Processing methods for high-amylase corn: Impact on ruminal digestion and feedlot cattle performance

by

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Abstract

Three studies evaluated effects of high-amylase corn (Enogen® Feed Corn, EFC) on ruminal digestive characteristics using different processing methods; a 4th study evaluated performance of finishing cattle fed steam-flaked EFC. In study 1, mill-run corn (CON) and EFC were ground through 9-, 6-, or 4 -mm screens, blended to contain 0, 33.3, 66.7, or 100% EFC, and heated to 50, 75, or 100°C (plus a non-heated control). No 2- or 3-way interactions occurred ($P > 0.05$). Increasing EFC in blends linearly improved *in situ* dry matter disappearance (ISDMD; $P < 0.01$) and *in vitro* gas production (IVGP; $P = 0.02$). Study 2 used blends of EFC and CON containing 0, 25, 50, 75, or 100% EFC, which were reconstituted to 27, 30, or 33% moisture, and ensiled. High-amylase corn did not affect ISDMD ($P = 0.19$) but IVGP increased linearly ($P < 0.01$) in response to greater amounts of EFC. Acetate:propionate ratio and total volatile fatty acid (VFA) production by *in vitro* fermentation improved linearly by increasing EFC ($P < 0.02$). Study 3 used the same blends of EFC and CON as study 2. Grains were tempered with 0, 3, or 6% moisture, steam conditioned 15, 30, or 45 min, and flaked. No 2- or 3-way interactions occurred. Starch availability, ISDMD, IVGP, and *in vitro* production of acetate, propionate, and total VFA increased linearly ($P < 0.01$) with greater flaked EFC in blends. Study 4 fed finishing diets consisting of steam-flaked CON, or EFC, for 136 d to 700 crossbred beef heifers (394 ± 8.5 kg initial BW). Compared to CON, cattle fed EFC had similar DMI ($P = 0.78$) but had greater ADG ($P < 0.01$) and a 5% improvement in feed efficiency ($P < 0.01$). Carcass weight was 6 kg greater for EFC cattle ($P < 0.01$), which also had 8% fewer liver abscesses ($P = 0.03$) than CON. Marbling score was greater in CON cattle ($P = 0.04$) than EFC; no differences in USDA Quality Grade ($P = 0.33$), Yield Grade ($P = 0.13$), LM area ($P = 0.89$), or 12th-rib fat thickness ($P = 0.21$) were evident. Improvements in digestion associated with EFC are likely confined to that

component of grain mixtures due to a lack of quadratic effects. Improvements in feed efficiency, carcass weight, and potential liver abscess mitigation may be of advantageous use for producers.

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CHAPTER 1

Literature Review

Introduction

For feedlot cattle, feed accounts for 75 to 80% of total production cost (Richards and Hicks, 2007). Corn is used as a primary dietary energy source followed by other cereal grains in a majority of feeding operations. A variety of methods are used to enhance feeding value of corn, to use less feed and still improve weight gain. This review will focus on characteristics of corn, digestion of starch, and processing methods to improve finishing cattle performance.

Morphology of Corn Grain

While varieties of feed corn have similar structural components, small differences in their physical characteristics can influence digestibility. Four primary parts comprising a corn kernel are: pericarp, endosperm, germ, and tip cap (Singh and Johnston, 2004). Pericarp is the outermost layer of the kernel. Serving as protective layer, it is semi-resistant to penetration by water (Smith, 2004). Corn pericarp is comprised primarily of cellulose and hemicellulose, in addition to some waxes and oils. Eckhoff (2010) states that pericarp is generally greater than 91% neutral detergent fiber (NDF), and analyses containing less than this amount are likely contaminated by attached endosperm. Pericarp contributes approximately 51% of total kernel fiber (Eckhoff, 2010), while comprising just 6-8% of kernel dry weight (Smith, 2004).

Endosperm represents the major component of a corn kernel, and consists principally of starch. It also contains a variable amount of protein (8 to 10%), and small amounts of oil, fiber, and ash (Singh and Johnston, 2004). Surrounding corn endosperm is aleurone, a protein layer that includes oils, lipids, and mineral. The aleurone layer primarily serves to generate energy for

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a growing seedling by secreting amylases and proteases which break down endosperm into individual amino acids and sugars (Becraft and Yi, 2011). Starch is stored in two distinct structural forms, i.e., hard or soft endosperm (Eckhoff, 2010). This also can be described by the vitreousness of the endosperm, with hard being more vitreous, and soft being more floury. This difference is determined by the degree of starch association with a protein matrix (McAllister et al., 2008). Harder, more vitreous endosperm has tightly packed starch granules with greater association to protein. Barley and wheat have mainly floury endosperm with looser protein matrix association, which contributes to their being more rapidly digested. Two common variations among corn hybrids are flint and dent. Flint corn is comprised primarily of hard endosperm, while dent corn has a vitreous starch layer around the sides of the grain, and a more flowery endosperm in the center of the kernel (Brown et al., 1985). Upon drying, floury endosperm dissociates with protein, wherein the grain collapses, causing a dent to develop on the crown (top) of the kernel. This is where dent corn gets its name, making it visually distinguishable from flint corn. Decreased vitreousness observed in dent hybrids results in improved ruminal digestion (Philippeau and Monredon, 1999) compared to flint corn.

Germ, or embryo, represents roughly 11 to 12% of kernel weight; it contains energy-rich oils for plant growth, and would develop into roots and leaves if planted (Singh and Johnston, 2004; Eckhoff, 2010). Similar to the aleurone layer, germ contains enzymes (amylases, proteases, and lipases) which break down starch, endosperm protein matrix, and triglycerides, yielding substrates for embryo growth (Eckhoff, 2010).

The final major component of a corn kernel is the primarily fibrous tip cap. It is the attachment site between the kernel and the cob, and is the sole means of nutrient transport into the seed (Singh and Johnston, 2004; Eckhoff, 2010). Eckhoff (2010) states that during corn

drying, a hilum or black layer forms just beneath the tip cap in an attempt to seal it. Pericarp and kernel tip cap are two physical barriers to microbial digestion of energy dense endosperm and germ.

Properties of Starch

Chemical and Physical Characteristics of Starch

Starch is composed of glucose polymers, in which structure and shape are determined by location of certain chemical linkages. It is composed of amylose and amylopectin. Amylose is a linear chain linked by glycosidic α-1, 4 D-glucose bonds, and is less abundant in most cereal grains (20 to 30%; Rooney and Pflugfelder, 1986; Ratnayake and Jackson, 2008) compared to amylopectin. Amylopectin is usually the predominant polymer, and is characterized by α-1, 6 branch points which expand starch granules. Granules assemble by the formation of crystalline and amorphous growth rings. Amorphous regions are less-organized and contain more amylopectin branch points, whereas the double-helical structure of starch, similar in appearance to DNA, is shaped in crystalline regions (McAllister et al., 2008). Amorphous starch can be penetrated by water and is much more susceptible to enzymatic hydrolysis compared to crystalline starch. For this reason, starch digestion always begins in amorphous regions (Rooney and Pflugfelder, 1986). Starch granules in corn take on a spherical or polyhedral shape, and range in size from 2 to 30 μm (Tester et al., 2004).

An important physical characteristic of starch to note is birefringence, which is a property that be used to measure extent of organization or crystallinity in starch granules. When viewed under polarized light, starch may display a characteristic pattern referred to as a "Maltese cross" (Rooney and Pflugfelder, 1986; Ratnayake and Jackson, 2008). When present, birefringence

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signifies a high level of organization within glucose polymers of the starch granule. Birefringent starch is considered to be in the native, or unaltered state. When a light ray passing through crystalline starch refracts into two rays, it means it's anisotropic, with characteristics differing in alternate directions. Radial anisotropy in semi-crystalline starch is responsible for the Maltese cross (Ratnayake and Jackson, 2008).

Gelatinization and Retrogradation of Starch

Many theories exist, but complete understanding of starch gelatinization still requires further research. Essentially, the swelling of starch granules under application of water and heat hydrolyzes intermolecular bonds in crystalline starch, causing loss of birefringence (Rooney and Pflugfelder, 1986; Ratnayake and Jackson, 2008). Initial swelling in amorphous areas disrupts crystalline structures allowing further penetration of water. Amylose is released from the granules, and the previously crystalline regions become much more susceptible to enzymatic breakage of starch polymers. Temperature at which gelatinization occurs varies across all cereal grains or starchy foods. Xing et al. (2018) speculate that critical temperature thresholds for amorphous swelling and gelatinization of crystalline structures in corn starch are between 70 and 76ºC, with time at these temperatures also bearing significant effect. This is irreversible in the sense that starch granules that have gelatinized can no longer revert to native structures; however, reformation of crystalline starch will occur via retrogradation.

When gelatinized starch cools, bound water is released, and starch polymers may re-associate into crystalline structures. Rate of retrogradation is influenced by a multitude of factors, such as temperature, moisture, total starch concentration, and presence of other agents such as lipids (Rooney and Pflugfelder, 1986). Reversal of retrogradation is more easily achieved in amylopectin than amylose, as the retrograded form is stable up to 120ºC. Zinn et al.

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(2002) describes retrogradation as a "glue-like hardening", decreasing permeability of the starch matrix and limiting rehydration potential and substrate availability for enzymatic breakdown. Retrograded portions form much tighter bonds than native crystalline starch and reduce digestibility in those regions.

Enzymatic Hydrolysis of Starch

A variety of amylases are responsible for enzymatic breakdown of starch into monosaccharides. These enzymes are specialized and function differently, cleaving polysaccharide chains in specific locations. Endoamylases work on the interior of amylose or amylopectin chains, cleaving α-1, 4 glycosidic bonds. α-Amylase is the primary, and most active endoamylase. This produces α-configured oligosaccharides and α-limit dextrins (Van Der Maarel et al., 2002). Exoamylase enzymes cleave external units of glucose from amylose or amylopectin chains, leaving glucose, maltose, or β-limit dextrin as products. β-Amylase is an exoamylase only capable of breaking α-1, 4 glycosidic bonds, whereas amyloglucosidase (or glucoamylase) and α-glucosidase cleave both α-1, 4 and α-1, 6 glycosidic bonds (Van Der Maarel et al., 2002).

Debranching enzymes are the final group of specialized starch degraders; they focus on splitting α-1, 6 bonds of amylopectin, leaving linear, unbranched polysaccharides. One isoamylase hydrolyzes only α-1, 6 bonds. Pullulan is a compound made of repeating α-1, 6 linked maltotriose units that can be cleaved by the debranching enzyme pullulanase (type I). Pullulanases can also cleave the same α-1, 6 glycosidic bonds as isoamylase. Type II pullulanases have the added ability to hydrolyze α-1, 4 glycosidic bonds; a common example is amylopullanase, leaving end products of maltose or maltotriose (Van Der Maarel et al., 2002). While certain enzymes have a wide functional range in terms of hydrolytic potential, or the

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ability to cleave glycosidic bonds in multiple forms, most native forms of starch in cereal grains require the collective action of these enzymes for rapid or complete degradation.

Ruminal Starch Digestion

Digestion of non-structural starch from cereal grains occurs rapidly in the rumen. Corn processing method alters site and extent of starch digestion significantly in ruminants; differences between processing methods are discussed in greater detail later. Owens and Soderlund (2006) list a range of ruminal starch digestion of corn between 64 and 87% as a percent of intake (range dependent on processing method). Total tract starch digestion ranges between 87 and 99%, meaning the clear majority of digestion occurs ruminally. The bulk of starch fermentation is performed by bacteria, with protozoa and fungi also contributing. Ruminal microbes produce all the necessary enzymes to completely degrade starch into glucose molecules; bacteria performing the greater part of this task are classified as amylolytic bacteria. Cotta (1988) lists *Streptococcus bovis*, *Selenomonas ruminantium*, *Ruminobacter amylophilus*. *Butyrivibrio fibrisolvens*, *Succinimonas amylolytica*, and *Prevotella ruminicola* as main bacteria exhibiting amylolytic activity. Less than 10% of ruminal bacteria have been successfully cultured under laboratorial anaerobic conditions. This suggests that many amylolytic species, or others contributing to starch digestion have yet to be characterized (McAllister et al., 2008). Attachment of bacteria to a starch granule is prerequisite to fermentation, as up to 75% of starch digestion occurs with bacteria either tightly or loosely associated to the matrix (Huntington, 1997). Unlike wheat and barley, digestion of starch granules from corn proceed "inside out", with amylolytic bacteria tunneling into the granule's center, then working to break down the matrix in an outward fashion (McAllister et al., 2008). This difference is not typically the

primary factor explaining rate and extent of digestion differences between these grains, as major differences are apparent in their physical structures.

Amylolytic bacteria produce all exo- and endo-amylase enzymes required for starch digestion. Generally speaking, however, individual bacterial species do not produce the entire range of enzymes required for fermentation (Huntington, 1997). This means that cross-feeding and cooperation between ruminal microbes are required for complete starch fermentation in order to yield greatest bacterial growth rates (Cotta, 1992). Protozoa can affect rate and extent of starch digestion in the rumen, and have important effects modulating ruminal pH, especially in high-grain diets. Both Holotrich and Entodiniomorph protozoal species can degrade starch, and can reduce rate of starch digestion through predation of amylolytic bacteria and by engulfing starch granules, thus limiting total ruminal starch available for bacterial fermentation (Kotarski et al., 1992; Nagaraja et al., 1992). Huntington et al. (2006) cites ruminal protozoa requiring up to 36 h to completely metabolize a starch granule. While overall impact of ruminal fungi is minor when it comes to starch digestion, they may have greater influence in the digestion of more vitreous starch, as fungal rhizoids can penetrate through protein matrixes in corn, creating more access points for bacterial starch digestion (McAllister et al., 2008).

Rate and degree of starch digestion rely on a multitude of factors that have been well explored. These factors include diet, starch source, meal size, mechanical or chemical processing, and ruminal microbial population due to diet adaptation (Huntington, 1997). On high-concentrate diets, large amounts of starch are fermented to produce VFA and non-volatile acids (most significantly lactate) which decrease ruminal pH, making ruminal acidosis a concern if improperly managed. Roughage included in these diets helps to stimulate rumen motility and saliva secretion to buffer rumen acidity, but this will not affect rate of starch digestion.

Huntington (1997) describes common management practices to mitigate this issue, including consumption regulation, feed additives, and extent of grain processing. Reducing total starch consumed at one time by feeding smaller meals more frequently has proven to stabilize ruminal pH (Choat et al., 2002). Ionophores, which are feed additives, alter microbial microbiota, and can also regulate feed intake patterns (Burrin et al., 1988; Stock et al., 1990). More recently, use of probiotics to increase utilization of lactic acid accumulation during starch fermentation has proven to be beneficial for adapting cattle to high-concentrate diets (Drouillard et al., 2012; Miller, 2013). Other methods to control rate and extent of carbohydrate fermentation are increased dietary fat inclusion (Zinn, 1988; Krehbiel et al., 1995) and chemical treatment with phenolics or formaldehyde (Fluharty and Loerch, 1989).

Starch intake seems to have no effect on microbial capacity to digest starch, which is seemingly limitless, except when conversion to organic acids is excessive, causing digestive disorders (acidosis, bloat, founder, liver abscessation etc.; Huntington, 1997). This, in turn, is possibly the greatest limiting factor for degree of, and advancements in corn processing (Owens et al., 1997). Greater detail on common processing methods and how they impact digestibility is reviewed in the sections to follow.

Intestinal Starch Digestion

Enzymatic hydrolysis of starch in the small intestine of ruminants functions in a manner similar to monogastric species. The majority of amylase present in the ruminant small intestine is produced in the pancreas, with a minor amount also produced by intestinal mucosa (Owens et al., 1986). Pancreatic α-amylase catalyzes conversion of amylose and amylopectin into limit dextrins, and linear oligosaccharides (Huntington, 1997). For final conversion into single

glucose units which can be absorbed, ruminants rely on maltase and isomaltase activity, as they possess no detectible amount of sucrase (Harmon, 1993), a common carbohydrase in the monogastric small intestine. Harmon et al. (2004) stated that glucose absorption in the small intestine is energetically favorable compared to ruminal metabolism. This raises the question why feeds are not developed to achieve more starch bypassing the rumen, in order to achieve more digestion in the small intestine. Owens et al. (1986) summarized data from 11 experiments, and reports 55% of starch was digestible in the small intestine. A review by Harmon et al. (2004) found a similar value of 62% small intestinal starch digestibility. However, regression equations yielded an adjusted mean of only 40%. This is because the regressed relationship between digestibility and starch input was negative, meaning small intestinal starch digestion decreased with increasing starch intake. Limitations within the the ruminant small intestine for starch digestion have been a focus of many experiments and reviews.

Many hypotheses have been researched over the years to explain poor starch digestion in the small intestine. These hypotheses include limited activity of amylolytic enzymes or presence of amylase inhibitors, limited capacity for glucose absorption, time limitation for complete starch digestion, and poor accessibility for enzymatic hydrolysis of starch granules exiting the rumen as these granules may be less penetrable (Owens et al., 1986). Today it appears that the dominant factor is insufficient production of pancreatic amylase, explaining why small intestinal starch digestion is reduced with increased intake (Owens et al., 1986; Kreikemeier et al., 1991; Huntington, 1997; Harmon et al., 2004). Corn processing method will also yield marked differences in small intestinal capacity for starch digestion, as accessibility of amylases to degrade starch may be limited in poorly processed grains. Steam-flaking, in particular, has the

ability to increase small intestinal digestion (Zinn et al., 2002), and perhaps capitalize on the energetic improvement of intestinal glucose absorption when compared to the rumen.

Optimizing energetic advantages in small intestinal starch digestion is difficult simply due to enzymatic limitations. No research in the literature was found exploring effects of adding amylolytic enzymes post-ruminally and whether this would improve small intestinal starch digestion. At the very least, limiting the amount of starch reaching the large intestine should be a goal when considering how best to process grains. Large intestinal starch digestion may aid in compensating for incomplete starch digestion of whole or poorly processed grains (Theurer, 1986). Owens et al. (1986) states that starch fermentation in the large intestine is undesirable, as it's the least efficient site of digestion, and the animal cannot make use of microbial cell protein. They reported 30 to 60% of starch leaving the ileum is digested in the large intestine. Until methods are found to improve limitations of intestinal starch digestion, maximizing ruminal digestion without increasing digestive disorders will continue to be the goal of cattle feeders, largely through grain processing techniques.

Impact of Grain Processing on Cattle

This review will focus on dry-rolling/grinding, high-moisture ensiling, and steam-flaking corn as the three primary methods of processing grains to improve feed efficiency in feedlot cattle. Conceptually, processing corn serves to degrade digestive barriers such as pericarp and protein matrixes so that microbes have greater access to starchy endosperm. Additionally, by using any of these methods, particle size is reduced and/or surface area increased, yielding more attachment sites for ruminal microbes and enzymatic attack (McAllister et al., 2008). Mechanisms for each method are discussed in greater detail later.

Owens and Soderlund (2006) reported improved total tract starch digestibility over whole corn when employing any of the three aforementioned processing methods. Of the three, steam-flaking consistently yields the greatest improvements in cattle performance. When compared to dry-processed grains, Zinn et al. (2002) found a 5.4% improvement in ADG, and a 12% improvement in feed efficiency using steam-flaked corn (SFC). The next sections of this review will focus on individual processing methods and their mechanisms for improvement.

Dry-processed Grain

When referring to dry-processed grains, dry-rolled corn (DRC) and ground corn (if coarse) are often used as synonyms, and generally compiled into one category when being compared to other grain processing methods. Dry-processing is a common method used in a variety of feeding systems and, depending on feedlot size, may offer the best balance between cost and cattle performance. In order to cover cost of feeding high-moisture corn (HMC) over DRC in a 5,000-head feedlot, feed efficiency would need to improve by 2.4% in an 85% corn diet (Macken et al., 2006). Using these same parameters, feed efficiency would need to improve by 6.1% to cover costs if feeding SFC. Simply put, smaller operations tend to utilize dry-processed grains over more advanced techniques due to cost.

Milling Corn

McKinney (2006) identifies four basic principles of grinding/particle size reduction as: compression, impact, attrition, and shear. Most equipment will utilize a combination of these principles. Hammermills and roller mills are the two most commonly used pieces of equipment for cattle feeding. Hammermills use a greater proportion of impact and attrition when compared to roller mills, which employ more compression and shear (McKinney, 2006). In some cases

moisture is added (tempering), mainly for dust control and reduction of fine particles, with some improvement in microbial digestion and animal performance as a possible added benefit (Zinn et al., 1998; Zinn et al., 2011). It is important when operating these machines to consider effects of particle size. As more kernel surface area is exposed, a greater proportion of nutritional components are available for enzymatic or microbial action, and improvements in animal performance are anticipated (Koch, 2002). Over-processing, however, results in excess fine material that may predispose animals to digestive disorders, along with unneeded wear on equipment. Particle size can certainly be adjusted for type of feeding or intended use of the final product.

In a hammermill, grain entering the system is impacted by rotating steel bars, or "hammers", causing the kernel to fracture into many pieces. Particle size is largely controlled by a screen (with holes specific to the largest sized particle that could pass through), but also is influenced by speed of hammer rotation (Koch, 2002). Particles produced in a hammermill generally have a spherical shape; after shattering of a kernel, abrasion smooths down sharp edges. This is largely the reason for more dust generation with this method (McKinney, 2006). Particle size distribution tends to have more variation around the mean geometric diameter, meaning there are many large particles produced, as well as many fines (Koch, 2002).

