Utilization of a high-amylase corn hybrid in beef cattle finishing diets

by

Adrian Neal Baker

B.S., Kansas State University, 2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

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Abstract

Four experiments were conducted to evaluate Enogen Corn (EC) for production of corn silage and steam-flaked corn (SFC). In experiment 1, whole corn plant material from EC and a commercial corn hybrid utilized for corn silage (CON) were harvested and ensiled for 30, 60, 90, 120, or 240 d. Ensiled forages were analyzed for dry matter, volatile fatty acids (VFA), lactate, and aerobic stability. Silages produced from EC had greater concentrations of acetate compared to CON silages (144 vs 128 mM, $P<0.01$) and remained aerobically stable for a longer duration than CON silage ($P<0.05$). A second experiment was conducted to evaluate the initial 30-d of ensiling EC compared to CON. Freshly chopped corn plant samples were ensiled for 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 27, or 30 d. After ensiling, samples were analyzed for pH, VFA and lactate concentrations. An effect of forage type was observed, with silage produced from EC having greater lactate concentrations compared to CON (332.0 vs 291.7 mM, $P<0.01$). Experiment 3 evaluated starch gelatinization and retrogradation characteristics from ground whole corn (EC or CON) at moisture levels of 20, 30, 40, 50, 60, 70, or 80%. There were no grain type by moisture interactions observed for gelatinization temperatures or enthalpy ($P>0.10$). Retrogradation was affected by the interaction of grain type by moisture content, with EC at 70 and 80% moisture having lower recrystallization of starch compared to EC at lower moisture levels and CON grain at all moisture levels ($P<0.05$). Experiment 4 compared steam flaking EC versus CON at high-moisture. Deionized water was added at a 50% w/w to whole grains to measure water absorption and tempered to achieve moisture levels greater than commonly utilized in industry. Grains were flaked to a common bulk density and starch solubles were measured. Starch solubles were greater for steam-flaked EC compared to CON (4.7 vs 2.3 %, respectively; $P<0.05$). Three experiments were conducted to evaluate the performance and/or

digestibility of EC as corn silage, SFC, and a combination dry-rolled corn (DRC)/ high-moisture corn (HMC). Experiment 5 was conducted to evaluate performance, carcass characteristics, and meat quality of steers fed EC or CON as corn silage and/or SFC in a 2 x 2 factorial treatment arrangement. Steers (n=960; 388± 7.4 kg) were fed finishing diets for 138, 152, or 166 d. Steers fed EC silage and CON grain had greater hot carcass weights compared to other treatments (P<0.05) and longissimus lumborum steaks from steers consuming EC grain were observed to contain greater poly-unsaturated fat content compared to steaks from the CON grain treatment (P<0.05). Two experiments were conducted to compare digestibility of EC versus CON utilizing different grain processing methods. Experiment 6 utilized cannulated steers (n=7) fed finishing diets comprised the DRC/HMC combinations as either EC or CON in a replicated cross over design. Digesta samples were collected at h 0, 8, and 16 on d 11 through 14, advancing 2-h each day to represent a 24-h post-feeding cycle. Grain type did not affect total-tract digestion (P>0.10), but small intestinal nitrogen digestion tended to be greater with steers fed EC grain combination compared to CON grain (69.8 vs 59.5; P=0.09). Experiment 7 utilized the same cannulated steers in a similar study design as previously described; however, fed finishing diets containing SFC produced from either EC or CON grain flaked to 390 g/L and 360 g/L, respectively. Grain type did not affect intestinal or total-tract starch digestion (P>0.10). Ruminal fluid of steers fed steam-flaked EC were observed with greater butyrate content compared to steers fed steam-flaked CON grain (23.3 vs 18.7 mM, respectively; P<0.01). Ensiling EC modifies VFA, and lactate produced and improves aerobic stability of the silage produced from that forage type. Furthermore, gelatinized EC grain may be less susceptible to retrogradation due to amylolytic activity on the starch. Enogen corn fed as corn silage improves performance and meat produced from cattle consuming EC as grain have greater poly-unsaturated lipid content

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Major Professor
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Chapter 1 - Literature review

A survey of consulting nutritionists listed corn as the primary grain source, and corn silage as the primary roughage source in finishing diets (Samuelson et al., 2016). Corn as either the grain itself or chopped whole corn plant may be utilized to provide energy in diets due to starch in the kernels. In beef cattle finishing diets, roughage inclusion is generally between 6 to 12% of the diet (Samuelson et al., 2016). This makes utilization of starch a primary interest in finishing cattle diets. Several factors may affect the digestibility of corn starch in ruminants' gastro-intestinal tract. Grains are commonly processed by steam flaking, dry rolling, or ensiling at high moisture. These processing methods have been shown to alter site and extent of digestion and thus affecting performance of the cattle (Owens et al., 1997; Owens and Zinn, 2005). A review by Owens et al. (1986) concluded that the small intestine of ruminants has limited ability to digest starch, with lack of amylolytic enzymes to hydrolyze starch as a possible cause.

Recently, a corn hybrid has been developed to reduce or eliminate the need for addition of exogenous enzymes that aid in the hydrolysis of starch to improve fermentation of starch to produce fuel ethanol. This corn hybrid has been genetically modified to have increased concentrations of α-amylase within the kernel. There may be benefits in utilizing this corn hybrid in finishing diets due to limited enzymatic activity in the ruminant's small intestine. This review will evaluate the current knowledge of corn (starch) and limitations to digestibility of starch in the rumen and small intestine. In addition, it will review current processing methods utilized to improve digestibility or add value to corn as either grain or chopped whole corn plant.

Corn kernels and starch morphology

As previously stated, corn grain is commonly utilized as a concentrate source in feedlot diets, this is due to the energy value of the starch. Overall, each kernel contains approximately 70

to 75% starch, 8 to 9% crude protein, and approximately 4% lipids (Garcı´a-Lara et al., 2019). Kernels are comprised of a pericarp, germ, tip cap, and endosperm (Hoseney, 1986). With the main component of the kernel being the endosperm, accounting for 80-82% of the kernel dry weight and being comprised of approximately 89% starch (Garcı´a-Lara et al., 2019; Li et al., 2007).

Development of starch granules

Endosperm may be divided into two parts: vitreous and floury endosperm (Hoseney, 1986). The vitreous endosperm is found in the peripheral layer within the endosperm and is connected to the pericarp, while floury endosperm resides in the inner portion of the endosperm, near the corn germ. Starch granules in each kernel are embedded within a protein matrix comprised of zein proteins, which gives the endosperm structure (Garcı´a-Lara et al., 2019). The protein/starch matrix of the endosperm is what gives the corn its hardness and determines floury versus vitreous endosperm (Hoseney, 1986). Although these two types of endosperms differ physically, the granules are comprised of glucose molecules. Corn plants utilize photosynthesis to produce mainly sucrose as a source of energy and convert excess sucrose into glucose in the amyloplasts of endosperm cells within each kernel (Garcı´a-Lara et al., 2019). Granules are formed in amyloplasts, with only one granule per amyloplast (Hoseney, 1986). Granules may be round or polyhedral in shape and are approximately 15 nm in diameter (Lineback, 1986).

As previously stated, starch is comprised of glucose molecules and these molecules are found linked together to form glucose chains. Starch may be further distinguished by two distinct structures found in each granule: amylose and amylopectin. Although, these two polysaccharides are commonly found in cereal grain and in corn are generally observed in these proportions: approximately 25-30% amylose and 70-75% amylopectin (Blennow et al., 2013). Amylose may

contain 100-10,000 glucose chain unit lengths, while amylopectin may contain 100 times more glucose molecules (Blennow et al., 2013). Chain length of amylopectin can vary depending on location within the kernel. Lin et al. (2002) determined that amylopectin chains in the vitreous endosperm are greater in length than amylopectin in the floury endosperm by examining flour and grit fractions following dry milling. Furthermore, surface gelatinization studies have shown that amylopectin chain length differs in the granule itself, with longer chain lengths toward the hilum (center) of the granule and becoming shorter as they near the periphery of the granule (Jane, 2009).

Amylose and amylopectin are comprised of linked glucose molecules, but differ in bonds found within each structure. Glucose may be linked in linear chains from the 1^{st} carbon on the reducing end of a glucose molecule to the 4^{th} carbon on the non-reducing end of another glucose to form a linear starch molecule known as amylose. Amylopectin contains the α-1,4 bonds similar to amylose, but can have additional glucose molecules linked to the 6^{th} carbon on a glucose molecule to form a branched structure (Damager et al., 2010). During starch synthesis glucose is added to the non-reducing end of an amylopectin or amylose chain at the hilum of the granule (Damager et al., 2010). The synthesis of amylose and amylopectin is dependent on enzymes to form the starch granule and grow by apposition. Blennow et al. (2013) described synthesis of amylose and amylopectin with the first step being phosphorylation of glucose to form glucose-1-phosphate, which is then transported into the amyloplast. ADP-glucose pyrophosphorylase enzyme in conjunction with ATP, dephosphorylates the glucose to form ADP-glucose. The synthesis of amylose and amylopectin rely on ADP-glucose as the substrate. Amylose is then synthesized by granule bound starch synthase enzyme that attaches a glucose to a malto-oligosaccharides primer for chain elongation (Blennow et al., 2013). Similarly, during

synthesis of amylopectin, branching enzymes utilize ADP-glucose to connect glucose to a malto-oligosaccharide primer to form pre-amylopectin. Pre-amylopectin is debranched by a debranching enzyme that transforms the amylopectin in to semi-crystalline clusters (Blennow et al., 2013). Amylose and amylopectin are observed in proportions specific to each grain type; however, regulation of synthesis of these starch structures is unknown (Huang et al., 2021).

Starch structure and composition

Amylose and amylopectin are arranged in a semi-ordered crystalline structure, although there are opposing theories regarding the structure starch (Bertoft, 2004; Jane, 2009). The cluster model described by Jane (2009) appears to more accurately represent the structure of starch as will be described. Amylopectin is synthesized into clusters that may be connected from a reducing end on one cluster to the nonreducing end of another cluster, with the connection of the two clusters known as branch points. Each cluster of amylopectin have several nonreducing ends with only one reducing end at the beginning of the cluster. Clusters are connected by crosslinking with amylose to form a ring (Jane, 2009). Each ring of clusters is referred to as crystalline lamellae, which are layered within each granule. The area between each ring that contains the branch points from the amylopectin clusters is known as the amorphous lamellae (Blennow et al., 2013; Huang et al., 2021; Myers et al., 2000). The crystalline and amorphous lamellae form growth rings for each starch granule, which gives the order or crystalline structure to starch. The majority of amylose in a starch granule resides in the amorphous lamellae of the starch structure in linear chains among the branch points of the amylopectin and between amylopectin clusters (Robin et al., 1974; Jane, 2009).

Gelatinization of starch

Polarized light that is passed through a native or ungelatinized starch granule will form a Maltese Cross. The Maltese Cross is evidence of birefringence and a crystalline state of the starch. Lineback (1986) describes gelatinization as a loss of birefringence. Several factors may affect starch gelatinization, such as water content, heating rate, and amylose:amylopectin ratio. Several potential transition phases occur during gelatinization, but it is generally accepted gelatinization occurs when starch is heated in conjunction with excess water over 55ºC (Ratnayake and Jackson, 2009). The most accepted description of gelatinization begins in the amorphous region of the granule with disruption of hydrogen bonds and diffusion of amylose from the granule (Ratnayake and Jackson, 2006). Further heating swells the granule and separates the amylopectin clusters, resulting in a loss of birefringence (BeMiller, 2007; Ratnayake and Jackson, 2009, 2006).

