diets with high (28.7% of starch, 24.6% of corn, and 2.5% of citrus pulp on DM basis) or low (21.5% of starch, 14.1% of corn, and 13.8% of citrus pulp on DM basis) corn concentration. There were no statistical differences between AAC and control on DMI and milk yield, but AAC in combination with low corn diet had the highest feed efficiency (energy corrected milk yield/DMI) among treatments (1.54 vs. 1.48) (Silva *et al.*, 2025). Furthermore, AAC tended to increase total tract NDF digestibility (49.9 vs. 48.0% of NDF intake) with no effects on starch digestibility (98.5 vs. 98.5% of starch intake) and ruminal fermentation profile (Silva *et al.*, 2025). The combination of AAC and a high corn concentration diet reduced milk urea-N and plasma urea-N compared to AAC in a low corn concentration diet, resulting in lower N concentrations in feces and urine relative to the other treatments (Silva *et al.*, 2025). In the same study, cows fed AAC with a high corn diet exhibited reduced meal frequency compared to those fed AAC with a low corn diet, while AAC supplementation had no major impact on rumination, chewing, or sorting behaviors (Silva et al., 2025).

Supplementation of dairy cows with whole plant corn silage from AAC in diets with 40% of corn silage (on DM basis), 24.6% of starch (on DM basis), and 13.8% of finely ground corn kernels (on DM basis) increased milk yield (38.8 vs 40.8 kg/day) without affecting DMI (26.5 vs 26.3 kg/day), resulting in an improvement in feed efficiency (1.47 vs 1.55) (Cueva *et al.*, 2021). In the same study, AAC did not affect total tract starch (97.7 vs 97.6% of starch intake) and NDF (52.8 vs 53.4% of NDF intake) digestibility, and N partition, but tended to increase DM digestibility (71.3 vs 72.5% of DMI) (Cueva *et al.*, 2021). There were no major effects of AAC on ruminal fermentation profile, except for a decrease in butyrate (Cueva *et al.*, 2021).

In a similar way, Cueva *et al.* (2024) provided AAC as whole plant corn silage in diets with 40% of corn silage (on DM basis) and ground corn as the starch source in the concentrate. In this experiment, control diets had 23.6% of starch (on DM basis) and AAC diets had high (22.6% of DM) or low (20.7% of DM) starch concentration (Cueva *et al.*, 2024). Treatments diets did not affect DMI and milk yield, but AAC reduced milk urea-N (Cueva *et al.*, 2024). The association of AAC and low starch diet decreased milk protein N and increased unaccounted N relative to control diet (Cueva *et al.*, 2024). Cows fed AAC in a high starch diet increased total tract NDF (50.3 vs 47.7% of NDF intake) and acid detergent fiber (47.2 vs 43.6% of acid detergent fiber intake) digestibility relative to control with no effects on ruminal fermentation profile.

Krogstad and Bradford (2023) evaluated the effects of feeding dairy cows with whole plant corn silage from AAC in diets containing 40% of corn silage (on DM basis) and either 26.2% (low starch) or 30.6% (high starch) dietary starch. The inclusion of AAC improved the digestibilities of DM, NDF, and CP, and tended to increase starch digestibility when whole plant corn silage was stored for a short period (44 ± 3 days), but not after a long storage period (86 ± 3 days). Furthermore, corn type had no significant effect on milk yield and DMI, and no interactions between corn type and dietary starch concentration were observed for lactation performance.

Rebelo *et al.* (2023) investigated the effects of AAC inclusion in diets containing 31.6% starch (on DM basis), 50.4% whole plant corn silage (on DM basis), and 18.1% ground corn (on DM basis). Supplementation of AAC as a forage and/or concentrate source increased milk yield (35.3 vs 33.1 kg/day) and DMI (26.7 vs 25.1 kg/day) relative to control treatment (Rebelo *et al.*, 2023). However, when AAC was included only as a forage source, it tended to increase milk yield (36.5 vs 34.1 kg/day), improved feed efficiency (1.37 vs 1.28), and decreased milk fat concentration (3.32 vs 3.79%) relative to cows fed both whole plant corn silage and ground corn from AAC. Furthermore, AAC decreased total tract starch digestibility (97.4 vs 98.3% of starch intake) relative to control, probably because of higher DMI in AAC fed cows. A tendency for lower total tract starch digestibility was also observed when AAC was included as both forage and concentrate (97.1 vs 97.6% of starch intake) compared to inclusion as forage only (Rebelo *et al.*, 2023). No major effects of treatments on ruminal fermentation profile were observed (Rebelo *et al.*, 2023).

Oliveira *et al.* (2024) evaluated the fermentation profile *in vitro* of conventional flint ground corn, and AAC ground corn from a vitreous or floury corn hybrid. The utilization of AAC increased the proportions of propionate, butyrate, isovalerate and valerate, and decreased the proportion of acetate relative to flint corn without the technology, with an improvement on DM disappearance and reduction in methane concentration with AAC corn (Oliveira *et al.*, 2024). In the same study, AAC from flint corn reduced the proportion of acetate, valerate and ammonia, and had lower DM degradation relative to AAC from dent corn (Oliveira *et al.*, 2024).

Table 1. Lactation performance and nutrient digestibility responses in dairy cows fed an alpha-amylase enabled corn compared to isogenic control corn

Study	Source[1]	SL[2] (days)	SC[2] (% of DM)	DMI[2] (kg/day)	MY[2] (kg/day)	FE[2]	DMD[2] (% of intake)	StarchD[2] (% of intake)	NDFD[2] (% of intake)
Cueva *et al.,* 2021	WPCS	220	24.6	26.3 vs. 26.5 ($P = 0.70$)	40.8 vs. 38.8 ($P < 0.01$)	1.55 vs. 1.47 ($P < 0.01$)	72.5 vs. 71.3 ($P = 0.08$)	97.6 vs. 97.7 ($P = 0.43$)	53.4 vs. 52.8 ($P = 0.68$)
Krogstad and Bradford, 2023	WPCS	44	28.4	27.4 vs. 27.2 ($P = 0.75$)	38.9 vs. 39.6 ($P = 0.38$)	1.52 vs. 1.53 ($P = 0.79$)	+3.4 ($P < 0.01$)	+0.4 ($P = 0.07$)	45.5 vs. 37.4 ($P < 0.01$)
Krogstad and Bradford, 2023	WPCS	86	28.4	27.4 vs. 27.2 ($P = 0.75$)	38.9 vs. 39.6 ($P = 0.38$)	1.52 vs. 1.53 ($P = 0.79$)	($P = 0.22$)	($P = 0.41$)	44.5 vs. 42.0 ($P < 0.01$)
Rebelo *et al.,* 2023	WPCS + GC	40	31.6	26.7 vs. 25.1 ($P < 0.01$)	35.3 vs. 33.1 ($P = 0.05$)	1.32 vs. 1.32 ($P = 0.88$)	60.8 vs. 62.5 ($P = 0.13$)	97.4 vs. 98.3 ($P < 0.01$)	38.0 vs. 40.8 ($P = 0.46$)
Cueva *et al.,* 2024	WPCS	145	23.1	26.5 vs. 25.2 ($P > 0.05$)	36.0 vs. 36.5 ($P > 0.05$)	1.36 vs. 1.47 ($P > 0.05$)	67.0 vs. 65.8 ($P > 0.05$)	95.8 vs. 95.7 ($P > 0.05$)	50.3 vs. 47.7 ($P < 0.05$)
Silva *et al.,* 2025[3]	REC	28	28.7	22.7 vs. 22.8 ($P = 0.32$)	34.1 vs. 34.2 ($P = 0.56$)	1.47 vs. 1.48 ($P > 0.05$)	72.1 vs. 71.3 ($P = 0.13$)	98.5 vs. 98.5 ($P = 0.94$)	49.9 vs. 48.0 ($P = 0.09$)
Silva *et al.,* 2025[3]	REC	28	21.5	22.7 vs. 22.8 ($P = 0.71$)	34.1 vs. 34.2 ($P = 0.89$)	1.54 vs. 1.49 ($P \leq 0.05$)	72.1 vs. 71.3 ($P = 0.13$)	98.5 vs. 98.5 ($P = 0.94$)	49.9 vs. 48.0 ($P = 0.09$)

[1]WPCS = whole plant corn silage; GC = ground corn; REC = rehydrated and ensiled corn.
[2]SL = storage length; SC = starch content of the diet; DMI = dry matter intake; MY = milk yield; FE = feed efficiency; DMD = total tract dry matter digestibility; StarchD = total tract starch digestibility; NDFD = total tract neutral detergent fiber digestibility.
[3]Feed efficiency was calculated as energy corrected milk/dry matter intake.

The alpha-amylase present in AAC appears to be activated under high-temperature conditions (>80°C), exhibiting only approximately 10% of its maximum activity at 30°C (APHIS, 2008). This indicates that ruminal conditions are not optimal for maximizing the enzymatic activity of AAC. Similarly, no differences in ruminal amylase activity were observed when exogenous amylases were supplemented in dairy cow diets (Hristov et al., 2008). Hu, Persia, and Kung Jr. (2010) observed an increase in *in vitro* starch digestibility when AAC was incubated at 65°C relative to 40°C. Furthermore, there is evidence that the amylase activity of AAC may be reduced during silage fermentation (Cueva *et al.*, 2024), suggesting that the alpha-amylase enzyme could be degraded during silage storage. Therefore, the ensiling process of AAC may result in different responses in dairy cows performance relative to ground AAC.

The utilization of AAC in lactating dairy cows diets may have beneficial effects on performance when starch digestibility is impaired. Corn kernel processing is essential to improve alpha-amylase activity of AAC, since the enzyme can not act on the starch in the intact kernel (APHIS, 2008). Given that alpha-amylase is present in the kernel endosperm, physical disruption of the pericarp is necessary to promote effective contact between the enzyme and starch granules in the rumen fluid. This suggests a potential interaction between the effects of AAC supplementation and the degree of corn kernel processing on animal response. The effects of AAC corn seem to be dependent on diet composition in ingredients and nutrients, corn kernel processing, and when provided as silage, the length of storage period. The incorporation of AAC technology has been performed in its majority in dent corn hybrids, and all the effects of AAC on dairy cow performance are associated with corn hybrids of low vitreousness. To date, no studies have evaluated the impact of incorporating AAC technology into vitreous corn hybrids on dairy cow performance.

Therefore, the objective of this project was to evaluate the effects of AAC, included as a concentrate source, on the performance of lactating dairy cows under different dietary compositions that reflect practical feeding strategies used by field nutritionists. Furthermore, the associative effects of AAC in diets with different corn concentration (Experiment 1) and corn kernel processing (Experiment 2) were evaluated in the project. The study aimed to assess cow responses in terms of milk yield, DMI, nutrient digestibility, ruminal fermentation profile, N partition, and eating and rumination behavior.

SECOND PART – PUBLISHED PAPER

This paper is published in the Journal of Dairy Science.

DOI: https://doi.org/10.3168/jds.2024-25522

INTERPRETIVE SUMMARY

Effect of ensiled alpha-amylase enabled corn grain at two concentrations in the diet on lactation performance, chewing activity, ruminal fermentation, nutrient digestibility and nitrogen partition of dairy cows. *By Silva et al.* Silage of alpha-amylase enabled corn (AAC) grain can increase feed efficiency and reduce nitrogen excretion of dairy cows, but the effect may depend on the concentration in the diet. There was a tendency for ensiled AAC to increase the total tract neutral detergent fiber digestibility, but starch digestibility did not change. Type of ensiled corn did not affect chewing activity and ruminal fermentation. The reduction in the dietary concentration of ensiled corn grain by partial replacement with citrus pulp (28.7 vs 21.5% starch in diet dry matter) increased energy-corrected milk yield due to increased milk fat yield. The AAC in combination with low starch induced the highest feed efficiency and AAC with high starch reduced the proportion of consumed nitrogen in excreta.

ALPHA-AMYLASE ENABLED CORN FOR DAIRY COWS

Effect of ensiled alpha-amylase enabled corn grain at two concentrations in the diet on lactation performance, chewing activity, ruminal fermentation, nutrient digestibility and nitrogen partition of dairy cows

Wesley R. Silva[a], Ana Júlia C. Silva[a], Mariane A. Tiengo[a], Rayana B. Silva[b], Renata A. N. Pereira[c], Trevor J. DeVries[d], and Marcos N. Pereira[a*]

[a]Universidade Federal de Lavras, Faculdade de Zootecnia e Medicina Veterinária, Lavras, MG 37203-202, Brazil

[b]Better Nature Research Center, Lavras, MG 37203-016, Brazil

[c]Empresa de Pesquisa Agropecuária de Minas Gerais, Unidade Regional Epamig Sul de Minas, Lavras, MG 37200-970, Brazil

[e]University of Guelph, Department of Animal Biosciences, Guelph, ON N1G 2W1, Canada

*Corresponding author: M. N. Pereira. Universidade Federal de Lavras, Departamento de Zootecnia, Lavras, MG, 37200-900, Brazil. E-mail: mpereira@ufla.br

ABSTRACT

This experiment evaluated the effect of alpha-amylase enabled corn (AAC) on cows fed ensiled mature kernels at 2 concentrations in the diet. Twenty individually housed Holstein cows, arranged in 4 × 4 Latin squares (with 21-d periods), were exposed to each of 4 treatments in a 2 × 2 factorial combination of corn concentration (C): High (28.7% starch, 24.6% corn, 2.5% citrus pulp) vs Low (21.5% starch, 14.1% corn, 13.8% citrus pulp) and type (T): AAC (48.8% vitreousness) vs isogenic control (CTL. 51.1% vitreousness). Kernels were ground weekly, hydrated (62.2 ± 1.3% DM on AAC and 63.1 ± 1.4% DM on CTL) and ensiled for 28 ± 3 d. The statistical model had the effects of square, cow(square), period, C, T, and the C × T interaction. No effect of treatment was detected for milk yield (34.1 kg/d) and DMI (22.8 kg/d). High corn reduced the daily yield of fat (1.209 vs 1.297 kg/d) and ECM (33.2 vs 34.4 kg/d) and increased milk protein concentration (3.12 vs 3.09%). When fed with Low, AAC increased ECM/DMI relative to CTL (1.54 vs 1.49). Meal frequency was reduced on High-AAC (9.8 meals/d) relative to Low-AAC (10.3 meals/d). Cows fed High corn had greater proportion of daily intake in the morning (49.9 vs 44.4%) and longer duration of the first daily meal (69.7 vs 62.0 min) than cows fed Low. There was a tendency for AAC to increase the total tract NDF digestibility (49.9 vs 48.0%), but starch digestibility did not differ (98.4%). Ruminal microbial yield did not differ (335 mmol/d of allantoin in urine). High corn reduced the ruminal acetate to propionate ratio (2.45 vs 2.95) and pH (6.59 vs 6.74) relative to Low corn. Cows fed High-AAC had lower MUN than Low-AAC (17.4 vs 18.8 mg/dL) and plasma urea-N was lower on High-AAC than on High-CTL and Low-AAC (17.2, 19.0, and 18.7 mg/dL, respectively). High starch reduced urine-N excretion (g/d) and partition (% of daily N intake) relative to Low starch and also tended to reduce fecal N excretion and partition. Overall, Low starch increased milk solids yield, N loss in urine and feces, and the ruminal acetate to propionate ratio. Cows fed AAC and Low had the highest feed efficiency and cows fed AAC and High had the lowest proportion of N intake in total excreta.

Key words: Amylase, Enzyme, Feed Efficiency, Starch

INTRODUCTION

Alpha-amylase enabled corn (**AAC**) was primarily developed to improve ethanol production efficiency from corn fermentation and has a microbial gene capable of increasing the expression of alpha-amylase in the endosperm (APHIS, 2008). Ensiling AAC may affect silage fermentation (Heinzen Jr et al., 2024) and may have similar effects of exogenous amylase on ruminal and intestinal digestibility in dairy cows (Pech-Cervantes et al., 2022). The AAC has been shown to increase BW gain and feed efficiency of beef cattle when fed as whole plant corn silage (**WPCS**), but had no effect on animal performance when fed as dry-rolled kernels (Johnson et al., 2019). Dairy cows fed AAC as WPCS had improvements in the yields of milk, protein, and lactose and in feed efficiency, had reduced MUN, and had no difference on DMI, ruminal fermentation, and N partition relative to cows fed silage of the isogenic conventional hybrid (Cueva et al., 2021). Rebelo et al. (2023) detected a positive effect of AAC as WPCS on milk, protein, and lactose yields, DMI, feed efficiency, and ruminal microbial yield of dairy cows. However, in that same study, when AAC as corn grain was added to a diet containing AAC corn silage there was a tendency for lower yields of milk, protein, and lactose, and reduced feed efficiency than that with isogenic conventional corn grain. The utilization of AAC as WPCS or ground corn had no effect on ruminal fermentation profile and N partition (Rebelo et al., 2023). These results suggest that AAC in silage or grain can affect lactation performance and feed efficiency, but the results may depend on diet composition.

The effect of AAC on the total tract starch digestibility of dairy cows seems to vary, since an increase (Krogstad and Bradford, 2023), no effect (Cueva et al., 2021), or reduction (Rebelo et al., 2023) have been observed *in vivo*. When AAC or the isogenic hybrid were ensiled as WPCS and earlage, the *in situ* starch degradation at 0 and 6 h was lower on AAC than the isogenic corn (Heinzen Jr et al., 2024). Increased ruminal starch degradation with AAC could reduce N loss in excreta, capable of reducing the negative impact of ruminants on the environment (Koehler et al., 2022). The meta-analysis of Volk et al. (2021) indicates that the positive effect of AAC on feed efficiency of finishing beef cattle is diet dependent. In that analysis, diets with high concentration of dry-rolled corn had greater improvement in feed efficiency when AAC was fed than diets with high concentration of distiller grains. Likewise, cattle performance on AAC and conventional corn was similar when diets had high concentration of high moisture corn (Volk et al., 2021).

There is not enough research evaluating the effect of the variation in the concentration of AAC and starch in the diet of dairy cows, this may be important since high starch may increase the risk of ruminal acidosis and may also impose limitation to starch digestion (Oba and Allen, 2003). Krogstad and Bradford (2023) fed lactating cows with AAC or conventional WPCS (40% of diet DM) in diets with high (30.7% of

DM) or low (26.1% of DM) dietary starch concentration and observed no interactions between AAC and starch level in the diet, except for tendencies on milk fat content and MUN (both were highest on low starch-conventional corn). In that experiment WPCS was ensiled for at least 41 d and the effect of AAC on nutrient digestibility was greater on experimental wk 1 than wk 6, suggesting a larger effect of AAC on silage digestibility with short duration of storage. Dietary starch concentration and source may be involved in the effect of AAC on dairy cows.

Therefore, the objective of this experiment was to evaluate the effect of ground, hydrated, and ensiled mature corn kernels of AAC or the isogenic control (**CTL**) under short storage duration (28 d) at two starch concentrations in the diet on lactation performance, chewing activity, ruminal fermentation, digestibility, and N partition of dairy cows. The goal was to replace 10% of ensiled corn grain (on DM basis) with pellets of citrus pulp (a common byproduct feed) to lower the dietary starch concentration with a source of highly digestible fiber and residual organic matter (NASEM, 2021) rich in pectin (galacturonic acid) and sugars (Miron et al., 2001), while other feed ingredients were kept at constant concentration in the diet. Our hypothesis was that AAC would be more beneficial to feed efficiency when fed with low starch concentration in the diet.

MATERIALS AND METHODS

Feeds

A corn hybrid (E116K4-3000gt. Syngenta Seeds LLC) had a microbial gene incorporated in its DNA (Event 3272) to produce the AAC corn, and the same hybrid was used as the CTL corn, but without Event 3272. Both varieties were cultivated in Polk County (Nebraska, USA), and after harvesting, 20,507 kg of corn kernels from each type were packed in 20 kg paper bags and shipped (sea freight) in containers to

Brazil to be used as feeds during the experiment. During the experiment, kernels from mature AAC (48.8% vitreousness. Syngenta Enogen® Feed Corn) and its isogenic counterpart (51.1% vitreousness) were weekly ground with a stationary hammer mill (Nogueira TN-8, Nogueira Máquinas Agrícolas) using a 6-mm mesh diameter screen. Only the amount of corn kernels necessary to feed the cows for one week was ground on each griding day, and the remaining corn bags were stored in containers throughout the entire experiment. After grinding, ground corn was hydrated to achieve 35% moisture (Gomes et al., 2020) (37.8 ± 1.29% of moisture on AAC and 36.9 ± 1.36% of moisture on CTL) by mixing in a stationary vertical mixer (Unimix 1200; Casale) for 3 min. The hydrated corn was ensiled for 28 ± 3 d in 200 L plastic buckets with caps. A microbial inoculum (Kerasil, Kera Nutrição Animal. 4.0 × 10^{10} ufc/g *Lactobacillus plantarum* and 4.0 × 10^{10} ufc/g *Propionibacterium acidipropionici*) was added to each batch at a ratio of 0.149 g/kg of hydrated corn.

Weekly samples of dry ground corn were collected for particle size distribution and geometrical mean particle size evaluation using a sieve shaker (ROTAP–MA 750/YTLHZ, Marconi Equipamentos para Laboratórios) with 8 sieves (6,700, 4,750, 3,350, 2,360, 1,700, 1,180, 600, and 250 μm) and pan. Samples (50 g) were placed in the top sieve of the equipment for 10 min. The geometrical mean particle size was calculated as described in Baker and Herrman (2002). To calculate the loss of DM during ensiling, the weight of each bucket was obtained immediately after ensiling and at silo opening and corn samples were obtained simultaneously. Samples were dried in a forced air oven at 105°C for 24 h for non-volatile DM evaluation.

The vitreousness of each corn type was the mean of 5 kernels sampled at random from an experiment composite sample, replicated 3 times (15 kernels/corn type). Corn kernels were immersed in 50 mL of distilled water for 5 min, dried in paper towel, and the pericarp and germ were removed with a scalpel to obtain the endosperm. The endosperm was weighed and dried in a forced air oven at 105°C for 12 h and the floury endosperm was removed with a scalpel. The vitreous endosperm was weighed and expressed as a percentage of the total endosperm. Corn kernels of AAC and CTL were collected once throughout the experiment (1 sample/corn type) and the presence of the amylase protein (Event 3272) was evaluated in both samples by a commercial qualitative test kit (QuickStix for Enogen, EnviroLogix Inc.), and the AAC tested positive as the CTL tested negative for the protein.

Forty-eight buckets of each corn type were prepared for the feeding experiment. Samples of high-moisture corn grain from each bucket were collected immediately after opening and frozen for further analysis. Samples were thawed and 10 g were weighed and thoroughly mixed with 90 mL of distilled water for 1 min for pH determination (Phmetro Digimed DM 20. Digicrom Analítica). From d 15 to 21 of each experimental period, samples of buckets opened throughout the week were collected immediately after opening for microbiology evaluation. Samples were refrigerated and sent to the laboratory within 3 h. Sequential 10-fold dilutions (10^{-1} – 10^{-6}) were prepared with peptonized water and aqueous extracts were prepared. Filamentous fungi and yeast growth were evaluated in Dichloran Rose Bengal agar Chloramphenicol (K25-1160 DRBC, Kasvi) incubated at 28°C for 168 h and for 72 h, respectively. For enumeration of spore forming bacteria the aqueous extract was maintained at 90°C for 10 min, and further decimal dilutions were evaluated in Plate Count Agar medium (K25-1060 Agar Nutriente, Kasvi). Plates were incubated at 30°C for 24 h. Lactic acid bacteria growth was evaluated in MRS agar medium (K25-1043 Agar MRS, Kasvi) incubated for 48 h at 32°C. Counts of cfu was performed by visual observation of each plate and data were log transformed for analyses.

Samples of feed ingredients and orts per cow were collected daily and frozen during the last week of each experimental period and composite samples were formed per period. Samples were dried in a forced-air oven at 55°C for 72 h and ground to pass a 1-mm diameter mesh screen in a grinder (Star FT 80, Fortinox Indústria, Comércio e Serviços). The DM concentration was determined in a forced air oven at 105°C for 24 h and ash was evaluated by incineration at 600°C for 4 h. The CP concentration was determined with a micro Kjeldahl steam distillatory (method 990.03; AOAC International, 2006). The NDF concentration was evaluated using an adaptation of AOAC International, 2006 method 2002-04 (Mertens, 2002), with heat-stable α-amylase (Termamyl, Novozymes), without sodium sulfite and were manually filtered using crucibles. Starch plus free glucose was measured with α-amylase and amyloglucosidase and colorimetry for glucose as described in Fernandes et al. (2022), adapted from Hall et al. (2015). Composite samples of rehydrated and ensiled corn kernels were obtained from d 15 to 21 of each treatment period and analyzed with NIRS curves at 3r Ribersolo/Rock River laboratory (Ribeirão Preto, Brazil).

The particle size distribution of WPCS and alfalfa haylage were measured with the Penn State Particle Separator with two sieves (19 mm and 8 mm diameter) and pan. Composite samples from each screen of the Penn State separator of WPCS and alfalfa haylage were formed per period and the DM, NDF, and starch concentrations were analyzed as previously described. Physically effective NDF (**$_{pe}$NDF$_{>8}$**) was calculated from individual feed analysis and diet ingredient composition assuming that only WPCS, alfalfa haylage, and whole cottonseeds would contribute to NDF greater than 8 mm.

Experimental Design, Cows, and Treatments

Twenty Holstein cows (33.8 ± 5.0 kg/d milk yield, 124 ± 56 DIM, 594 ± 93 kg BW, including 8 primiparous) were used in the experiment. Cows formed squares of 4 animals based on similarity in parity and milk yield. Within a square, cows were randomly allocated to a sequence of 4 treatments using a 4 × 4 Latin square design, with 21-d periods (with 14-d adaptations), balanced for carryover effects by assigning a treatment in sequence to the other 3 treatments the same number of times within a square (Williams, 1949). Treatments were a 2 × 2 factorial arrangement of dietary corn grain inclusion: High (24.6% ensiled corn grain and 28.7% dietary starch in DM) vs Low (14.1% ensiled corn grain and 21.5% dietary starch in DM), and corn type: AAC vs CTL (Table 1). Ration formulation was based on NASEM (2021) recommendations for the average cow (milk yield, DIM, BW, BCS, and parity) immediately before the start of the experiment.

Cows were individually housed and fed in an open-walled, sand-bedded tie stall barn with fans and high-pressure sprinklers and milked 3 ×/d, starting at 0500, 1300, and 2000 h, in an adjacent herringbone parlor. The TMR was mixed once daily in a 1.2 m^3 stationary vertical mixer (Unimix 1200; Casale) and cows were fed at 0700 h. Feed was pushed-up manually with a broom at least 10 ×/d. The daily feed refusal per cow, on an as-fed basis, was 15.0 ± 5.4% of offered (mean ± SD). Samples of WPCS, alfalfa haylage, and ensiled corn grain were collected weekly for DM determination with a microwave oven (by drying for 5 min and then in 3 min steps until a stable weight was obtained) and the TMR was adjusted accordingly, adjusting the amount of each feed ingredient to maintain the % in diet DM constant.

Lactation Performance and Intake

Milk yield and DMI were recorded daily throughout the experiment and the means of d 15 to 21 of each treatment period were used to compare treatments. On d

17 to 21 of each period, 0.5% of the milk produced in each milking was collected (i.e., a cow produced 15 L of milk in the morning milking, a sample of 75 mL was collected) and a composite daily sample from the 3 milkings was performed for each cow. Composite samples were stored in flasks containing 2-bromo-2-nitropropane-1-3 diol under refrigeration. All composite samples (5 samples/cow/period) were sent to a commercial laboratory (Laboratory of the Paraná State Holstein Breeders Association) and the concentrations of CP, casein, fat, lactose, total solids, SCC, urea-N (**MUN**), and fatty acids (**FA**) were analyzed by mid-infrared analysis (Bentley Instruments Inc.). Milk energy secretion (Mcal/d) was calculated (NRC, 2001): [(0.0929 × % fat) + (0.0547 × % protein) + (0.0395 × % lactose)] × kg of milk. The yield of energy corrected milk (**ECM**. kg/d) was calculated as: Milk energy secretion/0.70 (assumes 0.70 Mcal/kg of milk with 3.7% fat, 3.2% protein, and 4.6% lactose). Feed efficiencies were calculated as milk yield/DMI and ECM/DMI. Body weight was measured on d 17, 19 and 21 with an individual walk-over weigh scale (Eziweigh2. Tru-Test) and BCS (1 to 5, thin to fat; Wildman et al., 1982) was the mean of 3 independent evaluators on d 21 of each treatment period.

Total-Tract Apparent Digestibility and Ruminal Microbial Yield

During d 19 to 21 of each treatment period, total collection of feces and urine was performed during three, 8-h collection periods, with 8-h intervals starting 8 h late on each day, to represent a 24-h collection. Feces and urine were collected in buckets simultaneously to excretion by at least 7 people from 2100 to 0500 h (1st collection period), 1300 to 2100 h (2nd collection period), and 0500 to 1300 h (3nd collection period) with minimum disturbance and total maintenance of the daily cow routine. During data collection, cows were moved in groups of 3 to the milking routine in a 2 × 3 herringbone parlor and immediately returned to their ties stalls after milking to reduce the daily time without sampling. The milking routine of each group of 3 cows lasted less than 20 min. Fecal samples were immediately weighed and frozen throughout the collection period. A composite sample was formed per cow per period. Samples were dried at 55°C for 72 h. The concentrations of DM, NDF, ash, and starch were measured as previously described. The total tract apparent digestibilities of DM, OM, NDF, starch, and the non-NDF OM were calculated. Digestible OM intake (**DOMI**) was estimated and the ECM/DOMI ratio was calculated as a measure of energy usage efficiency. Urine samples were mixed to a 4% sulfuric acid solution (1:5) and frozen for allantoin determination according to Chen and Gomes (1992). The daily excretion of allantoin was determined in urine and used as an indirect estimate of ruminal microbial yield (Valadares et al., 1999; Pereira et al., 2021). The efficiency of ruminal microbial growth was estimated as Allantoin/DOMI (mmol/kg).

