

Evaluation of ground grain sorghum as an alternative to dry-rolled corn in finishing steer diets.

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Abstract

Interest in the ability of grain sorghum production to conserve water by reduced crop irrigation compared to corn, while also meeting nutritional demands of the feedlot industry has motivated the following evaluations. One finishing cattle study and a complementary *in vitro* experiment were conducted to evaluate use of ground grain sorghum as an alternative to dry-rolled corn in finishing steer diets. The finishing cattle study was conducted to evaluate feedlot performance and carcass characteristics, utilizing yearling steers ($n=300$; 346 ± 11 kg initial body weight). Cattle were allocated randomly to feedlot pens (30 pens; 10 steers/pen), and pens were assigned randomly to dietary treatments within blocks (15 blocks). Steers were fed once daily and feed amounts were allocated to result in only traces of feed remaining in the bunk the following day. Fresh fecal pats were collected from pen surfaces on day 120 and analyzed for concentrations of acid insoluble ash, starch, and organic matter to estimate diet digestibility. Steers were harvested after 182 d on feed and carcass data were collected after 96 h of refrigeration. Muscle tissue samples from the *infraspinatus* and *semitendinosus* muscles were collected from a subset of 32 carcasses to be analyzed for concentrations of fatty acid methyl esters. Corn and sorghum diets yielded similar digestibilities ($P > 0.10$) of organic matter (79.0 vs 78.3%, SEM=2.29) and starch (91.4% vs 88.5%, SEM=1.85). Cattle fed corn consumed less DM but had greater ADG and G:F compared to those fed sorghum (DM intake: 10.93 vs 11.54 kg/d; ADG: 1.61 vs 1.46 kg/d; G:F: 0.1533 vs 0.1327; $P < 0.01$). Calculated NEm (2.24 vs 2.08 Mcal/kg) and NEg (1.55 vs 1.42 Mcal/kg) were greater for corn compared to sorghum ($P < 0.03$). Heavier carcasses with greater marbling and superior quality grades were observed for cattle fed corn produced compared to those fed sorghum, resulting in \$112 greater carcass value for cattle fed corn ($P < 0.01$). Meat tissues from corn-fed steers were characterized by greater

concentration of total fatty acids (FA) and monounsaturated FA, specifically in the infraspinatus ($P < 0.05$). Meat tissues from grain sorghum-fed steers contained greater polyunsaturated FA (PUFA) proportion in the *semitendinosus* ($P < 0.01$). In the second experiment, *in vitro* fermentation characteristics including cumulative gas production, volatile fatty acid (VFA) profiles, and *in vitro* dry matter disappearance of grain sorghum and corn for beef steers were evaluated. A randomized complete block design was utilized to evaluate three grain sorghum and three corn samples (hybrids unknown) from the feedlot study. Compared to sorghum, IVDMD tended to be greater for corn (59.18% vs 62.31%; $P = 0.07$), but concentrations of VFAs were not different ($P < 0.01$). Cultures with grain sorghum as substrate produced less gas compared to those containing corn as substrate ($P < 0.01$), but rates of gas production did not differ ($P > 0.10$). These results indicate that when compared to diets containing dry-rolled corn, diets containing ground grain sorghum are inferior. Diets cause feedlot steers to underperform and produce inferior carcasses.

Key words: feedlot, milo, digestibility, performance

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Chapter 1 - Literature Review

Introduction

Feedlot cattle production makes significant contributions to agricultural economies in the High Plains region of the United States, but its future is threatened by depletion of groundwater resources used for crop irrigation. The current state of the Ogallala Aquifer that supplies water to the area is driven by over-withdrawal at a rate that exceeds its natural replenishing capacity [1]. The two most commonly fed grains in cattle feedlot diets are corn and sorghum respectively [2], but corn has long been the preferred energy source due to its wide availability and suitable effects on animal growth. However, grain sorghum is broadly adapted to yield stability under water limitation, potentially providing means for conservation of water while still meeting demands of the feedlot industry [3]. The variability in grain sorghum, including kernel size, genetic variety, polyphenolic content, and starch chemistry, requires research to evaluate these cumulative effects on beef cattle performance when compared to corn if it is to become more commonly fed. Sorghum's unique attributes include various benefits and anti-nutritional factors which are discussed here.

Sorghum physical structure and characteristics

Grain sorghum's tolerance to drought is largely due to its root structure and degree of branching. The primary root system of sorghum rapidly grows to soil depths of 1 to 1.5 meters shortly after emergence and contains very little branching. A more expansive secondary root system develops next which extends further to depths of up to 2 meters by the heading stage [3]. This extensive root system allows sorghum to access deeper soil with greater moisture, contributing to its superior tolerance compared to other cereal grains, like corn. Another water-

efficient characteristic is that sorghum can recover quickly following periods of water stress and can endure drought without permanent damage to yield potential.

The U.S. Grain Council developed a kernel hardness index to monitor grain sorghum hardness. Harder kernels may be more resistant to insects and disease but are also harder to process as they produce coarser and larger particles [4]. This makes grain sorghum more costly and inefficient to process and feed to livestock. While sorghum grains vary widely in shape, size, color, and hardness, they often have one flattened surface on the mostly round grain. Seeds can range 3 to 80 mg in weight [5] and are comprised of: pericarp, endosperm, and germ [6]. The pericarp is made of multiple layers known as the epicarp, mesocarp, and endocarp. Sorghum is the only cereal to have starch granules present in the pericarp. It contains a starchy endosperm divided into peripheral, vitreous and floury endosperm. Waxy sorghum is characterized by reduced amylose and has been found to contain less peripheral endosperm, which may contribute to its improved starch digestibility. The endosperm contains proteins and most of the lipids, vitamins and minerals of the grain [7].

Sorghum utilization and attributes

First cultivated in Northeastern Africa approximately 4,000 years ago [8], sorghum (*Sorghum bicolor* L. Moench) is a monocotyledonous cereal within the Poaceae family. It is an abundant crop ranking fifth in the world in overall grain production behind maize, rice, wheat, and barley. The demand for cereal grains that increase animal feed efficiency, address global malnutrition, and meet niche-markets dictate the necessity for improvements in grains that have been largely over-looked [9]. Sorghum is characterized by a high degree of genetic diversity which influences differences in chemistry and structure of sorghum, including its color and size. This creates a lack of uniformity, which without preserving genetic identity, may limit its uses.

Many parts of the world consume sorghum in traditional foods and beverages where maintaining variety integrity is less essential [6]. It is utilized as livestock feed in other areas, and increasingly for ethanol and biofuel production in the United States.

The nutrient profile of grain sorghum may vary, but generally contains around 88.7% DM (dry matter), 86% TDN (total digestible nutrients), 11.6% CP (crude protein), and 7.2% NDF (neutral detergent fiber) [10]. It is interesting to note the DM and CP values are greater for grain sorghum than corn, which may impact animal performance. The difference in energy value between grain sorghum and corn is slight, but varies depending on processing method, with net energy for gain of sorghum being 97% that of dry-rolled corn [10]. This indicates a slight advantage for corn that may not be detectable in animal trials, especially when the grains are processed in a similar manner.

With use of irrigation, sorghum can achieve yields between 3.5 to 5 T/ha at 12 to 15% moisture content. The water utilization efficiency for harvested yield ranges from 0.6 to 1 kg/m³, reflecting its ability to produce a substantial amount of crop with less water input compared to corn) [3]. Critical growth stages specifically establishment, flowering, and early grain formation, are especially sensitive to moisture deficits. The drought tolerance of sorghum is mainly attributed to its physical structure which is characterized by a deep extending, and well branched root system [3].

Starch chemistry and digestibility of grain sorghum

Sorghum has gained attention for its high starch content, which accounts for over 80% of the total grain weight [8]. Starch is the primary storage carbohydrate in plants and is found in high concentrations within cereal grains. It is one of six fractions of carbohydrates that are categorized based on their rates of digestion and ruminal availability [10]. The five other

fractions are organic acids (OA), water-soluble carbohydrates (WSC), neutral detergent-soluble fiber (NDSF), available NDF, and unavailable NDF.

The structure of sorghum starch granules are hierarchical, with a hilum at the core consisting of a loosely packed central region characterized by a low degree of organization. Kaufman et al. [11] reported that individual granules range from 3 to 35 micrometers in size and that certain environmental conditions, such as increased temperature during growth, can affect morphology by reducing granule diameter. This structure and nature is identified through X-ray diffraction. Granules from vitreous and corneous endosperm are generally more compact than those from floury endosperm. The surface and internal structure of sorghum starch granules are closely associated with proteins and lipids. Sorghum proteins are found on the external surface as well as within the granules. Depending on the variety, protein content of the endosperm ranges from 0.2% to 1.2%, while lipid content ranges from 0.2% to 1.4% [12]. Sorghum starch contains three types of lipids: non-starch lipids bound to surface proteins, surface lipids that penetrate during steeping, and native starch lipids embedded within the granule structure [13]. Each of these lipids contribute to reduced starch digestibility via interferences with enzyme access to the granules themselves.

Starch granules contain growth rings composed of blocks organized into alternating amorphous and crystalline lamellae that contribute to overall crystalline nature. Crystalline domains are composed of amylopectin and amorphous domains consist of amylose and non-crystalline amylopectin [14]. Amylose is a linear glucose polymer linked by α -(1,4) glycosidic bonds, whereas amylopectin is a highly branched polymer that contains both α -(1,4) and α -(1,6) linkages. Most cereal starches are composed of approximately 16 to 35% amylose and 65 to 84% amylopectin [15], although waxy starch varieties can contain amylopectin concentrations

exceeding 84%. High-amylose starches, conversely, may contain over 40% amylose. For example, waxy grain sorghum contains 0% amylose, whereas non-waxy varieties contain around 24% amylose. Grain sorghum contains approximately 71%, while dry-rolled corn contains about 72% [4]. The amylose-to-amylopectin ratio increases with corn kernel maturity and decreases with elevated growing temperatures and decreases starch digestibility [16]

Cereal grain starch exhibits unique physical properties that allow them to reorganize their structure. Upon heating in the presence of water, sorghum starch granules swell as they absorb water. Amylose may dissolve in the water during this process, contributing to gelatinization—an irreversible transformation characterized by granule swelling and loss of crystallinity. Following gelatinization, starch may then undergo retrogradation which occurs when it is cooled below its crystalline melting point. This results in the realignment and reassociation of amylose and amylopectin polymers, which increases paste viscosity, turbidity, gel formation, and water exudation [12], all of which are important factors to consider during grain processing.

Starch digestibility in cereal grains is influenced by several structural and compositional factors. The process matrix is especially robust in sorghum which effectively reduces its digestibility relative to other grains. Key factors affecting starch digestibility include plant species, the extent of starch–protein interactions, granule morphology, tannin or inhibitor content, and the type of starch itself [17]. Among cereal grains, sorghum typically exhibits the poorest starch digestibility which can be attributed to dense peripheral endosperm and stronger protein matrix. Although, greater feeding value of some varieties, like waxy sorghum, have been reported due to enhanced starch and protein digestibility from increased amylopectin and loosely packed starch granules [18]. The endosperm of grain sorghum contains prolamins storage proteins that adhere to the starch granule surface. Each grain type expresses a unique form of

prolamin—zein in corn and kafirin in sorghum [10]. These prolamins comprise 50% to 60% of endosperm protein in cereal grains. The physical and chemical properties of prolamins directly impact ruminal degradability. Zein, for instance, is highly insoluble in the rumen, which reduces the digestibility of the protein and the starch it encompasses [19]. Other chemical components such as tannins may also impact grain digestion.

Fatty acids and their impact on meat quality

There are two fatty acids that are essential for ruminants as they cannot be synthesized endogenously. They are linoleic acid (an omega-6 fatty acid) and alpha-linolenic acid (an omega-3 fatty acid). These play a crucial role in many physiological processes like the production of long-chain polyunsaturated fatty acids (PUFA), and are commonly found in plant oils and forages [20]. They may be present as end-products of fermentation in silage or as plant-derived OA in fresh forage and hay. Lactic acid is the dominant OA produced during silage fermentation, ranging from 4–6% of DM in well-fermented cereal silages [10]. Once consumed, lactic acid is quickly converted to propionate in the rumen and subsequently to glucose in the liver [21].

Most fatty acids are supplied by diet feedstuffs. The fatty acid composition of different grain sorghum varieties is characterized by a total oil content ranging from 5.0–8.2%, with the amount of PUFA generally more abundant than that of monounsaturated (MUFA) [22]. Oleic, linoleic, and palmitic acids are the major components, while some varieties may contain rare saturated fatty acids (SFAs) that may exhibit antibacterial properties by way of membrane disruption and inhibition to cell wall synthesis [23].