Dry-rolled corn is produced in a roller mill. When grain flows into this system, it will pass through one or more pairs of rollers spinning in opposite directions. Rolls rotate at different speeds, causing grains to be sheared and compressed (Koch, 2002). Corrugated or grooved rolls also utilize tearing and grinding, and size and shape of corrugations will impact particle size reduction. Other factors impacting particle size are roll speed, and gap size (between two rolls). Kernels processed through this system are reduced to more irregular cubic shaped pieces that are

more uniform in their particle size distribution compared to grain processed through a hammermill (Koch, 2002; McKinney, 2006).

While particle shape is unlikely to affect digestive efficiency of the product or animal performance, it should be taken into account that it does have major impact on handling and mixing (McKinney, 2006). Due to shape, roller mill ground grain does not pack as well as grain processed through a hammermill, and thus does not mix as easily with supplements (vitamin, mineral, protein, etc.). This is also evidenced by the fact that rolled grain generally has a 5 to 15% lower bulk density than hammermilled grain, which can impact volumetric mixing of ingredients (Koch, 2002; McKinney, 2006). Grain moisture can also be heavily impacted by mill type. Impact and shattering effects present in a hammermill cause a tremendous amount of kinetic energy that yields heat. In particular, kernels containing more moisture absorb the impact to some extent, requiring more pressure to shatter, and causing a greater amount of heat when breaking. This then dries the grain, ultimately making a lower moisture product than was first introduced (McKinney, 2006). Roller mills generally perform better with higher moisture grains and produce less heat.

Impact of Particle Size

Particle size is perhaps the greatest influencing factor of DRC feeding value. It is widely accepted that smaller particle sizes increase rate and extent of ruminal digestion, but may cause decreased dry matter intake (DMI) and digestive disorders (Owens et al., 1997). Coarser grinds reduce ruminal digestion and shift more towards post-ruminal digestion (Owens et al., 1986). In order to evaluate effects of DRC particle size on starch digestibility and finishing cattle performance, Schwandt et al. (2016) used 360 crossbred steers, and fed diets containing approximately 65% coarse, medium, or fine ground corn (mean geometric particle size: 4882,

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3760, or 2359 μm respectively). *In situ* dry matter disappearance over 24 h increased linearly and quadratically with decreasing particle size. Fecal starch decreased linearly with decreasing particle size. Performance differences during the feeding phase were increased DMI over the final 5 weeks for cattle feed coarse DRC, and tendency for a quadratic improvement in carcass adjusted final body weight (BW), as steers feed the medium grind were heaviest. It should also be noted, steers fed the medium grind had the greatest average daily gain (ADG) and gain:feed (G:F) during the 23-d step-up period (quadratic effect).

Using a similar approach with three different particle sizes of DRC, Secrist et al. (1995) evaluated effects of particle size on finishing steer performance and carcass characteristics. Coarse (2600 μm; note this is finer than most corn processed for feedlot diets), medium (1500 μm), and fine (800 μm) grinds were fed at 85% of diet DM. Over the complete period, there were linear and quadratic trends for ADG and G:F; with cattle fed the medium grind being optimal. Using regression equations, they estimated DMI to be optimal with mean particle size of 2300 μm; while ADG was optimal at 1700 μm, and feed efficiency was optimal at 1600 μm. They also observed a single significant difference in carcass traits, this being that cattle fed medium ground corn had numerically greater marbling scores. Similar to the previous study, Secrist et al. (1995) noted that total tract digestibility tended to be lower for coarse DRC. Fine particles had greater starch digestibility than coarse and tended to be greater than medium particles. Fecal starch also was lower in the fine particle group. While digestibility was improved using fine particles, this doesn't always translate to improved animal performance; the authors attribute this difference to possible digestive disorders, likely sub-acute acidosis in cattle fed fine ground corn.

Lundy et al., (2015) used two particle size distributions to evaluate finishing steer performance. Their coarse ground corn averaged 2400 μm, and fine had a 500 μm mean geometric particle size. This was fed at a rate of 45% DM, in an 87% concentrate diet (assuming 30% concentrate present in corn silage). Steers fed fine ground corn had reduced DMI by 0.69 kg, and reduced ADG by 0.17 kg. Steers fed coarse ground corn had 12 kg heavier carcasses. Like previous trials, total tract starch digestion was greater in cattle fed fine ground corn.

It is of importance to note that in this study (Lundy et al., 2015), wet distillers grains plus solubles were fed at 35% of diet DM; and in the experiment by Schwandt et al. (2016), wet distillers grains were fed at approximately 20% of the diet. Even with additional grain byproduct, effects of DRC particle size are still very clear. There likely isn't a correct answer as to what the ideal particle size in a feedlot diet is; further, the effects of particle size will vary by other factors such as proportion of DRC in the diet and characteristics of other ingredients.

High-moisture Corn

High-moisture corn is a common ingredient in feedlot diets, especially in certain regions of the US. In some cases, it is fed in combination with another form of processed corn (generally DRC), primarily due to storage cost or space (Macken et al., 2006). Many factors go into HMC that can influence cattle performance, such as moisture, storage form (whole or ground), bunker or silo storage, and fermentation inoculants to name a few (Stock et al., 1991; Owens et al., 1997). To this day, HMC can be highly variable in its makeup, and thus in its nutritive value for cattle feeding.

Process and Chemistry of High-moisture Corn

High-moisture corn is made through ensiling, defined as preservation of a perishable feedstuff for future use (Rutherford, 2014). Preservation is achieved with reduction of pH via production of organic acids which occur from bacterial fermentation of soluble sugars. Lactic acid is responsible for the greatest proportion of this pH drop, followed by acetate. Other commonly reported compounds in the literature are propionate, butyrate, and ethanol (Goodrich et al., 1975; Wardynski et al., 1993; Rutherford, 2014). In some cases, volatile fatty acids (VFA) or microbial inoculants are added while harvesting in attempt to more rapidly facilitate the pH drop (Tonroy et al., 1974; Wardynski et al., 1993). Moisture content of HMC typically ranges from 25 to 36% and has marked effect on feeding value of the final product.

For ideal fermentation to occur, making a product less prone to spoilage with fewer yeasts and molds, oxygen must be limited so anaerobic processes can dominate. In lower oxygen environments such as vertical silos or plastic ensiling bags that can promote handling ease, grains are often stored at a lower moisture content (below 26%; Owens et al., 1997; Mader and Rust, 2006). If bunkers or silos are used, grains are often harvested at higher moisture and coarsely ground or rolled to permit better packing, oxygen removal, and less space for air to penetrate.

Rutherford (2014) described six stages that occur during the ensiling process. Stage 1 is immediately after harvest, is aerobic, and can last several days after packing. Heat, water, and carbon dioxide are produced from conversion of water-soluble carbohydrates by aerobic microbes. When oxygen or soluble carbohydrates are depleted, respiration and production of heat, water, and carbon dioxide ceases; however, oxidation will still occur anywhere oxygen can still penetrate, causing proliferation of yeasts, molds, and thus weight loss.

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When oxygen is depleted, stage 2 can begin. Fermentation during this phase is called heterofermentation, meaning a variety of products are being formed. Lactate, acetate, and ethanol are the primary products, in addition to other VFAs, non-volatile fatty acids, and alcohols in minor proportion. Bacteria responsible for this stage of fermentation are heat-tolerant due to excessive heat produced during stage 1 (~32ºC); but only produce small amounts of fermentative products. These bacteria are inhibited once pH drops below 5, which shifts fermentation to stage 3 (Rutherford, 2014).

Stage 3 is when formation of lactic acid dominates, as homofermentative bacteria thriving below pH 5 almost exclusively shift fermentation to production of lactate (Rutherford, 2014). This is a transitional stage that may only last a day. During this transition the ensiled mass cools, and a new group of lactic acid producing bacteria proliferate and stage 4 begins. *Lactobacillus sp.* dominate phase 4; they are more tolerant of pH at or below 4 and cooler temperatures (29ºC). Lactate is produced exponentially, further reducing pH, preserving nutrient content of grains, and can be converted into energy when consumed by a ruminant. Following stage 4, pH is now low enough to inhibit growth of other microbes present in the silage, and the product remains in a preserved state with little to no change in pH. Stage 5 is considered stable; without disruption, properties of the contents remain fairly constant until opening. Some changes may occur, depending on a multitude of characteristics such as microbial populations at harvest time, and types of lactate producers, which can still alter organic acid profile in the final product (Rutherford, 2014).

Stage 6 occurs when the final product is opened and a proportion removed for feeding. Upon exposure to air, organic acids will be converted to carbon dioxide and water by yeasts and other anaerobic microbes (Rutherford, 2014), pH increases, and spoilage occurs over time.

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Microbial inoculants such as *Lactobacillus plantarum* and *Lactobacillus buchneri* have been used to reduce spoilage.

Effects on Fermentative Characteristics and Cattle Performance

In most cases, HMC will outperform its DRC counterpart, especially regarding digestibility. Starch digestion of HMC shifts towards the rumen when compared to DRC. High-moisture corn has 26% more starch digested in the rumen, and 8.3% greater total tract starch digestion compared to DRC (Owens and Soderlund, 2006). However, HMC has been shown to reduce DMI, and doesn't show consistent improvements in ADG compared to DRC (Owens et al., 1997).

Variability among HMC production and final product have displayed marked differences on cattle performance, requiring producers to make decisions on what the best balance between HMC production/storage and feeding value is. Moisture content arguably has the greatest impact on fermentative end products and cattle performance. Goodrich et al. (1975) compared moisture contents of 21.5, 27.5, 33%, and their effects on fermentation characteristics in HMC. The highest moisture treatment had the greatest dry matter loss, greater energy loss than 21.5% treatment, and greatest gas production, all indicating an increased amount of fermentation. All treatments produced increasing concentrations of ethanol with increasing moisture content. More lactate, acetate, and propionate were produced using 33% moisture than with the low moisture treatment. It also produced the most butyrate. Moderate moisture resulted in increased propionate and lactate compared to the low moisture treatment. When reporting effects on cattle performance, Owens et al. (1997) found that feedlot cattle had lower DMI with increased moisture in HMC. Using regression equations from all studies included, they found that ADG and metabolizable energy (ME) would be optimum between 30 and 31% moisture.

One aspect not yet mentioned is reconstituted HMC. This is where dried corn has water added back to it to reach a target moisture value. By this method, feeding value of the corn may change compared to traditional ensiling. In terms of fermentative characteristics, (Goodrich et al., 1975) observed lower dry matter loss and increased gas production using reconstituted corn. However, production of lactate and butyrate was lower in reconstituted grain; no differences occurred for acetate, propionate, or ethanol. When comparing four experiments, Tonroy et al. (1974) found that reconstituted corn increased DMI, but had no effect on ADG when compared to traditional HMC. Feed efficiency was more variable among experiments but tended to be (and was always numerically) poorer in cattle fed reconstituted corn. While differences can arise when reconstituting HMC, it still appears to be a viable method and may provide producers added flexibility, without needing to work around harvesting schedules, and greater ability to accurately adjust target moisture.

Steam-flaked Corn

Benefits of steam flaking corn have been well established; it is the premier method to increase the feeding value of corn today. Start-up cost to invest in a steam-flaker is the reason this processing method is generally limited to high-capacity feedlots (Macken et al., 2006). A compilation of studies have shown cattle performance benefits over whole or DRC. Zinn et al. (2002) showed improvement in ADG by over 5%, reduction in DMI of 6.1%, and 12% improved feed efficiency. Their summary of literature values show SFC improving ME by 0.47 Mcal/kg. Compared to DRC, SFC in feedlot cattle diets improves ruminal, post-ruminal, and total tract starch digestion by 32, 31, and 9% respectively (Owens and Soderlund, 2006). Starch digestion is similar between HMC and SFC. Neutral detergent fiber (NDF) digestion using SFC is less

than DRC, but greater than HMC (Owens and Soderlund, 2006). Steam-flaking corn has also

consistently proven to increase post-ruminal protein digestion (Zinn, 1990a; Barajas and Zinn,

1998; Zinn et al., 1998). Processing mechanics, reasons for improved starch availability, and

factors influencing flake quality and digestibility will be discussed in detail.

Processing Mechanics

Zinn et al. (2002) suggests 5 principle factors influencing SFC quality: steam chest

temperature, time conditioned with steam, roll corrugation, roll gap, and roll tension. Corn is

steam treated typically for 30 to 60 min in a stainless-steel cylinder (or "steam chest"). When

steam is applied, corn DM increases from around 13% moisture to 18 to 25%. Heated grains

with feeder-controlled flow drop down onto a set of corrugated rolls spinning in opposite

directions, which then crush kernels into a flake. With adequate moisture, the flake will keep its

shape and not break into multiple crumbles. Flake density, moisture, and endosperm structure of

the corn hybrid are likely the main contributing factors for reduction of fine particles (Zinn,

1990b; Sindt et al., 2006b; Zinn et al., 2011). Flake thickness is typically determined by a bulk

density measurement (g/L, or lb/bu), as the relationship between the two remains relatively

constant (although amount of fines can have an impact). Flake thickness, along with starch

solubility, moisture content, and nutrient release, can be used to estimate degree of starch

gelatinization using enzymatic procedures (Zinn et al., 2002; Sindt, 2004). In a survey of feedlot

consulting nutritionists, Vasconcelos and Galyean (2007) evaluated preferred methods to

measure starch availability. Preference was given to the enzymatic method, followed by gas

production, and then measurement of gelatinization. Approximately 24% of survey respondents

used a combination of these three methods, as well as the Flake Color Index System (Lextron

Inc., Greeley, CO), and the Kansas State University refractive index (Sindt et al., 2000). It appears recommended target starch availability varies by method.

Physical and Chemical Changes

Heat and moisture applied from steam cause swelling and gelatinization of starch granules. Varieties of corn have differences in their swelling power, with waxy species being greatest, and high-amylose species being poorest (Zinn et al., 2002). These differences may also influence steaming time. Harbers (1975) used electron microscopy to observe changes to protein matrixes during flaking; flaking action caused flattening of starch granules, and protein matrixes to stretch. The physical action of flaking combined with gelatinization is what gives ruminal microbes greater access to degradable substrate.

Like all forms of gelatinized starch, retrogradation occurs to some extent. Ward and Galyean (1999) determined there is a 40% loss in enzymatic starch availability for corn stored in a bin compared to when it was first flaked and sampled just under the rolls. However, *in vitro* digestion of these two products did not differ. Zinn and Barrajas (1997) observed no differences in ruminal or total tract starch digestion when comparing freshly flaked corn to SFC that had been air dried for 5 days. While there is evidence retrogradation occurs, minimal impact has been shown on its effect on digestibility and cattle performance. Additionally, in a commercial setting there likely isn't enough time between when corn is flaked to when it's consumed to observe any effect on cattle performance (McAllister et al., 2008).

Processing Alterations Impacting Digestibility and Cattle Performance

Sometimes corn is tempered with water prior to being flaked, increasing final moisture content of the product. Also, a topical surfactant may be added along with water. At a range including several intervals between 6 and 14% added water (w/w), multiple studies confirmed

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there were little to no effects on feedlot cattle performance (Zinn et al., 2002; Sindt et al., 2006b; Sindt et al., 2006a; Gutierrez et al., 2018). It was confirmed however that tempering (with or without surfactant) and a topical surfactant both improved flake durability (Sindt et al., 2006b; Sindt et al., 2006a). Tempering at 0, 6, or 12% moisture caused a linear decrease in particle size and tended to linearly increase enzymatic starch availability with increasing amounts of added water. Tempering with moisture prior to steaming is effective at adding moisture to grains and may reduce amount of steam required for adequate conditioning.

Arguably the greatest factor when steam-flaking corn impacting digestive characteristics is bulk density (flake thickness). Density on a steam-flaker is adjusted by the roll gap; thinner flakes have a lower density then thick flakes. In a report compiling survey answers from 24 feedlot nutritionists, the recommendation for mean bulk density of flaked corn was 350 g/L, with a minimum of 320 g/L, and a maximum of 360 g/L (Samuelson et al., 2016). Recommended starch availability averaged 59.8%, with a minimum of 52.5, and a maximum of 65.0% (different methods to determine starch availability were used). Testing flake densities of 310, 335, and 360 g/L, Sindt et al. (2006a) reported linear increases in flake durability and *in vitro* gas production, with decreasing bulk density. When comparing flake densities of 310 and 360 g/L, Sindt et al. (2006b) observed an increase in starch availability, and decrease in particle size using lighter density flakes.

Relationships between starch availability, *in vitro* measurements, and how they correlate to *in vivo* digestion and cattle performance are often unclear. Zinn (1990a) reports linear increases in total tract digestion of organic matter (OM), starch, and nitrogen as flake density decreased from 420 to 300 g/L. Results from Theurer et al. (1999) would correspond, explaining that degree of processing increases extent of ruminal starch digestion due to the relationship

between flake density and starch solubility (Zinn et al., 2002). Despite these differences, impact of density on feedlot cattle performance and carcass characteristics appears minimal (Zinn, 1990a; Owens et al., 1997; Sindt et al., 2006b; Gutierrez et al., 2018). Zinn et al. (2002) explains that flaking below a density of 310 g/L may be counterproductive, as increasing starch solubility can reduce DMI, and more rapid rates of fermentation may induce digestive disorders. So long as producers flake within a reasonable range (approximately 310 to 360 g/L, based on values extrapolated from literature), cattle performance will likely be similar. When considering a flake density to operate with, one may also need to account for wear on rolls, and electrical energy use when processing to a greater extent, as there may be a breaking point between cattle performance and flaker maintenance/use.

Summary

Characteristics of size and type, of a feeding operation likely dictates the most appropriate method of grain processing. It's clear that some form of processing yields substantial efficiency benefits over feeding whole corn. Until methods are found to increase digestive capacity of starch in the small intestine, improving ruminal digestion is preferred. Extent of processing must be thoroughly considered, as over-processed grains lead to rapid fermentation and accumulation of organic acids, leading to digestive disorders and reduced cattle performance. Dry-rolling corn allows greater proportions of starch to be digested ruminally over whole corn, and this can be further enhanced with HMC or SFC. To justify using HMC, or especially SFC, producers must be able to account and make up for added costs. If this can be done, utilization of energy from corn can be maximized. Enzymatic or other methods to determine starch availability or gelatinization can be useful tools to evaluate SFC quality;

however, they don't always fully reflect what occurs during ruminal or post-ruminal digestion, and thus can't necessarily be used to predict cattle performance. Each method of processing corn is designed to gain more value from the product being fed, and each has a functional role in cattle feeding today. Methods or certain corn hybrids can still be developed to enhance corn processed through each of these systems, and potentially improve digestion through increased enzymatic activity, both ruminally and post-ruminally.

Literature Cited

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

Becraft, P. W., and G. Yi. 2011. Regulation of aleurone development in cereal grains. J. Exp. Bot. doi:10.1093/jxb/erq372.

Brown, W. L., M. S. Zuber, L. L. Darrah, and D. V. Glover. 1985. Origin, adaptation, and types of corn. Natl. Corn Handb. 1–6.

Burrin, D. G., R. A. Stock, and R. A. Britton. 1988. Monensin level during grain adaptation and finishing performance in cattle. J. Anim. Sci. 66:513–521. doi:10.2527/jas1988.662513x.

Choat, W. T., C. R. Krehbiel, M. S. Brown, G. C. Duff, D. A. Walker, and D. R. Gill. 2002. Effects of restricted versus conventional dietary adaptation on feedlot performance, carcass characteristics, site and extent of digestion, digesta kinetics, and ruminal metabolism. J. Anim. Sci. 80:2726–2739. doi:10.2527/2002.80102726x.

Cotta, M. A. 1988. Amylolytic activity of selected species of ruminal bacteria. Appl. Environ. Microbiol. 54:772–776.

Cotta, M. A. 1992. Interaction of ruminal bacteria in the production and utilization of maltooligosaccharides from starch. Appl. Environ. Microbiol. 58:48–54.

Drouillard, J. S., P. H. Henning, H. H. Meissner, and K. J. Leeuw. 2012. Megasphaera elsdenii on the performance of steers adapting to a high-concentrate diet, using three or five transition diets. South African J. Anim. Sci. 42:195–199. doi:10.4314/sajas.v42i2.13.

Eckhoff, S. 2010. Correct terminology usage in corn processing. Cereal Foods World. 55:144–147.

Fluharty, F. L., and S. C. Loerch. 1989. Chemical treatment of ground corn to limit ruminal starch digestion. Can. J. Anim. Sci. 69:173–180.

Goodrich, R. D., F. M. Byers, and J. C. Meiske. 1975. Influence of moisture content, processing and reconstitution on the fermentation of corn grain. J. Anim. Sci. 41:876–881. doi:10.2527/jas1975.413876x.

Gutierrez, B. H., E. G. Alvarez, M. F. Montano, J. Salinas-Chavira, N. G. Torrentera, and R. A. Zinn. 2018. Influence of flake density and tempering on the feeding value of steam-flaked corn for feedlot cattle. J. Appl. Anim. Res. 46:155–158. doi:10.1080/09712119.2017.1278699.

Harbers, L. H. 1975. Starch granule structural changes and amylolytic patterns in processed sorghum grain. J. Anim. Sci. 41:1496–1501.

Harmon, D. L. 1993. Nutritional regulation of postruminal digestive enzymes in ruminants. J. Dairy Sci. 76:2102–2111. doi:10.3168/jds.S0022-0302(93)77545-1.

