Nutritional strategies for improved beef cattle performance: A focus on corn utilization

by

Stefan Knecht

D.V.M. National University of Asuncion, 2021

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Approved by:

Major Professor
Dr. James S. Drouillard

Copyright

Abstract

The pursuit of optimizing feed efficiency in beef cattle has led to investigations into various corn hybrids and processing methods. This study aimed to evaluate the impact of Enogen corn, known for its increased expression of alpha-amylase, in comparison to conventional corn, and to assess effects of dry-rolled versus steam-flaked processing on feedlot cattle performance. Two complementary experiments were conducted. Experiment 1 was an animal trial involving 960 crossbred beef steers, in a randomized complete block design with a 2 x 2 factorial arrangement, where steers were randomly assigned to one of four treatment groups. Experiment 2 was an *in vitro* investigation examining ruminal fermentation dynamics using a 2 x 2 factorial design. In Experiment 1, it was hypothesized that Enogen corn would improve growth performance, and feed efficiency, and evaluate if EC when dry-rolled would lessen the need for steam flaking. Results indicated that cattle fed steam-flaked either EC or CC, had the heavier final body weight ($P<0.01$). Enogen corn when steam-flaked or dry-rolled, and steam-flaked CC presented improved average daily gain ($P=0.012$), and G:F ratio ($P<0.01$) compared to the conventional corn when it is dry-rolled. Heavier carcasses ($P<0.01$) were observed for the treatments that included steam-flaked EC or CC. Dry matter intake was similar among cattle fed different diets ($P=0.269$). Experiment 2, focused on *in vitro* fermentation of Enogen corn processed through dry rolling or steam flaking, and compared to conventional corn. Results indicated that steam-flaked EC or CC presented lower gas production ($P=0.043$). Enogen corn when steam flaked had the lowest $t_{1/2}$ ($P=0.027$). Conventional corn when steam-flaked had the highest acetate to propionate ratio ($P<0.01$). Volatile fatty acid production was not affected by the interaction. In conclusion, dry-rolled Enogen corn is an alternative to steam-flaking while reducing the cost of the steam

flake process. Similarly, in an *in vitro* system, dry-rolled Enogen corn performed similarly to steam-flaking.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to express my sincere appreciation to Jim Drouillard for his guidance, and patience, and for providing me the opportunity to pursue a master's degree at Kansas State University. Your advice and insights have been helpful in my journey, my research and academic skills, which will be instrumental in my future career.

My deepest thanks to my parents, Horst and Maria Elena, and my sister for their unwavering support and love. You were always there for me, and I will always be grateful for it.

Also, I would like to thank my lab mates, Ludmila, Ross, Firman, Harleigh, and Elizabeth, for their support, assistance, and collaboration over the years. You make this experience enjoyable, and easier, I am thankful for crossing paths in Kansas.

My profound gratitude to my committee members, Logan Thompson and Yong Cheng Shi, for your advice and assistance.

Lastly, I would like to thank my friends, especially Santiago and Jovani. It has been years, and your support has been essential in my journey, I am thankful for making my time here enjoyable.

Chapter 1 - Literature Review – Optimizing corn starch utilization in feedlot cattle: A review of nutritional and environmental implications.

1.1 Introduction

Corn starch is a cornerstone of beef cattle nutrition, playing an important role in their growth and overall performance. And with the global demand for beef on the rise, getting starch utilization right is more important than ever. Corn starch, thanks largely to its branched structure from amylopectin, influences on how ruminants digest feed and, ultimately, on cattle health [1].

Ruminants, with their complex digestive systems, ferment starch into volatile fatty acids (VFAs) within the rumen. However, the speed of this fermentation can vary, depending on starch's physical and chemical structure, how it's processed, and the overall diet. Striking a balance in fermentation, by getting enough energy while avoiding ruminal metabolic disorders, is a key challenge [2,3]. So, a solid understanding of starch digestion, covering its composition and how it's processed, is crucial.

Processing methods, such as grinding, rolling, and steam-flaking, are used to improve starch digestibility by changing the structure of those starch granules and increasing their surface area. Steam-flaking, for example, is great for disrupting the starch-protein matrix, which improves gelatinization and makes energy more available [4]. These processing methods have a significant impact on corn starch's nutritional value and how well beef cattle perform.

Recent advances, like Enogen corn, hybrid that incorporates alpha-amylase enzyme, further improve starch digestion. This sort of "pre-digestion" right inside the corn kernel itself ramps up microbial fermentation in the rumen and enzymatic digestion later on in the small intestine. Studies suggest Enogen corn can improve feed efficiency, weight gain, and nutrient utilization, offering a promising avenue for more sustainable beef production [5].

1.2 Structure and biochemistry of starch

A. Molecular structure of starch

Starch is a carbohydrate composed of long chains of glucose monomers bound by glycosidic bonds. These chains form two distinct types of polymers: amylose and amylopectin. Starch is stored in plants in the form of granules, primarily within the endosperm of corn kernels. In fact, digestible starch makes up approximately 80% of a corn kernel. The ratio of amylose to amylopectin in corn starch is typically around 20:80, although this can vary among different corn varieties [6].

Amylose

Amylose is a polysaccharide composed of glucose monomers linked by α-1,4 glycosidic bonds, forming a linear molecule with a molecular mass between 10^5 and 10^6 Da. Due to its helical structure, amylose can form complexes with lipids and may exist in both free and complex forms [7]. Its helical structure makes it less accessible to enzymatic action compared to amylopectin, resulting in slower digestion in the rumen and reduced fermentability by ruminal microbes [8].

Amylopectin

On the other hand, amylopectin is a branched structure that employs a collection of amylose helices that are organized in parallel to define the granule's fundamental structure, the helices are sequentially connected by α-1,6 bonds, and thus provide multiple sites for enzymatic activity making it more easily digestible, its increased solubility and susceptibility to hydrolysis allows rapid fermentation in the rumen, leading to the production of volatile fatty acids (VFA's), which are crucial energy sources for ruminants [8,9]. Amylopectin is the predominant component of starch, and it is also larger in size compared to amylose [10].

B. Physical properties of starch

The physical properties of starch granules, such as size, shape, and crystallinity, are key factors influencing their behavior during digestion and processing. Corn starch granules typically range between 5 to 25 micrometers in diameter and exhibit a polygonal shape [11–13]. These granules contain both crystalline and amorphous regions, with the first being more resistant to enzymatic degradation [10]. Crystallinity in starch is primarily determined by the arrangement of amylopectin chains, which form a densely packed, ordered structure known as an A-type crystalline structure in corn starch. This structure is particularly relevant for its resistance to digestion in the rumen and affects the overall digestibility of starch in ruminants [14,15].

C. Functional properties of starch

Gelatinization is one of the most critical functional properties of starch, particularly in the context of its digestibility for ruminants. Gelatinization occurs when the starch granules are heated in the presence of water, leading to the disruption of hydrogen bonds within the granules, causing them to swell and lose their crystalline structure [16]. This process significantly increases the enzymatic accessibility of starch, enhancing its digestibility [4]. In feedlot ruminants, the degree of gelatinization directly impacts the efficiency of starch utilization, making it more accessible to ruminal and pancreatic enzymes, as it affects the rate at which starch is fermented in the rumen and subsequently absorbed in the small intestine [17–19].

Retrogradation, a related phenomenon, occurs when gelatinized starch molecules reassociate upon cooling, forming a more ordered structure. This can reduce the digestibility of starch, as the newly formed crystalline regions are more resistant to enzymatic breakdown, therefore an undesirable process. The extent of retrogradation depends on several factors, including the amylose content and the conditions under which the feed is processed and stored. Controlling the

gelatinization and retrogradation processes is crucial in feed preparation to maximize the nutritional value of starch-based diets for ruminants, therefore, water content, storage conditions, and the utilization of feed additives are crucial [20,21].

1.3 Corn as a source of starch for ruminants

A. Overview of corn

Corn (Zea mays) is the most widely produced feed grain in the United States according to the USDA [22], and one of the most cultivated crops globally according to the FAO [23], serving as a staple food source, an essential industrial raw material, and a primary feed ingredient in livestock production [24]. For ruminant nutrition, corn is particularly valued for its high starch content, making it a crucial energy source in feedlot diets [25]. Maize's versatility and adaptability allow it to be grown in a wide range of environments, contributing to its prominence as a key feed ingredient. Corn can be classified into five types based on kernel characteristics, including dent corn, flint corn, soft corn, sweet corn, and popcorn according to Sturtevant's classification and described by Anderson and Cutler [26]. Dent corn, characterized by its soft starch content, is commonly used in livestock feed, owing to its favorable digestibility and ease of processing [27]. Based on the starch composition, maize can be categorized into three classes: (1) waxy maize, which contains almost 100% amylopectin; (2) high-amylose maize, containing amylose between 40-70%; and (3) sugary maize, with lower starch content and a higher level of sucrose. The maize grain is composed of endosperm ($\approx$82%), germ ($\approx$6%), pericarp ($\approx$11%), and tip cap ($\approx$1%) [1]. The starch in corn is primarily located in the endosperm, which constitutes around 80% of the kernel [6,28]. The high starch content and the potential to be nearly 100% digested are critical for meeting the energy requirements of feedlot ruminants, supporting rapid weight gain and efficient feed conversion [29]. Besides starch, the whole maize grain also has

protein between 6 and 11 %, and fat between 4 and 5.5 %, respectively. However, the primary focus in ruminant nutrition is on optimizing starch utilization, given its impact on animal performance and feed efficiency. Corn is typically processed in different ways, aiming to increase starch availability and digestibility, and to do so, these processing methods break down the outer pericarp and disrupt the endosperm, increasing the surface area available for microbial fermentation in the rumen [30,31].

Feeding high levels of corn starch in ruminant diets requires careful management to prevent digestive disorders such as acidosis. The rapid fermentation of corn starch in the rumen can lead to the production and accumulation of large quantities of VFAs and other acids such as lactic acid and with it a drop in rumen pH, which can negatively impact animal health [2,3]. Therefore, balancing corn starch with effective fiber and other nutrients is critical to maintaining optimal rumen function and preventing metabolic disorders.

B. Corn starch characteristics

Corn starch being a highly digestible and fermentable source of energy for ruminants, is considered the most important in feedlot diets. Starch is primarily composed of amylose and amylopectin, with the latter making up around 80% and amylose around 20% as mentioned before. The high amylopectin content in corn is associated with rapid fermentation in the rumen, leading to a quick release of energy that supports growth and feed efficiency [1].

The digestibility of corn starch can vary based on factors like corn variety, processing methods, and granule structure. For example, when steam-flaking and fine grinding, significantly improve starch digestibility by breaking down the granule structure, and changing the physical structure of the grain, making it easier for rumen microbes to access and ferment the starch [25].

1.4   Digestion and utilization of starch in ruminants

A. Starch digestion in the rumen

In ruminants, starch digestion starts in the rumen, where microbial fermentation plays a central role. Once ingested, starch from the feed is exposed to a diverse microbial population primarily consisting of bacteria that specialize in breaking down carbohydrates, by microbial attachment to feed particles and enzyme production [32]. These microbes produce enzymes, such as amylases, pullulanase and isoamylase, maltase, and others, that hydrolyze starch into simpler sugars like maltose and glucose, which further fermented into VFAs, primarily acetate, propionate, and butyrate [8,33]. This process of starch fermentation allows bacteria to produce adenosine triphosphate (ATP), which is essential for microbes to accomplish their metabolic processes and produce waste products, VFAs, which is the main energy source for ruminants, with it constitutes a symbiotic relationship between microbes and ruminants [34].

The rate and extent of ruminal starch digestion depend on factors, such as the source of starch; processing method; physical and chemical properties of starch [25]. Corn starch is less rapidly degraded in the rumen than starch from other sources such as barley and wheat, as well as the passage rate, this slower fermentation rate is advantageous in feedlot diets, as it helps in the prevention of rapid acid production which can lead to ruminal acidosis [35].

Managing starch digestion in the rumen becomes a crucial task for nutritionists for maintaining rumen health, if the rate of starch degradation is high, VFA and other acids such as lactic will accumulate causing ruminal pH to drop and leading to subacute ruminal acidosis (SARA), and other problems for the host. To avoid this, the nutritionist's challenge is to balance a starch-rich diet with adequate fiber, helping fermentation rates and maintaining a stable rumen environment.

B. Post-ruminal starch digestion

After undigested starch escapes the rumen, it enters the small intestine, where it undergoes further digestion and absorption. The importance of post-ruminal starch digestion increases when the extent of ruminal digestion is low and more of the dietary starch is flushed into the intestines. Huntington et al. [36], describe the digestion and absorption of starch in the small intestine of ruminants in three phases, the first, luminal phase begins in the lumen of the duodenum, where pancreatic α-amylase initiates the breakdown of starch, through this process various products such as maltose, and branched-chain products called limit dextrins are produced; the next phase, brush border membrane phase occurs at the brush border membrane of the small intestine where carbohydrases like maltase and isomaltase breakdown maltose and limit dextrins into simpler sugars; the third phase is the glucose transport phase, which involves the transport of glucose out of the intestinal lumen into the circulation portal.

1.5   Corn processing methods and their impact on starch utilization.

The path of the maize from the field to the feed bunk is marked by a variety of processing methods, each developed to enhance the nutritional factors of starch for ruminants, to make the most of its potential. Raw corn presents a formidable challenge to the microbial ecosystem within the rumen. The kernel's outer layer limits the accessibility of starch to microbes and microbial enzymes. Therefore, processing becomes essential to enhance digestibility and with it animal performance.

A. Processing techniques

Dry milling:

Dry milling is a relatively straightforward approach that involves grinding corn kernels into smaller particles. This process increases the surface of the area exposed to microbial activity,

thereby accelerating starch digestion. However, the extent of starch gelatinization is limited in dry milling, affecting the overall digestibility.

Wet milling:

Wet milling, on the other hand, is a more complex process that involves stepping the corn kernels into water and sulfur dioxide to loosen the bran and the germ from the endosperm, facilitating the separation of starch. The resulting starch is utilized for further processing, such as gelatinization, enhancing its digestibility, through this process starch can be labeled with a more predictable digestion profile [37]

Steam flaking:

Steam flaking combines the benefit of heat and pressure. Through this method, corn kernels are exposed to steam, and with it hydrates and softens the starch inside, the next step involves passing the softened corn kernels through rollers, creating thin flakes. This process induces partial gelatinization and disrupts the crystalline structure of starch, making it available for microbial activity. When steam flaking, the density of the final flake has a large impact on rumen digestibility [31].

High-moisture corn:

Harvesting corn at a higher moisture content, and then storing it under anaerobic conditions, allows fermentation processes to occur, often this method results in the partial breakdown of the corn kernel increasing starch availability. During the fermentation process, organic acids are produced, which would further enhance digestibility [38].

B. Effect of processing on starch digestibility.

As we have explored various corn processing methods employed in feedlot operations aiming to enhance digestibility through physical and chemical modifications on the structure of starch. These alterations will determine the accessibility of starch granules to microbial activity, influencing the extent and rate of starch digestion.

Increased surface area:

Techniques like dry rolling and grinding primarily increase the surface area of corn, exposing more starch molecules to microbial enzymes, these physical modifications of corn accelerate the stages of starch digestion, as the enzymes have grater greater access to the substrate. However, increasing surface area does not gelatinize the starch, and limits the overall extent of digestion.

Gelatinization and disruption of crystalline structure:

Methods like steam flaking and wet milling induce gelatinization, a process that disrupts the crystalline structure of starch granules making them more susceptible to enzymatic breakdown. This transformation is crucial for maximizing starch digestion in the rumen, however, the extent of gelatinization varies depending on specific processing parameters like flake density that directly correlates with the amount of starch gelatinized.

Variability and individuality:

Factors like corn variety, different hybrids, corn moisture content, and processing conditions can change the final product since the effects of processing on starch digestibility can be uniform. Additionally, animal individual variation (age, diet, health status), the rumen microbiome, and digestive capabilities, like rumen size, can influence how starch is effectively utilized.