A sample from the first defecation of each cow was collected on d 21 for viscosity determination. Viscosity was evaluated according to an adaptation of the methodology of Cannon et al. (2010). Samples of fresh feces (100 g) were diluted in 120 mL of water. The solution was homogenized with a metal spatula for 30 s and filtered through 2 layers of cheesecloth. The viscosity of the solution was measured with a rotational viscometer (Model DV-E. Brookfield Engineering Laboratories) at 100 rpm, with spindle LV-1 at room temperature. Fecal samples (10 g), from the composite samples obtained per period, were diluted in 100 mL of distiller water for pH determination (Phmetro Digimed DM 20. Digicrom Analítica).

Chewing Activity Behavior

During d 15 to 17 of each treatment period rumination and eating behaviors were monitored by visual observation at 5-min intervals, continuously for each 24 h period across those 3 d. Buccal activities were classified as rumination, ingestion, drinking, and idleness. Individual feeding observations were combined and separated into meals using a meal criterion (i.e., the minimum duration of time between meals) calculated for each cow. Meal criteria were calculated for each cow using methods described by DeVries et al. (2003), using a software package (MIX 3.1.3; MacDonald and Green, 1988). The number of different meals in a day was termed meal frequency (meals/d). Total meal time (min/d) was the total eating time (eating observations/d × 5 min), plus all the nonfeeding intervals shorter than the length of the meal criterion of each cow. Meal duration (min/meal) was calculated as the total daily meal time divided by the meal frequency. Finally, meal size (kg/meal) was the ratio between daily DMI (kg/d) and meals per day. The duration and moment post-feeding of the longest daily meal was calculated. The duration of the first daily meal was measured with a stopwatch. A person observed the behavior of all cows, individually, after offering feed at 0700 h until the last cow finished its first meal.

Sorting behavior in periods of the day was evaluated from d 15 to 17 of each treatment period with the Penn State particle size separator with the 19 mm and 8 mm mesh diameter screens and pan. The particle distribution of the TMR available to each cow was measured at 0700 h (offered TMR), 1200 h, 1900 h, and 0500 h. The predicted intake (as-fed basis) of particles on each screen was: % TMR retained on screen × kg of TMR consumed. The observed intake of particles was: % TMR retained on screen × kg of TMR offered - % orts retained on screen × kg of orts. The selection index (Leonardi and Armentano, 2003) was: 100 × (observed intake/predicted intake). Sorting values below 100% represent selective refusal, above 100% represent preferential intake, and equal to 100% represent no selection. A mean value was generated per cow per period. On d 15, 16, and 17 of each period, the proportion of daily intake in the morning (0700 to 1200 h), afternoon (1200 to 1900 h), and night (1900 to 0700 h) were determined.

Ruminal Fermentation

Ruminal fluid samples were obtained on d 21 of each period with a flexible oro-gastric tube coupled with a vacuum pump at 11.7 ± 0.13 h after morning feeding, to represent the moment of the day of lowest ruminal pH and highest VFA concentration (Pereira and Armentano, 2000). The first 100 mL were discarded to reduce saliva contamination. Cows were randomly sampled within squares and ruminal pH was measured (Phmetro Digimed DM 20. Digicrom Analítica). A sample was frozen in liquid nitrogen to suppress fermentation and stored at -20°C. Ruminal fluid samples were thawed and prepared as described by Ferreira et al. (2016) for analysis of VFA by gas-liquid chromatography (CG HP 7890A; Injetor HP 7683B, Agilent Technologies) equipped with a capillary column HP-FFAP (1909F-112; 25 m; 0,32 mm; 0,5 µm; JeW Agilent Technologies). For protozoa counting, another 10 mL sample of rumen fluid was diluted (50% v/v) in a 36% (v/v) formaldehyde solution and mixed. The sample was stained according to Dehority (1984) and total protozoa was enumerated with an optical microscope in a Neubauer chamber (Warner, 1962). Samples of ruminal fluid were also refrigerated for viscosity determination. Samples of 50 mL of ruminal fluid were diluted in 100 mL of water for viscosity evaluation. The solution was homogenized with a metal spatula for 30 s and filtered through 2 layers of cheesecloth. The viscosity of the solution was measured with a rotational viscometer (Model DV-E. Brookfield Engineering Laboratories) at 100 rpm, with spindle LV-1 at room temperature.

Blood and N Partition

Blood samples were collected on d 18 of each treatment period from the coccygeal vessels at 0 and 2 h relative to the end of the morning, afternoon, and night milkings and return to the feed bunk. Samples were collected in tubes with EDTA and were centrifuged at 2,000 × *g* for 10 min at room temperature. Plasma was obtained and frozen at -20°C for urea-N determination (**PUN**) with a commercial kit (Urea Enzimática, Bioclin). On d 18 and 21, blood samples were collected with tubes containing sodium fluoride. After sampling, tubes were immediately centrifuged (2,000 × *g* for 10 min) for glucose determination. Samples were collected 10.9 ± 0.32 h post-feeding on d 18 and 11.7 ± 0.13 h post-feeding on d 21.

Fecal and urine N concentrations were determined with a Kjeldahl steam distillator (method 990.03; AOAC International, 2006) as described for feed analyses. The N concentration in milk was: Milk CP/6.38 (Ipharraguerre and Clark, 2005). The proportion of N intake (% of daily intake) and excretion (g/d) of urine-N, milk-N, and fecal-N were calculated.

Statistical analysis

Data were analyzed with the MIXED procedure of SAS (SAS University Edition, Cary, USA). The model had the random effects of square (1 to 5) and cow(square) (1 to 20) and the fixed effects of period (1 to 4), corn concentration (High, Low), and corn type (AAC, CTL), and the interaction between corn concentration (**C**) and corn type (**T**). The model used to evaluate silage pH, microbiology, and DM loss had only the fixed effect of T.

Degrees of freedom were calculated using the Kenward-Roger option. The PUN was analyzed as repeated measures over time. The fixed effect of time and the 2- and 3-way interactions between time, C, and T were added to the previous model. The interactions between cow, period, C, and T were defined as random. The best covariance structure was chosen based on the lowest value for the Akaike's Information Criterion. Covariance structures tested were first order autoregressive, compound symmetry, and unstructured and unstructured was the one of choice. Statistical significance was declared at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$. Treatment means were compared with a Tukey correction when a $P \leq 0.10$ was detected for the interaction between main effects.

RESULTS

Feeds and treatment diets

The TMR ingredient and nutrient composition are reported in Table 1. The composition of the AAC and CTL diets was similar, the only major change was the source of starch from ensiled corn grain. Ensiled corn grain was 24.6% of DM in High and 18.3% of DM in Low. The starch concentration of the consumed diet was 28.7% of DM in High and 21.5% of DM in Low, NDF was 32.2% and 33.8%, and non-starch NFC was 12.1% and 17.9%, respectively. The particle size of corn before ensiling did not differ (Table 2) and other feedstuff composition is described in Table 3. The proportion of dietary starch coming from ensiled corn grain was 64% of total starch in High and 49% in Low and the proportion of total starch coming from WPCS was 34% and 45%, respectively. The processing of the WPCS is described in Supplemental Table S1. The experiment induced changes in concentration in the diet and nature of dietary starch and non-starch NFC, without affecting FNDF and ${}_{pe}NDF_{>8}$ concentrations.

The type of corn had no effect on grain silage nutrient composition, microbiology, pH, and DM loss ($P \geq 0.13$) (Table 4). The measures of ruminal starch degradation obtained by NIRS are reported on Supplemental Table S2.

Lactation performance and intake

Table 5 reports lactation performance, DMI, and feed efficiency. No differences were detected in milk yield (34.1 kg/d) and DMI (22.8 kg/d) ($P \geq 0.32$). Energy corrected milk yield was lower on High than Low (33.1 vs 34.8 kg/d; $P < 0.01$), due to a reduction in milk fat concentration (3.82 vs 3.60%; $P < 0.01$) and milk fat yield (1.209 vs 1.297 kg/d; $P < 0.01$) with more starch in the diet. The partial replacement of corn grain silage with citrus pulp pellets increased total milk solids concentration and yield ($P < 0.04$). Cows fed high starch had higher concentration of protein (3.12 vs 3.09%; $P = 0.04$) and casein (2.43 vs 2.37%; $P < 0.01$) in milk, but the yields of protein and casein did not differ ($P \geq 0.24$). Low starch increased the efficiency of energy utilization (ECM/DOMI) from 2.14 to 2.21 relative to High ($P = 0.03$).

Corn type had no effect on lactation performance and DMI (Table 5), except for a significant interaction ($P < 0.01$) between corn concentration and type for milk lactose content. Cows fed AAC had higher milk lactose concentration than CTL only when fed Low starch. A tendency ($P = 0.07$) for the interaction C × T was also detected for feed efficiency measured as ECM/DMI. Similarly to milk lactose concentration, the AAC silage increased feed efficiency only when added to the Low starch diet. The Low-AAC diet increased ECM/DMI by 4.05% compared to the other 3 treatment combinations.

The profile of FA in milk fat is reported in Table 6. Diets Low increased the concentrations in milk (g/100 g milk) of Preformed, Mixed, and De Novo FA relative to High. Low starch also increased the yield (g/d) of Preformed and De Novo FA. The daily yield of Mixed FA was increased by AAC only when fed with Low ($P = 0.09$ for the interaction C x T). Treatments had no effect on milk fat FA profile (g/100 g FA), except for a tendency for High to increase the proportion of Preformed FA relative to

Low. There was also a tendency (*P* = 0.09) for AAC to increase the concentration of palmitic acid in milk (1.384 vs 1.329 g/100 g of milk). Although concentrations in milk and daily yield of both saturated and unsaturated FA were depressed by High, the profile of FA in milk fat (g/100 g FA) was not affected by starch concentration and corn type (*P* ≥ 0.15). The effect of corn type on milk FA profile was minor compared to the effect of starch concentration in the diet.

Chewing Activity Behavior

The intake of NDF is in Table 7. The intakes of FNDF (0.87% of BW; *P* ≥ 0.63) and $_{pe}NDF_{>8}$ (0.88% of BW; *P* ≥ 0.51) did not differ across diets. However, total NDF intake as a proportion of BW was higher on Low than High starch (1.27 vs 1.21%; *P* < 0.01), due to the replacement of corn grain with NDF from citrus pulp.

There was an interaction C × T for meal frequency (*P* = 0.04) and tendencies (*P* = 0.07) for this interaction were also detected for meal size (kg/meal) and duration (min/meal) (Table 7). Cows fed High-AAC had lower meal frequency than cows fed Low-AAC (9.8 vs 10.3 meals/d) and there was a tendency (*P* ≤ 0.10) for meal size to be higher on High-AAC than Low-AAC (2.37 vs 2.25 kg DM/meal), suggesting that meal frequency and size were affected by starch concentration only when AAC was fed. No difference among treatment means were detected for meal duration (*P* ≥ 0.10).

No effects of AAC on rumination and eating behaviors were detected (*P* ≥ 0.12; Table 7). Cows fed AAC tended (*P* ≤ 0.10) to have lower rumination time (min/d) compared to cows fed CTL only when fed Low (432 vs 445 min/d; *P* = 0.07 for the interaction C × T). There was a tendency (*P* = 0.07) for the moment of the longest meal of the day to be later relative to the morning daily feeding on Low than High (350 vs 287 min following feed delivery). Diet Low also reduced first meal duration compared to High (69.7 vs 62.0 min; *P* = 0.04) and the proportion of daily intake in the morning (44.4 vs 49.9%; $P < 0.01$) and increased the proportion of intake in the afternoon (37.7 vs 35.4%; $P < 0.01$) and night (17.9 vs 14.9%; $P < 0.01$).

Cows fed AAC tended ($P \leq 0.10$) to sort more in favor of small TMR particles (< 8 mm) in the morning than cows fed CTL only when fed Low (108 vs 105%; $P = 0.05$ for the interaction C × T) (Table 7). In the afternoon, cows fed AAC tended to sort against particles retained on the 8-19 mm screen of the Penn State and cows fed CTL select in favor of particles retained on this screen (99 vs 101; $P = 0.08$). Likewise, in the afternoon Low increased sorting in favor of particles retained on the 8-19 mm screen relative to High (101 vs 99; $P = 0.03$) and at night the same sorting behavior was observed (99 vs 95; $P = 0.02$).

Digestibility, ruminal fermentation, and N partition

The change in dietary starch affected digestion and ruminal fermentation (Table 8). Low starch increased urine excretion (27.0 vs 24.1 L/d; $P = 0.02$). There was no effect of starch concentration on the total tract starch, NDF, and OM digestibilities (98.5, 49.0, and 73.9% of intake, respectively; $P \geq 0.15$), although a tendency was observed for CP digestibility to be higher on High than Low (77.3 vs 76.3% of intake; $P = 0.08$). Cows fed High had greater digestible starch intake (6.4 vs 4.8 kg/d; $P < 0.01$), but fecal starch concentration was similar (1.4% of DM; $P = 0.13$). High starch reduced the ruminal acetate to propionate ratio (2.45 vs 2.95; $P < 0.01$) and pH (6.59 vs 6.74; $P = 0.05$), but ruminal microbial yield and efficiency and protozoa concentration did not differ ($P \geq 0.47$). The reduction in the acetate to propionate ratio was driven by an increase in propionate (25.1 vs 21.9%; $P < 0.01$) and a decrease in acetate (59.1 vs 62.5%; $P < 0.01$) proportions of total VFA. There was a tendency ($P = 0.10$) for fecal pH to be lower on High than Low only when CTL corn grain silage was fed (7.72 vs 7.83). High starch increased the viscosity of ruminal fluid ($P = 0.01$)

and had no effect on fecal viscosity (P = 0.60). Plasma glucose did not differ by treatment diet ($P \geq 0.38$).

There was a tendency for AAC to increase NDF digestibility relative to CTL (49.9 vs 48.0% of intake; P = 0.09), but there was no change in total tract starch digestibility (P = 0.94) (Table 8). Ruminal fermentation profile, microbial yield, and protozoa concentration did not differ by corn type ($P \geq 0.14$), except for a reduction in the molar proportion of isobutyrate on cows fed AAC relative to CTL (0.92 vs 0.98% of total VFA; P = 0.03). Fecal and ruminal pH and viscosity and plasma glucose were not affected by corn type ($P \geq 0.32$).

The partition on N intake into milk, urine and feces is reported in Table 9. Corn type had no effect on N partition in g/d or % of daily intake ($P \geq 0.25$). High reduced MUN and PUN only when AAC corn was fed ($P \leq 0.05$ for the interaction C × T). Cows fed High had lower excretion of N in urine (221.2 vs 232.6 g/d; P = 0.01) and there was a tendency for reduced N excretion in feces (135.0 vs 141.4 g/d; P = 0.08), inducing a reduction in N in total excreta (346.1 vs 374.0 g/d; $P < 0.01$) relative to Low, at similar N intake and secretion in milk ($P \geq 0.89$). Cows fed High-AAC had the lowest proportion of N intake in excreta relative to the other 3 treatment diets (56.5 vs 62.1% of daily intake; $P \leq 0.05$ and P = 0.10 for the interaction C × T).

DISCUSSION

Corn grain silage composition

Silages of AAC and CTL were similar in nutrient composition, fermentation profile, and predicted starch degradability. There was no evidence for increased NH_3-N or reduced starch degradability on AAC relative to CTL, as observed by Heinzen Jr. et al. (2024) in 4 L laboratory silos of WPCS and earlage. The DM loss of the rehydrated and ensiled mature corn stored in 200 L bucket silos was around 1.6% of ensiled. There was no evidence for AAC to have an effect on nutrient composition and particle size of corn, suggesting that the effect of AAC on dairy cows in our experiment was driven by the presence of exogenous amylase in kernel endosperm.

Cow performance

The experimental diets differing in starch concentration were similar in DM concentrations of $_{pe}NDF_{>8}$ and FNDF. This is consistent with the lack of a treatment effect on rumination behavior, at constant DMI. The partial replacement of ensiled corn grain with citrus pulp pellets at 14% of diet DM had no effect on daily DMI, although citrus pulp in replacement to corn could reduce DMI (Leiva et al., 2000; Broderick et al., 2002; Salvador et al. 2008), suggesting that the reduction in dietary starch concentration was beneficial to intake. Even though daily DMI was similar, starch concentration had a significant effect on the proportion of daily intake in periods of the day. Cows fed more corn grain silage had greater proportion of daily intake in the morning, longer duration of the first daily meal, and lower proportions of intake in the afternoon and night than cows fed with more citrus pulp, suggestive of greater propensity for concentrated intake and faster feed rates in the morning on High starch. Slug feeding of the high starch diet in the morning may have contributed to the lower ruminal pH on High than on Low (Stone, 2004), in addition to the difference in the NFC profile of the diet. Diets with high starch concentration can increase meal frequency and reduce meal size by a mechanism involving propionic acid (Oba and Allen, 2003). Meal frequency was higher on Low-AAC than on High-AAC (10.3 vs 9.8 meals/d) and meal size did not differ, independently of the higher proportion of propionate in ruminal VFA on High relative to Low. Our data suggests that short term regulation of feed intake by propionic acid was not the major driver of eating behavior when citrus pulp replaced ensiled corn in the diet.

Cows sorted in favor particles retained on the 8 mm screen of the Penn State particle separator, suggesting that cows sorted in favor of citrus pulp pellets that are completely retained on the 8 mm mesh screen. Citrus pulp is known to have high water holding capacity compared to other non-forage fiber sources (Ramanzin et al., 1994). Broderick et al. (2002) observed that the partial replacement of high moisture corn with citrus pulp reduced DMI, but had no effect on digesta volume and mass of dairy cows due to an increase in ruminal volume and mass per unit of DMI. Short-term ruminal fill by hydrated citrus pulp may have modulated eating behavior in our experiment, independently of ruminal fermentation profile. Overall, the type of corn had no effect on chewing activity behavior, but diet carbohydrate profile modulated eating behavior despite having no effect on daily DMI and rumination.

There was no effect of AAC on energy efficiency (ECM/DOMI). The Low starch diet increased milk fat yield and the efficiency of energy usage. The gain in ECM/DOMI with Low was associated with a higher ruminal acetate to propionate ratio, indicating that the efficiency of converting metabolizable energy into milk fat may have been improved by low starch in the diet. The capacity of the ruminal epithelium to absorb VFA may have been increased or there was less inhibition of milk fat synthesis by lipid biohydrogenation intermediates when cows were fed Low starch and had more consistent DMI throughout the day, probably increasing the uptake of acetate by the mammary gland. Low starch increased the concentration in milk and daily yields of Mixed and De Novo FA, denoting some advantage in ruminal lipid biohydrogenation, probably as the result of less ruminal acidosis (Baumgard et al., 2002; Ferlay et al., 2017). Ruminal pH and the acetate to propionate ratio, evaluated once per day around 11.7 h post-feeding, were higher on Low than High, denoting that citrus pulp was less acidogenic to the rumen than ensiled corn grain. Similarly, Ben-Ghedalia et al. (1989)

observed that the substitution of barley with citrus pulp increased ruminal pH and microbial yield in sheep.

Preformed FA were also increased in cows fed Low, suggesting that more digested FA may have been incorporated into milk fat when cows were fed the diet with reduced starch. The similarity across treatments in BW and BCS does not support that the change in milk preformed FA was due to a difference in lipid mobilization from the adipose tissue. Reduced insulin secretion may have promoted less tissue accretion (Piccioli-Cappelli et al., 2014), and a greater proportion of digested FA may have been directed to the mammary gland, explaining at least part of the gain in milk fat yield and in energy efficiency of the Low starch diets.

There was no effect of starch concentration on the total tract digestibility of starch. The experimental variation in dietary starch concentration from ensiled corn grain did not affect the capacity of the digestive tract to digest starch, suggesting that amylase supply from AAC did not support the digestion in a situation with deficiency in amylase activity relative to starch intake. The highest ECM/DMI ratio was observed on diet Low-AAC, denoting that exogenous amylase was more beneficial to feed efficiency (+4%) when citrus pulp partially replaced ensiled corn grain and diet starch concentration was reduced from 29 to 21% of DM. This increase in feed efficiency in response to AAC is comparable to that previously observed in dairy cows (+5.4%; Cueva et al., 2021) and beef cattle (+5%; Jolly-Breithaupt et al., 2018; Horton et al., 2020).

Mixed FA yield (kg/d) was the highest on Low-AAC, associated with the numerically higher C16:0 yield for cows fed Low-AAC relative to the other diets (+10%), the same trend as the response in ECM/DMI. The change in Mixed FA suggests that Low-AAC may have affected ruminal biohydrogenation intermediates or acetate supply for de novo synthesis of C16:0, although ruminal acetate concentration did not differ among treatments. The AAC also tended to reduce rumination (min/d) only on diet Low, but no other interaction between corn type and concentration in the diet was detected for eating and rumination behaviors. There was no evidence, based on ruminal fermentation and milk FA metrics, that ruminal fermentation was excessive when AAC was fed with High starch.

Fecal pH did not change in response to the concentration of starch in the diet or type of corn. This agrees with the lack of a treatment effect on the total tract starch digestibility and fecal starch concentration. The higher starch intake on High than Low did not penalize starch digestibility when ensiled corn grain was the starch source. Rehydrated and ensiled corn grain was capable of increasing flint corn digestibility and is a highly fermentable starch source (Castro et al., 2019). The amylase trait in corn kernels had to improve the digestibility of highly digestible starch (98.5% of intake) of cows with low fecal starch concentration (1.4% of DM) (Fredin et al., 2014). The effect of AAC on more resistant starch sources requires further evaluation.

The interactions between starch concentration and corn type were highly significant for the concentrations of MUN and PUN, suggesting that AAC may have increased the capturing of N as microbial protein when it was fed with High starch. Exogenous amylase supplementation reduced PUN (Andreazzi et al., 2018) and AAC WPCS reduced MUN (Cueva et al., 2021) of dairy cows. Krogstad and Bradford (2023) observed that AAC WPCS reduced MUN more on a diet with low than on high starch concentration from finely ground conventional corn grain. There was a reduction of isobutyrate molar proportion with AAC, suggestive of lower ruminal valine deamination (Nakae and Elliot, 1965; Hultquist and Casper, 2016). Although there was no difference on ruminal microbial yield, the data seems to be suggestive that protein deamination and ruminal N loss may have been reduced by AAC corn type.

Unaccounted N, a gross measure of N retention, was affected by diet composition. On cows fed High-AAC, unaccounted N retention (g/d) and the proportion retained (% of daily intake) were higher than on cows fed Low-AAC and Low-CTL. The mean across treatments for unaccounted N was 71.7 g/d in our experiment, higher than the mean value of 38.8 g/d of a recent meta-analysis of experiments adopting total collection of urine and feces in dairy cows (Spanghero and Kowalski, 2021). However, the SD was ± 43.2 g/d in this meta-analysis, emphasizing the large amount of variability in N balance studies. Fecal-N may have been underestimated in our experiment, since fecal samples were dried before N analysis and some N volatilization may have occurred (Morris et al., 2019). The reduction in urine-N excretion apparently induced the increase in the efficiency of N usage with AAC associated with High starch. While the Low-AAC diet was the most effective to increase feed efficiency (ECM/DMI) and the Low starch diets improved energy efficiency (ECM/DOMI), the High-AAC diet was the most effective to promote N retention relative to both Low starch diets and had the lowest proportion of N intake in excreta.

Exogenous amylases supplementation has improved N metabolism of dairy cows, being capable of increasing milk CP concentration (Gencoglu et al., 2010; McCarthy et al., 2013; Pech-Cervantes et al., 2022) and decreasing urinary N excretion (Zilio et al., 2019). However, results are not always consistent (Weiss et al., 2011; Nozière et al., 2014; Andreazzi et al., 2018). Cueva et al. (2021) and Rebelo et al. 2023) observed no effect of AAC WPCS on N partition of dairy cows. No consistency on the response in N partition seems to occur for both exogenous amylase and AAC corn type.

The High starch diets reduced urine N by 9.24% (-21.5 g/d) of the excretion of cows fed Low starch. This is similar in magnitude to the reduction in urine-N induced by tannin supplementation (Oliveira et al., 2023), a frequently used feed additive to reduce N excretion. There was also a tendency for High starch to reduce fecal-N by 4% (-6.29 g/d) relative to Low starch. Increased digestible starch intake may have increased N recycling to the gastrointestinal tract (Theurer et al., 2002; Souza and White, 2021), supporting the reduction of N excretion in urine and feces. Unaccounted N was increased by 62% (+36.5 g/d) on High-AAC compared to the Low starch diets, denoting that the possible increase in N retention with High-AAC was not totally accounted for by the reduction in N in total excreta and some gain in the efficiency of metabolic protein deposition may have occurred.

There was a tendency for the total tract NDF digestibility to be higher on AAC than CTL, without affecting starch digestibility. The same response to AAC WPCS was observed by Krogstad and Bradford (2023). Amylase can potentially increase the total tract NDF digestibility, supposedly by increasing the ruminal availability to fiber digesting bacteria of starch hydrolysis products (Cotta, 1988). Improvement in total tract NDF digestibility of cows fed exogenous amylase has been previously described (Gencoglu et al., 2010; Weiss et al., 2011), but results are not consistent (Nozière et al., 2014; Andreazzi et al., 2018). In a recent meta-analysis, Pech-Cervantes et al. (2022) did not report any difference in the total tract digestibility of NDF when exogenous amylase was fed to lactating dairy cows, although improvements were detected in the digestibilities of starch (+0.5% percentage points) and DM (+1.0% percentage points).

The gene added to AAC is from a bacteria genus that have optimal growth in environments with high temperature (> 80°C) (APHIS, 2008), suggesting that alpha amylase synthesized by AAC corn needs a high temperature to increase its activity. The AAC corn has been developed to improve the efficiency of ethanol production on ethanol plants, a process that includes a high temperature (> 100°C) fermentation to break down the starch (APHIS, 2008). Hu et al. (2010) observed an increase in *in vitro* starch digestibility when AAC was incubated at 65°C relative to 40°C. Ruminal environment temperature is not as high as the temperature of 65°C used by Hu et al. (2010) and the ethanol plants, which could result in reduced alpha amylase activity in the digestive tract of ruminants. In agreement, the utilization of exogenous amylases may not affect the amylase activity in the ruminal fluid (Hristov et al., 2008). Besides that, the amylase activity of AAC in the rumen may be influenced by the previous processing or conservation method adopted on the farm. Therefore, a better understanding of how temperature, and maybe pH, affect the alpha amylase activity of AAC corn can optimize the utilization of this technology in the diets of ruminants.

CONCLUSIONS

High corn concentration in the diet decreased milk fat content and daily ECM yield. The partial replacement of ensiled corn grain with citrus pulp pellets reduced first meal duration and the proportion of daily intake in the morning, at the same daily DMI. There were no major effects of AAC on dairy cow performance, but when combined with Low corn concentration, the AAC induced the highest ECM/DMI. High corn concentration induced the lowest proportion N intake in excreta.

Supplemental Table S1 and Supplemental Table S2 are available in the Mendeley Data repository at: https://data.mendeley.com/datasets/ysdydh5ff4/2.

NOTES

Experimental procedures were approved by the University of Lavras Bioethic Committee in Utilization of Animals (Protocol 002/22). The authors are grateful to Minas Gerais State Agency for Research and Development (FAPEMIG), Brazilian Federal Foundation for Support and Evaluation of Graduate Education (CAPES), and Grupo do Leite of University of Lavras. We would like to thank Syngenta Seeds for partially funding this experiment and for the cultivation and delivery of corn kernels. W. R. Silva received a CAPES/PRINT grant "Sandwich Doctorate" at the University of Guelph (88887.839615/2023-00). The authors have not stated any conflicts of interest.

REFERENCES

Andreazzi, A. S. R., M. N. Pereira, R. B. Reis, R. A. Pereira, N. N. Morais Júnior, T. S. Acedo, R. G. Hermes, and C. S. Cortinhas. 2018. Effect of exogenous amylase on lactation performance of dairy cows fed a high-starch diet. J. Dairy Sci. 101:7199–7207. https://doi.org/10.3168/jds.2017-14331.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International.

APHIS. 2008. Environmental Assessment Alpha-Amylase Maize Event 3272. Accessed December 05, 2023. https://www.aphis.usda.gov/brs/aphisdocs/05_28001p_ea.pdf.

Baker, S., and T. Herrman. 2002. Evaluating particle size. Publication MF-2051. Kansas State Univ., Manhattan.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163. https://doi.org/10.3168/jds.S0022-0302(02)74294-X.

Ben-Ghedalia, D., E. Yosef, J. Miron, and Y. Est. 1989. The effect of starch and pectin-rich diets on quantitative aspects of digestion in sheep. Anim. Feed Sci. Technol. 24:289–298. https://doi.org/10.1016/0377-8401(89)90150-8.

Broderick, G. A., D. R. Mertens, and R. Simons. 2002. Efficacy of carbohydrate sources for milk production by cows fed diets based on alfalfa silage. J. Dairy Sci. 85:1767–1776. https://doi.org/10.3168/ jds.S0022-0302(02)74251-3.