Beside water content, amylose and amylopectin appear to have the greatest impact on starch gelatinization. Hsieh et al. (2019) demonstrated using RVA that starches consisting primarily of amylopectin have greater viscosity and lower gelatinization temperatures compared to normal corn starch containing approximately 25% amylose. Chung et al. (2011) evaluated gelatinization of rice starches with varying proportions of amylose and determined that amylose increases cooking temperatures required to gelatinize starch. The authors attribute this to amylose decreasing swelling of granules during gelatinization by interfering with water penetration into the cluster.

Following gelatinization of starches granules or complete disruption of the granule, the starch becomes soluble in water, which differs from native starches that are insoluble in water (Ratnayake and Jackson, 2009). Individual granules in a group do not gelatinize at the same time

or temperature and once gelatinization occurs in a starch granule, it is considered irreversible (BeMiller, 2007). Gelatinized starches may reform into a different semi-crystalline structure when cooled, a process known as retrogradation.

Retrogradation of gelatinized starch

Following disruption of the crystalline structure by gelatinization, the starch when cooled may retrograde into an insoluble state (BeMiller, 2007). Amylopectin reassociates into a different crystalline structure than previously found in the native starch. Similar factors that influence gelatinization also affect retrogradation, such as time, water content, and amylopectin: amylose ratio. Amylose retrogrades more rapidly than amylopectin, with amylose retrograding within minutes, while amylopectin may take hours to days (BeMiller, 2007). Storage temperature following cooking of the starch can affect retrogradation. As gelatinized starch reaches the glass transition temperature (approximately -5ºC for cereal starch), nucleation or reformation of the starch molecules into crystalline structures will occur (Slade and Levine, 1991). The propagation of starch crystallites is further intensified, as the starch is reheated to near the melting point (approximately 50ºC) of the starch crystallites. The initial reformation of the starch crystallites must occur before the growth of crystallites. Total retrogradation is the sum of both the nucleation and propagation of the starch (Slade and Levine, 1991). Chain length (degree of polymerization, DP) may also impact the amount of retrogradation, with DP between 14-24 glucose units being more prone to retrograde (Shi and Seib, 1992). This largely is due to the DP length of required to form double helices during retrogradation. Zhou et al. (2011) observed as water content of the starch decreased the impact of amylose on starch retrogradation decreased. Generally, retrogradation is perceived as undesirable in nutrition due to decreased susceptibility of starches to enzymatic hydrolysis by pancreatic enzymes (Zhou and Lim, 2012).

Hydrolysis of starch

Englyst et al**.** (1992) grouped starches by their rate of digestibility or ability to be hydrolyzed in the small intestine of humans: rapidly digestible starch, slowly digestible starch, and resistant starch. These classifications were determined enzymatically *in vitro* and utilized to suggest probability of digestion in the small intestine with freshly cooked starches being classified as rapidly digestible, uncooked starch being considered slowly digestible, and resistant starches physically inaccessible for enzymatic hydrolysis. The classifications were initially developed for use in human nutrition, but may be applicable to ruminant nutrition.

There are several enzymes capable of hydrolyzing starch. Alpha-amylase is an important endoenzyme that hydrolyzes the α-1,4 bonds within the internal structure of amylose and amylopectin. This produces varied lengths of linear oligosaccharides and oligosaccharides that contain branch points, commonly known as α-limit dextrins. In conjunction with amyloglucosidase, α-amylase increases the rate of hydrolysis of starch by amyloglucosidase, which hydrolyzes the α-1,6 branch points and α-1,4 bonds from non-reducing end of amylose and amylopectin to release one unit of glucose. This action occurs on the external glucose unit in the starch chain, which classifies amyloglucosidase as an exoenzyme. Beta-amylase is another exoenzyme similar to amyloglucosidase in that it cleaves the α-1,4 bonds on amylose and amylopectin chains, but releases maltose from the nonreducing end of the glucose chain (BeMiller, 2007).

A group of enzymes that hydrolyze the α-1,6 bonds are referred to as debranching enzymes. Two common debranching enzymes are pullulanase and isoamylase. Pullulanase specifically hydrolyzes α,1-6 bonds in pullulans, which is a repeating chain of maltotriose linked

by α,1-6 bonds. Isoamylase has action similar to that of pullulanase, acting on α,1-6 bonds, though isoamylase specifically degrades amylopectin (Van Der Maarel et al., 2002).

Starch digestion in ruminants

Ruminants are foregut fermenters, which differentiates them from monogastric animals. This means they have multiple sites of digestion for starch. The two primary sites of starch digestion are the rumen and small intestine, with the majority of starch digestion taking place in the rumen (Orskov, 1986).

Ruminal starch digestibility

The rumen allows for a symbiotic relationship between bacteria, protozoa, fungi, and the animal (Huntington, 1997). Starch is fermented in the rumen by microbes to produce volatile fatty acids which are absorbed by the animal through the ruminal epithelium, and gases that are eructated from the animal, including methane and carbon dioxide. Cattle consuming high starch diets have been reported to have greater propionate concentrations in the rumen, which consequently produce less methane than cattle fed forage-based diets due to propionate being a hydrogen sink (Orskov, 1986). Most ruminal fermentation of starch is accomplished by bacteria as they have the quickest growth rate (Huntington, 1997).

Fermentation of starch is based on several factors such as source of starch, diet composition, and extent of starch processing (Huntington, 1997). A review by Kotarski et al. (1992) lists several ruminal bacteria with amylolytic activity. Bacteria either secrete amylases into the ruminal media or produce surface associated enzymes to hydrolyze starch. The authors demonstrated the impact of starch disappearance in *in vitro* cultures with or without protease addition. The authors concluded degradation of the starch/protein matrix may be integral in starch digestion by bacteria.

McAllister et al. (1993) evaluated three ruminal fungi strains (*Orpinomyces joyonii*, *Neocallimastix patriciarum*, and *Piromyces communis*) impact on starch degradation following incubation *in vitro* for 96 hours. In this experiment, all fungi strains degraded the protein matrix and observed a decreased in granule size. This suggests extra cellular amylases may have a role in fungal degradation of starch by hydrolyzing the peripheral portion of the granule. The role of fungi in starch fermentation in the rumen is still unknown due to the slow rate of disappearance, with less than 20% starch disappearance after 24-hours of incubation.

Some bacterial species do not contain enzymes needed to completely hydrolyze starch. Cross-feeding is the occurrence between bacterial species during hydrolysis of starch produces intermediates that are utilized by other species (Cotta, 1992). Cotta (1992) demonstrated cross-feeding by utilizing a co-culture system that contained amylolytic and dextrin-utilizing bacteria. Individually, *S. ruminantium* was unable to ferment starch due to its preference of dextrins, but with addition of an amylolytic bacteria both were able to grow. The concept of cross-feeding may be further extrapolated to explain the fermentation of starch by rumen microbial populations. As previously described, fungi may have a role in the degradation of proteins in the starch matrix, which potentially allows access for bacterial attachment to the granules.

The impact of the starch/protein matrix on starch fermentation was further investigated by Allen et al. (2021). The authors evaluated corn endosperm type and grind size on ruminal fermentation in dairy cows. Finer ground endosperm improved ruminal apparent starch digestibility by 52%; moreover, floury endosperm had greater ruminal apparent starch digestibility compared to vitreous endosperm. This is likely due to less starch/protein matrix found in floury endosperm compared to vitreous, which allows more accessibility to starch by bacterial amylolytic enzymes.

Protozoa have a different role in ruminal fermentation of starch. Feedlot cattle fed high starch diets contain have smaller populations of protozoa, which may influence ruminal fermentation of starch (Towne et al., 1990). Mendoza et al. (1993) evaluated starch fermentation in defaunated sheep. Defaunation increased ruminal starch digestion and reduced starch digestion in the small intestine by limiting starch flow. Protozoa may slow ruminal fermentation of starch by ingesting starch granules or through predation of bacteria (Kotarski et al., 1992).

Several microbial populations are involved in starch fermentation. It is clear that ruminal fermentation of starch is essential in finishing diets. Owens et al. (1986) estimated that 65 to 85% of starch intake is digested in the rumen and following fermentation, starch flow to the small intestine may be in the form of microbial polysaccharides or intake starch that was not fermented. Intensive processing of corn can increase ruminal fermentation of starch; however, if ruminal fermentation is too rapid there can be negative impacts on performance and health. Accumulation of volatile fatty acids and organic acids can reduce ruminal pH and subsequently cause ruminal acidosis (Nagaraja and Lechtenberg, 2007). Acidosis is divided into two categories: acute and subacute. Subacute acidosis presents no clinical signs, but is characterized with a pH of 5.00 to 5.50, 0 to 5 mM of lactic acid, and high ruminal concentrations of volatile fatty acids (150 to 225 mM; Nagaraja and Titgemeyer, 2007). Acute acidosis presents clinical signs and is characterized with ruminal pH below 5.00, below normal volatile fatty acid concentrations, and high lactic acid concentrations. Fermentation of starch is largely dependent on grain processing methods and the effect of grain processing on digestion will be discussed further in the review.

Intestinal digestibility of starch

Starch and microbial polysaccharides from the rumen flow to the small intestine for further digestion and then finally to the large intestine. Absorption of glucose in the small intestine is beneficial as it is energetically more efficient than fermentation. Harmon and McLeod (2001) estimated that 13-18% of energy is lost due to ruminal fermentation. Owens et al. (1986) estimated 82% of the starch that reached the small intestine is digested and provides 42% more energy compared to starch fermented in the rumen.

Digestion of starch in the small intestine of ruminants has been suggested to be limited by: lack of presence of amylolytic enzymes (specifically α-amylase); limited glucose absorption; passage rate; or limited access of enzymes to starch particles in the small intestine (Owens et al., 1986). The lack of amylolytic enzymes in the small intestine has been largely studied due to being considered first limiting. Owens et al. (1986) reviewed a series of experiments and concluded α-amylase secreted from the pancreas into the small intestine is deficient. Alpha-amylase released by the pancreas is an integral endo-enzyme in starch digestion by hydrolyzing polysaccharides (5 α-1,4 linked glucose units) to maltose, maltotriose, or branched limit dextrins (Brake and Swanson, 2018). This initial step in the digestion allows for further hydrolysis of polysaccharides to glucose, which occurs on the intestinal epithelium by brush boarder enzymes, such as, isomaltase, maltase, and glucoamylase (Brake and Swanson, 2018). Glucose is absorbed in the small intestine by sodium dependent transporters (Harmon and McLeod, 2001). Absorption of glucose by ruminants has been thought to be deficient due to the lack of upregulation in response to carbohydrate entry into the small intestine; however, net portal glucose absorption from infusing glucose at a rate 60 g/h was 94% (Harmon and McLeod, 2001; Kreikemeier et al., 1991).

Kreikemeier and Harmon (1995) infused corn starch or corn dextrin in the duodenum, and observed oligosaccharides were present at the ileum, suggesting lack of amylase or brush border enzyme activity. This coincides with conclusions drawn from several authors (Harmon et al., 2004; Huntington, 1997; Owens et al., 1986). A recent review concluded lack of sucrase activity in the ruminant small intestine could be the limiting component to starch digestion (Trotta et al., 2021a). This enzyme is responsible for the hydrolysis of maltose to glucose.

Starch digestion is thought to be limited in the small intestine. Harmon et al. (2004) estimate approximately 79.5% of starch that entered the small intestine was digested. Huntington et al. (2006) modeled the g/d of starch digested in the small intestine and concluded when entry is less than 700 g/d, digestibility of starch is above 70%. Additionally, at 1,500 g/d digestibility would be approximately 45%. This coincides with results from Kreikemeier and Harmon (1995), who infused 1600 g/d of starch and observed approximately 50% of the starch disappeared as glucose in the portal vein.