The "Sweet Spot" of processing:

The process of gelatinization is important, but not necessarily to gelatinize completely starch, some amount should be able to escape from ruminal digestion, as starch digested in the small intestine can provide a more efficient source of glucose for the ruminant [39]. Therefore, finding the optimal balance between ruminal and post-ruminal starch digestion is crucial for maximizing feed efficiency and animal performance.

Implications for feedlot management:

For feedlot managers to make the most efficient decision it is important to understand the effects of processing on starch digestibility, regarding feed selection and processing methods. By optimizing starch utilization, through various processing methods of corn, producers can improve feed efficiency, reduce feed costs, and enhance animal performance.

1.6   Enogen corn for feedlot ruminants.

As producers explored new ways to improve ruminant nutrition, Syngenta, a global company that seeks sustainable agriculture through crop protection, seed production, and modification for adaptability and enhanced production through improved genetics, first developed Enogen feed corn in the early 2000s. Enogen feed corn was first developed for the ethanol production industry, a corn hybrid characterized by a high alpha-amylase expression, and rapidly gaining attention for its potential to be capable of enhancing starch digestion in feedlot cattle.

Traditional corn processing methods, while effective, often face limitations to achieve consistent or optimal starch digestibility. Enogen corn, by introducing alpha-amylase enzyme within the kernel, was meant to offer an advantage, this enzyme, typically produced by microbes, is

responsible for starting the breakdown of starch into smaller and simpler sugar units, making it available for microbial fermentation.

Enhanced starch digestibility:

The presence of alpha-amylase in Enogen corn significantly enhances starch digestibility. Recent research has shown that cattle-fed Enogen corn has greater performance indicating higher ruminal starch digestibility compared to a conventional corn hybrid, some recent authors like Volk et al. [5], Glaser et al. [40], and Jolly-Breithaupt et al. [41]. This improved digestibility of starch to greater energy available for the animal, leading to improved feed efficiency and growth performance as concluded by the authors mentioned before.

Reduced use of external enzyme supplementation:

Feedlot diets often include exogenous enzyme supplements to enhance starch digestion, for example, exogenous alpha-amylase, Enogen corn, by providing the enzyme within the corn kernel, reduces the need for utilization of this external supplement, assuming so lower cost and simplification of the diet formulation.

Flexibility in processing:

Enogen corn has been shown to offer flexibility in processing methods, while it can be used in traditional processing techniques like steam flaking, and dry rolling, and it also performs well as whole-shelled corn [5]. This adaptability provides feedlot managers with greater control over feed processing and diet formulation.

The "Bigger Picture" of sustainability:

As various researchers concluded, Enogen corn has been demonstrated to improve feed efficiency, besides animal performance, which could contribute to reducing manure output and

greenhouse gas emissions, aligning with the growing emphasis on sustainable livestock production, thus this corn hybrid holds a potential for environmental sustainability.

1.7   Nutritional and performance impacts

A. Energy contribution of starch

Starch, as we have explored, plays a primordial role in providing available energy to ruminants, its fermentation end product in the rumen are volatile fatty acids, primarily acetate, propionate, and butyrate, which serve as the primary energy source for growth and maintenance [42]. The efficiency of starch utilization directly influences performance metrics in feedlot operations.

- **Weight gain:** Increased digestibility through processing methods or by different corn hybrids shows greater energy available for growth. Studies have consistently shown a positive correlation between starch intake and the average daily gain in feedlot cattle, however, this relationship is not always linear and excessive starch intake can cause potential health issues and lead to diminished returns.

- **Feed conversion:** Improving starch utilization enhances feed efficiency, as more energy is utilized from the diet, resulting in lower feed conversion, meaning that less feed is required to produce a unit of weight gain. An enhanced feed conversion not only benefits the producers economically but also has implications for environmental sustainability by reducing resource use and waste output.

- **Overall performance:** Starch also contributes to overall animal performance by enhancing other physiological functions when supplied adequately like immune system, reproduction, lactation, maintenance, and muscle development. Furthermore, starch also influences carcass characteristics, such as fat deposition and marbling score, which are important factors in determining meat quality.

B. Health considerations

While starch is the primary source of energy offered in feedlot diets, its utilization when formulating requires careful consideration to maintain animal health and well-being.

- **Rumen health:** The microbiome of the rumen is sensitive to changes in the diet composition, a sudden increase in starch intake can disrupt the balance of the rumen microbes, followed by a drop of pH, leading to acidosis. This ruminal disorder causes effects on rumen function, feed intake, and overall animal health. Additionally, excessive starch intake can lead to other metabolic disorders, such as fatty liver, which negatively impact animal health and productivity. Therefore, a step-up ration allows ruminants to gradually adapt to the final high starch diet and also ensure an adequate fiber content to maintain rumen health.

- **Acidosis risk:** Factors such as type of starch, processing method, or feeding management, can increase the risk of causing ruminal acidosis. Processing methods that increase starch gelatinization or highly fermentable starches like those from finely ground corn can also increase the risk of acidosis. Therefore, carefully formulating rations, and including an appropriate amount of forage, and bunk management strategies are crucial to prevent and mitigate ruminal acidosis.

C. Economic implications

The utilization of corn as the principal starch source in diets has a significant economic impact on feedlot operations.

- **Cost-Effectiveness:** Corn is an effective source of energy for ruminants when compared to other feed ingredients based on its cost, however, it can fluctuate depending on market

conditions and geographical location. Nutritionists, managers, and producers evaluate the cost of corn relative to its energy content, its potential for digestibility, and other benefits to determine its economic viability.

- **Feed efficiency and profitability:** Improved feed efficiency through enhanced starch utilization can be reflected in the economic gains, summarizing, by reducing the amount of feed per unit of gain, producers can lower feed costs and improve profitability. Furthermore, enhanced animal performance, such as increased weight gain, and improved carcass characteristics, such as fat deposition and marbling for better scoring, also contribute to the producer's economic returns.

- **Sustainability:** While corn's economic benefits are important, it is also important to consider sustainability, for example, the environmental impact of corn production, including resource use, especially water, and greenhouse gas emissions, must be taken into account into the economic evaluation. Sustainable practices, such as optimizing corn yields, utilizing byproducts, or developing hybrids to better adapt, can contribute to the long-term economic viability of corn starch.

1.8   Environmental Considerations

The increased use of starch-based diets, particularly those that depend on corn, has formulated important environmental questions. To make ruminant production sustainable, we need to look closely at how our choices impact the environment.

- **Resource use:** Corn production demands significant resources like land, water, and fertilizers, the demand for these resources places pressure on ecosystems. Deforestation and habitat loss occur when the land is converted for agriculture, especially large-scale corn production. Furthermore, in arid regions, irrigation for corn crops can deplete water

resources, which would impact human and ecological needs. Additionally, the use of fertilizers in corn production creates concerns about nutrient runoff into the water and potentially harm aquatic life. It is clear that adopting sustainable practices is no longer just an ethical practice but also a necessity to ensure the long-term viability of corn-based feed production.

- **Soil health and erosion:** Continuous monoculture, where corn is grown repeatedly in the same field, can deplete soil organic matter and biodiversity. This makes the soil more susceptible to erosion, especially in regions with heavy rainfall or strong winds, soil erosion also contributes to sedimentation in rivers and streams, which harms the aquatic ecosystems. Innovative practices have been developed and applied to mitigate this impact, such as no-till farming, cover crops, and crop rotation.

- **Emissions and climate change:** Ruminants, particularly cattle, are significant contributors to greenhouse gas emissions, primarily methane. Diets high in rapidly fermentable starch can lead to increased methane production. Therefore, strategies to optimize starch digestion and reduce methane emissions are critical for mitigating the environmental impact of ruminants. Emerging research that certain feed additives can reduce enteric methane emission, Duin et al.[43] through an *in vitro* study and Melgar et al.[44]] through an animal trial, demonstrated that 3-NOP (3-nitrooxypropanol) effectively reduces methane emissions from ruminants. Furthermore, lubabegron has been shown to reduce ammonia gas emissions as demonstrated by Teeter et al.[45]. Further research of these and other innovative approaches is crucial to the carbon footprint of livestock production.

1.9 Corn and Starch Byproducts for Enhanced Sustainability

To accomplish an efficient and sustainable ruminant feeding system, it is important to consider the full utilization of corn and starch, which includes not only the whole grain but also the byproducts generated during corn processing, such as corn gluten feed, distiller grains, and corn germ meal. Byproducts can offer economic and environmental benefits, contributing to a more holistic and sustainable approach to ruminant nutrition.

- **Variety of resources:** The corn processing industry generates a variety of by-products, each with its nutritional profile and potential for its utilization in ruminant diets, these byproducts can be gluten feed, distiller grains, corn germ meal, and corn steep liquor. Corn gluten feed is a byproduct of wet milling, rich in protein and fiber, and can be used as a supplement for growing and lactating animals [46]. Distiller grains are the corn's residues of ethanol production and high in energy and protein and can be incorporated into feedlot diets to replace a portion of the corn percentage [47]. Corn germ meal is rich in oil and protein, and corn steep liquor is a concentrated liquid byproduct used as a feed supplement or as a biostimulant for agriculture [48,49].

- **Economic and environmental advantages:** Utilizing byproducts offers advantages. From an economic standpoint, these products are offered at a lower cost compared to whole corn grain, this can help reduce feed costs and improve profitability, particularly when corn prices are high. From an environmental perspective, finding valuable uses to byproducts reduces waste and with it minimizes the environmental impact associated with corn processing and livestock production.

- **Challenges and considerations:** As corn and starch byproducts offer benefits, these are associated with challenges and considerations when used. The nutritional composition of

the byproducts can vary depending on the source and processing methods, which requires careful analysis and formulation to ensure balanced diets, some byproducts may have limitations in terms of digestibility, requiring further processing or limiting its inclusion to the diets. Additionally, the availability and transportation of byproducts may also increase the costs which will vary depending on geographic location and market conditions.

1.10   Future Directions and Research

While our understanding of starch in ruminant nutrition has grown, the quest for optimal utilization continues. Technologies like micronization or precision nutrition emerged, aiming to enhance starch digestion and animal performance. Further research is needed to better understand the rumen microbiome, starch structure, and fermentation, this will help future generations to further develop sustainable feeding strategies, aiming to maximize efficiency while minimizing environmental impact.

1.11   Conclusion

This review has highlighted the important role that starch plays in ruminant nutrition, particularly in feedlot systems. We have explored the complexities of starch digestion, from its molecular structure to the impacts of processing methods. To optimize starch utilization a careful approach needs to be taken into account, factors such as corn hybrid, processing methods, and feeding management. Moreover, the development of genetically modified corn varieties, such as Enogen, offers enhanced digestibility and feed efficiency. Furthermore, the economic and environmental implications of corn starch-based diets underscore the need for sustainable practices that balance profitability with resource conservation and ecological responsibility. Moving forward, more

research and innovation will be important to better understand starch digestion, develop feed

technologies, and ensure the long-term viability of ruminant production systems.

Literature cited

1. Singh, N.; Kaur, A.; Shevkani, K. Maize: Grain structure, composition, milling, and starch characteristics. In *Maize: Nutrition Dynamics and Novel Uses*; Chaudhary, D. P., Kumar, S., Langyan, S., Eds.; Springer India: New Delhi, 2014; pp 65–76. https://doi.org/10.1007/978-81-322-1623-0_5.

2. Slyter, L. L. Influence of acidosis on rumen function. *J. Anim. Sci.* **1976**, *43* (4), 910–929. https://doi.org/10.2527/jas1976.434910x.

3. Owens, F. N.; Secrist, D. S.; Hill, W. J.; Gill, D. R. Acidosis in cattle: a review. *J. Anim. Sci.* **1998**, *76* (1), 275–286. https://doi.org/10.2527/1998.761275x.

4. Svihus, B.; Uhlen, A. K.; Harstad, O. M. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Anim. Feed Sci. Technol.* **2005**, *122* (3), 303–320. https://doi.org/10.1016/j.anifeedsci.2005.02.025.

5. Volk, S. M.; Wilson, H. C.; Hanford, K. J.; MacDonald, J. C.; Erickson, G. E. Impact of feeding Syngenta Enogen® feed corn compared to control corn in Different diet scenarios to finishing beef cattle. *Animals* **2021**, *11* (10), 2940. https://doi.org/10.3390/ani11102940.

6. Rooney, L. W.; Pflugfelder, R. L. Factors affecting starch digestibility with special emphasis on sorghum and corn1. *J. Anim. Sci.* **1986**, *63* (5), 1607–1623. https://doi.org/10.2527/jas1986.6351607x.

7. Schmiele, M.; Sampaio, U. M.; Pedrosa Silva Clerici, M. T. Chapter 1 - Basic Principles: Composition and properties of starch. In *Starches for Food Application*; Silva Clerici, M. T. P., Schmiele, M., Eds.; Academic Press, 2019; pp 1–22. https://doi.org/10.1016/B978-0-12-809440-2.00001-0.

8. Kotarski, S. F.; KWaniska, R. D.; Thurn, K. K. Starch hydrolysis by the ruminal microflora. *J. Nutr.* **1992**, *122* (1), 178–190. https://doi.org/10.1093/jn/122.1.178.

9. Moran, EdwinT. Starch: Granule, amylose-amylopectin, feed preparation, and recovery by the fowl's gastrointestinal tract. *J. Appl. Poult. Res.* **2019**, *28* (3), 566–586. https://doi.org/10.3382/japr/pfy046.

10. Bertoft, E. Understanding starch structure: Recent progress. *Agronomy* **2017**, *7* (3), 56. https://doi.org/10.3390/agronomy7030056.

11. Sandhu, K. S.; Singh, N.; Kaur, M. Characteristics of the different corn types and their grain fractions: physicochemical, thermal, morphological, and rheological properties of starches. *J. Food Eng.* **2004**, *64* (1), 119–127. https://doi.org/10.1016/j.jfoodeng.2003.09.023.

12. Mir, S. A.; Bosco, S. J. D.; Bashir, M.; Shah, M. A.; Mir, M. M. Physicochemical and structural properties of starches isolated from corn cultivars grown in Indian temperate climate. *Int. J. Food Prop.* **2017**, *20* (4), 821–832. https://doi.org/10.1080/10942912.2016.1184274.

13. Li, W.; Wu, P.; Zhang, D.; Yan, S. Granule size distribution and pasting properties of starch in normal, waxy and sweet maize kernels. *Bangladesh J. Bot.* **2020**, *49* (4), 949–956. https://doi.org/10.3329/bjb.v49i4.52504.

14. Martens, B. M. J.; Gerrits, W. J. J.; Bruininx, E. M. A. M.; Schols, H. A. Amylopectin structure and crystallinity explains variation in digestion kinetics of starches across botanic sources in an in vitro pig model. *J. Anim. Sci. Biotechnol.* **2018**, *9* (1), 91. https://doi.org/10.1186/s40104-018-0303-8.

15. Cai, L.; Shi, Y.-C. Preparation, structure, and digestibility of crystalline A- and B-type aggregates from debranched waxy starches. *Carbohydr. Polym.* **2014**, *105*, 341–350. https://doi.org/10.1016/j.carbpol.2014.01.075.

16. Schirmer, M.; Jekle, M.; Becker, T. Starch gelatinization and its complexity for analysis. *Starch - Stärke* **2015**, *67* (1–2), 30–41. https://doi.org/10.1002/star.201400071.

17. Han, C.; Guo, Y.; Cai, X.; Yang, R. Starch properties, nutrients profiles, in vitro ruminal fermentation and molecular structure of corn processed in different ways. *Fermentation* **2022**, *8* (7), 315. https://doi.org/10.3390/fermentation8070315.

18. Kokić, B.; Dokić, L.; Pezo, L.; Jovanović, R.; Spasevski, N.; Kojić, J.; Hadnađev, M. Physicochemical changes of heat-treated corn grain used in ruminant nutrition. *Animals* **2022**, *12* (17), 2234. https://doi.org/10.3390/ani12172234.

19. Cooper, R. J.; Milton, C. T.; Klopfenstein, T. J.; Scott, T. L.; Wilson, C. B.; Mass, R. A. Effect of corn processing on starch digestion and bacterial crude protein flow in finishing cattle. *J. Anim. Sci.* **2002**, *80* (3), 797–804. https://doi.org/10.2527/2002.803797x.