Cannon, S. J., G. C. Fahey Jr, M. R. Murphy, C. L, Dikeman, B. L, Miller, and J. K. Drackley. 2010. Inclusion of psyllium in milk replacer for neonatal calves. Effects on growth, digesta viscosity, rate of passage, nutrient digestibilities, and metabolites in blood. J. Dairy Sci. 93:3652–3660.

Castro, L. P., M. N. Pereira, J. D. Dias, D. V. Lage, E. F. Barbosa, R. P. Melo, F. Karla, J. T. R. Carvalho, F. F. Cardoso, and R. A. Pereira. 2019. Lactation performance of dairy cows fed rehydrated and ensiled corn grain differing in particle size and proportion in the diet. J. Dairy Sci. 102:9857-9869. https://doi.org/10.3168/jds.2019-16559.

Chen, X. B., and M. J. Gomes. 1992. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of Technical Details. International Feed Resources Unit, Rowett Research Institute.

Cotta, M. A. 1988. Amylolytic activity of selected species of ruminal bacteria. Appl. Environ. Microbiol. 54:772–776. https://doi.org/10.1128/aem.54.3.772-776.1988.

Cueva, S. F., H. Stefenoni, A. Melgar, S. E. Räisänen, C. F. A. Lage, D. E. Wasson, M. E. Fetter, A. M. Pelaez, G. W. Roth, and A. N. Hristov. 2021. Lactational performance, rumen fermentation, and enteric methane emission of dairy cows fed an amylase-enabled corn silage. J. Dairy Sci. 104:9827–9841. https://doi.org/10.3168/ jds.2021-20251.

Dehority, B. A. 1984. Evaluation of subsampling and fixation proce dures used for counting rumen protozoa. Appl. Environ. Microbiol. 48:182–185. https://doi.org/10.1128/aem.48.1.182-185.1984.

DeVries, T. J., M. A. G. von Keyserlingk, D. M. Weary, and K. A. Beauchemin. 2003. Measuring the feeding behavior of lactating dairy cows in early to peak lactation. J. Dairy Sci. 86:3354–3361. https://doi.org/10.3168/jds.S0022-0302(03)73938-1.

Ferlay, A., L. Bernard, A. Meynadier, and C. Malpuech-Brugère. 2017. Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: A review. Biochim. 141:107-120. https://doi.org/10.1016/j.biochi.2017.08.006.

Fernandes, T., C. L. S. Ávila, M. N. Pereira, and L. F. Ferraretto. 2018. Effect of washing method, grinding size, and the determination of an indigestible fraction on in situ degradation of starch in mature corn grain. J. Dairy Sci. 101:9052–9057. https://doi.org/10.3168/jds.2018-14870.

Fernandes, T., D. T. da Silva, B. F. Carvalho, R. F. Schwan, R. A. N. Pereira, M. N. Pereira, and C. L. S. Ávila. 2022. Effect of amylases and storage length on losses, nutritional value, fermentation, and microbiology of silages of corn and sorghum kernels. Anim. Feed Sci. Technol. 285:115227. https://doi.org/10.1016/j.anifeedsci.2022.115227.

Ferraretto, L. F., R. D. Shaver, M. Espineira, H. Gencoglu, and S. J. Bertics. 2011. Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. J. Dairy Sci. 94:1490-1499. https://doi.org/10.3168/jds.2010-3736.

Ferreira, E. M., A. V. Pires, I. Susin, M. V. Biehl, R. S. Gentil, M. D. O. M. Parente, D. M. Polizel, C. V. M. Ribeiro, and E. Almeida. 2016. Nutrient digestibility and ruminal fatty acid metabolism in lambs supplemented with soybean oil partially replaced by fish oil blend. Anim. Feed Sci. Technol. 216:30-39. http://dx.doi.org/10.1016/j.anifeedsci.2015.09.007.

Firkins, J. L., A. N. Hristov, M. B. Hall, G. A. Varga, and N. R. St-Pierre. 2006. Integration of ruminal metabolism in dairy cattle. J. Dairy Sci. 89:E31-E51. https://doi.org/10.3168/jds.S0022-0302(06)72362-1.

Fredin, S. M., L. F. Ferraretto, M. S. Akins, P. C. Hoffman, and R. D. Shaver. 2014. Fecal starch as an indicator of total-tract starch digestibility by lactating dairy cows. J. Dairy Sci. 97:1862–1871. https://doi.org/10.3168/jds.2013-7395.

Gencoglu, H., R. D. Shaver, W. Steinberg, J. Ensink, L. F. Ferraretto, S. J. Bertics, J. C. Lopes, and M. S. Akins. 2010. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. J. Dairy Sci. 93:723–732. https:// doi.org/10.3168/jds.2009-2673.

Gomes, A. L., A. V. Bueno, F. A. Jacovaci, G. Donadel, L. F. Ferraretto, L. G. Nussio, C. C. Jobim, J. L. Daniel. 2020. Effects of processing, moisture, and storage length on the fermentation profile, particle size, and ruminal disappearance of reconstituted corn grain. J. Anim. Sci. 98:1-9. https://doi.org/10.1093/jas/skaa332.

Hall, M. B., J. Arbaugh, K. Binkerd, A. Carlson, T. Doan, T. Grant, C. Heuer, H. D. Inerowicz, B. Jean-Louis, R. Johnson, J. Jordan, D. Kondratko, E. Maciel, K. McCallum, D. Meyer, C. A. Odijk, A. Parganlija-Ramic, T. Potts, L. Ruiz, S. Snodgrass, D. Taysom, S. Trupia, B. Steinlicht, and D. Welch. 2015. Determination of dietary starch in animal feeds and pet food by an enzymatic-colorimetric method: Collaborative study. J. AOAC Int. 98:397–409. https://doi.org/10.5740/jaoacint.15-012.

Heinzen Jr, C., M. R. Pupo, L. G. Ghizzi, E. C. Diepersloot, and L. F. Ferraretto. 2024. Effect of a genetically-modified corn hybrid with alpha-amylase and storage length on fermentation profile and starch disappearance of whole-plant corn silage and earlage. J. Dairy Sci. 107:3631-3641. https://doi.org/10.3168/jds.2023-24228.

Horton, L. M., C. L. Van Bibber-Krueger, H. C. Müller, and J. S. Drouillard. 2020. Effects of high-amylase corn on performance and carcass quality of finishing beef heifers. J. Anim. Sci. 98:1-10. https://doi.org/10.1093/jas/skaa302.

Hristov, A. N., C. E. Basel, A. Melgar, A. E. Foley, J. K. Ropp, C. W. Hunt, and J. M. Tricarico. 2008. Effect of exogenous polysaccharide-degrading enzyme preparations on ruminal fermentation and digestibility of nutrients in dairy cows. Anim. Feed Sci. Technol. 145:182-193. https://doi.org/10.1016/j.anifeedsci.2007.05.051.

Hultquist, K. M., and D. P. Casper. 2016. Effects of feeding rumen-degradable valine on milk production in late-lactating dairy cows. J. Dairy Sci. 99:1201-1215. https://doi.org/10.3168/jds.2015-10197.

Hu, W., M. E. Persia, and Jr. L. Kung. 2010. In vitro ruminal fermentability of a modified corn cultivar expressing a thermotolerant α-amylase. J. Dairy Sci. 93:4846-4849. https://doi.org/10.3168/jds.2009-2321.

Ipharraguerre, I. R., and J. H. Clark. 2005. Varying protein and starch in the diet of dairy cows. II. Effects on performance and nitrogen utilization for milk production. J. Dairy Sci. 88:2556–2570. https://doi.org/10.3168/jds.S0022-0302(05)72932-5.

Jolly-Breithaupt, M. L., M. E. Harris, B. L. Nuttelman, D. B. Burken, J. C. MacDonald, M. K. Luebbe, T. K. Iragavarapu, and G. E. Erickson. 2019. Effects of Syngenta Enogen Feed Corn containing an α-amylase trait on finishing cattle performance and carcass characteristics. Transl. Anim. Sci. 3:504-512. https://doi.org/10.1093/tas/txy121.

Johnson, M. A., T. Spore, S. P. Montgomery, W. R. Hollenbeck, R. N. Wahl, E. D Watson, D. A. Blasi. 2019. Syngenta Enogen Feed Corn silage containing an alpha amylase expression trait improves feed efficiency in growing calf diets. Kansas Agricultural Experiment Station Research Reports. 5(Suppl. 1):3. (Abstr).

Koehler, T. J., J. T. Axtell, J. P. Goeser, M. M. Miller, R. D. Shaver, and E. D. Watson. 2022. Enogen corn hybrids can improve feed efficiency and reduce environmental impact in dairy production. J. Dairy Sci. 105(Suppl. 1):308. (Abstr).

Krogstad, K. C., and B. J. Bradford. 2023. The effects of feeding α-amylase enhanced corn silage with different dietary starch concentrations to lactating dairy cows on milk production, nutrient digestibility, and blood metabolites. J. Dairy Sci 106:4666-4681. https://doi.org/10.3168/jds.2022-23030.

Leiva, E., M. B. Hall, and H. H. Van Horn. 2000. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. J. Dairy Sci. 83:2866–2875. https://doi.org/10.3168/jds.S0022-0302(00)75187-3.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557–564. https://doi.org/10.3168/jds.S0022 -0302(03)73634-0.

MacDonald, P. D. M., and P. E. Green. 1988. User’s Guide to Program MIX: An Interactive Program for Fitting Mixtures of Distributions. Release 2.3, January 1988. Ichthus Data Systems.

McCarthy, M. M., M. A. Engstrom, E. Azem, and T. F. Gressley. 2013. The effect of an exogenous amylase on performance and total-tract digestibility in lactating dairy cows fed a high-byproduct diet. J. Dairy Sci. 96:3075-3084. https://doi.org/10.3168/jds.2012-6045.

Mertens, D. R.; Collaborators: M. Allen, J. Carmany, J. Clegg, A. Davidowicz, M. Drouches, K. Frank, D. Gambin, M. Garkie, B. Gildemeister, D. Jeffress, C. S. Jeon, D. Jones, D. Kaplan, G. N. Kim, S. Kobata, D. Main, X. Moua, B. Paul, J. Robertson, D. Taysom, N. Thiex, J. Williams, and M. Wolf. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. of AOAC Int. 85:1217-1240. https://doi.org/10.1093/jaoac/85.6.1217.

Miron, J., E. Yosef, and D. Ben-Ghedalia. 2001. Composition and in vitro digestibility of monosaccharide constituents of selected byproduct feeds. J. Agric. Food Chem. 49:2322-2326. https://doi.org/10.1021/jf0008700.

Morris, D. L., A. W. Tebbe, W. P. Weiss, and C. Lee. 2019. Effects of drying and analytical methods on nitrogen concentrations of feeds, feces, milk, and urine of dairy cows. J. Dairy Sci. 102:5212-5218. https://doi.org/10.3168/jds.2019-16256.

Nakae, T., and J. A. Elliott. 1965. Production of volatile fatty acids by some lactic acid bacteria. II. Selective formation of volatile fatty acids by degradation of amino acids. J. Dairy Sci.48:293-299. https://doi.org/10.3168/jds.S0022-0302(65)88219-4.

NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. Natl. Acad. Press.

Nozière, P., W. Steinberg, M. Silberberg, and D. P. Morgavi. 2014. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. J. Dairy Sci. 97:2319-2328. https://doi.org/10.3168/jds.2013-7095.

Oba, M., and M. S. Allen. 2003. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174-183. https://doi.org/10.3168/jds.S0022-0302(03)73598-X.

Oliveira, L. N., M. A. Pereira, C. D. Oliveira, C. C. Oliveira, R. B. Silva, R. A. Pereira, T. J. DeVries, and M. N. Pereira. 2023. Effect of low dietary concentrations of Acacia mearnsii tannin extract on chewing, ruminal fermentation, digestibility, nitrogen partition, and performance of dairy cows. J. Dairy Sci.106:3203-3216. https://doi.org/10.3168/jds.2022-22521.

Pech-Cervantes, A. A., L. F. Ferrarretto, and I. M. Ogunade. 2022. Meta-analysis of the effects of the dietary application of exogenous alpha-amylase preparations on performance, nutrient digestibility, and rumen fermentation of lactating dairy cows. J. Anim. Sci. 100:skac189. https://doi.org/10.1093/jas/skac189.

Pereira, M. N., and L. E. Armentano. 2000. Partial replacement of forage with nonforage fiber sources in lactating cow diets. II. Digestion and rumen function. J. Dairy Sci. 83:2876-2887. https://doi.org/10.3168/jds.S0022-0302(00)75188-5.

Pereira, M. N., N. N. Morais Júnior, R. Caputo Oliveira, G. G. S. Salvati, and R. A. N. Pereira. 2021. Methionine precursor effects on lactation performance of dairy cows fed raw or heated soybeans. J. Dairy Sci. 104:2996–3007. https://doi.org/10.3168/jds.2020-18696.

Piccioli-Cappelli, F., J. J. Loor, C. J. Seal, A. Minuti, and E. Trevisi. 2014. Effect of dietary starch level and high rumen-undegradable protein on endocrine-metabolic status, milk yield, and milk composition in dairy cows during early and late lactation. J. Dairy Sci. 97:7788–7803. https://doi.org/10.3168/jds.2014-8336.

Ramanzin, M., L. Bailoni, and G. Bittante. 1994. Solubility, water-holding capacity, and specific gravity of different concentrates. J. Dairy Sci. 77:774-781. https://doi.org/10.3168/jds.S0022-0302(94)77012-0.

Rebelo, L. R., M. L. Eastridge, J. L. Firkins, and C. Lee. 2023. Effects of corn silage and grain expressing α-amylase on ruminal nutrient digestibility, microbial protein synthesis, and enteric methane emissions in lactating cows J. Dairy Sci. 106:3932-3946. https://doi.org/10.3168/jds.2022-22770.

Salvador, S. C., M. N. Pereira, J. F. Santos, L. Q. Melo, and M. L. Chaves. 2008. Response of lactating cows to the total replacement of corn by citrus pulp and to the supplementation of organic trace minerals. I: Intake and digestion. Arq. Bras. Med. Vet. Zootec. 60:682–690. https://doi.org/10.1590/S0102-09352008000300024.

Spanghero, M., and Z. M. Kowalski. 2021. Updating analysis of nitrogen balance experiments in dairy cows. J. Dairy Sci. 104:7725-7737. https://doi.org/10.3168/jds.2020-19656.

Stone, W. C. 2004. Nutritional approaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. J. Dairy Sci. 87(E. Suppl.):E13–E26. https://doi.org/10.3168/jds.S0022-0302(04)70057-0.

Valadares, R. F. D., G. A. Broderick, S. C. Valadares Filho, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686–2696. https://doi.org/10 .3168/jds.S0022-0302(99)75525-6.

Volk, S. M., H. C. Wilson, K. J. Hanford, J. C. MacDonald, and G. E. Erickson. 2021. Impact of Feeding Syngenta Enogen® Feed Corn Compared to Control Corn in Different Diet Scenarios to Finishing Beef Cattle. Anim. 11:2940. https://doi.org/10.3390/ani11102940.

Zilio, E. M., T. A. Del Valle, L. G. Ghizzi, C. S. Takiya, M. S. Dias, A. T. Nunes, G. G. Silva, and F. P. Rennó. 2019. Effects of exogenous fibrolytic and amylolytic enzymes on ruminal fermentation and performance of mid-lactation dairy cows. J. Dairy Sci. 102:4179-4189. https://doi.org/10.3168/jds.2018-14949.

Warner, A. C. 1962. Enumeration of rumen microorganisms. J. Gen. Microbiol. 28:119–128. https://doi.org/10.1099/00221287-28-1-119.

Weiss, W. P., W. Steinberg, and M. A. Engstrom. 2011. Milk production and nutrient digestibility by dairy cows when fed exogenous amylase with coarsely ground dry corn. J. Dairy Sci. 94:2492–2499. https://doi.org/10.3168/jds.2010-3766.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, J. H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to standard production characteristics. J. Dairy Sci. 65:495–501. https://doi.org/10.3168/jds .S0022-0302(82)82223-6.

Williams, E. J. 1949. Experimental designs balanced for the estimation of residual effects of treatments. Aust. J. Sci. Res. 2:149–168. https://doi.org/10.1071/CH9490149.

Table 1. Ingredient composition of offered diets and nutrient composition of the consumed diets (offered – orts)

Corn concentration[1]	High		Low	
Corn type	AAC	CTL	AAC	CTL
Ingredients, % of DM				
Alfalfa haylage	10.9	10.9	10.9	10.8
Whole plant corn silage	34.3	34.1	34.0	33.9
Citrus pulp pellets	2.5	2.5	13.8	13.8
Soybean meal	15.5	15.4	15.4	15.4
Ensiled AAC corn grain	24.4		14.0	
Ensiled Isogenic corn grain		24.7		14.2
Whole cottonseeds	9.3	9.3	9.3	9.3
Limestone	0.5	0.5		
Premix[2]	2.7	2.7	2.6	2.6
Nutrients, % of DM				
CP	16.5	16.4	16.4	16.4
RDP[3]	11.8	11.8	11.9	11.9
RUP[3]	4.6	4.6	4.6	4.6
NDF	32.3	32.0	33.8	33.7
Forage NDF (alfalfa haylage + corn silage)	23.2	23.0	23.0	22.9
$_{pe}NDF_{>8}$[4]	23.4	23.3	23.2	23.2
Ether extract	4.2	4.2	4.1	4.1
Ash	6.5	6.5	6.4	6.5
NFC[5]	40.5	40.9	39.3	39.4
Starch	28.7	28.6	21.5	21.4
Starch from ensiled corn grain	18.3	18.2	10.5	10.5
Starch from whole plant corn silage	9.7	9.6	9.6	9.6
Non-starch NFC[6]	11.8	12.3	17.8	18.0
NEL, Mcal/kg of DM[3]	1.80	1.78	1.79	1.79
MP supply, g/d[3]	2144	2142	2139	2156
MP balance, g/d[3]	95	91	80	102
DM, % of as-fed	52.6	52.8	54.1	54.4

[1]High = high corn concentration, Low = low corn concentration, AAC = rehydrated and ensiled alpha-amylase corn, CTL = rehydrated and ensiled isogenic control corn.
[2]Premix (on DM basis): 9.4% limestone, 37.7% sodium bicarbonate, 15.1% magnesium oxide, 9.4% NaCl, and 28.3% minerals and vitamins (23.8% Ca, 16.9% P, 1.9% Mg, 2.3% S, 100 mg/kg Co, 1,231 mg/kg Cu, 3,080 mg/kg Mn, 7323 mg/kg Zn, 51 mg/kg Se, 124 mg/kg I, 615,385 IU/kg Vit. A, 153,600 IU/kg Vit. D; 3825 IU/kg Vit E; 2000 mg/kg monensin).
[3]Estimated with NASEM (2021) for dairy cattle model.
[4]Physically effective fiber ($_{pe}NDF_{>8}$) calculated from feed analysis and diet composition assuming that only whole plant corn silage, alfalfa haylage, and whole cottonseeds contributed to NDF > 8 mm.

[5]NFC = 100 - (CP + NDF + ether extract + ash).
[6]Non-starch NFC = NFC - Starch.

Table 2. Effect of grinding control (CTL) or (AAC) amylase-enabled corn kernels on the particle size distribution and geometric mean particle size (GMPS) of ground corn grain (n = 12/corn type)

Corn type	AAC	CTL	SEM	*P*-value[1]
% retained on screen				
4750 µm	0.30	0.40	0.111	0.53
3350 µm	5.19	4.60	0.502	0.42
2360 µm	15.39	15.23	1.113	0.92
1700 µm	21.20	22.15	0.330	0.16
1180 µm	15.28	15.74	0.419	0.44
600 µm	19.88	20.20	0.773	0.77
250 µm	16.03	14.77	0.638	0.18
Pan	6.75	6.91	0.375	0.76
GMPS[2], µm	814	815	36.1	0.98

[1]*P*-value for corn type.

[2]Kansas State University: MF-2051. Baker and Herrman (2002).

Table 3. Composition of feed ingredients. Mean ± SD of 4 composite samples from each experimental period

Item[1]	DM % of as-fed	CP % of DM	EE % of DM	NDF % of DM	Ash % of DM	NFC[2] % of DM	Starch % of DM	Non-starch NFC[3] % of DM
Alfalfa haylage	40.8 ± 5.1	18.0 ± 1.5	2.4 ± 0.4	43.0 ± 2.0	11.0 ± 0.6	25.6 ± 2.5	1.8 ± 0.7	23.8 ± 1.9
Whole plant corn silage	36.8 ± 2.6	7.2 ± 0.5	2.9 ± 0.5	53.7 ± 4.4	2.4 ± 0.2	33.8 ± 4.9	28.3 ± 1.8	5.5 ± 4.8
Citrus pulp pellets	89.0 ± 1.2	7.1 ± 0.3	2.6 ± 0.3	24.3 ± 1.2	5.3 ± 1.2	60.8 ± 2.3	5.4 ± 1.5	55.5 ± 1.3
Soybean meal	88.0 ± 1.9	51.1 ± 1.6	1.8 ± 0.2	10.7 ± 0.1	6.9 ± 0.5	29.5 ± 2.2	2.1 ± 0.6	27.4 ± 2.0
Whole cottonseeds	92.6 ± 0.6	22.4 ± 2.9	18.3 ± 0.8	48.9 ± 8.2	3.6 ± 0.2	6.8 ± 2.9	1.0 ± 0.8	5.8 ± 3.1

[1]Samples were analyzed by wet chemistry.
[2]NFC = 100 – (CP + NDF + EE + ash).
[3]Non-starch NFC = NFC - Starch.

Table 4. Effect of rehydration and ensiling alpha-amylase enabled corn (AAC) or isogenic control (CTL) corn grains on nutrient composition, microbial count, pH, and DM loss of rehydrated and ensiled corn grain

Corn type	AAC	CTL	SEM	*P*-value[1]
DM, % of as-fed[2]	62.2	63.1	0.66	0.35
Nutrients, % of DM[2]				
CP	7.6	7.6	0.08	1.00
NDF	9.5	8.9	1.19	0.71
Ash	1.4	1.4	0.06	1.00
NFC	77.9	78.5	1.27	0.72
Starch	74.8	73.8	1.05	0.50
Non-starch NFC	3.0	4.7	1.05	0.29
Log_{10} cfu/g[3]				
Yeast	3.65	3.09	0.254	0.13
Spore forming aerobic bacteria	3.41	2.99	0.259	0.26
Filamentous fungi	1.98	1.74	0.333	0.61
Lactic acid bacteria	5.48	5.20	0.132	0.15
pH[4]	3.93	3.99	0.051	0.43
DM loss, % of ensiled[5]	1.53	1.71	0.357	0.70
Mass at ensiling, kg of as-fed[5]	202.2	198.1	2.31	0.18
Mass at opening, kg of as-fed[5]	201.0	197.9	2.18	0.29
Mass at ensiling, kg of DM[5]	125.7	123.5	1.46	0.27
Mass at opening, kg of DM[5]	123.7	121.4	1.37	0.20

[1]*P*-value for corn type.
[2]Data obtained by wet chemistry. N = 4 samples obtained during the last week of each period/corn type.
[3]cfu = colony forming units. N = 18 silos/corn type.
[4]N = 41 silos/corn type.
[5]N = 25 silos/corn type.

Table 5. Effect of corn concentration and type on DMI, digestible OM intake (DOMI), lactation performance, feed efficiency, linear SCC, BW, and BCS of dairy cows

Corn concentration[1]	High		Low			*P*-value[2]		
Corn type	AAC	CTL	AAC	CTL	SEM	C	T	C×T
kg/d								
DMI	22.6	22.6	22.8	23.0	0.83	0.32	0.71	0.61
DOMI	15.8	15.6	15.8	15.7	0.60	0.82	0.50	0.98
Milk	33.8	34.2	34.4	34.1	1.33	0.56	0.89	0.32
ECM[3]	33.1	33.3	34.8	34.0	1.10	< 0.01	0.45	0.26
Fat	1.211	1.207	1.319	1.275	0.0428	< 0.01	0.28	0.37
Protein	1.053	1.057	1.057	1.049	0.0375	0.88	0.89	0.63
Casein	0.820	0.825	0.808	0.808	0.0303	0.24	0.81	0.85
Lactose	1.565	1.596	1.609	1.582	0.0596	0.44	0.91	0.14
Total solids	4.150	4.185	4.313	4.230	0.1407	0.04	0.62	0.23
% of milk								
Fat	3.62	3.58	3.87	3.77	0.103	< 0.01	0.23	0.63
Protein	3.13	3.10	3.08	3.09	0.050	0.04	0.37	0.13
Casein	2.44	2.42	2.35	2.38	0.043	< 0.01	0.68	0.13
Lactose	4.64[b]	4.67[ab]	4.69[a]	4.65[b]	0.050	0.10	0.69	< 0.01
Total solids	12.34	12.29	12.60	12.47	0.158	< 0.01	0.12	0.50
Milk/DMI	1.49	1.51	1.50	1.48	0.036	0.41	0.76	0.16
ECM/DMI	1.47[b]	1.48[b]	1.54[a]	1.49[b]	0.028	0.02	0.19	0.07
ECM/DOMI	2.12	2.15	2.22	2.19	0.049	0.03	0.99	0.34
Ln SCC,[4] 0 to 9	1.14	0.84	1.32	1.41	0.385	0.18	0.70	0.49
BW, kg	602	600	604	603	20.4	0.27	0.48	0.98
BCS, 1 to 5	3.15	3.15	3.15	3.14	0.072	0.69	0.75	0.69

[1]High = high corn concentration, Low = low corn concentration, AAC = rehydrated and ensiled alpha-amylase corn, CTL = rehydrated and ensiled isogenic control corn.
[2]C × T = interaction between corn concentration (C) and type (T). [a,b]Means in a row with differing superscripts differ at $P \leq 0.05$ by Tukey.
[3]ECM =energy corrected milk.
[4]Equivalency of the Ln SCC: 1.14 = 27,550 cells/mL; 0.84 = 22,380 cells/mL; 1.32 = 31,210 cells/mL; 1.41 = 33,220 cells/mL.

Table 6. Effect of corn concentration and type on milk fatty acids profile[1]

Corn concentration[2]	High		Low			*P*-value[3]		
Corn type	AAC	CTL	AAC	CTL	SEM	C	T	C × T
g/100 g milk								
Preformed[4]	1.142	1.130	1.260	1.191	0.0329	< 0.01	0.12	0.28
Mixed[5]	1.192	1.184	1.361	1.263	0.0432	< 0.01	0.12	0.18
De Novo[6]	0.599	0.600	0.655	0.648	0.0233	< 0.01	0.84	0.76
Monounsaturated	0.547	0.533	0.581	0.561	0.0226	0.03	0.23	0.80
Polyunsaturated	0.032	0.031	0.031	0.032	0.0040	0.88	0.88	0.66
Saturated	2.242	2.211	2.472	2.378	0.0760	< 0.01	0.16	0.48
Unsaturated	0.559	0.542	0.602	0.581	0.0263	0.02	0.25	0.88
Palmitic	1.308	1.274	1.460	1.383	0.0552	< 0.01	0.09	0.49
Stearic	0.295	0.289	0.331	0.315	0.0105	< 0.01	0.16	0.52
Oleic	0.398	0.390	0.426	0.410	0.0175	0.03	0.26	0.71
kg/d								
Preformed	0.381	0.382	0.430	0.402	0.0133	< 0.01	0.15	0.13
Mixed	0.398[b]	0.400[b]	0.464[a]	0.427[b]	0.0167	< 0.01	0.13	0.09
De Novo	0.201	0.204	0.224	0.220	0.0097	< 0.01	0.89	0.47
Monounsaturated	0.181	0.176	0.197	0.188	0.0055	< 0.01	0.13	0.59
Polyunsaturated	0.011	0.010	0.011	0.011	0.0014	0.40	0.31	0.45
Saturated	0.751	0.749	0.841	0.807	0.0319	< 0.01	0.28	0.33
Unsaturated	0.184	0.178	0.204	0.194	0.0067	< 0.01	0.14	0.71
Palmitic	0.438	0.431	0.496	0.470	0.0213	< 0.01	0.14	0.38
Stearic	0.097	0.096	0.113	0.106	0.0034	< 0.01	0.12	0.28
Oleic	0.132	0.129	0.145	0.137	0.0040	< 0.01	0.11	0.47
g/100 g fatty acids								
Preformed	39.06	38.80	38.55	38.47	0.346	0.09	0.49	0.71
Mixed	40.53	40.52	41.34	40.61	0.388	0.17	0.26	0.27
De Novo	20.41	20.67	20.11	20.92	0.504	0.95	0.20	0.52
Monounsaturated	19.44	19.12	19.00	18.86	0.534	0.15	0.37	0.70
Polyunsaturated	1.11	1.06	1.00	1.05	0.125	0.25	0.98	0.34
Saturated	80.35	80.82	80.42	80.59	0.612	0.79	0.31	0.63
Unsaturated	19.65	19.18	19.58	19.41	0.612	0.79	0.31	0.63
Palmitic	44.72	44.03	44.51	45.05	1.081	0.61	0.92	0.43
Stearic	9.97	9.88	10.15	10.21	0.246	0.24	0.96	0.74
Oleic	13.54	13.33	13.15	13.22	0.518	0.46	0.84	0.67

[1]Samples analyzed by mid-infrared reflectance spectroscopy.

[2]High = high corn concentration, Low = low corn concentration, AAC = rehydrated and ensiled alpha-amylase corn, CTL = rehydrated and ensiled isogenic control corn.

[3]C×T = interaction between corn concentration (C) and type (T). [a, b]Means in a row with differing superscripts differ at $P \leq 0.05$ by Tukey.