The process of fatty acid synthesis within plant cells begins with the conversion of acetyl-CoA to malonyl-CoA, by acetyl-CoA carboxylase (ACCase). This is followed by cyclic reactions

in which two-carbon units from acetate are condensed with malonyl-acyl carrier protein (ACP) at each step (Cox, 2013). According to Rawsthorne [24] while some acetate may serve as a carbon source for plastidial fatty acid synthesis, other studies suggest that glucose 6-phosphate, malate, phosphoenolpyruvate, and pyruvate are more effective at supporting high rates of fatty acid synthesis in isolated plastids. The acetyl-CoA itself is generated through metabolism in the plastid, utilizing endogenous sources of fixed carbon supplied by the photosynthesis pathway in the chloroplast. The elongating fatty acid chain remains attached to the ACP, which facilitates solubility and processivity. This elongation typically continues until the chain reaches 16 or 18 carbon atoms in length. While some palmitic acid (16:0) is released from the synthesis pathway, the majority is further elongated to stearic acid (18:0) and desaturated to oleic acid (18:1) by desaturase enzymes in the plastids [25]. As a result, 16:0 and 18:1 are commonly the predominant fatty acids synthesized in plant plastids.

Overall, carbon flux into fatty acid synthesis is divided into two key components: the enzymatic systems that synthesize or utilize acetyl-CoA within the plastid, and the transport and conversion of cytosolic metabolites into usable substrates for those enzymes [24]. Compared to wheat and rice, sorghum has a higher crude fat content but contains less lipid than corn. While PUFA are commonly the most dominant fatty acid type, certain varieties vary in their abundant fatty acid category (SFA or MUFA vs. PUFA), which may have effects on nutritional value. Diets rich in MUFAs serve as functional alternatives to low-fat diets for humans by improving lipid metabolism, immune response, and membrane function [22].

Fatty acids may also be derived through *de novo* synthesis in various tissues. Volatile fatty acids (VFA), contribute significantly to ruminal energy supply. VFA such as acetate, butyrate, and propionate are end products of ruminal fermentation that account for up to 80% of

metabolizable energy for ruminants [26]. The predominant VFAs are acetate, propionate and butyrate which Acetate is typically the most abundant VFA found in fermented feeds, comprising 1–3% of DM. By contrast, butyrate and propionate generally measure below 0.5% DM and, when elevated, can indicate sub-optimal fermentation [27]. Production of VFA is primarily influenced by diet composition, particularly grain type and processing method of that grain. Franks et al. [28] completed a study evaluating the relationship of VFAs to type of grain used in feedlot diets. They concluded that grain type did not significantly alter total rumen VFA levels. Although, feeding rapidly fermentable barley grain resulted in an increase in molar proportions of butyrate with decreased proportions of propionate, while feeding grain sorghum and corn had minimal direct influence on overall fatty acid concentrations in the rumen.

Fatty acid composition contributes to beef's nutritional profile and sensory qualities [29]. Fatty acids found in meat melt between 25°C and 50°C with saturated fatty acids melting at higher temperatures than polyunsaturated fatty acids [30]. Variance in fatty acid profile and melting point may contribute to differences in eating experience by things like tenderness and cutability. It also affects its oxidative stability during retail processing before it even reaches a consumer. Polyunsaturated fatty acids are conserved in muscle in ruminant animals and are most susceptible to oxidative breakdown according to Wood et al. [31]. While factors like vitamin E and antioxidant content also affect meat spoilage, fatty acid profile is an important factor to consider. The composition in livestock adipose and muscle tissues is largely influenced by the total fat content within the animal. In cattle PUFA, particularly linoleic acid (18:2n-6), are conserved in muscle phospholipids and incorporated into tissues at levels proportional to dietary intake. Linoleic acid and its elongation product, arachidonic acid (20:4n-6), compete for integration into phospholipid membranes. PUFA are more susceptible to oxidative breakdown,

affecting shelf life and spoilage of meat [32]. The relative proportion of linoleic acid declines as overall fat deposition increases. This can be a result of a decrease in phospholipids relative to total muscle lipids and a subsequent rise in SFA and MUFA [31]. This is of interest because oleic acid (18:1cis-9), a primary component of neutral lipids, along with linoleic acid is considered to be nutritionally important component for human diets. In contrast, alpha-linolenic acid (18:3n-3), a major fatty acid found in grasses, has less efficient incorporation into muscle and adipose tissue due to the extended rumen transit time of forages compared to grains that contain more linoleic acid. These differences in fatty acid profiles have notable implications for meat quality. The composition of fatty acids from differing grain sources influences the firmness and oiliness of adipose tissue, as well as the oxidative stability, flavor, and color of muscle tissue.

Characterization and impacts of polyphenolic compounds in grain sorghum

While the majority of commodity sorghum grown in the U.S has been genetically bred to no longer contain tannins [33], some varieties grown in other countries contain concentrations of tannins including polyphenolics, anthocyanins, phenolic acids, and other antioxidants. Ferulic acid is the dominant phenolic acid in sorghum and is found in other grains used as feed as well [4]. Tannins are polyphenols of differing chemical and physical complexity that are synthesized during secondary metabolism and largely reside in the pigmented testa just underneath the pericarp of the grain [34]. These tannins play a major role in plant protection from pests and pathogens while also preventing toxicity by binding to polymers of protein or carbohydrates [35]. There are two major groups of tannins- hydrolysable and condensed. Sorghum polyphenols are comprised of condensed tannins which are oligomers and polymers of subclasses catechins and epicatechins. These make up a broader class of polyphenolic

compounds called procyanidins [36]. Phenolic compounds are of interest because they can exhibit positive effects such as antioxidant and antimicrobial activity which can support shelf-life stability and mitigate lipid oxidation of retail meat [37].

Phenolic compounds also contribute to potentially negative effects that include interference with starch digestion. Awika et al. [38] utilized the oxygen radical absorbance capacity (ORAC) method to measure the ability of antioxidants to protect protein from damage by free radicals. It was found that phenol contents of sorghum correlated strongly with their antioxidant activity. These authors reported coefficients of determination for phenol vs ORAC, ($R^2=0.96$) and phenol vs ABTS (a reagent used to measure antioxidant activity) ($R^2=0.96$). This confirms phenols as mainly responsible for sorghum's antioxidant activity. There are significant differences in phenol concentrations and antioxidant activity of sorghum and sorghum by-products that are mainly influenced by tannin components. Tannins are known to have greater antioxidant activity compared to other phenols. Brown tannin-containing sorghums including Sumac and Hi tannin, were observed to have greater antioxidant activities compared to other varieties containing less tannins [38] Black sorghum contains no tannins but instead has high levels of anthocyanins, which also have substantial antioxidant activity. Sorghum varieties with the least antioxidant activities have white pericarp that contains no tannins or anthocyanins and very low phenol concentrations. Sorghum bran has been reported to have 3 to 5 times the antioxidant activity of grains [39].

Antimicrobial properties of tannins may aid in reducing methane production by ruminants [35]. Grain sorghum varieties containing greater tannin concentration could potentially be used to modify ruminal fermentation. A study performed by Gisele de Oliveira et al. [40], evaluated impact of sorghum tannin content on beef cattle performance and methane

emission. Cannulated steers were fed ensiled sorghum varieties with known tannin levels. Methane content of eructate gas was measured by the authors observed no effect of tannins on methane emissions. Cardoso-Gutierrez et al. [35] fed diets of tannin-containing tropical plants as well as one containing supplemented tannins. They reported a reduction in total methane emissions from ruminal fermentation. They also noted that the inclusion level of tannins varies greatly depending on plant or grain source, geographic location, and processing method, as well as total diet composition. Similarly, Patra and Saxena [41] found that inclusion of tannins, or plant extracts containing tannins, resulted in a 50% reduction in methane production from ruminal fermentation. Observations of Jayanegara et al. [42] supported this finding noting that tannins decreased methane concentration linearly and quadratically.

The cause of methane production reduction is speculated to be attributed to the deleterious effect that tannins may have on methanogenic organisms. The deleterious effects of tannins on methanogens is due to the binding of microbial cell proteins and enzymes [43]. Condensed tannins have greater ability to bind with nutrients than hydrolysable tannins, and their extensive polymerization interferes with rumen digestion [42].

Tannins have anti-nutritional factors that can result in negative effects on grain digestibility by interacting with the starch and protein matrix. This could result in decreased dry matter intake and reduced protein and fiber digestion [40]. The degree of these effects depends on tannin concentration, as well as the type of tannin, animal species and its physiological state, and diet composition [44]. Tannins are thought to bind to macromolecules including proteins, structural components, carbohydrates, and starch which may decrease extent of ruminal digestion [45]. A study by Mkandawire et al. [46] reported a decrease in α -amylase activity caused by the presence of tannins reducing, which effectively decreased amylopectin hydrolysis. Interestingly,

when the same variety of sorghum was grown and harvested over two consecutive years during the study, there was great variation in tannin chemistry, showing the impact that environment has on the crop.

Processing and its effects on digestion

Grain processing plays a critical role in ruminal fermentation and starch utilization in feedlot cattle by altering both the site and extent of starch digestion by increasing surface area which encourages increased microbial digestion [47]. Grains respond differently to methods of processing, with wheat and barley benefiting the least. Corn and grain sorghum require the pericarp to be disrupted before it can be properly digested [48]. Responses to processing vary due to differences in grain and starch structure, as well as the interaction of the starch-protein matrix. The latter is a particular challenge in grain sorghum. To combat starch-protein matrix interference with digestibility, processing techniques that disrupt the starch-protein matrix may be utilized. Sorghum kernels contain a greater proportion of vitreous (flint) endosperm compared to corn, which is associated with lower ruminal starch availability and decreased total tract digestion [6]. Through processing methods, disruptions to the protein matrix and endosperm organization [49] may alleviate some of these disadvantages.

The impact of grain processing depends on factors including grain type, processing conditions, characteristics of the animal, and diet composition. Dry processing techniques, including grinding or dry rolling, mechanically compress or fracture the fibrous hull and pericarp of the grain. This not only increases the surface area for microbial attachment but also enables more direct access to the internal endosperm by ruminal microbes and enzymes [49]. In a study by Galyean et al. [50] total starch digestion of whole corn was 88.2%, but when dry processed to three smaller particle sizes that percentage increased to 93.5% to 94.5%. In a review of

literature, Owens et al. [51] estimated the percentage of dry rolled sorghum digested in the rumen was 69.4%. Alternatively, the ruminal digestibility of steam flaked grain sorghum was estimated to increase to 85.9%. This emphasizes the positive impacts on feed value of wet processing grain sorghum.

Wet processing methods including steam flaking and steam rolling are more extensive and require longer conditioning times. Steam flaking, the most commonly used grain processing method in U.S. feedlots [2], involves applying 3–6% water to the grain, followed by steeping for 15 minutes to 24 hours. This results in steamed flakes with moisture contents ranging from 19% to 24% [47]. These effects are by way of starch gelatinization which is described by swelling of starch granules as they absorb water, followed by disruption of the protein matrix and crystalline structure, then dissolution and diffusion of polysaccharides from ruptured granules [14].

According to the Beef Feeding Guide [52], this is what makes steam flaking the most effective processing method for grain sorghum. Conditioning times for grain sorghum range from 40 to 80 minutes compared to 20 to 60 minutes for corn. Alternatively, steam rolling can be completed in less than 15 minutes [47]. Adequate flake densities measure between 309 and 412 g/L, with optimal performance reported at 260 to 386 g/L [53,54]. In a 2016 survey of consulting feedlot nutritionists Samuelson et al. [2] reported an average flake density of 350 g/L for steam-flaked corn and 340 g/L for steam-flaked sorghum in U.S. feedlots. The benefits of steam flaking on feedlot performance are widely accepted. Zinn et al. [16] reported that replacing dry-rolled corn with steam flaked corn improved ADG by 6.3% and reduced DMI by 5%. Similarly, steam flaked grains improved ruminal, post-ruminal, and total tract starch digestion. Owens et al. [51] reported that high-moisture corn supported even greater ruminal

starch digestion than steam-flaked corn, but total tract digestibility remained similar between the two processing methods.

High-moisture ensiling and reconstitution/ensiling improve digestibility of corn and sorghum compared to dry processed grains. High-moisture processing involves harvesting grain at greater moisture levels between 24% and 33%, followed by anaerobic fermentation. Reconstitution requires rehydrating harvested dry grain to approximately 30% moisture and then fermenting it under oxygen-free conditions. Both methods significantly increase ruminal starch availability and extent of digestion. Zinn et al. [16] reported the following descending order for total tract starch digestibility for the major grain processing techniques: high-moisture grain; steam flaking; dry grinding; coarse cracking or dry rolling. When highly processed grains (flaked or ensiled) are used in feedlot diets, total tract starch digestion typically exceeds 98%, further emphasizing the value of processing for optimizing energy utilization in feedlot cattle [10].

The site of starch digestion in cattle can shift depending on the extent of grain processing. When grains are extensively processed, a greater proportion of starch is fermented in the rumen, enhancing microbial protein synthesis. However, this rapid fermentation may lead to an excessive accumulation of VFAs which in turn increases the risk of ruminal acidosis. Although ruminal fermentation plays a key role in energy absorption, enzymatic starch digestion in the small intestine is more energetically efficient than fermentation in the rumen, and significantly more efficient than digestion in the large intestine [10]. Shifting starch digestion to the small intestine is only beneficial when digestibility is not limited by factors such as particle size or insufficient digestive enzyme activity. When these limitations occur, undigested starch bypasses

the small intestine and is either fermented in the large intestine or excreted. This is commonly observed with whole or coarsely processed grains.