Undigested starch flow to the large intestine may be fermented similar to starch fermented in the rumen. Approximately 47% of starch entering the large intestine is digested (Harmon et al., 2004). Unlike ruminal fermentation of starch, microbial polysaccharides and protein are lost to fecal excretion. The large intestine is the least energetically efficient site of starch digestion.

Corn processing

As previously discussed, corn is a primary source of energy in feedlot diets. The previous sections discussed starch and the impact of processing on the starch. This section will focus on current grain processing methods utilized in the feedlot industry and their impact on digestion.

The main processing methods to improve digestion of grains are steam flaking, followed by dry-rolling, and then ensiling at high moistures (Owens & Zinn, 2005).

Steam flaking corn

Steam flaking corn is the most recommended method for processing grains by consulting nutritionists (Samuelson et al., 2016). Steam flaking is the most costly of processing methods utilized by feedlots. This is due to the need for steam production, increased electrical demand, and a large amount of infrastructure (Heiman, 2005). Steam flaking grains also is more economically beneficial for feedlot operations compared to other processing methods (Macken et al., 2006). This is due to starch gelatinization that occurs during steaming and rolling of the grain, which improves digestibility. During the flaking process, the grain is flattened and alters the bulk density of the corn. Density of the flakes is commonly measured to determine the degree of gelatinization in the process, due to measurements being able to be taken real time with ease (Zinn et al., 2002). In addition to bulk density, flakes may be analyzed for starch availability. This measures the degree of susceptibility to enzymatic hydrolysis and is used as an indicator of digestibility (Richards and Hicks, 2007). Bulk density and starch availability have been correlated for determining digestibility (Zinn et al., 2002). Due to ease of measurement, bulk density is measured frequently to ensure repeatability of the process that will be described below.

Typically, steam flaking involves transferring whole corn into a vessel, open to atmospheric pressure (i.e., steam chest). During transfer, grain may be tempered with the addition of water and potentially with a surfactant. This increases the moisture of the grain and tempering generally adds 4 to 8% moisture to the grain (Zinn et al., 1998). A survey of 17 commercial feedlots showed 16 add water to grain in either a mixing auger during transfer to the

steam chest or in a soak tank above the steam chest (Schwandt et al., 2016). In the same survey the authors reported 3 of the 16 feedlots use a surfactant in addition to the water addition.

Corn is steamed within the steam chest by injecting steam into the chest to further hydrate and heat the kernels for 30 to 40 min, and excess steam is vented from the top of the chest (Richards and Hicks, 2007; Zinn et al., 2002). During steaming, kernel moisture may increase approximately 5% (Zinn, 1990a). Steaming is essential to gelatinization due to the moisture migration into the kernel and swelling of the starch granules. In addition to granule swelling, there is partial disruption of the starch/ protein matrix in the endosperm (Richards and Hicks, 2007). Following conditioning of the grain in the steam chest, the grain is then rolled between two large diameter rolls. This allows for a larger nip angle to increase pressure applied to the conditioned grain. The distance between the rolls determines the bulk density of the corn and the roll speed differential of 1:1 provides little shear, but mechanically flattens the grain and alters the starch (Heiman, 2005; Richards and Hicks, 2007). As discussed previously, bulk density is commonly used to determine degree of gelatinization. Two surveys of commercial feedlot operations report the mean flake density is 0.35 kg/L (Samuelson et al., 2016; Schwandt et al., 2016). Starch availabilities between the surveys differ, with Samuelson et al. (2016) reporting a mean of 59.9% and Schwandt et al. (2016) reporting a mean of 50.6%.

Ramirez et al. (1985) fed steamed whole corn and steam-flaked corn to feedlot cattle and determined steaming grain in conjunction with the rolling has the greatest impact on digestibility. The heating and hydration of corn, prior to adding mechanical disruption from the roller mill appears to have the largest impact on gelatinization of the starch in the kernels. The greatest effects on starch granules occurs after cooking the granule and adding mechanical energy to the grain, which results in shorter polymer chain lengths (Mason, 2009).

Steam flaking is the one of the few processing method that involves gelatinization of starch to improve digestibility and has the potential for negative effects of retrogradation. Ward and Galyean (1999) investigated the impact of starch retrogradation on *in vitro* dry matter disappearance of steam-flaked corn. The authors collected samples immediately from the rolls and from the steam-flaked corn bin, assuming differences in starch availabilities from the two collection sites would be retrogradation. They determined starch availabilities decreased at the bin collection site, but *in vitro* dry matter disappearance did not differ between collection sites. The authors concluded that retrogradation would not affect digestibility of the starch. Similarly Trotta et al. (2021a) sampled steam-flaked corn immediately following flaking and subjected a portion of steam-flaked corn to temperatures at 55ºC for 3 days to compare starch availabilities. They observed lower starch availability with the flaked corn incubated at 55ºC for 3 days compared freshly processed flakes. In an additional experiment, Trotta et al. (2021b) hypothesized starch retrogradation may slow the rate of ruminal fermentation of starch. Utilizing similar methodology as the previous study, the authors compared freshly steam-flaked corn to corn incubated at 55°C for 3 days using *in situ* method. They determined that steam-flaked corn stored at 55ºC lowered ruminal starch disappearance compared to the freshly processed steam-flaked corn. There appears to be limited research to determine the impact of starch retrogradation in beef cattle diets. A review of starch digestion in human literature suggests retrograded starch is not completely resistant to bacterial fermentation (Han et al., 2006; Patel et al., 2017).

Retrograded starch is of considerable interest in human nutrition due to potential health benefits. Starch that has been retrograded is also known as resistant starch and is studied for its lack of digestibility in the human small intestine. The inability of digestive enzymes to hydrolyze the starch for absorption glucose has been shown to reduce glycemic index in humans compared

to consuming gelatinized starches (Han et al., 2006; Patel et al., 2017). Unhydrolyzed starch from the small intestine is then able to be fermented in the large intestine. Moreau et al. (2004) evaluated volatile fatty acid uptake in rats consuming resistant starch. The authors observed an increase in butyrate uptake in intestinal mucosa from bacterial fermentation of the retrograded starch.

Dry corn processing

Another common method for feeding grain is by grinding or dry rolling the grain. Grinding generally refers to grain that has been ground with a hammer mill. Grain flows into the grinding chamber, and several hammers are attached to a rotor that spin at speed up to 7,750 m/min (Heiman, 2005). The chamber is enclosed by screens, which can be sized to suite the desired particle size. The hammers contact the grain and break the kernels into smaller pieces. The larger particles will be impacted again if they are too large to fall through the screen of the mill (Heiman, 2005).

Roller mills reduce particle size of the grain by passing between corrugated rolls, similar to steam flaking. The two main difference between steam flaking and dry rolling is moisture at which grain is processed and differential speed at which the rolls operate. The differential rotation of the rolls allows for a shearing of the kernel and reduces the particle size. The gap and roll configuration are utilized to control particle size. Particle size distribution is generally less for dry-rolled grain compared to grain ground by a hammer mill (Heiman, 2005). Dry rolling of grains is more commonly utilized in feedlot operations (Richards and Hicks, 2007).

High-moisture corn

High-moisture grain differs from the other processes in that the grain is harvested earlier than typical corn when the kernels are between 25 and 30% moisture. Challenges occur with

harvest, as moisture content that may decrease at a rate of approximately 1% per day (Mader and Rust, 2006). Harvest moisture is critical because corn that is ensiled at less than 25% moisture can be subject to spoilage. Corn is ground or rolled prior to storage in a bunker (Richards and Hicks, 2007). Ground grains allow for better packing density and eliminate oxygen in the grain pile, but using a roller mill to crack the grain provides more uniform particle size during feeding of the grain to cattle (Richards and Hicks, 2007). During storage the grain undergoes fermentation that allows for preservation of the grain. This is on the condition that the storage methods eliminate oxygen from the corn to allow for anaerobic fermentation and elimination of aerobic deterioration (Mader and Rust, 2006). Moisture content of the grain influences production of organic acids during fermentation and ultimately, the reduction of pH (Goodrich et al., 1975). Adequate ensiling is essential for reducing dry matter losses (Mader and Rust, 2006).

Ensiling alters digestibility of the corn components. Cooper et al. (2002) compared bacterial crude protein flow from the rumen, with diets containing dry-rolled, high-moisture, and steam-flaked corn. The authors observed a 29% increase in ruminal bacterial flow and greater ruminal digestibility of high-moisture corn compared to the other processing methods. Hoffman et al. (2011) hypothesized that ensiling increases starch susceptibility to enzymatic hydrolysis due to separation of the zein proteins from the starch granules. The authors observed a reduction in zein protein subunits following ensiling, thus hypothetically improving bacterial fermentation of starch.

Effect of corn processing on digestibility and performance of finishing cattle

The effects of processing on digestibility and performance have been extensively investigated. One method of feeding corn that has not been previously discussed in this review is feeding whole corn. This is practice is not commonly utilized in commercial feedlots and has

limited total-tract digestion compared to dry-rolled or steam-flaked corn (Owens and Zinn, 2005; Samuelson et al., 2016). Processing method affects total tract starch digestion, with steam flaking and high-moisture having similar disappearance (~99%) and to a lesser extent dry rolled (~89%) in the diet (Owens and Zinn, 2005). Site of digestion differs depending on processing method. For instance, ruminal disappearance of starch is greatest with high-moisture corn compared to other processing methods (Owens and Zinn, 2005).

Dry rolling is a common method of grain processing and exposes endosperm for digestion (Zinn et al., 2011). The greatest impact of dry rolling is due to particle size reduction. Scott et al. (2003) compared dry-rolled corn (4,619 µm), finely-rolled corn (3,977 µm), and finely ground corn (715 µm) in two separate experiments utilizing steers fed finishing diets. In the first experiment, they compared dry-rolled corn versus finely ground corn and observed similar average daily gain, but the steers consuming the dry-rolled corn had greater dry matter intake. This allowed steers consuming the finely ground corn to be more efficient. The second experiment compared dry-rolled corn versus finely-rolled corn and observed no difference in performance. Although, the processed corn's particle only differed by approximately 600 µm.

In a study comparing dry matter intakes of cattle fed dry-rolled corn versus cattle fed high-moisture corn, cattle consuming high-moisture corn tended to have lower intakes; however, no effect of grain processing was observed for average daily gain (Ladely et al., 1995). The authors reported a tendency for improved efficiency in cattle fed high-moisture corn compared dry-rolled corn. Ensiling corn at high-moisture increases rapidly degradable fraction of starch in corn compared to dry-rolled corn (Philippeau and Michalet-Doreau, 1998). As previously discussed, this may be due to degradation of the starch/protein matrix during ensiling. Additionally, moisture content of the high-moisture corn appears to impact dry matter intake and

efficiency. Cattle fed ground high-moisture corn greater than 27% moisture have an 8% lower dry matter intake and are more efficient than cattle fed 18 to 22% moisture corn (Owens et al., 1997). Stock et al. (1991) conducted several experiments evaluating moisture, particle size, and duration of ensiling. The authors determined that rate of starch digestion increases with increased length of storage, smaller particle size, and higher moisture content. All of these variables can potentially affect ruminal starch fermentation, potentially contribute to digestive disorders, which makes high-moisture corn a highly variable concentrate source.