20. Wang, S.; Li, C.; Copeland, L.; Niu, Q.; Wang, S. Starch retrogradation: A comprehensive review. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14* (5), 568–585. https://doi.org/10.1111/1541-4337.12143.

21. Gudmundsson, M. Retrogradation of starch and the role of its components. *Thermochim. Acta* **1994**, *246* (2), 329–341. https://doi.org/10.1016/0040-6031(94)80100-2.

22. *USDA ERS - Corn and other feed grains*. https://www.ers.usda.gov/topics/crops/corn-and-other-feed-grains/ (accessed 2024-08-20).

23. *Home | Food and Agriculture Organization of the United Nations*. FAOHome. https://www.fao.org/home/en (accessed 2024-08-20).

24. Erenstein, O.; Jaleta, M.; Sonder, K.; Mottaleb, K.; Prasanna, B. M. Global maize production, consumption and trade: trends and R&D implications. *Food Secur.* **2022**, *14* (5), 1295–1319. https://doi.org/10.1007/s12571-022-01288-7.

25. Huntington, G. B. Starch utilization by ruminants: from basics to the bunk2. *J. Anim. Sci.* **1997**, *75* (3), 852–867. https://doi.org/10.2527/1997.753852x.

26. Anderson, E.; Cutler, H. C. Races of Zea Mays: I. Their recognition and classification. *Ann. Mo. Bot. Gard.* **1942**, *29* (2), 69–88. https://doi.org/10.2307/2394331.

27. García-Lara, S.; Chuck-Hernandez, C.; Serna-Saldivar, S. O. Chapter 6 - Development and structure of the corn kernel. In *Corn (Third Edition)*; Serna-Saldivar, S. O., Ed.; AACC International Press: Oxford, 2019; pp 147–163. https://doi.org/10.1016/B978-0-12-811971-6.00006-1.

28. Kikuchi, K.; Takatsuji, I.; Tokuda, M.; Miyake, K. Properties and uses of horny and floury endosperms of corn. *J. Food Sci.* **1982**, *47* (5), 1687–1692. https://doi.org/10.1111/j.1365-2621.1982.tb05012.x.

29. Loy, D. D.; Lundy, E. L. Chapter 23 - Nutritional properties and feeding value of corn and its coproducts. In *Corn (Third Edition)*; Serna-Saldivar, S. O., Ed.; AACC International Press: Oxford, 2019; pp 633–659. https://doi.org/10.1016/B978-0-12-811971-6.00023-1.

30. Knowlton, K. F.; Glenn, B. P.; Erdman, R. A. Performance, ruminal fermentation, and site of starch digestion in early lactation cows fed corn grain harvested and processed differently. *J. Dairy Sci.* **1998**, *81* (7), 1972–1984. https://doi.org/10.3168/jds.S0022-0302(98)75771-6.

31. Zinn, R. A.; Owens, F. N.; Ware, R. A. Flaking corn: processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. *J. Anim. Sci.* **2002**, *80* (5), 1145–1156. https://doi.org/10.2527/2002.8051145x.

32. McAllister, T. A.; Bae, H. D.; Jones, G. A.; Cheng, K.-J. Microbial attachment and feed digestion in the rumen. *J. Anim. Sci.* **1994**, *72* (11), 3004–3018. https://doi.org/10.2527/1994.72113004x.

33. Owens, F. N.; Basalan, M. Ruminal Fermentation. In *Rumenology*; Millen, D. D., De Beni Arrigoni, M., Lauritano Pacheco, R. D., Eds.; Springer International Publishing: Cham, 2016; pp 63–102. https://doi.org/10.1007/978-3-319-30533-2_3.

34. Hungate, R. E. The rumen microbial ecosystem. *Annu. Rev. Ecol. Syst.* **1975**, *6*, 39–66.

35. Offner, A.; Bach, A.; Sauvant, D. Quantitative review of in situ starch degradation in the rumen. *Anim. Feed Sci. Technol.* **2003**, *106* (1), 81–93. https://doi.org/10.1016/S0377-8401(03)00038-5.

36. Huntington, G. B.; Harmon, D. L.; Richards, C. J. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle1. *J. Anim. Sci.* **2006**, *84* (suppl_13), E14–E24. https://doi.org/10.2527/2006.8413_supplE14x.

37. Morris, C. F. Grain quality attributes for cereals other than wheat. In *Encyclopedia of Food Grains (Second Edition)*; Wrigley, C., Corke, H., Seetharaman, K., Faubion, J., Eds.; Academic Press: Oxford, 2016; pp 257–261. https://doi.org/10.1016/B978-0-12-394437-5.00248-5.

38. Stock, R. A.; Sindt, M. H.; Cleale, R. M., IV; Britton, R. A. High-moisture corn utilization in finishing cattle1. *J. Anim. Sci.* **1991**, *69* (4), 1645–1656. https://doi.org/10.2527/1991.6941645x.

39. 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. *J. Dairy Sci.* **1991**, *74* (10), 3598–3629. https://doi.org/10.3168/jds.S0022-0302(91)78552-4.

40. Glaser, M. A.; Montgomery, S. P.; Vahl, C. I.; Titgemeyer, E. C.; Kubick, C. S.; Glaser, G. I.; Spore, T. J.; Hollenbeck, W. R.; Wahl, R. A.; Blasi, D. A. Effects of feeding corn containing an alpha-amylase gene on the performance and digestibility of growing cattle. *Transl. Anim. Sci.* **2022**, *6* (1), txac013. https://doi.org/10.1093/tas/txac013.

41. Jolly-Breithaupt, M. L.; Harris, M. E.; Nuttelman, B. L.; Burken, D. B.; MacDonald, J. C.; Luebbe, M. K.; Iragavarapu, T. K.; Erickson, G. E. Effects of Syngenta Enogen feed corn containing an α-amylase trait on finishing cattle performance and carcass characteristics1. *Transl. Anim. Sci.* **2019**, *3* (1), 504–512. https://doi.org/10.1093/tas/txy121.

42. Bergman, E. N. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* **1990**, *70* (2), 567–590. https://doi.org/10.1152/physrev.1990.70.2.567.

43. Duin, E. C.; Wagner, T.; Shima, S.; Prakash, D.; Cronin, B.; Yáñez-Ruiz, D. R.; Duval, S.; Rümbeli, R.; Stemmler, R. T.; Thauer, R. K.; Kindermann, M. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. *Proc. Natl. Acad. Sci.* **2016**, *113* (22), 6172–6177. https://doi.org/10.1073/pnas.1600298113.

44. Melgar, A.; Lage, C. F. A.; Nedelkov, K.; Räisänen, S. E.; Stefenoni, H.; Fetter, M. E.; Chen, X.; Oh, J.; Duval, S.; Kindermann, M.; Walker, N. D.; Hristov, A. N. Enteric methane emission, milk production, and composition of dairy cows fed 3-nitrooxypropanol. *J. Dairy Sci.* **2021**, *104* (1), 357–366. https://doi.org/10.3168/jds.2020-18908.

45. Teeter, J. S.; Werth, S. J.; Gruber, S. L.; Kube, J. C.; Hagenmaier, J. A.; Allen, J. B.; Herr, C. T.; Brown, M. S.; Boler, D.; Dilger, A. C.; Zhao, Y.; Pan, Y.; Mitloehner, F. M. Effects of feeding lubabegron on gas emissions, growth performance, and carcass characteristics of

beef cattle housed in small-pen environmentally monitored enclosures during the last 3 mo of the finishing period. *J. Anim. Sci.* **2021**, *99* (12), skab338. https://doi.org/10.1093/jas/skab338.

46. Fleck, A. T.; Lusby, K. S.; Owens, F. N.; McCollum, F. T. Effects of corn gluten feed on forage intake, digestibility and ruminal parameters of cattle fed native grass hay. *J. Anim. Sci.* **1988**, *66* (3), 750–757. https://doi.org/10.2527/jas1988.663750x.

47. Liu, K. Chemical composition of distillers grains, a review. *J. Agric. Food Chem.* **2011**, *59* (5), 1508–1526. https://doi.org/10.1021/jf103512z.

48. Perry, T. W.; Weatherly, W. H. Supplemental corn steep liquor, soap stock and soybean oil for finishing beef steers. *J. Anim. Sci.* **1976**, *42* (4), 1002–1007. https://doi.org/10.2527/jas1976.4241002x.

49. Garcia-Sanchez, F.; Camara-Zapata, J. M.; Navarro-Morillo, I. Use of corn steep liquor as a biostimulant in agriculture. *Horticulturae* **2024**, *10* (4), 315. https://doi.org/10.3390/horticulturae10040315.

Chapter 2 - Evaluating Enogen® and conventional corn grain processed by dry-rolling and steam-flaking for energetic efficiency and animal performance.

2.1 Abstract

This study investigated the impact of corn hybrid (Enogen [EC] vs. conventional [CC]) and processing method (dry-rolled vs. steam-flaked) on feedlot cattle performance and carcass characteristics. Crossbred beef steers (n=960; initial body weight 427.21 ± 10.76 kg) were stratified by weight and assigned to one of four treatment groups in a randomized complete block design. Cattle were fed for a total of 155 or 162 days. The finishing diets consisted of corn silage (14% of diet DM) with steam-flaked or dry-rolled corn (78.18% of diet DM). Weights were recorded at the beginning and the end of the study, and steers were harvested at a commercial abattoir where carcass data were collected. We hypothesized that Enogen corn, with its enhanced starch digestibility, would improve growth performance and feed efficiency, and Enogen corn when dry-rolled could perform similarly to steam-flaked corn. Dry matter intake (DMI) was similar across all treatments (P=0.269). An interaction between grain processing and grain type was observed for average daily gain (ADG; P=0.01) and feed efficiency (P<0.01), where cattle fed dry-rolled CC performed poorly compared to other treatments, the remaining three treatments were not different (P>0.05). Dry-rolled Enogen corn performed comparably to, and could be an alternative to steam-flaking.

Keywords: Corn, processing, Enogen, hot carcass weight, steam-flaking, dry-rolling.

2.2   Introduction

Corn processing has been an important tool used by producers to achieve improvements in digestion and animal performance. Corn is the primary grain used by nutritionists [1,2]. In the past, a challenge for the production manager has been to adjust processing techniques and diet formulation to accommodate characteristics of the available sources of starch [3]. To improve the digestibility of starch in ruminants, various processing methods have been utilized such as ensiling, grinding, dry-rolling, and steam-flaking, all of which increase starch availability [4,5].

Syngenta, introduced Enogen® feed corn (EC) in 2011 [6], a corn hybrid that is genetically enhanced, containing a thermotolerant alpha-amylase enzyme that afforded advantages for the dry-milling ethanol process [7]. Enogen® corn has been tested for its capacity in different diet formulations. Johnson et al.[8] concluded that calves fed Enogen® corn had improved feed efficiency when compared to control (yellow) corn fed calves. Cueva et al.[9] fed Enogen® corn as silage to dairy cows and evaluated lactational performance, concluding that cows fed the high-amylase corn silage had greater milk production, milk protein content, lactose yields, and improved feed efficiency. Studies have been conducted to demonstrate benefits and effectiveness of Enogen® corn grain compared to conventional corn using different processing methods. When Enogen® corn was fed as steam-flaked to beef heifers, Horton et al.[10] observed improvements in cattle performance and carcass characteristics. Schoonmaker et al.[11] found no differences in performance and carcass characteristics of feedlot steers fed ground Enogen corn compared to those fed a diet were up to 20% of a non-genetically modified graund corn was replaced. Similarly, Baker et al. [12] reported no improvement on average daily gain (ADG) or dry matter intake (DMI) for feedlot finishing steers fed steam-flaked Enogen® corn compared to the control,

but they observed that animals fed the genetically modified grain were less efficient. Objectives of the present study were to evaluate animal performance, and carcass characteristics utilizing two grain types processed by two methods, either steam flaking or dry rolling.

2.3   Materials and Methods:

The procedures used in this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC).

Experimental design

A randomized complete block design with a 2 x 2 factorial arrangement of treatments with twelve replicates was used, with feedlot pen as the experimental unit. Crossbred beef steers obtained from a commercial source (n=960; 427.2 ± 10.76 kg initial body weight) were selected from a population of 1,000 cattle, and blocked by weight within the receiving group. Each block was randomly assigned to one of four treatments with two factors. Factor 1 consisted of grain type, including Enogen corn (EC) or conventional corn (CC). Factor 2 was grain processing method, which included dry-rolled corn (DR) or steam-flaked corn (SF). Cattle were housed in 48 pens, which measured approximately 10.15×30.48 meters (15 cattle/pen; 24 pens) or 10.15 x 45.72 meters (25 cattle/pen; 24 pens). Animals had *ad libitum* access to feed and were fed once daily approximately 11:00 am throughout the study. Bunks were monitored daily to facilitate feed management such that cattle were nominally allowed *ad libitum* access to diets but with a minimum of unconsumed feed remaining in the bunks the following day. Municipal water was available throughout the study from in-pen automatic waterers that were shared between adjacent pens.

Initial processing

Initial processing of steers included a dewormer (Dectomax; Zoetis, Parsippany, NJ) for internal and external parasites; vaccination with Bovishield Gold-5 (Zoetis) and Ultrabac 7 (Zoetis); and administration of probiotic Lactipro (Axiota Animal Health; Fort Collins, CO). Steers were implanted with Component TE-200 (Elanco Animal Health, Greenfield, IN). Cattle were identified with uniquely numbered ear tags and radio frequency identification tags. At the onset of the study, full body weight per pen was determined on day 0 (pretreatment), establishing a baseline measurement for each group of animals.

Finishing diet, formulation, and preparation

Basal diets pre-trial consisted of a mixture of corn silage, steam-flaked corn, and supplements to provide approximately equal proportions of concentrate and roughage. During the trial phase, the forage component of finishing diets consisted of corn silage (14% of diet dry matter) with steam-flaked or dry-rolled corn (78.18% of diet DM). Enogen grain was sourced from the study sponsor and CC was sourced from a producer near Manhattan, KS. Diets were balanced for protein using a combination of urea and soybean meal and contained supplemental vitamins, minerals, and monensin as presented in Table 1. During the final 42 days on feed (DOF), cattle received 300 mg/animal daily of ractopamine hydrochloride (Optaflexx; Elanco Animal Health). Steam-flaked corn was processed daily. Control corn was processed to a bulk density of 360 g/L (28 lb/bu), and Enogen corn was processed to a density of 386 g/L (30 lb/bu). The final moisture content of flaked grains was approximately 20%. Starch availabilities, flaked density, moisture content, and particle size distribution were determined daily on freshly processed material. For the steam flaking process, water was applied to grains before steam conditioning with a moisture applicator (SarTec; Anoka, MN) to obtain a final moisture content

of 20%, for Control and the Enogen corn. Corn was then conditioned for 30 to 45 minutes before processing in a steam-flaker (R & R Machine Works; Dalhart, TX) equipped with 46 cm dia × 91 cm long corrugated rolls (6.3 corrugations/cm). Corn conditioning commenced daily at 05:30 am. Rollers were adjusted for each corn type to achieve the desired bulk density with the goal of achieving similar starch availability for the grain types. Samples of steam-flaked corn for each hybrid were retained at the beginning of the daily processing and analyzed immediately according to standard operating procedures for bulk density, particle size, and starch availability. Particle size was measured using 7 sieves stacked according to sizes (9.5mm > 6.7mm > 4.75mm > 3.35mm > 2.36mm > 1.7mm > 1.18mm and a pan); 200 grams of steam flaked corn were placed on the first sieve and the stack of sieves was placed onto a Ro-Tap shaker (W.S. Tyler, Mentor, OH) for five min, and residues within each sieve were weighed. Starch availability was determined for each corn hybrid by mixing 25 g of the steam-flaked corn, collected 15 minutes after starting the flaking process, with 100 mL of buffered 2.5% amyloglucosidase solution. The mixture was placed in waterbath for 15 minutes at 55 °C [13], the solution was filtered using Whatman hardened ashless filter paper (Whatman International Limited, Kent, England) and several drops were placed on the prism of a handheld refractometer and the percentage of solubles was measured and recorded. The dry matter of each corn hybrid was measured by placing a representative amount in an aluminum pan and placed into a 105 °C forced-air oven for 24 h. Percent of solubles and dry matter values were utilized to determine starch availability as described by Sindt et al. [13]. Dry-rolled corn was processed approximately weekly to achieve similar particle sizes for Enogen and control grain. Corn was processed at the O.H. Kruse Feed Technology Center weekly. Dry-rolled corn was delivered to the research center twice weekly and stored in a covered commodity storage barn. Amounts of rations delivered each day were

based on a visual assessment of bunks each morning. All ingredients for each diet were mixed in a Roto-Mix feed truck (ROTO-MIX, Dodge City, KS), and then delivered to the corresponding feed bunks beginning at approximately 0800 h each day.