[4]Preformed = Fatty acids ≥ 18 C.

[5]Mixed = Fatty acids with 16 or 17 C.

[6]De novo = Fatty acids with 4 to 15 C.

Table 7. Effect of corn concentration and type on intakes of physically effective fiber (${}_{pe}NDF_{>8}$), forage NDF (FNDF), and total NDF, meal and chewing behavior, proportion of intake during the day, and sorting behavior of dairy cows

Corn concentration[1]	High		Low			*P*-value[2]		
Corn type	AAC	CTL	AAC	CTL	SEM	C	T	C × T
${}_{pe}NDF_{>8}$,[3] kg/d	5.3	5.2	5.3	5.4	0.20	0.63	0.99	0.38
${}_{pe}NDF_{>8}$,[3] % of BW	0.88	0.88	0.87	0.89	0.020	0.85	0.51	0.22
FNDF, kg/d	5.2	5.2	5.2	5.2	0.19	0.91	0.72	0.72
FNDF, % of BW	0.87	0.87	0.87	0.87	0.020	0.81	0.88	0.63
NDF, % of BW	1.22	1.21	1.27	1.28	0.029	< 0.01	0.93	0.55
Meal behavior								
Meal frequency, meals/d	9.8^{b}	10.0^{ab}	10.3^{a}	9.9^{ab}	0.32	0.14	0.69	0.04
Meal size, kg DM/meal	2.37^{A}	2.28^{AB}	2.25^{B}	2.35^{AB}	0.092	0.62	0.95	0.07
Meal duration, min/meal	36.9	35.0	35.2	36.3	1.60	0.78	0.64	0.07
Meal time, min/d	350	345	355	354	11.7	0.17	0.62	0.68
Longest meal duration, min/meal	81.6	80.0	77.6	79.5	4.69	0.56	0.96	0.66
Longest meal timing,[4] min	311	263	332	367	49.3	0.07	0.86	0.24
First meal duration, min/meal	68.5	70.8	60.7	63.3	5.77	0.04	0.50	0.96
Chewing activity behavior								
Eating, min/d	314	311	321	320	11.3	0.12	0.64	0.81
Rumination, min/d	444^{AB}	436^{AB}	432^{B}	445^{A}	7.4	0.79	0.62	0.07
Chewing activity,[5] min/d	758	747	752	765	13.1	0.46	0.92	0.14
Eating, min/kg DMI	14.0	14.0	14.0	14.0	0.84	0.96	0.84	0.95
Rumination, min/kg DMI	19.7	19.4	18.9	19.5	0.87	0.32	0.62	0.23
Chewing activity, min/kg DMI	33.7	33.4	32.9	33.5	1.58	0.54	0.83	0.42
Eating, min/kg ${}_{pe}NDF_{>8}$	63	62	63	62	3.6	0.70	0.52	0.88
Rumination, min/kg ${}_{pe}NDF_{>8}$	88	86	85	87	3.8	0.34	0.83	0.17
Chewing activity, min/kg ${}_{pe}NDF_{>8}$	151	149	147	149	6.8	0.41	0.85	0.40
Eating, min/kg FNDF	63	63	63	63	3.7	0.88	0.64	0.97
Rumination, min/kg FNDF	88	87	85	88	3.8	0.46	0.73	0.20
Chewing activity, min/kg FNDF	152	150	148	150	6.9	0.57	0.99	0.40
% of daily intake								
0700-1200 h	49.2	50.5	43.8	45.0	1.55	< 0.01	0.17	0.93
1200-1900 h	36.1	34.6	37.9	37.5	1.13	< 0.01	0.27	0.52
1900-0700 h	14.8	14.9	18.3	17.5	1.38	< 0.01	0.72	0.60
Sorting, Observed/Predicted × 100%[6]								
0700-1200 h								
> 19 mm[7]	81	80	79	85	4.4	0.72	0.52	0.26
8-19 mm	99	98	99	99	1.2	0.56	0.56	0.74
< 8 mm	106^{AB}	107^{AB}	108^{A}	105^{B}	1.7	0.96	0.54	0.05
1200-1900 h								
> 19 mm	81	79	82	78	3.7	1.00	0.29	0.67
8-19 mm	98	100	100	101	1.0	0.03	0.08	0.73
< 8 mm	109	108	108	107	1.6	0.37	0.68	0.84
1900-0700 h								
> 19 mm	89	96	84	89	4.3	0.18	0.12	0.78
8-19 mm	96	94	100	97	1.8	0.02	0.13	0.93
< 8 mm	112	109	111	111	2.9	0.91	0.38	0.45

[1]High = high corn concentration, Low = low corn concentration, AAC = rehydrated and ensiled alpha-amylase corn, CTL = rehydrated and ensiled isogenic control corn.

[2]C × T = interaction between corn concentration (C) and type (T). [a,b]Means in a row with differing superscripts differ at $P \le 0.05$ by Tukey. [A,B]Means in a row with differing superscripts differ at $P \le 0.10$ by Tukey.

[3]Physically effective fiber calculated from feed analysis and diet composition assuming that only whole plant corn silage, alfalfa haylage, and whole cottonseeds contributed to NDF > 8 mm.

[4]Minutes from first daily feeding at 0700 h.

[5]Chewing activity: eating + rumination.

[6]Sorting: > 100 = preferential intake; < 100 = refusal; 100 = no sorting.

[7]Screens of the Penn State Particle Separator.

Table 8. Effect of corn concentration and type on urine excretion, total tract apparent digestibility, digestible starch intake, urinary allantoin and allantoin/digestible OM intake (DOMI), ruminal molar VFA proportions, pH and viscosity of feces and ruminal fluid, ruminal protozoa concentration, and plasma glucose of dairy cows

Corn concentration[1]	High		Low			*P*-value[2]		
Corn type	AAC	CTL	AAC	CTL	SEM	C	T	C × T
Urine, L/d	23.8	24.3	26.4	27.6	1.71	0.02	0.47	0.79
Digestibility, % of intake								
DM	72.1	71.7	72.1	70.9	0.68	0.50	0.13	0.50
OM	74.3	73.7	74.3	73.2	0.61	0.60	0.15	0.70
NDF	49.2	47.1	50.6	48.9	1.29	0.18	0.09	0.83
Non-NDF OM	87.3	87.4	87.5	86.8	0.36	0.56	0.27	0.22
Starch	98.5	98.6	98.4	98.3	0.18	0.15	0.94	0.69
CP	77.6	76.9	76.5	76.1	0.58	0.08	0.32	0.72
Digestible starch intake, kg/d	6.4	6.4	4.8	4.8	0.20	< 0.01	0.78	0.86
Fecal starch, % of DM	1.5	1.4	1.3	1.3	0.18	0.13	0.72	0.76
Fecal starch, kg/d	0.095	0.096	0.081	0.083	0.0126	0.17	0.90	0.92
Allantoin, mmol/d[3]	322	336	357	326	21.8	0.51	0.65	0.23
Allantoin/DOMI, mmol/kg	20.7	21.8	22.6	20.9	1.26	0.63	0.79	0.24
Molar % of total VFA								
Acetate	59.2	59.0	62.3	62.7	0.87	< 0.01	0.89	0.57
Propionate	25.0	25.1	22.2	21.5	0.91	< 0.01	0.58	0.51
Butyrate	11.1	10.7	11.0	11.4	0.33	0.39	0.95	0.22
Isobutyrate	0.97	1.01	0.87	0.94	0.030	< 0.01	0.03	0.56
Valerate	1.79	2.13	1.57	1.63	0.198	0.07	0.31	0.48
Isovalerate	1.93	1.98	1.63	1.75	0.077	< 0.01	0.14	0.51
Acetate/Propionate	2.47	2.43	2.92	2.97	0.122	< 0.01	0.90	0.51
pH								
Rumen	6.54	6.63	6.70	6.77	0.089	0.05	0.32	0.89
Feces	7.78^{AB}	7.72^{B}	7.74^{AB}	7.83^{A}	0.045	0.37	0.73	0.10
Viscosity, cP[4]								
Rumen	7.58	7.41	7.05	7.10	0.161	0.01	0.69	0.51
Feces	45.4	42.6	44.7	44.9	1.85	0.60	0.38	0.32
Protozoa, × 10^5/mL	5.82	4.75	4.57	4.98	0.795	0.47	0.65	0.30
Glucose, mg/dL								
Day 18	61.1	62.1	61.2	60.8	1.04	0.57	0.78	0.55
Day 21	58.2	58.4	57.6	58.0	1.11	0.60	0.76	0.95
Mean	59.7	60.2	59.4	59.4	0.71	0.38	0.64	0.66

[1]High = high corn concentration, Low = low corn concentration, AAC = rehydrated and ensiled alpha-amylase corn, CTL = rehydrated and ensiled isogenic control corn.
[2]C × T = interaction between corn concentration (C) and type (T). [A,B]Means in a row with differing superscripts differ at $P \leq 0.10$ by Tukey.
[3]Equivalency of Allantoin (mmol/d): 322 mmol/d= 255 g/d of microbial N; 336 mmol/d = 266 g/d of microbial N; 357 mmol/d = 282 g/d of microbial N; 326 = 258 g/d of microbial N. Duodenal purine

base flow (mmol/d) = 17.0 + 1.037 × urinary allantoin (Firkins et al., 2006). Microbial N (g/d) = (Duodenal purine base flow × 70) / (0.116 × 83 × 1000) (Chen and Gomes, 1992).

[4]cP = centipoise.

Table 9. Effect of corn concentration and type on MUN, plasma urea-N (PUN), and N partition of dairy cows

Corn concentration[1]	High		Low			*P*-value[2]		
Corn type	AAC	CTL	AAC	CTL	SEM	C	T	C × T
MUN, mg/dL	17.4^{b}	18.1^{ab}	18.8^{a}	18.2^{ab}	0.65	0.02	0.88	0.05
PUN,[3] mg/dL	17.2^{b}	19.0^{a}	18.7^{a}	17.9^{ab}	0.65	0.69	0.25	< 0.01
N, g/d								
Intake	597.1	594.6	597.4	599.1	21.71	0.76	0.95	0.79
Milk[4]	165.0	165.7	165.7	164.4	5.88	0.88	0.89	0.63
Urine	203.9	218.4	235.3	230.0	10.68	0.01	0.58	0.24
Fecal	132.7	137.4	140.5	142.2	5.69	0.08	0.38	0.67
Total excreta[5]	336.6	355.7	375.8	372.1	14.09	< 0.01	0.40	0.22
Unaccounted N	95.5^{a}	73.1^{ab}	56.0^{b}	62.0^{b}	11.77	< 0.01	0.37	0.10
N, % of daily intake								
Milk	27.8	28.0	27.9	27.7	0.57	0.57	0.95	0.55
Urine	34.1	37.1	39.8	38.8	1.46	< 0.01	0.44	0.13
Fecal	22.4	23.1	23.5	23.9	0.58	0.08	0.32	0.72
Total excreta[5]	56.5^{b}	60.2^{a}	63.3^{a}	62.7^{a}	1.53	< 0.01	0.25	0.10
Unaccounted N	15.6^{a}	11.2^{ab}	8.8^{b}	9.6^{b}	1.70	< 0.01	0.28	0.10

[1]High = high corn concentration, Low = low corn concentration, AAC = rehydrated and ensiled alpha-amylase corn, CTL = rehydrated and ensiled isogenic control corn.

[2]C × T = interaction between corn concentration (C) and type (T). ^{a,b}Means in a row with differing superscripts differ at $P \leq 0.05$ by Tukey.

[3]Samples obtained at 0 and 2 h relative to the end of morning (immediately before feeding), afternoon, and night milkings. 6 ×/d. $P < 0.01$ for time of sampling (Ti), $P = 0.60$ for C × Ti, $P = 0.33$ for T × Ti, and $P = 0.16$ for C × T × Ti.

[4]Milk crude protein/6.38.

[5]Urine + feces.

Supplemental Table S1. Whole plant corn silage particle size, NDF and starch distributions and concentrations of DM, NDF, and starch on fractions of the Penn State Particle Separator

	Whole plant corn silage
Particle size distribution,[1] % of as-fed	
> 19 mm	11.2 ± 3.27
8-19 mm	66.1 ± 15.63
< 8 mm	22.7 ± 5.70
Particle size distribution,[1] % of DM	
> 19 mm	10.4 ± 1.91
8-19 mm	67.0 ± 1.94
< 8 mm	22.6 ± 2.30
NDF distribution,[2] % of DM	
> 19 mm	7.7 ± 1.47
8-19 mm	30.7 ± 0.91
< 8 mm	13.1 ± 1.31
Starch distribution,[2] % of DM	
> 19 mm	1.0 ± 0.20
8-19 mm	22.2 ± 2.54
< 8 mm	5.1 ± 0.67
DM of fraction retained,[1] % of as-fed	
> 19 mm	32.8 ± 1.52
8-19 mm	36.0 ± 1.56
< 8 mm	35.8 ± 3.36
NDF of fraction retained,[2] % of DM	
> 19 mm	74.2 ± 1.51
8-19 mm	46.0 ± 1.20
< 8 mm	57.7 ± 1.36
Starch of fraction retained,[2] % of DM	
> 19 mm	9.2 ± 1.15
8-19 mm	32.2 ± 3.56
< 8 mm	21.8 ± 1.56

[1]Mean ± SD of 12 samples of each ingredient obtained throughout the experiment.
[2]Mean ± SD of 4 composite samples from each experimental period.

Supplemental Table S2. Mean and SD of soluble protein, NH_3-N, ruminal degradation of starch, and fermentation profile of rehydrated and ensiled corn (NIRS data[1]). 4 samples obtained during the last week of each experimental period per corn type

Corn type[2]	AAC	CTL
Soluble protein, % of CP	31.2 ± 2.41	33.0 ± 3.09
NH_3-N, % of DM	0.20 ± 0.039	0.21 ± 0.044
NH_3-N, % of CP	2.63 ± 0.482	2.67 ± 0.543
Starch degradation, % of starch		
0 h (A fraction)	25.2 ± 4.05	22.5 ± 1.08
7 h	57.8 ± 1.93	57.2 ± 0.66
kd of starch, %/h	11.7 ± 0.66	11.6 ± 0.27
ERD, % of starch[3]	65.5 ± 1.56	64.9 ± 0.52
Organic acids, % of DM		
Lactic acid	1.88 ± 0.042	1.87 ± 0.172
Acetic acid	0.19 ± 0.026	0.23 ± 0.022

[1]3r ribersolo/Rock River (Ribeirão Preto, Brazil).

[2]AAC = rehydrated and ensiled alpha-amylase corn. CTL = rehydrated and ensiled isogenic control corn.

[3]ERD = effective ruminal degradation. The ERD was calculated with a 1-pool starch model (ERD = Starch × kd / (kd + kp). Tylutki et al., 2007) assuming a fractional passage rate (kp) of 7%/h (Seo et al., 2006) and the fractional degradation rate (kd) of the NIRS analysis.

THIRD PART – SUBMITTED MANUSCRIPT

This manuscript is in review to publication according to the Journal of Dairy Science submission guidelines

INTERPRETIVE SUMMARY

Effect of ensiled or finely ground alpha-amylase enabled corn grain on lactation performance, chewing, ruminal fermentation, digestibility and nitrogen partition of dairy cows. *By Silva et al.* Lactation performance of dairy cows fed alpha-amylase enabled corn (AAC) may vary by kernel processing method. Treatment diets included 13.0% dry matter from AAC or control corn as rehydrated and ensiled (REC) or finely ground kernels, 9.6 % of diet dry matter as starch from each treatment. Feeding REC tended to reduce milk fat content and to increase feed efficiency, while corn type did not affect lactation performance. Diets with AAC tended to improve the total tract starch digestibility and to reduce fecal starch content. Diets with REC lowered ruminal pH, and AAC showed a similar tendency. Cows fed REC had smaller, shorter, and more frequent meals. Nitrogen partitioning was unaffected, though REC tended to increase the proportion of nitrogen intake secreted in milk. Results showed few significant interactions between corn type and processing, suggesting that the main mode of action of AAC was by improving digestion, independently of ensiling.

ALPHA-AMYLASE ENABLED CORN FOR DAIRY COWS

Effect of ensiled or finely ground alpha-amylase enabled corn grain on lactation performance, chewing, ruminal fermentation, digestibility and nitrogen partition of dairy cows

Wesley R. Silva[a], Ana Julia C. Silva[a], Mariane A. Tiengo[a], João Pedro A. Rezende[a], Stefania P. Souza[a], Renata A. N. Pereira[b], Trevor J. DeVries[c], and Marcos N. Pereira[a*]

[a]Universidade Federal de Lavras, Faculdade de Zootecnia e Medicina Veterinária, Lavras, MG 37203-202, Brazil

[b]Empresa de Pesquisa Agropecuária de Minas Gerais, Unidade Regional Epamig Sul de Minas, Lavras, MG 37200-970, Brazil

[c]University of Guelph, Department of Animal Biosciences, Guelph, ON N1G 2W1, Canada

*Corresponding author: M. N. Pereira. Universidade Federal de Lavras, Departamento de Zootecnia, Lavras, MG, 37200-900, Brazil. E-mail: mpereira@ufla.br

ABSTRACT

Alpha-amylase enabled corn (AAC) and ensiling of corn kernels can improve starch digestion, feed efficiency, and N partition into milk of dairy cows. This experiment evaluated the effect of AAC on cows fed finely ground (GRC) or rehydrated and ensiled (REC) corn kernels on lactational performance, DMI, ruminal fermentation, digestibility, chewing behavior and N partition of dairy cows. Twenty-four, individually-fed Holstein cows (37.1 ± 4.8 kg/d milk yield, 143 ± 100 DIM, 633 ± 64 kg body weight), arranged in 4 × 4 Latin squares (with 21-d periods), were assigned to 4 treatment sequences. Treatments were arranged in a 2 × 2 factorial with two corn processing methods (P: GRC vs. REC) and two corn types (T: AAC, 48.8% vitreousness vs. CTL, 51.1% vitreousness). Kernels were weekly ground, hydrated (61.7 ± 0.55% DM for AAC and 61.7 ± 0.45% of DM for CTL) and ensiled to maintain a constant storage duration (28 ± 3 d). Diets had 13.0% corn grain from each treatment, 22.4% total starch, and 9.6% starch from each treatment, on DM basis. No effect of treatments was detected for milk yield (36.1 kg/d) and DMI (22.4 kg/d). Milk fat tended to be lower on cows fed REC compared to GRC (3.68 vs 3.74%). The ECM/DMI did not differ between treatments, but REC tended to increase Milk/DMI compared to GRC (1.63 vs 1.61). Cows fed REC had shorter meals than cows fed GRC (35.9 vs. 37.5 min/meal). Meal frequency was lowest with GRC-CTL than the other treatments (9.3 vs. 10.4 meals/d). Meal size was reduced in cows fed REC-CTL (2.2 kg DM/meal) compared to GRC-CTL (2.5 kg DM/meal), whereas no difference was detected between REC-AAC (2.3 kg DM/meal) and GRC-AAC (2.4 kg DM/meal). Cows fed AAC tended to have higher total tract starch digestibility (93.7 vs 92.9% of starch intake) and lower fecal starch concentration (4.3 vs 4.8% of DM) compared to CTL. No effect of treatment was detected on NDF digestibility (48.6% of NDF intake), ruminal microbial yield and molar proportions of VFA. Ruminal pH was lower on cows fed REC compared with GRC (6.72 vs 6.83) and tended to be lower on AAC compared to CTL (6.74

vs 6.82). Fecal pH did not differ between treatments. Cows fed REC tended to have a greater proportion of N intake in milk compared to GRC (34.2 vs 33.7%), but fecal-N and urine-N excretions (g/d and % of N intake), and urea-N in milk and in plasma did not differ. Results showed few significant interactions between corn type and processing, suggesting that the mode of action of AAC was in the digestive tract, independently of ensiling. Overall, providing AAC to lactating dairy cows may enhance starch digestibility without affecting DMI or milk yield, regardless of the corn kernel processing method used.

Key words: Amylase, Enogen corn, Silage, Starch

INTRODUCTION

A transgenic corn capable of expressing alpha-amylase in the kernel endosperm was developed to improve the efficiency of corn ethanol production (APHIS, 2008). Apha-amylase enabled corn (**AAC**) has the potential to improve starch utilization and performance of dairy (Cueva et al., 2021; Silva et al., 2025) and beef (Johnson et al., 2019; Volk et al., 2021) cattle. Dairy cows fed AAC as whole plant corn silage (**WPCS**) have shown improvements in the yields of milk and components and in feed efficiency. Rebelo et al. (2023) observed that cows fed diets containing 31.6% starch and 51.0% of AAC as WPCS (DM basis) had increased milk, protein and lactose yields. Similarly, Cueva et al. (2021) reported that a diet with 40.0% AAC WPCS and 24.6% starch (DM basis) resulted in a 2 kg/d increase in milk yield and a 5.4% improvement in feed efficiency.

In addition to the gain in animal performance, AAC may have a beneficial effect on N partitioning, measured as decreased MUN and PUN and the proportion of N intake in excreta (Cueva et al., 2021, 2024; Krogstad and Bradford, 2023). The possible action of amylase on ruminal starch degradation could increase N retention in ruminal microbial protein and reduce N in excreta of dairy cows, although it has not been demonstrated in vivo when exogenous amylase was supplemented to dairy cows with duodenal and ruminal cannulas (Nozière et al., 2014). The total tract NDF digestibility can be improved when AAC is fed to dairy cows (Cueva et al., 2024; Silva et al., 2025), but this outcome is not consistent (Cueva et al., 2021; Rebelo et al., 2023). Likewise, the effect of AAC on the total tract starch digestibility *in vivo* is variable, since an increase (Krogstad and Bradford, 2023), no effect (Cueva et al., 2021; 2024; Silva et al., 2025), or reduction (Rebelo et al., 2023) have all been observed.

There are studies whereby AAC has been evaluated as WPCS (Cueva et al., 2021; 2024; Krogstad and Bradford, 2023), but fewer experiments have been conducted to evaluate AAC as a concentrate feed source for dairy cows (Rebelo et al., 2023; Silva et al., 2025). Furthermore, rehydrated and ensiled corn has been widely adopted in Brazil as an alternative to store grain at low cost at the farm, reduce the labor and energy costs associated with fine grinding of mature flint corn (Pereira et al., 2013), enhance profitability (Mundim, 2024), and improve starch digestibility in vitreous corn through degradation of the starch-protein matrix during ensiling (Hoffman et al., 2011; Junges et al., 2017). These factors highlight the need for further evaluate the effect of AAC when provided as rehydrated and ensiled corn. Krogstad and Bradford (2023) observed that the improvement in the total tract digestibility of DM, NDF, CP and starch with AAC as WPCS occurred in short duration of silage storage (44 ± 3 d), but not in longer storage (86 ± 3 d), despite differences in the starch content of the diets.

The concentration of starch in the diet is a factor capable of affecting the effect of AAC on lactating dairy cows (Volk et al., 2021). In a recent study, Silva et al. (2025) observed an increase of 4% in energy corrected feed efficiency (ECM/DMI) of cows fed AAC as REC (28 ± 3 d of storage) in a low starch diet (21.5% of DM), but the positive effect of AAC on energy corrected feed efficiency was not detected when dietary starch concentration was increased to 28.7% of DM. The AAC can affect animal performance (Cueva et al., 2021; Rebelo et al., 2023) by affecting silage fermentation profile and starch digestibility during ensiling (Heinzen Jr. et al., 2024) or by direct action of the amylase in kernel endosperm in the digestive tract of ruminants, possibly by a mode of action similar to the observed when exogenous amylase is supplemented to dairy cows (Pech-Cervantes et al., 2022). The positive effect of AAC on starch digestibility may be enhanced in silages because the longer duration of silage storage time may allow for more extensive enzyme action compared with shorter retention time of the digesta in the digestive tract. Furthermore, the increase in silage temperature during the aerobic phase of ensiling may also contribute to enzyme action, since the ruminal temperature may not be ideal for maximum amylase activity (Hu et al., 2010). Changes in ruminal starch degradation can influence both the eating (Allen, 2000; Krause et al., 2003; Oba and Allen, 2003a) and the sorting behaviors of dairy cows (DeVries et al., 2008; Maulfair et al., 2013). Supplementation with exogenous alpha-amylase has been shown to decrease chewing time and meal frequency in one experiment, while it increased sorting in favor of long feed particles (Andreazzi et al., 2018). Further research is warranted to clarify the effects of AAC on eating and feed sorting behaviors of dairy cows.

To date, only one study has evaluated the response of dairy cows to AAC fed as rehydrated and ensiled corn (Silva et al., 2025). Therefore, the objective of this experiment was to evaluate the effect of corn kernel processing (silage or finely ground) from AAC or an isogenic control (**CTL**) on lactation performance, DMI, feed efficiency, total tract digestibility, ruminal microbial yield and fermentation profile, eating and rumination behaviors, and N partition of dairy cows. Our hypothesis was that under low dietary starch concentration and short duration of silage storage the AAC would improve feed efficiency more when fed as grain silage than as finely ground corn, due to the potential additive effect of the enzyme on starch degradation during ensiling and in the digestive tract. Kernel processing of AAC or CTL as ground or ensiled corn kernels allowed for an *in vivo* evaluation of whether the effect of alpha-amylase in corn endosperm was primarily due to action in the digestive tract, as with ground corn, or to the potential combined effect of the enzyme on silage fermentation and in digestion, as with grain silage.

MATERIALS AND METHODS

Experimental Design, Cows, and Treatments

Twenty-four Holstein cows (37.1 ± 4.8 kg/d milk yield, 143 ± 100 DIM, 633 ± 64 kg BW) were used in the experiment. Cows were blocked within squares of 4 animals based on similarity in parity and milk yield. Four primiparous cows were used, all assigned to the same Latin square. Within a square, cows were randomly allocated to a sequence of 4 treatments using a 4 × 4 Latin square design, with 21-d periods (with 14-d of adaptation), balanced for carryover effects by assigning a treatment in sequence to the other 3 treatments the same number of times within a square (Williams, 1949). Treatments were a 2 × 2 factorial arrangement of dietary corn processing: finely ground corn (**GRC**) vs rehydrated and ensiled corn (**REC**), and corn type: AAC vs CTL (Table 1). Experimental diets had 13.0% of DM as corn kernels, 9.6% of DM of starch from each treatment, and 22.4% of DM of total starch (Table 1). Ration formulation was based on NASEM (2021) recommendations for the average cow (milk yield: 36.9 kg/d, DIM: 144, BW: 625 kg, BCS: 3.0, and parity: 0.83) immediately prior to the start of the experiment.

Cows were individually housed and fed in an open-walled, sand-bedded tie stall barn with fans and high-pressure sprinklers and milked 3 ×/d, starting at 0500, 1200, and 1900 h, in an adjacent herringbone parlor. A TMR was mixed 1 ×/d in a 1.2 m^3 stationary vertical mixer (Unimix 1200; Casale) and cows were fed at 0700 h. Feed was pushed-up manually with a broom at least 10 ×/d. Feed refusal was targeted at 10 to 15% of the as-fed intake per cow per day, and the daily observed refusal during the 3rd week of each experimental period was 13.4 ± 5.3% of the TMR offered (mean ± SD). Samples of WPCS, alfalfa haylage, and ensiled corn grain were collected weekly for DM determination with a microwave oven (by drying for 5 min and then in 3 min steps until a stable weight was obtained). Diets were adjusted accordingly by modifying the amount of each feed ingredient to maintain their proportions constant on a DM basis.

Feeds

The corn hybrid (E116K4-3000gt. Syngenta Seeds LLC) used in the present study had a microbial gene incorporated in its DNA (Event 3272) to produce the AAC, and the same hybrid was used as the CTL corn, but without Event 3272. Both corn types were cultivated in Polk County (Nebraska, USA), and after harvesting, 20,507 kg of corn kernels from each type were packed in 20 kg paper bags and shipped (sea freight) in containers to Brazil to be used as feed during the experiment. During the experiment, kernels from mature AAC (48.8% vitreousness. Syngenta Enogen® Feed Corn) and its isogenic counterpart (51.1% vitreousness) were weekly ground with a stationary hammer mill (Nogueira TN-8, Nogueira Máquinas Agrícolas) using a 3-mm mesh diameter screen. Only the amount of corn kernels necessary to feed the cows for 1 wk was ground on each grinding day, and the remaining corn bags were stored in containers throughout the experiment. After grinding, ground corn was hydrated (38.3 ± 0.55% of moisture on AAC and 38.3 ± 0.45% of moisture on CTL) by mixing in a stationary vertical mixer (Unimix 1200; Casale) for 3 min. The hydrated corn was ensiled for 28 ± 3 d in 200 L plastic buckets with caps. Each silo lasted for 3 to 4 d, with two silos used per experimental week. To ensure consistency in storage duration, a new silo was systematically opened every Monday and the residual silage from the previous week was fed to the lactating herd. A microbial inoculum (Kerasil, Kera Nutrição Animal. 4.0 × 10^{10} cfu/g Lactobacillus plantarum and 4.0 × 10^{10} cfu/g Propionibacterium acidipropionici) was added to each batch at a ratio of 0.15 g/kg of hydrated corn.

Weekly samples of ground corn were collected for particle size distribution and geometrical mean particle size (**GMPS**) evaluation using a sieve shaker (ROTAP–MA

750/YTLHZ, Marconi Equipamentos para Laboratórios) with 8 sieves (6,700, 4,750, 3,350, 2,360, 1,700, 1,180, 600, and 250 µm) and pan. Samples (50 g) were placed in the top sieve of the equipment for 10 min. The GMPS was calculated as described in Baker and Herrman (2002). The GRC used in the experiment was ground weekly with a 3-mm mesh diameter screen in the same day and time of the ground corn used in REC treatments, and GMPS of GRC was evaluated as previously described. To calculate the loss of DM during ensiling, the weight of each bucket was obtained immediately after ensiling (0 d of ensiling) and at silo opening (± 28 d of ensiling) and corn samples were obtained simultaneously. Samples were dried in a forced air oven at 105°C for 24 h for non-volatile DM evaluation.