Similar to high-moisture corn, steam flaking may produce varied results depending on processing parameters. Steaming time and flake density can affect digestibility and therefore effects feedlot performance. In a digestibility study, Zinn (1990a) evaluated steam conditioning times on corn for 34, 47, and 67 minutes prior to flaking and observed no improvement in total tract starch digestibility from additional steaming time past 34 minutes, which was approximately 99.4%. Overall, steam flaking improved total tract starch digestion by approximately 8% compared to dry-rolled corn. Extensive processing of corn may negatively impact feedlot performance. Zinn (1990b) performed two studies evaluating impact of three flake densities on digestibility and performance in finishing cattle. Decreasing flake density improved starch availabilities and improved total tract digestion of starch in diets fed at 85% of *ad libitum* intake, but linearly decreased pH. Furthermore, the authors fed steam-flaked corn at the same densities to finishing cattle and observed that at the lowest flake density (300 g/L) feedlot cattle decreased average daily gain and dry matter intake compared to those fed corn flaked at a greater densities. Zinn (1990b) hypothesized that the lowest flake density caused digestive disturbances, which reduced dry matter intake. A review by Zinn et al. (2011) assessed several studies that compared digestibility of processing methods. They concluded that total tract

digestibility of steam-flaked and high-moisture corn is at approximately 99%, while dry-rolled and ground corn are approximately 90 and 92%, respectively. High-moisture corn had the greatest ruminal starch digestion (91%) compared to dry-rolled (61%) and steam-flaked (84%). Additionally, it appears that steam-flaked corn has the greatest post-ruminal starch digestion (94% of flow) and the majority of starch disappearance is from small intestinal digestion (88% of flow; Owens and Zinn, 2005). This would suggest that steam flaking is more energetically efficient grain processing method compared to other methods.

Macken et al. (2006) compared the three processing methods in 5,000 and 20,000 capacity feedlots and used the average increase in feed efficiency for high-moisture corn (5.6%) and steam-flaked corn (12.6%) compared to dry-rolled corn. The improvements in feed efficiency the authors utilized were averaged from several experiments and used to compare profitability of the three-processing method. The authors concluded for a 20,000 head feedlot a 4.2% improvement in feed efficiency would be needed to offset the cost of steam-flaking and 1.7% improvement to in feed efficiency to be profitable feeding high-moisture corn.

It is apparent that grain processing method affects digestibility and performance of finishing cattle. Differences in total tract and site of digestion can impact feed efficiency. The improvements in energy utilization can be observed when evaluating NEg for the steam-flaked corn (1.67 Mcal/kg), dry-rolled corn (1.49 Mcal/kg), and high-moisture corn (1.56 Mcal/kg; NASEM, 2016). Extensive processing of grain can have adverse effects on performance, as a result of predisposition to digestive disturbances. It appears that steam-flaking corn, if processing variables are constant, has the greatest improvement in feed efficiency compared to the other processing methods and is large enough to offset the cost of flaking.

Corn silage

Ensiling is the process of preserving forages for use during seasons when fresh forages are not available. The primary objective of ensiling is to preserve the harvested material until fed by preventing losses from spoilage or dry matter loss, which ultimately affects profitability. As previously stated, corn silage is the primary roughage source recommended by consulting nutritionist (Samuelson et al., 2016). Differences in corn forage characteristics can affect ensiling and may impact digestibility during feed out. This section of the literature will discuss ensiling, plant properties that effect ensiling, inoculants, aerobic stability of silage, and the use of silage in finishing diets.

Ensiling

Ensiling forages may be separated into four phases. Production of corn silage involves growing and harvesting the whole corn plant. Once harvested, during the time until anaerobic conditions are achieved (aerobic phase), the silage is prone to degradation. Preservation (ensiling phase) is the primary purpose of ensiling forages, as it maintains forages at high moistures for extended periods of time (storage phase). Similar to the aerobic phase, silage is subject to degradation due to exposure to air during removal of silage from storage or feed out phase. In addition, inoculating chopped whole corn plant prior to ensiling can aid fermentation and/or improve aerobic stability.

Aerobic phase

The first phase of the ensiling process is developing an anaerobic environment for microorganisms to develop, which preserves the forage. Prior to this, the whole corn plant is chopped in the field and transported to a storage site. Anaerobic environments are achieved by loading the chopped forage into storage containers. Silage is generally stored in bunkers, upright

silos, or plastic tubes. These storage containers are intended to be sealed, so there is no entry of air. Packing of the forage reduces porosity, which reduces the movement of oxygen throughout the silage mass (Muck and Holmes, 1999). Increased packed silage density decreases dry matter losses and improves aerobic stability during feed out (Ruppel et al., 1995). Pack density may also be increased with mechanical processing. Silage harvesters are often equipped with kernel processors. These processors reduce particle size and have been shown to increase pack density (Johnson et al., 2002). Current recommended pack density for corn silage is 224 kg/m^3 (Muck and Holmes, 1999). In a bunker, silage is spread evenly in approximately 15 cm layers. Farm equipment is driven on each layer to pack the silage thoroughly. Packing the silage to the recommended pack density will ensure oxygen has been removed from the mass and begin creating the anerobic environment. Ruppel et al.(1995) observed a negative correlation between silo filling rate and preservation. Filling rate is defined by the amount of time to fill the silo until it is sealed. It has been shown that delayed filling rates increases oxygen exposure to the forage and growth of unwanted microorganisms (Ruppel et al., 1995).

Once the silo is sealed, plant enzymes use the remaining oxygen in the mass by respiratory enzymes, which further facilitates development of an anaerobic environment (McDonald et al., 1991). This is referred to as aerobic respiration, although respiration will continue to occur if filling or sealing is delayed. During respiration, sugars in the presence of oxygen, are hydrolyzed by plant enzymes to produce carbon dioxide, water, and heat. Excess respiration depletes substrates utilized for fermentation, which will be discussed during the ensiling phase section, and creates excess heat in the silage. If extended respiration occurs, excess heat may denature protein and fiber components of the silage and decrease digestibility

(Muck, 1988). Limiting oxygen by adequately packing and sealing the silo, in a timely manner, reduces the potential for excess respiration and dry matter loss.

Ensiling phase

The anaerobic environment provided by packing, sealing the silo, and respiration, allows for anaerobic microbe populations to develop and grow. Corn plants have a variety of microorganisms, such as bacteria, molds, fungi, and yeasts, on their surfaces. The plant material in conjunction with the harvest equipment spread and transport these microorganisms to the silo or bunker (McDonald and Whittenbury, 1973; Stirling and Whittenbury, 1963). Lactic acid bacteria are commonly found on corn plants and are facultative anaerobes, meaning they grow in anaerobic conditions. If conditions are adequate, such as in a silage mass, these bacteria grow and are the primary contributors to the ensiling process (Bolsen et al., 1996).

The fermentation of water-soluble carbohydrates produces organic acids, primarily lactic acid, which preserve the forage. The acidification of the silage is essential to preservation. Lactic acid has a low pKa and such, lowers the pH of the ensiled material to inhibit growth of aerobic microorganisms (McDonald et al., 1991). Lactic acid producing bacteria can be divided into two categories: homofermentative and heterofermentative bacteria. They differ in fermentation products produced, homofermentative lactic acid bacteria produce 2 moles of lactic acid with every mole of glucose fermented. While, heterofermentative lactic acid bacteria produce lactic acid, ethanol, and carbon dioxide from the fermentation of glucose (McDonald et al., 1991). Homofermentative fermentation is more rapid and is responsible for the rapid reduction in pH. For corn silage, a pH of less than 4.00 is optimal for reducing growth of undesirable microorganisms (McDonald et al., 1981).

If a suitable pH is not achieved rapidly, then secondary fermentation can occur from undesirable organism, such as clostridia. Clostridia such as *Clostridium tyrobutyricum,* ferment lactic acid in silage to produce butyric acid and carbon dioxide (Driehuis and Elferink, 2000). The fermentation of lactic acid by this bacterium will subsequently cause an increase in pH, which further promotes growth of undesirable microorganisms and causes spoilage. Some clostridia have been shown to have proteolytic activity causing further nutrient loss in the silage (Driehuis and Elferink, 2000).

Storage phase

It appears little occurs during the storage phase assuming air is excluded from the silage mass due to the primary goal of ensiling is to preserve the composition of the forage (Bolsen et al., 1996). Inoculants, such as *L. buchneri* have been observed with silage stored for longer than 120-d fermenting lactic acid to acetic acid, which the benefits of this will be discussed later in this review (Muck et al., 2018). Silages that had limited substrate during fermentation may ferment sugars produced from the degradation of hemicellulose during storage (Bolsen et al., 1996). Populations of undesirable microorganisms, such as yeast and fungi, have been identified in silages during the storage phase (Storm et al., 2010). The low pH and lack of oxygen present limit their activity; however, during the period of feed out, the introduction of oxygen to the silage mass allow these once dormant organisms to flourish.

Feed out/aerobic phase

Similar to the aerobic phase, exposure to air may degrade the silage. Aerobic stability has been used to describe the time that the silage remains unspoiled following exposure to air. The primary contributor to deterioration in the silage is yeasts. Yeasts are commonly found on the whole corn plant prior to chopping. During ensiling the low pH inhibits their activity, but during

feed out in the presence of oxygen concentrations of organic acids decrease due to yeast activity. Lactic acid utilizing yeasts metabolize lactic acid as an energy source and produce carbon dioxide and heat (Woolford, 1990). Aerobic stability of corn silage can be determined by the length time for temperature to increase above its ensiled temperature (Ranjit and Kung, 2000). The greatest issue with silages that are not aerobically stable is losses in dry matter and potentially adverse effects on feed intake. The initial pack density of the silage will inhibit air flow into the silage during feed out if removal does not disturb the mass beyond the silage face (Kung, 2010). Woolford (1990) estimates surface waste and aerobic deterioration may attribute up to 10% of energy loss in the silage.

Role of inoculants in ensiling and aerobic fermentation

The use of inoculants to improve aerobic stability has been extensively reviewed (Muck et al., 2018). In this review, the authors separated bacterial inoculants into 3 groups: homofermentative lactic acid bacteria, heterofermentative lactic acid bacteria, and combination of hetero- and homofermentative lactic acid bacteria.

The most common inoculant is homofermentative lactic acid producing bacteria. These bacteria aid in the preservation of silage by increasing lactic acid production during the ensiling phase. Lactic acid has little effect on aerobic stability during feed out as lactate utilizing yeast are the principle cause of deterioration (Muck et al., 2018; Woolford, 1990).

Heterofermentative bacteria, such as *Lactobacillus buchner* have been shown to improve aerobic stability when applied as inoculants (Ranjit and Kung, 2000). Silages inoculated with this bacterium show increases in acetic acid concentrations. Moon (1983) evaluated the inhibition of yeasts by combination of acetic acid, propionic acid, and lactic acid and lactic acid by itself offers little inhibition in yeast growth. Lactic acid in conjunction with propionic acid has

greater capacity to inhibit of yeasts, and to a lesser extent the combination lactic and acetic acid when compared to lactic acid alone. Ranjit and Kung (2000) observed lower yeast counts of silages inoculated with *L. buchner* after exposure to air versus uninoculated silages. However, one issue with heterofermentative bacteria is the slow rate of fermentation, which could cause delayed preservation and prolong the aerobic phase (McDonald et al., 1981).

Reich and Kung (2010) evaluated combinations of homofermentative lactic acid producing bacteria with *L. buchner*. In this study, the authors combined *L. buchner* with either *Lactobacillus plantarum*, *Pediococcus acidilactici* or *Pediococcus pentosaceus* and evaluated fermentation characteristics. After ensiling for 215 days, both untreated and inoculated silages had a pH below 4.00, but silages treated with an inoculant had almost 3-fold improvement in aerobic stability.

Inoculants have been shown to improve fermentation and aerobic stability. Muck (1988) describes the best attributes of an inoculant is a rapid growth rate to decrease pH and a population density to rival naturally occurring bacteria found on the forage. Heating and spoilage of silage due to a slow rate of fermentation or aerobic deterioration can lead to dry matter losses and poor performance of the cattle consuming the silage (Kung, 2010).