Grain processing methods demonstrate notable effects on performance despite having limited impact on absolute VFA concentrations. Shaw et al. [55] reported that steam flaking sorghum resulted in a significant increase in feed intake and live weight gain compared to coarse grinding or dry rolling it, while fine grinding of the grain was associated with decreased intake. The acetate to propionate (A:P) ratio varies with processing method, but overall is inversely correlated with average daily gain, feed intake, and feed efficiency. This indicates that lower A:P ratios may support more efficient energy utilization. It was also reported that changes in this ratio were predictive of performance outcomes, while Franks et al. [28] showed little evidence to support this. Although VFA concentrations in the rumen could be correlated with animal performance, they do not appear to be reliable predictors of feedlot efficiency, demonstrating their ineffectiveness when used independently of feeding trials. Franks et al. [28] This suggests that while VFA profiles offer valuable insights into fermentation dynamics and nutrient utilization, direct feeding and growth performance evaluation remains essential to accurately assess the impacts of grain type and processing method on performance outcomes.

Combination feeding of grain sorghum

As corn is the number one fed grain in beef cattle finishing diets in the U.S., most studies have compared sorghum to corn. While the benefits of feeding grain sorghum alone may be limited, there are considerable benefits when it is fed in combination with other grains. Huck et al. [56] evaluated effects of feeding combinations of steam-flaked grain sorghum (SFGS) with various forms of processed corn, including steam-flaked (SFC), dry-rolled (DRC), and high-moisture corn (HMC). Cattle were fed diets with varying proportions of these grains to assess

growth and carcass outcomes. In heifers, increasing SFGS in place of SFC led to linear decreases in average daily gain feed efficiency, and final carcass weights ($P < 0.05$), with no impact on dry matter intake, backfat, quality grade, or liver abscess rates. A diet containing 75:25 SFC:SFGS blend yielded superior performance, although it was not statistically significant. The same study reported that for steers, feeding SFC alone resulted in 7% greater ADG and feed efficiency compared to DRC or HMC. Notably, combining SFGS with DRC or HMC at a 67:33 ratio produced 5 to 6% positive associative effects for performance without compromising carcass traits. These findings align with earlier studies from Stock et al. [57], which reported improved performance when feeding grain sorghum with corn compared to when sorghum alone is fed.

Similarly, Axe et al. [58] fed steers diets of 100% wheat, 100% sorghum, and a 50:50 wheat-to-sorghum blend and evaluated their effects on ruminal fermentation, digesta flow, and site of digestion. Since wheat is a more rapidly fermentable grain, increasing its inclusion in diets lowered ruminal pH and acetate-to-propionate ratio, while increasing concentrations of L-lactate, propionate, valerate, and total volatile fatty acids. Wheat also increased ruminal liquid outflow and reduced retention time when compared to sorghum. Gross et al. [59] utilized the same wheat and sorghum diet compositions to evaluate their effects on net portal nutrient flux and found that increasing the percentage of sorghum in diets increased arterial urea nitrogen and isobutyrate levels. Arterial glucose concentrations also increased when fed sorghum. Ruminal digestion of dry matter and organic matter were greater for cattle fed diets containing sorghum than for those containing wheat. The 100% sorghum and 50:50 diets resulted in a shift of digestion to the intestines, dry matter intake declined, and starch and nitrogen digestibility were less than for the wheat-based diet. When wheat was fed, it promoted greater ruminal

fermentation, reflected in lower pH and higher volatile fatty acid production. It also prompted an increase in duodenal flow of non-ammonia nitrogen. Net energy absorption was found to be primarily from acetate and propionate (57–66%), with minimal contribution from glucose, especially in sorghum-based diets, reaffirming VFAs as the major energy substrates produced during fermentation.

A common observation among studies designed to evaluate grain mixtures is that associative effects are greatest when rapidly fermented grains are combined with more slowly fermentable grains. These combinations may optimize ruminal and post-ruminal digestion. As hypothesized by Huck et al. [56], these positive associative effects may be due to reduced acidosis risk as a result of moderating the rate of ruminal fermentation. Although intestinal starch digestion is more energetically efficient than ruminal fermentation [60], intestinal digestibility of sorghum is poor in comparison to other grains. Still, feeding grain combinations may enhance energy utilization and support microbial protein synthesis, especially for sorghum. While marbling and carcass quality generally remains unaffected [56], combining grains offers a cost-effective way to improve gain and efficiency, especially when sorghum is sold at a discount relative to corn.

***In vitro* gas production of cultures containing grain sorghum**

Livestock production contributes 14 to 15% of global greenhouse gas emissions, which are mostly caused by feed production, ruminal fermentation, animal waste, and land usage [61]. Ruminants produce 115 million tons of methane per year carried out by the microbial population involved in ruminal fermentation [61]. It is also a source of energy loss that is diverted from other biological pathways in the animal [23]. *In vitro* laboratory studies provide adequate means to measure gas production from dietary substrates without sacrificing integrity and accuracy of

the evaluation. They are less costly and labor intensive than *in vivo* studies and require fewer animals and other resources. First described by Tilley et al. [63] as a means to assess forage digestibility, *in vitro* techniques utilize ruminal fluid from fistulated donor animals, which is mixed with phosphate buffer [64] to emulate actions of saliva. Substrates are added and cultures are incubated under anaerobic conditions at 39 degrees C. Pepsin can then be added, followed by a secondary incubation period. A proper pH range of 6.7-6.9 is essential, along with adequate filtration of the ruminal fluid to separate out sediments.

Certain varieties of grain sorghum contain high tannin concentration, which can have considerable effects on gas production by ruminal fermentation modification [35]. An *in vitro* study by Jayanegara et al. [42] reported that the effects of methane reduction were greater for plants containing hydrolysable tannins than those rich in condensed tannins. An additional *in vitro* study by Salami et al [65] reported a reduction in total VFA production by consequence of a reduction in acetate production when fed 16 g/kg DM of extract from quebracho, which is a high-tannin tropical plant. These effects were not observed when the inclusion amount was less than 2 g/Kg DM. Patra and Saxena [41] have suggested that there are varying results when measuring effects of tannins due to several factors such as microbial adaptation to tannins by formation of a protective exo-layer, and modification of cell membranes.

When compared to *in vivo* techniques such as respiration capturing systems like GreenFeed systems (C-Lock Inc.), *in vitro* gas production is measured with reasonable accuracy and precision [66]. Danielsson et al. [67] reported numerous causes for variation in end-product estimates, including composition of diets fed to donor animals, amount of buffer added to the substrate, and composition of flushing gas (CO₂ vs N₂ vs H₂).

Conclusion

There is significant geographical overlap of feedlot cattle and grain sorghum production in the High Plains, yet sorghum is seldom utilized in feedlot operations. Minimizing variations in grain processing and genetic identity preservation help to alleviate the shortcomings of grain sorghum compared to corn. Increased use of grain sorghum in feedlot diets may contribute to less overall water use for feed production [68]. With net energy for gain value of just over 97% that of dry-rolled corn [10], this indicates the potential for grain sorghum to be accepted as an alternative energy source for finishing cattle, which would likely increase acceptance by cattle feeders especially when coupled with adequate processing. Sorghum's ability to grow under conditions of water stress, its antimicrobial and antioxidant properties, manipulation of site and extent of starch digestion, combination feeding benefits, and potential to decrease methane emissions from ruminal fermentation [69], are all attributes in favor of the use of grain sorghum in cattle diets. It is important to note, however, the antinutritional factors that may exist when feeding certain varieties of sorghum to ruminants due to the inhibitory effects on digestion caused by tannins. These findings provide groundwork for future efforts in optimizing grain sorghum as a feedstuff for finishing beef cattle.

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Chapter 2- Effects of feeding ground grain sorghum or dry-rolled corn on growth performance, carcass characteristics, and muscle tissue fatty acid composition of finishing beef steers.

Abstract

Feedlot cattle production makes significant contributions to agricultural economies of the High Plains, but its future is threatened by depletion of groundwater resources used for crop irrigation. Grain sorghum is broadly adapted to yield stability under water-limitation, potentially providing means for conservation of water resources while still meeting demands of the feedlot industry [1]. The objective of this study was to evaluate ground grain sorghum as an alternative to dry-rolled corn in cattle finishing diets with respect to feedlot performance and carcass characteristics. Yearling steers (n=300; 346 ± 11 kg initial body weight) were randomly allocated to feedlot pens (30 pens; 10 steers/pen) and pens were assigned randomly to dietary treatments within blocks (15 blocks). Cattle were vaccinated against viral and clostridial pathogens, treated against internal and external parasites, implanted with estradiol/trenbolone acetate, and then transitioned to finishing diets using a series of three step-up diets over three weeks. Steers were fed once daily and feed amounts were allocated to result in only traces of feed remaining in the bunk the following day. Fresh fecal pats were collected from pen surfaces on day 120 and analyzed for concentrations of acid insoluble ash, starch, neutral detergent fiber (NDF), and organic matter to estimate diet digestibility. Steers were harvested after 182 d on feed and DM intake, average daily gain (ADG), feed efficiency (G:F), and carcass weight were determined. Carcass data were collected after 96 h of refrigeration. Muscle samples from the *infraspinatus* and *semitendinosus* were collected from a subset of 32 carcasses to be analyzed for

concentrations of fatty acid methyl esters. Data were analyzed as mixed models with diet as a fixed effect and block as a random effect. Cattle fed corn consumed less DM but had greater ADG and G:F compared to those fed sorghum (DM intake: 10.93 vs 11.54 kg/d; ADG: 1.61 vs 1.46 kg/d; G:F: 0.1533 vs 0.1327; $P < 0.01$). Corn and sorghum diets yielded similar digestibilities ($P > 0.10$) of organic matter (79.0 vs 78.3%, SEM=2.29) and starch (91.4% vs 88.5%, SEM=1.85). Calculated NEm (2.24 vs 2.08 Mcal/kg) and NEg (1.55 vs 1.42 Mcal/kg) were greater for corn compared to sorghum ($P < 0.03$). Heavier carcasses with greater marbling and superior quality grades were observed for cattle fed corn compared to those fed sorghum, resulting in \$112 greater carcass value for cattle fed corn ($P < 0.01$). Meat tissues from corn-fed steers were characterized by greater concentration of total fatty acids (FA) and monounsaturated FA, specifically in the *infraspinatus* ($P < 0.05$). Meat tissues from grain sorghum-fed steers contained greater polyunsaturated FA (PUFA) concentrations in the *semitendinosus* ($P < 0.01$). Further efforts to improve feed value of grain sorghum through processing methods and(or) selective breeding are warranted for sorghum to be a viable alternative to corn in finishing cattle diets for today's beef market.

Keywords: milo, digestibility, feedlot, carcass

Introduction

The High Plains region of the US is a bountiful crop producing area. Much of this cropland is irrigated from water supplied by the Ogallala aquifer. Irrigation has allowed crops to be grown across a wide range of soil types and climates that would otherwise be unsuitable for farming most crops. Although, this finite source of water is threatened as it is being withdrawn at a rate that exceeds its natural ability to replenish. A study by Deines et al. [2] concluded that 24% of irrigated land that utilizes water from the Ogallala aquifer may be unable to support irrigated crops by 2100. The area of the High Plains that produces sorghum is referred to as the “Sorghum Belt” and it ranks first as the largest producer of grain sorghum in the world [3]. Grain sorghum thrives in this region, as it is broadly adapted to semi-arid climates due to its extensive root structure, reduces its need for irrigation [1].

Feedlot cattle production is a major contributor to the agricultural economies of the High Plains, but its future is threatened indirectly by depletion of groundwater resources for crop production. Corn is the most widely utilized grain used in feedlot diets followed by grain sorghum [4]. It has been long accepted that grain sorghum is inferior to corn when fed to beef cattle; however, grain sorghum’s ability to produce grain under drought conditions may provide means for conservation of water resources while still meeting demands of the feedlot industry [1]. After years of efforts to improve nutritive value of grain sorghum, this study aimed to evaluate feeding ground grain sorghum in beef finishing diets as an alternative to dry-rolled corn with respect to its impacts on feedlot performance, carcass characteristics, and muscle tissue fatty acid profiles.

Materials and Methods

All procedures in this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee.