Final body weights and harvesting

Cattle were weighed twice during the study, first at the beginning the day prior to receiving the finishing diet, and finally the night prior to sending the steers to harvest. Cattle were divided into two groups of 24 pens (based on initial weight and block) and were harvested one week apart. Blocks four, five, and six were sent after 155 DOF and the remaining 24 pens were sent the week after for a total of 162 DOF. On the last day of the study, the weight of each pen of animals was determined using a group scale, and cattle were loaded onto trucks and transported 450 km to a commercial abattoir in Holcomb, Kansas. Once at the plant, all cattle were unloaded and placed into pens where they were sprayed with water to facilitate cooling, subjected to an ante-mortem inspection, and presented for slaughter after a lairage of approximately 6 h. Ear tag numbers and EID numbers were recorded to establish order of harvest. Carcasses were identified with unique tag numbers after hide removal. With the visible tags on the carcasses, livers were scored. After 48 h in the cooler, carcasses were graded using a camera imaging system (VBG 2000; E+V Technology GmbH & Co. KG, Oranienburg, Germany), and other data such as hot carcass weight (HCW), ribeye area (REA), backfat, quality grade, and kidney, pelvic and heart fat percentage (KPH).

Liver scores followed the Elanco scoring system (0 = healthy; A− = one or two small abscesses; A = two to four well-defined abscesses; A+ = more than four abscesses active and large; Elanco, USA). Marbling scores followed USDA scoring system (200 to 299 = Trace; 300 to 399 = Slight; 400 to 499 = Small; 500 to 599 = Modest; 600 to 699 = Moderate; 700 to 799 = Slightly

Abundant; 800 to 899 = Moderately Abundant; USDA). Yield grades were calculated with a formula according to USDA ($Yield\ grade\ (YG) = 2.50 + (2.5 \times adjusted\ fat\ thickness) + (0.2 \times KPH\ fat\ percentage) + (0.0038 \times HCW) - (0.32 \times REA)$; USDA).

Statistical analyses

All data were analyzed using the GLIMMIX procedure of the Statistical Analysis System (SAS version 9.4; SAS Inst. Inc., Cary, NC). Fixed effects included treatment (corn hybrid type, corn processing, and their interaction). Experimental unit was feedlot pen and block was the random effect. Statistical significance was declared at $P \leq 0.05$ and tendencies for an effect if $0.05 < P < 0.10$. Average daily gain was calculated using initial and final body weights, divided by days on feed. Dry matter intake was calculated using the percentage of the diet ingredients as-fed and the dry matter was corrected with the weekly analyses conducted across the study for these ingredients. Gain:feed was computed as ADG/DMI.

2.4   Results & Discussion

Diet composition and nutrient analyses are presented in Table 1. Analysis of monthly composites of the ingredients showed fluctuations in the nutrient composition, mainly corn; therefore, some minor differences can be observed among treatments. Daily steam-flaked corn samples for analyses (starch availability, particle size and bulk density), showed that while both corn hybrids were flaked to different densities to achieve similar starch availability, EC was higher (*P*<0.01). Geometric particle size means, and standard deviations are presented in Table 2.

Animal performance

Results of this experiment are presented in Table 3. The interactions between corn processing by grain type did not affect DMI (*P*=0.269) across treatments. An effect for the interaction for cattle

fed steam-flaked CC and steam-flaked EC was observed exhibiting greater final BW ($P<0.01$) compared to dry-rolled corn diets. Interestingly, no differences were observed when EC was steam-flaked or dry-rolled. Steers fed steam-flaked CC did not differ from those receiving steam-flaked EC, but they had heavier final body weights compared to dry-rolled CC, and dry-rolled EC. Steam-flaked EC was not different from dry-rolled EC ($P>0.05$). Average daily gain ($P=0.01$) was greater for steers fed steam-flaked diets (EC or CC) and dry-rolled EC compared to animals fed dry-rolled CC. Similarly, G:F ($P<0.01$) was greater for cattle fed steam-flaked corn (EC or CC) and dry-rolled EC compared to those fed dry-rolled CC diets. When comparing steam-flaked diets (EC or CC) no differences were observed for G:F, which aligns with results reported by Jolly-Breithaupt et al. [14]. The findings of the current study align with previous research by Theurer et al. [15], which reported an increase in ADG and gain efficiency for SFC compared to DRC. This can also be explained by the fact that steam-flaked corn has greater starch digestibility in the rumen and total tract compared to dry-rolled corn [15]. Dry-rolled EC was not different compared to the steam-flaked diets, but outperformed CC when dry-rolled, as demonstrated by Zerby et al.[16] cattle fed dry-whole corn with exogenous alpha-amylase improved gain efficiency compared to the control. Alpha-amylase is an enzyme that breaks down starch to simpler sugars for digestion, while Enogen corn expressing high concentrations of this enzyme had an advantage over dry-rolled CC. In contrast, DiLorenzo et al.[17] conducted an experiment comparing steam-flaked corn with dry-rolled corn with or without inclusion of exogenous amylase, where no difference in DMI, ADG and G:F were observed for the interaction of grain processing by amylase supplementation. Contrary to the results presented in this study, Miller et al.[18] reported an increase in DMI for finishing beef steers fed dry-rolled Enogen corn compared to the control, but no improvements were reported for ADG or G:F.

There were no differences between steam-flaked control and steam-flaked Enogen corn ($P>0.05$) across this study. As demonstrated through previous research by Corona et al. [19], diets containing SFC, when compared to DRC, exhibited greater ruminal, post-ruminal, and total tract starch digestion in finishing steers.

Effects of processing were evident insofar as steers fed steam-flaked corn exhibited greater ADG ($P < 0.01$), and heavier FBW ($P < 0.01$) compared to those fed dry-rolled corn. This can be explained by the fact that steam-flaking increases energy value of grains [20,21]. By processing corn through this method, producers enhance its digestibility through increased starch digestibility, which improves cattle performance [22–24]. Researchers demonstrated in two trials that steam-flaking reduces feed intake and improves feed conversion [25]. Furthermore, Harrelson et al.[26] reported a 24% improvement in ruminal starch digestibility with SFC compared to DRC. Similarly, Plascencia et al.[21] found that steam-flaking corn increased ruminal, postruminal and apparent total tract starch digestion. Contrary to the findings of the present study where the main effect of processing showed that steam-flaked corn performed better than dry-rolled corn, González-Vizcarra et al. [27] substituted steam-flaked corn for dry-rolled corn and concluded that it enhances ADG, gain efficiency, and dietary net energy.

An effect of grain type was observed for G:F ($P = 0.03$), as shown in Table 3. Cattle fed Enogen corn had greater G:F independent of processing method compared to CC. This result aligns with findings of previous researchers [7] comparing Enogen to conventional corn, demonstrating improved gain efficiency. Horton et al. [10], reported that heifers fed high-amylase corn exhibited improved feed efficiency and carcass quality compared to the control. Similarly, Jolly-Breithaupt et al. [28] compared a control diet containing conventional corn, a diet containing Enogen corn, and a diet combining conventional corn with a commercial α-amylase enzyme

supplement. They observed that cattle fed Enogen corn or the conventional corn combined with α-amylase enzyme supplement had improved ADG and feed efficiency compared to animals fed the control diet. Johnson et al.[8], by feeding growing steers either EC or control processed by dry-rolled or whole-shelled, observed a 5.5% improvement in feed efficiency for cattle-fed EC compared to those fed conventional corn. The current study showed a tendency ($P = 0.09$) of EC to have greater ADG compared to CC fed steers, contrary to findings reported by Jolly-Breithaupt et al. [28]. Through two experiments Glaser et al. [29], showed in the first experiment that EC improved feed efficiency, showing a 5.5% improvement compared to the control. The second experiment used cannulated steers to evaluate intake and digestibility and the authors observed greater dry matter and organic matter digestibility for the EC fed group, as well as faster fermentation for EC-fed animals. On the other hand, Scilacci et al.[30] did not find differences when replacing conventional corn with Enogen corn.

Carcass characteristics

Going back to the cattle performance results (Table 3), while no difference was observed for the initial weight among treatments, cattle fed CC specifically when steam-flaked tended ($P=0.06$) to have heavier initial weight. For the current study an approximately 7 kg difference was observed for the steers fed steam-flaked CC, this advantage when starting the experiment impacted in the final body weight and later HCW.

Interaction between corn processing by grain type was observed for HCW, cattle fed steam-flaked corn, either CC or EC, had heavier HCW ($P<0.01$) compared to the other treatments. As mentioned before, and concluded by Corona et al.[31], steam-flaked corn improves carcass yield. This is in contrast with Huck et al.[32], and Baker et al.[12], who did not observe differences in carcass characteristics. Given the difference in initial weight impacting on the HCW we assumed

a common dressing percentage (DP) among treatments set at 50%, the estimated HCW gain

would be 289, 297, 305 and 301 for dry-rolled CC, dry-rolled EC, steam-flaked CC and steam-

flaked EC respectively. By computing these assumptions and values statistically analyzed, we

would observe an interaction effect for HCW gain ($P<0.01$; SEM:3.2), where dry-rolled CC

treatment would present the lightest carcasses, steam-flaked CC would present the heavier

carcasses but not different from steam-flaked EC, and dry-rolled EC intermediate but not

different from EC when steam-flaked. No differences were observed for REA ($P = 0.249$), KPH

($P = 0.719$), liver abscesses ($P = 0.777$), quality grades or yield grades 1, 2, 3, 4 ($P>0.05$). A

tendency for interaction was observed for cattle fed steam-flaked diets (EC or CC) and dry-rolled

EC to have greater marbling scores ($P=0.079$) compared to those fed dry-rolled CC. Similarly,

backfat deposition tended ($P=0.088$) to be greater for steers fed steam-flaked diets (EC or CC)

and dry-rolled EC, as Wertz et al.[33] showed a linear relationship between backfat and marbling

score. Interestingly, when comparing results between DRC treatments, these were different for

the items described before, EC when dry-rolled outperformed dry-rolled CC. Conversely,

Brinton et al.[34] observed no differences between dry-rolled EC and CC, concluding no benefit

of EC dry-rolled over the control. Approximately 80% of the carcasses among each treatment

graded Choice, and by breaking it down, carcasses corresponding to treatments including steam-

flaked corn (EC or CC), and dry-rolled EC tended ($P=0.098$) to have more carcasses grading

premium Choice, but no differences were observed among treatments for low Choice ($P=0.289$).

Approximately 55% of the carcasses graded USDA yield grade 3 for each treatment, but

treatments did not differ ($P=0.638$). An interaction effect was observed for number of carcasses

categorized Yield Grade 5 for each ($P=0.04$), where steers fed dry-rolled EC, did not grade in

this group compared to the other treatments. Around 14% of livers corresponding to each

treatment presented abscesses, although no differences (P=0.777) were observed among treatments.

Effects of processing were observed for HCW, REA and backfat. Steers fed with steam-flaked corn, exhibited heavier carcasses (P<0.01), a greater rib eye area (P=0.02), and more backfat deposition (P=0.01) compared to those fed dry-rolled corn.

Cost of gain to target weight

Due to the difference in the initial weight we decided to compare the treatments to the same target gain set at 215.5 kg (approximately the weight gained by steers fed steam-flaked CC). Results are presented in Table 5. We assumed that the approximately 7 kg initial weight advantage in one treatment group did not affect DMI and ADG; therefore, the raw values for each treatment were utilized. Feed cost of each ration was calculated on a daily basis per steer accounting for the grain, processing method, silage, soybean meal, and supplement. Days on feed were calculated by dividing target gain by ADG, where fewer DOF were observed for cattle fed steam-flaked CC compared to other treatments. Furthermore, the feed cost per animal to the target gain was calculated, and finally, the cost to produce one kilogram of gain. The analysis of cost of gain revealed that, while animals fed dry-rolled EC were estimated to be 5 days more compared to those fed steam-flaked CC, and no differences in DMI or ADG were observed between this treatment group and steam-flaked EC or CC, the cost to produce one kilogram was less compared to the other treatments. When comparing the cost of gain for each treatment with the group of steam-flaked CC, dry-rolled CC showed 0.05 dollars more. Treatment of steam-flaked EC while DMI, ADG was not different from steam-flaked CC, a 0.01-dollar difference was observed. Finally, Enogen corn when dry-rolled exhibited 0.07 dollars less to produce one kilogram compared to steam-flaked CC.

2.5　Conclusion

This study highlights the significant impact of corn hybrid and processing choices on feedlot cattle performance. Our results suggest that Enogen corn, with its higher alpha-amylase content, offers a promising approach for improving feed efficiency and potentially boosting growth rates. While steam flaking may offer some advantages, dry-rolled Enogen corn performed comparably to steam-flaked Enogen corn, suggesting that dry-rolled Enogen may be a viable alternative to steam flaking without capital expenditures necessary for instalation of steam-flaking equipment. While various studies have been conducted comparing dry-rolled with steam-flaked corn, our results suggest that future research should focus on evaluating the economic implications of Enogen corn processed through different methods, particularly when compared to other grain types in terms of costs and benefits.

Literature cited

1. Oliveira, C. A.; Millen, D. D. Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. *Anim. Feed Sci. Technol.* **2014**, *197*, 64–75. https://doi.org/10.1016/j.anifeedsci.2014.08.010.

2. Samuelson, K. L.; Hubbert, M. E.; Galyean, M. L.; Löest, C. A. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey1. *J. Anim. Sci.* **2016**, *94* (6), 2648–2663. https://doi.org/10.2527/jas.2016-0282.

3. Huntington, G. B. Starch utilization by ruminants: from basics to the bunk. *J. Anim. Sci.* **1997**, *75* (3), 852–867. https://doi.org/10.2527/1997.753852x.

4. Hale, W. H. Influence of processing on the utilization of grains (starch) by ruminants. *J. Anim. Sci.* **1973**, *37* (4), 1075–1080. https://doi.org/10.2527/jas1973.3741075x.

5. Owens, F. N.; Secrist, D. S.; Hill, W. J.; Gill, D. R. Acidosis in cattle: a review. *J. Anim. Sci.* **1998**, *76* (1), 275–286. https://doi.org/10.2527/1998.761275x.

6. *Syngenta | United States*. https://www.syngenta-us.com/ (accessed 2024-07-16).

7. Volk, S. M.; Wilson, H. C.; Hanford, K. J.; MacDonald, J. C.; Erickson, G. E. Impact of feeding Syngenta Enogen® feed corn compared to control corn in different diet scenarios to finishing beef cattle. *Animals* **2021**, *11* (10), 2940. https://doi.org/10.3390/ani11102940.

8. Johnson, M.; Spore, T.; Montgomery, S.; Weibert, C.; Garzon, J.; Hollenbeck, W.; Wahl, R.; Watson, E.; Blasi, D. Syngenta enhanced feed corn (Enogen) containing an alpha amylase expression trait improves feed efficiency in growing calf diets. *Kans. Agric. Exp. Stn. Res. Rep.* **2018**, *4* (1). https://doi.org/10.4148/2378-5977.7543.