The vitreousness of each corn type was the mean of 5 kernels sampled at random from the weekly composite samples obtained in each griding day, replicated 3 times (15 kernels/corn type). Vitreousness was evaluated by manual dissection of corn kernels as proposed by Dombrink-Kurtzman and Bietz (1993). Corn kernels were immersed in 50 mL of distilled water for 5 min, dried in paper towel, and the pericarp and germ were removed with a scalpel to obtain the endosperm. The endosperm was weighed and dried in a forced air oven at 105°C for 12 h and the floury endosperm was removed with a scalpel. The vitreous endosperm was weighed and expressed as a percentage of the total endosperm. Corn kernels of AAC and CTL were collected once throughout the experiment (1 sample/corn type) and the presence of the amylase protein (Event 3272) was evaluated in both samples by a commercial qualitative test kit (QuickStix for Enogen, EnviroLogix Inc.), and the AAC tested positive, while the CTL tested negative for the protein. Samples of REC were obtained from d 15 to 21 of each treatment period and one composite sample per corn type was analyzed with NIRS curves by 3r Ribersolo/Rock River laboratory (Ribeirão Preto, Brazil) (Supplemental Table S1).

Twenty-four buckets of each corn type were fed in the experiment. Samples from each bucket were collected immediately after opening and frozen for further analysis. Samples were thawed and 10 g were weighed and thoroughly mixed with 90 mL of distilled water for 1 min for pH determination (Phmetro Digimed DM 20. Digicrom Analítica). From d 15 to 21 of each experimental period, samples of buckets opened throughout the week were collected immediately after opening for microbiology evaluation (7 silos/corn type). Samples were refrigerated and sent to the laboratory within 3 h of opening. Sequential 10-fold dilutions (10^{-1} – 10^{-6}) were prepared with peptonized water and aqueous extracts were prepared. Filamentous fungi and yeast growth were evaluated in Dichloran Rose Bengal agar Chloramphenicol (K25-1160 DRBC, Kasvi) incubated at 28°C for 168 h and for 72 h, respectively. For enumeration of spore forming bacteria the aqueous extract was maintained at 90°C for 10 min, and further decimal dilutions were evaluated in Plate Count Agar medium (K25-1060 Agar Nutriente, Kasvi). Plates were incubated at 30°C for 24 h. Lactic acid bacteria growth was evaluated in MRS agar medium (K25-1043 Agar MRS, Kasvi) incubated for 48 h at 32°C. Counts of cfu were performed by visual observation of each plate and data were log transformed for analyses.

Samples of feed ingredients and orts per cow were collected daily and frozen during the last week of each experimental period and composite samples were formed per period. On each day, orts were thoroughly mixed, and a sample of approximately 300 g was collected. At the end of the sampling week daily ort samples were mixed to form a composite sample per cow (290 ± 43.0 g). Samples of orts and feed ingredients were dried in a forced-air oven at 55°C for 72 h and ground to pass a 1-mm diameter mesh screen in a grinder (Star FT 80, Fortinox Indústria, Comércio e Serviços). The DM concentration was determined in a forced air oven at 105°C for 24 h and ash was evaluated by incineration at 600°C for 4 h. The CP concentration was determined with a micro Kjeldahl steam distillatory (method 990.03;

AOAC International, 2006). The NDF concentration was evaluated using an adaptation of AOAC International (2006), method 2002-04 (Mertens, 2002), with heat-stable α-amylase (Termamyl, Novozymes), without sodium sulfite and were manually filtered using crucibles. Starch plus free glucose was measured with α-amylase and amyloglucosidase and colorimetry for glucose as described in Fernandes et al. (2022), adapted from Hall et al. (2015). The nutrient composition of feed ingredients is described in Table 2.

From d 15 to 17 of each period, one daily sample of WPCS and alfalfa haylage was collected to evaluate particle size distribution using the Penn State Particle Separator with two sieves (19 mm and 8 mm) and a pan (Lammers et al., 1996). Particle size distribution was assessed on an as-fed basis using three samples per period for each forage. Material retained on each sieve of the Penn State Particle Separator across the three sampling days was pooled to form a composite sample per sieve and period. Composite samples of WPCS were analyzed for DM, NDF, and starch concentrations as previously described (Supplemental Table S2). Additionally, DM and NDF concentrations were determined in the composite samples of alfalfa haylage. Physically effective NDF ($_{pe}\mathbf{NDF}_{>8}$; Lammers et al., 1996) was calculated from individual feed analysis and diet ingredient composition assuming that only WPCS, alfalfa haylage, and whole cottonseeds would contribute to NDF greater than 8 mm.

Lactation Performance and Intake

Milk yield and DMI were recorded daily throughout the experiment and the means of d 15 to 21 of each treatment period were used to compare treatments. On d 17 to 21 of each period, 0.5% of the milk produced in each milking was collected (i.e., a cow produced 15 L of milk in the morning milking, a sample of 75 mL was collected) and a composite daily sample from the 3 milkings was performed for each cow. Composite samples were subsampled (45 mL) and stored in flasks containing 2-bromo-2-nitropropane-1-3 diol under refrigeration for 5 d prior analysis. All composite samples (5 samples/cow/period) were sent to a commercial laboratory (Laboratory of the Paraná State Holstein Breeders Association) and the concentrations of CP, casein, fat, lactose, total solids, SCC, urea-N (**MUN**), and fatty acids (**FA**) were analyzed by mid-infrared spectroscopy (Bentley Instruments Inc.). The N concentration in milk was: Milk CP/6.34 (Karman and van Boekel, 1986) as proposed in NASEM (2021). The proportion of N intake (% of daily intake) and excretion (g/d) of urine-N, milk-N, and fecal-N were calculated. Milk energy secretion (Mcal/d) was calculated (NASEM, 2021): [(0.0929 × % fat) + (0.055 × % crude protein) + (0.0395 × % lactose)] × kg of milk. The yield of energy corrected milk (**ECM**, kg/d) was calculated as: Milk energy secretion/0.70 (assumes 0.70 Mcal/kg of milk with 3.7% fat, 3.2% protein, and 4.6% lactose). Feed efficiencies were calculated as milk yield/DMI and ECM/DMI. Body weight was measured on d 17, 19 and 21 of each treatment period with an individual walk-over weigh scale (Eziweigh2. Tru-Test) and BCS (1 to 5, thin to fat; Wildman et al., 1982) was the mean of 3 independent evaluators on d 21 of each treatment period.

Total Tract Apparent Digestibility and Ruminal Microbial Yield

During days 19 to 21 of each treatment period, fecal and urine collection was performed continuously in three separate 8-h collection periods, with 8-h intervals between each, to represent the 24 hours of a day. Feces and urine were collected in buckets simultaneously to excretion by at least 7 people from 2100 to 0500 h (1^{st} collection period), 1300 to 2100 h (2^{nd} collection period), and 0500 to 1300 h (3^{rd} collection period) with minimum disturbance and maintenance of the daily cow routine. During data collection, cows were moved in groups of 3 to the milking routine in a 2 × 3 herringbone parlor and immediately returned to their individual tie stalls after milking to reduce the daily time without sampling. The milking routine of each group of 3 cows lasted less than 20 min. Fecal samples were collected, immediately weighed, sampled (1% of the weight), and frozen throughout the sampling period. A composite sample was formed per cow per period. Samples were dried at 55°C for 72 h. The concentrations of DM, NDF, ash, and starch were measured as previously described. The total tract apparent digestibilities of DM, OM, NDF, starch, CP, and the non-NDF OM were calculated. Digestible OM intake (**DOMI**) was estimated and the ECM/DOMI ratio was calculated as a measure of energy usage efficiency. Immediately after collection, urine volume was measured with a graduated beaker, and a 3% sample was stored under refrigeration. Urine samples were mixed to a 4% sulfuric acid solution (1:5) and frozen for allantoin determination according to Chen and Gomes (1992). The daily excretion of allantoin in urine and used as an indirect estimate of ruminal microbial yield (Valadares et al., 1999; Pereira et al., 2021). The efficiency of ruminal microbial growth was estimated as Allantoin/DOMI (mmol/kg). Fecal and urine N concentrations were determined with a Kjeldahl steam distillator (method 990.03; AOAC International, 2006) as described for feed analyses.

A sample from the first defecation of each cow was collected on d 21 for viscosity determination. Viscosity was evaluated according to an adaptation of the methodology of Cannon et al. (2010). Samples of fresh feces (100 g) were diluted in 120 mL of water. The solution was homogenized with a metal spatula for 30 s and filtered through 2 layers of cheesecloth. The viscosity of the solution was measured with a rotational viscometer (Model DV-E. Brookfield Engineering Laboratories) at 100 rpm, with spindle LV-1 at room temperature. Fecal samples (10 g), from the composite samples obtained per period, were diluted in 100 mL of distiller water for pH determination (Phmetro Digimed DM 20. Digicrom Analítica).

Chewing Behavior

During d 15 to 17 of each treatment period rumination and eating behaviors were monitored by visual observation at 5-min intervals, continuously for each 24-h period across those 3 d. Buccal activities were classified as rumination, ingestion, drinking, and idleness.

Individual feeding observations were combined and separated into meals using a meal criterion (i.e., the minimum duration of time between meals) calculated for each cow. Meal criteria were calculated for each cow using methods described by DeVries et al. (2003), using a software package (MIX 3.1.3; MacDonald and Green, 1988). The number of different meals in a day was termed meal frequency (meals/d). Total meal time (min/d) was the total eating time (eating observations/d × 5 min), plus all the nonfeeding intervals shorter than the length of the meal criterion of each cow. Meal duration (min/meal) was calculated as the total daily meal time divided by the meal frequency. Finally, meal size (kg/meal) was the ratio between daily DMI (kg/d) and meals per day. The duration and moment post-feeding of the longest daily meal was calculated. The duration of the first daily meal was measured with a stopwatch. A person observed the behavior of all cows, individually, after offering feed at 0700 h until the last cow finished its first meal.

Sorting behavior in periods of the day was evaluated from d 15 to 17 of each treatment period with the Penn State particle size separator with the 19 mm and 8 mm mesh diameter screens and pan. The particle distribution of the TMR available to each cow was measured at 0700 h (offered TMR), 1200 h, 1900 h, and 0500 h. The predicted intake (as-fed basis) of particles on each screen was: % TMR retained on screen × kg of TMR consumed. The observed intake of particles was: % TMR retained on screen × kg of TMR offered - % orts retained on screen × kg of orts. The selection index (Leonardi and Armentano, 2003) was: 100 × (observed intake/predicted intake). Sorting values below 100% represent selective refusal, above 100% represent preferential intake, and equal to 100% represent no selection. A mean value was generated per cow per period. On d 15, 16, and 17 of each period, the proportion of daily intake in the morning (0700 to 1200 h), afternoon (1200 to 1900 h), and night (1900 to 0700 h) were determined.

Ruminal Fermentation

Ruminal fluid samples were obtained on d 21 of each period with a flexible oro-gastric tube coupled with a vacuum pump at 11.6 ± 0.07 h after the morning feeding, to represent the moment of the day of lowest ruminal pH and highest VFA concentration (Pereira and Armentano, 2000). The first 100 mL were discarded to reduce saliva contamination. Cows were randomly sampled within squares and ruminal pH was measured (Phmetro Digimed DM 20. Digicrom Analítica). A sample was frozen in liquid nitrogen to suppress fermentation and stored at -20°C. Ruminal fluid samples were thawed and prepared as described by Ferreira et al. (2016) for analysis of VFA by gas-liquid chromatography (CG HP 7890A; Injetor HP 7683B, Agilent Technologies) equipped with a capillary column HP-FFAP (1909F-112; 25 m; 0,32 mm; 0,5 µm; JeW Agilent Technologies). For protozoa counting, another 10 mL sample of rumen fluid was diluted (50% v/v) in a 36% (v/v) formaldehyde solution and mixed. The sample was stained according to Dehority (1984) and total protozoa was enumerated with an optical microscope in a Neubauer chamber (Warner, 1962). Samples of ruminal fluid were also refrigerated for viscosity determination. Samples of 50 mL of ruminal fluid were diluted in 100 mL of water for viscosity evaluation. The solution was homogenized with a metal spatula for 30 s and filtered through 2 layers of cheesecloth. The viscosity of the solution was measured with a rotational viscometer (Model DV-E. Brookfield Engineering Laboratories) at 100 rpm, with spindle LV-1 at room temperature.

Blood Analysis

Blood samples were collected on d 18 of each treatment period from the coccygeal vessels at 0 and 2 h relative to the end of the morning, afternoon, and night milkings and return to the feed bunk. Samples were collected in tubes with EDTA and were centrifuged at 2,000 × *g* for 10 min at room temperature. Plasma was obtained and frozen at -20°C for urea-N determination (**PUN**) with a commercial kit (Urea Enzimática, Bioclin). On d 18 and 21, blood samples were collected with tubes containing sodium fluoride. After sampling, tubes were immediately centrifuged (2,000 × *g* for 10 min) for glucose determination by a colorimetric enzymatic test (Glicose Monoreagente, Bioclin). Samples were collected 12.0 ± 0.07 h post-feeding on d 18 and 11.6 ± 0.07 h post-feeding on d 21.

Statistical Analysis

Data were analyzed with the MIXED procedure of SAS (SAS University Edition, Cary, USA). The Latin square model had the random effects of square (1 to 6) and cow(square) (1 to 24) and the fixed effects of period (1 to 4), corn processing (GRC, REC), and corn type (AAC, CTL), and the interaction between corn processing (**P**) and corn type (**T**). Data describing silage pH, microbiology, and DM loss were analyzed as a completely randomized design with silage buckets as the experimental unit with a model containing only the fixed effect of T (AAC vs. CTL). The same statistical model was used for particle size evaluation, in which weekly samples collected on each grinding day were used as the experimental unit (12/corn type).

Degrees of freedom were calculated using the Kenward-Roger option. The PUN was analyzed as repeated measures over time. The fixed effect of time and the 2- and 3-way interactions between time, P, and T were added to the previous model. The interactions between cow, period, P, and T were defined as random. The best covariance structure was chosen based on the lowest value for the Akaike's Information Criterion. Covariance structures tested were first order autoregressive, compound symmetry, and unstructured; unstructured was chosen for all models based on the lowest Akaike information criterion. Statistical significance was declared at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$. Treatment means were compared with a Tukey correction when a $P \leq 0.10$ was detected for the interaction between main effects.

RESULTS

Feeds and Treatment Diets

The ingredient and nutrient composition of diets were similar, but DM content was +1.1 percentage points higher on GRC diets than on REC diets. The protein content of the analyzed diet (14.8% of DM) was lower than expected (16.3% of DM) and MP balance estimated with NASEM (2021) was negative in all diets. The CP concentration of the actual diet was lower than the formulated diet due to reductions of 1.7, 3.4, and 5.2 percentage points of CP in the experiment composite samples of alfalfa haylage, soybean meal, and whole cottonseeds, respectively.

Mature and dry ground corn kernels from AAC had larger GMPS than CTL before ensiling (530 vs 450 µm; P = 0.05) and lower proportion of particles retained below the 250 µm sieve screen (6.53 vs 9.08%; P = 0.05) (Table 3). There was no detected difference in nutrient composition, microbial population, and DM loss between silages of rehydrated AAC and CTL corn ($P \geq 0.27$) (Table 4).

Lactation Performance and Intake

Cows fed with GRC diets tended to have higher concentrations in milk of fat (3.74 vs 3.68%; P = 0.06) and TS (12.61 vs 12.55%; P = 0.05) than cows fed on REC diets (Table 5). Feed efficiency (Milk/DMI) tended to be lower on GRC than REC (1.61 vs 1.63; P = 0.06), but no differences in ECM/DMI and ECM/DOMI were detected ($P \geq 0.36$). There was no detected effect of corn type on lactation performance, intake and feed efficiency ($P \geq 0.16$) (Table 5).

The GRC diets induced higher concentrations in milk than REC ($P \leq 0.04$) of FA de novo (0.863 vs 0.844 g/100 g of milk), monounsaturated (0.692 vs 0.670 g/100 g of milk), polyunsaturated (0.043 vs 0.039 g/100 g of milk) and unsaturated (0.704 vs 0.677 g/100 of milk) and tended to increase stearic (0.404 vs 0.395 g/100 g of milk; P = 0.08) (Table 6). The concentration of oleic FA in milk was higher ($P < 0.05$) on GRC-CTL (0.480 g/100 g of milk) than REC-CTL (0.451 g/100 g of milk). Cows fed GRC-CTL also had higher proportions of milk fat of FA unsaturated (18.07 vs 17.18 g/100 g of FA) and oleic (11.96 vs 11.37 g/100 g of FA) and lower proportion of saturated (81.93 vs 82.82 g/100 g FA) than REC-CTL (*P* < 0.05).

There were tendencies (*P* ≤ 0.09) for AAC diets to decrease preformed FA (37.48 vs 37.82 g/100 g FA) and to increase mixed FA (41.04 vs 40.74 g/100 g FA) relative to CTL, with no detected difference due to corn type on the de novo FA content of milk fat (21.47 g/100 g FA; *P* = 0.83) (Table 6). Preformed, mixed and de novo FA concentrations in milk (g/100 g milk) and secretion (g/d) did not differ by corn type (*P* ≥ 0.36). The replacement of CTL by AAC on REC diets tended to increase the proportion of palmitic FA in milk fat (49.51 vs 48.29 g/100 of FA; *P* < 0.10).

Chewing Behavior

There were tendencies for GRC to increase the refusal of medium size particles (8-19 mm) in the afternoon (84 vs 89% of the predicted intake; *P* = 0.10) and to increase the selection in favor of small feed particles (< 8 mm) at night (106 vs 105% of the predicted intake; *P* = 0.07) relative to REC, suggestive of refusal of citrus pulp pellets in the afternoon (retained on the 8 mm screen) and in favor of ground corn at night (recovered in pan) (Table 7).

There was no detected effect (*P* ≥ 0.13) of corn type or processing on intake of NDF, forage NDF, and ${}_{pe}NDF_{>8}$ (Table 7), reflecting the similarity in daily DMI (Table 5). However, differences were detected in eating behavior (Table 7). Meal duration was shorter (35.4 vs 37.5 min/meal; *P* = 0.01), meal frequency was higher (10.3 vs 9.7 meals/d; *P* = 0.02) and meal size was smaller (2.2 vs 2.5 kg DM/meal; *P* < 0.01) on REC than GRC. The GRC-CTL diet had the lowest meal frequency among all treatment diets (9.3 vs 10.2 meals/d; *P* < 0.05). Meal size was higher on GRC-CTL than REC-AAC and REC-CTL (2.5, 2.3 and 2.2 kg

DM/meal, respectively), but GRC-CTL did not differ from GRC-AAC ($P > 0.05$). Similarly, REC-CTL and REC-AAC did not differ ($P > 0.05$). No differences in meal time, eating and rumination time and rate, the longest meal duration and timing, and first meal duration were detected for corn type or processing ($P \geq 0.12$).

The proportion of daily intake in the morning tended to be greater on AAC than CTL (39.6 vs 38.0%; $P = 0.08$), but no differences in the proportions of daily intake in the afternoon and night were detected ($P \geq 0.16$). Cows fed AAC selected in favor and cows fed CTL refused long particles (> 19 mm) in the morning (103 vs 91% of the predicted intake; $P < 0.01$). The AAC diets also induced less selection in favor of medium size particles (8-19 mm) than CTL in the morning (102 vs 104% of the predicted intake; $P = 0.04$). In the afternoon, cows fed AAC refused long particles (83 vs 90% of the predicted intake; $P < 0.01$) and were more selective in favor of medium size particles (103 vs 101% of the predicted intake; $P = 0.02$) than CTL. No effect of corn type was detected on particle sorting behavior at night ($P \geq 0.19$).

Digestibility, Ruminal Fermentation, and N Partition

No effect of corn type or processing was detected for total tract apparent digestibility of NDF (48.6% of intake; $P \geq 0.53$) (Table 8). There was a tendency for the total tract starch digestibility to be higher on AAC than CTL (93.7 vs 92.9% of intake; $P = 0.07$), associated with lowered fecal starch concentration on AAC (4.31 vs 4.78% of DM; $P = 0.08$). The CP digestibility did not differ by corn type or processing ($P \geq 0.30$). Diet REC-AAC had higher digestibility of the non-NDF OM than GRC-AAC (83.4 vs 81.3% of intake; $P < 0.05$). No effect of treatment was detected for the intake of digestible starch (4.55 kg/d; $P \geq 0.29$).

Ruminal pH tended to be lower on cows fed AAC than CTL (6.74 vs 6.82; $P = 0.06$) and it was lower on REC than GRC (6.73 vs 6.83; $P < 0.01$) (Table 9). Fecal pH did not differ (7.92; $P \geq 0.71$), but there was a tendency for REC to reduce fecal viscosity relative to GRC

(40.01 vs 37.33 cP; $P = 0.08$). Ruminal VFA proportions, microbial yield, and protozoa concentration were not detected to differ ($P \geq 0.12$), except for an increase in ruminal isovalerate molar proportion on REC-AAC relative to GRC-AAC and REC-CTL ($P < 0.05$) (Table 9). Plasma glucose concentration did not differ by corn type or processing ($P \geq 0.16$) (Table 10).

There was no detected treatment effect on MUN (15.5 mg/dL; $P \geq 0.77$) (Table 5) and PUN (16.0 mg/dL; $P \geq 0.15$) (Table 10). Although N intake did not differ ($P \geq 0.18$), there was a tendency for cows fed REC to have a greater proportion of N intake in milk than cows fed GRC (34.5 vs 34.0% of daily intake; $P = 0.09$), but no effects of corn type or processing were detected for the proportions of N intake in feces and urine ($P \geq 0.30$) (Table 11).

DISCUSSION

Corn processing and nutrient composition of silages and diets

Ground corn kernels of AAC and CTL had GMPS of 530 μm and 450 μm, respectively, both of which fall below the threshold for fine grinding (1,250 μm) defined by NASEM (2021). The higher GMPS of ground AAC than ground CTL corn was not expected, since the same stationary hammer mill with a 3-mm mesh diameter screen was used for both corn types and vitreousness was similar (50% of endosperm). In agreement with our findings, Rebelo et al. (2023) observed an increase of 400 μm in mean particle size for AAC relative to CTL when corn kernels were ground with roller mills with a 700 μm gap (GMPS of 1050 and 650 μm, respectively). In a recent experiment with the same hybrids and methodology as in this study (Silva et al., 2025), no difference in GMPS was observed between AAC and CTL corn kernels were ground in a stationary hammer mill with a 6-mm mesh diameter screen. The only difference in corn processing from Silva et al. (2025) was the smaller particle size at grinding in this experiment (3-mm mesh diameter screen and GMPS 490 μm vs 6-mm mesh diameter screen and GMPS 815 μm). Although data on the typical particle size of ground AAC are limited, our observation suggests that AAC seems to exhibit higher grinding resistance than its isogenic counterpart, but only when fine grinding was adopted. The 3-mm mesh diameter screen is the finest used in practice to grind corn for REC, although the grinding rate at this particle size is much slower than the mostly used coarser particle sizes, usually with sieves from 5 to 9 mm (Castro et al., 2019).

Although there was a difference in corn particle size before ensiling depending on corn type, this difference did not affect silage composition or recovery, since there was no difference in nutrient content, microbiology and DM loss between silages of AAC and CTL, in accordance with Silva et al. (2025). The fermentation profile of REC stored for a short period (28 ± 3 d) was not affected by the presence of alpha-amylase in the corn endosperm. Corn type, inherently confounded with particle size in our experiment, did not affect silage fermentation, composition, and DM loss.

The DM loss was 2.3% of the DM ensiled, 0.7%-units higher than in Silva et al. (2025), in both cases, REC was stored in 200 L buckets for 28 d under similar condition. The DM loss during silage storage in our experiment was lower than the 3.1 % of the DM ensiled at 30 d of storage and 8.9 % at 180 d of storage for 519 µm flint corn ensiled in polyvinyl chloride mini-silos (Fernandes et al., 2022). Short duration of storage reduces the DM loss during storage. However, short storage could potentially reduce the positive time dependent effect of ensiling on starch digestibility, although the greatest rate of increase in starch digestibility during ensiling occurs in the first 30 d of storage (Kung Jr. et al., 2018). Long storage may increase starch digestibility, but it can reduce the recovery from the silo of degradable DM (Fernandes et al., 2022). The loss of starch during storage in mini-silos was increased from 12.4% to 25.1% of ensiled starch when duration of storage was increased from 30 to 180 d (Fernandes et al., 2022). In contrary to high moisture corn (corn kernels harvested ideally from 35 to 40% moisture), in which the duration of storage may be as long as the length of the interval between crops (usually 1 year for corn), in REC the duration of storage can be programmed by the farm, since there is no need to ensile the yearly supply of corn grain at once. The ensiling of REC can improve farm finances (Mundim, 2024). Castro et al. (2019), using 200 L bucket silos identical to ours, observed a silage loss of 11.3% of DM for REC ensiled from 205 to 289 days, independently of particle size. The low level of DM loss in our experiment was probably the combination of the short duration of storage, storage in 200 L tick plastic buckets and good silage management, probably representing an achievable DM loss relative to the observed in the practice of dairy farms. We are unaware of any research that reliably quantitated the loss of DM during the ensiling of REC in Brazilian dairy farms, although we have learned that farm size, storage duration and silage management are major factors on silage recovery at the farm level.

The reduced N concentration of protein feeds during the experiment relative to the pre-experimental values, especially for whole cottonseeds, reduced diet CP concentration of all consumed diets, although they remained iso-nitrogenous (14.8% CP in DM). The reason for such variation was unclear to us. The same batch of all concentrate feedstuffs and silages were fed during the entire experiment. Probably some laboratory grinding issue may have induced the greatest reduction in the CP content of whole cottonseeds. Replicate samples of each feed were analyzed on each experimental period and feed composition was consistent across replicates.

However, although the actual CP concentration in the diets was below the formulated diet (14.8% vs 16.3% of DM) and the calculated MP balance was negative (-281 g/d, on average), the RDP concentration (10.5% of diet DM) was above the minimum recommended by NASEM (2021) to maximize the ruminal microbial yield (10-12% of DM range). The MUN was similar across all treatments, and the mean value was 15.5 mg/dL, suggesting that N availability was not limiting ruminal function (Broderick and Clayton, 1997). The total tract

NDF digestibility coefficient (48.6% of NDF intake) was numerically like the 49.0% coefficient of a previous experiment (Silva et al., 2025), conducted with similar diets, the same batch of WPCS, and the same experimental protocol as this experiment. There was no evidence to suggest that the lower than predicted dietary CP concentration negatively affected ruminal function and the total tract digestibility of NDF and starch. The low CP content of the diets certainly explain the high efficiency of N usage in all treatments (Milk N = 34.2% of N intake, on average) (Colmenero and Broderick, 2006; Powell et al., 2010). Although it was not a predicted scenario, the treatments had to affect N partition of cows with high efficiency of N usage.

The interaction between corn type and processing

The experimental design evaluated AAC and CTL kernels processed as GRC or REC in order to assess whether the *in vivo* effects of alpha-amylase on dairy cows were driven by the enzyme activity in the gastrointestinal tract, as in GRC, or also by an additional effect of the enzyme on silage fermentation, as in REC. Feed efficiency was expected to be improved with AAC, particularly under the low starch diet used in this study. We had previously observed that AAC corn improved feed efficiency more when ensiled corn kernels were fed on a low rather high starch diet (Silva et al., 2025). Additionally, we hypothesized that the effects of AAC on feed efficiency would be more pronounced when corn grain was ensiled, since the enzyme action would occur in the silo and in the digestive tract of the animal. However, no interaction between corn processing and corn type was detected for feed efficiency and very few interactions were detected for other variables (FA profile of milk fat and meal size and frequency only), suggesting that the major mode of action of the amylase in corn kernels was by action in the digestive tract, independently of previous ensiling.

The enzyme action may not have been potent enough in the silo, since both temperature and pH values in the silo are most of the time not ideal for amylase action. The amylase activity of AAC appears to be optimal at 80°C, with activity reduced to approximately 10% of its maximum at 30°C (APHIS, 2008). In agreement, Hu et al. (2010) reported greater *in vitro* starch digestibility when AAC was incubated at 65°C compared to 40°C. Considering that silage temperature during the aerobic phase of ensiling can range from 29 to 41°C (Kung, 2011) and can be influenced by the environment (Borreani et al., 2018), it could potentially enhance the amylase activity in AAC silages for short duration and immediately after ensiling. There is also some evidence that the amylase activity of AAC may decline during silage fermentation (Cueva et al., 2024), possibly due to enzyme degradation. By this rationale, greater enzymatic activity would be expected when AAC was fed as GRC rather than REC, but it was not supported by our data. Another possibility for the lack of significant P × T interactions was the low dietary starch concentration of the diets, possibly reducing the impact of the treatments on the total diet. Our hypothesis of a potential interaction between corn type and processing could not be supported by our data.

Overall, the low occurrence of interactions between corn type and processing and the similarity in silage composition and fermentation profile suggest that the corn type effect was not dependent on ensiling, but to the direct action of the enzyme in the digestive tract. This has practical implication, suggesting that AAC in concentrates may increase starch digestibility in the cow. Since very few interactions were detected, the main effects of corn type and processing are discussed separately.