Experimental design and randomization to treatments

A randomized complete block design (15 blocks of two treatments) was conducted at the Kansas State University Southeast Research and Extension Center between March and September of 2024. Black-hided, crossbred yearling steers ($n=300$; 346.3 ± 11.11 kg initial body weight) were procured from a rye grazing operation in Lyons, Kansas and transported 323 km to the research center. Upon arrival, cattle were placed into pens with access to hay and water and allowed 24 hours rest before processing. At induction, cattle were identified using their existing ranch tags and with individually numbered radio frequency identification ear tags; vaccinated (Pyramid® 5 +Presponse®; Boehringer Ingelheim Animal Health USA Inc., Duluth, GA); treated for internal and external parasites (Valbazen® drench and Dectomax® injectable; Zoetis Animal Health, Parsippany, NJ); implanted with long-acting steroidal implants (Revalor®-XS; Merck Animal Health, Rahway, NJ), and weighed to determine an initial weight using a hydraulic restraining chute equipped with an electronic scale.

Cattle were randomly allocated to feedlot pens (10 animals/pen) based on order of processing, such that the first 20 animal processed were allocated to block one, the second twenty animals to block two, and so on until all 15 blocks were complete. Pens within block were then allocated randomly to treatments. Cattle were housed in open, earthen-surfaced feedlot pens measuring 15.24 x 30.48 meters. Each pen of cattle had access to approximately 15 meters (150 cm/ steer) of feed bunk space. A concrete apron extended from the bunk

approximately 3.5 m into the pen. Water was made available to animals through automatic, energy-free ball waterers shared between two adjacent pens.

Dietary treatments

Dietary treatments consisted of finishing diets containing grain sorghum or corn. Corn was dry rolled to a mean geometric particle size of 2,510 μm . Sorghum grain was ground to a mean geometric particle size of 588 μm . Corn utilized was a Channel[®] (Bayer, Leverkusen, Germany) variety, and grain sorghum was of non-specific variety and unknown origin, procured from the local elevator in Bartlett, Kansas. Cattle were transitioned to their final finishing diets using a series of three transition diets (7 d each) containing progressively more concentrate and less corn silage (60, 44, and 28% silage, DM). Final finishing diets contained 12% silage, 79.37% grain, and the balance as supplement. Supplements provided monensin at 36 mg/kg diet DM (Rumensin[®]; Elanco USA, Greenfield, IN) and 8 mg/kg diet DM of tylosin (Tylan[®]; Elanco USA, Greenfield, IN). Additionally, ractopamine hydrochloride (Optaflexx 500[®]; Elanco USA, Greenfield, IN) was incorporated into the supplement at the rate of 300 mg/animal daily for the final 37 days on feed. Diets were fed once daily and feed bunks were scored daily and feed amounts were allocated to result in only traces of feed remaining in the bunk the following day. Dry matter contents of ingredients were determined weekly using composites of each ingredient. Unconsumed feed was removed from feed bunks every Monday and Thursday and dried at 55 degrees C for 48 h, making it possible to determine actual DMI for each 3- or 4-day interval.

Feedlot performance

Individual body weights were measured on days 1, 28, and 56 of the study to calculate dry matter intake (DMI), average daily gain (AGD), and feed efficiency (G:F). Thereafter, pens of cattle (n=10) were weighed on a large platform scale. On days 1, 28, 56, 84, 112, 140, and

168, three blocks of cattle (i.e., 6 feedlot pens) were relocated to a separate section of the feedlot that was equipped with a dual comb laser spectroscopy system for estimation of enteric methane and ammonia emissions. Emissions data are presented in a separate manuscript.

Net energy and nutrient concentrations of treatments

Fecal samples were collected from 10 manure pats per pen 24 hours after feeding. Samples were mixed thoroughly, heat sealed in Mylar bags (DuPont Tyvek[®], Richmond, VA), and frozen until ready for shipment to Servitech Inc. laboratories (Dodge City, KS) where they were analyzed for concentrations of acid detergent insoluble ash (internal marker), starch, crude protein (CP), dry matter, organic matter (OM), and NDF. Total mixed ration (TMR) samples of each diet also were sent to the laboratory and subjected to the same analyses. Performance adjusted energy content of the corn and grain sorghum were calculated through an iterative procedure using California net energy system [5] which included DMI values of cattle as well as ingredient nutrient concentrations, and energy calculations for medium-framed yearling steers as described in Nutrient Requirements of Beef Cattle [6].

Carcass measurements

At the end of the finishing period (182 days), pens of cattle were loaded onto trucks and transported 561 km to a commercial abattoir (Tyson Fresh Meats, Holcomb, KS). Final body weight was estimated as hot carcass weight divided by a common dressing percentage of 61%. Hot carcass weight and the incidence and severity of abscessed livers were recorded the day of harvest. Livers were scored in accordance with the Elanco liver abscess scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health: 0=no abscess; A-=one or two small abscesses or inactive scars; A0=one or two large abscesses or several small abscesses; A+=multiple large abscesses). After approximately 96 h of refrigeration, 12th rib subcutaneous

fat thickness; marbling score; percent kidney, pelvic, and heart fat (KPH); longissimus muscle area (LMA); USDA quality grade; and USDA yield grade were acquired via a camera imaging system (VBG 2000; E+V Technology GmbH & Co. KG, Oranienburg, Germany). Total carcass value was determined for carcasses using base price, as well as discounts and premiums reported by USDA [7] during the week of harvest.

Fatty acid analyses

After approximately 96 h of refrigeration, muscle tissue samples were collected from a subset of 32 carcasses (16 per treatment) during fabrication at the packing plant, to be analyzed for concentrations of fatty acid methyl esters. Tissue samples were taken from both the *infraspinatus* and *semitendinosus* muscles of each carcass. These tissue samples, along with four ground corn and four ground sorghum grain samples (weekly samples collected during live animal feeding), and 1 corn total mixed ration (TMR) and 1 sorghum TMR underwent analysis to determine concentration of fatty acid methyl esters as described by O'Fallon et al. [8]. The grain and TMR samples were ground through a 1-mm screen using a Thomas-Wiley Laboratory Mill, Model 4 (Thomas Scientific, Swedesboro, NJ) and stored in zipper storage bags for preservation. Then, 1.0 gram (DM basis) of corn, sorghum, or freeze-dried tissue sample were transferred to 16 x 125 mm Pyrex® screw-cap culture tubes to allow for proper permeation, dissolution, and hydrolyzation of the samples according to the procedure described by O'Fallon et al. [8]. After extraction and derivatization of fatty acid methyl esters (FAME), tubes were centrifuged for five minutes at 1500 x g and the final hexane supernatant layer was transferred to a gas chromatography (GC) vial. Vials were capped with screw top lids and stored at -20° C until analysis. Concentrations of FAME were analyzed and quantified through gas chromatography on an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA)

using an SP-2560 fused silica capillary column (100m x 0.25mm x 0.2µm film thickness; Supelco, St Louis, MO) with hydrogen as carrier gas. Fatty acids were identified by comparing their retention times with the FAME 37 standard (Supelco 37 Component FAME Mix; Sigma-Aldrich, St Louis, MO, USA).

Statistical analysis

Performance data were analyzed as mixed models (SAS, ver 9.4) where pen was the experimental unit, treatment was the fixed effect, replicate was the random effect, and days on feed was the repeated measure where appropriate. Compound d symmetry was utilized as the covariance structure. For carcass data, models included treatment as fixed effect and replicate as the random effect. Incidence and severity of abscessed livers, USDA quality grade, and USDA yield grade were analyzed as multinomial distributions.

Fatty acid content data from grains and muscle tissues were analyzed separately as mixed models (SAS, ver 9.4) where animal was the experimental unit, treatment was the fixed effect and replicated was the random effect. For nutrient digestibilities and energy content of the grains, grain type was included as fixed effect and replicate as random effect. Statistical significance was declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P < 0.10$. Least-squares means were separated using the predicted difference function of SAS.

Results

One steer in the corn treatment group was removed from study and subsequently died. Cause of death at necropsy was inconclusive. Calculations for feedlot performance of the pen were based on nine total steers rather than ten.

Nutrient digestibility and fatty acid content of grains

Nutrient digestibility of dry-rolled corn and ground grain sorghum were analyzed via fecal samples using ADIA as an internal marker and are presented in Table 1.5. The grains yielded very similar component digestibilities, resulting in differences in digestibility of starch, CP, OM, or NDF between the grains ($P < 0.1$). Starch digestibility of grain sorghum was found to be 97% that of the starch digestibility of corn.

There was no difference in amount of total FA between grains ($P > 0.10$). There also were no differences in amount of SFA, PUFA, or omega-6 FA between corn and grain sorghum ($P > 0.10$). There was, however, a greater amount of omega-3 FA present in grain sorghum than corn ($P < 0.01$).

Feedlot performance

There were differences observed for ADG, DMI, and G:F between the two dietary treatments ($P < 0.01$). Feedlot performance data are presented in Table 1.2. Body weights of steers fed the grain sorghum diet were consistently numerically decreased compared to the steers fed the corn diet at each of the 28-d periods when pen weights were recorded. This resulted in an effect of grain and day. An interaction between grain and day was also observed for body weights ($P < 0.01$). The dry-rolled corn group had a numerically increased ADG (Figure 1.2) compared to the ground grain sorghum group at every time point except for day 84, when they measured 1.32 and 1.46 kg/d respectively; however, this difference was not significant ($P > 0.10$). This resulted in an effect of grain and day, as well as an interaction of grain by day ($P < 0.01$). Although the ground grain sorghum steers had a decreased ADG, they consumed more feed (Figure 1.3) compared to dry-rolled corn steers ($P < 0.01$) resulting in an effect of grain and day. This corresponds with the greater overall G:F (Figure 1.4) for the dry-rolled corn treatment

group ($P < 0.01$) compared to the sorghum treatment which resulted in effects of grain and day, as well as an interaction between the two.

Carcass characteristics

Carcass characteristics results are presented in Table 1.3. There were no differences between treatments observed for 12th rib fat thickness or KPH ($P > 0.10$).

Greater HCW, marbling score, and REA were observed for the dry-rolled corn treatment group carcasses ($P < 0.01$).

There was a tendency ($P = 0.057$) for more carcasses from cattle fed corn to grade Prime compared to those fed sorghum, which translated to 4.6% more carcasses than sorghum. There was a difference observed for percentage of Premium Choice graded carcasses, with more corn carcasses grading in the category ($P < 0.01$) than sorghum carcasses. Most carcasses were graded Low Choice with a tendency for more sorghum carcasses to be graded in the category (48.0% and 35.6% of carcasses for sorghum and corn, respectively; $P = 0.055$). More carcasses from cattle fed sorghum graded Select compared to those fed corn ($P < 0.05$).

There were no differences observed for yield grade ($P > 0.10$) and the majority of carcasses were awarded yield grades two or three. Incidence of abscessed livers also was not different ($P > 0.10$), and overall incidence was relatively low.

Calculated carcass value for the sorghum treatment averaged \$2,379.1 which was less than that of carcasses from cattle fed corn at \$2,491.6 ($P < 0.01$). The \$112.5 difference can be attributed to the greater base pay from increased HCW, as well as greater premiums and fewer discounts associated with quality grades in carcasses from cattle fed corn.

Calculated energy density of grains

Energy values for both dry-rolled corn and ground grain sorghum were calculated from the diets and cattle performance observed in the feeding trial and are presented in Table 1.4.

Dry-rolled corn was found to contain more energy than ground grain sorghum, which is consistent with what was previously known about the grains [6]. Calculated energy values for metabolizable energy (ME), net energy for maintenance (NE_m), and net energy for gain (NE_g) were greater for dry-rolled corn than ground grain sorghum ($P < 0.01$). When comparing net energy of gain, ground grain sorghum was calculated to be 92% that of dry-rolled corn.

Fatty acid content of muscle tissues

Total concentration of fatty acids measured in the *infraspinatus* and *semitendinosus* are presented in Figure 1.5 and were different for steers fed dry-rolled corn or ground grain sorghum ($P < 0.05$). Individual fatty acids (FA) are expressed as percentages of total fatty acid content of muscle tissue samples and are displayed in Table 1.7. *Infraspinatus* tissue contained more fatty acids than *semitendinosus* tissue ($P < 0.01$). There was no difference in the total amount of saturated fatty acids (SFA) measured in *infraspinatus* or *semitendinosus* tissues ($P = 0.094$). Monounsaturated fatty acids (MUFA) occurred most in the *infraspinatus* tissue ($P < 0.01$). *Semitendinosus* muscle tissue contained greater proportions of polyunsaturated fatty acids (PUFA), omega-6 FA, and omega-3 FA ($P < 0.01$).

Transitioning to differences in grain treatment of the muscle FA content, there was greater concentration of total fatty acids in muscle tissues from the corn treatment group ($P < 0.05$). There was no difference in grain type with respect to total amount of saturated fatty acids (SFA) in *infraspinatus* or *semitendinosus* muscles ($P > 0.10$). Monounsaturated fatty acids were more abundant in muscles from cattle fed grain sorghum ($P < 0.05$). Muscle tissues from corn-

fed cattle, contained the greatest amounts of PUFA ($P < 0.01$) compared to those from cattle fed sorghum. There were grain by treatment interactions for individual FA of C15:0, C17:0, C18:2c9t11n6, and C24:0 ($P < 0.05$).