Harmon, D. L., R. M. Yamka, and N. A. Elam. 2004. Factors affecting intestinal starch digestion in ruminants: A review. Can. J. Anim. Sci. 84:309–318. doi:10.4141/A03-077.

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

Huntington, G. B., D. L. Harmon, and C. J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84:E14–E24. doi:10.2527/2006.8413_supplE14x.

Koch, K. 2002. Hammermills and Roller Mills. Kansas State Univ. Agric. Exp. Stn. Coop. Ext. Serv. MF-2048:1–5.

Kotarski, S. F., R. D. Waniska, and K. K. Thurn. 1992. Starch hydrolysis by the ruminal microflora. J. Nutr. 122:178–190. doi:10.1093/jn/122.1.178.

Krehbiel, C. R., R. A. Stock, D. H. Shain, C. J. Richards, G. A. Ham, R. A. McCoy, T. J. Klopfenstein, R. A. Britton, and R. P. Huffman. 1995. Effect of level and type of fat on subacute acidosis in cattle fed dry-rolled corn finishing diets. J. Anim. Sci. 73:2438–2446. doi:10.2527/1995.7382438x.

Kreikemeier, K. K., D. L. Harmon, R. T. Brandt, T. B. Avery, and D. E. Johnson. 1991. Small intestinal starch digestion in steers: effect of various levels of abomasal glucose, corn starch and corn dextrin infusion on small intestinal disappearance and net glucose absorption. J. Anim. Sci. 69:328–338. doi:10.2527/1991.691328x.

Lundy, E. L., B. E. Doran, E. E. Vermeer, D. D. Loy, and S. L. Hansen. 2015. Influence of corn particle size on steer performance and carcass characteristics when fed diets with moderate inclusions of wet distillers grans plus solubles. Anim. Ind. Rep. AS 661. doi:10.15232/pas.2015-01387.

van der Maarel, M. J. E. C., B. Van Der Veen, J. C. M. Uitdehaag, H. Leemhuis, and L. Dijkhuizen. 2002. Properties and applications of starch-converting enzymes of the α-amylase family. J. Biotechnol. 94:137–155. doi:10.1016/S0168-1656(01)00407-2.

Macken, C. N., T. J. Klopfenstein, and G. E. Erickson. 2006. The cost of corn processing for finishing cattle. Prof. Anim. Sci. 22:23–32. doi:10.15232/S1080-7446(15)31057-3.

Mader, T., and S. Rust. 2006. High moisture grains: harvesting, processing, and storage.

Oaklahoma State Beef Ext. Conf. Proc. 88–92.

McAllister, T. A., D. J. Gibb, K. A. Beauchemin, and Y. Wang. 2008. Starch type, structure and ruminal digestion. 30–41.

McKinney, L. J. 2006. Grain Processing: Particle size reduction methods. Proc. Cattle Grain Proccessing Symp. 42–45.

Miller, K. A. 2013. Utilizing Lactipro (Megasphaera elsdenii NCIMB 41125) to accelerate adaptation of cattle to high-concentrate diets and improve the health of high risk calves. PhD Diss. Kansas State Univ., Manhattan.

Nagaraja, T. G., G. Towne, and A. A. Beharka. 1992. Moderation of ruminal fermentation by ciliated protozoa in cattle fed a high-grain diet. Appl. Environ. Microbiol. 58:2410–2414.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle: A review. J. Anim. Sci. 75:868–879.

Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. J. Anim. Sci. 63:1634–1648. doi:10.2527/jas1986.6351634x.

Owens, F., and S. Soderlund. 2006. Ruminal and postruminal starch digestion by cattle. Cattle grain Process. Symp. 116–128. doi:10.1017/CBO9781107415324.004.

Philippeau, C., and F. L. D. De Monredon. 1999. Relationship between ruminal starch degradation and the physical characteristics of corn grain. J. Anim. Sci. 77:238–243.

Ratnayake, W. S., and D. S. Jackson. 2008. Chapter 5 Starch Gelatinization. Adv. Food Nutr. Res. 55:221–268. doi:10.1016/S1043-4526(08)00405-1.

Richards, C. J., and B. Hicks. 2007. Processing of corn and sorghum for feedlot cattle. Vet. Clin. North Am. - Food Anim. Pract. 23:207–221. doi:10.1016/j.cvfa.2007.05.006.

Rooney, L. W., and R. L. Pflugfelder. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. J. Anim. Sci. 63:1607–1623. doi:10.2527/jas1986.6351607x.

Rutherford, W. M. 2014. Fermentation Aids for High Moisture Corn. Oaklahoma Beef Ext. 99–105.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico state and Texas tech university survey. J. Anim. Sci. 94:2648–2663. doi:10.2527/jas.2016-0282.

Schwandt, E. F., J. J. Wagner, T. E. Engle, S. J. Bartle, D. U. Thomson, and C. D. Reinhardt. 2016. The effects of dry-rolled corn particle size on performance, carcass traits, and

starch digestibility in feedlot finishing diets containing wet distiller' s grains. J. Anim. Sci. 94:1194–1202. doi:10.2527/jas2015-9408.

Secrist, D. S., W. J. Hill, F. N. Owens, and S. D. Welty. 1995. Effects of corn particle size on feedlot steer performance and carcass characteristics. Anim. Sci. Res. Rep. 99-10.

Sindt, J. J. 2004. Factors influencing the utilization of steam-flaked corn. PhD Diss. Kansas State Univ., Manhattan.

Sindt, J. J., J. S. Drouillard, S. P. Montgomery, and E. R. Loe. 2006a. Factors influencing characteristics of steam-flaked corn and utilization by finishing cattle. J. Anim. Sci. 84:154–161.

Sindt, J. J., J. S. Drouillard, E. C. Titgemeyer, S. P. Montgomery, E. R. Loe, B. E. Depenbusch, and P. H. Walz. 2006b. Influence of steam-flaked corn moisture level and density on the site and extent of digestibility and feeding value for finishing cattle. J. Anim. Sci. 84:424–432.

Sindt, J. J., S. P. Montgomery, T. B. Farran, and J. S. Drouillard. 2000. Refractive index: a rapid method for determination of starch availability in grains. Kansas Agric. Exp. Stn. Res. Reports. 0:62–64.

Singh, V., and D. Johnston. 2004. An enzymatic process for corn wet milling. Adv. Food Nutr. Res. 48:151–171. doi:10.1016/S1043-4526(04)48003-6.

Smith, C. W. 2004. Corn: Origin, History, Technology, and Production. Volume 4. (C. W. Smith, B. Javier, and C. A. E. Runge, editors.). John Wiley & Sons.

Stock, R. A., M. H. Sindt, R. M. Cleale, and R. A. Britton. 1991. High-moisture corn utilization in finishing cattle. J. Anim. Sci. 69:1645–1656. doi:10.2527/1991.6941645x.

Stock, R. A., M. H. Sindt, J. C. Parrott, and F. K. Goedeken. 1990. Effects of grain type, roughage level and monensin level on finishing cattle performance. J. Anim. Sci. 68:3441–3455. doi:10.2527/1990.68103441x.

Tester, R. F., J. Karkalas, and X. Qi. 2004. Starch - Composition, fine structure and architecture. J. Cereal Sci. 39:151–165. doi:10.1016/j.jcs.2003.12.001.

Theurer, C. B. 1986. Grain processing effects on starch utilization by ruminants. J. Anim. Sci. 63:1649–62. doi:10.2527/jas1986.6351649x.

Theurer, C. B., O. Lozano, A. Alio, A. Delgado-Elorduy, M. Sadik, J. T. Huber, and R. A. Zinn. 1999. Steam-processed corn and sorghum grain flaked at different densities alter ruminal, small intestinal, and total tract digestibility of starch by steers. J. Anim. Sci. 77:2824–2831.

Tonroy, B. R., T. W. Perry, and W. M. Beeson. 1974. Dry, ensiled high-moisture, ensiled reconstituted high-moisture and volatile fatty acid treated high moisture corn for growing-finishing beef cattle. J. Anim. Sci. 39:931–936.

Vasconcelos, J. T., and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: The 2007 Texas Tech University survey. J. Anim. Sci. 85:2772–2781. doi:10.2527/jas.2007-0261.

Ward, C. F., and M. L. Galyean. 1999. The relationship between retrograde starch as measured by starch availability estimates and in vitro dry matter disappearance of steam-flaked corn. Burnett Center Internet Progress Report. No. 2, 1-3.

Wardynski, F. A., S. R. Rust, and M. T. Yokoyama. 1993. Effect of microbial inoculation of high-moisture corn on fermentation characteristics, aerobic stability, and cattle performance. J. Anim. Sci. 71:2246–2252. doi:10.2527/1993.7182246x.

Xing, J. jie, D. Li, L. jun Wang, and B. Adhikari. 2018. Temperature thresholds and time-temperature dependence of gelatinization for heat-moisture treated corn starch. J. Food Eng. 217:43–49. doi:10.1016/j.jfoodeng.2017.08.019.

Zinn, R. A. 1988. Comparative feeding value of supplemental fat in finishing diets for feedlot steers supplemented with and without monensin. J. Anim. Sci. 66:213–227. doi:10.2527/jas1988.661213x.

Zinn, R. A. 1990a. Influence of flake density on the comparative feeding value of steam-flaked corn for feedlot cattle. J. Anim. Sci. 68:767–775.

Zinn, R. A. 1990b. Influence of steaming time on site of digestion of flaked corn in steers. J. Anim. Sci. 68:776–781. doi:10.2527/1990.683776x.

Zinn, R. A., E. G. Alvarez, M. F. Montano, A. Plascencia, and J. E. Ramirez. 1998. Influence of tempering on the feeding value of rolled corn in finishing diets for feedlot cattle. J. Anim. Sci. 76:2239–2246.

Zinn, R. A., and R. Barrajas. 1997. Comparative ruminal and total tract digestion of a finishing diet containing fresh vs air-dry steam-flaked corn. J. Anim. Sci. 75:1704–1707. doi:10.2527/1997.7571704x.

Zinn, R. A., A. Barreras, L. Corona, F. N. Owens, and A. Plascencia. 2011. Comparative effects of processing methods on the feeding value of maize in feedlot cattle. Nutr. Res. Rev. 24:183–190. doi:10.1017/S0954422411000096.

Zinn, R. A., F. N. Owens, and R. A. Ware. 2002. Flaking corn: Processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. J. Anim. Sci. 80:1145–1156. doi:10.2527/2002.8051145x.

CHAPTER 2

Effects of dry-processing and reconstituted high-moisture ensiling of high-amylase corn on ruminal *in situ* and *in vitro* digestive characteristics

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ABSTRACT

High-amylase corn (Enogen[®] Feed Corn, EFC) is characterized by amplified expression of heat stable amylase. Objectives of Exp. 1 were to evaluate effects of particle sizes, dry-heat conditioning, and proportion of EFC on *in situ* and *in vitro* digestion of cereal grain mixtures. High-amylase corn and mill-run corn (CON) were processed (Wiley Mill) with 4, 6, or 9 mm screens; blended to produce mixtures containing 0, 33.3, 66.7, or 100% EFC; and then heated in a forced-air oven, to achieve internal temperatures of 50, 75, or 100ºC (plus a non-heated control). The 3 x 4 x 4 factorial was completed in duplicate. There were no 2- or 3-way interactions among factors ($P > 0.05$). Reducing screen size from 9 to 4 mm linearly increased *in situ* dry matter disappearance (ISDMD; $P < 0.01$), and *in vitro* gas production (IVGP; $P < 0.01$). Decreasing screen size reduced acetate:propionate (A:P) ratio (linear and quadratic; $P = 0.02$), and increased total volatile fatty acid (VFA) production linearly ($P = 0.05$). Heating to 75ºC improved ISDMD (quadratic; $P < 0.01$), but had no effect on IVGP ($P = 0.25$), and minimal effect on VFA production. Greater inclusion of EFC linearly improved ISDMD ($P < 0.01$) and IVGP ($P = 0.02$), but had no effects on VFA profile ($P > 0.05$). Experiment 2 evaluated effects of moisture in high-moisture blends of EFC on ensiling and digestive characteristics. High-amylase corn and CON were blended to contain 0, 25, 50, 75, or 100% EFC. Blends were reconstituted with water to achieve final moisture content of 27, 30, or 33%, and were ensiled 346 d. Silos were opened and grain was mixed with deionized water, steeped

30 min, measured for pH, filtered, and liquid extract was frozen. Liquid extract was analyzed for VFA and lactate. Moisture x EFC interactions occurred; the pH of liquid extract had minimal changes when increasing EFC amount in blends that were 30% moisture. pH was reduced in 27% moisture blends with 50% EFC, but in blends containing 33% moisture, pH increased with 50% EFC (moisture x EFC; $P < 0.01$). This may be a response to lactic acid, as blends with 27% moisture had increased lactate when 50% EFC was used, and blends with 33% moisture had decreased lactate with 50% EFC (moisture x EFC; $P < 0.01$). Liquid extract acetate increased when 27% moisture was used with 50 and 75% EFC; but in 30 or 33% moisture blends this response wasn't observed (moisture x EFC; $P < 0.01$). High-amylase corn had minimal impact on isobutyrate concentration in 27% moisture blends, but in 30% moisture corn, isobutyrate increased when using 75 and 100% EFC; this response was even greater in 33% moisture blends as isobutyrate rose at every increase in EFC proportion in grain mixtures (moisture x EFC; $P < 0.01$). Valerate was only present in liquid extract of 33% moisture blends containing 75 or 100% EFC (moisture x EFC; $P < 0.01$). Liquid extract heptanoate decreased with 50% EFC in 30 and 33% moisture blends but was followed by a sharp increase with 75% EFC inclusion; the opposite was true in grain mixtures with 27% moisture, as heptanoate increased with 50% EFC, followed by a sharp decrease with 75% EFC (moisture x EFC; $P = 0.02$). Increasing moisture from 27 to 33% linearly increased propionate, butyrate, and total VFA during ensiling ($P < 0.01$). Increasing moisture also increased ISDMD, IVGP, and *in vitro* production of all VFA linearly ($P < 0.01$), and quadratically ($P < 0.02$) for acetate, valerate, and total VFA. *In vitro* production of propionate with 27% moisture corn decreased with 50% EFC inclusion; when using 33% moisture, propionate rose from 0 to 50% EFC, but then dropped when 75% EFC was used; in 30% moisture corn propionate production rose steadily with every increase of EFC amount

(moisture x EFC; $P = 0.02$). In 30% moisture corn, *in vitro* production of butyrate decreased when 25% EFC was included; in 33% moisture corn, butyrate increased when 25% EFC was included, but dropped with 100% EFC (moisture x EFC; $P = 0.04$). Total VFA in liquid extract increased linearly with greater EFC concentration ($P = 0.05$). Proportion of EFC in grain mixtures did not affect ISDMD ($P = 0.19$), but IVGP increased linearly ($P < 0.01$) with greater EFC content. Increasing EFC proportion linearly improved A:P ratio, and total VFA ($P < 0.02$). Lack of quadratic effects indicate digestive improvements by EFC are confined to that component of grain mixtures.

Keywords: Amylase, dry-processed, high-moisture, *in situ*, *in vitro*

Abbreviations: High-amylase corn/Enogen Feed Corn, EFC; mill-run corn, CON; *in situ* dry matter disappearance, ISDMD; *in vitro* gas production, IVGP; volatile fatty acid, VFA; high-moisture corn, HMC; acetate:propionate ratio, A:P; average daily gain, ADG; Kansas State University Beef Cattle Research Center, KSU BCRC; Kansas State University Pre-Harvest Food Safety Laboratory, KSU PHFSL; gas chromatography, GC; dry-rolled corn, DRC; organic matter, OM; body weight, BW.

1. Introduction

Still new in its use as a feedstuff, Enogen® Feed Corn (Syngenta Seeds, LLC) is a genetically modified corn, characterized by increased expression of heat-stable α-amylase in the kernel endosperm. Initial use of EFC was for ethanol production and is now being identified as a potential livestock feed. Corn is the primary grain fed to meet energy requirements in U.S. cattle diets. Grains with increased amylase content may be beneficial as ruminants have limited capacity for secretion of pancreatic amylase (Harmon et al., 2004). Our experiments focus on the impacts during ruminal digestion.

Dry-processing or high-moisture ensiling are common methods to enhance feeding value of corn (Owens et al., 1997). Preliminary work with dry-processed EFC has shown improvements in feed efficiency greater than 5% (Harris et al., 2016; Johnson et al., 2018). However, when fed as HMC, EFC has had negative effects on feed efficiency and ADG (Jolly-Breithaupt et al., 2016a). Actions of EFC when fed to cattle are still unclear and require more research. Grain processing method likely has substantial impact on digestive efficiency of EFC. Horton et al. (2017) used steam-flaking as a method to activate the enzyme within EFC, and observed improvements in feedlot performance and microbial digestion. Using 2 experiments our objectives were to: (1) evaluate effects of dry-heat and particle size on digestive characteristics of dry-processed EFC, and (2) evaluate effects of moisture content on high-moisture ensiling and digestive characteristics of EFC.

2. Materials and methods

2.1. Experiment 1

2.1.1. Experimental design

Whole CON and EFC were ground in a Wiley Mill using screen sizes of 9, 6, or 4 mm. Grains were then blended (w/w) to contain 0, 33.3, 66.7, or 100% EFC with CON making up the balance. Samples (1 kg) prepared in duplicate were then heated in a forced-air oven to achieve internal temperatures of 50, 75, or 100ºC, which were maintained for 5 min. A non-heated control was also used. The 3 x 4 x 4 factorial arrangement of treatments was evaluated in duplicate.

2.1.2. Determination of particle size

Approximately 200 g dry-processed corn (prior to blending) from each grain type and screen size were placed onto a set of sieves with screen openings of: 2,360; 1,700; 1,180; 850; 600; 425; 300 μm; and a solid pan. Sieves were placed in a Ro-Tap orbital shaker (RX-30; W. S. Tyler, Mentor, OH) using a 5-min rotary tapping cycle. Grain fractions from each sieve were then weighed, and values entered into a spreadsheet (Baker and Herrman, 2002) calculating mean geometric particle size and standard deviation, using equations developed by Pfost and Headley (1976).

2.1.3. In situ dry matter disappearance

A fraction from each grain sample (3 g DM) was weighed and sealed into Dacron bags, prepared in triplicate. Dry-weights of bags were recorded prior to addition of grain. Analyses were repeated over 3 d using 6 fistulated Jersey steers housed at the KSU BCRC. Blocks were animal within day. Bags were incubated ruminally for 14 h, after which bags were removed, rinsed with water, dried 24 h at 105ºC, and weighed to calculate dry matter disappearance. Blank bags with no grain were used to adjust values.

2.1.4. In vitro gas production and VFA profiles

In vitro gas production and VFA profiles were measured in duplicate for each sample over 4 runs, with 1 replicate per run. Ruminal fluid was collected from 2 fistulated steers at the KSU BCRC, then transported to the KSU PHFSL; it was then strained through 8 layers of cheese cloth into a separatory flask, degassed using nitrogen, and placed in a 39ºC incubation chamber. Strained ruminal fluid then separated into 3 distinct layers over approximately 1 h. While waiting for separation, 140 mL McDougall's buffer was poured into 250-mL fermentation bottles containing pre-weighed samples (3 g DM) of grain. Two blanks were used per run to later adjust for background concentrations of VFA contributed by the ruminal inoculum.

The bottom fraction in the separatory flask was discarded, and the center, microbe rich fraction was used to inoculate each bottle with 10 mL mixed ruminal microbes. Immediately after inoculation, initial pH was recorded, the bottle was degassed with nitrogen, and capped with an ANKOM pressure sensing module (ANKOM Technologies, Macedon, NY). ANKOM modules recorded cumulative gas production from microbial cultures every 15 min. Once a bottle was capped it was placed into an incubation chamber (39ºC) equipped with an orbital shaker for continuous light agitation of substrate and incubated 24 h. Following incubation, each bottle was opened, a final pH reading taken, and 4 mL supernatant were combined with 1 mL 25%-metaphosphoric acid, and frozen. Frozen samples were later thawed, vortexed, and centrifuged to remove the top layer for VFA analyses by GC. A GC (Aglient Technologies, Santa Clara, CA) with a Nukol capillary column (15 m x 0.35 mm, d_f 0.50 μm; Supelco, Bellefonte, PA) was used to identify and quantify VFAs. Standards were prepped to identify acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate, isocaproate, and heptanoate.

2.1.5. Statistical analyses

All data were analyzed using the MIXED procedure of the Statistical Analysis System (SAS version 9.4; SAS Inst. Inc., Cary, NC). Fixed effects were percent EFC in grain blends, mill screen size, heat treatment, and all 2- and 3-way interactions. A random effect of block was used, and orthogonal contrasts were run to examine linear and quadratic effects of treatments.

2.2. Experiment 2

2.2.1. Experimental design

Whole EFC and CON were processed in a Wiley Mill using a 9 mm screen. Grains were then blended to contain 0, 25, 50, 75, or 100% EFC with CON as the remainder. Corn blends were then put into glass jars, and reconstituted with deionized water to achieve final moisture content of 27, 30, or 33%. Jars were placed on rollers for 30 min, ensuring adequate mixing and moisture distribution. After reconstitution, 1 kg grain was placed into foil bags equipped with a one-way valve and sealed under vacuum to remove nearly all oxygen. Sealed bags were placed in coolers with atmospheric temperature of approximately 24ºC, and preserved by fermentation for 346 d prior to being opened. When opened, two sub-samples from each bag were taken to determine DM by heating in a forced-air oven at 105ºC, and the remaining portion was frozen. The 5 x 3 factorial arrangement of treatments was prepared in duplicate as a randomized complete block design.