Properties of whole corn plant

Several characteristics of the chopped whole corn plant determine the ability to ensile, store, and its aerobic stability during feed out. Bolsen et al. (1996) describes corn as the "nearly perfect" crop for ensiling. This is due to the dry matter content, water soluble carbohydrates, and buffering capacity of the forage.

Dry matter

Several aspects of silage quality are determined by dry matter. As previously discussed, removal of oxygen or air from the forage is critical to achieving anaerobic conditions. Dry matter of the silage impacts the ability of the forages to be packed. There is a balance of dry matter needed to achieve the recommended dry pack density and moisture required for fermentation (Muck and Holmes, 1999). Generally, dry matter may be utilized by a producer for determining appropriate time to harvest the forage. Johnson et al. (2003) harvested corn silage at hard dough, 1/3 milk line, and 2/3 milk line. In these experiments, dry matter increased with maturity and a lower pH was observed following ensiling with less mature forage or forage with lower dry matter. Additionally, with increasing dry matter, the authors observed a decrease in water soluble carbohydrate content. Although, starch concentrations increases with corn maturity, with reaching maximum content at full milk line (Andrae et al., 2001).

Water soluble carbohydrates

Soluble carbohydrates in forages include glucose, disaccharides, oligosaccharides, and fructans (McDonald et al., 1991). During the aerobic and fermentation phase, these sugars are utilized by plant enzymes or microorganisms. Water soluble carbohydrates are the primary source of substrate utilized by lactic acid-producing bacteria. Therefore, reducing losses in water soluble carbohydrates during the aerobic phase provides more substrate utilization by bacteria during the fermentation phase (Bolsen et al., 1996). Active fermentation occurs during 7 to 14 days unless a low pH of the silage inhibits fermentation or water soluble carbohydrates are depleted (Bolsen et al., 1996). Johnson et al. (2003) measured water-soluble carbohydrates in 3 stages of corn plant maturity and initial concentrations were greatest for corn plants in the dough stage. Additionally, they continued to measure concentrations every 10 day throughout 60 days

of ensiling. Water soluble carbohydrates of the fresh forage ranged from 4 to 8% of the dry matter and by day 10 reached 1% of dry matter. This coincides with the review by Bolsen et al. (1996) that stated active fermentation is within 7 to 14 d or until the water soluble carbohydrates are depleted.

Buffering capacity

Ensiling is dependent on lowering the pH to a point that limits microbial activity. The forage's ability to buffer the pH change from lactic acid produced prolongs the fermentation process. Buffering capacity is a greater concern in grass silages than in corn silage (Bolsen et al., 1996). One common incident that can increase buffering capacity of silage is the addition of soil. This is due to cutting height during harvest. This can reduce the acidification of the silage and aerobic microorganisms will remain active until the pH is reduced to approximately 4.00 (Dunière et al., 2013).

Corn silage in beef cattle finishing diets

The addition of roughage to finishing diets has been shown to reduce digestive disturbances. Typically, recommended finishing diets contain 6 to 12% roughage (Samuelson et al., 2016). A review of roughage level in finishing diets concluded that roughage level accounts for approximately 70% of the variation in dry matter intake. Additionally, finishing diets containing lower roughage levels generally have greater NEg and thus improved performance (Galyean and Defoor, 2003).

An experiment conducted by Mader et al. (1987) compared roughage sources in dry-rolled and high-moisture corn diets. In this experiment, diets containing corn silage formulated at 11.5% (DM basis) of the diet were compared to diets containing alfalfa hay at 9.2% (DM basis) of the diet. Net energy for gain was calculated using NRC tabular values and did not differ

between diets. However, steers were more efficient with diets containing corn silage compared to alfalfa hay throughout the first 56 days, but final body weights did not differ. This would suggest corn silage has greater energy value compared to alfalfa. A similar study conducted by Uwituze et al. (2010) compared corn silage and alfalfa hay as a roughage source in diets with or without dried distillers grains. In this experiment, diets were formulated to compare 11% corn silage inclusion versus 6% alfalfa inclusion. The authors justified this due to the corn silage containing approximately 40% grain and thus 60% roughage. Heifers fed corn silage had greater dry matter intake, but average daily gain and efficiency did not differ. The authors attributed this to inaccurately characterizing the corn silage prior to the initiation of the experiment due to underestimating roughage level of the corn silage. It would seem accounting for grain level in corn silage would be appropriate when formulating finishing diets to maximize energy intake and to reduce incidence of digestive disturbances.

Hamilton et al. (2019) conducted a study comparing corn silage inclusion levels of 0, 7.5, and 15% versus 7.5% alfalfa. Decreasing corn silage inclusion from 15% to 7.5% numerically improved average daily gain and feed efficiency, but differences among treatments were not significant. From this study, it appears that considerations for roughage or grain level in silage may be futile. Although, the treatment containing no roughage did not differ from diets containing roughage as either corn silage or alfalfa. This may be a consequence of the experimental procedure and not adequately predict outcomes in industry. A digestibility study was conducted utilizing the same treatments, and recorded higher average ruminal pH in steers consuming the 15% corn silage inclusion level compared to steers consuming the 7.5% inclusion. This would have been expected due to roughage would have been replaced with concentrate, thus reducing ruminal pH.

A corn silage containing approximately 35% starch, would have a grain content of approximately 50% or would be comprised of 50% roughage. As previously discussed, analyzing starch content of silage can be useful in determine roughage content of the forage. This can be utilized when formulating diets to achieve a predetermined roughage level. In the previous studies discussed, digestibility of the starch in the silage was not discussed. Digestibility of corn content of the silage can be improved using kernel processors. Andrae et al. (2001) compared starch disappearance in processed corn silage and unprocessed silage. The authors observed increased *in situ* starch disappearance after 24 and 48 h of ruminal incubation for processed silage, but observed a decrease in fiber disappearance due to processing.

Corn silage may be utilized in finishing diets as a roughage source and adequately characterizing the starch content of the silage is critical to determining the usage of corn silage as roughage in the diet. Kernel processing may negatively affect fiber digestion, but roughage in feedlot diets is utilized to stimulate rumination, and improving fiber digestion offers little benefit.

Summary

Corn may be utilized as grain or fed as ensiled chopped whole corn plant in finishing diets. Processing method of grain can influence digestibility and subsequently, affect feedlot performance. Steam flaking appears to have the greatest benefit in feedlots diets compared to other processing methods due to its ability to maximize starch utilization. Considerations need to be taken in determining grain processing method and controls to increase repeatability of the process to limit digestive disturbances. Utilization of corn silage requires adequate characterization of the silage to limit digestive issues and improve efficiency in finishing diets. Producing quality corn silage is dependent on harvesting forages at the appropriate dry matter,

limiting exposure to air, and rapidly reducing pH to limit dry matter losses. Overall, it is apparent that maximizing intake of NEg improves feedlot performance and profitability.

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Chapter 2 - The effect of a high-amylase corn hybrid on fermentation characteristics and aerobic stability of corn silage

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Abstract

Amylase is an enzyme involved in the hydrolysis of starch. Enogen, which is a high-amylase corn hybrid (EC; Syngenta Seeds, LLC) was utilized to conduct two experiments to evaluate EC as corn silage. Experiment 1 utilized a 2 × 5 factorial treatment arrangement with EC and a corn hybrid commonly used for silage (CON), with ensiling periods of 30, 60, 90, 120, or 240 d. Both forage types were planted in adjacent fields and harvested on consecutive days. Whole corn plant material was chopped using a commercial harvester to a length of 1.5 cm and with a kernel processor set to 2 mm. The fresh chopped whole corn plant was packed into experimental silos, each containing approximately 40 kg of fresh plant material, with each forage type and day of ensiling having 4 replicates. Fresh and ensiled forages were characterized with respect to dry matter (DM), starch, and fiber. Forage samples were then compressed, and exudate was collected for determining concentrations of alcohols, lactate, and volatile fatty acids. Additionally, a subsample of ensiled forage was removed from each silo and placed, without packing, into an insulated container designed to allow for entry of ambient air. A recording thermocouple probe was inserted into the center of the silage mass and changes in temperature were recorded hourly over a period of 14 d. Forage type influenced acetate concentrations, with EC producing greater concentrations than CON (144 vs. 128 mM, $P<0.01$). Aerobic stability was characterized by time to reach 2ºC difference from ambient. Forage type by hour interactions were observed, with EC remaining within 2ºC from ambient for a longer duration than CON ($P<0.01$). Experiment 2 consisted of approximately 0.5 kg of each EC or CON fresh forage material sealed in vacuum bags equipped with check valves and ensiled for 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 27, or 30 d, with four replicates per ensiling period. At the end of each ensiling period, bags were opened, samples were pressed to obtain a liquid exudate, pH was measured, and exudate was

characterized as in experiment 1. An effect of forage type was observed, with lactate concentrations produced from EC tending to be greater than CON (332 vs. 292 mM, $P<0.10$). Silage produced from EC produces greater concentration of acetate and thus decreases DM loss due to improved aerobic stability.

Keywords: Amylase, Corn silage, Enogen Corn

Abbreviations: Enogen Corn, EC; control hybrid, CON; Kansas State University Beef Cattle Research Center, BCRC; water soluble carbohydrate, WSC; volatile fatty acids, VFA; dry matter, DM; gas chromatography, GC; acid detergent fiber, ADF; alpha-amylase neutral detergent fiber, aNDF;

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Introduction

The practice of ensiling chopped whole corn plant is commonly performed on beef and dairy operations as a storage method for high-moisture forages. Several studies have been conducted with fibrolytic and/or proteolytic enzyme addition to forages prior to ensiling to improve fermentation and nutritional composition of forages (Muck et al., 2018). Starch degrading enzymes also have been evaluated as additives for production of high-moisture ensiled grains, yielding varied results (Kung et al., 2007; Oliveira et al., 2019). During the ensiling process anaerobic bacteria utilize sugars, mainly water-soluble carbohydrates, to produce lactate and volatile fatty acids that preserve the forage (Bolsen et al., 1996).

A high-amylase corn hybrid (Enogen Corn; Syngenta Seeds, LLC), was initially developed for use in the fuel ethanol industry. Horton et al. (2020) utilized *in vitro* analysis to measure fermentation characteristics of steam-flaked EC and determined increasing proportions of EC alters ruminal fermentation and *in vitro* gas production. Furthermore, a feeding study

conducted utilizing corn silage produced from EC improved feed efficiency in growing steers (Johnson et al., 2019).

Enogen corn alters ruminal fermentation and may also alter fermentation during ensiling by increasing the availability of fermentable substrates compared to forages typically used for silage. Therefore, our objective was to evaluate fermentation characteristics during ensiling and aerobic stability of corn silage produced from EC compared to a corn hybrid commonly utilized for corn silage production. This was achieved by conducting two studies: the first of which evaluated fermentation characteristics and aerobic stability of silage from EC over 240 d, and a second evaluated compositional changes during fermentation of EC during the first 30 d of ensiling.

Materials and methods

Experiment 1

A 2 × 5 factorial experiment was conducted in a randomized complete block design to compare silages produced from EC and a commercial corn hybrid commonly used for silage production. The two corn varieties were planted in adjacent fields near the Beef Cattle Research Center at Kansas State University to minimize variability in growing conditions. Forages were sampled after harvesting and after 30, 60, 90, 120 and 240 d of ensiling. Experimental silos were utilized for storage (57-L polyurethane containers; Grief Inc., Delaware, OH) and each ensiling length was replicated in quadruplicate. Enogen Corn and CON forages were harvested on consecutive days utilizing a commercial harvester (John Deere, Model 7980; Moline, IL) with chop length set to 1.59 cm and incorporating a kernel processor set to 2 mm opening.