Discussion

Steers fed ground grain sorghum for 182 d consumed more feed but gained less weight, resulting in reduced feed efficiency compared to steers fed dry-rolled corn. Overall, corn-fed cattle gained 10.27% faster than those fed grain sorghum. Effects of grain, day, and grain $\times$ day interactions for ADG, DMI, and G:F appear to be driven by cattle increasing or maintaining intake over time while gaining weight at a slower rate—an effect that was more pronounced in the sorghum group. Fox et al. [9] reported that DMI decreases 2.7% for each 1% increase in body fat. Figure 1.1 depicts the trend of the corn-fed steers gaining weight quicker than the grain sorghum fed steers, from which we can speculate that they gained fat quicker as well. Along with the elevated energy content of corn grain, this may help explain why the corn-fed treatment group had reduced DMI compared to cattle fed sorghum.

Reduced live body weight and ADG in sorghum-fed steers were reflected in carcass traits, with lighter HCW and leaner carcasses compared to cattle fed corn. Corn-fed steers produced larger ribeyes, though yield grade distribution was similar between treatments ($P > 0.10$). Condemned livers are an area of great economic loss at meat packing plants [10]. Liver abscess incidence did not differ between treatments

There was a major difference in carcass value observed due to differences in HCW, and premiums and discounts associated with yield grade and quality grades. Corn-fed steer carcasses averaged \$112.50 greater total value compared to those from sorghum-fed steers. This

is consistent with Schake [11], who reported that lighter HCW and reduced dressing percentage resulted in reduced carcass value for sorghum-fed cattle compared to corn-fed. Our results reflect reduced ADG, lighter carcasses, and decreased carcass value in sorghum-fed steers, though unlike Schake [11], we observed increased marbling scores for corn-fed cattle ($P < 0.01$) without differences in 12th rib fat thickness ($P > 0.10$).

Our grain energy calculations aligned with observations of Fox [9], who reported greater net energy content in corn-based diets compared to sorghum-based diets. The values that we calculated for ground grain sorghum including ME, NE_m , and NE_g , were slightly lower than the values presented in NASEM [6]. These discrepancies could be due to different growth conditions of the crop, degree of processing, grain variety, or overestimation/underestimation of energy content of other diet components. Given the NE_g of ground grain sorghum diet was approximately 92% that of corn diet, it is likely sorghum-fed steers increased DMI in an attempt to compensate for lower energy density but were ultimately unable to match the growth performance of the corn group, thus finishing 15 kg lighter. Similar trends were reported by Goldy [12], where cattle fed various grain sorghum varieties underperformed compared to corn-fed cattle. The author also observed reduced weight gain, decreased fat deposition, and reduced energy retention (Mcal) in cattle finished on grain sorghum, findings that are consistent with our observations.

Despite differences in energy content, overall nutrient digestibility was comparable. Grain sorghum is known for decreased starch digestibility compared to other cereal grains [13] and our data supported this, with digestibility of starch for grain sorghum reaching 97% that of corn ($P > 0.10$). Numerically decreased digestibilities of CP, OM, and NDF, though not statistically different, also were observed for grain sorghum compared to corn. These modest

differences in nutrient digestibilities likely contributed to reduced energy content of diets and overall observed carcass differences.

The *infraspinatus* and *semitendinosus* muscle tissues were selected for analysis because of their differing fat content. Sahar and Dufour [14] reported that in Charolais cattle, the *infraspinatus* contains 4.8% fat while the *semitendinosus* contains 1.6%. As expected, the *infraspinatus* muscle tissue contained greater total FA than *semitendinosus* muscle tissue ($P < 0.01$). Elevated PUFA concentrations, when expressed as a percent of total FA content, were observed in the leaner *semitendinosus*, compared to the more marbled *infraspinatus* ($P < 0.01$). This is consistent with Wood et al. [15], who reported that ruminants tend to conserve PUFA in muscle tissue rather than in adipose tissue.

Grain sorghum contained a greater total amount of fatty acids ($P < 0.01$), while no measurable differences were detected in MUFA concentrations between corn and sorghum ($P > 0.10$). Fatty acid composition of the grains reflected typical plant lipid profiles reported by Mehmood et al. [18], where C18:2n-6 (linoleic acid) was present in the greatest concentration, followed by C18:1c9 (oleic acid) and C16:0 (palmitic acid). These FA were numerically the most abundant in both muscle tissues across both grain treatments in our study.

Muscle tissues from the corn treatment group contained a greater concentration of total FA ($P < 0.01$). This may be attributed to the elevated HCW and marbling score of carcasses from cattle fed corn ($P < 0.01$). Muscle tissues from corn-fed cattle contained greater proportions of MUFA in the *infraspinatus* ($P < 0.01$), which is consistent with Huerta-Liedenz et al. [16], who reported that MUFA concentrations increase as carcass fatness and marbling increase. While there was no difference in amount of PUFA present in the grains, the sorghum treatment muscles contained greater concentrations of PUFA compared to corn treatment

muscles ($P < 0.01$). In our study cattle consumed rye pasture prior to entering the feedlot. Forages contain greater amounts of omega-3 and PUFA compared to concentrate diets [15]. As the steers grow and accumulate more fat, PUFA that were accumulated during the backgrounding phase is diluted with accretion of additional body fat. Our carcass data support this, as the grain sorghum treatment produced leaner carcasses with reduced intramuscular fat, perhaps contributing to greater proportions of PUFA in muscle tissue for cattle from this treatment. This is of note as PUFA are more susceptible to oxidative breakdown, affecting shelf life and spoilage of meat [17].

Conclusion

Steers fed a finishing diet containing dry-rolled corn outperformed those fed ground grain sorghum in terms of gain, efficiency, and overall carcass value. These differences may be attributed to the lower net energy content of grain sorghum, which was approximately 92% that of corn. These observations emphasize the importance of improving feed value of grain sorghum through methods such as processing or selective breeding to become a viable alternative to corn in finishing cattle diets.

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Table 2.1 Characterization of dry-rolled corn and ground grain sorghum fed in finishing steer diets for 182 d.

Item	Treatment		SEM ¹	P-value
	Corn	Sorghum		
Digestibility ² , %				
Starch	91.4	88.8	1.31	0.189
Crude protein	68.7	68.5	1.97	0.951
Organic matter	79.0	78.3	1.61	0.764
Neutral detergent fiber	45.9	45.0	3.54	0.851
Fatty acid ³ , µg/g				
C16:0	226.85	282.04	23.043	0.125
C18:0	33.08	28.00	2.451	0.177
C18:1c9	457.04	593.39	41.131	0.044
C18:2n6	882.05	869.61	92.220	0.926
C18:3n3	24.38	39.84	2.464	<0.01
Total fatty acids	1623.41	1810.87	160.880	0.427
Saturated fatty acids	259.93	310.04	25.482	0.198
Monounsaturated fatty acids	457.04	593.39	41.131	0.044
Polyunsaturated fatty acids	906.44	909.44	94.654	0.983
Particle size distribution ⁴ , µm				
D _{gw}	2,510	588	-	-
S _{gw}	2.61	2.71	-	-

¹ Standard error of mean.

² Analyzed from fecal grab samples using acid detergent insoluble ash (ADIA) as internal marker.

³ Analyzed via gas chromatography on Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA), with hydrogen as carrier gas and utilizing fatty acid methyl ester 37 standard (Supelco 37 Component FAME Mix; Sigma-Aldrich, St Louis, MO, USA).

⁴ Particle size analyzed on four samples of dry-rolled corn and four samples of ground grain sorghum using a series of 13 sieves on a Ro-tap (W.S.Tyler, Mentor, OH) shaker; D_{gw}: mean geometric diameter; S_{gw}: standard deviation of geometric diameter.

Table 2.2 Composition of dietary treatments containing dry-rolled corn or ground grain sorghum fed to finishing steers for 182 d (DM basis).

Item	Treatment ¹	
	Corn	Sorghum
Ingredient, %		
Dry-rolled corn	79.37	-
Ground grain sorghum	-	79.37
Corn silage	12.00	12.00
Supplement ²	8.63	8.63
Analyzed nutrient composition ³ , %		
Crude protein	11.60	11.50
Neutral detergent fiber	13.80	12.50
Organic matter	4.20	4.60
Starch	68.90	68.50

¹ Treatment amounts fed once daily based on DM of weekly ingredient samples and slick bunk management, such that only crumbs remained at the following feeding.

² Supplement contained soybean meal, urea, minerals, vitamins, and feed additives and was formulated to provide (total diet dry matter basis) 0.7% calcium, 0.7% potassium, 0.3% salt, 33 g/ton monensin, 8 g/ton tylosin, and the following added amounts of trace elements and vitamins: 2204.62 IU/ kg vitamin A; 15.43 IU/ kg vitamin E; 0.1 ppm cobalt; 10 ppm copper; 0.5 ppm iodine; 40 ppm manganese; 0.2 ppm selenium, and 40 ppm zinc. Ractopamine hydrochloride was included at 25 g/ton of the diet dry matter for the final 37 days on feed.

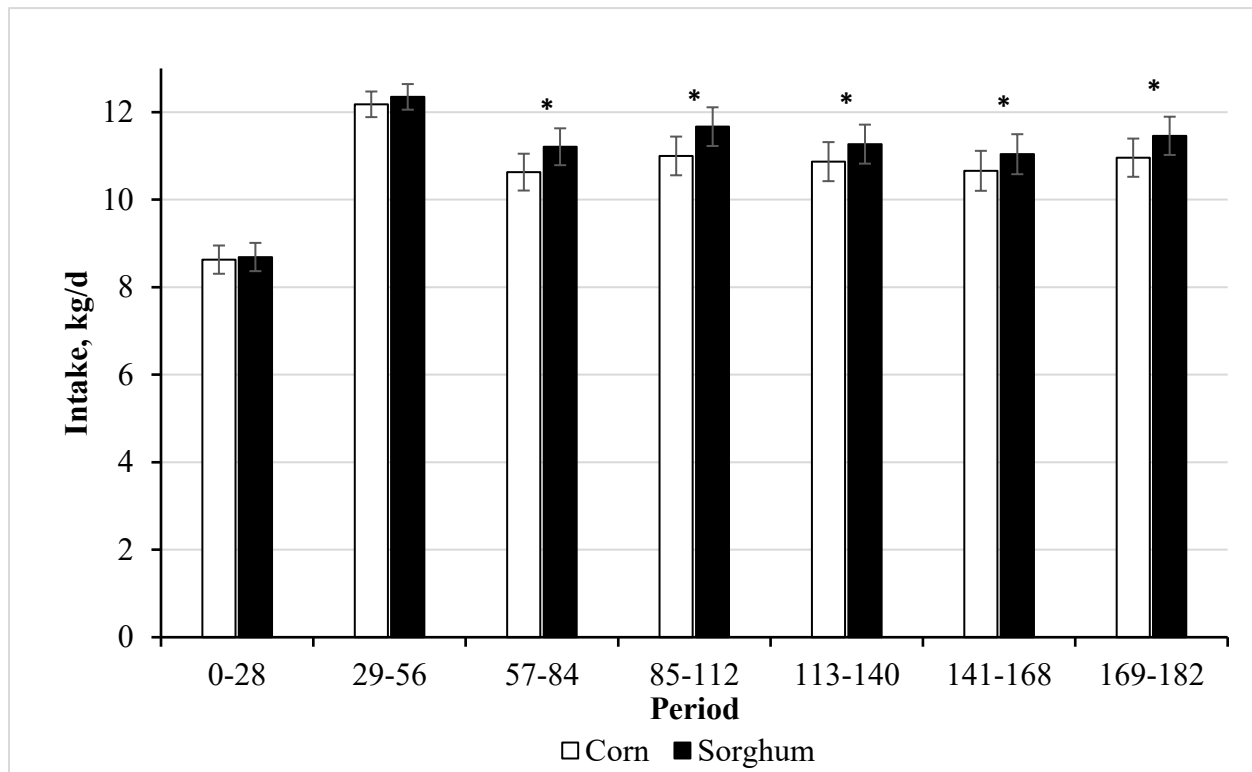
³ Analyzed from total mixed rations.

Table 2.3 Feedlot performance of steers fed dry-rolled corn or ground grain sorghum for 182 d.¹

Item	Treatment			P-value		
	Corn	Sorghum	SEM	Grain	Day	Grain × Day
Dry matter intake, kg/d	10.8	11.2	0.360	<0.01	<0.01	0.230
Average daily gain, kg	1.74	1.67	0.067	<0.01	<0.01	<0.01
Gain:Feed	0.166	0.153	0.002	<0.01	<0.01	<0.01

¹ Interim weights were recorded for each pen of cattle in 28-day increments, except for day 182 which followed a 14-day increment in order to coordinate with harvest facility. Day 182 data are based on carcass adjusted weight which was calculated utilizing the following equation: carcass adjusted weight=(average carcass weight)/(dressing percentage).