Ensiled corn vs ground corn

This is the first experiment that compared corn grown in the USA that was rehydrated and ensiled with ground corn. The rehydration and ensiling of mature ground corn kernels has been widely used in Brazil, mainly to store corn grain in the farm and to reduce the labor and energy of fine grinding mature flint corn (Pereira et al., 2013). In a survey of dairy farms in Brazil, the 51 farms feeding REC had lower concentrate cost and increased profitability than 64 feeding ground corn (Mundim, 2024). Rehydration and ensiling can improve starch digestibility of flint corn by starch-protein matrix degradation (Hoffman et al., 2011; Junges et al., 2017). As USA corn is floury and more digestible than Brazilian flint corn (Correa et al. 2002), it is expected that the gain in starch digestibility induced by ensiling should be proportionally lower on floury corn than on flint corn (Andrade Filho et al., 2010), although corn vitreousness was not extremely low in this experiment (50% vitreous endosperm). In this experiment REC of USA corn was used as a model to evaluate the effect of alpha-amylase in corn endosperm as silage or ground corn grain.

Providing a more fermentable starch source is expected to reduce DMI and improve feed efficiency (Ferraretto et al., 2013), which aligns with the tendency of REC to increase Milk/DMI in the present study, although corn processing did not affect DMI or milk yield. The reduction in ruminal pH can alter the ruminal biohydrogenation processes leading to a reduction in short chain FA synthesis in the mammary gland (Baumgard et al., 2002; Ferlay et al., 2017). This mechanism supports the concept that the reduced concentration of De novo FA (g/100 g of milk) on cows fed REC was the result of the lowered ruminal pH on this treatment. Increasing ruminal starch degradation can improve propionate synthesis, increase meal frequency, and reduce meal size and duration (Allen, 2000; Krause et al., 2003; Oba and Allen, 2003a). Overall, the changes observed in ruminal pH, milk FA and eating behavior are suggestive of higher ruminal starch degradation on REC than GRC, although no differences in total tract starch digestibility were detected. The similarity in total tract digestibility suggests that intestinal starch digestion may have compensated for a lower ruminal starch degradation of GRC relative to REC (Oba and Allen 2003b; Ferraretto et al., 2013; Nozière et al., 2014). Fecal pH also did not differ, suggesting that the lower tract starch fermentation was not significantly affected by corn processing.

Corn processing had no detected effects on milk protein yield and concentration, ruminal microbial yield, MUN, PUN and N partition, except for a tendency of increase in the proportion of N intake going into milk on REC than GRC. The diets had low starch concentration and adequate particle size, and it may have precluded a strong effect of corn processing on ruminal fermentation (Castro et al., 2019). There was no evidence that the ensiled corn could reduce N in excreta relative to GRC when this floury corn (50% of vitreousness) was fed at 9.6% starch in diet DM.

Alpha-amylase enabled corn vs isogenic control

There were no detected effects of corn type on milk and components secretion, DMI and feed efficiency. Some researchers have shown the effect of AAC from WPCS on lactation performance of dairy cows (Cueva et al., 2021, 2024; Krogstad and Bradford, 2023), but to our knowledge, there are only two other studies where AAC as a concentrate feed source for dairy cows has been evaluated, one as mature ground corn (Rebelo et al., 2023) and the other as REC (Silva et al., 2025).

The similarity in feed efficiency due to corn type in our experiment was unexpected, although the effect of AAC on feed efficiency of dairy cows has been variable. Improvement (Cueva et al., 2021; Silva et al., 2025), reduction (Cueva et al., 2024) or no effect (Krogstad and Bradford, 2023; Rebelo et al., 2023) on feed efficiency have all been reported. Silva et al. (2025) observed that REC of the same AAC hybrid used in this experiment induced an improvement of 4% in feed efficiency (ECM/DMI) only when fed in a low starch diet (22% of DM) and had no effect on a high starch diet (29% of DM). Based on our previous study (Silva et al., 2025), feed efficiency was expected to increase on AAC since we fed a low starch diet (22.3% of DM) in the present study. The low starch diet evaluated by Silva et al. (2025) contained 44.8% forage, 13.8% citrus pulp pellets, and 33.0% NDF, whereas the diets in the present study included 57.4% forage, 5.7% citrus pulp pellets, and 36.5% NDF (DM basis).

Despite these differences in dietary composition, the total tract NDF digestibility coefficient was similar between studies (49.0% and 48.6% of NDF intake). In Silva et al. (2025) a greater proportion of the total NDF in the diet came from citrus pulp NDF. The difference in diet carbohydrate profile and particle size may explain the variation in feed efficiency response due to AAC (Pech-Cervantes et al., 2022).

The total tract starch digestibility was 0.8 percentage points higher and fecal starch concentration tended to be lower when AAC was fed, with no effect of corn type on the digestibility of other nutrients. The supplementation of dairy cows with AAC was expected to improve nutrient digestibility, particularly with the short silage storage duration adopted in this experiment. The tendency for increased total tract starch digestibility with AAC relative to CTL is in agreement with the findings of Krogstad and Bradford (2023). These authors reported a + 0.4 percentage points increase in total tract starch digestibility when AAC WPCS was stored for 44 ± 3 d, but no improvement in starch digestibility was observed when storage duration was longer (86 ± 3 d). Throughout silage storage, microbial and plant enzymes degrade the starch–protein matrix in corn kernel endosperm (Hoffman et al., 2011; Junges et al., 2017), thereby improving starch digestibility (Kung Jr. et al., 2018). The mode of action by which AAC enhances nutrient digestibility in dairy cows fed on silage of grain or whole plant are not fully understood. Unfortunately, we did not measure the enzyme activity of silages in our experiment. In a recent meta-analysis, Pech-Cervantes et al. (2022) concluded that exogenous alpha-amylase supplementation can increase the total tract starch digestibility by 0.5 percentage points, an improvement of smaller magnitude than the response detected in this experiment. Although ground AAC had a larger GMPS than CTL before ensiling, the former was more digestible, suggesting that the larger particles of AAC than CTL did not impair starch digestibility when 50% vitreousness corn was ensiled for 28 d.

The total tract starch digestibility in dairy cows is usually above 95% of starch intake (Fredin et al., 2014). The mean total tract starch digestibility in the current study was 93.3% of starch intake in all treatment diets. The WPCS fed to the cows was harvested with a pull-type forage harvester without a kernel processor. Corn kernel processing was not ideal in this experiment, as suggested by the higher proportion of starch retained above the 8 mm screen of the Penn State Particle Separator (Supplemental Table S2). The deficient kernel processing of WPCS may have contributed to the low total tract starch digestibility observed in the present study. A positive effect of AAC on the total tract starch digestibility has not been observed when starch digestibility of the CTL diet was already greater than 95% (Cueva et al., 2021, 2024; Silva et al., 2025). Similarly to our findings, in the meta-analysis by Pech-Cervantes et al. (2022), the mean total tract starch digestibility was 94.1% of starch intake, and supplementation with exogenous alpha-amylase was associated with improved starch digestibility. Although the improvement in total tract starch digestibility with AAC was modest compared to the effects typically observed with changes in corn processing (Nocek and Tamminga, 1991; Firkins et al., 2001; Ferraretto et al., 2013), it falls within the range reported for exogenous alpha-amylase supplementation (Pech-Cervantes et al., 2022) and for AAC fed as WPCS (Krogstad and Bradford, 2023), suggesting that AAC may exert a mild positive effect on starch digestibility. These data suggest that AAC may be beneficial to starch digestibility only on diets with low starch digestibility, although this hypothesis was not formally evaluated in this experiment.

The AAC had no detected effect on ruminal fermentation profile, but a reduction in ruminal pH tended to occur in cows fed AAC. This is suggestive that AAC improved ruminal starch degradation. The increase in the dietary proportion of flaked corn from AAC *in vitro* linearly increased the production (mmol/g of substrate DM) of acetate, propionate, valerate and total VFA (Horton et al., 2020). Oba and Allen (2003a, b) reported that replacing ground corn with high-moisture corn increased ruminal starch degradation, resulting in greater estimation of the daily production (moles/d) of propionate, acetate, and total VFA, without altering their concentrations in ruminal fluid. Similarly, in the present study, it is plausible that AAC enhanced daily ruminal VFA production without affecting their molar proportions. The AAC reduced ruminal pH at the same intakes of DM, digestible OM, $_{pe}NDF_{>8}$, and digestible starch and similar rumination as CTL, suggesting that it was more ruminally fermentable. The high digestibility of starch in USA corn (Correa et al., 2002) may have precluded a pronounced effect of corn type on ruminal fermentation. In other research the accumulation of VFA after 6 h of ruminal *in vitro* fermentation was increased when exogenous amylase was added to flint corn, but had no effect on floury corn (Klingerman et al., 2009). The effects of AAC on ruminal fermentation and starch degradation could have been more pronounced if the technology was incorporated into corn with vitreous endosperm rather than floury endosperm. Furthermore, in the present study, approximately 12.0% (on a DM basis) of the starch in the diet came from WPCS produced from a vitreous Brazilian corn hybrid, which may have contributed to the positive effect of AAC on total tract starch digestibility. Although only one sample per corn type of REC was analyzed, AAC showed numerically greater starch degradation at 0 h (34.0 vs 22.9% of starch) and 7 h (58.5 vs 54.7% of starch), as well as higher fractional degradation rate (12.1 vs 10.8%/h) and effective ruminal starch degradation (75.8 vs 69.7% of starch) compared to CTL (Supplemental Table S1). Overall, ruminal starch degradation was apparently enhanced by AAC corn, although the effect was apparently subtle.

Cows fed AAC selected in favor of long feed particles in the morning, while cows fed CTL selected against long particles, although cows fed AAC had higher degree of rejection of long particles in the afternoon than cows fed CTL. Andreazzi et al. (2018) observed that dairy cows fed an exogenous alpha-amylase increased their preferential intake of long feed particles and the refusal of short particles. Ruminal acidosis can affect the sorting behavior of dairy cows, increasing the selection in favor of long particles (DeVries et al., 2008; Maulfair et al., 2013). Sorting in favor of long feed particles may have contributed to the lack of a corn type effect on ruminal fermentation profile. Increased intake of long particles can influence ruminal fermentation dynamics by enhancing rumen buffering capacity, thereby attenuating reductions in ruminal pH and minimizing alterations in VFA profile (Mertens, 1997; Zebeli et al., 2012). However, cows fed AAC tended to have a greater proportion of daily intake in the morning, denoting some alteration in eating pattern throughout the day induced by corn type.

The similarity in ruminal fermentation profile, despite the tendency of lower ruminal pH on AAC than CTL, is in line with the similarity in ruminal microbial yield and protozoa concentration, MUN and PUN. The partition of N consumed into urine, milk and feces was not affected by corn type. The estimated MP deficiency was approximately 281 g/d (NASEM, 2021). Previous studies have demonstrated that a reduction in MP supply, due to lower dietary CP concentration, can increase N usage efficiency (milk protein N/N intake) and reduce urinary and fecal N excretion (Colmenero and Broderick, 2006; Letelier et al., 2022; Erickson et al., 2023). The efficiency of converting consumed N into milk N was high in this experiment (> 33.9% of daily N intake) and tended to be higher on REC than GRC. Treatments had to improve N usage efficiency in a scenario of low N intake. An increase in dietary starch concentration from REC reduced N in excreta of dairy cows, but, similar to the observed in this experiment, the replacement of CTL with AAC in a diet with low starch concentration had no detectable effect on N partition in a previous experiment (Silva et al., 2025).

CONCLUSIONS

When floury corn was fed at low dietary starch concentration there was no substantial interaction between corn processing and type, as we hypothesized. Corn type and processing exerted only modest effects on lactation performance, DMI, feed efficiency, ruminal fermentation profile, and N partition in dairy cows. Milk fat concentration tended to be reduced, Milk/DMI tended to be increased, and ruminal pH was reduced by REC relative to GRC. The AAC tended to increase the total tract starch digestibility and to reduce fecal starch concentration, associated with a tendency for lowered ruminal pH. Eating behavior was affected by corn processing, but was not altered by corn type. The effect of AAC on dairy cows was apparently driven by the effect of the amylase in corn endosperm in the digestive tract, independently of ensiling.

Supplemental Table S1 and S2 are available in the Mendeley Data repository at: https://data.mendeley.com/datasets/vvt4sdv2vk/1.

NOTES

Experimental procedures were approved by the University of Lavras Bioethic Committee in Utilization of Animals (Protocol 003/23). The authors are grateful to Minas Gerais State Agency for Research and Development (FAPEMIG), Brazilian Federal Foundation for Support and Evaluation of Graduate Education (CAPES), and Grupo do Leite of University of Lavras. We would like to thank Syngenta Seeds for partially funding this experiment and for the cultivation and delivery of corn kernels. W. R. Silva received a CAPES/PRINT grant “Sandwich Doctorate” at the University of Guelph (88887.839615/2023-00). The authors have not stated any conflicts of interest.

REFERENCES

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624. https://doi.org/10.3168/jds.S0022-0302(00)75030-2.

Andrade Filho, R., R. B. Reis, M. N. Pereira, and M. Antenor. 2010. Degradabilidade ruminal in situ de grãos de milho maduros do tipo flint ou dentado, secos ou reconstituídos e ensilados. In Proceedings of the 47th Meeting of the Brazilian Society of Animal Science, Salvador, Bahia, Brazil. The Brazilian Society of Animal Science. (Abstr).

Andreazzi, A. S. R., M. N. Pereira, R. B. Reis, R. A. Pereira, N. N. Morais Júnior, T. S. Acedo, R. G. Hermes, and C. S. Cortinhas. 2018. Effect of exogenous amylase on lactation performance of dairy cows fed a high-starch diet. J. Dairy Sci. 101:7199–7207. https://doi.org/10.3168/jds.2017-14331.

AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International.

APHIS. 2008. Environmental Assessment Alpha-Amylase Maize Event 3272. Accessed December 05, 2023. https://www.aphis.usda.gov/brs/aphisdocs/05_28001p_ea.pdf.

Baker, S., and T. Herrman. 2002. Evaluating particle size. Publication MF-2051. Kansas State Univ., Manhattan.

Baumgard, L. H., E. Matitashvili, B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002. Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. J. Dairy Sci. 85:2155-2163. https://doi.org/10.3168/jds.S0022-0302(02)74294-X.

Borreani, G., E. Tabacco, R. J. Schmidt, B. J. Holmes, and R. A. Muck. 2018. Silage review: Factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101:3952-3979. https://doi.org/10.3168/jds.2017-13837.

Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80:2964-2971. https://doi.org/10.3168/jds.S0022-0302(97)76262-3.

Cannon, S. J., G. C. Fahey Jr, M. R. Murphy, C. L, Dikeman, B. L, Miller, and J. K. Drackley. 2010. Inclusion of psyllium in milk replacer for neonatal calves. Effects on growth, digesta viscosity, rate of passage, nutrient digestibilities, and metabolites in blood. J. Dairy Sci. 93:3652–3660.

Castro, L. P., M. N. Pereira, J. D. Dias, D. V. Lage, E. F. Barbosa, R. P. Melo, F. Karla, J. T. R. Carvalho, F. F. Cardoso, and R. A. Pereira. 2019. Lactation performance of dairy cows fed rehydrated and ensiled corn grain differing in particle size and proportion in the diet. J. Dairy Sci. 102:9857-9869. https://doi.org/10.3168/jds.2019-16559.

Chen, X. B., and M. J. Gomes. 1992. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of Technical Details. International Feed Resources Unit, Rowett Research Institute.

Colmenero, J. O., and G. A. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704-1712. https://doi.org/10.3168/jds.S0022-0302(06)72238-X.

Correa, C. E. S., R. D. Shaver, M. N. Pereira, J. G. Lauer, and K. Kohn. 2002. Relationship between corn vitreousness and ruminal in situ starch degradability. J. Dairy Sci. 85:3008-3012. https://doi.org/10.3168/jds.S0022-0302(02)74386-5.

Cueva, S. F., D. E. Wasson, L. F. Martins, S. E. Räisänen, T. Silvestre, and A. N. Hristov. 2024. Lactational performance, ruminal fermentation, and enteric gas emission of dairy cows fed an amylase-enabled corn silage in diets with different starch concentrations. J. Dairy Sci. 107:4426-4448. https://doi.org/10.3168/jds.2023-23957.

Cueva, S. F., H. Stefenoni, A. Melgar, S. E. Räisänen, C. F. A. Lage, D. E. Wasson, M. E. Fetter, A. M. Pelaez, G. W. Roth, and A. N. Hristov. 2021. Lactational performance, rumen fermentation, and enteric methane emission of dairy cows fed an amylase-enabled corn silage. J. Dairy Sci. 104:9827–9841. https://doi.org/10.3168/ jds.2021-20251.

Dehority, B. A. 1984. Evaluation of subsampling and fixation proce dures used for counting rumen protozoa. Appl. Environ. Microbiol. 48:182–185. https://doi.org/10.1128/aem.48.1.182-185.1984.

DeVries, T. J., F. Dohme, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feed sorting. J. Dairy Sci. 91:3958– 3967. https://doi.org/10.3168/jds.2008-1347.

DeVries, T. J., M. A. G. von Keyserlingk, D. M. Weary, and K. A. Beauchemin. 2003. Measuring the feeding behavior of lactating dairy cows in early to peak lactation. J. Dairy Sci. 86:3354–3361. https://doi.org/10.3168/jds.S0022-0302(03)73938-1.

Dombrink-Kurtzman, M. A., and J. A. Bietz. 1993. Zein composition in hard and soft endosperm of maize. Cereal Chem. 70:105–108.

Erickson, M. G., T. Barros, M. J. Aguerre, J. O. Colmenero, S. J. Bertics, and M. A. Wattiaux. 2024. Reducing dietary crude protein: Effects on digestibility, nitrogen balance, and blood metabolites in late-lactation Holstein cows. J. Dairy Sci. 107:4394-4408. https://doi.org/10.3168/jds.2023-24079.

Ferlay, A., L. Bernard, A. Meynadier, and C. Malpuech-Brugère. 2017. Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: A review. Biochim. 141:107-120. https://doi.org/10.1016/j.biochi.2017.08.006.

Fernandes, T., D. T. da Silva, B. F. Carvalho, R. F. Schwan, R. A. N. Pereira, M. N. Pereira, and C. L. S. Ávila. 2022. Effect of amylases and storage length on losses, nutritional value, fermentation, and microbiology of silages of corn and sorghum kernels. Anim. Feed Sci. Technol. 285:115227. https://doi.org/10.1016/j.anifeedsci .2022.115227.

Ferraretto, L. F., P. M. Crump, and R. D. Shaver. 2013. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. J. Dairy Sci. 96:533-550. https://doi.org/10.3168/jds.2012-5932.

Ferreira, E. M., A. V. Pires, I. Susin, M. V. Biehl, R. S. Gentil, M. D. O. M. Parente, D. M. Polizel, C. V. M. Ribeiro, and E. Almeida. 2016. Nutrient digestibility and ruminal fatty acid metabolism in lambs supplemented with soybean oil partially replaced by fish oil blend. Anim. Feed Sci. Technol. 216:30-39. http://dx.doi.org/10.1016/j.anifeedsci.2015.09.007.

Firkins, J. L., A. N. Hristov, M. B. Hall, G. A. Varga, and N. R. St-Pierre. 2006. Integration of ruminal metabolism in dairy cattle. J. Dairy Sci. 89:E31-E51. https://doi.org/10.3168/jds.S0022-0302(06)72362-1.

Firkins, J. L., M L. Eastridge, N R. St-Pierre, and S. M. Noftsger. 2001. Effects of grain variability and processing on starch utilization by lactating dairy cattle. Anim. Sci. 79:E218-E238. https://doi.org/10.2527/jas2001.79E-SupplE218x.

Fredin, S. M., L. F. Ferraretto, M. S. Akins, P. C. Hoffman, and R. D. Shaver. 2014. Fecal starch as an indicator of total-tract starch digestibility by lactating dairy cows. J. Dairy Sci. 97:1862–1871. https://doi.org/10.3168/jds.2013-7395.

Hall, M. B., J. Arbaugh, K. Binkerd, A. Carlson, T. Doan, T. Grant, C. Heuer, H. D. Inerowicz, B. Jean-Louis, R. Johnson, J. Jordan, D. Kondratko, E. Maciel, K. McCallum, D. Meyer, C. A. Odijk, A. Parganlija-Ramic, T. Potts, L. Ruiz, S. Snodgrass, D. Taysom, S. Trupia, B. Steinlicht, and D. Welch. 2015. Determination of dietary starch in animal feeds and pet food by an enzymatic-colorimetric method: Collaborative study. J. AOAC Int. 98:397–409. https://doi.org/10.5740/jaoacint.15-012.

Heinzen Jr, C., M. R. Pupo, L. G. Ghizzi, E. C. Diepersloot, and L. F. Ferraretto. 2024. Effect of a genetically-modified corn hybrid with alpha-amylase and storage length on fermentation profile and starch disappearance of whole-plant corn silage and earlage. J. Dairy Sci. 107:3631-3641. https://doi.org/10.3168/jds.2023-24228.

Hoffman, P. C., N. M. Esser, R. D. Shaver, W. K. Coblentz, M. P. Scott, A. L. Bodnar, R. J. Schmidt, and R. C. Charley. 2011. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J. Dairy Sci. 94:2465-2474. https://doi.org/10.3168/jds.2010-3562.

Horton, L. M., C. L. Van Bibber-Krueger, H. C. Müller, and J. S. Drouillard. 2020. Effects of high-amylase corn on performance and carcass quality of finishing beef heifers. J. Anim. Sci. 98:1-10. https://doi.org/10.1093/jas/skaa302.

Hu, W., M. E. Persia, and Jr. L. Kung. 2010. In vitro ruminal fermentability of a modified corn cultivar expressing a thermotolerant α-amylase. J. Dairy Sci. 93:4846-4849. https://doi.org/10.3168/jds.2009-2321.

Johnson, M. A., T. Spore, S. P. Montgomery, W. R. Hollenbeck, R. N. Wahl, E. D Watson, D. A. Blasi. 2019. Syngenta Enogen Feed Corn silage containing an alpha amylase expression trait improves feed efficiency in growing calf diets. Kansas Agricultural Experiment Station Research Reports. 5(Suppl. 1):3. (Abstr).

Junges, D., G. Morais, M. H. F. Spoto, S. P. Santos, A. T. Adesogan, L. G. Nussio, and J. L. P. Daniel. 2017. Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. J. Dairy Sci. 100:9048-9051. https://doi.org/10.3168/jds.2017-12943.

Karman, A. H., and A. J. S. van Boekel. 1986. Evaluation of the Kjeldahl factor for conversion of the nitrogen content of milk and milk products to protein content. Net. Milk Dairy J. 40:315-336.

Klingerman, C. M., W. Hu, E. E. McDonell, M. C. DerBedrosian, and L. Kung Jr. 2009. An evaluation of exogenous enzymes with amylolytic activity for dairy cows. J. Dairy Sci. 92:1050-1059. https://doi.org/10.3168/jds.2008-1339.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2003. Effects of increasing levels of refined cornstarch in the diet of lactating dairy cows on performance and ruminal pH. J. Dairy Sci. 86:1341-1353. https://doi.org/10.3168/jds.S0022-0302(03)73719-9.

Krogstad, K. C., and B. J. Bradford. 2023. The effects of feeding α-amylase enhanced corn silage with different dietary starch concentrations to lactating dairy cows on milk production, nutrient digestibility, and blood metabolites. J. Dairy Sci. 106:4666-4681. https://doi.org/10.3168/jds.2022-23030.

Kung, L., Jr. 2011. Silage temperatures: how hot is too hot? Available online at: https://cdn.canr.udel.edu/wp-content/uploads/2014/ 02/HowHotisTooHot-2011.pdf. Accessed on July 15, 2025.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928. https://doi.org/10.3168/jds.S0022-0302(96)76442-1.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557–564. https://doi.org/10.3168/jds.S0022 -0302(03)73634-0.

Letelier, P., G. I. Zanton, and M. A. Wattiaux. 2022. Production performance of Holstein cows at 4 stages of lactation fed 4 dietary crude protein concentrations. J. Dairy Sci. 105:9581-9596. https://doi.org/10.3168/jds.2022-22146.

MacDonald, P. D. M., and P. E. Green. 1988. User's Guide to Program MIX: An Interactive Program for Fitting Mixtures of Distributions. Release 2.3, January 1988. Ichthus Data Systems.

Maulfair, D. D., K. K. McIntyre, and A. J. Heinrichs. 2013. Subacute ruminal acidosis and total mixed ration preference in lactating dairy cows. J. Dairy Sci. 96:6610–6620. https://doi.org/10.3168/jds.2013-6771.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481. https://doi.org/10.3168/jds.S0022-0302(97)76075-2.

Mertens, D. R.; Collaborators: M. Allen, J. Carmany, J. Clegg, A. Davidowicz, M. Drouches, K. Frank, D. Gambin, M. Garkie, B. Gildemeister, D. Jeffress, C. S. Jeon, D. Jones, D. Kaplan, G. N. Kim, S. Kobata, D. Main, X. Moua, B. Paul, J. Robertson, D. Taysom, N.

Thiex, J. Williams, and M. Wolf. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. of AOAC Int. 85:1217-1240. https://doi.org/10.1093/jaoac/85.6.1217.

Mundim, G. G. 2024. Uso de silagem de grão úmido/reidratado de milho sobre indicadores técnicos e econômicos em bovinos de leite. MSc Thesis. Universidade Federal de Lavras, Lavras, Brazil.

NASEM (National Academies of Sciences, Engineering, and Medicine). 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. Natl. Acad. Press.

Nocek, J. E., and S. Tamminga. 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. J. Dairy Sci. 74:3598-3629. https://doi.org/10.3168/jds.S0022-0302(91)78552-4.

Nozière, P., W. Steinberg, M. Silberberg, and D. P. Morgavi. 2014. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. J. Dairy Sci. 97:2319-2328. https://doi.org/10.3168/jds.2013-7095.

Oba, M., and M. S. Allen. 2003a. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174-183. https://doi.org/10.3168/jds.S0022-0302(03)73598-X.

Oba, M., and M. S. Allen. 2003b. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. J. Dairy Sci, 86:184-194. https://doi.org/10.3168/jds.S0022-0302(03)73599-1.

Pech-Cervantes, A. A., L. F. Ferrarretto, and I. M. Ogunade. 2022. Meta-analysis of the effects of the dietary application of exogenous alpha-amylase preparations on performance, nutrient digestibility, and rumen fermentation of lactating dairy cows. J. Anim. Sci. 100:skac189. https://doi.org/10.1093/jas/skac189.

Pereira, M. N., and L. E. Armentano. 2000. Partial replacement of forage with nonforage fiber sources in lactating cow diets. II. Digestion and rumen function. J. Dairy Sci. 83:2876-2887. https://doi.org/10.3168/jds.S0022-0302(00)75188-5.

Pereira, M. N., N. N. Morais Júnior, R. Caputo Oliveira, G. G. S. Salvati, and R. A. N. Pereira. 2021. Methionine precursor effects on lactation performance of dairy cows fed raw or heated soybeans. J. Dairy Sci. 104:2996–3007. https://doi.org/10.3168/jds.2020-18696.

Pereira, M. N., R. A. N. Pereira, L. L. Bitencourt, G. S. Dias Júnior, N. M. Lopes, and O. D. F. Zacaroni. 2013. Rehydrated corn silage in dairy cattle. Inf. Agropec. 34:7–18.

Powell, J. M., C. J. P. Gourley, C. A. Rotz, and D. M. Weaver. 2010. Nitrogen use efficiency: A potential performance indicator and policy tool for dairy farms. Environ. Sci. Policy. 13:217-228. https://doi.org/10.1016/j.envsci.2010.03.007.

Rebelo, L. R., M. L. Eastridge, J. L. Firkins, and C. Lee. 2023. Effects of corn silage and grain expressing α-amylase on ruminal nutrient digestibility, microbial protein synthesis, and enteric methane emissions in lactating cows J. Dairy Sci. 106:3932-3946. https://doi.org/10.3168/jds.2022-22770.

Silva, W. R., A. J. C. Silva, M. A. Tiengo, R. B. Silva, R. A. N. Pereira, T. J. DeVries, and M. N. Pereira. 2025. Effect of ensiled alpha-amylase enabled corn grain at two concentrations in the diet on lactation performance, chewing activity, ruminal fermentation, nutrient digestibility and nitrogen partition of dairy cows. J. Dairy Sci. 108:4878–4894. https://doi.org/10.3168/jds.2024-25522.

Valadares, R. F. D., G. A. Broderick, S. C. Valadares Filho, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686–2696. https://doi.org/10 .3168/jds.S0022-0302(99)75525-6.

Volk, S. M., H. C. Wilson, K. J. Hanford, J. C. MacDonald, and G. E. Erickson. 2021. Impact of Feeding Syngenta Enogen® Feed Corn Compared to Control Corn in Different Diet Scenarios to Finishing Beef Cattle. Anim. 11:2940. https://doi.org/10.3390/ani11102940.

Zebeli, Q., J. R. Aschenbach, M. Tafaj, J. Boguhn, B. N. Ametaj, and W. Drochner. 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J. Dairy Sci. 95:1041-1056. https://doi.org/10.3168/jds.2011-4421.

Warner, A. C. 1962. Enumeration of rumen microorganisms. J. Gen. Microbiol. 28:119–128. https://doi.org/10.1099/00221287-28-1-119.