9. Cueva, S. F.; Stefenoni, H.; Melgar, A.; Räisänen, S. E.; Lage, C. F. A.; Wasson, D. E.; Fetter, M. E.; Pelaez, A. M.; Roth, G. W.; Hristov, A. N. Lactational performance, rumen fermentation, and enteric methane emission of dairy cows fed an amylase-enabled corn silage. *J. Dairy Sci.* **2021**, *104* (9), 9827–9841. https://doi.org/10.3168/jds.2021-20251.

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

11. Schoonmaker, J. P.; Persia, M. E.; Beitz, D. C. Effect of feeding corn modified to contain a unique amylase on performance and carcass characteristics of feedlot steers. *Prof. Anim. Sci.* **2014**, *30* (5), 561–565. https://doi.org/10.15232/pas.2014-01322.

12. Baker, A.; Veloso, V. de A.; Baker, A.; Barros, L. 343 Feedlot performance and carcass characteristics of steers fed diets containing steam-flaked grain and corn silage from Enogen® Feed Corn. *J. Anim. Sci.* **2019**, *97* (Supplement_2), 137. https://doi.org/10.1093/jas/skz122.243.

13. Sindt, J. J.; Drouillard, J. S.; Titgemeyer, E. C.; Montgomery, S. P.; Loe, E. R.; Depenbusch, B. E.; Walz, P. H. Influence of steam-flaked corn moisture level and density on the site and extent of digestibility and feeding value for finishing cattle. *J. Anim. Sci.* **2006**, *84* (2), 424–432. https://doi.org/10.2527/2006.842424x.

14. Jolly-Breithaupt, M. L.; Bittner, C. J.; Hilscher, F. H. H.; Erickson, G. E.; MacDonald, J. C.; Luebbe, M. K. Impact of Syngenta Enogen feed corn on finishing cattle performance and carcass characteristics. *Neb. Beef Cattle Rep.* **2018**.

15. Theurer, C. B.; Lozano, O.; Alio, A.; Delgado-Elorduy, A.; Sadik, M.; Huber, J. T.; Zinn, R. A. Steam-processed corn and sorghum grain flaked at different densities alter ruminal, small

intestinal, and total tract digestibility of starch by steers. *J. Anim. Sci.* **1999**, *77* (10), 2824–2831. https://doi.org/10.2527/1999.77102824x.

16. Zerby, H. N.; Bard, J. L.; Loerch, S. C.; Kuber, P. S.; Radunz, A. E.; Fluharty, F. L. Effects of diet and Aspergillus oryzae extract or Saccharomyces cervisiae on growth and carcass characteristics of lambs and steers fed to meet requirements of natural markets. *J. Anim. Sci.* **2011**, *89* (7), 2257–2264. https://doi.org/10.2527/jas.2010-3308.

17. DiLorenzo, N.; Smith, D. R.; Quinn, M. J.; May, M. L.; Ponce, C. H.; Steinberg, W.; Engstrom, M. A.; Galyean, M. L. Effects of grain processing and supplementation with exogenous amylase on nutrient digestibility in feedlot diets. *Livest. Sci.* **2011**, *137* (1), 178–184. https://doi.org/10.1016/j.livsci.2010.11.003.

18. Miller, J. L.; Wilke, K.; Erickson, G. E.; Loza, P. L. 163 Effect of Enogen feed corn inclusion in conventional and natural finishing cattle diets. *J. Anim. Sci.* **2023**, *101* (Supplement_2), 208–209. https://doi.org/10.1093/jas/skad341.231.

19. Corona, L.; Owens, F. N.; Zinn, R. A. Impact of corn vitreousness and processing on site and extent of digestion by feedlot cattle. *J. Anim. Sci.* **2006**, *84* (11), 3020–3031. https://doi.org/10.2527/jas.2005-603.

20. Trotta, R. J.; Kreikemeier, K. K.; Royle, R. F.; Milton, T.; Harmon, D. L. Corn processing, flake density, and starch retrogradation influence ruminal solubility of starch, fiber, protein, and minerals. *J. Anim. Sci.* **2022**, *100* (6), skac149. https://doi.org/10.1093/jas/skac149.

21. Plascencia, A.; Bermúdez, R. M.; Cervantes, M.; Corona, L.; Dávila-Ramos, H.; López-Soto, M. A.; May, D.; Torrentera, N. G.; Zinn, R. A. Influence of processing method on comparative digestion of white corn versus conventional steam-flaked yellow dent corn in

finishing diets for feedlot cattle. *J. Anim. Sci.* **2011**, *89* (1), 136–141. https://doi.org/10.2527/jas.2010-3116.

22. Zinn, R. A.; Owens, F. N.; Ware, R. A. Flaking corn: processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. *J. Anim. Sci.* **2002**, *80* (5), 1145–1156. https://doi.org/10.2527/2002.8051145x.

23. Franco-Hernández, M. A.; Corona, L.; Plascencia, A.; Avery Zinn, R. Practical parameters for assessing starch digestion and feeding value of steam-flaked corn in finishing diets for feedlot cattle. *J. Appl. Anim. Res.* **2023**, *51* (1), 24–30. https://doi.org/10.1080/09712119.2022.2149537.

24. Barajas, R.; Zinn, R. A. The feeding value of dry-rolled and steam-flaked corn in finishing diets for feedlot cattle: influence of protein supplementation. *J. Anim. Sci.* **1998**, *76* (7), 1744–1752. https://doi.org/10.2527/1998.7671744x.

25. Zinn, R. A. Influence of lasalocid and monensin plus tylosin on comparative feeding value of steam-flaked versus dry-rolled corn in diets for feedlot cattle. *J. Anim. Sci.* **1987**, *65* (1), 256–266. https://doi.org/10.2527/jas1987.651256x.

26. Harrelson, F. W.; Erickson, G. E.; Klopfenstein, T. J.; Jackson, D. S.; Clark, P. M.; Fithian, W. A. Influence of corn hybrid, kernel traits, location, and dry rolling or steam flaking on ruminal digestibility in beef cattle. *Appl. Anim. Sci.* **2019**, *35* (1), 8–19. https://doi.org/10.15232/aas.2018-01778.

27. González-Vizcarra, V.; Plascencia, A.; Ramos-Aviña, D.; Zinn, R. Influence of substituting steam-flaked corn for dry rolled corn on feedlot cattle growth performance when cattle are allowed either ad libitum or restricted access to the finishing diet. *Asian-Australas. J. Anim. Sci.* **2017**, *30*, 1563–1567. https://doi.org/10.5713/ajas.17.0185.

28. Jolly-Breithaupt, M. L.; Harris, M. E.; Nuttelman, B. L.; Burken, D. B.; MacDonald, J. C.; Luebbe, M. K.; Iragavarapu, T. K.; Erickson, G. E. Effects of Syngenta Enogen feed corn containing an α-amylase trait on finishing cattle performance and carcass characteristics. *Transl. Anim. Sci.* **2019**, *3* (1), 504–512. https://doi.org/10.1093/tas/txy121.

29. Glaser, M. A.; Montgomery, S. P.; Vahl, C. I.; Titgemeyer, E. C.; Kubick, C. S.; Glaser, G. I.; Spore, T. J.; Hollenbeck, W. R.; Wahl, R. A.; Blasi, D. A. Effects of feeding corn containing an alpha-amylase gene on the performance and digestibility of growing cattle. *Transl. Anim. Sci.* **2022**, *6* (1), txac013. https://doi.org/10.1093/tas/txac013.

30. Scilacci, M.; Johnson, M.; Titgemeyer, E.; Montgomery, S.; Tarpoff, A.; Watson, E.; Hollenbeck, W.; Blasi, D. Syngenta Enogen corn fed as corn grain and corn silage in diets containing corn coproducts did not enhance growth performance of growing heifers. *Kans. Agric. Exp. Stn. Res. Rep.* **2022**, *8* (1). https://doi.org/10.4148/2378-5977.8228.

31. Corona, L.; Rodriguez, S.; Ware, R. A.; Zinn, R. A. Comparative effects of whole, ground, dry-rolled, and steam-flaked corn on digestion and growth performance in feedlot cattle. *Prof. Anim. Sci.* **2005**, *21* (3), 200–206. https://doi.org/10.15232/S1080-7446(15)31203-1.

32. Huck, G. L.; Kreikemeier, K. K.; Kuhl, G. L.; Eck, T. P.; Bolsen, K. K. Effects of feeding combinations of steam-flaked grain sorghum and steam-flaked, high-moisture, or dry-rolled corn on growth performance and carcass characteristics in feedlot cattle. *J. Anim. Sci.* **1998**, *76* (12), 2984–2990. https://doi.org/10.2527/1998.76122984x.

33. Wertz, A. E.; Berger, L. L.; Walker, P. M.; Faulkner, D. B.; McKeith, F. K.; Rodriguez-Zas, S. L. Early-weaning and postweaning nutritional management affect feedlot performance, carcass merit, and the relationship of 12th-rib fat, marbling score, and feed efficiency among

Angus and Wagyu heifers. *J. Anim. Sci.* **2002**, *80* (1), 28–37.

https://doi.org/10.2527/2002.80128x.

34. Brinton, M.; Boyd, B.; Hilscher, F.; McPhillips, L.; MacDonald, J.; Erickson, G. E.

    Evaluating Syngenta Enogen feed corn silage or grain on growing beef cattle performance.

    Neb. Beef Cattle Rep. **2020**.

Table 1. Composition of experimental diets (dry matter basis)

Item[+]	Dry-rolled corn		Steam-flaked corn	
	Control	Enogen	Control	Enogen
Corn hybrid				
No.2 yellow corn	78.18	-	78.18	-
Enogen corn	-	78.18	-	78.18
Corn silage	14	14	14	14
Dehulled soybean meal, 47.5%	4	4	4	4
Supplement[1]	3.82	3.82	3.82	3.82
Analyzed composition[2], %DM				
CP	13.6	13.7	14.0	14.2
NPN	3.5	3.5	3.7	3.5
NDF	13.7	14.8	14.5	14.4
Salt	0.28	0.27	0.29	0.28
Fat	2.9	3.3	3.6	3.3
Ca	0.66	0.65	0.69	0.67
K	0.64	0.65	0.67	0.69

[+]CP: crude protein; NPN: non-protein nitrogen; NDF: neutral detergent fiber; Ca: calcium; K: potassium.

[1]Consisted of urea, salt, limestone, trace mineral premix, vitamin premix, and KCl to provide (on a total diet DM basis): 0.10 mg/kg cobalt, 10 mg/kg copper, 0.50 mg/kg iodine, 40 mg/kg manganese, 0.10 mg/kg selenium, 40 mg/kg zinc, 2205 IU/kg vitamin A, 22 IU/kg vitamin E, and 33.3 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN).

[2]Analyzed nutrient composition of ingredients in total diet.

*Optaflexx (Elanco Animal Health, Greenfield, IN) was fed at 300 mg ractopamine–HCl per steer daily for the final 42 days on feed.

Table 2. Starch Availability, bulk density, and particle size distribution

Item	Grain type		SEM	P-value
	Control	Enogen		
Steam-flaked corn				
Starch availability[#], %	50.50	56.70	0.4155	<0.01
Bulk density, g/L	360	386	0	<0.01
Dg, mm$^\nabla$	6.79	5.91	0.057	<0.01
Sg, mm$^\phi$	1.64	1.72	0.010	<0.01
Dry-rolled corn				
Dg, mm$^\nabla$	2.62	2.56	0.001	<0.01
Sg, mm$^\phi$	1.57	1.59	0.006	0.04

$^\nabla Dg$ = the geometric mean diameter calculated using the log-transformed particle size data.

$^\phi Sg$ = geometric standard deviation.

*Steam-flaked Con=107 samples; Steam-flaked Eno=101 samples; Dry-rolled Con=22 samples; Dry-rolled Eno=22 samples.

[#]Starch availability was analyzed with an enzymatic method as described by Sindt et al. [13].

$^\Psi$Particle size was analyzed using a series of sieves on a Ro-tap (W.S.Tyler, Mentor, OH) shaker (steam-flaked: 9.5, 6.7, 4.75, 3.35, 2.36, 1.70, and 1.18 mm sieves; dry-rolled: 6.7, 4.75, 3.35, 2.36, 1.70, 1.18, and 0.85 mm sieves).

Table 3. Feedlot performance of animals fed dry-rolled and steam-flaked grains.

Item*	Dry-rolled corn		Steam-flaked corn		SEM	Probability values		
	Control corn	Enogen corn	Control corn	Enogen corn		Grain	Processing	Interaction
Initial weight, kg	426.5	424.9	432.3	425.2	10.518	0.069	0.1962	0.233
Final weight, kg	616.8[c]	629.7[b]	644.9[a]	635.1[ab]	6.9627	0.669	<0.01	<0.01
DMI, kg/d	10.18	9.93	10.07	10.12	0.16	0.447	0.764	0.269
ADG, kg	1.36[b]	1.46[a]	1.51[a]	1.49[a]	0.0345	0.093	<0.01	0.012
Gain:feed	0.1342[b]	0.1471[a]	0.1500[a]	0.1474[a]	0.00429	0.038	<0.01	<0.01
Feed:gain	7.452[b]	6.798[a]	6.667[a]	6.784[a]	0.20544	0.038	<0.01	<0.01

* Means within a row without a common superscript letter are different (P<0.05). DMI: dry matter intake; ADG: average daily gain.

Table 4. Carcass characteristics of steers fed dry-rolled and steam-flaked grains

Item*	Rolled corn		Flaked corn		SEM	Probability values		
	Control corn	Enogen corn	Control corn	Enogen corn		Grain	Processing	Interaction
Hot carcass weight, kg	385.5 [c]	393.5 [b]	403.0 [a]	396.9 [ab]	4.35	0.669	<0.01	<0.01
Ribeye area, cm^2	82.2	83.0	84.4	83.8	0.70	0.864	0.023	0.249
Marbling score[‡]	522	540	546	534	8.1	0.701	0.27	0.079
Backfat, cm	1.34	1.43	1.48	1.45	0.039	0.356	0.014	0.088
KPH, %	1.87	1.89	1.88	1.89	0.012	0.379	0.837	0.719
Quality grade								
Prime, %	7.3	10.1	10.3	8.2	2.21	0.881	0.829	0.278
Premium Choice, %[§]	44.1	53.2	52.7	50.8	3.35	0.271	0.347	0.098
Low Choice, %	35.8	30.7	30.3	32.1	3.17	0.600	0.533	0.289
Select, %	7.9	5.5	5.2	7.6	1.85	0.983	0.868	0.165
Others, %[Δ]	4.9	0.5	1.5	1.3	0.46	0.016	0.539	0.176
USDA Yield Grade 1	1.8	0.7	0.9	1.6	0.70	0.748	0.982	0.183
USDA Yield Grade 2	22.3	20.2	15.7	16.3	2.84	0.797	0.071	0.623
USDA Yield Grade 3	58.5	54	56.3	55	3.29	0.382	0.857	0.638
USDA Yield Grade 4	16.4	25.1	26.7	25.4	3.62	0.288	0.132	0.148
USDA Yield Grade 5	1.0 [ab]	0.0 [b]	0.4 [ab]	1.7 [a]	0.58	0.77	0.362	0.043
Abscessed livers, %	14.6	13.1	14.1	14.1	2.91	0.759	0.915	0.777

*Means within a row without a common superscript letter are different (P<0.05). KPH: kidney, pelvic and heart fat.

[#]Carcasses were graded using a camera imaging system (VBG 2000; E+V Technology GmbH & Co. KG, Oranienburg, Germany).

[∇]Liver scores followed the Elanco scoring system (0 = healthy; A− = one or two small abscesses; A = two to four well-defined abscesses; A+ = more than four abscesses active and large; Elanco, USA), results represent the percentage of abscessed livers.

[‡]Marbling score of 200 to 299 = Trace; 300 to 399 = Slight; 400 to 499 = Small; 500 to 599 = Modest; 600 to 699 = Moderate; 700 to 799 = Slightly Abundant; 800 to 899 = Moderately Abundant.

[§]Premium Choice and Low Choice percentages are based on marbling scores only.

[Δ]Others = Not graded; dark cutter; mature carcasses; blood splash.