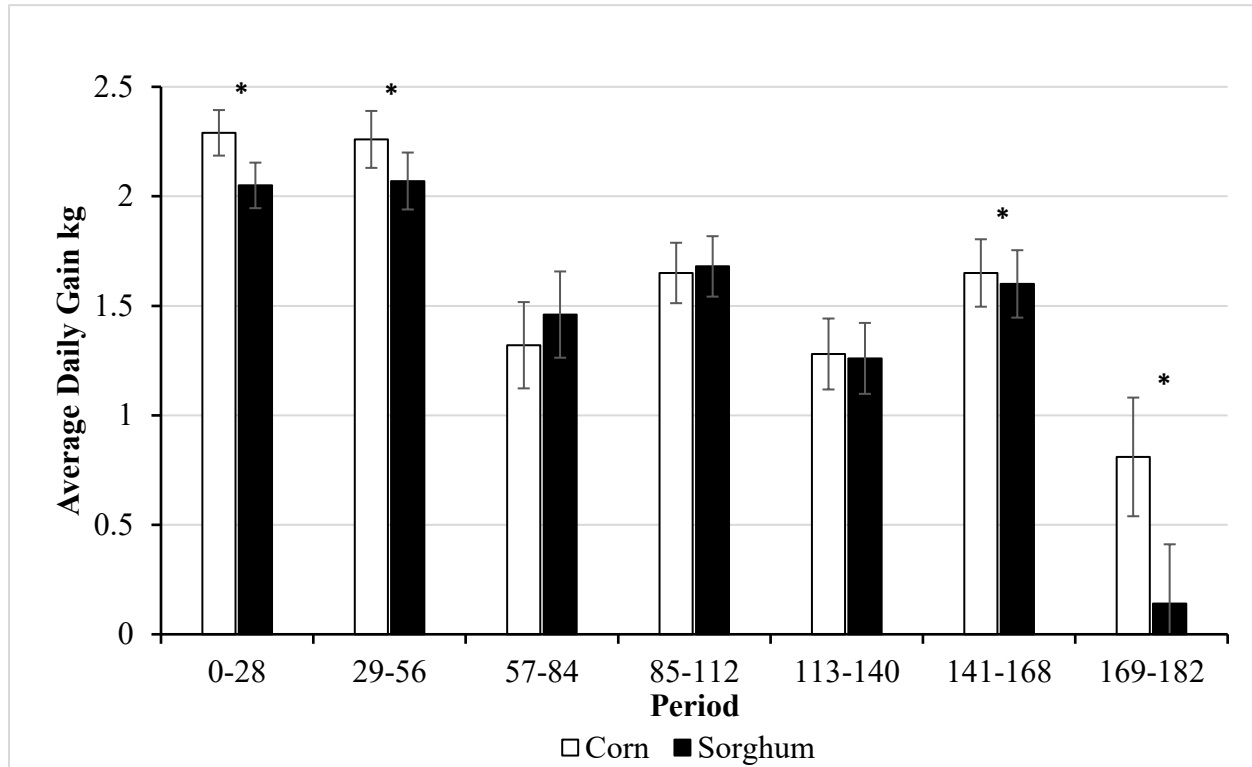
Figure 2.1 Dry matter intake by steers fed dry-rolled corn or ground grain sorghum finishing diets for 182 d.^{1,2}



¹ * indicates values within period are different.

² Effect of diet: ($P < 0.01$); effect of day: ($P < 0.01$); effect of diet x day: ($P > 0.10$).

Figure 2.2 Average daily gain of steers fed dry-rolled corn or ground grain sorghum finishing diet for 182 d.^{1,2,3}

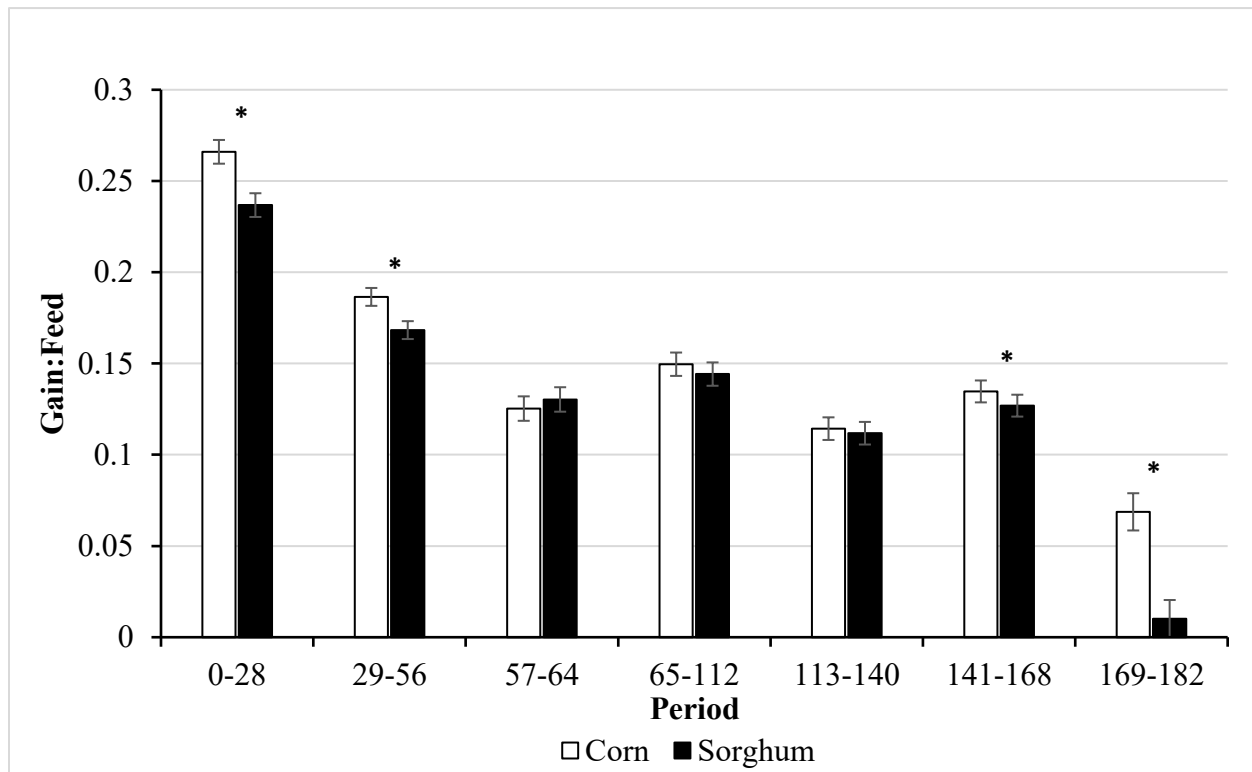


* indicates values within period are different.

² Effect of diet: ($P < 0.01$); effect of day: ($P < 0.01$); effect of diet x day: ($P < 0.01$).

³ Interim weights were recorded for each pen of cattle in 28-day increments for performance calculations, except for day 182 which followed a 14-day increment in order to coordinate with harvest facility. Day 182 data are based on carcass adjusted weights which were calculated utilizing the following equation: carcass adjusted weight=(average carcass weight)/(0.61).

Figure 2.3 Efficiency of feed utilization by steers fed dry-rolled corn or ground grain sorghum finishing diets for 182 d.^{1,2}



* indicates values within period are different.

² Effect of diet: ($P < 0.01$); effect of day: ($P < 0.01$); effect of diet x day: ($P < 0.01$).

Table 2.4 Carcass characteristics of steers fed dry-rolled corn or ground grain sorghum-based finishing diet for 182 d.

Item	Treatment		SEM ¹	P-value
	Corn	Sorghum		
Hot carcass weight, kg	398.2	383.5	7.12	<0.01
Marbling score ²	519	467	8.00	<0.01
12 th rib fat thickness, cm	1.4	1.4	0.02	0.188
Longissimus muscle area, cm ²	85.3	83.5	0.10	<0.01
KPH ³ , %	1.9	1.9	0.02	0.677
Quality grade, %				
Prime	5.3	0.7	1.59	0.057
Premium Choice	47.6	29.3	3.89	<0.01
Low Choice	35.6	48.0	4.19	0.055
Select	11.5	21.3	3.08	0.034
Sub-Select	0.0	0.7	0.47	0.334
Yield grade, %				
Grade 1	1.4	0.0	0.68	0.165
Grade 2	24.4	30.0	3.99	0.335
Grade 3	52.8	54.0	4.91	0.867
Grade 4	15.3	20.1	3.50	0.280
Grade 5	1.3	0.7	0.80	0.564
Abscessed livers ⁴ , %	8.7	6.0	2.03	0.314
Total carcass value ⁵ , \$	2,491.6	2,379.1	19.06	<0.01

¹ Standard error of mean

² Slight amount of marbling, 300 to 399; Small amount of marbling, 400 to 499; Modest amount of marbling, 500 to 599; Moderate amount of marbling, 600 to 699.

³ Kidney, pelvic, and heart fat

⁴ Elanco scoring system (Elanco US, Inc., Greenfield, IN); 0 for no abscess; A- for one or two small abscesses or inactive scars; A for one or two large abscesses or several small abscesses; A+ for multiple large abscesses.

⁵ Based on average price of premiums and discounts reported by USDA on Sept. 13, 2024 (www.ams.usda.gov/mnreports).

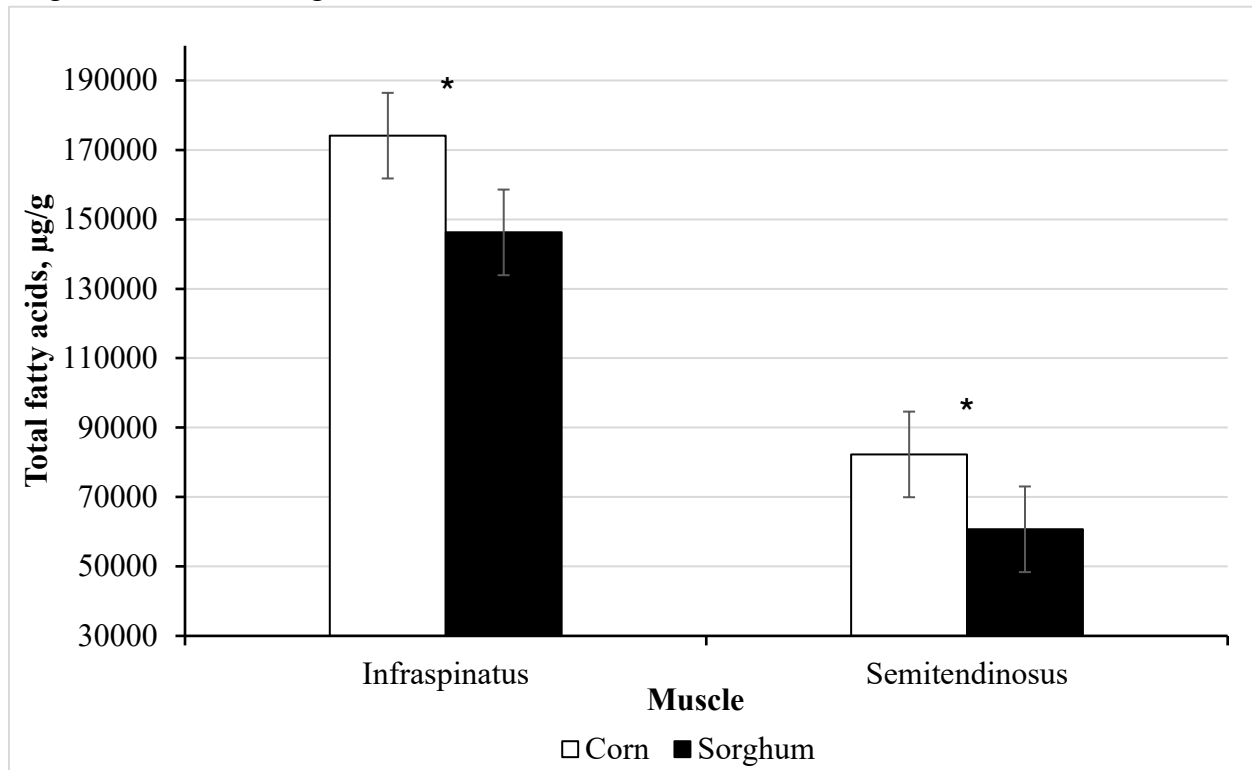
Table 2.5 Calculated energy density of dry-rolled corn and ground grain sorghum fed to finishing steers for 182 d.¹

Item, Mcal/kg	Treatment		SEM²	P-value
	Corn	Sorghum		
Metabolizable energy	3.25	3.06	0.32	<0.01
Net energy for maintenance	2.24	2.08	0.02	<0.02
Net energy for gain	1.55	1.42	0.03	<0.03

¹ Performance-adjusted energy values calculated via procedure described by Knox et al. (1973) using grain nutrient concentrations and energy calculation for medium-framed yearling steers reported by NASEM (2016).

² Standard error of mean.

Figure 2.4 Total fatty acids in *infraspinatus* or *semitendinosus* muscles of beef steers fed corn or sorghum-based finishing diets for 182 d.¹



¹ Analyzed via gas chromatography on Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA), with hydrogen as carrier gas and utilizing FAME 37 standard (Supelco 37 Component FAME Mix; Sigma-Aldrich, St Louis, MO, USA).