Chopped whole corn plant material was transported to the BCRC and weighed in 40.8-kg aliquots and pressed into experimental silos to achieve a wet density of 0.72 kg/L. Experimental

silos were sealed with a lid equipped with a Bunsen valve, allowing gas produced from fermentation to escape from the silo without entry of ambient air. Additionally, experimental silos selected for the 240 d of ensiling treatments were sealed with a thermocouple probe (Model PT-960; Pace Scientific, Inc., Mooresville, NC) placed in the geometric center of the silage mass. Ambient air temperature was monitored by placing a thermocouple probe in an empty container. Silos were stored in a temperature-controlled room at approximately 21ºC. Respiration losses were quantified by weighing silos after d 10, 20, 30, 60, 90, 120, and 240 of ensiling.

Samples of freshly chopped forage were taken immediately after harvest for compositional analyses. On scheduled sampling days, silos were emptied and the contents mixed for uniform sampling. Samples were collected immediately after opening and placed into sterile sample bags (B01063; Whirl-Pak, Madison, WI) for mold and yeast counts. Samples were stored at 4ºC and shipped to Rock River Labs (Watertown, WI) for analyses. Approximately 500 g were retained for determination of pH and starch content, WSC, DM, VFA, lactate, and alcohol concentrations at the Pre-Harvest Food Lab at Kansas State University.

A 4.5 kg sub-sample from each silo was loosely placed into an insulated 18-L polyethylene container with an insulated liner for aerobic stability testing. The containers were constructed to include an opening in the lid and bottom of the container, allowing air to circulate through the silage mass. Temperature probes (Model PT-960) were placed in the geometric center of the silage, and temperature was recorded at hourly intervals for 14 d. Additionally, a temperature probe was placed into an identical, but empty container to record ambient temperatures. Containers were weighed at the start of the aerobic stability test and on d 7 and 14 . After monitoring temperature for 14 d, forages were removed from each container, mixed, and a

representative sample was collected for further analysis. Length of aerobic stability was determined as time to reach 2ºC difference from ambient temperature.

Analytical procedures

Freshly harvested and ensiled samples were sent to Rock River Labs (Watertown, WI) for analyses of water-soluble carbohydrates by near infrared spectroscopy. Furthermore, samples of fresh and ensiled forages were lyophilized (Genesis Model 35EL; SP Scientific, Warminster, PA) to determine DM content, and then air equilibrated prior to being ground in a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ) equipped with a 1-mm screen. Approximately 1 g of ground sample was dried (in duplicate) in 105ºC forced air oven for 24 h to determine laboratory DM and then placed into a 450ºC muffle oven for 8 h to determine ash content. Ground samples were utilized to determine aNDF and sequential ADF using the Ankom filter bag technique (Ankom Technologies, Macedon, NY). Additionally, lyophilized samples were also utilized for determining amylase activity and starch content.

Amylase activity was determined for the initial fresh forage using a kit (K-Cera; Megazyme Ireland International, Ltd., Bray, Ireland) with modifications to the recommended procedures. Samples were incubated in the supplied extraction buffer at 85ºC for 30 min to extract amylase enzyme from the forage samples. The samples were then filtered through glass filter paper (Whatman GF/A; Cytiva, Marlbrorough, MA) and filtrate was transferred into glass test tubes as per the instructions and incubated with the amylase HR reagent provided in the kit, for 10 min in a 55ºC water bath. The stopping reagent was added to terminate the reaction and a 250-µL aliquot was transferred into a 96-well plate to read absorbance at 400 nm. The Mega-Calc software (Megazyme) provided with the kit was utilized to determine amylase activity from absorbance values.

Starch content was determined as described by Richard et al. (1995) and Saldana et al. (1989). Samples were weighed in duplicate and into vials containing 25 mL of a 0.1M acetate buffer. Heat stable alpha-amylase (FAA α-amylase enzyme; Ankom Technologies, Macedon, NY) was added (50 µL) to each vial and incubated in a 95ºC water bath for 30 min. Vials were allowed to cool prior to adding 100 µL of glucoamylase solution (Validase GA; Valley Research, South Bend, IN) and incubated in a 60ºC water bath overnight. Hydrolyzed samples were then centrifuged at 1000 × *g* for 10 min. Supernatant was collected and analyzed for glucose concentrations by a colorimetric assay kit (AutoKit Glucose; ThermoFisher Scientific, Rockford, IL) in a 96-well plate. Absorbances were read at 505 nm and compared to standards prepared from the glucose kit to determine glucose concentration of samples. Starch concentration was calculated from determined glucose concentrations divided by the DM weight of sample.

Analyses of fermentation products

Portions of samples retained from fresh forages, experimental silos, and aerobic stability containers were compressed using a manual arbor press to collect liquid exudate. The pH of liquid extracts was measured using a portable pH meter (Thermo Scientific Orion 3-star portable pH meter, Waltham, MA) and transferred to two containers. One container held a 25% m-phosphoric acid solution for analyses of volatile fatty acids and one was an unacidified container for analyses of alcohols. Acidified were frozen for determination of VFA and lactate. The unacidified samples were frozen for determination of alcohol concentrations. Acidified samples were thawed, vortexed, and centrifuged. Supernatant was transferred into GC vials for analyses by gas chromatography using a flame ionization detector (Agilent Technologies, Santa Clara, CA). The chromatograph was equipped with a Nukol capillary column (15 m length × 0.35 mm diameter × 0.50 µm film thickness; Supelco, Bellefonte, PA). Hydrogen was used as carrier gas

with a flow rate of 3.4 mL/min and utilizing a 10:1 split ratio with injection size of 1 µL. Inlet and detector temperatures were maintained at 300ºC and oven temperature started at 80ºC and increased to a final temperature of 200ºC at a rate of 10ºC/min. Samples were compared to known standards (Volatile Fatty Acid Mix; Sigma-Aldrich, St. Louis, MO) for identification and quantification of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate, isocaproate, and heptanoate.

Lactate concentrations were quantified using a GC as previously described for VFA analysis. Gas chromatography method differed by increasing hydrogen flow rate to 4.7 mL/min and using a split ratio of 80:1. Samples for lactate analysis utilized supernatant from samples containing 25%-metaphosphoric acid, and were derivatized by adding 0.4 mL of 50% sulfuric acid and 2 mL methanol, and heated in a 55ºC water bath for 30 min. After incubating, 1 mL of chloroform and 2 mL deionized water were added into each vial. Samples were homogenized using a vortex mixer, then centrifuged for 5 min at 500 × *g*. The chloroform layer was removed and transferred into GC vials for analysis. Samples were compared to prepared standards (Lithium L-lactate; Sigma-Aldrich, St. Louis, MO).

Alcohols were determined using non-acidified samples. Samples were centrifuged at 15,000 × *g* for 20 min and supernatant was transferred to GC vials for analysis of alcohols: ethanol, 1-propanol, 1,2-propanediol, and 2,3 butanediol. Determination of alcohol concentration utilized a GC equipped with a capillary column (Agilent DB-624 UI, 30 m length × 0.53 mm diameter × 3.0 µm film thickness) to compare against known individual standards purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen was used as a carrier gas at a flow rate of 4 mL/min with a 100:1 split. Inlet and detector temperatures were maintained at 250ºC and 300ºC,

respectively. Oven temperature started at 40ºC and increased to a final temperature of 260ºC at a rate of 10ºC/min and final temperature was held for 3 min.

Experiment 2

A 2 × 13 factorial experiment was conducted to evaluate compositional changes in forages through the first 30 d of ensiling. Whole chopped corn plant material was received from the study sponsor to the BCRC and 0.5 kg of either EC or CON forages were sealed into vacuum seal foil bags (Uline, S-18228SIL, Pleasant Prairie, WI). The vacuum bags were constructed with a one-way valve to allow gas from fermentation to discharge. Samples were stored in insulated containers for periods of 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 27, and 30 d in quadruplicate.

Dry matter, lactate and VFA concentrations were evaluated for fermentation characteristics in this experiment. Analyses of VFAs and lactate concentrations were as described in experiment 1. Initial samples were taken to characterize the fresh forage. Dry matter was measured by placing samples in a 105ºC forced air oven for 24 h.

Statistical analyses

The MIXED procedure of SAS (Version 9.4; SAS Inst., Cary, NC) was used to analyze data from both experiments. Volatile fatty acids, silage composition, alcohols, and lactate were analyzed using forage type, days of ensiling, and the interaction of forage type and days of ensiling as effects in the model. Linear and quadratic contrast statements for days of ensiling and the interaction between main effects were included in the analyses, and replicate was included in the models as a random effect. The PDIFF function was utilized to determine differences in means. For analysis of ensiling and aerobic stability temperatures, the main effects of forage type and time were utilized in the model. Compound symmetry covariance structure was utilized for analyses, with the repeated measure defined as hour and subject as experimental silo. The slice

function was used to determine differences between forage type by hour. Significance was determined with a *P*-value less than 0.05 and a tendency with less than 0.10.

Results

Experiment 1

Fresh forages were analyzed for pH, WSC, ADF, aNDF, DM, ash and starch concentration as shown in Table 2.1. Dry matter content was greater ($P<0.01$) for forages produced from EC compared to CON. Similarly, starch content was 19.6% greater for EC forages compared to CON forages. Acid detergent fiber, aNDF, WSC, and pH did not differ between forage types ($P>0.05$). Amylase activity was greater for forages produced from EC compared to CON (1.39 vs. 0.37 U/g of DM; $P<0.01$).

Compositions of ensiled forages are presented in Table 2.2. An interaction was observed between days of ensiling and forage type for WSC concentration. Control silage ensiled 30, 90, 120, and 240 d had greater WSC concentration than all days of ensiling EC ($P<0.05$). A linear and quadratic contrast was observed for days of ensiling for both forage types. The effect of forage type was observed with CON silage containing 15% greater WSC concentrations than EC silage ($P<0.01$). The effect of days of ensiling did not affect DM, ADF, aNDF, ash, and starch concentrations ($P>0.10$). Similar to the composition of the fresh forages, effect of forage type was observed for DM, ash content, and starch concentrations of ensiled forages ($P<0.01$). Ensiled EC forages had a greater DM content compared to CON silages (337.9 vs. 308.8 g/kg DM, respectively; $P<0.01$). Starch concentrations were also affected by forage type, which EC ensiled forages having 11% greater starch concentrations compared to CON (334.3 vs. 300.9 g/kg DM, respectively; $P<0.01$). Additionally, the effect of forage type was observed for ADF

and aNDF (P<0.05). Concentrations of ADF and aNDF were observed to be 7% and 4% greater for silages produced from CON compared to silage produced from EC (P<0.05), respectively.

Ensiling temperatures are presented in Figure 2.1. Respiration losses were quantified by weighing the experimental silos at the start of the experiment and at intervals of 10, 20, 30, 60, 90, 120, and 240 d of ensiling. Percentage differences from the initial starting weight of experimental silos are presented in Table 2.3. No interactions were observed between forage type and days of ensiling. There was a linear effect of days of ensiling as respiration losses increased with length of ensiling (P<0.05). Additionally, an effect of forage type was observed with silage produced from CON having greater DM loss compared to EC (0.61 vs. 0.51 % DM loss, respectively; P<0.01).