2.2.2. Grain fermentative characteristics after ensiling

Deionized water (35 mL) was combined with 25 g (DM) from each sample, and steeped 30 min with intermittent stirring. A pH reading was recorded, and liquid was then squeezed from each sample through filter paper into scintillation vials and frozen. Volatile fatty acids were measured by GC using the same procedure as Exp. 1 (*2.1.4.*), after combining 4 mL liquid extract with 1 mL 25%-metaphosphoric acid.

Lactic acid was measured using a colorimetric procedure adapted from Barker and Summerson (1941). Standards were prepared to contain 0, 10, 20, 30, 40, and 50 mg/dL lactate, in triplicate. Liquid extract from corn samples were again acidified with 25%-metaphosphoric acid and diluted with deionized water 20:1. Prepared in duplicate, 0.5 mL liquid extract from corn samples were combined with 0.5 mL 20%-$CuSO_4 \cdot 5H_2O$, 4 mL DI water, and 0.5 g $Ca(OH)_2$ powder. This mixture was vortexed vigorously and remained at room temperature for 30 min with occasional shaking. Tubes were then centrifuged at 1000 x g for 10 min. Supernatant (0.5 mL) was transferred to a different glass tube, wherein 25 mL μL 4%-$CuSO_4$ was added and mixed. This was followed by a 3.0 mL addition of sulfuric acid, after which tubes were vortexed. Tubes were then placed in a boiling water bath for 5 min, then placed in cold water and cooled until below 20°C. Once cooled, 50 μL p-hydroxydiphenyl was added to tubes and quickly vortexed. Tubes were then placed in a 30°C water bath for 30 minutes, mixing gently after 10 and 20 min. After this period, tubes were put back in the boiling water bath for 1.5 min, destroying excess alkali as purple color develops. Tubes were again cooled in cold water until reaching room temperature. Each tube was vortexed, 30 μL of final substrate were pipetted into a plate reader, and absorbance at 560nm was determined. Values for each sample were assessed using software based off the regression line generated from the 6 standards.

2.2.3. In situ dry matter disappearance

In situ dry matter disappearance was performed using the same procedure as Exp. 1 (*2.1.3.*). Bags were again prepared in triplicate with 3 g (DM) grain and incubated ruminally for 14 h. Three fistulated steers were used and analyses were repeated once over 2 d.

2.2.4. In vitro gas production and VFA profiles

Procedures for IVGP and VFA production were identical to Exp 1. (*2.1.4.*); the only difference being samples for this experiment were analyzed in triplicate over 3 runs.

2.2.5. Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (version 9.4). Fixed effects were percent EFC in grain mixture, moisture, and interactions between the two, which were examined using linear and quadratic contrasts. Block was used as a random effect.

3. Results

3.1. Experiment 1

No 2- or 3-way interactions among factors were observed ($P < 0.05$), thus only main effects are reported.

3.1.1. Particle size distribution

Particle size between EFC and CON did not differ ($P = 0.73$; Table 2.1), nor did particle size standard deviation ($P = 0.47$); it's distribution across sieves can be viewed in Figure 2.1. Averaged between corn type, particle sizes were 1070, 1543, and 1855 µm d_{gw} for corn processed through screen sizes of 4, 6, and 9 mm respectively; this was a linear ($P < 0.01$) and quadratic ($P = 0.03$) response (Table 2.2). Distribution of particles weighed from each sieve size is displayed in Figure 2.2. Standard deviation of particle size tended to differ (linear effect; $P = 0.10$), as screen sizes of 6 and 9 mm resulted in greater numerical S_{gw}.

3.1.2. Effects of particle size

When decreasing particle size through use of smaller screen openings (9, 6, or 4 mm) during milling, ISDMD and IVGP both increased in a linear fashion ($P < 0.01$; Table 2.3). Reducing particle size resulted in increased *in vitro* production of propionate (linear; $P < 0.01$),

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butyrate (linear; $P = 0.03$), valerate (linear; $P < 0.01$), and total VFA (linear; $P = 0.05$).

Acetate:propionate ratio had a linear ($P < 0.01$) response, as decreasing screen size from 9 to 4

mm resulted in lower numerical ratios. There also was a quadratic response ($P = 0.02$), as there

was a greater numerical decrease in A:P ratio when reducing screen size from 6 to 4 mm, as

opposed to decreasing from 9 to 6 mm. Decreasing screen size approached a linear tendency ($P = 0.11$) to improve acetate production. Particle size had no effect on production of minor VFAs,

isobutyrate ($P = 0.32$) or isovalerate ($P = 0.33$).

3.1.3. Effects of heat conditioning

Dry heat treatment of corn blends resulted in a quadratic ($P < 0.01$) improvement of

ISDMD; heating grains to 75ºC was optimal, but heating to 100ºC reduced ISDMD (Table 2.4).

There were no effects on IVGP ($P = 0.25$), or *in vitro* production of acetate ($P = 0.43$),

propionate ($P = 0.56$), butyrate ($P = 0.48$), valerate ($P = 0.42$), or total VFA ($P = 0.40$). By

increasing temperature applied to grains, A:P ratio tended to decrease linearly ($P = 0.09$).

Production of minor VFAs was suppressed using the 100ºC temperature endpoint; isobutyrate

decreased linearly ($P = 0.02$) and quadratically ($P = 0.02$) due to the largest difference being the

drop between 75 and 100ºC. The same could be said for isovalerate, which had a linear ($P < 0.01$) and quadratic ($P = 0.02$) response, as there was reduced production with the greatest heat

treatment.

3.1.4. Effects of high-amylase corn

By increasing the proportion of EFC blended with CON, ISDMD increased linearly ($P < 0.01$; Figure 2.3), as did IVGP ($P = 0.02$; Figure 2.4). These effects were not as pronounced

when analyzing *in vitro* VFA profile (Table 2.5). High-amylase corn did not affect production of

acetate ($P = 0.84$), propionate ($P = 0.39$), butyrate ($P = 0.88$), isobutyrate ($P = 0.24$), or total

VFA ($P = 0.86$). Acetate:propionate ratio tended to decrease linearly ($P = 0.09$) as greater amounts of EFC were utilized. By increasing inclusion of EFC, production of valerate tended to increase (linear; $P = 0.6$), while isovalerate tended to decrease (linear; $P = 0.07$).

3.2. Experiment 2

3.2.1. Effects of moisture on fermentative characteristics from ensiling

Values in Table 2.6 reflect the composition of liquid extract obtained by methods described in section *2.2.2*. Interactions between moisture x EFC occurred ($P < 0.01$) for pH and lactate. When using 27% moisture, 50% EFC had the lowest numerical pH, but when using 33% moisture, 50% EFC was greatest. Using 50% EFC had seemingly little impact on pH in blends with 30% grain moisture. This trend was somewhat reflective in liquid extract lactate, in that one may expect greater concentrations of lactate in instances where pH is reduced. When using 27% moisture, 50% EFC had the greatest concentration of lactic acid, but when using 33% moisture, 50% EFC had the lowest concentration. Using 50% EFC again had little to no impact on lactate when 30% moisture was used. There was a moisture x EFC interaction for acetate ($P < 0.01$); when using 30 or 33% moisture, concentration of acetate dropped as greater amounts of EFC were included, but when using 27% moisture, acetate increased when using 50 and 75% EFC. Isobutyrate resulted in another moisture x EFC interaction ($P < 0.01$). Isobutyrate was minimal when using 27% moisture and any proportion of EFC in grain mixtures. When using 30% moisture, isobutyrate was again minimal until 75 and 100% EFC were included, as a large increase in isobutyrate concentration occurred. The effect of EFC increasing concentration of isobutyrate was even more pronounced when using 33% moisture, as isobutyrate continually increased as greater amounts of EFC were incorporated. Valerate was only present in blends that were 33% moisture and contained 75 or 100% EFC (moisture x EFC; $P < 0.01$). The final

moisture x EFC interaction occurred in heptanoate ($P = 0.02$). When using either 27 or 30% moisture, heptanoate concentration decreased when 50% EFC was included, but then increased with 75 and 100% EFC. When using 33% moisture the response to EFC was more erratic; at this moisture, heptanoate was numerically greatest with 0% EFC, then decreased when 25% EFC was included, increased with 50% EFC, but dropped to its lowest concentration when 75% EFC was used, and again increased with 100% EFC. Concentration of propionate and butyrate increased linearly ($P < 0.01$) as corn moisture from reconstitution increased from 27 to 33%. Isovalerate also tended to increase with greater amounts of moisture (linear; $P = 0.10$). While there was a tendency for a moisture x EFC interaction ($P = 0.10$), total VFA in reconstituted high-moisture corn blends increased linearly ($P < 0.01$) as moisture increased.

3.2.2. Effects of moisture on in situ and in vitro digestion

As moisture increased from 27 to 33% in corn blends, ISDMD responded as a linear increase ($P < 0.01$), as did IVGP ($P < 0.01$; Table 2.7). *In vitro* gas production also resulted in a quadratic effect ($P < 0.01$), as the increase was much greater when increasing moisture from 27 to 30%, than it was between 30 and 33%. Moisture x EFC interactions occurred (Table 2.8) during *in vitro* VFA production of propionate ($P = 0.02$), butyrate ($P = 0.04$), isovalerate ($P = 0.04$), as well as a tendency for interaction for valerate production ($P = 0.10$). When using 27% moisture, propionate increased initially from 0 to 25% EFC, but decreased with 50% EFC; it then increased with 75% EFC, and again with 100% EFC. Propionate production increased with every increase in EFC inclusion when 30% moisture was used. When using 33% moisture, propionate increased from 0 to 50% EFC, but then dropped when using 75 and 100% EFC. The moisture x EFC interaction for butyrate production is likely due to 25% EFC increasing butyrate in 33% moisture blends but decreasing butyrate when using 30% moisture. Also, when using

33% moisture, 100% EFC resulted in decreased butyrate, which was not observed in either 27 or 30% moisture treatments. *In vitro* production of isovalerate with 30 and 33% moisture responded similarly to increasing increments of EFC; both dropped initially between 0 and 50% EFC, then increased with 75% EFC, and was followed by another drop in isovalerate when 100% EFC was used. In contrast, using 27% moisture resulted in a slight increase in 50% EFC blends, followed by a sharper decrease when 75% EFC was included. By increasing moisture in blends, production of acetate (linear and quadratic effect; $P < 0.01$), isobutyrate (linear; $P < 0.01$), valerate (linear and quadratic effect; $P = 0.02$), and caproate (linear; $P < 0.01$) all increased. Production of total VFA also resulted in both linear ($P < 0.01$) and quadratic ($P < 0.01$) improvements due to increasing moisture content.

3.2.3. *Effects of high-amylase corn on fermentative characteristics from ensiling*

When analyzing composition of liquid extract from reconstituted high-moisture corn blends, moisture x EFC interactions were again: pH, lactate, acetate, isobutyrate, valerate, and heptanoate (see *3.2.1.*; Table 2.6). When increasing EFC content in blends from 0 to 100%, greater amounts of EFC tended to linearly increase propionate concentration ($P = 0.10$; Table 2.6). There was a tendency for a quadratic response for butyrate ($P = 0.08$), as it appears its production was reduced when including 50% EFC. High-amylase corn had no effect on isovalerate concentration ($P = 0.14$). Keeping in mind there was a tendency for a moisture x EFC interaction when analyzing total VFA in liquid extract ($P = 0.10$), using greater concentrations of EFC increased total VFA (linear; $P = 0.05$). There also was a quadratic effect ($P = 0.02$), where including 50% EFC in corn blends reduced total VFA.

3.2.4. *Effects of high-amylase corn on in situ and in vitro digestion*

Concentration of EFC in reconstituted high-moisture corn blends did not affect ISDMD ($P = 0.19$; Table 2.9). However, IVGP increased linearly ($P < 0.01$) with greater inclusion of EFC. As stated in section *3.2.2.*, moisture x EFC interactions for *in vitro* VFA profile were: propionate, butyrate, isovalerate, and an interactive tendency for valerate production (Table 2.8). High-amylase corn had no effect ($P = 0.41$) on acetate production, but linearly decreased ($P < 0.01$) A:P ratio as inclusion of EFC increased from 0 to 100%. Isobutyrate and caproate production was less in blends containing greater proportions of EFC than those having CON as the dominant grain (linear; $P < 0.01$). High-amylase corn caused a quadratic response ($P = 0.02$) in valerate production, as there was a marked increase when increasing EFC content from 0 to 25%. Total VFA produced by *in vitro* fermentation improved linearly ($P = 0.02$), as greater amounts of EFC were included in corn blends.

4. Discussion

Identifying overall impact of EFC on ruminal digestive characteristics was part of the objective for both experiments, but including potential interactive factors that may induce changes in EFC digestion were also implemented. This was not the case in Exp. 1 as there were no 2- or 3-way interactions. Differences in particle size when feeding DRC have been well researched and match our findings for increased *in situ* and *in vitro* digestion. Ruminal digestion of starch is increased in both rate and extent when feeding smaller particle sizes (Owens et al., 1997). Ruminal digestion may be shifted post-ruminally to a greater extent with coarser grinds (Owens et al., 1986).

The improvement in *in situ* digestion when heating grains up to 75ºC was independent of corn type, and likely had no effect on amylase activity within EFC. This means that to amplify

activity of the enzyme likely requires a combination of moisture and heat. This impact was less pronounced during *in vitro* digestion. Research has evaluated dry heating of grains, but is specific in targeting drying of grains with much higher initial moisture content than what we used (12 to 13% vs moisture greater than 30%). In a review, Odjo et al. (2015) acknowledges the drying and heating of corn changes structural components of starch and protein, likely affecting digestibility. They state there's no consensus on how dry heating changes feeding value of corn, likely due to the wide difference of conditions in experimental procedures. Reduction in digestibility when applying dry heat reaching 100°C may be explained by reduced swelling power and reduced capacity to bind water due to increased structural rigidity (Malumba et al., 2010, 2009). This interferes with ability of starch to gelatinize. When applying dry heat, Rooney and Pflugfelder (1986) state dextrinization can occur which reduces starch digestibility. There also is the potential for maillard reactions to occur, a reaction between a reducing sugar and amino group of a protein, reducing solubility and digestibility (Hedegaard and Skibsted, 2013). Maillard reactions can occur at almost any temperature, but require heat before meaningful changes occur, with 100 to 110°C being a critical threshold for peptide degradation and rapid crosslinking of peptides with sugars (Lan et al., 2010). Mechanisms to explain improvement in ISDMD when heating corn blends up to 75°C are less clear. It's possible that what little moisture is in corn fragments may cause partial gelatinization when subjected to heat. Gelatinization of starch is largely responsible for improvements in digestibility using more advanced corn processing methods like steam-flaking (Huntington, 1997; Svihus et al., 2005). However, what gelatinization could occur is likely minimal; Altay and Gunasekaran (2006) found corn starch that was dried at either 20 or 100°C required a minimum 21 or 29% water respectively to gelatinize.

With EFC still an emerging feedstuff for cattle feeding, literature to compare with is somewhat limited. Our observations of improved ISDMD and IVGP using dry-processed EFC indicate increased microbial digestion with EFC. As no quadratic effects occurred, actions of EFC are likely confined to that component of the grain mixture, and did not impact digestibility of CON portions. High-amylase corn fed whole or dry-rolled has resulted in a 5.5% increase in feed efficiency in growing cattle (Johnson et al., 2018). Harris et al. (2016) had mixed results in two experiments feeding dry-rolled EFC to finishing cattle; 1 experiment showed no difference in feed efficiency, while the second showed a 5.4% improvement. This is perhaps due to composition of other ingredients included in the diets. While we observed improved ruminal digestion, Jolly-Breithaupt et al. (2016b) reported no differences in ruminal digestibility of dry-rolled EFC. However, they did observe tendencies for improved postruminal digestion of OM and starch, and total-tract digestion of DM, OM, and starch were all greater in cattle fed EFC compared to their control. There's enough evidence to suggest feeding dry-processed EFC results in improved digestive efficiency when compared to respective control corn.

Results from Exp. 2, especially fermentative characteristics from ensiling are more difficult to interpret due to numerous EFC x moisture interactions. A major abnormality in our findings was substantial production of butyrate. Principally, lactic acid is the predominant factor that decreases pH during ensiling to preserve the final product (Rutherford, 2014). After lactate, acetate is generally the organic acid found in the next greatest concentration. While we generally found lactate to be in the greatest amount, butyrate was numerically higher in 27% moisture and 0% EFC blends. Grain type had no effect on butyrate production, so it can't be identified as a trait of EFC fermentation. Butyrate can be formed through 4 different pathways, and can be performed by many bacterial families (Vital et al., 2014). With ensiled corn it's unlikely

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pathways such as lysine, glutarate, or 4-aminobutyrate would have much or any impact; in high-moisture corn butyrate likely follows the acetyl-CoA pathway for formation by the breakdown of polysaccharides in starch (Rutherford, 2014; Vital et al., 2014). In many cases butyrate isn't reported in the literature, however, some studies have found butyrate in varying quantities (Goodrich et al., 1975; Schaefer et al., 1989). Goodrich et al. (1975) found butyrate increased as moisture content of grains increased from 21.5 to 33.1% (lactate and acetate also increased similarly to our findings). Butyrate surpassed propionate using 27.5 and 33.1% moisture, and at 33.1%, butyrate concentration was 36% less than acetate. Still, no studies have approached the levels of butyrate we observed, and it's unclear if a specific bacterial species caused the spike in butyrate, or what other mechanisms could have come into play.

Effects of moisture on total VFA produced during ensiling, where increasing grain moisture resulted in greater quantities of VFA agree with results from Goodrich et al. (1975). They however did not observe a similar increase in propionate formation between 27.5 and 33.1% moisture. Increasing moisture improved *in situ* and *ruminal* digestion. Presence of quadratic effects in IPGP and total VFA suggest that there is a greater difference between corn that's 27 and 30% moisture, then there is from increasing moisture content from 30 to 33%. Through use of regression equations, Owens et al. (1997) suggests ADG and ME is optimal when corn moisture is between 30 and 31%, and DMI reduction can be expected with increasing amounts.

Including 50% EFC in blends reduced total VFA in liquid extract obtained post-ensiling. This is primarily due to it having the numerically lowest measurement of butyrate. There still was a linear effect where utilization of 75 or especially 100% EFC increased total VFA when compared to blends with less or zero EFC. This is the first research examining ensiling

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characteristics of EFC, and actions causing an increase in VFA during ensiling are still speculative. Presence of amylase may be responsible for more extensive/rapid breakdown of sugars yielding greater fermentative potential; but the suppression of total VFA when using 50% is unclear and an additional caveat.

High-amylase HMC improved *in vitro* fermentation, as greater concentrations of EFC improved IVGP, A:P ratio, valerate, and total VFA production. This indicates more efficient microbial digestion of grain. However, ISDMD was not affected by inclusion of EFC in corn mixtures. High-moisture corn is digested rapidly/and extensively in the rumen, much more so than other corn processing methods (dry-processing, steam-flaking; Owens and Soderlund, 2006). It may be that by 14-h ruminal incubation, grains had already been thoroughly degraded to where any effect of EFC had diminished. Jolly-Breithaupt et al. (2016a) found that feeding high-moisture EFC decreased feed efficiency in finishing steers by 2.1% compared to their control. Feeding dry-rolled EFC resulted in the greatest final BW and ADG, while those fed EFC as HMC were lower. If EFC fed as HMC is more rapidly degraded in the rumen, animals could be more prone to digestive disorders which could explain the decrease in live cattle performance (Huntington, 1997). More research likely needs to examine actions of EFC during ensiling, such as enzyme activity during fermentation and if it's still present at the time of feeding. Limited quadratic effects during microbial digestion again suggest actions of EFC are isolated to that component of grain mixtures.

5. Conclusions

Dry-heating corn to a moderate degree may be an effective strategy to create digestive improvements. This form of heat however had presumably no impact on EFC enzyme activity,

as there were no interactions between heat and EFC. High-moisture ensiling of EFC at different moisture levels likely requires more work for a better understanding of interactions. Microbial digestion using EFC has more clear effects, as its use dry-processed or as HMC both were more productive in either *in situ* or *in vitro* digestion when compared to CON. Use of EFC in cattle diets may provide feed efficiency benefits, and overall contribution of the amylase component likely needs quantified.

References

Altay, F., and Gunasekaran, S., 2006. Influence of drying temperature, water content, and heating rate on gelatinization of corn starches. J. Agric. Food Chem. 54:4235–4245. https://doi.org/10.1021/jf0527089

Baker, S., Herrman, T., 2002. Evaluating particle size. Kansas State Univ. Dep. Grain Sci. Ind. MF-2051, 1–6.

Barker, S., Summerson, W., 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138:535–554.

Goodrich, R.D., Byers, F.M., Meiske, J.C., 1975. Influence of moisture content, processing and Reconstitution on the fermentation of corn grain. J. Anim. Sci. 41:876–881. https://doi.org/10.2527/jas1975.413876x

Harmon, D.L., Yamka, R.M., Elam, N.A., 2004. Factors affecting intestinal starch digestion in ruminants: A review. Can. J. Anim. Sci. 84:309–318. https://doi.org/10.4141/A03-077

Harris, M.E., Jolly-Breithaupt, M.L., Nuttelman, B.L., Burken, D., Erickson, G.E., Rush, I.G., Luebbe, M.K., 2016. Evaluating syngenta enhanced feed corn on finishing cattle performance and carcass characteristics. Nebraska Beef Cattle Rep. 135–138.