² * indicates values within muscle are different.

³ Effect of grain: ($P < 0.05$); effect of muscle: ($P < 0.01$); effect of grain x muscle: ($P > 0.10$).

Table 2.6 Fatty acid methyl esters (FAME) expressed as a percentage of total fatty acids in infraspinus and semitendinosus tissues from cattle fed corn or sorghum.

Item ¹	Infraspinus		Semitendinosus		SEM ²	P-value ³		
	Corn	Sorghum	Corn	Sorghum		Grain	Muscle	GxM
C12:0	0.024	0.018	0.001	0.002	0.0008	0.661	<0.01	0.559
C13:0	0.350	0.421	0.888	0.920	0.0703	0.364	<0.01	0.797
C14:0	3.256	3.104	2.868	2.497	0.1457	<0.05	<0.01	0.308
C14:1	0.853	0.774	0.918	0.723	0.0651	<0.01	0.890	0.242
C15:0	0.480	0.479	0.438	0.343	0.0306	0.076	<0.01	<0.05
C15:1	0.002	0.000	0.000	0.000	0.0021	0.347	0.348	0.348
C16:0	27.602	27.518	29.442	28.828	0.5145	0.485	<0.01	0.595
C16:1	0.697	0.163	4.422	3.989	0.1936	<0.01	<0.01	0.743
C17:0	0.502	0.716	1.258	0.123	0.0974	0.606	<0.01	<0.05
C17:1	1.312	1.209	0.622	0.323	0.0652	<0.01	<0.01	0.086
C18:0	13.139	13.807	11.737	13.807	0.4928	0.170	<0.01	0.681
C18:1t9	1.599	2.052	1.030	1.329	0.1817	<0.05	<0.01	0.662
C18:1c9	46.669	45.175	37.852	36.634	0.9352	0.090	<0.01	0.856
C18:2c9t11n6	0.104	0.126	0.030	0.005	0.0154	0.927	<0.01	<0.05
C18:2n6	0.671	1.067	4.587	5.623	0.4278	<0.05	<0.01	0.235
C20:0	0.053	0.030	0.004	0.000	0.0060	<0.05	<0.01	0.109
C20:1c11	0.208	0.204	0.098	0.072	0.0165	0.141	<0.01	0.220
C18:3n3	0.211	0.280	0.125	0.274	0.0171	<0.01	<0.01	0.014
C21:0	0.267	0.255	0.083	0.044	0.0235	0.228	<0.01	0.518
C20:2c11c14	0.112	0.124	0.030	0.031	0.0154	0.593	<0.01	0.668
C22:0	0.034	0.023	0.000	0.001	0.0063	0.300	<0.01	0.230
C20:3c8c11c14n6	0.271	0.370	0.412	0.584	0.0471	<0.01	<0.01	0.347
C23:0	0.932 ^c	1.212 ^c	1.592 ^b	2.204 ^a	1.5923	<0.01	<0.01	0.199
C20:4c5c8c11c14n6	0.014 ^c	0.007 ^b	0.004 ^a	0.003 ^a	0.0054	0.111	<0.01	0.178
C24:0	0.0004 ^c	0.012 ^c	0.564 ^b	0.912 ^a	0.0777	<0.01	<0.01	<0.01
C20:5n3	0.019	0.019	0.000	0.001	0.0048	0.889	<0.01	0.844
C24:1c15	0.182	0.253	0.196	0.219	0.0322	0.101	0.717	0.390
C22:6n3	0.243 ^b	0.336 ^{ab}	0.701 ^b	0.849 ^a	0.0702	<0.05	<0.01	0.614
Saturated fatty acids	46.405	47.378	47.998	48.371	0.8848	0.394	0.094	0.696
Monounsaturated fatty acids	51.483	49.791	45.091	43.250	1.0391	<0.05	<0.01	0.929
Polyunsaturated fatty acids	1.633	2.335	5.880	7.455	0.5041	<0.01	<0.01	0.216

^{a,b,c} Means in row within muscle type without common superscript letter are different ($P < 0.05$).

¹ Analyzed via gas chromatography on Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA), with hydrogen as carrier gas and utilizing FAME 37 standard (Supelco 37 Component FAME Mix; Sigma-Aldrich, St Louis, MO, USA).

² Standard error of mean.

³ GxM: Grain x muscle interaction.

Chapter 3 - *In vitro* fermentation characteristics of corn and grain sorghum with respect to volatile fatty acid profile, dry matter disappearance, and gas production.

Abstract

Corn and grain sorghum are the two most commonly fed grains in feedlot diets in the United States [1]. While corn is more widely utilized, feedlot cattle and grain sorghum production exhibit significant geographical overlap in the High Plains region of the U.S. Both cattle feeding and farming make significant contributions to agricultural economies of the region, but their future is threatened by depletion of groundwater resources. Due to the nature of grain sorghum's root structure, it is broadly adapted to yield stability under water-limitation [2], potentially providing means for conservation of water resources while still meeting demands of the feedlot industry. The objective of this study was to evaluate *in vitro* fermentation characteristics of corn and grain sorghum. A randomized complete block design was utilized to evaluate three grain sorghum and three corn samples (hybrids unknown). Samples were ground through a 1-mm screen using a Thomas-Wiley Laboratory Mill, Model 4 (Thomas Scientific, Swedesboro, NJ) before being combined with buffered ruminal fluid from a cannulated donor steer consuming a concentrate diet. Each culture was fitted with an Ankom RF gas production module (Ankom Technology, Macedon, NY) to measure cumulative gas production and incubated at 39 degrees C for 48 h with constant, light agitation. At the end of the incubation period, a portion of the culture fluid was reserved for measurement of volatile fatty acid (VFA) profiles, and the remaining residue was dried to determine *in vitro* dry matter disappearance

(IVDMD). Gas production was modeled using non-linear regression (Lay-Modified Gompertz function) and resulting model parameters, along with terminal pH, and VFA profiles were analyzed as mixed models using grain type as the fixed effect and replicate as the random effect. Compared to sorghum, IVDMD tended to be greater for corn (59.18% vs 62.31%; $P = 0.069$), but concentrations of VFAs were not different ($P > 0.01$). Cultures with grain sorghum produced less gas compared to those containing corn as substrate ($P < 0.01$), but rates of gas production did not differ ($P > 0.10$). These results indicate that the extent of ruminal digestibility and enhanced fermentation favor corn compared to sorghum. Further efforts to increase grain sorghum digestibility through processing methods and(or) selective breeding are necessary for grain sorghum to be a viable alternative to corn in finishing cattle diets.

Keywords: milo, digestibility, beef cattle

Introduction

The High Plains region is home to the highest concentration of feedlot cattle in the U.S. [3], but its future is threatened by depletion of groundwater resources used for crop irrigation. Much of the cropland in the region is irrigated by water supplied by the Ogallala aquifer. Irrigation has allowed crops to be produced across a wide range of soil types and climates that would otherwise be unsuitable for most farming practices. This finite source of water is threatened by depletion, as it is being withdrawn at a rate that exceeds its natural ability to replenish. A study by Deines et al., (2020) [2] concluded that 22,000 km² of irrigated land that utilizes water from the Ogallala aquifer may be unable to support irrigated crops by 2100. While corn is the most widely utilized grain in feedlot diets, grain sorghum ranks second [1]. Historically, grain sorghum was the predominant grain source in finishing diets in states of Texas and Kansas, but wide variations in grain type and digestibility have led to perceptions of inferiority compared to corn. Grain sorghum is broadly adapted to semi-arid climates due to its extensive root structure, which reduces its need for irrigation [4]. Grain sorghum's ability to produce grain under conditions of water stress may provide means for conservation of water resources in areas like the Ogallala, while still meeting demands of the feedlot industry.

In recent years, the livestock industry has been under increasing scrutiny for its contributions to greenhouse gas emissions, but certain polyphenolic compounds in sorghum varieties may work to mediate gas production compared to corn [5]. *In vitro* analysis of grains and feedstuffs has been proven to provide a means of measuring ruminal fermentation characteristics, including gas production, without compromising the accuracy that live animal studies provide, while also being more cost-effective [6]. Years of selective breeding and few updated research studies is why a thorough comparative evaluation of the feeding value of corn

and grain sorghum is due. This study aimed to evaluate ruminal fermentations characteristics of commercial corn and grain sorghum of an unknown variety in relation to VFA profile, IVDMD, and *in vitro* gas production for finishing beef steers.

Materials and Methods

Experimental design

Grain samples from a feedlot performance study were evaluated using an *in vitro* gas production procedure to estimate extent of gas production and fermentative end products. Three *in vitro* fermentation runs were completed. Each run (replicate) contained seven blocks consisting of one blank (no added substrate) and three samples of each grain representing the beginning, middle, and end of the performance trial. Corn utilized was a Channel[®] (Bayer, Leverkusen, Germany) variety, and grain sorghum was of non-specific variety and unknown origin, procured from the local elevator in Bartlett, Kansas. A randomized complete block design was utilized to conduct comparisons of gas production, volatile fatty acid (VFA) concentrations, and *in vitro* dry matter disappearance (IVDMD) of the samples. Ankom RF gas production modules (Ankom Technology, Macedon, NY) were used to measure cumulative gas production. Grain samples were first ground through a 1-mm screen using a Thomas-Wiley Laboratory Mill, Model 4 (Thomas Scientific, Swedesboro, NJ). Ground grains (1.5 g DM basis) were measured into 250-mL glass Pyrex[®] bottles fitted with a screw cap and stored until ready for use in the experiment.

Laboratory analyses

Ruminal fluid was collected from a single cannulated steer the day of the analysis. The donor was a beef steer consuming a corn grain-based diet (70% concentrate and 30% roughage).

Ruminal fluid was strained through two layers of cheesecloth (1.5-1.8 mm² pore size) once when collected from the donor and placed into warmed containers (1 L), and once when transferred to separatory funnels in the laboratory where it was flushed with nitrogen gas to displace oxygen in the head space. After 30 minutes in the separatory funnel, ruminal fluid segregated into three distinct layers. The bottom layer of ruminal fluid sediment was discarded and the microbe-rich layer (1.25 L) was collected for use as the inoculant. McDougal's buffer [7] (6.8 pH) was prepared and warmed to 39 degrees Celsius and combined with 1.25 L of ruminal fluid to create 6.25 L of buffered ruminal fluid (BFR) in a 1:4 ratio. Nitrogen gas was constantly flowing into the flask of BFR to displace oxygen. Pyrex® bottles containing either corn, sorghum, or no substrate were then filled with 150 mL BFR via a liquid dispensing machine (Desktop Digital Liquid Filling Machine GFK-160, Foshan, Guangdong, China). Blanks, which consisted of only BRF and no substrate, were included in each block to account for DM contributions of BRF for subsequent calculations of IVDMD. Nitrogen gas was dispensed into bottles to displace oxygen, then bottles were fitted with gas production measurement modules (Ankom RF Wireless Gas Production System, Macedon, NY) and placed into an incubator (Forma Scientific Orbital Shaker, Thermo Fisher Scientific, Waltham, MA). The bottles were incubated for 48 h with constant light agitation (25 RPM). At the end of the incubation time, bottles were placed in an ice bath for 20 minutes to cool contents to room temperature. Terminal pH of each bottle was recorded post-incubation and 4 mL of culture liquid were added to 12 x 75 mm culture tubes containing 1 mL of 25% (w/v) meta-phosphoric acid for determination of volatile fatty acid concentrations. Tubes were frozen, thawed, homogenized with a vortex mixer, centrifuged at 15,000 x g for 15 minutes, and 1-mL of supernatant was then collected and placed into a 2-mL gas chromatography vial. Concentrations of VFA were measured using an Agilent 7890A gas

chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector, and a 20m x 180µm wax capillary column (DB Wax; Agilent Technologies, Palo Alto, CA). The injection and detector temperatures were set at 325 and 350° C, respectively. Initial oven temperature was 50° C and increased to 240 degrees at the rate of 25 degrees/min. Inlet split was 50:1 and hydrogen was utilized as the carrier gas. Column flow rate was 40 cm/sec. Acetate, propionate, and butyrate concentrations were measured and expressed as millimolar concentrations.

The remaining post-fermentation residue was transferred from the fermentation bottles into aluminum pans and dried at 55°C for 72 h. Dried samples and pans were weighed to determine sample weight. Weights of dried samples were utilized in the following formula to calculate IVDMD, expressed as percent of original sample weight.

$$IVDMD = \frac{1[-[(Dried\ residue\ weight, g - Pan\ weight, g) - Blank\ weight, g]]}{Initial\ grain\ weight, g\ (DM)}$$

$$Blank\ weight, g = Dried\ blank\ residue, g - Pan\ weight, g$$

Calculation of gas production parameters

In vitro gas production data were evaluated using nonlinear regression procedure NLIN (SAS, ver 9.4) for each *in vitro* culture. Logistic, log-logistic and Gompertz functions were employed to model gas production data as they are commonly used to describe population growth.