Volatile fatty acids, lactate, and alcohols concentrations produced during ensiling were determined and are presented in Table 4. An interaction between forage type and days of ensiling was observed for isocaproate concentrations and pH for both EC and CON silages, pH was below 4.00 after 30 d of ensiling and remained below 4.00 thereafter. Enogen corn ensiled for 240 d had the greatest concentration of isocaproate (P<0.01) compared to the other treatments. Duration of ensiling or forage type did not affect lactate concentrations (P>0.10). Acetate concentrations were affected by the main effects of forage type and days of ensiling. Silages produced from EC had a greater concentration of acetate than silages produced from CON (P<0.05). Forages ensiled for 30, 60, 90, and 120 d contained similar concentrations of acetate, but forages ensiled for 240 d produced the greatest acetate concentrations (P<0.05). No interactions were observed between days of ensiling and forage type for butyrate, isovalerate, valerate, caproate, and heptanoate (Table 2.4) and no propionate was detected. Enogen corn

tended to have greater proportions of butyrate (P=0.07); however, there was an effect of ensiling duration, with silage stored for 240 d containing the greatest concentration of butyrate (P<0.01).

No interactions of forage type and days of ensiling were observed for alcohol content of silages. Forage type affected the ethanol concentrations produced, with CON silages containing approximately 43% greater concentrations of ethanol than EC (238.3 vs. 166.3 mM, respectively; P<0.01). Similar to other variables analyzed, the effect of ensiling length was observed for ethanol concentration, with silage ensiled for 240 d having greater ethanol concentrations compared to the shorter ensiling durations (P<0.05). Quantities of ethanol were greater in comparison to the other alcohols, and 2,3-butanediol was the second most abundant alcohol quantified. An effect of days of ensiling was observed for 2,3-butanediol, day 60 and 240 had greater concentrations compared to 30, 90, and 120 d of ensiling (P<0.05). Concentrations of 1-propanol, 2-propanol, and 2-butanol were found in small quantities and there were no effects of forage type or days of ensiling on aforementioned alcohols except, an effect of forage type was determined with concentrations of propylene glycol tending to be greater for silage produced from EC than CON (P=0.06).

Aerobic stability was determined by the length of time required for silages to reach a temperature of 2ºC above ambient temperature. A forage type by days of ensiling interaction was observed for hours to reach the 2ºC threshold (P<0.01) and data are presented in Figure 2.2. The interaction of forage type × hour to reach the threshold is illustrated in Figure 2.3. Control silage exceeded the 2ºC threshold by 102 h, but only tended to differ from EC (P=0.08). After 109 h, CON silage temperature was significantly greater than that of EC (P<0.05). Enogen silage did not reach the 2ºC threshold until 127 h.

Both forage types underwent substantial changes with exposure to air. As with the ensiled forage samples, analyses were also performed on the liquid extract from forages after 14 d of exposure to air. Volatile fatty acids, lactate, and alcohol concentrations observed from the aerobic stability test are shown in Table 2.5. An interaction between forage type and days of ensiling was observed for heptanoate concentrations ($P<0.05$). Concentrations of heptanoate were greatest for both forage types ensiled for 240 d compared to the either forage type stored for shorter durations ($P<0.05$); however, EC ensiled for 240 d had the greatest concentration of heptanoate compared to CON ensiled for 240 d (1.6 vs. 0.9 mM, $P<0.05$). Lactate concentrations varied as presented in Table 2.5, an interaction between forage type and days of ensiling were observed for lactate content in the silages exposed to air ($P<0.05$). Additionally, acetate concentrations did not differ between forage type following exposure to air ($P>0.10$) as they previously did prior to the aerobic stability test.

Respiration losses during aerobic stability testing were quantified by weighing containers on d 0, 7, and 14. Percent difference from the initial weights to the d 7 and 14 weights are presented in Table 2.6. From d 0 to 7 there was an effect of forage type and a linear effect of ensiling duration. Respiration losses increased linearly with silages that were exposed to air for longer durations ($P<0.05$). Following 14 d of exposure to air an effect of forage type, day of ensiling, and an interaction was observed ($P<0.05$). EC silage ensiled for 90 d had less loss in DM compared to CON silage ($P<0.05$), but was similar to losses from EC silage ensiled for 60 or 90 d. There was a main effect of forage type, with EC having less respiration loss compared to CON following 14 d of exposure to air (3.23 vs. 3.75%, $P<0.05$).

Experiment 2

Silages produced from EC had greater DM than CON ($P<0.01$) as illustrated in Table 2.7. A linear and quadratic interaction was observed between forage type and days of ensiling for pH ($P<0.01$, Figure 2.4.). Silage from EC had a greater starting pH than CON, but decreased to a pH below the 4.00 threshold by d 6 compared to CON silage which reached the threshold by d 10 ($P<0.05$). Lactate concentrations tended to differ with interaction of forage type by day ($P=0.08$) as presented in Figure 2.5. An effect of forage type was observed with EC silage having greater lactate concentrations than CON silage (332.0 vs. 291.7 mM, $P<0.01$). A linear and quadratic effect of days of ensiling was observed with lactate concentrations increasing with days of ensiling ($P<0.01$). A linear interaction of forage type by days of ensiling was observed with acetate concentration as illustrated in Figure 2.6 ($P<0.01$). Acetate concentrations did not differ between forage types ($P=0.66$). Acetate concentrations were similar between forage types for d 0 ($P>0.05$), but were greater for EC on d 21, 24, and 30 ($P<0.05$). An interaction between forage type and days of ensiling was observed for valerate, caproate, and heptanoate concentrations ($P<0.05$) as shown in Table 2.7. Butyrate concentrations were greater for silages produced from EC compared to silage produced from CON (3.68 vs. 2.33 mM, $P<0.01$).

Discussion

Dry matter and WSC content are essential to ensiling forages (Bolsen et al., 1996). In experiment 1, initial forage composition between forage types differed in DM, ash, and starch concentrations. Forages produced from EC in experiment 1, yielded a DM approximately 9% greater than CON. Differences in DM may have impacted the dry pack density for CON, as it would have been approximately 221 kg/m^3 and EC silage would have been approximately 242 kg/m^3. Control silage dry density was slightly lower than the minimum of 224kg/m^3

recommended by Muck et al. (1999). This could have potential implications with experimental silos achieving anaerobic conditions. Although in a well-sealed silo, oxygen levels rapidly decrease due to residual plant respiration during the aerobic phase of ensiling (Sprague, 1974). This likely was not an issue in experiment 1, due to no differences observed with mold or yeast counts between forage types and days of ensiling. Similarly, DM was affected by forage type in experiment 2, with silage produced from EC having a greater DM compared to CON. Dry matter content from forages in experiment 2 were greater than experiment 1; however, both experiments are in the 55 to 75% moisture range recommended by Bolsen et al. (1996) for adequate ensiling.

Achieving anaerobic conditions is the first step in ensiling and thus allows LAB to ferment WSC into lactic acid, which reduces the pH of the forage (McDonald et al., 1991). In industry, it is common to apply inoculants containing LAB to forages prior to ensiling but, in both experiments, forages were not inoculated prior to ensiling. However, LAB are commonly found as part of the epiphytic bacteria on whole corn plant (Stirling and Whittenbury, 1963). Due to both forage types being planted in adjacent fields, it was assumed epiphytic bacteria populations would be similar.

The production and accumulation of lactate reduces the pH, which in turn inhibits fermentation of LAB due to pH being below 4.00, thus preserving the forage (Bolsen et al., 1996). It was hypothesized that EC may alter the sugars utilized by bacteria in the forages. Modeling of amylase addition to corn silage predicted a lower pH when WSC are limited (Pitt, 1990). Water soluble carbohydrate concentrations in the initial fresh forages from experiment 1 did not differ between forage types and thus likely provide similar substrate quantities. This can be observed by both forage types reaching a pH below 4.00 by d 30 of ensiling in experiment 1 and both contained similar lactate concentrations throughout 240 d of storage. Water soluble

carbohydrate concentrations were not measured in experiment 2; however, there was an effect of forage type, with EC silage lactate concentrations being greater than CON silages, although concentrations did not differ from d 21 through 30. Furthermore, CON silage reached the previously defined pH threshold of 4.00 after 10 d days of ensiling compared to 6 d for EC silage. This suggests forages from EC ensile more rapidly compared to CON silage in these experiments.

Fermentation products produced during ensiling may indicate the microbial activity during ensiling. Homofermentative and heterofermentative LAB produce different fermentation products depending on substrate utilized and if oxygen is present. Homolactic LABs mainly produce lactic acid, while heterolactic LAB fermentation of sugars is a slower pathway and produces lactate, ethanol and acetate. (McDonald et al., 1991). Experiment 2 illustrates the different fermentation pathways utilized to ferment EC forage compared to CON forage. Acetate concentrations were initial greater for CON silage during the first 12 d of ensiling. Similar concentrations of lactate concentrations were observed in experiment 1, but in experiment 2, EC silage had greater lactate content at d 4 of ensiling compared to CON silage. Additionally, CON silage exhibited increased ethanol concentrations compared to EC, but lower acetate concentrations. This suggests EC forages promote homolactic LAB during the fermentation phase of ensiling. The increase in acetate concentrations and a tendency to increase propylene glycol concentrations for EC could be caused by the fermentation of lactate by *L. buchneri* as described by Elferink et al. (2001). Additionally, this is demonstrated with acetate concentrations increasing linearly with duration of ensiling. Similarly, Kleinschmit and Kung (2006) observed increased acetate concentrations with longer ensiling durations after inoculating with *L. buchneri*.

Hererolactic fermentation produces ethanol and acetate and reduces the yield of DM. In addition to these products, a carbon dioxide molecule is produced and it contributes to DM loss (McDonald et al., 1991; Rooke and Hatfield, 2003). Enogen corn silage in experiment 1, had 12% greater acetate and 41% lower ethanol concentrations than CON silage suggesting a different fermentation pathway. The increase in ethanol likely contributed to the increased respiration losses observed during ensiling CON. Similarly in experiment 2, acetate concentrations varied between days of ensiling and forage type, with CON silages having greater acetate concentrations than EC and inversely after 15 d of ensiling EC silages having greater acetate concentrations. This potentially suggests during the initial days of ensiling, EC silage promotes homolactic fermentation of sugars, which increases accumulation of lactate during the initial phase of ensiling.

Silage produced from EC remained below the 2ºC threshold for determining aerobic stability for a greater length of time compared to CON. A review by Woolford (1990) determined the greatest cause of aerobic deterioration in silage are yeasts. Yeast counts were similar during the entire ensiling periods for both forage types in experiment 1, however yeasts can remain dormant during storage until feed out (Woolford, 1990). Moon (1983) demonstrated that yeast growth may be inhibited by lactate in combination with acetate. Acetate was observed in liquid extract from both experiments, with greater concentrations of acetate from silages produced from EC. Ranjit and Kung (2000) observed silages inoculated with *L. buchn*eri produced greater acetate concentrations compared to the uninoculated silage and thus improved aerobic stability. Furthermore, when evaluating DM loss during exposure to air there was a forage type effect from the initial weight until d 7 with EC silage having lower DM loss.

Measuring products from aerobic deterioration only showed an effect of forage type in 2,3-butanediol and propylene glycol concentrations, this is possibly due to the 14-d experimental time. Both forage types had reached the 2ºC threshold by d 6. Any differences due to an effect of forage type was probably missed due to the extended aerobic deterioration. Many of the reported differences were an effect of ensiling duration and it is possible this an effect of the time of year. The containers used for aerobic stability testing were stored in a temperature-controlled room. It is feasible that ambient microflora changes depending on seasons. The 90- and 120-d silos were opened in the winter months. Silages produced from EC were more aerobically stable than CON silages in these experiments, in contrast to observations of DM loss and time to reach the threshold temperature of 2°C.

Conclusion

Silage produced from EC appears to ferment more rapidly than CON. Increased lactate accumulation during the initial fermentation phase lowered pH more rapidly in EC silage than CON. During the storage phase EC produces a greater amount of acetate and less ethanol than CON, suggesting a heterofermentative pathway not utilized in fermentation of CON forages. This allows for silage produced from EC to have improved aerobic stability during exposure to air, and limit the DM loss.