Westreicher-Kristen, E., K. Robbers, R. Blank, A. Tröscher, U. Dickhoefer, S. Wolffram, and A. Susenbeth. 2018. Postruminal digestion of starch infused into the abomasum of heifers with or without exogenous amylase administration. Anim. Sci. 96:1939-1951. https://doi.org/10.1093/jas/sky082.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, J. H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to standard production characteristics. J. Dairy Sci. 65:495–501. https://doi.org/10.3168/jds .S0022-0302(82)82223-6.

Williams, E. J. 1949. Experimental designs balanced for the estimation of residual effects of treatments. Aust. J. Sci. Res. 2:149–168. https://doi.org/10.1071/CH9490149.

Table 1. Ingredient composition of offered diets[1] and nutrient composition of the consumed diets (offered – orts)

	GRC		REC	
Item	AAC	CTL	AAC	CTL
Ingredients, % of DM				
Alfalfa haylage	8.9	8.9	8.9	8.9
Whole plant corn silage	48.5	48.5	48.5	48.5
Citrus pulp pellets	5.7	5.7	5.7	5.7
Soybean meal	13.3	13.3	13.3	13.3
GRC AAC	13.0			
GRC CTL		13.0		
REC AAC			13.0	
REC CTL				13.0
Whole cottonseeds	8.0	8.0	8.0	8.0
Premix[2]	2.6	2.6	2.6	2.6
Nutrients, % of DM				
CP	14.8	14.8	14.8	14.7
RDP[3]	10.5	10.5	10.6	10.5
RUP[3]	4.2	4.2	4.2	4.2
NDF	36.6	36.4	36.2	36.1
Forage NDF (alfalfa haylage + corn silage)	28.1	28.0	28.1	28.0
${}_{pe}NDF_{>8}$[4]	23.6	23.5	23.5	23.5
Ether extract	4.0	4.0	4.0	3.9
Ash	7.3	7.2	7.3	7.2
NFC[5]	37.8	37.8	38.3	38.2
Starch	22.4	22.3	22.4	22.4
Starch from corn kernels	9.6	9.6	9.6	9.7
Starch from whole plant corn silage	12.0	11.9	12.0	11.9
Nonstarch NFC[6]	17.3	17.3	17.9	17.8
NE_L, Mcal/kg of DM[3]	1.76	1.76	1.77	1.77
MP supply, g/d[3]	1918	1918	1927	1918
MP balance, g/d[3]	-284	-273	-273	-292
DM, % of as-fed	38.3	38.8	37.4	37.6

[1]Corn processing: GRC = finely ground corn and REC = rehydrated and ensiled corn; Corn type: AAC = alpha-amylase enabled corn and CTL = isogenic control corn.
[2]Premix: 28.3% limestone, 33.3% sodium bicarbonate, 13.3% magnesium oxide, 8.3% NaCl, and 16.7% minerals and vitamins (23.8% Ca, 16.9% P, 1.9% Mg, 2.3% S, 100 mg/kg Co, 1,231 mg/kg Cu, 3,080 mg/kg Mn, 7323 mg/kg Zn, 51 mg/kg Se, 124 mg/kg I, 615,385 IU/kg Vit. A, 153,600 IU/kg Vit. D; 3825 IU/kg Vit E; 2000 mg/kg monensin).
[3]Estimated with NASEM (2021) for dairy cattle model.
[4]Physically effective fiber (${}_{pe}NDF_{>8}$) calculated from feed analysis and diet composition assuming that only whole plant corn silage, alfalfa haylage, and whole cottonseeds contributed to NDF > 8 mm.
[5]NFC = 100 - (CP + NDF + ether extract + ash).
[6]Nonstarch NFC = NFC - Starch.

Table 2. Composition of feed ingredients. Mean ± SD of 4 composite samples from each experimental period

Item[1]	DM % of as-fed	CP % of DM	EE[2] % of DM	NDF % of DM	Ash % of DM	NFC[3] % of DM	Starch % of DM	Non-starch NFC[4] % of DM
Alfalfa haylage	37.2 ± 2.5	16.5 ± 1.0	2.4	48.5 ± 3.7	7.7 ± 1.6	25.0 ± 5.5	1.8 ± 0.9	23.2 ± 5.6
Whole plant corn silage	26.6 ± 0.1	8.4 ± 0.3	2.9	48.8 ± 1.4	4.7 ± 1.0	35.3 ± 1.8	24.6 ± 2.7	10.7 ± 3.9
GRC AAC[5]	87.2 ± 0.5	7.4 ± 0.3	3.8	12.5 ± 0.9	1.4 ± 0.1	75.1 ± 0.9	73.8 ± 1.4	1.1 ± 1.0
GRC CTL[5]	86.6 ± 0.8	7.4 ± 0.5	3.8	12.1 ± 1.2	1.4 ± 0.1	75.6 ± 1.4	74.1 ± 0.6	1.3 ± 0.8
Citrus pulp pellets	86.0 ± 3.7	7.4 ± 0.6	2.6	26.1 ± 1.2	7.0 ± 0.9	57.0 ± 1.5	2.9 ± 1.0	54.1 ± 1.8
Soybean meal	88.5 ± 1.9	46.9 ± 2.0	1.8	12.3 ± 0.5	7.1 ± 0.6	31.9 ± 2.3	2.6 ± 1.7	29.4 ± 2.7
Whole cottonseeds	93.4 ± 1.5	19.5 ± 2.1	18.3	46.8 ± 1.7	4.0 ± 0.4	11.4 ± 2.8	1.4 ± 0.4	10.0 ± 3.1

[1]Samples were analyzed by wet chemistry.
[2]Obtained from NASEM (2021).
[3]NFC: 100 – (CP + NDF + EE + ash).
[4]Nonstarch NFC = NFC - Starch.
[5]GRC = finely ground corn, AAC = alpha-amylase enabled corn, CTL = isogenic control corn.

Table 3. Effect of grinding control (CTL) or amylase-enabled corn (AAC) kernels on the particle size distribution and geometric mean particle size (GMPS) of ground corn grain (n = 12/corn type)

Corn type	AAC	CTL	SEM	*P*-value[1]
% retained on screen				
4750 µm	0.12	0.25	0.094	0.33
3350 µm	0.32	0.56	0.136	0.21
2360 µm	1.72	1.69	0.279	0.95
1700 µm	7.50	6.31	0.797	0.30
1180 µm	19.08	17.84	0.987	0.38
600 µm	35.25	36.12	0.841	0.47
250 µm	29.48	28.15	1.307	0.48
Pan	6.53	9.08	0.848	0.05
GMPS[2], µm	530	450	27.2	0.05

[1]*P*-value for corn type.
[2]Kansas State University: MF-2051. Baker and Herrman (2002).

Table 4. Effect of rehydration and ensiling alpha-amylase enabled corn (AAC) or isogenic control (CTL) corn grains on nutrient composition, microbial count, pH, and DM loss of rehydrated and ensiled corn grain

Corn type	AAC	CTL	SEM	*P*-value[1]
DM, % of as-fed[2]	61.7	61.7	0.25	0.91
Nutrients, % of DM[2]				
CP	7.4	7.3	0.23	0.95
NDF	9.0	9.3	0.28	0.39
Ash	1.6	1.4	0.19	0.32
NFC	78.4	78.4	0.53	0.94
Starch	73.5	74.3	0.53	0.32
Non-starch NFC	5.0	4.1	0.60	0.34
pH[3]	3.72	3.73	0.009	0.37
Log_{10} cfu/g[4]				
Yeast	2.48	1.82	0.408	0.29
Spore forming aerobic bacteria	1.14	1.35	0.487	0.77
Filamentous fungi	1.96	1.25	0.424	0.27
Lactic acid bacteria	5.21	4.70	0.341	0.31
DM loss, % of ensiled[5]	2.24	2.38	0.32	0.75
Mass at ensiling, kg of as-fed[5]	212.4	214.5	2.12	0.50
Mass at opening, kg of as-fed[5]	211.8	213.6	2.16	0.55
Mass at ensiling, kg of DM[5]	129.2	130.8	1.36	0.40
Mass at opening, kg of DM[5]	126.4	127.8	1.37	0.48

[1]*P*-value for corn type.
[2]Data obtained by wet chemistry. N = 4 samples obtained during the last week of each period/corn type.
[3]N = 24 silos/corn type.
[4]cfu = colony forming units. N = 7 silos/corn type.
[5]N = 24 silos/corn type.

Table 5. Effect of corn processing and type[1] on DMI, digestible OM intake (DOMI), lactation performance, MUN, feed efficiency, linear SCC, BW, and BCS of dairy cows

	GRC		REC			*P*-value[2]		
Item	AAC	CTL	AAC	CTL	SEM	P	T	P×T
kg/d								
DMI	22.4	22.4	22.4	22.3	0.55	0.64	0.82	0.96
DOMI	14.3	14.3	14.5	14.3	0.40	0.62	0.64	0.55
Milk	35.8	35.9	36.1	36.4	1.02	0.22	0.63	0.64
ECM[3]	35.8	36.0	35.9	36.1	0.88	0.75	0.48	0.88
Fat	1.318	1.332	1.316	1.326	0.0377	0.78	0.40	0.87
Protein	1.141	1.333	1.140	1.151	0.0299	0.43	0.87	0.36
Casein	0.911	0.905	0.909	0.918	0.0239	0.49	0.84	0.37
Lactose	1.666	1.675	1.682	1.694	0.0515	0.29	0.51	0.94
TS	4.495	0.508	0.508	0.544	0.1117	0.54	0.55	0.78
% of milk								
Fat	3.72	3.75	3.68	3.68	0.101	0.06	0.50	0.66
Protein	3.21	3.18	3.18	3.18	0.062	0.18	0.16	0.14
Casein	2.56	2.54	2.53	2.54	0.049	0.12	0.19	0.13
Lactose	4.64	4.66	4.66	4.66	0.040	0.58	0.28	0.29
TS	12.60	12.61	12.54	12.55	0.159	0.05	0.66	0.94
MUN, mg/dL	15.6	15.6	15.5	15.5	0.49	0.66	0.92	0.77
Milk/DMI	1.60	1.61	1.62	1.64	0.037	0.06	0.23	0.54
ECM/DMI	1.60	1.61	1.61	1.63	0.031	0.36	0.21	0.78
ECM/DOMI	2.52	2.52	2.50	2.56	0.060	0.95	0.39	0.40
Ln SCC,[4] 0 to 9	1.77	1.61	1.60	1.85	0.373	0.79	0.77	0.17
BW, kg	659	657	659	657	13.9	0.88	0.23	0.93
BCS, 1 to 5[5]	3.27	3.24	3.27	3.25	0.061	0.78	0.25	0.99

[1]Corn processing: GRC = finely ground corn and REC = rehydrated and ensiled corn; Corn type: AAC = alpha-amylase enabled corn and CTL = isogenic control corn.

[2]P × T = interaction between corn processing (P) and type (T).

[3]ECM =energy corrected milk. ECM = {[(0.0929 × % fat) + (0.055 × % crude protein) + (0.0395 × % lactose)] × kg of milk}/0.70 (NASEM, 2021).

[4]Equivalency of the Ln SCC: 1.77 = 42,630 cells/mL; 1.61 = 38,150 cells/mL; 1.60 = 37,900 cells/mL; 1.85 = 45,070 cells/mL.

[5]BCS was scored on a 1 (thin) to 5 (obese) scale according to Wildman et al. (1982).

Table 6. Effect of corn processing and type[1] on milk fatty acids[2]

	GRC		REC			*P*-value[3]		
Item	AAC	CTL	AAC	CTL	SEM	P	T	P×T
g/100 g milk								
Preformed[4]	1.488	1.506	1.480	1.485	0.040	0.27	0.36	0.61
Mixed[5]	1.640	1.625	1.614	1.607	0.0469	0.14	0.46	0.77
De Novo[6]	0.861	0.865	0.853	0.835	0.0298	0.04	0.45	0.25
Monounsaturated	0.687	0.696	0.678	0.661	0.0334	0.03	0.66	0.17
Polyunsaturated	0.042	0.044	0.040	0.037	0.0056	<0.01	0.79	0.14
Saturated	3.172	3.188	3.136	3.159	0.0816	0.22	0.46	0.88
Unsaturated	0.696	0.712	0.688	0.666	0.0403	0.03	0.83	0.13
Palmitic	1.948	1.939	1.902	1.936	0.0580	0.25	0.57	0.33
Stearic	0.402	0.405	0.398	0.392	0.0123	0.08	0.82	0.36
Oleic	0.468^{ab}	0.480^{a}	0.467^{ab}	0.451^{b}	0.0263	0.08	0.81	0.10
kg/d								
Preformed	0.528	0.535	0.531	0.535	0.0152	0.83	0.38	0.87
Mixed	0.582	0.577	0.580	0.577	0.0166	0.90	0.57	0.87
De Novo	0.304	0.308	0.304	0.302	0.0110	0.47	0.77	0.48
Monounsaturated	0.243	0.248	0.242	0.239	0.0126	0.21	0.85	0.32
Polyunsaturated	0.015	0.016	0.015	0.014	0.0023	0.06	0.93	0.13
Saturated	1.125	1.132	1.124	1.137	0.0304	0.87	0.40	0.80
Unsaturated	0.247	0.254	0.246	0.241	0.0151	0.16	0.87	0.22
Palmitic	0.691	0.689	0.680	0.696	0.0216	0.86	0.43	0.29
Stearic	0.143	0.145	0.143	0.142	0.0057	0.41	0.75	0.53
Oleic	0.167	0.171	0.167	0.163	0.0099	0.25	0.89	0.22
g/100 g FA								
Preformed	37.38	37.75	37.58	37.88	0.313	0.39	0.07	0.85
Mixed	41.18	40.64	40.89	40.83	0.294	0.77	0.09	0.18
De Novo	21.44	21.61	21.54	21.30	0.348	0.48	0.83	0.18
Monounsaturated	17.57^{ab}	17.76^{a}	17.61^{ab}	17.14^{b}	0.546	0.11	0.44	0.07
Polyunsaturated	1.10	1.12	1.07	0.98	0.143	0.04	0.47	0.16
Saturated	82.23^{ab}	81.93^{b}	82.18^{ab}	82.82^{a}	0.717	0.09	0.50	0.06
Unsaturated	17.76^{ab}	18.07^{a}	17.82^{ab}	17.18^{b}	0.717	0.09	0.50	0.06
Palmitic	49.03^{AB}	48.71^{AB}	48.29^{B}	49.51^{A}	1.036	0.95	0.33	0.09
Stearic	10.09	10.20	10.07	10.03	0.204	0.19	0.66	0.26
Oleic	11.65^{ab}	11.96^{a}	11.75^{ab}	11.37^{b}	0.486	0.14	0.85	0.04

[1] Corn processing: GRC = finely ground corn and REC = rehydrated and ensiled corn; Corn type: AAC = alpha-amylase enabled corn and CTL = isogenic control corn.

[2]Samples analyzed by mid-infrared reflectance spectroscopy.

[3]P × T = interaction between corn processing (P) and type (T). [a, b]Means in a row with differing superscripts differ at $P \leq 0.05$ by Tukey. [A, B]Means in a row with differing superscripts differ at $0.05 < P \leq 0.10$ by Tukey.

[4]Preformed = Fatty acids ≥ 18 C.

[5]Mixed = Fatty acids with 16 or 17 C.

[6]De novo = Fatty acids with 4 to 15 C.

Table 7. Effect of corn processing and type[1] on intakes of physically effective fiber ($_{pe}NDF_{>8}$), forage NDF (FNDF), and total NDF, meal and chewing activity behavior, proportion of intake during the day, and sorting behavior of dairy cows

	GRC		REC			*P*-value[2]		
Item	AAC	CTL	AAC	CTL	SEM	P	T	P×T
$_{pe}NDF_{>8}$,[3] kg/d	5.30	5.26	5.25	5.24	0.129	0.43	0.59	0.78
$_{pe}NDF_{>8}$, % of BW	0.81	0.80	0.80	0.80	0.017	0.41	0.64	0.75
FNDF, kg/d	6.31	6.28	6.27	6.26	0.153	0.58	0.64	0.88
FNDF, % of BW	0.96	0.96	0.96	0.95	0.020	0.70	0.65	0.90
NDF, % of BW	1.25	1.24	1.23	1.23	0.026	0.13	0.63	0.85
Meal behavior								
Meal frequency, meals/d	10.1^{a}	9.3^{b}	10.2^{a}	10.4^{a}	0.48	0.02	0.36	0.07
Meal size, kg DM/meal	2.4^{ab}	2.5^{a}	2.3^{bc}	2.2^{c}	0.09	< 0.01	0.96	0.03
Meal duration, min/meal	37.6	37.4	35.9	34.8	1.60	0.01	0.43	0.61
Meal time, min/d	359	339	346	346	11.9	0.73	0.27	0.26
Longest meal duration, min/meal	75.2	72.3	72.3	71.1	3.41	0.33	0.34	0.68
Longest meal timing,[4] min	367	382	310	394	37.6	0.48	0.12	0.28
First meal duration, min/meal	54.2	54.1	56.8	54.1	3.00	0.49	0.48	0.49
Chewing activity behavior								
Eating, min/d	316	312	314	318	7.7	0.62	0.95	0.30
Rumination, min/d	415	414	421	413	8.6	0.57	0.33	0.39
Chewing activity,[5] min/d	731	726	734	732	12.0	0.49	0.54	0.84
Eating, min/kg DMI	14.0	13.9	14.0	14.1	0.42	0.49	0.92	0.80
Rumination, min/kg DMI	18.4	18.8	18.9	18.5	0.75	0.74	0.97	0.10
Chewing activity, min/kg DMI	32.3	32.7	33.0	32.5	1.05	0.56	0.97	0.29
Eating, min/kg $_{pe}NDF_{>8}$	60	60	60	61	1.8	0.44	0.67	0.65
Rumination, min/kg of $_{pe}NDF_{>8}$	79	82	81	80	3.5	0.85	0.63	0.22
Chewing activity, min/kg of $_{pe}NDF_{>8}$	139	142	142	141	4.8	0.63	0.59	0.48
Eating, min/kg FNDF	51	50	50	51	1.5	0.68	0.66	0.51
Rumination, min/kg FNDF	66	68	68	67	2.8	0.93	0.65	0.25
Chewing activity, min/kg FNDF	117	119	118	118	3.9	0.80	0.60	0.58
% of daily intake								
0700-1200 h	39.6	39.0	39.5	36.9	1.61	0.23	0.08	0.27
1200-1900 h	37.6	38.5	37.8	39.3	1.04	0.56	0.16	0.70
1900-0700 h	22.8	22.5	22.7	23.8	1.30	0.39	0.65	0.34
Sorting, Observed/Predicted × 100%[6]								
0700-1200 h								
> 19 mm[7]	104	92	101	90	4.8	0.43	<0.01	0.84
8-19 mm	100	103	103	104	1.1	0.13	0.04	0.54
< 8 mm	98	100	98	98	1.2	0.21	0.24	0.72
1200-1900 h								
> 19 mm	80	88	85	92	4.1	0.10	<0.01	0.79
8-19 mm	103	101	102	101	0.8	0.45	0.02	0.97
< 8 mm	102	102	101	101	0.9	0.36	0.62	0.82
1900-0700 h								
> 19 mm	82	85	87	88	3.9	0.14	0.51	0.60
8-19 mm	100	99	98	100	0.9	0.44	0.39	0.19
< 8 mm	107	106	105	104	1.2	0.07	0.19	0.88

[1]Corn processing: GRC = finely ground corn and REC = rehydrated and ensiled corn; Corn type: AAC = alpha-amylase enabled corn and CTL = isogenic control corn.

[2]P × T = interaction between corn processing (P) and type (T). [a, b] Means in a row with differing superscripts differ at $P \leq 0.05$ by Tukey.

[3]Physically effective fiber calculated from feed analysis and diet composition assuming that only whole plant corn silage, alfalfa haylage, and whole cottonseeds contributed to NDF > 8 mm.

[4]Minutes from first daily feeding at 0700 h.

[5]Chewing = eating + rumination.

[6]> 100 = preferential intake; < 100 = refusal; 100 = no sorting.

[7]Screens of the Penn State Particle Separator.

Table 8. Effect of corn processing and type[1] on total tract apparent digestibility, digestible starch intake, urine excretion, urinary allantoin and allantoin/digestible OM intake (DOMI) of dairy cows

	GRC		REC			*P*-value[2]		
Item	AAC	CTL	AAC	CTL	SEM	P	T	P×T
Digestibility, % of intake								
DM	66.3	66.9	67.6	66.6	0.83	0.57	0.73	0.32
OM	68.6	69.0	70.0	69.0	0.77	0.36	0.71	0.34
NDF	49.0	48.4	49.0	48.0	1.28	0.86	0.53	0.89
Non-NDF OM	81.3[b]	82.3[ab]	83.4[a]	82.3[ab]	0.50	0.03	0.94	0.05
Starch	93.3	92.8	94.1	93.0	0.47	0.26	0.07	0.51
CP	69.6	69.5	70.9	69.9	0.83	0.30	0.48	0.60
Digestible starch intake, kg/d	4.70	4.64	4.69	4.65	0.121	0.93	0.29	0.89
Fecal starch, % of DM	4.45	4.86	4.17	4.70	0.291	0.41	0.08	0.82
Fecal starch, kg/d	0.333	0.348	0.296	0.330	0.0257	0.28	0.33	0.70
Urine, L/day	24.0	22.9	23.4	22.0	1.21	0.40	0.17	0.87
Allantoin, mmol/d[3]	225	216	199	211	18.7	0.42	0.95	0.56
Allantoin/DOMI, mmol/kg	15.8	15.1	14.1	14.7	1.30	0.43	0.96	0.60

[1]Corn processing: GRC = finely ground corn and REC = rehydrated and ensiled corn; Corn type: AAC = alpha-amylase enabled corn and CTL = isogenic control corn.

[2]P × T = interaction between corn processing (P) and type (T). [a, b]Means in a row with differing superscripts differ at $P \leq 0.05$ by Tukey.

[3]Equivalency of Allantoin (mmol/d): 225 mmol/d = 182 g/d of microbial N; 216 mmol/d = 175 g/d of microbial N; 199 mmol/d = 162 g/d of microbial N; 211 mmol/d = 171 g/d of microbial N. Duodenal purine base flow (mmol/d) = 17.0 + 1.037 × urinary allantoin (Firkins et al., 2006). Microbial N (g/d) = (Duodenal purine base flow × 70) / (0.116 × 83 × 1000) (Chen and Gomes, 1992).

Table 9. Effect of corn processing and type[1] on ruminal molar VFA proportions, pH and viscosity of feces and ruminal fluid, and ruminal protozoa concentration of dairy cows

	GRC		REC			*P*-value[2]		
Item	AAC	CTL	AAC	CTL	SEM	P	T	P×T
Molar % of total VFA								
Acetate	66.9	67.1	67.1	67.1	0.35	0.70	0.80	0.63
Propionate	19.6	19.2	19.2	19.3	0.25	0.67	0.48	0.20
Butyrate	9.5	9.6	9.5	9.6	0.16	0.84	0.39	0.97
Isobutyrate	0.89	0.92	0.88	0.88	0.018	0.12	0.29	0.24
Valerate	1.46	1.44	1.44	1.43	0.024	0.40	0.47	0.82
Isovalerate	1.71[b]	1.74[ab]	1.84[a]	1.68[b]	0.064	0.49	0.21	0.05
Acetate/Propionate	3.43	3.51	3.51	3.52	0.071	0.46	0.47	0.52
pH								
Rumen	6.78	6.88	6.70	6.75	0.055	< 0.01	0.06	0.50
Feces	7.92	7.91	7.93	7.91	0.032	0.82	0.71	0.80
Viscosity, cP[3]								
Rumen	6.10	6.10	6.22	6.21	0.084	0.13	0.99	0.97
Feces	41.22	38.08	36.78	37.88	1.592	0.08	0.45	0.12
Protozoa, × 10^5/mL	4.09	4.91	4.78	4.75	0.566	0.64	0.49	0.45

[1]Corn processing: GRC = finely ground corn and REC = rehydrated and ensiled corn; Corn type: AAC = alpha-amylase enabled corn and CTL = isogenic control corn.

[2]P × T = interaction between corn processing (P) and type (T). [a, b]Means in a row with differing superscripts differ at $P \leq 0.05$ by Tukey.

[3]cP = centipoise.

Table 10. Effect of corn processing and type[1] on plasma glucose and N concentration of dairy cows

	GRC		REC			*P*-value[2]		
Item	AAC	CTL	AAC	CTL	SEM	P	T	P×T
Glucose, mg/dL								
Day 18	60.9	62.2	58.8	61.5	1.53	0.32	0.16	0.60
Day 21	62.7	62.4	62.6	62.6	1.16	0.98	0.88	0.88
Mean	61.8	62.3	60.7	62.0	0.96	0.42	0.29	0.59
PUN,[3] mg/dL	15.8	16.4	15.7	16.0	0.50	0.47	0.15	0.70

[1]Corn processing: GRC = finely ground corn and REC = rehydrated and ensiled corn; Corn type: AAC = alpha-amylase enabled corn and CTL = isogenic control corn.

[2]P × T = interaction between corn processing (P) and type (T).

[3]PUN = plasma urea-N. Samples obtained at 0 and 2 h relative to the end of morning (immediately before the daily feeding), afternoon, and night milkings. 6 ×/d. $P < 0.01$ for time (Ti) of sampling, $P = 0.62$ for P × T, $P = 0.62$ for T × Ti, and $P = 0.32$ for P × T × Ti.

Table 11. Effect of corn processing and type[1] on N partition of dairy cows

	GRC		REC			*P*-value[2]		
Item	AAC	CTL	AAC	CTL	SEM	P	T	P×T
N, g/d								
Intake	531.3	528.5	529.5	526.8	12.84	0.68	0.52	0.99
Milk[3]	180.0	178.4	180.4	181.5	4.74	0.33	0.96	0.46
Urine	201.1	183.0	185.3	189.3	11.69	0.64	0.49	0.28
Fecal	160.9	161.8	153.4	157.3	5.58	0.18	0.59	0.75
Total excreta[4]	362.0	344.8	338.6	346.5	13.54	0.35	0.69	0.28
Unaccounted N	-10.7	4.7	9.9	-1.5	13.25	0.54	0.87	0.26
N, % of daily intake								
Milk	33.9	34.0	34.2	34.7	0.65	0.09	0.30	0.36
Urine	37.9	35.0	35.4	36.1	2.21	0.71	0.59	0.36
Fecal	30.4	30.5	29.1	30.1	0.83	0.30	0.48	0.60
Total excreta[4]	68.3	65.6	64.5	66.3	2.33	0.47	0.84	0.31
Unaccounted N	-2.2	0.4	1.4	-0.9	2.45	0.61	0.94	0.27

[1]Corn processing: GRC = finely ground corn and REC = rehydrated and ensiled corn; Corn type: AAC = alpha-amylase enabled corn and CTL = isogenic control corn.

[2]P × T = interaction between corn processing (P) and type (T).

[3]Milk crude protein/6.34.

[4]Urine + feces.

Supplemental Table S1. Soluble protein, NH_3-N, ruminal degradation of starch, and fermentation profile of rehydrated and ensiled corn[1]

Corn type[2]	AAC	CTL
Soluble protein, % of CP	20.4	19.3
NH_3-N, % of DM	0.13	0.14
NH_3-N, % of CP	1.72	1.69
Starch degradation, % of starch		
0 h (A fraction)	34.0	22.9
7 h	58.5	54.7
kd of starch, %/h	12.1	10.8
ERD, % of starch[3]	75.8	69.7
Organic acids, % of DM[2]		
Lactic acid	1.63	1.96
Acetic acid	0.25	0.49

[1]Data obtained by NIRS. N = 1 composite sample/corn type. Composite samples formed from samples collected during the last week of each experimental period.
[2]AAC = alpha-amylase enabled corn, CTL = isogenic control corn.
[3]ERD = effective ruminal degradation. The ERD was calculated with a 1-pool starch model (ERD = Starch × kd / (kd + kp); Tylutki et al., 2007) assuming a fractional passage rate (kp) of 7%/h (Seo et al., 2006) and the fractional degradation rate (kd) of the NIRS analysis.

Supplemental Table S2. Distribution of particles, NDF and starch in whole plant corn silage (WPCS) and concentrations of DM, NDF and starch of fractions of the Penn State Particle Separator

	Proportion or concentration
Particle distribution of WPCS,[1] % of as-fed	
> 19 mm	11.2 ± 0.73
8-19 mm	55.2 ± 0.36
< 8 mm	33.5 ± 0.95
Particle distribution of WPCS,[1] % of DM	
> 19 mm	10.8 ± 0.65
8-19 mm	57.8 ± 0.53
< 8 mm	31.4 ± 0.83
NDF distribution of WPCS,[2] % of NDF	
> 19 mm	7.4 ± 0.64
8-19 mm	25.0 ± 1.71
< 8 mm	14.3 ± 0.7
Starch distribution of WPCS,[2] % of starch	
> 19 mm	0.8 ± 0.11
8-19 mm	18.5 ± 1.90
< 8 mm	8.1 ± 0.55
DM of fraction retained,[1] % of as-fed	
> 19 mm	26.5 ± 0.89
8-19 mm	28.8 ± 1.11
< 8 mm	25.9 ± 1.01
NDF of fraction retained,[2] % of DM	
> 19 mm	68.6 ± 2.09
8-19 mm	43.1 ± 3.04
< 8 mm	45.5 ± 1.39
Starch of fraction retained,[2] % of DM	
> 19 mm	7.8 ± 0.77
8-19 mm	31.9 ± 3.07
< 8 mm	25.8 ± 1.20

[1]Mean ± SD of 12 samples obtained weekly throughout the experiment.
[2]Mean ± SD of 4 composite samples from the last week of each experimental period.