Logistic function

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

Gas production - Cumulative gas production (mL) at given time interval.

Time - Interval in hour increments of total time

K - Theoretical maximum cumulative gas production (mL).

β – Shape parameter

$t_{\frac{1}{2}}$ - Time to achieve half of K (hours)

The fit of these parameters were determined using logistic models in the non-linear procedure based on the cumulative gas production values of each culture measured by the Ankom measuring system.

Log-logistic function

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

Gas production - Cumulative gas production (mL) at given time interval

Time - Interval in hour increments of total time

K - Theoretical maximum cumulative gas production (mL).

r – Rate of gas production

$t_{\frac{1}{2}}$ - Time to achieve half of K (hours)

The fit of these parameters were determined using log-logistic models in the non-linear procedure based on the cumulative gas production values of each culture measured by the Ankom measuring system.

Lay-Modified Gompertz function

$$Gas\ production = A * e^{-\left(e^{\frac{C * e}{A(b-time)+1}}\right)}$$

Gas production- Cumulative gas production (mL) at given time interval

Time- Interval in hour increments of total time

A- Theoretical maximum cumulative gas production (mL)

b- displacement along x-axis (lag time)

C- Rate of increase of gas production

The fit of these parameters were determined using the Lay-Modified Gompertz model [8] in the non-linear procedure based on the cumulative gas production values of each culture measured by the Ankom measuring system.

All parameters generated through non-linear models underwent analyses through the GLIMMIX procedure of SAS and were used as dependent variables in linear mixed models to assess differences among treatments based on the cumulative gas production curves produced by the Ankom measuring system. The models estimated parameters using time as the independent variable and gas production as the dependent variable. The gas production parameters were then used to estimate the maximum extent of gas production potential (K or A), and the rate of gas production due to substrate fermentation (β , r , $t_{1/2}$, C). Maximum rate of gas production (m) was calculated using the response parameters from the models. Functions were used to calculate m in terms of the models.

Maximum slope (m) for logistic model

$$m = \beta * \left(\frac{K}{4}\right)$$

Maximum slope (m) for log-logistic model

$$m = \left[\left(1 - \frac{1}{r}\right) * \left(\frac{t_{1/2}}{1}\right)^r + \frac{1}{r}\right]^{-1} * \left[\frac{rK \left(1 - \frac{1}{r^2}\right)}{4}\right]$$

Maximum slope (m) for Gompertz model

$$m = \frac{A * C}{e}$$

The non-linear distribution that yielded the smallest average mean square error (MSE) was chosen as the best fit. Thus, the Gompertz function parameters was chosen to undergo further parameter analyses to access impact of grain type on gas production.

Statistical Analyses

In vitro gas production data were analyzed using the nonlinear regression procedure (NLIN, SAS ver 9.4). Logistic, log-logistic and Gompertz functions were used to model gas production curves for each culture, and best fit was identified as the model that yielded the lowest mean square error. The Gompertz model consistently aligned with the lowest mean square error and superior fit to the data, yielding parameters, A (theoretical maximum cumulative gas production), b (displacement along x-axis), and C (rate of increase of gas production). Gas production parameters, IVDMD, terminal pH, and VFA concentration were analyzed using the GLIMMIX procedure (SAS ver 9.4) with a fixed effect of grain type, and random effect of

replicate. Statistical significance was declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P < 0.10$.

Results

Data for VFA profiles, IVDMD, and *in vitro* gas production (A , b , and C) of cultures containing ruminal fluid with corn or grain sorghum are displayed in Table 2.1. There were no differences observed in VFA profiles of cultures containing corn and sorghum as substrate, including amounts of acetate, propionate, butyrate, isovalerate, or valerate ($P > 0.10$). When cultures containing sorghum are compared to those containing corn, IVDMD was greater for corn cultures ($P = 0.07$) (59.18% and 62.31% respectively). Acetate:propionate ratio and terminal pH of cultures were not different ($P > 0.10$) for corn and grain sorghum. Estimated *in vitro* gas production curves were generated utilizing the Gompertz function and are displayed in Figure 2.1. Cultures containing corn as substrate produced 4% more total gas compared to those containing sorghum as substrate ($P < 0.01$), but rates of gas production (C) and lag times (b) were not different ($P > 0.10$).

Discussion

There was no difference in VFA profile or acetate:propionate ratio so we would speculate that methane production would be not different between corn and grain sorghum from this. Formation of both acetate and butyrate contributes to hydrogen production. This is of particular interest as methanogenic bacteria utilize acetate and free hydrogen to produce methane [9]. The beef industry is under increasing scrutiny for its contributions to greenhouse gas emissions, therefore methods to reduce enteric methane production are of interest. Acetate:propionate ratio is another metric that can be used to estimate hydrogen production and subsequent methane

production. The formation of propionate liberates less hydrogen, therefore diverting it from methanogenesis [10]. Therefore, a decreased acetate:propionate ratio is more desirable for efficient energy utilization. The grain sorghum utilized in this study was of unknown variety, but certain varieties are characterized by elevated polyphenolic content which may also contribute to their ability to reduce methanogenesis [5].

In vitro gas production curves of corn and grain sorghum follow similar sigmoid curves that appear to plateau at approximately 45 h. Maximum *in vitro* gas production, A , is an estimation of the extent of total gas production from microbial digestion during the 48-h incubation period. Our observation of a difference in A reflects variation in the extent of microbial digestion of corn and grain sorghum. In a study by Thorn [11], *in vitro* evaluations were performed on numerous grain sorghum varieties and there was variation in gas production observed among hybrids. Since we utilized commercial grain sorghum that could contain numerous varieties that were stored together in a single bin, it is hard to explain these effects outside of what we know about the general composition of grain sorghum. Grain sorghum is characterized by a robust protein matrix and dense peripheral endosperm that interacts with starch granules, effectively decreasing digestibility and feed value of the grain [12]. We speculate that this may have contributed to differences in cumulative gas production for corn and grain sorghum. A greater extent of microbial digestion and greater ruminal fermentation activity corresponds with the greater maximum cumulative gas production of corn. Streeter et al. [13] conducted an *in vitro* analysis of various sorghum varieties and corn and concluded that gas production measured after 24 h of incubation was greater for sorghum hybrids than corn. There was a reduction in the magnitude observed for this difference as time increased.

Though there was no difference in b , lag time for gas production to begin or C , rate of increase in gas production for corn or grain sorghum in our study, these parameters further describe the susceptibility of grains to ruminal fermentation and degradation. Feed spends a finite amount of time in the rumen, so a grain that has an elevated b , value may also observe reduced IVDMD as a result of a low degree of microbial digestion.

Percent of IVDMD was slightly elevated for corn, which indicates greater susceptibility to microbial digestion. Streeter et al. [13] reported that IVDMD for various grain sorghum varieties was 12.2% lower than that of corn. While our difference was less magnified, this conclusion aligns with our findings. Both grains in our study were ground through a 1-mm screen, which likely minimized effects that may have been more evident if the grains had been processed in differing manners (i.e., ground or dry-rolled) before adding to *in vitro* cultures. While there was no difference in terminal pH of cultures (6.29 and 6.28 for corn and sorghum respectively), a lower pH may inhibit methanogens.

Conclusion

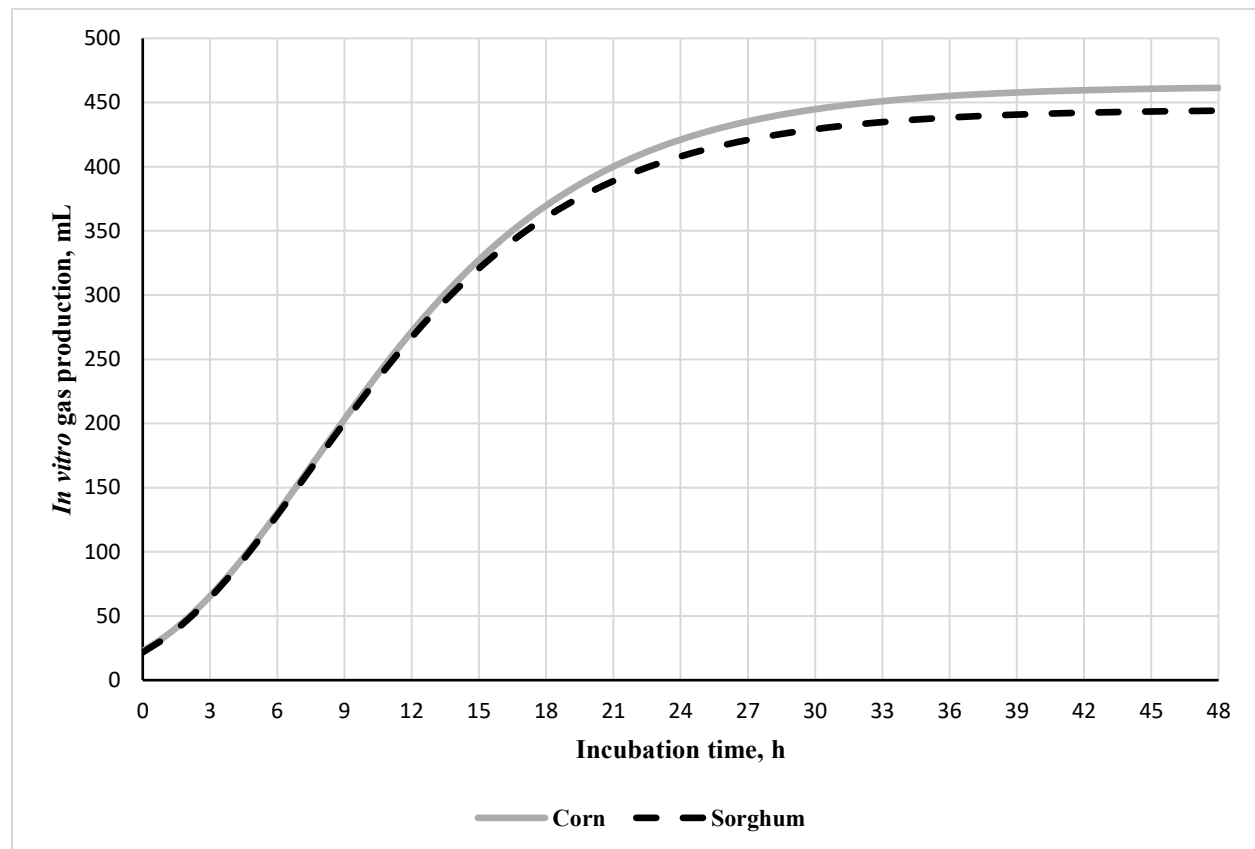
In vitro fermentation characteristics were similar for corn and grain sorghum, with no differences in methane or VFA production observed. Greater maximum gas production and elevated IVDMD were observed for corn, indicating enhanced microbial fermentation and digestion compared to grain sorghum. This is likely due to corn's less complex protein-starch matrix, contrary to grain sorghum's dense endosperm that can reduce starch availability. Despite similar gas production rates and terminal pH levels between the grains, the extent of digestion was in favor of corn, highlighting its superior ruminal fermentation when ground to 1-mm particle size and under similar rumen conditions.

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Figure 3.1 Gas production by *in vitro* cultures containing corn or grain sorghum as substrate and inoculated with strained ruminal contents from a concentrate-fed steer.¹



¹ Lay-Modified Gompertz function was used to model gas production curves from *in vitro* cultures containing corn or grain sorghum as substrate. Cultures were inoculated with ruminal contents extracted from a concentrate-fed cannulated steer. Non-linear regression yielded estimates for maximum gas production (A), time to achieve half of maximum gas production (b), and maximum rate of gas production (C). Data were then analyzed as mixed models with substrate as the fixed effect and replicate as the random effect. Effect of grain type on gas production parameters: A , $P < 0.01$; b , $P = 0.256$; C , $P = 0.273$.

Table 3.1 *In vitro* gas production parameters, volatile fatty acid profiles, and *in vitro* dry matter disappearance (IVDMD) values for cultures containing corn or grain sorghum as substrate and incubated for 48 h.

Item ¹	Treatment		SEM ²	P-value
	Corn	Sorghum		
A, mL	462.79	444.70	24.15	<0.01
b, h	0.72	0.71	0.553	0.962
C, mL/h	24.57	24.26	1.96	0.595
IVDMD, %	62.3	59.1	1.97	0.069
pH	6.29	6.28	0.041	0.967
Acetate, mM	55.5	56.9	1.45	0.468
Propionate, mM	32.0	33.3	4.99	0.370
Butyrate, mM	16.1	16.8	1.36	0.349
Isovalerate, mM	4.2	4.3	0.33	0.627
Valerate, mM	2.9	2.9	0.36	0.928
A:P	1.90	1.82	0.33	0.146

¹ A: theoretical maximum cumulative gas production; b: displacement along x-axis; C: rate of increase in gas production; IVDMD: *in vitro* dry matter digestibility.

² Standard error of mean