Hedegaard, R. V., Skibsted, L.H., 2013. Shelf-life of food powders, Handbook of Food Powders: Processes and Properties. Woodhead Publishing Limited, Cambridge, UK. https://doi.org/10.1533/9780857098672.2.409

Horton, L.M., Bibber-Krueger, C.L. Van, Muller, H.C., Katulski, S.L., Ellerman, T.J., Drouillard, J.S., 2017. 870 In vitro and in situ digestion characteristics and feedlot performance of cattle fed steam-flaked Enogen (high-amylase) feed corn. J. Anim. Sci. 95:371–372. https://doi.org/10.2527/asasann.2017.868

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

Johnson, M.A., Spore, T.J., Montgomery, S.P., Weibert, C.S., Garzon, J.S., Hollenbeck, W.R., Wahl, R.N., Watson, E.D., Blasi, D., 2018. syngenta enhanced feed corn (Enogen) containing an alpha amylase expression trait improves feed efficiency in growing calf diets. Kansas Agric. Exp. Stn. Res. Reports 4.

Jolly-Breithaupt, M.L., Bittner, C.J., Burken, D.B., Erickson, G.E., Macdonald, J.C., Luebbe, M.K., 2016a. 385 Effect of feeding a corn hybrid containing α amylase as dry rolled or high moisture corn on finishing performance and carcass characteristics. J. Anim. Sci. 94:180. https://doi.org/10.2527/msasas2016-384

Jolly-Breithaupt, M.L., Harding, J.L., Macdonald, J.C., Erickson, G.E., Luebbe, M.K., 2016b. Site and extent of digestion of finishing diets containing Syngenta enhanced feed corn. Nebraska Beef Cattle Rep. pp 139–142.

Lan, X., Liu, P., Xia, S., Jia, C., Mukunzi, D., Zhang, X., Xia, W., Tian, H., Xiao, Z., 2010. Temperature effect on the non-volatile compounds of Maillard reaction products derived from xylose-soybean peptide system: Further insights into thermal degradation and cross-

linking. Food Chem. 120:967–972. https://doi.org/10.1016/j.foodchem.2009.11.033

Malumba, P., Janas, S., Roiseux, O., Sinnaeve, G., Masimango, T., Sindic, M., Deroanne, C., Béra, F., 2010. Comparative study of the effect of drying temperatures and heat-moisture treatment on the physicochemical and functional properties of corn starch. Carbohydr. Polym. https://doi.org/10.1016/j.carbpol.2009.09.013

Malumba, P., Massaux, C., Deroanne, C., Masimango, T., Béra, F., 2009. Influence of drying temperature on functional properties of wet-milled starch granules. Carbohydr. Polym. 75:299–306. https://doi.org/10.1016/j.carbpol.2008.07.027

Odjo, S.D.P., Malumba, P.K., Beckers, Y., Béra, F., 2015. Impact of drying and heat treatment on the feeding value of corn. A review. Biotechnol. Agron. Soc. Environ. 19:301–312.

Owens, F.N., and Soderlund, S., 2006. Ruminal and postruminal starch digestion by cattle. Cattle grain Process. Symp. 116–128. https://doi.org/10.1017/CBO9781107415324.004

Owens, F.N., Secrist, D.S., Hill, W.J., Gill, D.R., 1997. The effect of grain source and grain processing on performance of feedlot cattle: A review. J. Anim. Sci. 75:868–879.

Owens, F.N., Zinn, R.A., Kim, Y.K., 1986. Limits to starch digestion in the ruminant small intestine. J. Anim. Sci. 63:1634–1648. https://doi.org/10.2527/jas1986.6351634x

Pfost, H., and Headley, V., 1976. Methods of determining and expressing particle size. Feed Manuf. Technol. II - Append. C. Am. Feed Manuf. Assoc. 512–520.

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

Rutherford, W.M., 2014. Fermentation aids for high moisture corn. Oklahoma Beef Ext. 99–105.

Schaefer, D.M., Brotz, P.G., Arp, S.C., Cook, D.K., 1989. Inoculation of corn silage and high-moisture corn with lactic acid bacteria and its effects on the subsequent fermentations and on feedlot performance of beef steers. Anim. Feed Sci. Technol. 25:23–38.

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

Vital, M., Howe, A.C., Tiedje, J.M., 2014. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. MBio 5. https://doi.org/10.1128/mBio.00889-14

Table 2.1. Effect of grain source on dry-processed corn particle size

Item	Grain source[1]		SEM	*P*-value
	CON	EFC		
$d_{gw}{}^{2}$, μm	1481	1498	35.3	0.74
$S_{gw}{}^{3}$	2.26	2.29	0.026	0.47

[1]Mill-run (CON) or high-amylase (EFC) corn processed through 4-, 6-, or 9-mm screens in a Wiley Mill prior to being blended.
[2]Mean geometric particle size.
[3]Standard deviation of particle size.

Table 2.2. Effect of screen size on dry-processed corn particle size

| Item | Screen size[1], mm | | | SEM | P-value | |
	4	6	9		Linear	Quadratic
d_{gw}[2], μm	1070	1543	1855	43.2	< 0.01	0.03
S_{gw}[3]	2.21	2.32	2.31	0.031	0.10	0.13

[1]Screen that processed grains passed through in Wiley Mill.
[2]Mean geometric particle size.
[3]Standard deviation of particle size.

Table 2.3. Effects of dry-processed corn particle size on *in situ* dry matter disappearance (ISDMD), *in vitro* gas production (IVGP) and volatile fatty acid profiles

Item	Screen size[1], mm			SEM	P-value	
	4	6	9		Linear	Quadratic
ISDMD[2], %	61.7	56.1	51.0	1.68	< 0.01	0.09
IVGP[3], mL	123.3	113.0	102.5	11.06	< 0.01	0.62
VFA[4], mmols/g substrate DM						
Acetate	1.86	1.83	1.73	0.40	0.11	0.78
Propionate	1.62	1.49	1.38	0.34	< 0.01	0.54
A:P[5]	1.17	1.23	1.25	0.04	< 0.01	0.02
Butyrate	0.38	0.37	0.34	0.07	0.03	0.76
Isobutyrate	0.012	0.012	0.011	0.003	0.53	0.32
Valerate	0.032	0.029	0.026	0.007	< 0.01	0.45
Isovalerate	0.014	0.015	0.014	0.005	0.91	0.33
Total	5.08	4.98	4.75	0.81	0.05	0.85

[1]Screen that processed grains passed through in Wiley Mill.
[2]Measured in triplicate by ruminally incubating 3 g (DM) grain in Dacron bags for 14-h.
[3]Mean gas production over 24-h incubation. Used 3 g (DM) grain as substrate, added to 10 mL mixed ruminal microbes in a solution with 140 mL McDougall's buffer at 39ºC in duplicate for each sample.
[4]Analysis of VFA production by mixed rumen microbes after 24-h *in vitro* fermentation.
[5]Acetate:propionate ratio.
[6]VFA = volatile fatty acid; DM = dry matter.

Table 2.4. Effects of dry heat treatment of dry-processed corn on *in situ* dry matter disappearance (ISDMD), *in vitro* gas production (IVGP) and volatile fatty acid profiles

Item	Heat treatment[1], ºC				SEM	*P*-value	
	No heat	50	75	100		Linear	Quadratic
ISDMD[2], %	55.7	56.6	59.2	53.5	1.71	0.45	< 0.01
IVGP[3], mL	114.5	114.6	115.3	107.4	11.21	0.28	0.25
VFA[4], mmols/g substrate DM							
Acetate	1.83	1.78	1.90	1.72	0.40	0.58	0.43
Propionate	1.48	1.46	1.58	1.46	0.34	0.87	0.56
A:P[5]	1.23	1.23	1.22	1.19	0.04	0.09	0.11
Butyrate	0.37	0.35	0.38	0.34	0.07	0.48	0.59
Isobutyrate	0.012	0.012	0.012	0.010	0.003	0.02	0.02
Valerate	0.029	0.028	0.031	0.027	0.007	0.42	0.45
Isovalerate	0.015	0.015	0.015	0.011	0.005	< 0.01	0.02
Total	4.96	4.87	5.15	4.76	0.81	0.63	0.40

[1]Internal temperature grains were heated to in a forced-air oven and maintained 5-min.
[2]Measured in triplicate by ruminally incubating 3 g (DM) grain in Dacron bags for 14-h.
[3]Mean gas production over 24-h incubation. Used 3 g (DM) grain as substrate, added to 10 mL mixed ruminal microbes in a solution with 140 mL McDougall's buffer at 39ºC in duplicate for each sample.
[4]Analysis of VFA production by mixed rumen microbes after 24-h *in vitro* fermentation.
[5]Acetate:propionate ratio.
[6]VFA = volatile fatty acid; DM = dry matter.

Table 2.5. Effects of high-amylase corn on *in vitro* production of volatile fatty acids (VFA) by mixed rumen microbes fed dry-processed corn as substrate

VFA[1], mmoles/g substrate DM	High-amylase corn[2], %				SEM	*P*-value	
	0	33.3	66.7	100		Linear	Quadratic
Acetate	1.79	1.85	1.75	1.84	0.40	0.93	0.84
Propionate	1.45	1.53	1.46	1.54	0.34	0.39	0.99
A:P[3]	1.23	1.22	1.20	1.20	0.04	0.09	0.90
Butyrate	0.36	0.37	0.35	0.37	0.07	0.98	0.88
Isobutyrate	0.012	0.012	0.011	0.011	0.003	0.24	0.81
Valerate	0.027	0.029	0.028	0.031	0.007	0.06	0.98
Isovalerate	0.015	0.015	0.014	0.013	0.005	0.07	0.86
Total	4.89	5.03	4.82	5.00	0.81	0.86	0.90

[1]Analysis of VFA production by mixed ruminal microbes after 24-h *in vitro* fermentation.
[2]Percent EFC blended with CON in grain mixtures.
[3]Acetate:propionate ratio.
[4]EFC = high-amylase corn; CON = mill-run corn; VFA = volatile fatty acid; DM = dry matter.

Table 2.6. Effect of high-amylase corn and moisture in reconstituted high-moisture corn blends on pH and composition of organic acids in liquid extract[1] collected after ensiling

EFC[2], %	27% Moisture					30% Moisture					33% Moisture					SEM	M*EFC[3]	Moisture[4]		EFC[5]	
	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100			L	Q	L	Q
pH[6]	4.76	4.71	4.59	4.78	4.88	4.49	4.50	4.53	4.53	4.61	4.42	4.20	4.38	4.28	4.56	0.031	< 0.01	< 0.01	0.17	< 0.01	< 0.01
Organic acids[7], mM																					
Lactate	8.84	12.66	17.16	10.42	9.35	17.79	22.96	22.43	21.61	24.78	21.82	31.39	24.61	36.76	26.33	1.580	< 0.01	< 0.01	0.03	< 0.01	< 0.01
Acetate	3.20	3.54	5.03	4.70	3.50	5.07	4.88	4.66	4.51	4.56	8.24	6.98	6.05	5.54	6.09	0.474	< 0.01	< 0.01	0.04	0.04	0.92
Propionate	1.21	1.21	0.94	1.74	1.75	2.03	2.22	2.42	2.74	2.76	2.37	3.65	3.02	2.88	2.69	0.392	0.46	< 0.01	0.17	0.10	0.53
Butyrate	14.07	12.33	11.97	11.94	10.42	18.96	19.02	13.25	15.51	15.68	23.36	20.20	16.46	25.08	24.67	2.959	0.40	< 0.01	0.66	0.42	0.08
Isobutyrate	nd	0.80	0.42	0.74	0.09	0.71	0.49	0.61	1.82	7.28	0.51	1.34	2.64	5.86	14.50	1.039	< 0.01	< 0.01	0.38	< 0.01	< 0.01
Valerate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.065	1.048	0.1050	< 0.01	< 0.01	0.06	< 0.01	< 0.01
Isovalerate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.050	0.053	0.0187	0.65	0.10	0.33	0.14	0.66
Heptanoate	0.87	0.89	0.78	1.02	1.05	0.75	0.70	0.67	0.88	0.84	0.74	0.52	0.64	0.42	0.68	0.087	0.02	< 0.01	0.86	0.09	< 0.01
Total VFA	19.34	18.75	19.12	20.13	16.80	27.51	27.30	21.82	25.46	31.12	35.22	32.67	28.79	39.97	49.73	4.138	0.10	< 0.01	0.46	0.05	0.02

[1]Determined by mixing 25 g (DM) grain with 35 mL deionized water into a slurry, steeping 30 min, and filtering liquid extract through filter paper.
[2]Proportion of EFC blended with CON (0, 25, 50, 75, or 100%) prior to reconstitution with water, and subsequent ensiling.
[3] P-value for moisture x EFC interaction.
[4]P-values for main effects of grain moisture.
[5]P-values for main effects of EFC in grain mixtures.
[6]Measurement of liquid extract[1] determined by pH probe.
[7]Lactic acid measurement in liquid extract obtained by filtering the slurry[1], and determined using a colorimetric procedure (Barker and Summerson, 1941) completed in duplicate for each sample. Volatile fatty acids measured by filtering liquid extract[1] and analyzing each sample in duplicate using gas chromatography.
[8]EFC = high-amylase corn; CON = mill-run corn; L = linear effect; Q = quadratic effect; nd = none detected.

Table 2.7. Effects of moisture in reconstituted corn blends on *in situ* dry matter disappearance (ISDMD) and *in vitro* gas production (IVGP)

Item	Moisture[1], %			SEM	*P*-value		
	27	30	33		M*EFC[2]	Linear	Quadratic
ISDMD[3], %	55.2	64.4	68.7	2.30	0.31	< 0.01	0.21
IVGP[4], mL	103.5	119.6	120.3	2.57	0.38	< 0.01	< 0.01

[1]Final moisture content of reconstituted high-moisture corn blends.
[2]Moisture x EFC interaction.
[3]Measured in triplicate by ruminally incubating 3 g (DM) grain in Dacron bags for 14-h.
[4]Mean gas production over 24-h incubation. Used 3 g (DM) grain as substrate, added to 10 mL mixed ruminal microbes in a solution with 140 mL McDougall's Buffer at 39ºC in duplicate for each sample.
[5]EFC = high-amylase corn; M = moisture.

0

Table 2.8. Effect of high-amylase corn and moisture in reconstituted high-moisture corn blends on volatile fatty acid profiles from *in vitro* fermentation[1] by mixed ruminal microbes fed corn as substrate

EFC[2], %	27% Moisture					30% Moisture					33% Moisture					SEM	M*EFC[3]	Moisture[4]		EFC[5]	
	0	25	50	75	100	0	25	50	75	100	0	25	50	75	100			L	Q	L	Q
VFA, mmoles/g substrate DM																					
Acetate	1.79	1.78	1.82	1.77	1.84	1.95	1.92	1.93	1.95	2.00	1.87	1.97	1.94	1.93	1.92	0.066	0.95	< 0.01	< 0.01	0.41	0.90
Propionate	1.64	1.72	1.64	1.76	1.83	1.82	1.86	1.93	1.97	2.10	1.88	1.94	2.03	1.92	1.93	0.064	0.02	< 0.01	< 0.01	< 0.01	0.92
A:P[6]	1.10	1.04	1.11	1.01	1.01	1.08	1.04	1.01	1.00	0.95	1.01	1.02	0.96	1.01	1.00	0.046	0.17	< 0.01	0.47	< 0.01	0.86
Butyrate	0.37	0.38	0.36	0.36	0.39	0.46	0.41	0.42	0.43	0.43	0.48	0.50	0.46	0.47	0.41	0.017	0.04	< 0.01	0.14	0.06	0.82
Isobutyrate	0.010	0.009	0.009	0.007	0.007	0.013	0.013	0.010	0.010	0.008	0.013	0.014	0.012	0.012	0.010	0.0010	0.53	< 0.01	0.36	< 0.01	0.20
Valerate	0.044	0.047	0.048	0.043	0.049	0.056	0.058	0.059	0.062	0.060	0.056	0.066	0.068	0.070	0.061	0.0029	0.10	< 0.01	0.02	0.06	0.02
Isovalerate	0.012	0.011	0.012	0.008	0.008	0.020	0.018	0.013	0.014	0.011	0.021	0.024	0.017	0.019	0.013	0.0017	0.04	< 0.01	0.50	< 0.01	0.28
Caproate	0.004	0.004	0.004	0.003	0.003	0.005	0.005	0.004	0.004	0.003	0.005	0.006	0.005	0.006	0.004	0.0006	0.19	< 0.01	0.85	< 0.01	0.20
Total VFA	3.87	3.95	3.89	3.95	4.12	4.33	4.29	4.37	4.44	4.61	4.33	4.51	4.54	4.42	4.33	0.106	0.16	< 0.01	< 0.01	0.02	0.94

[1]Used 3 g (DM) grain as substrate, fed to 10 mL mixed ruminal microbes in a solution with 140 mL McDougall's buffer at 39ºC in duplicate for each sample.   Volatile fatty acid production measured by gas chromatography after 24-h *in vitro* fermentation.

[2]Proportion of EFC blended with CON (0, 25, 50, 75, or 100%) prior to reconstitution with water, and subsequent ensiling.

[3] *P*-value for moisture x EFC interaction.

[4] *P*-values for main effects of grain moisture.

[5] *P*-values for main effects of EFC in grain mixtures.

[6]Acetate:propionate ratio.

[7]EFC = high-amylase corn; CON = mill-run corn; L = linear effect; Q = quadratic effect; nd = none detected; VFA = volatile fatty acid; DM = dry matter.

Table 2.9. Effects high-amylase corn in reconstituted high-moisture corn blends on *in situ* dry matter disappearance (ISDMD), *in vitro* gas production (IVGP) and volatile fatty acid (VFA) profile

| Item | High-amylase corn[1], % | | | | | SEM | P-value | | |
	0	25	50	75	100		M*EFC[2]	Linear	Quadratic
ISDMD[3], %	63.8	62.7	64.7	63.2	59.3	2.63	0.31	0.19	0.23
IVGP[4], mL	107.5	112.7	112.8	115.2	124.1	3.06	0.38	< 0.01	0.33