Table 5. Cost of gain to a target 215.5 kg of gain

Item	Dry-rolled		Steam-flaked	
	Control	Enogen	Control	Enogen
Dry matter intake, kg/d	10.18	9.93	10.07	10.12
Feed cost, $/head daily including processing[1]	1.78	1.73	1.90	1.89
Average daily gain, kg	1.36	1.46	1.51	1.49
Days on feed to achieve target gain (215.5 kg)	158	148	143	145
Feed cost, $/head to target gain	281.3	256.7	271.2	273.9
Cost of gain, $/kg	1.31	1.19	1.26	1.27
Cost of gain vs flaked control, $/kg	0.05	-0.07	---	0.01

[1]Feed costs using corn grain price of $0.17/kg at 86% DM; Corn silage $0.13/kg DM; Dehulled soybean meal $0.37/kg DM; Supplement $0.59/kg DM

Chapter 3 - In vitro ruminal fermentation of corn: Effects of processing, and grain type.

3.1   Abstract

The influence of processing methods and hybrid variations on corn's nutritional impact within ruminant diets remains a key area of study. The objective of this study was to evaluate Enogen corn, a corn genetically modified for elevated expression of heat-stable amylase, processed either through steam flaking (SF) or dry rolling (DR) using *in vitro* digestion methods. Two *in vitro* experiments were conducted with a 2 x 2 factorial design to evaluate gas production, dry matter disappearance, VFA profiles, and methane production by cultures of mixed ruminal microbes with corn as substrate. This allowed for a detailed examination of the synergistic effects of grain processing and grain type on microbial fermentation. Factor 1 consisted of grain type: conventional corn (CC) or Enogen corn (EC). Factor 2 consisted of grain processing method: SF or DR. For the first experiment, ruminal fluid was collected from a donor animal fed a high-concentrate diettypical of feedlot production, and for the second experiment ruminal fluid was obtained from a donor animal fed a mixed forage-concentrate diet more typical of a lactating dairy animal. All substrates were ground, weighed into individual Pyrex® bottles, and mixed with buffered ruminal fluid. An Ankom RF gas production module was utilized to seal the top of the bottles, which were placed into an incubator for 24 h. Results from the first experiment indicated that steam-flaked EC or CC presented lower gas production ($P=0.043$). Enogen corn when steam flaked had the lowest $t_{1/2}$ ($P=0.027$). Conventional corn when steam-flaked had the lowest acetate to propionate ratio ($P<0.01$). Volatile fatty acid production was not affected by the interaction, neither was methane yield ($P=0.596$; mL of methane produced per g of DM digested). The results of the second experiment showed faster $t_{1/2}$ ($P=0.04$) for cultures containing steam-flaked CC or EC compared to other treatments. Steam-flaked CC had the fastest rate of gas production ($P<0.01$) compared to other cultures. Overall, we observed that steam-flaking corn reduced lag times and increased rate of digestion compared to dry rolling. Moreover, Enogen grain, when dry rolled, was very similar to steam-flaked grain,

Keywords: in vitro fermentation, enogen, steam-flaking, dry-rolling, gas production.

3.2   Introduction

Corn remains a cornerstone of ruminant diets worldwide. It is the most important source of energy for feedlot cattle in the United States and it is prized for its energy density and broad applicability within diverse feed formulations. In 2023, U.S. corn reached a production record of 388.6 million tonnes and with it increased the production by 12% compared to the year 2022, according to the crop production summary 2023 [1]. The nutritional value of corn is influenced by processing methods and hybrid characteristics, which modulate starch accessibility and ruminal fermentation dynamics [2–4]. Traditional processing techniques, such as dry rolling (DRC) and steam flaking (SFC), have long been employed to enhance starch digestibility [5,6]. The development of genetically engineered grains, such as Enogen® corn (EC), which expresses high concentration of heat-stable alpha-amylase content in the corn kernel, can improve starch utilization. The extent to which this trait impacts starch utilization may be impacted by the method of grain processing that is employed.

Recent studies have highlighted the relationship between ruminal fermentation, methane emissions, production of volatile fatty acids, and feed [7–9]. While SFC is known to accelerate starch degradation and VFA production compared to DRC, the interaction between processing methods and corn hybrids, such as EC, is poorly understood [10].

Our objective in this study was to evaluate *in vitro* fermentation of two types of corn (conventional or Enogen) processed either through dry rolling or steam flaking, using a 2×2 factorial design. Measurements included gas production parameters, VFA profiles, and dry matter disappearance to better understand the interactive effects of grain processing and grain type on microbial fermentation.

3.3   Materials and Methods

All procedures were reviewed and approved by the Kansas State University IACUC committee before initiation of the study.

Experimental design

Two randomized complete block design experiments with four replicates in a 2×2 factorial arrangement were utilized to compare *in vitro* gas production, volatile fatty acid (VFA) concentrations, and *in vitro* dry matter disappearance (IVDMD). Factor 1 consisted of grain processing, including steam flaking or dry rolling (SF vs. DR). Factor 2 considered the grain type, including a conventional grain of unknown hybrid as the control and Enogen corn (CON vs. EN). Experiment 1 used ruminal fluid from a grain-fed donor animal, while experiment 2 used ruminal fluid from an animal fed approximately equal proportion of forage and concentrate.

Substrate

All substrates, which include two corn grains (DR or SF), were ground prior to the start of the experiment using a Wiley mill (Thomas Scientific, PA) equipped with a 1-mm screen. Samples were frozen before grinding, and for the grinding process samples were mixed with dry ice. Ground samples (1.5 grams of dry matter) were weighed into individual Pyrex® bottles and treatment diets were established as 85% corn and 15% corn silage. On the day of the experiment, Pyrex® bottles were prewarmed in an incubator to a temperature of 39°C.

In Vitro Gas Production

The McDougall's buffer [11], was prepared and mixed 4:1 with ruminal fluid (RF). Additionally, 0.6 g/L of tryptic soy broth was added to the buffer to provide cultures with a source of degradable protein.

Ruminal fluid was collected on the day of the experiment, prior to feeding donor animals and approximately one hour prior to start of the *in vitro* experiment. For the first experiment, fluid was obtained from a cannulated cow fed a diet consisting of 13% grass hay, 40% rolled corn, 40% wet corn gluten feed, and the balance as a protein, vitamin, mineral and feed additive (monensin) premix. For the second experiment, ruminal fluid was collected from a Holstein dairy cow fed a diet consisting of 20% wet corn gluten feed, 28% corn silage, 5% whole cottonseed, 13% alfalfa, and 34% of a grain-based concentrate mix. Ruminal contents were collected the morning prior to feeding and filtered through cheesecloth to separate large particles from the fluid phase. Once the collection was completed, ruminal fluid was transferred to the laboratory, re-filtered, placed into a separatory funnel, and sparged with nitrogen gas. After approximately 30 to 40 min, ruminal fluid stratified into three distinct layers. The bottom sediment layer, which consisted of protozoa and spent feed particles, was discarded, and the bacteria-rich middle layer was separated from the mat layer and used as the microbial inoculant. Ruminal fluid was combined with buffer in a 1:4 ratio to create a final buffered ruminal fluid (BRF).

To accomplish this experiment, the Ankom RF gas production system (Ankom Technology, Macedon, NY) was used. Ankom modules were programmed to record gas measurements every 15 min.

For the incubation, 150 mL of BRF was dispensed into 250-mL Pyrex® bottles and blanketed with nitrogen gas, aiming to displace the oxygen from inside the bottles and maintain an oxygen-free environment. Ankom RF gas production modules were used to seal the top of each bottle. Culture bottles were placed into an orbital shaker (model 4535; Forma Scientific Inc., Marietta, OH), with a constant temperature of 39 °C and agitation set at 25 rpm for a period of 24 hours.

The *in vitro* cultures were removed after 24 h of incubation and placed into an ice bath to cease fermentation. Final pH was measured and recorded for each bottle. From each *in vitro* culture, a 4-mL sample of fluid was collected and mixed in test tubes with 1 mL of 25% (w/v) meta-phosphoric acid solution and frozen for later quantification of volatile fatty acids. The remaining contents of each bottle were then flushed with deionized water into labeled aluminum trays and dried in a forced-air oven at 105 °C. Dry weights were used to determine *in vitro* dry matter disappearance (IVDMD). Blank bottles (without added substrate) were included and used to correct for background residue associated with ruminal fluid and buffer solution.

Calculation of Gas Parameters, VFA concentrations, and IVDMD

Gas production, VFA concentrations, and IVDMD were analyzed following each incubation. For each of the two *in vitro* incubations, a total of four replicates for each treatment combination were used. The liquid extract for volatile fatty acid analysis was centrifuged, 0.4 mL of supernatant was transferred to chromatography vials with 1.2 mL of pivalic acid solution as the internal standard, and concentrations of VFA were measured using an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID), and a wax (DB Wax, Agilent Technologies, Santa Clara, CA) capillary column with a length of 9.5 m, inside diameter of 100 μm, and a film thickness of 0.1 μm. Hydrogen was used as the carrier gas and column flow rate was 0.3 cm/sec. Samples were split injected with a ratio of 100:1. The initial temperature was 40 °C, held for 1 minute, and then increased at a ramp rate of 20 °C/min to the final temperature of 180 °C. Hydrogen, nitrogen, and airflow rates at the FID were set at 35, 25, and 400 mL/min, respectively. Acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, and heptanoate concentrations were measured and expressed as mM of VFA. Gas measurements from the Ankom system were recorded in psi and

converted to mL of gas produced. *In vitro* dry matter disappearance was calculated as described below.

$$IVDMD = \left[1 - \left[\frac{(Dried\ residue\ weight, g) - residue\ weight\ of\ blank}{Initial\ substrate\ weight, g} \right] \right]$$

Statistical Analyses

For each experiment, a logistic and a log-logistic regression model were applied to model cumulative gas production curves of each culture. For the logistic model parameters analyzed were K (maximum gas production), $t_{1/2}$ (time to achieve half of maximum gas production) and β, which describes the shape of the curve.

Logistic model

$$Gas\ production = \frac{K}{1 + \exp^{-\beta(Time - t_{\frac{1}{2}})}}$$

Gas production = Cumulative gas production (mL) at a given time interval (hour).

Time = Interval in quarter-hour increments of the total time (0.25 hours).

Log-logistic model

$$Gas\ production = \frac{K}{1 + (t_{\frac{1}{2}} \div Time)^r}$$

For the log-logistic model parameters analyzed were K (maximum gas production), $t_{1/2}$ (time to achieve half of maximum gas production) and *r*, which describes the shape of the curve.

Gas production = Cumulative gas production (mL) at a given time interval (hour).

Time = Interval in quarter-hour increments of the total time (0.25 hours).

These two models estimated each gas production parameter using time (independent variable) and gas production (dependent variable). These gas production parameters were used to estimate the extent of gas production potential (K) and the rate of gas production due to substrate digestion ($t_{1/2}$, β and r). Maximum rate of gas production (m) of each culture during both experiments was calculated using our response variables as inputs.

Max slope (m) for the logistical model

$$m = \beta \times {}^K\!/_4$$

Max slope (m) for the log-logistical model

$$m = \left[(1 - {}^1\!/_r) \times (t_{1/2})^r / 1 + {}^1\!/_r\right]^{-1/r} \times \left[rK\left(1 - {}^1\!/_{r^2}\right)/4\right]$$

When comparing the two models, those with the lowest mean square error were chosen for further analysis. Consistently, the log-logistic model provided a superior fit to the data; thus, K, r, $t_{1/2}$ and m were subjected to further analyses.

Predicted methane yield was estimated utilizing VFA concentrations and the equation Methane Yield (mL/g of dry matter digested) = 4.08×[Acetate (mol/100 mol)/Propionate (mol/100 mol)] + 7.05, described by Williams et al. [12].

In addition to the gas production parameters, pH, VFA concentrations, methane production and IVDMD were then analyzed using the GLIMMIX procedure of the Statistical Analysis System (SAS version 9.4; SAS Inst. Inc., Cary, NC). Fixed effects included donor (feedlot diet or mixed diet) and treatment (grain type, grain processing method, and the interaction), and the random effect was replicate. Statistical significance was declared at $P \leq 0.05$ and tendencies for an effect if $0.05 < P < 0.10$.

3.4   Results & Discussion

Results are presented separately by experiment, due to differences in composition of diets fed to each donor. This will reflect the impact of grain type and grain processing on a concentrate-fed diet and a mix-fed diet, reflecting the importance of choosing the correct donor for an experiment.

In vitro gas production parameters (IVGP)

Gas production for *in vitro* cultures using a high-concentrate diet fed donor is shown in Figure 2, and the parameters used to calculate the gas production are presented in Table 6. We did not observe a well-defined asymptote for parameter K, which likely would lead to an overestimation. While the current study measured gas production for 24 h, a defined asymptote might be observed with a longer measurement period, suggesting an extension of the incubation period. An effect for the interaction of grain type by grain processing method was observed for K (P=0.04); conventional corn when dry-rolled showed greater gas production compared to the other treatments, followed by Enogen corn when dry-rolled, contrary to the findings of Schiff et al. [13], reporting greater gas production for cultures containing steam-flaked corn mixed or not with alpha-amylase, compared to dry-rolled corn; however, treatments including steam-flaked, either CC or EC, produced less gas (P=0.04), but were not different from each other. A main effect of grain type (P<0.01) was observed for CC to have greater K compared to EC. A grain processing effect also was observed (P<0.01), as DR had greater K compared to SF. The time to reach the midpoint of K ($t_{1/2}$) was different for all four treatments (P=0.02). Enogen corn when steam-flaked reached $t_{1/2}$ faster compared to other treatments, indicating a more rapid microbial fermentation, followed by steam-flaked CC, these cultures fermented faster the substrate because of the process of gelatinization that is involved in steam flaking [14]. This was expected due to

the enhanced microbial fermentation that is attributed to this grain processing method, as supported by Theurer et al. [5], who reported that steam flaking consistently improves digestibility of starch by ruminal microbes over whole, ground, or dry-rolled processing of grains. Dry rolled CC took the longest time to reach $t_{1/2}$ compared to dry-rolled EC or steam-flaked grains. A grain type effect was observed ($P<0.01$) indicating shorter $t_{1/2}$ for EC compared to CC, Enogen corn breaks down faster the starch polymers due to a thermostable alpha-amylase contained in the kernel [15]. An effect of processing method also was observed ($P<0.01$), as $t_{1/2}$ was shorter for SF cultures compared to DR. A difference of parameter r was observed for the interaction ($P=0.04$), cultures containing steam-flaked either EC or CC were different form the other treatments, a variance of the peak growth rate was evidenced among treatments. Effects of grain type ($P<0.01$) and a processing method ($P<0.01$) were observed. Enogen corn presented greater r compared to CC, and SF was greater than DR. The maximum rate of gas production was not different ($P=0.352$) among the treatments. No difference of pH was observed ($P=0.326$) for the interaction, the system was buffered to prevent pH changes. There was an effect of grain type ($P=0.024$), for which CC had lower pH compared to EC, the difference might be significant but not meaningful. The procedure of *in vitro* gas production is useful for evaluating degradability of feedstuff, because they are directly related [16]. While this study did not observed differences in IVDMD ($P=0.391$), de Oliveira et al.[17] reported that Enogen corn increased the disappearance of dry matter. During the *in vitro* digestion, microbes proliferate and become part of the residue, which can lead to an overestimation of the undigested material and thus an underestimation of the actual digestibility of the substrate. Elwakeel et al. [18], through three experiments, observed an increased IVDMD but no changes in VFA production. McDermott et al. [19] through two experiments, also observed an increased IVDMD, and by

measuring the microbial crude protein (MCP) found a 27 and 44% decrease in MCP, suggesting that the microbial community was smaller and more efficient. Results of the gas production by cultures inoculated using ruminal contents of the mixed diet donor are shown in Figure 4 and parameters of gas production are summarized in Table 7. No interaction effect was observed for parameter K (P=0.296). The substrate provided in both runs was corn, and the microbiome of each donor was different, and as concluded by Amanzougarene et al. [20], the *in vitro* cultures seems to be more affected by the microbial inoculum than by the substrate. The time to reach midpoint K was affected by treatment (P=0.045), as expected the cultures containing steam-flaked either, CC or EC, had lower $t_{1/2}$, compared to the dry-rolled (CC or EC), also an effect of the processing method (P<0.01) could be observed, which indicates that cultures containing SF presented lower $t_{1/2}$ compared to DR. Parameter r was also affected by treatments (P=0.01), while the difference was small, control corn when either steam-flaked or dry rolled were different from other treatments, also a grain type (P<0.01) and a grain processing (P=0.02) effect were observed. Contrary to findings by Kang et al. [21], who reported a linear increase of r when the degree of gelatinization increased, while the results of the present study showed a higher parameter r for the interaction of steam-flaked CC, the lowest was observed for the cultures including steam-flaked EC (P=0.01). The pH was not different among treatments (P=0.680), but an effect of grain processing (P=0.01) showed that SF had lower pH compared to DR, as mentioned before the system was buffered thus the difference was significant but not meaningful. No differences were observed for *in vitro* dry matter disappearance (P = 0.177) among the treatments.