GENERAL CONCLUSION

Alpha-amylase enabled corn had no effect on DMI or milk yield in the present study. However, when supplemented in combination with a low starch diet, it improved feed efficiency (ECM/DMI). Total tract digestibility of starch and NDF may also be enhanced with AAC inclusion, although such effects were not consistently observed. There is a potential for AAC to influence eating behavior, including chewing and sorting, but these effects appear to be mild. Although AAC did not affect the molar proportion of the major ruminal VFA, it may alter branched-chain VFA profile, as observed in experiment 1. Nitrogen excretion in feces plus urine, as well as NUP and MUN, can be reduced when AAC is supplemented in high starch diets. The response of lactating dairy cows to AAC appears to be influenced by both nutrient profile and ingredient composition of the diet as suggested by the results observed in experiment 1. However, corn kernel processing did not exert a major effect on animal response to AAC supplementation as few interactions between corn processing and corn type were observed in experiment 2. The effects observed in the present study are specifically associated with AAC incorporated into a floury corn hybrid. Incorporation of this technology into a vitreous corn hybrid may elicit different responses, and further research is warranted to evaluate such interactions. Overall, the effects of AAC supplementation are comparable to those observed with exogenous alpha-amylase and may represent a viable strategy to improve feed efficiency and nutrient digestibility in dairy production systems when fed as a concentrate source.

REFERENCES

AHMADI, F. *et al*. Performance and feeding behavior of dairy cows fed high-concentrate diets containing steam-flaked or ground corn varying in particle size. **Journal of Dairy Science**, v. 103, n. 4, p. 3191-3203, 2020.

ALLEN, M. S.; BRADFORD, B. J.; OBA, M. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. **Journal of Animal Science**, v. 87, n. 10, p. 3317-3334, 2009.

ALLEN, M. S. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. **Journal of Dairy Science**, v. 83, n. 7, p. 1598-1624, 2000.

ALLEN, M. S.; LONGUSKI, R. A.; YING, Y. Effects of corn grain endosperm type and fineness of grind on feed intake, feeding behavior, and productive performance of lactating dairy cows. **Journal of Dairy Science**, v. 104, n. 7, p. 7630-7640, 2021a.

ALLEN, M. S.; LONGUSKI, R. A.; YING, Y. Effects of corn grain endosperm type and fineness of grind on site of digestion, ruminal digestion kinetics, and flow of nitrogen fractions to the duodenum in lactating dairy cows. **Journal of Dairy Science**, v. 104, n. 7, p. 7641-7652, 2021b.

ALLEN, M. S.; YING, Y. Effects of corn grain endosperm type and conservation method on feed intake, feeding behavior, and productive performance of lactating dairy cows. **Journal of Dairy Science**, v. 104, n. 7, p. 7604-7616, 2021a.

ALLEN, M. S.; YING, Y. Effects of corn grain endosperm type and conservation method on site of digestion, ruminal digestion kinetics, and flow of nitrogen fractions to the duodenum in lactating dairy cows. **Journal of Dairy Science**, v. 104, n. 7, p. 7617-7629, 2021b.

AMARAL, D. M. *et al*. Metabolism of propionate, glucose, and carbon dioxide as affected by exogenous glucose in dairy cows at energy equilibrium. **Journal of Dairy Science**, v. 73, n. 5, p. 1244-1254, 1990.

ANDRADE FILHO, R. *et al*. Degradabilidade ruminal in situ de grãos de milho maduros do tipo flint ou dentado, secos ou reconstituídos e ensilados. *In:* **Proceedings of the 47th Meeting of the Brazilian Society of Animal Science**, Salvador, Bahia, Brazil. Abstracts. The Brazilian Society of Animal Science, 2010.

ANDREAZZI, A. S. R. *et al*. Effect of exogenous amylase on lactation performance of dairy cows fed a high-starch diet. **Journal of Dairy Science**, v. 101, n. 8, p. 7199-7207, 2018.

AOAC International. Official Methods of Analysis. 18th ed. AOAC International, 2006.

APHIS. 2008. Environmental Assessment Alpha-Amylase Maize Event 3272. Accessado em 5 de Dezembro de 2023.

BAKER, S.; T. HERMAN. Evaluating particle size. Publication MF-2051. Kansas State Univ., Manhattan, 2002.

BANNINK, A. *et al.* Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. **Journal of Theoretical Biology**, v. 238, n. 1, p. 36-51, 2006.

BAUMGARD, L. H. *et al.* Trans-10, cis-12 conjugated linoleic acid decreases lipogenic rates and expression of genes involved in milk lipid synthesis in dairy cows. **Journal of Dairy Science**, v. 85, n. 9, p. 2155-2163, 2002.

BEN-GHEDALIA, D. *et al.* The effects of starch-and pectin-rich diets on quantitative aspects of digestion in sheep. **Animal Feed Science and Technology**, v. 24, n. 3-4, p. 289-298, 1989.

BORREANI, GIORGIO *et al.* Silage review: Factors affecting dry matter and quality losses in silages. **Journal of Dairy Science**, v. 101, n. 5, p. 3952-3979, 2018.

BRODERICK, G. A.; CLAYTON, M. K. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. **Journal of dairy science**, v. 80, n. 11, p. 2964-2971, 1997.

BRODERICK, G. A.; MERTENS, D. R.; SIMONS, R. Efficacy of carbohydrate sources for milk production by cows fed diets based on alfalfa silage. **Journal of Dairy Science**, v. 85, n. 7, p. 1767-1776, 2002.

CANNON, S. J. *et al.* Inclusion of psyllium in milk replacer for neonatal calves. 1. Effects on growth, digesta viscosity, rate of passage, nutrient digestibilities, and metabolites in blood. **Journal of Dairy Science**, v. 93, n. 8, p. 3652-3660, 2010.

CASTRO, L. P. *et al*. Lactation performance of dairy cows fed rehydrated and ensiled corn grain differing in particle size and proportion in the diet. **Journal of Dairy Science**, v. 102, n. 11, p. 9857-9869, 2019.

CHEN, X. B., AND M. J. GOMES. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of Technical Details. International Feed Resources Unit, Rowett Research Institute, 1992.

COLMENERO, J.J. O.; BRODERICK, G. A. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. **Journal of Dairy Science**, v. 89, n. 5, p. 1704-1712, 2006.

CORREA, C. E. S. *et al*. Relationship between corn vitreousness and ruminal in situ starch degradability. **Journal of Dairy Science**, v. 85, n. 11, p. 3008-3012, 2002.

COSTA, A. *et al*. Invited review: Milk lactose—Current status and future challenges in dairy cattle. **Journal of Dairy Science**, v. 102, n. 7, p. 5883-5898, 2019.

COTTA, M. A. Amylolytic activity of selected species of ruminal bacteria. **Applied and Environmental Microbiology**, v. 54, n. 3, p. 772-776, 1988.

CUEVA, S. F. *et al*. Lactational performance, rumen fermentation, and enteric methane emission of dairy cows fed an amylase-enabled corn silage. **Journal of Dairy Science**, v. 104, n. 9, p. 9827-9841, 2021.

CUEVA, S. F. *et al*. Lactational performance, ruminal fermentation, and enteric gas emission of dairy cows fed an amylase-enabled corn silage in diets with different starch concentrations. **Journal of Dairy Science**, v. 107, n. 7, p. 4426-4448, 2024.

DEHORITY, B. A. Evaluation of subsampling and fixation procedures used for counting rumen protozoa. **Applied and Environmental Microbiology**, v. 48, n. 1, p. 182-185, 1984.

DEVRIES, T. J.; DOHME, F.; BEAUCHEMIN, K. A. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feed sorting. **Journal of Dairy Science**, v. 91, n. 10, p. 3958-3967, 2008.

DEVRIES, T. J. *et al.* Measuring the feeding behavior of lactating dairy cows in early to peak lactation. **Journal of Dairy Science**, v. 86, n. 10, p. 3354-3361, 2003.

DIAS JUNIOR, G. S. *et al*. Relationship between processing score and kernel-fraction particle size in whole-plant corn silage. **Journal of Dairy Science**, v. 99, n. 4, p. 2719-2729, 2016.

Dombrink-Kurtzman, M. A.; BIETZ, J. A. Zein composition in hard and soft endosperm of maize. **Cereal Chemistry**, v. 70, n. 1, p. 105–108, 1993.

ERICKSON, M. G. *et al.* Reducing dietary crude protein: Effects on digestibility, nitrogen balance, and blood metabolites in late-lactation Holstein cows. **Journal of Dairy Science**, v. 107, n. 7, p. 4394-4408, 2024.

ESEN, A. A proposed nomenclature for the alcohol-soluble proteins (zeins) of maize (Zea mays L.). **Journal of Cereal Science**, v. 5, n. 2, p. 117-128, 1987.

FERLAY, A. *et al.* Production of trans and conjugated fatty acids in dairy ruminants and their putative effects on human health: A review. **Biochimie**, v. 141, p. 107-120, 2017.

FERNANDES, T. *et al*. Effect of amylases and storage length on losses, nutritional value, fermentation, and microbiology of silages of corn and sorghum kernels. **Animal Feed Science and Technology**, v. 285, p. 115227, 2022.

FERNANDES, T. *et al.* Effect of washing method, grinding size, and the determination of an indigestible fraction on in situ degradation of starch in mature corn grain. **Journal of Dairy Science**, v. 101, n. 10, p. 9052-9057, 2018.

FERRARETTO, L. F. *et al.* Influence of a reduced-starch diet with or without exogenous amylase on lactation performance by dairy cows. **Journal of Dairy Science**, v. 94, n. 3, p. 1490-1499, 2011.

FERRARETTO, L. F.; CRUMP, P. M.; SHAVER, R. D. Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. **Journal of Dairy Science**, v. 96, n. 1, p. 533-550, 2013.

FERREIRA, E. M. *et al.* Nutrient digestibility and ruminal fatty acid metabolism in lambs supplemented with soybean oil partially replaced by fish oil blend. **Animal Feed Science and Technology**, v. 216, p. 30-39, 2016.

FIRKINS, J. L. *et al.* Integration of ruminal metabolism in dairy cattle. **Journal of Dairy Science**, v. 89, p. E31-E51, 2006.

FIRKINS, J. L. *et al*. Effects of grain variability and processing on starch utilization by lactating dairy cattle. **Journal of Animal Science**, v. 79, n. suppl_E, p. E218-E238, 2001.

FREDIN, S. M. *et al.* Fecal starch as an indicator of total-tract starch digestibility by lactating dairy cows. **Journal of Dairy Science**, v. 97, n. 3, p. 1862-1871, 2014.

GENCOGLU, H. *et al*. Effect of feeding a reduced-starch diet with or without amylase addition on lactation performance in dairy cows. **Journal of Dairy Science**, v. 93, n. 2, p. 723-732, 2010.

GOMES, A. L. M. *et al.* Effects of processing, moisture, and storage length on the fermentation profile, particle size, and ruminal disappearance of reconstituted corn grain. **Journal of Animal Science**, v. 98, n. 11, p. skaa332, 2020.

HALL, M. B.; HEREJK, C. Differences in yields of microbial crude protein from in vitro fermentation of carbohydrates. **Journal of Dairy Science**, v. 84, n. 11, p. 2486-2493, 2001.

HALL, M. B.; Collaborators: ARBAUGH, J.; BINKERD, K.; CARLSON, A.; DOAN, T.; GRANT, T.; HEUER, C.; INEROWICZ H. D.; JEAN-LOUIS, B.; JOHNSON, R.; JORDAN, J.; KONDRATKO, D.; MACIEL, E.; MCCALLUM, K.; MEYER, D.; ODIJK, C. A.; PARGANLIJA-RAMIC, A.; POTTS, T.; RUIZ, L.; SNODGRASS, S.; TAYSOM, D.; TRUPIA, S.; STEINLICHT, B.; WELCH, D. Determination of dietary starch in animal feeds and pet food by an enzymatic-colorimetric method: Collaborative study. **Journal of AOAC International**, v. 98, n. 2, p. 397-409, 2015.

HAMAKER, B. R. et al. Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. **Cereal Chemistry**, v. 72, 1995.

HEINZEN JR, C. *et al*. Effects of a genetically modified corn hybrid with α-amylase and storage length on fermentation profile and starch disappearance of whole-plant corn silage and earlage. **Journal of Dairy Science**, v. 107, n. 6, p. 3631-3641, 2024.

HOFFMAN, P. C. *et al*. Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. **Journal of Dairy Science**, v. 94, n. 5, p. 2465-2474, 2011.

HOLDING, D. R. Recent advances in the study of prolamin storage protein organization and function. **Frontiers in Plant Science**, v. 5, p. 276, 2014.

HORTON, L. M. *et al*. Effects of high-amylase corn on performance and carcass quality of finishing beef heifers. **Journal of Animal Science**, v. 98, n. 10, p. skaa302, 2020.

HRISTOV, A. N. *et al*. Effect of exogenous polysaccharide-degrading enzyme preparations on ruminal fermentation and digestibility of nutrients in dairy cows. **Animal Feed Science and Technology**, v. 145, n. 1-4, p. 182-193, 2008.

HUNTINGTON, G. B. Starch utilization by ruminants: from basics to the bunk. **Journal of Animal Science**, v. 75, n. 3, p. 852-867, 1997.

HULTQUIST, K. M.; CASPER, D. P. Effects of feeding rumen-degradable valine on milk production in late-lactating dairy cows. **Journal of Dairy Science**, v. 99, n. 2, p. 1201-1215, 2016.

HU, W.; PERSIA, M. E.; KUNG JR, L. In vitro ruminal fermentability of a modified corn cultivar expressing a thermotolerant α-amylase. **Journal of Dairy Science**, v. 93, n. 10, p. 4846-4849, 2010.

IPHARRAGUERRE, I. R.; CLARK, J. H. Varying protein and starch in the diet of dairy cows. II. Effects on performance and nitrogen utilization for milk production. **Journal of Dairy Science**, v. 88, n. 7, p. 2556-2570, 2005.

JOLLY-BREITHAUPT, M. L. *et al*. Effects of Syngenta Enogen Feed Corn containing an α-amylase trait on finishing cattle performance and carcass characteristics. **Translational Animal Science**, v. 3, n. 1, p. 504-512, 2019.

JOHNSON, M. A. *et al.* Syngenta Enogen Feed Corn silage containing an alpha amylase expression trait improves feed efficiency in growing calf diets. **Kansas Agricultural Experiment Station Research Reports**, v. 5, n. 1, p. 3, 2019.

JUNGES, D. *et al*. Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. **Journal of Dairy Science**, v. 100, n. 11, p. 9048-9051, 2017.

KARMAN, A. H.; VAN BOEKEL, M. A. J. S. Evaluation of the Kjeldahl factor for conversion of the nitrogen content of milk and milk products to protein content. **Netherlands Milk and Dairy Journal**, v. 40, n. 4, p. 315-336, 1986.

KLINGERMAN, C. M. *et al.* An evaluation of exogenous enzymes with amylolytic activity for dairy cows. **Journal of Dairy Science**, v. 92, n. 3, p. 1050-1059, 2009.

Koehler, T. J. *et al.* Enogen corn hybrids can improve feed efficiency and reduce environmental impact in dairy production. *In:* 2022 **American Dairy Science Association (ADSA) Annual Meeting**, 2022, Kansas City, EUA. Abstract n. 2215T, p. 308, 2022.

KRAUSE, K. M.; COMBS, D. K.; BEAUCHEMIN, K. A. Effects of increasing levels of refined corn starch in the diet of lactating dairy cows on performance and ruminal pH. **Journal of Dairy Science**, v. 86, n. 4, p. 1341-1353, 2003.

KROGSTAD, K. C.; BRADFORD, B. J. The effects of feeding α-amylase-enhanced corn silage with different dietary starch concentrations to lactating dairy cows on milk production, nutrient digestibility, and blood metabolites. **Journal of Dairy Science**, v. 106, n. 7, p. 4666-4681, 2023.

KUNG JR, L. Silage temperatures: how hot is too hot? Available online at: https://cdn.canr.udel.edu/wp-content/uploads/2014/ 02/HowHotisTooHot-2011.pdf. Accessed on July 15, 2025. 2011.

KUNG JR, L. *et al*. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. **Journal of Dairy Science**, v. 101, n. 5, p. 4020-4033, 2018.

KUNG JR, L.; WINDLE, M. C.; WALKER, N. The effect of an exogenous protease on the fermentation and nutritive value of high-moisture corn. **Journal of Dairy Science**, v. 97, n. 3, p. 1707-1712, 2014.

KUNG JR, L. Silage temperatures: how hot is too hot? Available online at: https://cdn.canr.udel.edu/wp-content/uploads/2014/ 02/HowHotisTooHot-2011.pdf. 2011. Accessed on July 15, 2025.

LAMMERS, B. P.; BUCKMASTER, D. R.; HEINRICHS, A. J. A simple method for the analysis of particle sizes of forage and total mixed rations. **Journal of Dairy Science**, v. 79, n. 5, p. 922-928, 1996.

LAPIERRE, H. et al. Triennial Lactation Symposium: Mammary metabolism of amino acids in dairy cows. **Journal of Animal Science**, v. 90, n. 5, p. 1708-1721, 2012.

LAWTON, J. W. Zein: A history of processing and use. **Cereal Chemistry**, v. 79, n. 1, p. 1-18, 2002.

LEAN, I. J. et al. Meta-analysis to predict the effects of metabolizable amino acids on dairy cattle performance. **Journal of Dairy Science**, v. 101, n. 1, p. 340-364, 2018.

LEIVA, E.; HALL, M. B.; VAN HORN, H. H. Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. **Journal of Dairy Science**, v. 83, n. 12, p. 2866-2875, 2000.

LENDING, C. R. et al. Structure of maize protein bodies and immunocytochemical localization of zeins. **Protoplasma**, v. 143, p. 51-62, 1988.

LEONARDI, C.; ARMENTANO, L. E. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. **Journal of Dairy Science**, v. 86, n. 2, p. 557-564, 2003.

LOPES, J. C. et al. Type of corn endosperm influences nutrient digestibility in lactating dairy cows. **Journal of Dairy Science**, v. 92, n. 9, p. 4541-4548, 2009.

LETELIER, P.; ZANTON, G. I.; WATTIAUX, M. A. Production performance of Holstein cows at 4 stages of lactation fed 4 dietary crude protein concentrations. **Journal of Dairy Science**, v. 105, n. 12, p. 9581-9596, 2022.

MACDONALD, P. D. M.; GREEN, P. E. User’s Guide to Program MIX: An Interactive Program for Fitting Mixtures of Distributions. Release 2.3, January 1988. Ichthus Data Systems, 1988.

MAULFAIR, D. D.; MCINTYRE, K. K.; HEINRICHS, A. J. Subacute ruminal acidosis and total mixed ration preference in lactating dairy cows. **Journal of Dairy Science**, v. 96, n. 10, p. 6610-6620, 2013.

MCALLISTER, T. A. *et al*. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. **Journal of Animal Science**, v. 71, n. 1, p. 205-212, 1993.

MCCARTHY, M. M. *et al.* The effect of an exogenous amylase on performance and total-tract digestibility in lactating dairy cows fed a high-byproduct diet. **Journal of Dairy Science**, v. 96, n. 5, p. 3075-3084, 2013.

MERTENS, D. R.; Collaborators: ALLEN, M.; CARMANY, J.; CLEGG, J.; DAVIDOWICZ, A.; DROUCHES, M.; FRANK, K.; GAMBIN, D.; GARKIE, M.; GILDEMEISTER, B.; JEFFRESS, D.; JEON, C. S.; JONES, D.; KAPLAN, D.; KIM, G. N.; KOBATA, S.; MAIN, D.; MOUA, X.; PAUL, B.; ROBERTSON, J.; TAYSOM, D.; THIEX, N.; WILLIAMS, J.; WOLF, M. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. **Journal of AOAC International**, v. 85, n. 6, p. 1217-1240, 2002.

MERTENS, D. R. Creating a system for meeting the fiber requirements of dairy cows. **Journal of Dairy Science**, v. 80, n. 7, p. 1463-1481, 1997.

MIRON, J.; YOSEF, E.; BEN-GHEDALIA, D. Composition and in vitro digestibility of monosaccharide constituents of selected byproduct feeds. **Journal of Agricultural and Food Chemistry**, v. 49, n. 5, p. 2322-2326, 2001.

MORRIS, D. L. *et al.* Effects of drying and analytical methods on nitrogen concentrations of feeds, feces, milk, and urine of dairy cows. **Journal of Dairy Science**, v. 102, n. 6, p. 5212-5218, 2019.

MU-FORSTER, C.; WASSERMAN, B. P. Surface localization of zein storage proteins in starch granules from maize endosperm: Proteolytic removal by thermolysin and in vitro cross-linking of granule-associated polypeptides. **Plant Physiology**, v. 116, n. 4, p. 1563-1571, 1998.

MUNDIM, G. G. Uso de silagem de grão úmido/reidratado de milho sobre indicadores técnicos e econômicos em bovinos de leite. MSc Thesis. **Universidade Federal de Lavras**, Lavras, Brazil, 2023.

NAKAE, T.; ELLIOTT, J. A. Production of volatile fatty acids by some lactic acid bacteria. II. Selective formation of volatile fatty acids by degradation of amino acids. **Journal of Dairy Science**, v. 48, n. 3, p. 293-299, 1965.

NASEM - NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE. 2021. **Nutrient Requirements of Dairy Cattle**. 8th rev. ed. National Academies Press, 2021.

NOCEK, J. E.; TAMMINGA, S. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. **Journal of Dairy Science**, v. 74, n. 10, p. 3598-3629, 1991.

NOZIÈRE, P. *et al*. Amylase addition increases starch ruminal digestion in first-lactation cows fed high and low starch diets. **Journal of Dairy Science**, v. 97, n. 4, p. 2319-2328, 2014.

OBA, M.; ALLEN, M. S. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. **Journal of Dairy Science**, v. 86, n. 1, p. 174-183, 2003a.

OBA, M.; ALLEN, M. S. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. **Journal of Dairy Science**, v. 86, n. 1, p. 184-194, 2003b.

OLIVEIRA, D. S. *et al*. Effects of corn containing gene for high concentration of alpha amylase (Enogen Feed corn) on fermentative parameters, digestibility and methane in vitro. *In:* 2024 ASAS-CSAS-WSASAS ANNUAL MEETING, 2024, Alberta, Canadá. Abstracts [Resumo n PSXI-13]. Alberta: **Animal Science Association**, 2024. p. 754.

OLIVEIRA, L. N. *et al.* Effect of low dietary concentrations of Acacia mearnsii tannin extract on chewing, ruminal fermentation, digestibility, nitrogen partition, and performance of dairy cows. **Journal of Dairy Science**, v. 106, n. 5, p. 3203-3216, 2023.

OSORIO, J. S.; LOHAKARE, J.; BIONAZ, M. Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation. **Physiological Genomics**, v. 48, n. 4, p. 231-256, 2016.

PECH-CERVANTES, A. A.; FERRARRETTO, L. F.; OGUNADE, I. M. Meta-analysis of the effects of the dietary application of exogenous alpha-amylase preparations on performance, nutrient digestibility, and rumen fermentation of lactating dairy cows. **Journal of Animal Science**, v. 100, n. 8, p. skac189, 2022.

PEREIRA, M. N.; ARMENTANO, L. E. Partial replacement of forage with non-forage fiber sources in lactating cow diets. II. Digestion and rumen function. **Journal of Dairy Science**, v. 83, n. 12, p. 2876-2887, 2000.

PEREIRA, M. N. *et al.* Methionine precursor effects on lactation performance of dairy cows fed raw or heated soybeans. **Journal of Dairy Science**, v. 104, n. 3, p. 2996-3007, 2021.

PEREIRA, M. N. *et al.* Rehydrated corn silage in dairy cattle. **Informe Agropecuário**, v. 34, n. 277, p. 27–33, 2013.

PHILIPPEAU, C.; DE MONREDON, F. Le Deschault; MICHALET-DOREAU, B. Relationship between ruminal starch degradation and the physical characteristics of corn grain. **Journal of Animal Science**, v. 77, n. 1, p. 238-243, 1999.

PHILIPPEAU, C.; LANDRY, J.; MICHALET-DOREAU, B. Influence of the protein distribution of maize endosperm on ruminal starch degradability. **Journal of the Science of Food and Agriculture**, v. 80, n. 3, p. 404-408, 2000.

PHILIPPEAU, C.; MICHALET-DOREAU, B. Influence of genotype and ensiling of corn grain on in situ degradation of starch in the rumen. **Journal of Dairy Science**, v. 81, n. 8, p. 2178-2184, 1998.

PICCIOLI-CAPPELLI, F. *et al.* Effect of dietary starch level and high rumen-undegradable protein on endocrine-metabolic status, milk yield, and milk composition in dairy cows during early and late lactation. **Journal of Dairy Science**, v. 97, n. 12, p. 7788-7803, 2014.

POWELL, J. M. *et al*. Nitrogen use efficiency: A potential performance indicator and policy tool for dairy farms. **Environmental Science & Policy**, v. 13, n. 3, p. 217-228, 2010.

RAMANZIN, M.; BAILONI, L.; BITTANTE, G. Solubility, water-holding capacity, and specific gravity of different concentrates. **Journal of Dairy Science**, v. 77, n. 3, p. 774-781, 1994.

REBELO, L. R. *et al*. Effects of corn silage and grain expressing α-amylase on ruminal nutrient digestibility, microbial protein synthesis, and enteric methane emissions in lactating cows. **Journal of Dairy Science**, v. 106, n. 6, p. 3932-3946, 2023.

SALVADOR, S. C. *et al*. Resposta de vacas leiteiras à substituição total de milho por polpa cítrica e à suplementação com microminerais orgânicos I: Consumo e digestão. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 60, p. 682-690, 2008.

SILVA, W. R. *et al*. Effect of ensiled alpha-amylase enabled corn grain at two concentrations in the diet on lactation performance, chewing activity, ruminal fermentation, nutrient digestibility and nitrogen partition of dairy cows. **Journal of Dairy Science**, v. 108, p. 4878-4894, 2025.

SIMPSON, D. J. Proteolytic degradation of cereal prolamins—the problem with proline. **Plant Science**, v. 161, n. 5, p. 825-838, 2001.

SNIFFEN, C. J.; ROBINSON, P. H. Protein and fiber digestion, passage, and utilization in lactating cows. Microbial growth and flow as influenced by dietary manipulations. **Journal of Dairy Science**, v. 70, n. 2, p. 425-441, 1987.

SPANGHERO, M.; KOWALSKI, Z. M. Updating analysis of nitrogen balance experiments in dairy cows. **Journal of Dairy Science**, v. 104, n. 7, p. 7725-7737, 2021.

STONE, W. C. Nutritional approaches to minimize subacute ruminal acidosis and laminitis in dairy cattle. **Journal of Dairy Science**, v. 87, p. E13-E26, 2004.

TAYLOR, C. C.; ALLEN, M. S. Corn grain endosperm type and brown midrib 3 corn silage: Feeding behavior and milk yield of lactating cows. **Journal of Dairy Science**, v. 88, n. 4, p. 1425-1433, 2005a.

TAYLOR, C. C.; ALLEN, M. S. Corn grain endosperm type and brown midrib 3 corn silage: Ruminal fermentation and N partitioning in lactating cows. **Journal of Dairy Science**, v. 88, n. 4, p. 1434-1442, 2005b.

TAYLOR, C. C.; ALLEN, M. S. Corn grain endosperm type and brown midrib 3 corn silage: Site of digestion and ruminal digestion kinetics in lactating cows. **Journal of Dairy Science**, v. 88, n. 4, p. 1413-1424, 2005c.

VALADARES, R. F. D. *et al.* Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. **Journal of Dairy Science**, v. 82, n. 12, p. 2686-2696, 1999.

VARELA, J. et al. Effects of endosperm type and storage length of whole-plant corn silage on nitrogen fraction, fermentation products, zein profile, and starch digestibility. **Journal of Dairy Science**, v. 106, n. 12, p. 8710-8722, 2023.

VOLK, S. M. *et al.* Impact of feeding Syngenta Enogen® feed corn compared to control corn in different diet scenarios to finishing beef cattle. **Animals**, v. 11, n. 10, p. 2940, 2021.

ZEBELI, Q. et al. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. **Journal of Dairy Science**, v. 95, n. 3, p. 1041-1056, 2012.

ZILIO, E. M. C. *et al.* Effects of exogenous fibrolytic and amylolytic enzymes on ruminal fermentation and performance of mid-lactation dairy cows. **Journal of Dairy Science**, v. 102, n. 5, p. 4179-4189, 2019.

WARNER, A. C. I. Enumeration of rumen micro-organisms. **Microbiology**, v. 28, n. 1, p. 119-128, 1962.

WEISS, W. P.; STEINBERG, W.; ENGSTROM, M. A. Milk production and nutrient digestibility by dairy cows when fed exogenous amylase with coarsely ground dry corn. **Journal of Dairy Science**, v. 94, n. 5, p. 2492-2499, 2011.

WILDMAN, E. E. *et al.* A dairy cow body condition scoring system and its relationship to selected production characteristics. **Journal of Dairy Science**, v. 65, n. 3, p. 495-501, 1982.

WILLIAMS, E. J. Experimental designs balanced for the estimation of residual effects of treatments. **Australian Journal of Chemistry**, v. 2, n. 2, p. 149-168, 1949.

YODER, P. S. et al. Effects of jugular infused methionine, lysine, and histidine as a group or leucine and isoleucine as a group on production and metabolism in lactating dairy cows. **Journal of Dairy Science**, v. 103, n. 3, p. 2387-2404, 2020.