[1]Proportion of EFC blended with CON prior to reconstitution with water, and subsequent ensiling.
[2]Moisture x EFC interaction.
[3]Measured in triplicate by ruminally incubating 3 g (DM) grain in Dacron bags for 14-h.
[4]Mean gas production over 24-h incubation. Used 3 g (DM) grain as substrate, added to 10 mL mixed ruminal microbes in a solution with 140 mL McDougall's buffer at 39ºC in duplicate for each sample.
[5]EFC = high-amylase corn; M = moisture.
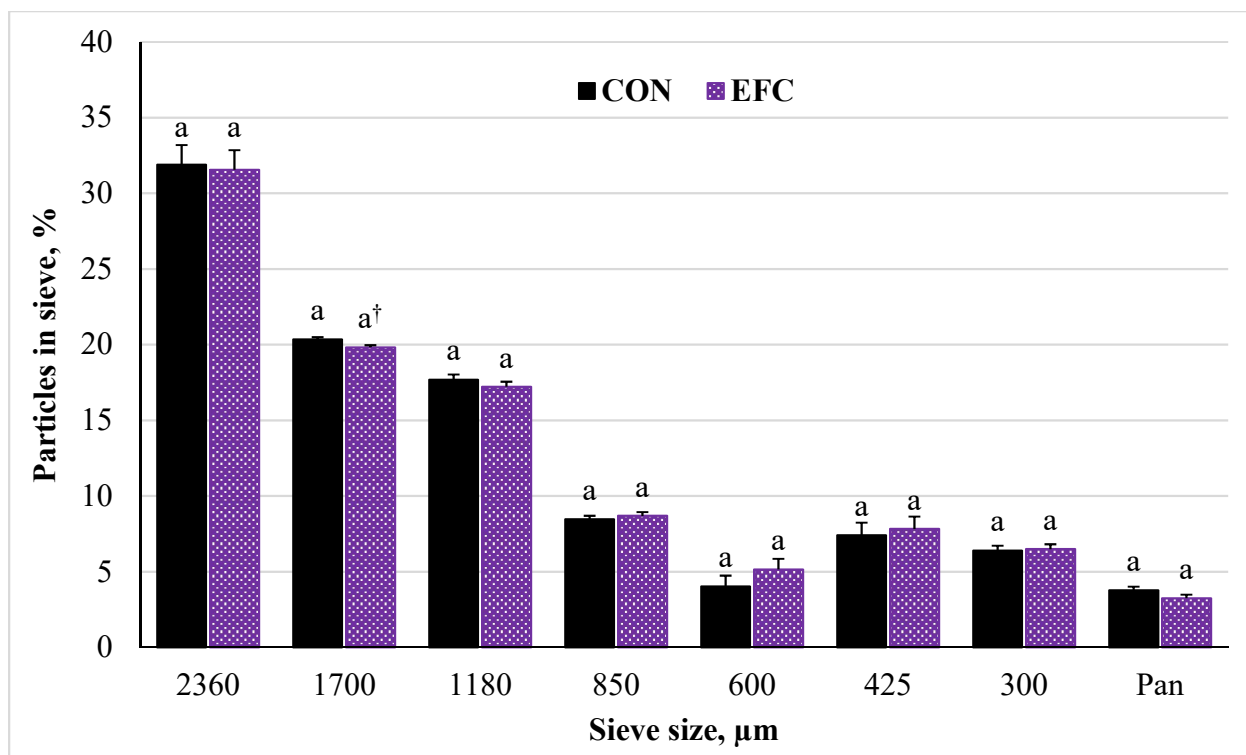
Figure 2.1. Effect of grain source on particle size distribution of dry-processed grains. Values are percent mill-run (CON) or high-amylase (EFC) corn weighed from each sieve and a solid pan, after 5-min cycle in a Ro-Tap shaker.
[a]Indicates difference ($P < 0.05$) within sieve size.
[†]Indicates tendency for difference ($P < 0.10$) within sieve size.
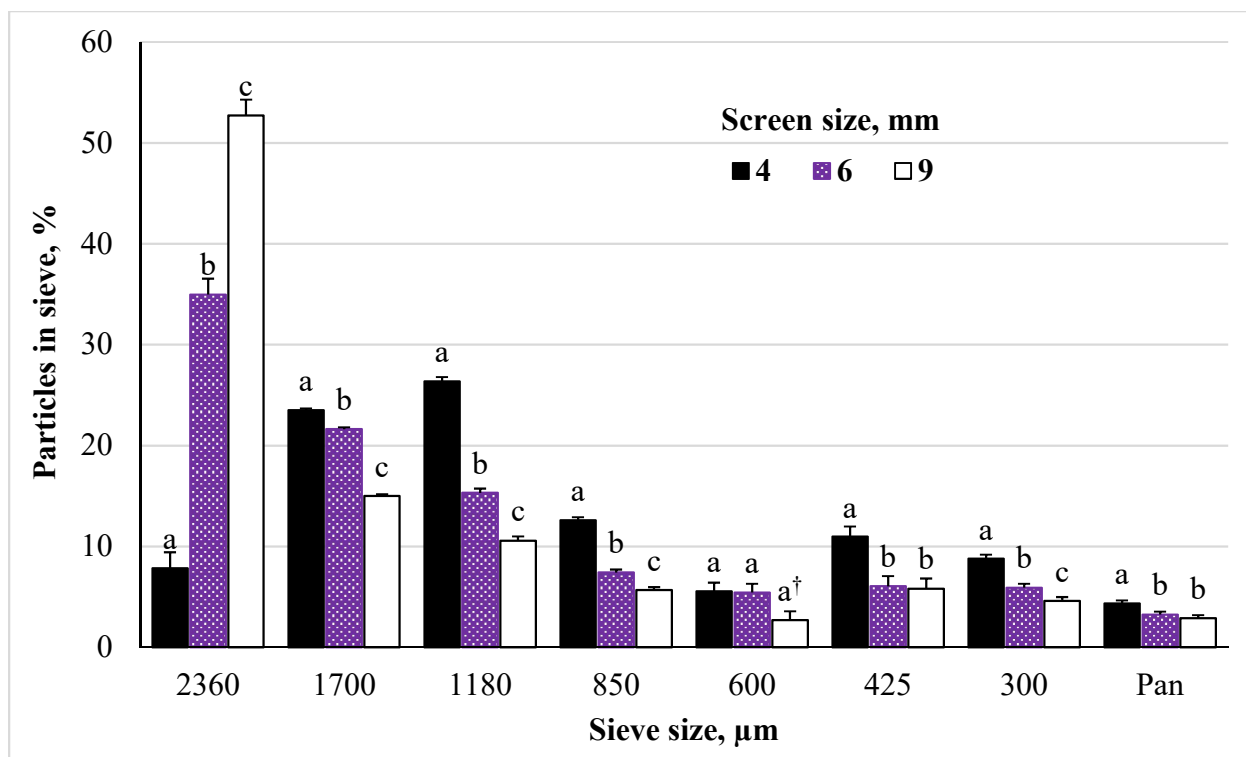
Figure 2.2. Effect of screen size used in Wiley Mill (4-, 6-, or 9-mm) on particle size distribution of dry-processed corn. Values represent percent of particles weighed from each sieve and a solid pan after 5-min cycle in a Ro-Tap shaker.
[a,b,c]Indicates difference ($P < 0.05$) within sieve size.
[†]Indicates tendency for difference ($P < 0.10$) within sieve size.
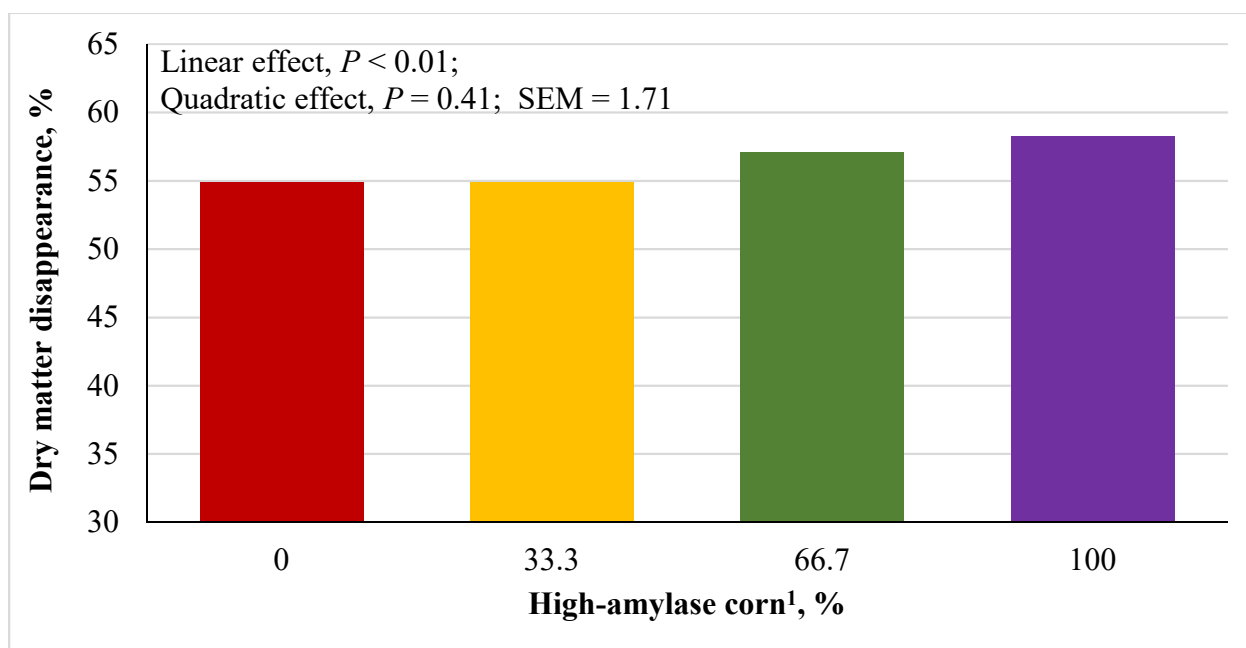
Figure 2.3. Effect of the proportion of high-amylase corn in dry-processed mixtures on *in situ* dry matter disappearance when incubated ruminally for 14 h.

[1]Percent high-amylase corn blended with mill-run corn in grain mixtures.
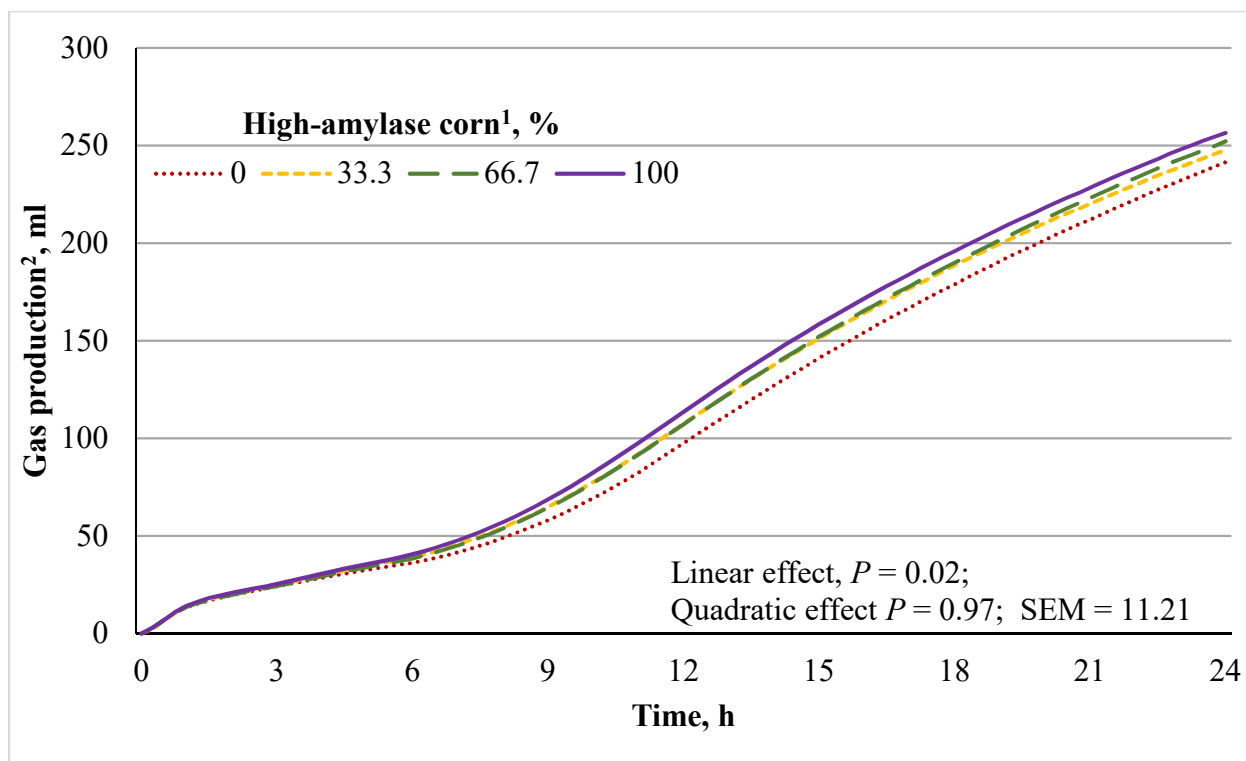
Figure 2.4. Effect of the proportion of high-amylase corn in dry-processed mixtures on fermentative gas production by *in vitro* cultures of mixed ruminal microbes over 24 h.
[1]Percent high-amylase corn blended with mill-run corn in grain mixtures.
[2]Cumulative gas production per *in vitro* culture bottle.

CHAPTER 3

Effects of high-amylase corn on performance and carcass quality of finishing beef heifers[1,2]

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ABSTRACT

Originally designed for ethanol production, high-amylase corn (Enogen Feed Corn, EFC) is being identified as a potential feed source for livestock. Two experiments evaluated digestive and steam-flaking characteristics of EFC, and effects on feedlot performance, carcass characteristics, and liver abscessation. Exp. 1 used blends of EFC and mill-run corn (CON) of 0, 25, 50, 75, or 100% EFC. Other factors were moisture addition prior to steam treatment (0, 3, or 6%), and steam conditioning time (15, 30, or 45 min). Grains were flaked to 360 g/L. A randomized complete block design (3 x 3 x 5 factorial) evaluated starch availability, *in situ* dry matter disappearance (ISDMD), *in vitro* gas production (IVGP), and VFA profile. No 2- or 3-way interactions among factors occurred. Adding increasing amounts of moisture improved starch availability (linear; $P < 0.01$), and tended to improve ISDMD (linear, $P = 0.06$) opposed to no moisture addition. Compared to 0 or 6%, adding 3% moisture reduced acetate production (quadratic; $P = 0.04$). Steam conditioning for 30 min improved starch availability, IVGP, acetate, propionate, butyrate, valerate, and total VFA production ($P < 0.01$) compared to 15 or 45 min. Starch availability, ISDMD, IVGP, acetate, propionate, valerate, and total VFA production increased, by increasing proportion of EFC in blends (linear, $P < 0.01$). Exp. 2 used 700 crossbred beef heifers (394 ± 8.5 kg initial BW) fed finishing diets with steam-flaked corn as CON or EFC for 136 d. Targeting similar starch availability, mill-run corn was steam-flaked to 360 g/L at a rate of ~6 tonne/h; EFC was flaked to 390 g/L at ~9 tonne/h. Heifers were blocked

by BW, stratified, then randomly assigned to 28 dirt-surfaced pens (25 animals/pen). Dry matter intake was similar between treatments ($P = 0.78$), but cattle fed EFC had greater ADG ($P < 0.01$), improving efficiency of feed utilization by 5% ($P < 0.01$). Hot carcass weight was 6 kg greater for EFC cattle ($P < 0.01$) than CON. No differences occurred for LM area ($P = 0.89$), 12th-rib fat thickness ($P = 0.21$), or USDA Yield Grade ($P = 13$). Cattle fed CON had greater marbling scores than EFC ($P = 0.04$), but this didn't affect USDA Quality Grade ($P > 0.33$). Cattle fed EFC had 8% fewer liver abscesses than CON ($P = 0.03$), due to fewer moderate ($P = 0.03$) and severe ($P = 0.11$) abscesses. High-amylase corn can be used to improve microbial digestion, mill-throughput, cattle performance, and may mitigate liver abscesses.

Key Words: amylase, high-amylase corn, steam-flaking

INTRODUCTION

Enogen Feed Corn (EFC; Syngenta Seeds, LLC) is characterized by high-amylase expression in kernel endosperm. It originally was developed and has been used extensively for production of ethanol. Corn is well established as the most dominant ingredient fed to finishing cattle, as starch provides a majority of dietary energy. Ruminants have limited capacity for pancreatic-amylase secretion, and consequently are limited in post-ruminal digestion of starch (Harmon et al., 2004). It has also been noted that what starch digestion does occur in the small intestine, is more energetically productive when compared to ruminal digestion (McLeod et al., 2007). It's plausible that any ruminally undigested starch, could be further degraded in the small intestine by α-amylase produced by the grain. This would be an energetic advantage.

Many consider steam-flaking corn to be the optimal processing method to maximize energy utilized from the grain, and improvements by this processing technique are extensively documented (Owens et al., 1997; Zinn et al., 2002). Limited literature is available on finishing cattle fed EFC, and in the case of steam-flaking EFC, this is the first such research to be performed. Actions of EFC enhance the flaking process, resulting in greater throughput, likely due to amylase increasing the rate of starch gelatinization. Furthermore, heat and moisture application by steam could be used to amplify the enzyme within EFC. Our objectives were to examine *in vitro* and *in situ* digestion, flaking characteristics of EFC when fed to finishing beef heifers, and the effects on feedlot performance, carcass characteristics, and liver abscess prevalence and severity.

MATERIALS AND METHODS

Experiment 1

Experimental Design and Grain Treatment

Grain mixtures were prepared using whole shelled EFC and mill-run corn (CON) as control. Grain types were blended in EFC:CON proportions of: 0:100, 25:75, 50:50, 75:25, and 100:0. Samples (2 kg) were placed into glass jars and water was applied at 0, 3, or 6% (w/w). Jars were put on a rotary device, spinning them 1 hour for adequate mixture of grains and moisture tempering. After mixing, grains were placed into perforated steel baskets and inserted into a custom fabricated steam table with 12 individual steam chambers. Randomized samples were then conditioned with steam for 15, 30, or 45 min. This was a 5 x 3 x 3 factorial, with all 45 treatments prepared in duplicate.

Immediately after steam conditioning, samples were flaked using a dual-drive roller mill (R & R Machine Works; Dalhart, TX) with 46 x 91 cm corrugated rolls. Samples were placed into the flaker through a conveyance system above the rolls, collecting flaked product simultaneously underneath. Grains were flaked to a bulk density of 360 g/L. Enzymatic starch availability was determined soon after by steeping 25 g corn flakes in 100 mL 2.5%-amyloglucosidase solution heated to 55ºC for 15 min (Sindt et al., 2006b). The liquid fraction is then filtered, and percent soluble sugars are viewed on a handheld refractometer. Percent solubles and DM are then converted to starch availability using regression equations. Another portion of grain was placed into a 105ºC forced-air oven for 24 h for determination of moisture content. All remaining grain was frozen and retained for future *in vitro* and *in situ* analyses. This process took place in September 2016 at the Kansas State University (KSU) Beef Cattle Research Center (BCRC), Manhattan KS.

In Situ Dry Matter Disappearance

Approximately 2-g (dry matter basis) of each flaked sample were weighed and sealed into Dacron bags, prepared in triplicate. Measurements were taken over 3 d. Randomized blocks

were animal within day, using 6 fistulated jersey steers housed at the BCRC. Bags (including blanks) were suspended ruminally for 14 h, after which bags were removed and rinsed. Bags were then dried at 105ºC in a forced-air oven for 24 h and weighed to calculate percent dry matter disappearance (ISDMD; values adjusted with blanks). Weighing and drying of samples took place at the KSU Pre-harvest Food Safety Laboratory (PHFSL).

In Vitro Gas Production and VFA Profile

In vitro studies were completed in duplicate for each flaked sample over 5 runs, each consisting of a single replicate. Although only 4 runs were required to complete in duplicate, the 5th run was performed due a computer communication problem causing incomplete gas production data during a previous run. Two blanks were used in each run to later adjust volatile fatty acid (VFA) values. Randomized samples (3 g DM whole corn flakes) were placed into 250 mL fermentation bottles equipped with ANKOM pressure sensing modules (ANKOM Technologies, Macedon, NY). Ruminal fluid was collected from 2 cannulated steers fed a 50:50 concentrate:roughage diet. Ruminal fluid strained through 8 layers of cheese cloth was poured into a separatory flask, degassed using nitrogen, capped, and placed into a 39ºC incubation chamber. After approximately 1-h, ruminal fluid separates into three distinct layers.

After complete separation of ruminal fluid, the center, microbe rich fraction was used as inoculant. Ten mL inoculum, combined with 140 mL McDougall's artificial saliva, and flaked corn were mixed, degassed with nitrogen, and capped with an ANKOM module. ANKOM modules measured cumulative *in vitro* gas production (IVGP) at 15 min intervals. Fermentation vessels were placed into an orbital shaker and incubated at 39ºC for 24 h with light agitation. Initial and final pH was taken to monitor adequate buffering capacity of the system. Following incubation, 4 mL liquid fraction from each bottle was combined with 1 mL 25% metaphosphoric

acid and frozen. When ready, frozen samples were thawed, vortexed, centrifuged, and the top

layer of supernatant was transferred into gas chromatography vials.

Volatile fatty acid analysis took place at the KSU PHFSL using an Aglient gas

chromatograph (Aglient Technologies, Santa Clara, CA) with a Nukol capillary column (15 m x

0.35 mm, d_f 0.50 μm). Analysis would be run to identify acetate, propionate, isobutyrate,

butyrate, isovalerate, valerate, isocaproate, caproate, and heptanoate.

Statistical Analyses

MIXED models procedure of the Statistical Analysis System (SAS version 9.4; SAS Inst.

Inc., Cary, NC) was used. Fixed effects were percent EFC, percent added moisture, steam

conditioning time, and all 2- and 3-way interactions. Random effect was block. Orthogonal

contrasts were used to identify linear and quadratic effects. Statistical significance declared at *P*

< 0.05, and tendency for significance at *P* < 0.10.

Experiment 2

The Kansas State University Institutional Animal Care and Use Committee approved all

protocols and procedures utilized in this study. The trial was initiated in December 2016, and

ended April 2017, taking place at the Kansas State University BCRC, Manhattan, KS.

Experimental Design

A randomized complete block design with 2 treatments was carried out using 700

crossbred beef heifers (394 kg ± 8.5 initial BW). Two lots of cattle, blocked separately, were

utilized in the trial. Three hundred fifty heifers received in June 2016, were used previously in a

receiving trial examining trace mineral supplementation. The second lot of cattle were received

in November 2016, targeting similar initial BW between lots at study initiation. Heifers were

blocked by lot, then BW, stratified, then randomly assigned to 1 of 28 dirt surfaced pens (25

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animals/pen). Treatments randomly assigned within block, consisted of mill-run corn (CON) as control, steam-flaked to 360 g/L; and high-amylase corn (EFC), steam-flaked to 390 g/L. Grain treatments were designed to target similar daily starch availability; based off Exp. 1 and additional preliminary work, a decision was made to flake EFC to a greater bulk density, and to flake with greater mill throughput to achieve this. Mill-run corn was flaked at approximately 6 tonne/h; EFC was flaked at approximately 9 tonne/h (50% increased mill throughput), decreasing steam-chest retention time.

Animal Processing, Housing, and Handling

Upon arrival at the Kansas State University Beef Cattle Research Center, heifers were given *ad libitum* access to alfalfa hay and water. Cattle were received on multiple dates for each lot, and were processed 24- to 48-h after arrival. Processing of lot 1 included vaccination using a 5-way viral vaccine (Bovishield Gold-5; Zoetis, Parsippany, NJ), a 7-way clostridial (Ultrabac 7/Somubac; Zoetis), and an antibiotic (Micotil; Elanco Animal Health, Greenfield, IN) to target respiratory disease, as heifers from this lot were of younger age and greater risk. Lot 2 vaccination was identical, except heifers from this lot were not treated with Micotil, and a topical parasiticide (Dectomax; Zoetis) was applied. During initial processing for both groups, animals were ear-tagged with a unique number for identification, and BW was recorded. On d 1 of trial initiation, starting BW was recorded as animals were sorted into pens, and received a trenbolone/estradiol implant (Component TE-IH with Tylan; Elanco Animal Health). On d 84 heifers were re-implanted (Component TE-200 with Tylan; Elanco Animal Health) and treated with a pour-on insecticide (Standguard; Elanco Animal Health).