Volatile fatty acid (VFA) production

Volatile fatty acids are end products of microbial fermentation in the rumen. No differences among treatments were observed for total VFA production (P=0.229) of cultures, including a high-concentrate diet fed donor. Aligning with Horton et al. [22], the current study did not observed differences among treatments for total VFA production, nor for the production of acetate (P=0.184), propionate (P=0.330), butyrate (P=0.282) or valerate (P=0.065), which is in contrast with results reported by Klingerman et al.[23]. A difference was observed in the A:P ratio (P<0.01) for the cultures that included steam-flaked CC compared to other treatments, while no differences were observed for acetate or propionate production. More cultures with more replicates may have revealed a statistical difference. Also, an effect of grain type (P=0.020) for CC with smaller ratio compared to EC. The results of the total VFA production, utilizing a mixed forage-concentrate fed donor (Table 7), were not different (P=0.101) among treatments. A tendency for interaction (P=0.061) was observed, with steam-flaked CC tending to produce more acetate compared to the other treatments. No further differences were observed for propionate (P=0.192), butyrate (P=0.140), or valerate (P=0.854). The A:P ratio was not different among treatments (P=0.109).

Methane production

Results of estimated methane production are presented as mL of methane produced per unit of DM digested (g), for cultures using a concentrate fed donor (Figure 1), no differences were observed for the interaction of grain type by processing method (P=0.596) and when a mixed fed donor was utilized (Figure 3) no differences were observed either (P=0.373). Rebelo et al. [24] observed decreased methane production using Enogen corn on non-cannulated dairy cows, while the current study used ruminal fluid from a mixed diet fed donor, no such effect was observed.

Methane production in the rumen is considered a major energy loss. Methanogens are the principal methane producing bacteria in the rumen, utilizing the available hydrogen, and with it making a more inefficient rumen from an energetic standpoint while contributing to global warming by increasing greenhouse gas emissions. The production of acetate and butyrate release H_2 which is utilized by methanogens to produce methane, on the other hand, propionate producing bacteria compete with methanogens for the available H_2. Reducing methane production by reducing acetate production through increasing the production of propionate would create a more efficient rumen while also reducing greenhouse gas emissions.

3.5 Conclusion

Results of this study have shown that conventional corn when dry-rolled resulted in greater gas production, while steam-flaked, corn regardless of the grain type, led to less total gas production. Enogen corn, when steam- flaked attained the midpoint of gas production fastest, while the longest time was for dry-rolled conventional corn. Overall, steam flaking resulted in less gas production, but had faster rates of gas production, which indicates a more efficient fermentation. These findings reflect the impact of grain type and processing method on microbial degradation while demonstrating the importance of the correct donor for the experiment.

Literature cited

1. *USDA*. https://www.usda.gov/ (accessed 2024-11-21).

2. Harrelson, F. W.; Luebbe, M. K.; Meyer, N. F.; Erickson, G. E.; Klopfenstein, T. J.; Jackson, D. S.; Fithian, W. A. Influence of corn hybrid and processing method on nutrient digestibility, finishing performance, and carcass characteristics1. *J. Anim. Sci.* **2009**, *87* (7), 2323–2332. https://doi.org/10.2527/jas.2008-1527.

3. Luebbe, M. K.; Erickson, G. E.; Klopfenstein, T. J.; Fithian, W. A. Influence of corn hybrid traits and processing method on nutrient digestibility. *Prof. Anim. Sci.* **2009**, *25* (4), 496–509. https://doi.org/10.15232/S1080-7446(15)30737-3.

4. Andrae, J. G.; Hunt, C. W.; Pritchard, G. T.; Kennington, L. R.; Harrison, J. H.; Kezar, W.; Mahanna, W. Effect of hybrid, maturity, and mechanical processing of corn silage on intake and digestibility by beef cattle. *J. Anim. Sci.* **2001**, *79* (9), 2268–2275. https://doi.org/10.2527/2001.7992268x.

5. Theurer, C. B. Grain processing effects on starch utilization by ruminants. *J. Anim. Sci.* **1986**, *63* (5), 1649–1662. https://doi.org/10.2527/jas1986.6351649x.

6. Theurer, C. B.; Lozano, O.; Alio, A.; Delgado-Elorduy, A.; Sadik, M.; Huber, J. T.; Zinn, R. A. Steam-processed corn and sorghum grain flaked at different densities alter ruminal, small intestinal, and total tract digestibility of starch by steers. *J. Anim. Sci.* **1999**, *77* (10), 2824–2831. https://doi.org/10.2527/1999.77102824x.

7. Janssen, P. H. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Anim. Feed Sci. Technol.* **2010**, *160* (1), 1–22. https://doi.org/10.1016/j.anifeedsci.2010.07.002.

8. Ellis, J. L.; Dijkstra, J.; Bannink, A.; Kebreab, E.; Archibeque, S.; Benchaar, C.; Beauchemin, K. A.; Nkrumah, J. D.; France, J. Improving the prediction of methane production and representation of rumen fermentation for finishing beef cattle within a mechanistic model. *Can. J. Anim. Sci.* **2014**, *94* (3), 509–524. https://doi.org/10.4141/cjas2013-192.

9. Johnson, J. R.; Carstens, G. E.; Krueger, W. K.; Lancaster, P. A.; Brown, E. G.; Tedeschi, L. O.; Anderson, R. C.; Johnson, K. A.; Brosh, A. Associations between residual feed intake and apparent nutrient digestibility, in vitro methane-producing activity, and volatile fatty acid concentrations in growing beef cattle1. *J. Anim. Sci.* **2019**, *97* (8), 3550–3561. https://doi.org/10.1093/jas/skz195.

10. DePeters, E. J.; Getachew, G.; Fadel, J. G.; Zinn, R. A.; Taylor, S. J.; Pareas, J. W.; Hinders, R. G.; Aseltine, M. S. In vitro gas production as a method to compare fermentation characteristics of steam-flaked corn. *Anim. Feed Sci. Technol.* **2003**, *105* (1), 109–122. https://doi.org/10.1016/S0377-8401(03)00042-7.

11. McDougall, E. I. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochem. J.* **1948**, *43* (1), 99–109.

12. Williams, S. R. O.; Hannah, M. C.; Jacobs, J. L.; Wales, W. J.; Moate, P. J. Volatile fatty acids in ruminal fluid can be used to predict methane yield of dairy cows. *Animals* **2019**, *9* (12), 1006. https://doi.org/10.3390/ani9121006.

13. Schiff, A. P.; Trotta, R. J.; Holder, V.; Kreikemeier, K. K.; Harmon, D. L. In vitro gas production kinetics are influenced by grain processing, flake density, starch retrogradation, and Aspergillus oryzae fermentation extract containing α-amylase activity. *J. Anim. Sci.* **2023**, *101*, skad031. https://doi.org/10.1093/jas/skad031.

14. Schirmer, M.; Jekle, M.; Becker, T. Starch gelatinization and its complexity for analysis. *Starch - Stärke* **2015**, *67* (1–2), 30–41. https://doi.org/10.1002/star.201400071.

15. Bothast, R. J.; Schlicher, M. A. Biotechnological processes for conversion of corn into ethanol. *Appl. Microbiol. Biotechnol.* **2005**, *67* (1), 19–25. https://doi.org/10.1007/s00253-004-1819-8.

16. Theodorou, M. K.; Williams, B. A.; Dhanoa, M. S.; McAllan, A. B.; France, J. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol.* **1994**, *48* (3), 185–197. https://doi.org/10.1016/0377-8401(94)90171-6.

17. De Oliveira, D. D. S.; Brixner, B. M.; Abdalla, A. L.; Polizel, D. M.; Drouillard, J. S. S.; Santos, F. A. P. PSXI-13 Effects of corn containing gene for high concentration of alpha amylase (Enogen feed corn) on fermentative parameters, digestibility and methane in vitro. *J. Anim. Sci.* **2024**, *102* (Supplement_3), 754–755. https://doi.org/10.1093/jas/skae234.851.

18. Elwakeel, E. A.; Titgemeyer, E. C.; Johnson, B. J.; Armendariz, C. K.; Shirley, J. E. Fibrolytic enzymes to Increase the nutritive value of dairy feedstuffs. *J. Dairy Sci.* **2007**, *90* (11), 5226–5236. https://doi.org/10.3168/jds.2007-0305.

19. McDermott, K.; Lee, M. R. F.; McDowall, K. J.; Greathead, H. M. R. Cross inoculation of rumen fluid to improve dry matter disappearance and its effect on bacterial composition using an in vitro batch culture model. *Front. Microbiol.* **2020**, *11*. https://doi.org/10.3389/fmicb.2020.531404.

20. Amanzougarene, Z.; Yuste, S.; Fondevila, M. Fermentation pattern of several carbohydrate sources incubated in an in vitro semicontinuous system with inocula from ruminants given

either forage or concentrate-based diets. *Animals* **2020**, *10* (2), 261.
https://doi.org/10.3390/ani10020261.

21. Kang, H.; Lee, M.; Jeon, S.; Lee, S. M.; Lee, J. H.; Seo, S. Effect of flaking on the
digestibility of corn in ruminants. *J. Anim. Sci. Technol.* **2021**, *63* (5), 1018–1033.
https://doi.org/10.5187/jast.2021.e91.

22. Horton, L.; Drouillard, J. 57 Effects of particle size and heat treatment on in situ and in vitro
digestion of dry-rolled Enogen® Feed Corn. *J. Anim. Sci.* **2018**, *96* (suppl_3), 391.
https://doi.org/10.1093/jas/sky404.857.

23. Klingerman, C. M.; Hu, W.; McDonell, E. E.; DerBedrosian, M. C.; Kung, L. An evaluation
of exogenous enzymes with amylolytic activity for dairy cows. *J. Dairy Sci.* **2009**, *92* (3),
1050–1059. https://doi.org/10.3168/jds.2008-1339.

24. Rebelo, L. R.; Eastridge, M. L.; Firkins, J. L.; Lee, C. 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.* **2023**, *106* (6), 3932–3946.
https://doi.org/10.3168/jds.2022-22770.

Table 6. Gas production parameters, volatile fatty acids profile, and IVDMD of *in vitro* cultures using corn as substrate and ruminal inoculum from a donor animal fed a high-concentrate diet.

| | Dry rolled | | Steam flaked | | | *P*-values | | |
Item	Control	Enogen	Control	Enogen	SEM	Grain	Processing	GxP♥
K, mL	569.74[a]	527.16[b]	477.30[c]	463.57[c]	6.725	<0.01	<0.01	0.043
$T_{1/2}$, h	11.65[a]	9.59[b]	7.90[c]	7.02[d]	0.281	<0.01	<0.01	0.027
r	1.19[a]	1.24[b]	1.35[c]	1.33[c]	0.017	0.202	<0.01	0.046
m^{ϕ}, mL/h	33.4	36.0	37.9	42.1	1.02	<0.01	<0.01	0.352
IVDMD♦, %	63.90	65.17	64.12	67.63	2.097	0.074	0.305	0.391
pH	6.17	6.18	6.15	6.17	0.006	0.024	0.083	0.326
Total VFA, mM	106.96	106.51	104.77	111.53	3.181	0.291	0.632	0.229
Acetate, mM	48.93	48.65	47.97	51.49	1.485	0.255	0.506	0.184
Propionate, mM	37.84	37.71	37.67	39.54	1.094	0.393	0.415	0.330
Butyrate, mM	14.21	14.21	13.50	14.31	0.407	0.285	0.426	0.282
Valerate, mM	2.75	2.81	2.69	3.03	0.096	0.013	0.266	0.065
A:P	1.29[a]	1.29[a]	1.27[b]	1.30[a]	0.005	0.020	0.403	<0.01

[a,b,c,d] Mean within a row without a common superscript letter are not different (*P*<0.05).

♥Interaction of grain type by grain processing method.

ϕMaximum rate of gas production, calculated as: $m = \left[\left(1 - 1/r\right) \times \left(t_{1/2}\right)^r / 1 + 1/r\right]^{-1/r} \times \left[rK\left(1 - 1/r^2\right)/4\right]$

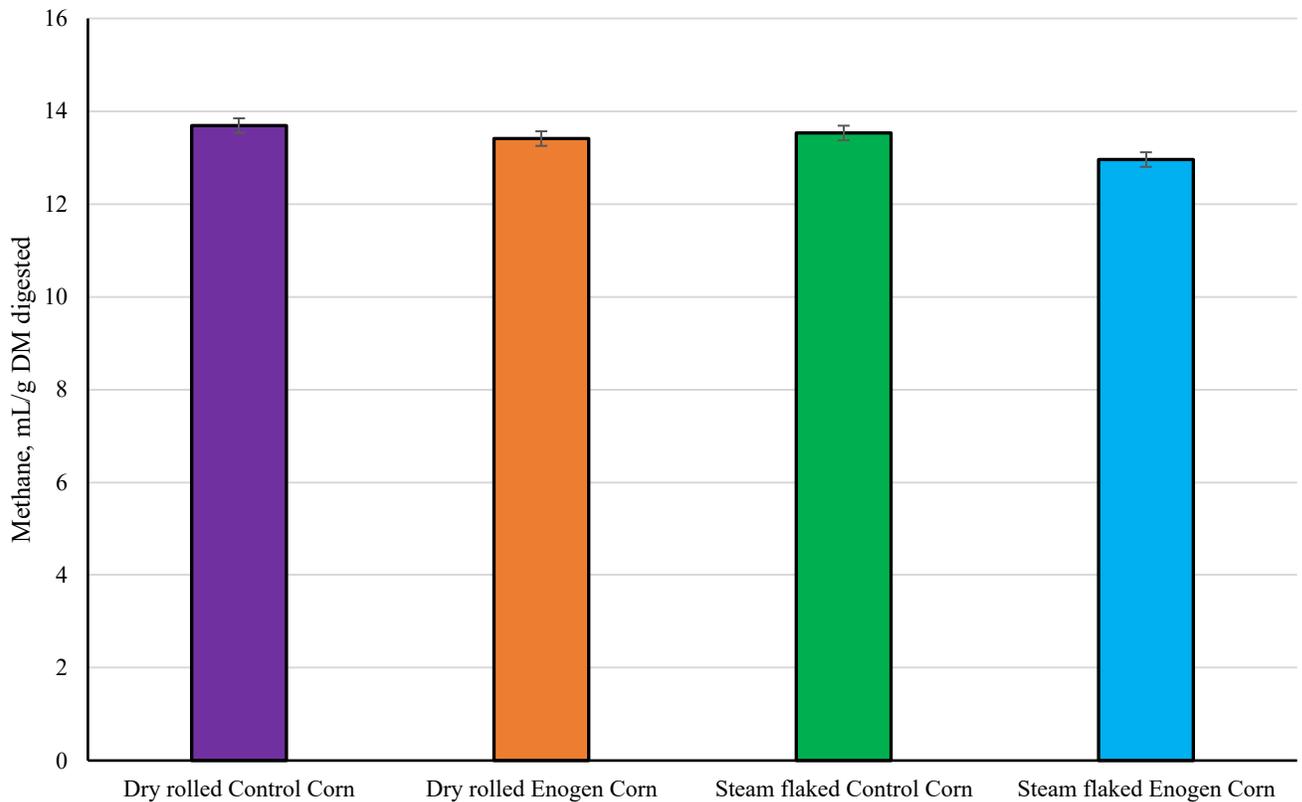♦IVDMD = *in vitro* dry matter disappearance.