Animals were housed in dirt surfaced pens that provided approximately 13 m^2 of surface area/animal; fences and gates were made of steel pipe and divided in 2 by an additional electric

fence. Automatic waterers allowing *ad libitum* access were shared between adjacent pens. Body weights were determined using a pen-scale and averaging pen-weight to determine mean BW for each pen.

Diet Preparation

Heifers were transitioned to finishing diets at the start of the trial over 21 d using 3 intermediate diets, with concentrate:roughage ratios of: 60:40, 71:29, and 92:18 (7 d/step) for gradual adaptation. Both grain types were steam-flaked daily, using the same flaking system as Exp. 1, equipped with a steam chest holding approximately 4.25 tonnes corn. Grain characteristics allow mill-run corn in this system to be flaked at approximately 6 tonne/h without grain build-up on the rolls; EFC however could be flaked at maximum mill capacity without any grain build-up (~9 tonne/h). A system to apply moisture to grains prior to steam conditioning (SarTec; Anoka, MN), allowed us to adjust grain conditioning so that flaked grain DM was equivalent between treatments. Composition of experimental diets are shown in Table 3.7. Diets were re-formulated for final 39 days to include 300 mg/d Optaflexx (Elanco Animal Health). Cattle were fed *ad libitum* rations which were mixed and delivered once daily, beginning at approximately 0800 h. Feed intakes were visually monitored and adjusted daily as-needed, so that only trace amounts of residual feed were in bunks each morning. Orts were collected as-needed to account for unconsumed feed, and dried at 55ºC for 48 h for accurate adjustment of DMI. Subsamples of each feed ingredient were collected weekly or upon arrival, dried at 55ºC for 48 h, and composited into monthly samples which were later analyzed for nutrient composition (SDK Labs; Hutchinson, KS).

Harvest

On d 136 all animals were weighed on a pen-scale immediately before shipping for slaughter. Final BW was calculated by multiplying the mean BW for each pen by 0.96 to account for 4% shrink during travel. Heifers were loaded onto trucks and transported approximately 440 km to a commercial abattoir in Lexington, NE. Records collected on the day of slaughter by trained Kansas State University personnel were: animal identification within kill-order, HCW, and liver abscess prevalence and severity using the Elanco scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health). Liver scoring gives grades of: 0 (no abscess), or A⁻, A, or A⁺ for mild, moderate, and severe liver abscesses respectively. Following a chill period over 24 h, LM area, 12th-rib subcutaneous fat, marbling score, USDA Yield Grade, and USDA Quality Grade data were collected. Dressing percentage was determined by averaging HCW within feedlot pen and dividing that value by final shrunk BW.

Grain Characteristics

Daily observations on DM, starch availability, and particle size were measured for both grain types. Grain DM was determined by drying in a forced-air oven set to 105°C for 24 h. If DM changes occurred, we would adjust the amount of moisture applied by the SarTec system accordingly to achieve equivalent moisture content between corn types. Enzymatic starch availability was determined using methodology described in Exp. 1. Particle size was determined by weighing approximately 200 g of flaked-corn poured onto a set of sieves, with decreasing screen sizes in the order: 4750, 3350, 2360, 1700, 1180, 850, 600 μm, and a solid pan. The stack is placed into a Ro-Tap shaker, with a rotary tapping cycle run for 5 min. Each individual sieve is cleaned, and particles weighed. Geometric particle size is calculated in a spreadsheet (Baker and Herrman, 2002) using equations described by Pfost and Headley (1976).

Statistical Analyses

Analyses of BW, DMI, ADG, and feed efficiency used the MIXED procedure of the SAS (version 9.4), with pen as the experimental unit, treatment as fixed effect, and block as a random effect. Categorical carcass traits (USDA Quality Grade, USDA Yield Grade, and liver abscess prevalence and severity) were analyzed with the GLIMMIX procedure of SAS, with the same parameters as above.

RESULTS AND DISCUSSION

Experiment 1

There were no 2- or 3-way interactions between factors for any experimental analyses ($P > 0.10$); only main effects of each treatment are shown. No caproate, isocaproate, or heptanoate were detected when analyzing *in vitro* VFA profiles.

Moisture Addition to Grains

Overall impact of adding moisture prior to steam conditioning corn appeared minimal. Starch availability, ISDMD, and IVGP effects can be seen in Table 3.1. There was a linear increase ($P < 0.01$) in enzymatic starch availability when the amount of tempered moisture increased from 0 to 6%. This is likely due to the increase in moisture that occurred to grains tempered with water, as the regressive index used in the procedure yields greater starch availability as grain DM decreases. A tendency for linear improvement ($P = 0.06$) of ISDMD occurred with increasing amounts of tempered moisture. Tempering with moisture did not affect IVPG. Table 3.2 details that adding 3% water to corn resulted in lower acetate production (quadratic; $P = 0.04$), and tendencies for reduced propionate (quadratic; $P = 0.07$) and total VFA (quadratic; $P = 0.08$) when compared to 0 or 6% treatments. Moisture addition prior to steam conditioning corn had no other effects on VFA profile.

These results appear reflective of previous research. Sindt et al. (2006a) observed no effect on IVGP when tempering with 6, 10, or 14% added moisture. Sindt et al. (2006b) showed a tendency for moisture addition (0, 6, or 12%) to increase enzymatic starch availability, and no effect on *in vivo* VFA profile. Moisture addition to corn prior to flaking has shown little to no impact on cattle performance (Zinn et al., 2002; Gutierrez et al., 2018), but may be used to improve flake durability and potentially reduce the amount of steam needed to condition corn (Sindt et al., 2006a).

Steam Conditioning Time

Impact of steam conditioning time is displayed in Tables 3.3 and 3.4. In most cases, treating corn with 30 min of steam was optimal when compared to 15 or 45 min, with this amount yielding the greatest numerical value for starch availability, IVGP, acetate, propionate, butyrate, valerate, and total VFA (quadratic; $P < 0.01$). Compared to 15 or 45 min, conditioning with 30 min of steam also reduced acetate:propionate (quadratic; $P < 0.01$). Looking at minor VFAs, 30 min improved isobutyrate (quadratic; $P = 0.03$), while 15 min of steam had the greatest production of isovalerate (linear; $P < 0.01$). Steam time had less impact on ISDMD, as there were no linear or quadratic differences.

These results reflect digestive properties of starch, as steam conditioning for a short time likely doesn't yield enough opportunity for starch gelatinization (Zinn et al., 2002; Huntington et al., 2006). Svihus et al. (2005) describes a linear relationship between degree of gelatinization, and digestibility. However, Kurakake et al. (1997) observed that starch granule swelling power and solubility decreased with higher temperature, implying more rigidity, and they state this is perhaps due to restructuring between amylose and amylopectin. We hypothesize that at 45 min of steam exposure, rearrangements such as this may occur, as well as maillard reactions where a

sugar reacts with an amino acid causing caramelization. It should be noted, with the steam-table

used for this experiment, in the small area grains are exposed to a much greater steam output then

would be present in a commercial steam chest. This means the meaningful conclusion that should

be made is: there's an optimal time to steam treat corn, and excessive conditioning could reduce

microbial digestion. Conditioning times used in this experiment likely aren't quite comparable to

a commercial flaking system, which generally range between 45 and 60 min.

High-amylase Corn Concentration

High-amylase corn improved starch solubility, and microbial digestion in every analysis

performed. Enzymatic starch availability improved linearly ($P < 0.01$) with increasing percentage

of EFC in flaked grain mixtures (Table 3.5). Also in Table 3.5, EFC increased ISDMD (linear; P

< 0.01), with 100% EFC resulting in an approximately 11% numerical improvement over CON.

This is a 32% increase in ISDMD. A visual graphic of IVGP improvement using EFC over time

is in Figure 3.1. Using greater proportions of EFC linearly improved IVGP ($P < 0.01$) over CON.

It appears separation of treatments had begun between 6 and 8 h and was maintained for the 24-h

duration. Table 3.6 displays effects of EFC on *in vitro* VFA profile. As may be expected based

off IVGP, increasing EFC concentration in grain blends linearly increased production of acetate,

propionate, valerate, and total VFA ($P < 0.01$). Acetate to propionate ratio decreased (linear; $P <$

0.01) with greater proportions of EFC. The only negative effect of EFC was a decrease in

isovalerate (linear; $P < 0.01$) by increasing EFC content.

Limited literature on EFC digestibility is available, none of which pertains to steam-flaked

corn. Jolly-Breithaupt et al. (2016b) observed numerical improvements in ruminal digestibility of

OM and starch when feeding a finishing diet using dry-rolled EFC to fistulated cattle. They saw

no effects on VFA production. It's likely processing method has substantial impact on EFC

digestibility, as application of moisture and heat from steam enhances EFC α-amylase activity. It's important to note that we observed no quadratic effects of EFC for any analysis; this means that the actions of EFC are likely confined to that portion of the grain mixture, and do not affect CON portion of the blends.

Experiment 2

Four animals were removed from the CON group for non-treatment related reasons; 3 due to calving, and 1 was found deceased due to respiratory disease. Four animals were also removed from the EFC group for non-treatment reasons; 1 due to a bacterial infection, 1 due to respiratory disease, 1 due to a displaced abomasum, and 1 due to a hip-injury, all of which caused severe weight loss.

Grain Characteristics

Laboratory analysis (SDK labs) of nutrient composition between CON and EFC are shown in Table 3.8. High-amylase corn had greater ADF ($P < 0.01$), and potassium ($P = 0.03$) components compared to CON. High-amylase corn also had a tendency ($P = 0.06$) for greater fat content (ether extract as measurement) as opposed to CON. No differences between grains were evident between flaked grains for protein, calcium, or phosphorus.

Characteristics of grains are presented in Table 3.9. By design, moisture content of both corn types had no difference after steam-flaking ($P = 0.55$), and starch availability was similar, although there was a tendency ($P = 0.08$) for EFC to yield a greater starch availability value than CON. Even though EFC was flaked to a greater bulk density (390 vs 360 g/L), it still resulted in a smaller mean particle size ($P < 0.01$) when compared to CON; Figure 3.2 displays particle size distribution across sieve sizes. There was no difference in standard deviation of grain particle size

($P = 0.99$). Sindt et al. (2006a) observed no effects on finishing performance or carcass characteristics when feeding different particle sizes of steam-flaked corn, this being 4,667 or 3,330 μm (smaller particle size achieved by 15 min of mixing, not by reducing flake density).

Feedlot Performance

Effects of EFC on gain and efficiency of feedlot heifers are found in Table 3.10. There was no difference in BW at trial initiation ($P = 0.52$), but cattle fed EFC were heavier than CON on the final day ($P < 0.01$). Thus, over the 136-d period, EFC cattle had improved ADG ($P < 0.01$). There was no difference in DMI between treatments ($P = 0.78$), which results in 5% greater feed efficiency for cattle fed EFC ($P < 0.01$) compared to CON. When comparing effects of EFC when dry-rolled (DRC) or as high-moisture corn (HMC), and combined with 1 of 2 different byproducts fed to finishing steers, Jolly-Breithaupt et al. (2016a) observed a numerical 3.9% increase in G:F when comparing EFC-DRC with modified wet distillers grains plus solubles (MDGS) apposed to their control. They also observed a 2.1% numerical improvement in G:F using EFC fed as HMC (with MDGS). When sweet bran (SB) was included instead of MDGS, EFC-HMC had the opposite effect, as feed efficiency decreased by 2.1%. Jolly-Breithaupt et al. (2016a) also presented a corn type x corn processing interaction, in which final BW ($P = 0.02$) and ADG ($P = 0.04$) were greatest in those fed EFC as DRC, and lowest when fed as HMC. These results suggest at least some similarities to what we observed when steam-flaking EFC. It also suggests feed effects of EFC may vary by corn processing method.

Other work has been performed to evaluate effectiveness of adding exogenous α-amylase to feedlot diets. In 2 experiments from Tricarico et al. (2007), α-amylase (Amaize, Alltech Inc., Nicholasville, KY) fed at a rate of 950 DU/kg DM (DU defined as: one dextrinizing unit is quantity needed to dextrinize soluble starch at 30ºC, pH 4.8, at 1 g/h) was first added to steam-

flaked corn diets using alfalfa or cottonseed hulls as roughage. The second experiment identified

amylase quantity effects when feeding cracked corn (CC) or HMC finishing diets. Experiment 1

showed no effects of added amylase, or amylase x roughage source interactions for any feedlot

performance data over the entire feeding period on beef steers. There were no interactive effects

between amylase and corn processing method in Exp. 2, where finishing beef heifers had optimal

ADG and DMI (quadratic effect, $P = 0.04$ and $P = 0.07$ respectively) when amylase was added to

diets at a rate of 580 DU/kg DM. They saw no effects on feed efficiency. While the increase in

ADG is comparable to our study, this was with CC and HMC; no effects were observed when

amylase was added to the steam-flaked corn diet. Amylase content of EFC grain varies with

hybrid and environmental conditions during the growing period. Furthermore, processing

temperatures needed for optimal amylase activities may be different for exogenous enzymes

compared to those contained within EFC.

Carcass Characteristics

Effects of EFC on carcass merit are shown in Table 3.11. Improved daily gain in cattle

fed EFC resulted in greater carcass weight, as heifers produced approximately 6 kg heavier

carcasses ($P < 0.01$) when compared to CON. No differences in LM area or 12th-rib fat were

evident. The CON diet yielded carcasses with a numerically greater marbling score ($P = 0.04$)

over EFC, but this did not impact USDA Quality Grades ($P > 0.33$). There also was a tendency

($P = 0.09$) for increased percentage of USDA Yield Grade 3 carcasses in heifers fed EFC

compared to CON. Jolly-Breithaupt et al. (2016a) evaluated impact of grain processing method

with EFC, and observed tendencies for interaction between corn type and processing method on

HCW ($P = 0.08$), 12th rib fat ($P = 0.07$), and marbling score ($P = 0.09$). When consuming EFC-

DRC, HCW and marbling score was greater than the control counterpart. When steers consumed

EFC-HMC on the other hand, HCW and marbling scores decreased compared to the HMC control. Twelfth-rib fat thickness was least for cattle fed EFC-DRC, and greatest for cattle fed EFC-HMC. The authors did not speculate regarding the possible cause for lower marbling scores and increased 12th rib fat thickness when EFC was fed as HMC. It is difficult to compare these results as differences in enzyme activation likely occur when comparing their processing methods to steam-flaked EFC. The increase in HCW when EFC was fed as DRC agrees with our findings, while the decrease in marbling score feeding EFC as HMC is an additional caveat. Jolly-Breithaupt et al. (2016b) do offer insight into the potential mechanism of action of high-amylase corn, citing increased total tract digestion of organic matter and starch for diets based on dry-rolled corn. For finishing diets based on steam-flaked corn, total tract starch digestion in flaked corn diets can be 99% or more (Sindt et al., 2006b), suggesting that opportunities to increase total tract starch digestibility are very limited. Altering site of digestion to favor ruminal or small intestinal digestion, may, therefore, be a more plausible mechanism of action.

Tricarico et al. (2007) observed no interactions between exogenous amylase addition and roughage source in finishing diets containing steam-flaked corn. Longissimus muscle area was greater ($P = 0.02$) for steers consuming the amylase diet compared to those fed the control diet. In a second study, these authors observed that HCW was optimal when cattle were fed 580 DU/kg DM amylase (quadratic effect, $P = 0.03$). Like Exp. 1, they observed a quadratic increase in LM area (quadratic effect, $P = 0.04$) with the 580 DU/kg DM concentration being optimal. A tendency for fat thickness to increase in response to increasing amylase concentration also was observed (linear effect, $P = 0.09$; quadratic effect, $P = 0.07$), and 580 DU/kg DM amylase resulted in the lowest numerical yield grade (quadratic effect, $P = 0.02$). The fact that HCW increased with added amylase would agree with our findings. However, differences in backfat,

LM area, and overall yield grade do not agree with our observations. The actions of exogenous amylase enzyme added to a finishing diet may be different than those of EFC, for which amylase is an inherent component of the grain. Meaningful comparisons at this stage again may be difficult with limited literature on finishing cattle with ECF, or adding amylase enzyme.

Liver abscess prevalence and severity are shown in Table 3.12. Note that tylosin was not included in experimental diets (Table 3.7). Finished beef heifers fed EFC had fewer total liver abscesses at slaughter than their CON counterparts ($P = 0.03$). This difference occurs due to fewer moderate ($P = 0.03$) and severe ($P = 0.11$) liver abscesses in the EFC group. No previous peer-reviewed literature has identified any liver abscess effects when feeding EFC (through any processing method), or by feeding with added amylase enzyme. Detrimental effects of liver abscesses on cattle gain (Potter et al., 1985) resulting in lighter, poorer quality carcasses (Brown and Lawrence, 2010) have been well established. The relationship between liver abscess severity and HCW for each treatment is shown in Figure 3.3. There were effects by diet ($P < 0.01$), liver abscess severity ($P < 0.01$), and tendency for a treatment x liver abscess interaction ($P = 0.09$). The EFC group maintained heavier carcasses, and did not display the same decrease in HCW when abscess severity increased that was observed for CON cattle. While mode of action for the effect steam-flaked EFC had on liver abscessation is unclear at this time, the implications could be substantial, as new methods for the prevention of liver abscesses are needed as use of antibiotics is increasingly discouraged.

Research aimed at determining site and extent of digestion of high-amylase hybrids that are steam-flaked may help to explain production responses observed in this experiment. Processing characteristics of EFC likely are attributable to presence of amylase enzyme, as we have not previously encountered corn grains that respond in this manner to flaking.

IMPLICATIONS

High-amylase corn could be used advantageously by producers to reduce production costs associated with steam-flaking. Reduced steam use, reduced grain processing (bulk density), and 50% increased mill throughput (reduced labor), are all benefits that occur prior to feed being delivered to bunks. Improvements in microbial digestion, ADG, feed efficiency, HCW, and liver abscess mitigation are potential benefits associated with feeding high-amylase corn.

REFRENCES

Baker, S., and T. Herrman. 2002. Evaluating Particle Size. Kansas State Univ. Dep. Grain Sci. Ind. MF-2051:1–6.

Brown, T. R., and T. E. Lawrence. 2010. Association of liver abnormalities with carcass grading performance and value. J. Anim. Sci. 88:4037–4043. doi:10.2527/jas.2010-3219.

Gutierrez, B. H., E. G. Alvarez, M. F. Montano, J. Salinas-Chavira, N. G. Torrentera, and R. A. Zinn. 2018. Influence of flake density and tempering on the feeding value of steam-flaked corn for feedlot cattle. J. Appl. Anim. Res. 46:155–158. doi:10.1080/09712119.2017.1278699.

Harmon, D. L., R. M. Yamka, and N. A. Elam. 2004. Factors affecting intestinal starch digestion in ruminants: A review. Can. J. Anim. Sci. 84:309–318. doi:10.4141/A03-077.

Huntington, G. B., D. L. Harmon, and C. J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. J. Anim. Sci. 84:E14–E24. doi:10.2527/2006.8413_supplE14x.

Jolly-Breithaupt, M. L., C. J. Bittner, D. B. Burken, G. E. Erickson, J. C. Macdonald, and M. K. Luebbe. 2016a. 385 Effect of feeding a corn hybrid containing α amylase as dry rolled or high moisture corn on finishing performance and carcass characteristics. J. Anim. Sci. 94:180. doi:10.2527/msasas2016–384.

Jolly-Breithaupt, M. L., J. L. Harding, J. C. Macdonald, G. E. Erickson, and M. K. Luebbe. 2016b. Site and extent of digestion of finishing diets containing syngenta enhanced feed corn. Nebraska Beef Cattle Rep. 139–142.

Kurakake, M., M. Noguchi, K. Fujioka, and T. Komaki. 1997. Effects on maize starch properties of heat-treatment with water – ethanol mixtures. J. Cereal Sci. 25:253–260.

McLeod, K. R., R. L. Baldwin VI, M. B. Solomon, and R. G. Baumann. 2007. Influence of ruminal and postruminal carbohydrate infusion on visceral organ mass and adipose tissue accretion in growing beef steers. J. Anim. Sci. 85:2256–2270. doi:10.2527/jas.2006–359.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1997. The effect of grain source and grain processing on performance of feedlot cattle: A review. J. Anim. Sci. 75:868–879.

Pfost, H., and V. Headley. 1976. Methods of determining and expressing particle size. Feed Manuf. Technol. II - Append. C. Am. Feed Manuf. Assoc. 512–520.

Potter, E. L., M. I. Wray, R. D. Muller, H. P. Grueter, J. McAskill, and D. C. Young. 1985. Effect of monensin and tylosin on average daily gain, feed-efficiency and liver-abscess incidence in feedlot cattle. J. Anim. Sci. 61:1058–1065.

Sindt, J. J., J. S. Drouillard, S. P. Montgomery, and E. R. Loe. 2006a. Factors influencing characteristics of steam-flaked corn and utilization by finishing cattle. J. Anim. Sci. 84:154–161.

Sindt, J. J., J. S. Drouillard, E. C. Titgemeyer, S. P. Montgomery, E. R. Loe, B. E. Depenbusch, and P. H. Walz. 2006b. Influence of steam-flaked corn moisture level and density on the site and extent of digestibility and feeding value for finishing cattle. J. Anim. Sci. 84:424–432.

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

Tricarico, J. M., M. D. Abney, M. L. Galyean, J. D. Rivera, K. C. Hanson, K. R. McLeod, and D. L. Harmon. 2007. Effects of a dietary Aspergillus oryzae extract containing α-amylase activity on performance and carcass characteristics of finishing beef cattle. J. Anim. Sci. 85:802–811. doi:10.2527/jas.2006–427.

Zinn, R. A., F. N. Owens, and R. A. Ware. 2002. Flaking corn: Processing mechanics, quality standards, and impacts on energy availability and performance of feedlot cattle. J. Anim. Sci. 80:1145–1156. doi:10.2527/2002.8051145x.

Table 3.1. Effects of moisture addition to grain (tempering) prior to steam conditioning on starch availability, *in situ* dry matter disappearance (ISDMD), and production of fermentative gasses by *in vitro* cultures of mixed ruminal microbes (IVGP)

Item	Tempered moisture[1], %			SEM	*P*-value	
	0	3	6		Linear	Quadratic
Starch availability[2], %	47.2	49.5	51.2	0.60	< 0.01	0.76
ISDMD[3], %	40.3	40.7	42.0	1.62	0.06	0.51
IVGP[4], mL	110.1	107.4	111.2	7.33	0.81	0.42

[1]Performed by adding water (w/w) with whole grains into glass jars. Jars were placed on rollers and mixed 60 min prior to steam conditioning.
[2]Measured using refractive index method (Sindt et al., 2006b) once per sample (90 observations, 30/treatment) soon after flaking.
[3]Measured over 3 d in triplicate by incubating 2.5 g (DM) corn flakes in Dacron bags ruminally for 14-h (270 observations, 90/treatment).
[4]Mean gas production during 24-h incubation period. Measured by incubating 3 g (DM) flaked corn as substrate, with 10 mL ruminal fluid inoculum, and 140 mL McDougall's Buffer at 39ºC. Repeated with 2 replicates per sample (180 observations, 60/treatment).

Table 3.2. Effects of moisture addition to grain (tempering) prior to steam conditioning on production of volatile fatty acids (VFA) by *in vitro* cultures of mixed ruminal microbes fed steam-faked corn as substrate

VFA, mmoles/g substrate DM	Tempered moisture[1], %				P-value	
	0	3	6	SEM	Linear	Quadratic
Acetate	1.60	1.54	1.60	0.063	0.91	0.04
Propionate	1.85	1.78	1.84	0.063	0.71	0.07
Acetate:propionate	0.87	0.87	0.88	0.048	0.31	0.50
Butyrate	0.52	0.51	0.52	0.067	0.72	0.60
Isobutyrate	0.003	0.002	0.002	0.0021	0.59	0.97
Valerate	0.063	0.063	0.064	0.0116	0.68	0.80
Isovalerate	0.006	0.006	0.005	0.0026	0.29	0.71
Total	4.05	3.90	4.02	0.082	0.79	0.08

[1]Performed by adding water (w/w) with whole grains into glass jars. Jars were placed on rollers and mixed 60 min prior to steam conditioning.

Table 3.3. Effects of steam conditioning time for flaked corn on starch availability, *in situ* dry matter disappearance (ISDMD), and production of fermentative gasses by *in vitro* cultures of mixed ruminal microbes (IVGP)

Item	Conditioning time, min			SEM	*P*-value	
	15	30	45		Linear	Quadratic
Starch availability[1], %	48.5	51.5	47.9	0.60	0.52	< 0.01
ISDMD[2], %	36.6	44.4	42.0	2.66	0.15	0.12
IVGP[3], mL	102.3	121.3	104.9	7.33	0.57	< 0.01

[1]Measured using refractive index method (Sindt et al., 2006b) once per sample (90 observations, 30/treatment) soon after flaking.

[2]Measured over 3 d in triplicate by incubating 2.5 g (DM) corn flakes in Dacron bags ruminally for 14-h (270 observations, 90/treatment).

[3]Mean gas production during 24-h incubation period. Measured by incubating 3 g (DM) flaked corn as substrate, with 10 mL ruminal fluid inoculum, and 140 mL McDougall's Buffer at 39ºC. Repeated with 2 replicates per sample (180 observations, 60/treatment).

Table 3.4. Effects of steam conditioning time on production of volatile fatty acids (VFA) from *in vitro* cultures of mixed ruminal microorganisms fed steam-flaked grains as substrate

VFA, mmoles/g substrate DM	Conditioning time, min				P-value	
	15	30	45	SEM	Linear	Quadratic
Acetate	1.56	1.67	1.51	0.063	0.13	< 0.01
Propionate	1.75	1.99	1.73	0.063	0.56	< 0.01
Acetate:propionate	0.89	0.85	0.88	0.048	< 0.01	< 0.01
Butyrate	0.50	0.58	0.48	0.067	0.16	< 0.01
Isobutyrate	0.002	0.004	0.001	0.0021	0.11	0.03
Valerate	0.058	0.073	0.058	0.0117	0.95	< 0.01
Isovalerate	0.007	0.005	0.005	0.0026	0.02	0.22
Total VFA	3.88	4.32	3.78	0.082	0.25	< 0.01

Table 3.5. Effects of increasing proportion of high-amylase corn (EFC) in grain mixtures on starch availability and *in situ* dry matter disappearance (ISDMD)

Item	0	25	50	75	100	SEM	Linear	Quadratic
	Proportion of grain mixture as EFC, %						P-value	
Starch availability[1], %	45.0	47.7	50.5	50.7	52.7	0.77	< 0.01	0.16
ISDMD[2], %	34.8	39.5	40.8	43.8	46.0	1.70	< 0.01	0.27

[1]Measured using refractive index method (Sindt et al., 2006b) once per sample (90 observations, 18/treatment) soon after flaking.

[2]Measured over 3 d in triplicate by incubating 2.5 g (DM) corn flakes in Dacron bags ruminally for 14-h (270 observations, 54/treatment).

Table 3.6. Effects of increasing proportion of high-amylase corn (EFC) in grain mixtures on production of volatile fatty acids by *in vitro* cultures of mixed ruminal microorganisms fed steam-flaked corn as substrate

VFA, mmoles/g substrate DM	Proportion of grain mixture as EFC, %					SEM	P-value	
	0	25	50	75	100		Linear	Quadratic
Acetate	1.49	1.51	1.61	1.63	1.66	0.067	< 0.01	0.64
Propionate	1.69	1.73	1.85	1.90	1.95	0.067	< 0.01	0.83
Acetate:propionate	0.89	0.88	0.87	0.87	0.86	0.048	< 0.01	0.76
Butyrate	0.53	0.48	0.52	0.51	0.54	0.067	0.21	0.06
Isobutyrate	0.004	0.000	0.003	0.003	0.002	0.0021	0.98	0.33
Valerate	0.053	0.056	0.067	0.063	0.076	0.0119	< 0.01	0.61
Isovalerate	0.008	0.005	0.007	0.005	0.004	0.0027	< 0.01	0.78
Total	3.77	3.78	4.05	4.12	4.24	0.097	< 0.01	0.93

Table 3.7. Composition of finishing diets fed to beef heifers[1]

Item	Control	EFC
Ingredient, % of DM		
Mill-run corn (steam-flaked)	85.40	0.00
High-amylase corn (steam-flaked)	0.00	85.40
Ground alfalfa	7.00	7.00
Soybean meal	1.64	1.64
Feed additive premix[2]	2.20	2.20
Supplement[3]	3.76	3.76
Analyzed composition[4], % DM		
CP	15.44	15.37
ADF	6.51	7.15
EE	3.58	4.16
Ca	0.60	0.59
P	0.23	0.24
K	0.64	0.68

[1]Optaflexx (Elanco Animal Health, Greenfield, IN) was fed at 300 mg ractopamine–HCl per heifer daily for the final 39 days on feed.

[2]Consisted of ground corn and 1% tallow, and provided 0.05 mg/kg melengestrol acetate (MGA; Zoetis, Parsippany, NJ) in total diet DM.

[3]Consisted of urea, salt, limestone, trace mineral premix, vitamin premix, and KCl to provide (on total diet DM basis) 0.15 mg/kg cobalt, 10 mg/kg copper, 0.50 mg/kg iodine, 20 mg/kg manganese, 0.10 mg/kg selenium, 30 mg/kg zinc, 2205 IU/kg vitamin A, 22 IU/kg vitamin E, and 36.4 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN).

[4]Analyzed nutrient composition of ingredients in total diet (SDK Labs; Hutchinson, KS). Each ingredient analyzed by month as composites from weekly samples (5 composites/ingredient).

Table 3.8. Nutrient analyses[1] of mill-run (CON) and high-amylase corn (EFC)

Item, % of DM	CON	EFC	SEM	P-value
CP	8.77	8.69	0.09	0.55
ADF	3.69	4.45	0.14	< 0.01
EE	3.58	4.27	0.18	0.06
Ca	0.016	0.014	0.002	0.59
P	0.226	0.246	0.008	0.13
K	0.334	0.374	0.008	0.03

[1]Analyzed by SDK Labs, Hutchinson, KS. Weekly samples (~1 kg) were composited into monthly samples which were then analyzed (5 composites/grain type).

Table 3.9. Characteristics of steam-flaked mill-run corn (CON) and steam-flaked high-amylase corn (EFC)

Item	CON	EFC	SEM	P-value
DM, %	78.8	78.9	0.70	0.55
Starch availability, %	51.3	52.1	0.30	0.08
d_{gw}[1], μm	4413	4292	24	< 0.01
S_{gw}[2]	1.50	1.50	0.017	0.99

[1]Mean geometric particle size.
[2]Standard deviation of particle size.

Table 3.10. Finishing performance of heifers fed diets containing steam-flaked mill-run corn (CON) or steam-flaked high-amylase corn (EFC)

Item	CON	EFC	SEM	P-value
Initial BW, kg	395	394	8.6	0.52
Final BW[1], kg	588	599	10.7	< 0.01
DMI, kg	10.00	10.07	0.196	0.78
ADG, kg	1.60	1.69	0.028	< 0.01
G:F	0.160	0.168	0.002	< 0.01

[1]Gross body weight (measured immediately prior to loading cattle onto trucks for transport to abattoir) multiplied by 0.96 to account for 4% shrink.

Table 3.11. Carcass characteristics of heifers fed diets containing steam-flaked mill-run corn (CON) or steam-flaked high-amylase corn (EFC)

Item	CON	EFC	SEM	P-value
HCW, kg	366	372	6.41	< 0.01
LM area, cm^2	94.7	94.6	1.02	0.89
12th rib fat thickness, cm	1.16	1.19	0.045	0.21
Marbling score[†]	605	589	10	0.04
USDA Prime, %	6.6	4.9	1.68	0.33
USDA Choice, %	68.7	70.4	4.44	0.62
USDA Select, %	10.7	11.4	2.58	0.79
USDA sub-Select[1], %	9.0	9.3	2.61	0.68
USDA Yield Grade	2.07	2.15	0.069	0.13
Yield Grade 1, %	23.2	19.5	3.77	0.22
Yield Grade 2, %	49.4	47.7	3.01	0.64
Yield Grade 3, %	25.1	30.9	3.07	0.09
Yield Grade 4, %	2.0	2.0	0.76	1.00
Yield Grade 5, %	0.3	0.0	0.20	0.32

[†]500 to 599 = Small degree of marbling; 600 to 699 = Modest degree of marbling.
[1]Carcasses graded as USDA Standard, Commercial, Utility, or Cutter.

Table 3.12. Liver abscess prevalence and severity[1] in heifers fed diets containing steam-flaked mill-run corn (CON) or steam-flaked high-amylase corn (EFC)

Item	CON	EFC	SEM	*P*-value
Total liver abscesses, %	34.4	26.6	2.47	0.03
Mild, %	11.9	12.7	1.80	0.73
Moderate, %	14.7	9.2	1.74	0.03
Severe, %	7.5	4.6	1.40	0.11

[1]Severity measured using Elanco scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health, Greenfield, IN)
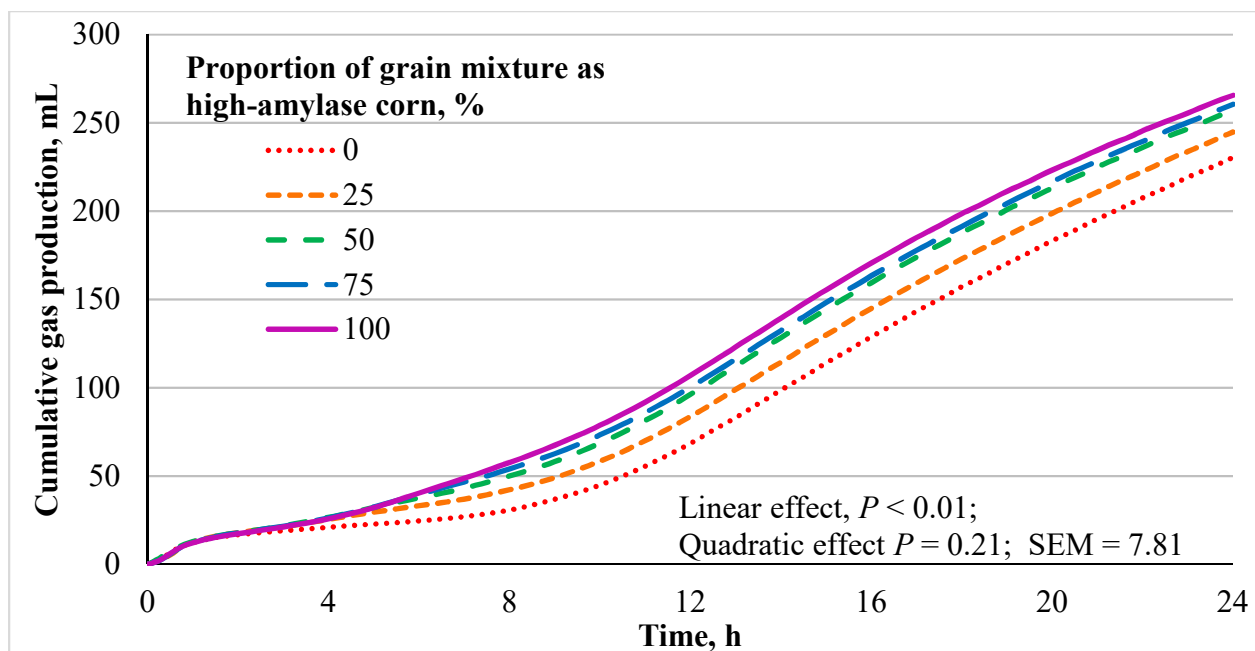
Figure 3.1. Effect of the proportion of high-amylase corn (EFC) in grain mixtures on production of fermentative gasses by *in vitro* cultures of mixed ruminal microbes fed steam-flaked grains as substrate.
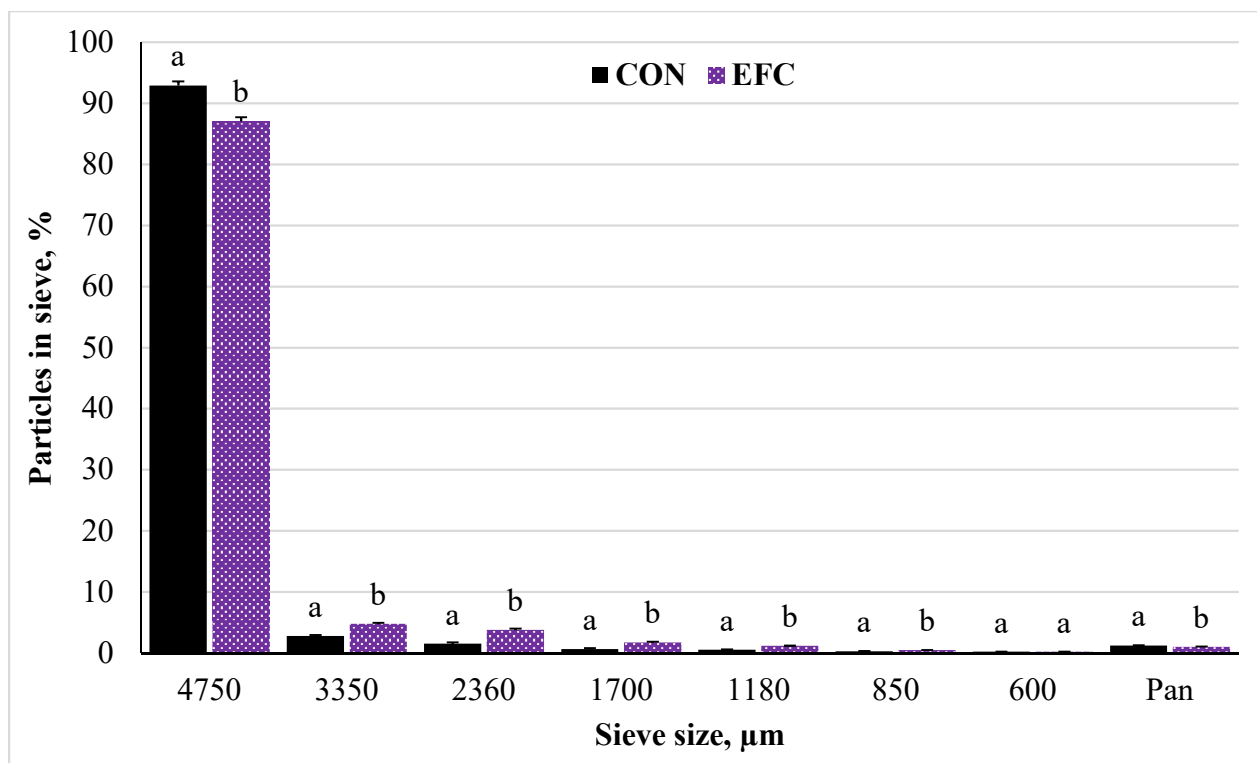
Figure 3.2. Effect of grain source on particle size distribution of flaked grains. Values are percent mill-run (CON) or high-amylase (EFC) corn weighed from each sieve and a solid pan, after 5-min cycle in a Ro-Tap shaker.
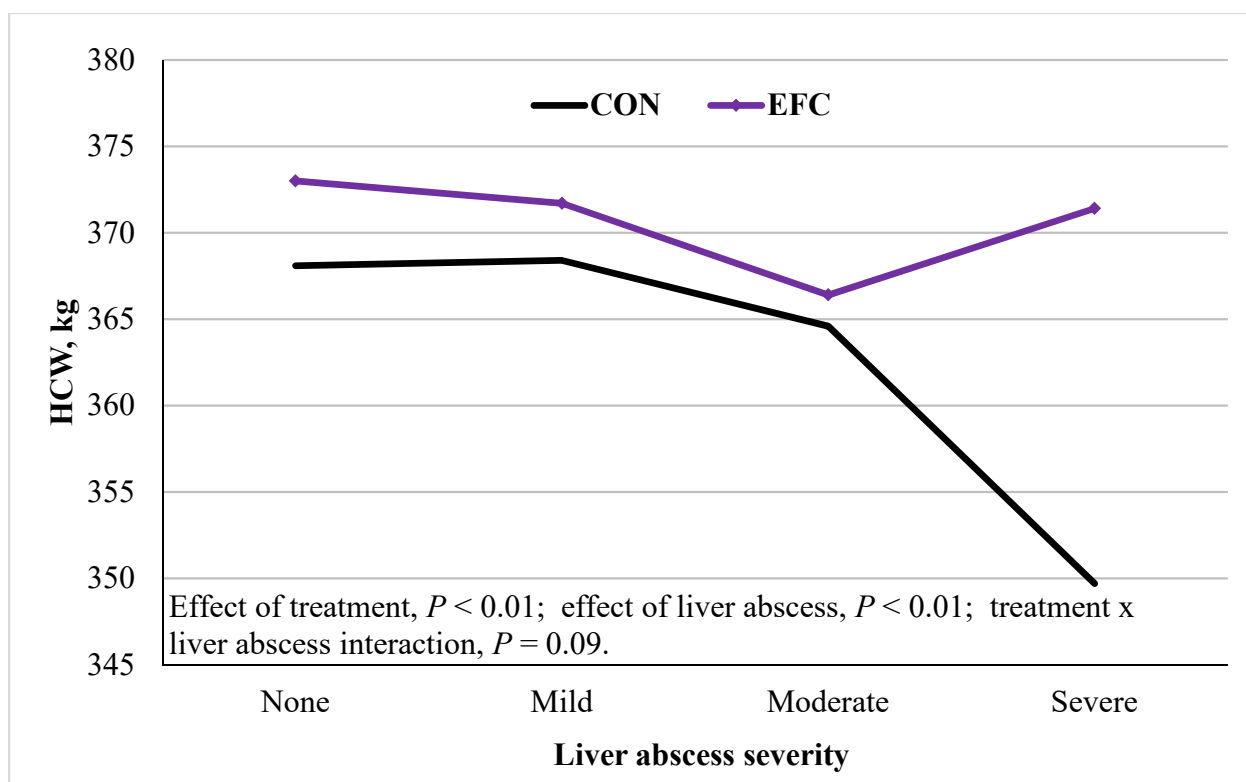[a,b]Indicates difference ($P < 0.05$) within sieve size.

Figure 3.3. Relationship between liver abscess severity and HCW for finishing heifers fed diets containing steam-flaked mill-run corn (CON) or steam-flaked high-amylase corn (EFC). Abscess severity measured using Elanco scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health, Greenfield, IN).