Figure 1. Estimated methane yield (high-concentrate fed donor).

Results of methane yield are presented as mL of methane produced per unit of DM digested (g).
Methane Yield = 4.08×[Acetate (mol/100 mol)/Propionate (mol/100 mol)] + 7.05, described by
Williams et al.[12]. Effect of grain type, P=0.130; effect of processing method, P=0.273; grain
type x processing interaction, P=0.596.
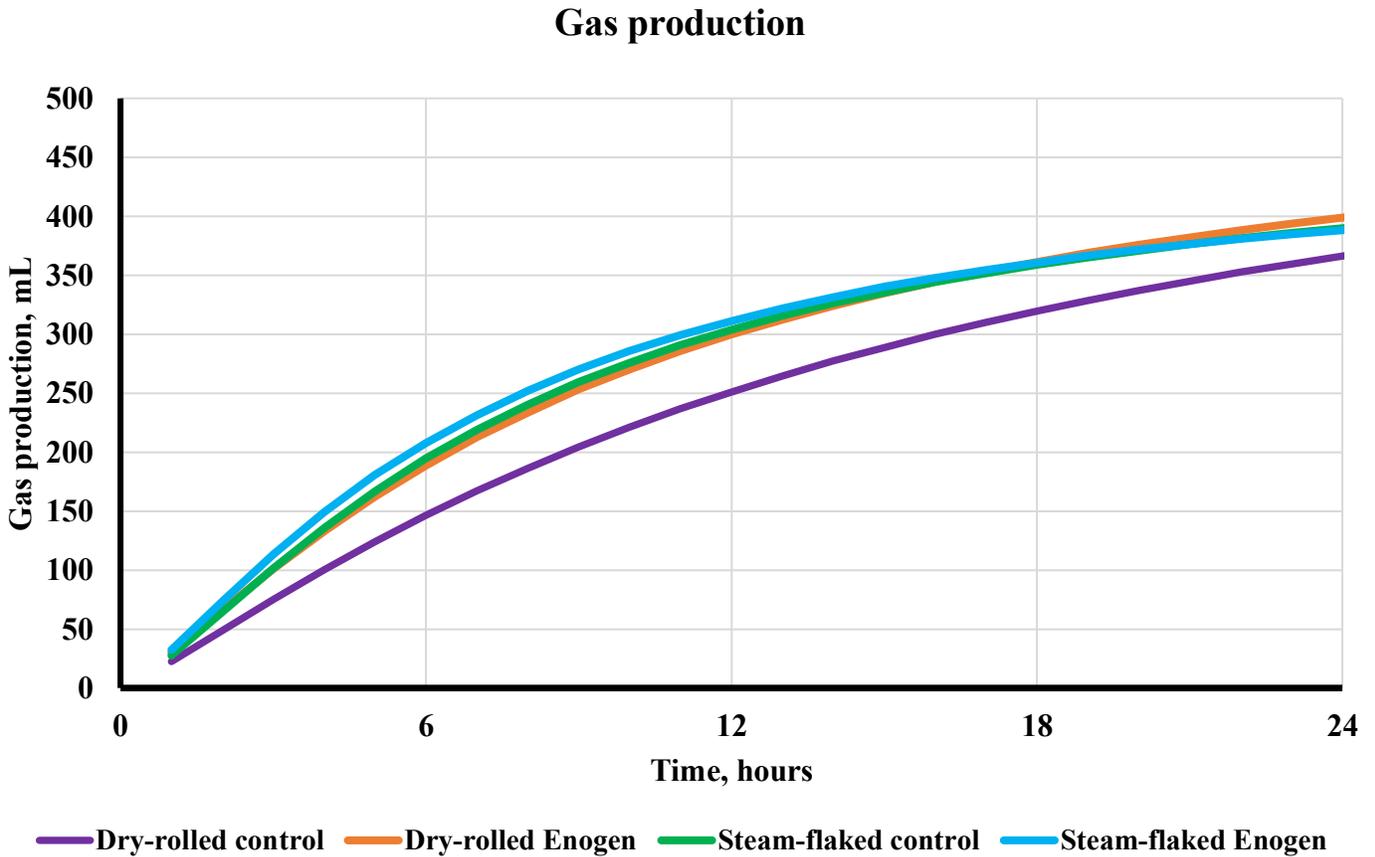
Gas production

Figure 2. Gas production from *in vitro* cultures with corn as substrate and ruminal inoculum from a donor animal fed a high-conentrate diet.

A log-logistic regression model was applied to model cumulative gas production curves for each culture, yielding parameters of K, $t_{1/2}$ and $\boldsymbol{r}$. These values were used to generate curves shown above using the equation: Gas Production = K / (1 + $(t_{1/2}/\text{time})^r$).
Variables analyzed included Enogen or a conventional (control) corn, when these were either dry-rolled or steam-flaked, in a 2x2 factorial arrangement.
K: effect of grain type, $P<0.01$; effect of grain processing method, $P<0.01$; interaction between grain type and grain processing method, $P=0.04$. $t_{1/2}$: effect of grain type, $P<0.01$; effect of grain processing method, $P<0.01$; interaction between grain type and grain processing method, $P=0.02$. $\boldsymbol{r}$: effect of grain type, $P<0.01$; effect of grain processing method, $P<0.01$; interaction between grain type and grain processing method, $P>0.05$.

Table 7 Gas production parameters, volatile fatty acids profile, and IVDMD of *in vitro* cultures using corn as substrate and ruminal inoculum from a donor animal fed a mixed forage:concentrate diet.

Item	Dry-rolled corn		Steam-flaked corn		SEM	P-values		
	Control	Enogen	Control	Enogen		Grain	Processing	GxP[♥]
K, mL	397.01	399.66	386.59	402.94	9.655	0.152	0.582	0.296
$T_{1/2}$, h	10.06 [a]	9.88 [a]	8.91 [b]	9.11 [b]	0.085	0.900	<0.01	0.045
r	2.85 [a]	2.74 [b]	2.85 [a]	2.62 [b]	0.043	<0.01	0.021	0.018
m[φ], mL/h	31.9	31.7	35.1	33.7	0.082	0.253	<0.01	0.328
IVDMD[♦], %	52.69	53.84	56.17	53.45	1.809	0.577	0.276	0.177
pH	6.43	6.44	6.40	6.41	0.020	0.357	0.019	0.680
Total VFA, mM	72.11	72.42	76.63	68.06	2.606	0.125	0.975	0.101
Acetate, mM	38.92	40.56	42.18	38.26	1.415	0.430	0.733	0.061
Propionate, mM	19.79	18.37	21.20	17.81	0.740	<0.01	0.572	0.192
Butyrate, mM	11.04	11.19	11.02	10.02	0.378	0.279	0.130	0.140
Valerate, mM	1.10	1.09	1.06	1.00	0.065	0.491	0.410	0.854
A:P, mM	1.97	2.20	1.99	2.15	0.029	<0.01	0.392	0.109

[a,b,c,d] Mean within a row without a common superscript letter are not different (*P*<0.05).

[♥]Interaction of grain type by grain processing method

[φ]Maximum rate of gas production, calculated as $m = \left[(1 - 1/r) \times (t_{1/2})^r / 1 + 1/r \right]^{-1/r} \times \left[rK \left(1 - 1/r^2 \right) / 4 \right]$

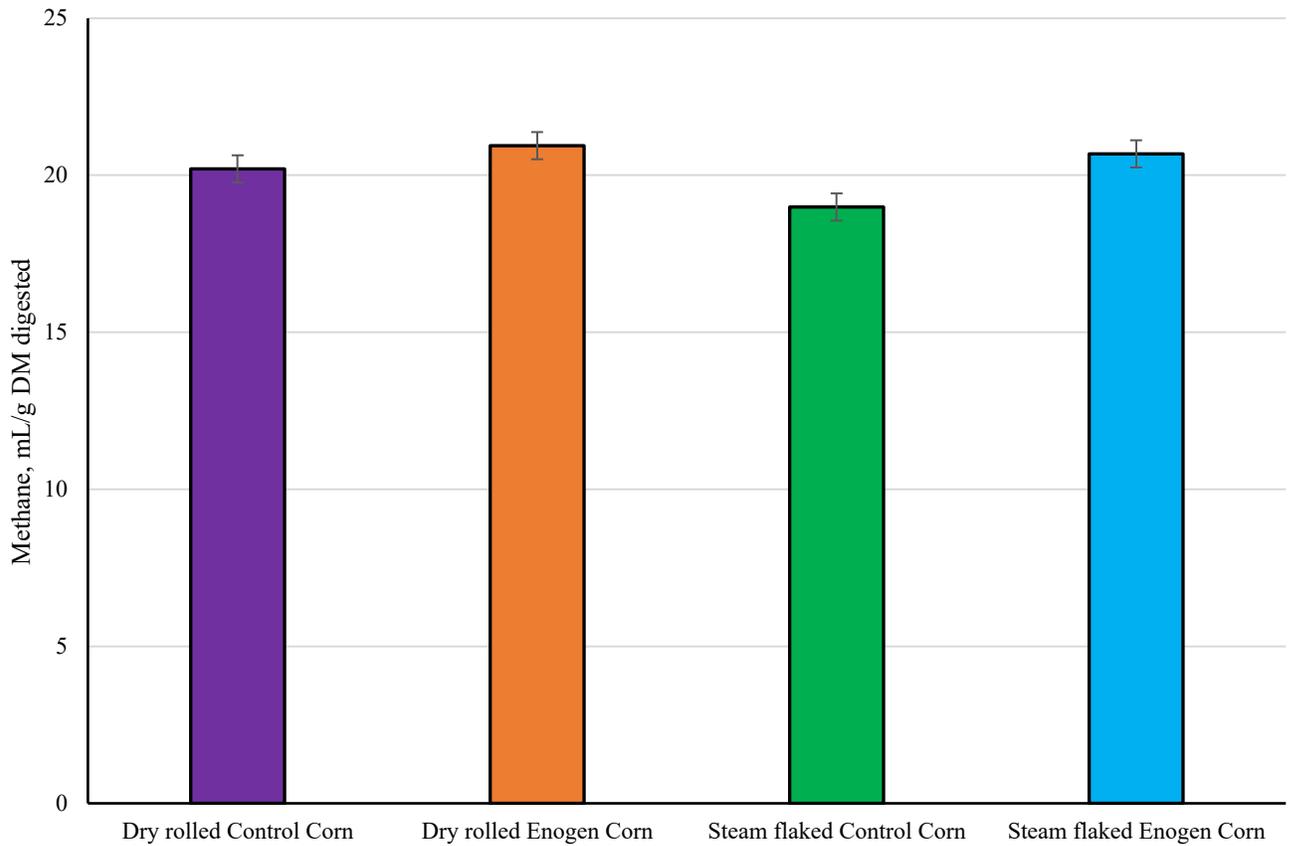[♦]IVDMD = *in vitro* dry matter disappearance

Figure 3. Estimated methane yield by *in vitro* cultures with corn as substrate and ruminal inoculum from a donor animal fed a mixed forage:concentrate diet..

Results of methane yield are presented as mL of methane produced per gram of DM disappearance.

Methane Yield = 4.08×[Acetate (mol/100 mol)/Propionate (mol/100 mol)] + 7.05, described by Williams et al.[12]. Effect of grain type, P=0.029; effect of grain processing method, P=0.173; Grain type by grain processing method interaction, P=0.373.
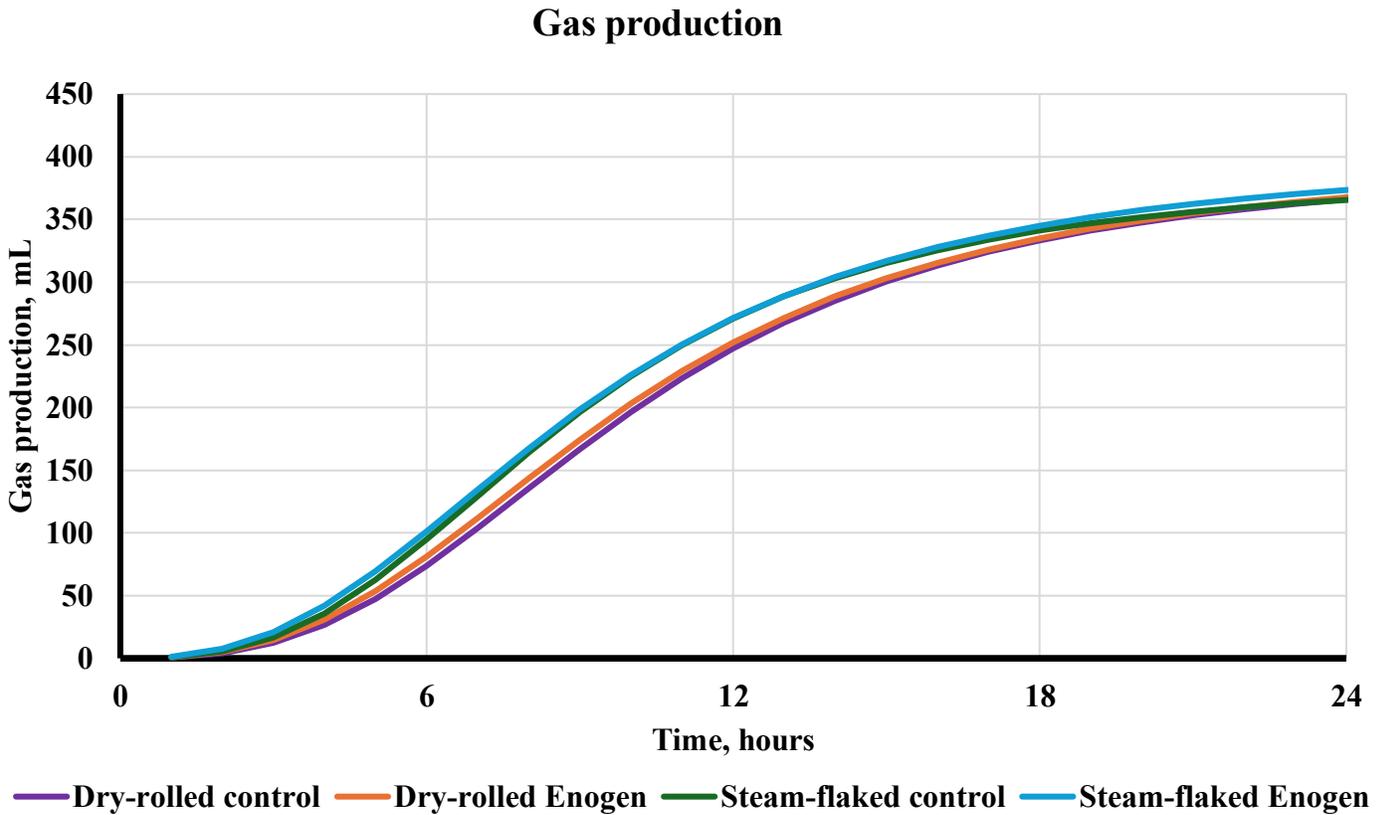
Figure 4. Gas production (mixed-fed donor).

A log-logistic regression model was applied to model cumulative gas production curves for each culture, yielding parameters of K, $t_{1/2}$ and r. These values were used to generate curves shown above using the equation: Gas Production = K / (1 + (t½/time)^r).

Variables analyzed included Enogen or a conventional (control) corn, when these were either dry-rolled or steam-flaked, in a 2x2 factorial arrangement.

K: effect of grain type, $P>0.05$; effect of grain processing method, $P>0.05$; interaction between grain type and grain processing method, $P>0.05$. $t_{1/2}$: effect of grain type, $P>0.05$; effect of grain processing method, $P<0.01$; interaction between grain type and grain processing method, $P=0.04$. r: effect of grain type, $P<0.01$; effect of grain processing method, $P=0.02$; interaction between grain type and grain processing method, $P=0.01$.