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Table 2.1. Composition of chopped whole corn plant material prior to ensiling in experiment 1.

	Forage type			
Item[a]	Control	Enogen	SEM	*P*-value
DM, g/kg	309.0	338.3	2.83	<0.01
ADF, g/kg of DM	215.8	204.3	6.28	0.27
aNDF, g/kg of DM	390.3	380.3	9.09	0.44
Ash, g/kg of DM	56.9	46.5	1.27	<0.01
Starch, g/kg of DM	302.3	361.8	10.89	<0.05
WSC, g/kg of DM	88.6	93.1	3.04	0.37
pH	5.68	5.66	0.312	0.53
Amylase activity, U/g of DM	0.37	1.39	0.048	<0.01

[a] DM= dry matter; ADF= acid detergent fiber; aNDF=alpha-amylase neutral detergent fiber; WSC=water soluble carbohydrates.

Table 2.2. Compositional changes to forages throughout 240 d of ensiling from a high-amylase corn hybrid in experiment 1.

	Control[a]					Enogen[a]						
Item[b]	30	60	90	120	240	30	60	90	120	240	SEM	*P*-value[c]
DM, g/kg	30.9	31.3	30.7	30.7	30.6	34.0	33.9	33.5	33.8	33.9	0.29	F
ADF, g/kg of DM	236.0	227.0	229.0	226.0	227.0	211.8	209.0	209.0	215.3	225.5	8.00	F
aNDF, g/kg of DM	398.3	387.5	383.0	378.3	383.8	370.5	364.8	360.0	372.5	382.5	13.19	F
Ash, g/kg of DM	58.0	54.7	57.4	57.3	59.2	49.5	48.5	49.0	51.3	47.0	1.79	F
Starch, g/kg of DM	294.5	323.3	295.5	303.0	288.0	308.5	357.5	336.8	325.3	343.5	16.06	F
WSC, g/kg of DM	44.3^{b,c}	42.6^{c,d}	46.0^{a,b}	48.4^{a}	47.5^{a}	40.0^{e}	33.7^{f}	42.2^{d,c,e}	41.8^{d,e}	41.1^{d,e}	0.83	F, LQ-D, I
Mold, log cfu/g of DM	3.18	3.33	3.50	3.68	3.30	2.70	3.03	3.18	3.33	3.03	0.443	NS
Yeast, log cfu/g of DM	3.09	3.07	2.70	3.75	2.70	3.28	3.28	3.22	3.20	2.70	0.414	NS

[a] Days of ensiling.

[b] DM = dry matter; ADF = acid detergent fiber; aNDF = alpha-amylase neutral detergent fiber; WSC = water soluble carbohydrates.

[c] F = Effect of forage type, $P<0.05$; D = Effect of days of ensiling, $P<0.05$; I = Effect of forage type by day interaction, $P<0.05$; L = Linear effect of days, $P<0.05$; Q = Quadratic effect of days, $P<0.05$; NS = Not significant, $P>0.05$.

[a, b, c, d, e, f, g] Means in the same row with different superscripts differ, P<0.05

Table 2.3. Cumulative dry matter losses during ensiling of a high-amylase corn hybrid expressed as difference from initial experiment silo weight throughout 240 d of ensiling in experiment 1.

	Forage type			
Item[a]	Control	Enogen	SEM	*P*-value[b]
10 d, %	0.45	0.37	0.021	F, L-D
20 d, %	0.51	0.42		
30 d, %	0.54	0.45		
60 d, %	0.62	0.52		
90 d, %	0.65	0.56		
120 d, %	0.69	0.59		
240 d, %	0.81	0.68		

[a] Days of ensiling

[b] F = Effect of forage type, $P<0.05$; D = Effect of days of ensiling, $P<0.05$; I = Effect of forage type by day interaction, $P<0.05$; L = Linear effect of days, $P<0.05$; Q = Quadratic effect of days, $P<0.05$; NS = Not significant, $P>0.05$.

Table 2.4. Effect of a high-amylase corn hybrid on fermentation products measured in liquid extract pressed[a] from ensiled forages in experiment 1.

	Control[b]					Enogen[b]						
Item	30	60	90	120	240	30	60	90	120	240	SEM	*P*-value[c]
pH	3.72[b,c,d]	3.69 [c,d,e]	3.66[e]	3.71[b,c,d]	3.74[a,b]	3.73[b,c]	3.68[d,e]	3.68[d,e]	3.58[f]	3.78[a]	0.014	LQ-D, Q-I
Volatile fatty acids, mM												
Acetate	107.49	128.99	111.72	111.50	180.60	116.56	120.48	127.59	137.97	217.68	9.374	F, LQ-D
Butyrate	0.41	0.37	0.47	0.25	1.94	0.44	0.47	0.46	0.84	2.06	0.150	LQ-D
Propionate	0.00	0.00	0.00	0.00	0.00	0.64	0.00	0.00	0.00	0.00	0.12	Q-D, Q-I
Isobutyrate	0.04	0.08	0.19	0.27	0.12	0.15	0.15	0.22	0.24	0.30	0.048	F, LQ-D
Isovalerate	0.03	0.09	0.10	0.22	0.47	0.07	0.00	0.00	0.15	0.47	0.046	L-D
Caproate	0.09	0.09	0.10	0.08	0.08	0.01	0.02	0.04	0.08	0.16	0.055	NS
Isocaproate	0.02 [c,d]	0.00[d]	0.05 [c,b,d]	0.16[c,b,d]	0.23[c,b]	0.07 [c,b,d]	0.10 [c,b,d]	0.07[c,b,d]	0.25[b]	0.77[a]	0.075	F, L-D, L-I
Heptanoate	0.08	0.08	0.08	0.15	0.21	0.10	0.10	0.10	0.09	0.23	0.033	L-D
Lactate, mM	415.2	394.9	432.7	446.5	415.0	434.1	442.7	409.4	440.7	443.1	16.22	NS
Alcohol, mM												
Ethanol	203.99	240.94	235.04	221.84	289.51	172.19	159.60	146.31	149.23	204.26	23.024	F, L-D
2,3-butanediol	6.63	8.91	7.91	6.22	12.90	6.27	15.40	9.93	8.40	18.25	2.716	L-D
Propylene glycol	0.50	0.33	0.82	0.00	0.30	1.91	12.59	2.04	1.35	2.73	2.954	NS
2-butandiol	0.00	0.00	0.00	0.00	0.41	0.00	0.17	0.00	0.00	0.00	1.117	NS
1-propanol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.026	NS

[a] Samples (8 samples per ensiling period) were collected by pressing samples using a press and collecting the liquid extract for analysis.
[b] Days of ensiling.
[c] F = Effect of forage type, $P<0.05$; D = Effect of days of ensiling, $P<0.05$; I = Effect of forage by day interaction, $P<0.05$; L = Linear effect of days, $P<0.05$; Q = Quadratic effect of days, $P<0.05$; NS = Not significant, $P>0.05$.
[a, b, c, d, e,f, g] Means in a row without a common differ, $P<0.05$.

Table 2.5. Effect of aerobic deterioration on fermentation products analyzed from liquid extracted[a] from silages exposed to air for 14 d in experiment 1.

	Control[b]					Enogen[b]						
Item	30	60	90	120	240	30	60	90	120	240	SEM	*P*-value[c]
pH	6.13^{b,c}	6.53^{a,b,c}	5.79^{c,d}	6.98^{a}	6.34^{a,b,c}	6.88^{a,b}	5.25^{d}	6.52^{a,b,c}	7.05^{a}	6.64^{a,b,c}	0.293	D, I
Volatile Fatty Acids, mM												
Acetate	16.4	11.7	23.4	20.4	19.8	18.0	20.7	13.7	12.3	33.2	5.01	NS
Butyrate	11.1	5.5	13.9	4.6	10.0	10.3	10.2	4.2	3.1	9.2	2.23	Q-D
Isobutyrate	0.6	0.4	0.3	0.2	0.2	0.8	0.6	0.1	0.0	0.1	0.77	LQ-D
Isovalerate	0.7	0.3	0.2	0.1	0.2	0.9	0.7	0.0	0.0	0.0	0.12	LQ-D
Isocaproate	0.2^{a}	0.0^{b,c}	0.1^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.2^{a}	0.1^{b}	0.0^{c}	0.0^{c}	0.02	L-D, Q-I
Caproate	0.1	0.1	0.00	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.02	LQ-D
Heptanoate	0.0^{c}	0.1^{c}	0.2^{c}	0.2^{c}	0.9^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.3^{c}	1.6^{a}	0.12	LQ-D, L-I
Lactate, mM	50.9^{b,c,d}	38.7^{b,c,d}	64.1^{b,c}	17.4^{d}	26.4^{c,d}	28.2^{b,c,d}	122.9^{a}	67.3^{b}	44.6^{b,c,d}	20.6^{d}	13.08	L-D, I
Alcohols, mM												
2-Propanol	8.5^{b,c,d,e}	1.9^{e}	20.1^{a}	3.3^{d,e}	16.4^{a,b,c}	9.7^{a,b,c,d,e}	13.3^{a,b,c,d}	6.0^{c,d,e}	7.0^{c,d,e}	19.4^{a,b}	3.85	L-D, I
Ethanol	7.7	3.4	25.2	26.9	22.1	6.5	16.3	15.0	5.0	11.5	6.48	NS
1-Propanol	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.06	S
2-Butandiol	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.03	NS
Propylene glycol	2.3	4.2	2.3	0.9	0.0	0.4	1.4	0.1	0.1	0.0	0.79	F, L-D
2,3-butanediol	120.1	82.1	137.8	101.2	130.3	123.6	80.7	30.9	30.7	79.8	30.92	F

[a] Samples (8 samples per ensiling period) were collected by pressing samples using a press and collecting the liquid extract for analysis.
[b]Days of ensiling
[c] F = Effect of forage type, $P<0.05$; D = Effect of days of ensiling, $P<0.05$; I = Effect of forage type by day interaction, $P<0.05$; L = Linear effect, $P<0.05$; Q = Quadratic effect, $P<0.05$; NS = Not significant, $P>0.05$.
[a-e] Means in a row without a common superscript differ, $P<0.05$

Table 2.6. The effect of a high-amylase corn hybrid on dry matter loss during exposure to aerobic conditions in experiment 1.

	Control[a]					Enogen[a]						
Item, %	30	60	90	120	240	30	60	90	120	240	SEM	*P*-value[b]
Initial to d 7	0.42	0.86	0.38	1.18	0.71	0.30	0.12	0.08	0.73	0.67	0.203	F, L-D
Initial to d 14	4.30^{a,b}	3.51^{a,b,c,d}	3.25^{b,c,d}	4.09^{a,b}	3.61^{a,b,c}	4.55^{a}	2.47^{d,e}	2.13^{e}	2.65^{d,c,e}	4.42^{a}	0.380	F, LQ-D, I
d 7 to d 14	3.89	2.68	2.88	2.95	2.92	4.68	2.58	2.21	1.94	3.77	0.377	Q-D

[a] Days of ensiling

[b] F = Effect of forage type, $P<0.05$; D = Effect of days of ensiling, $P<0.05$; I = Effect of forage type by day interaction, $P<0.05$; L = Linear effect of days, $P<0.05$; Q = Quadratic effect of days, $P<0.05$; NS = Not significant, $P>0.05$.

[a-e] Means in a row without a common superscript differ, $P<0.05$

Table 2.7. Dry matter and volatile fatty acid concentrations from a high-amylase hybrid during the first 30 d of ensiling in experiment 2.

	Days of ensiling														
Item[b]	0	2	4	6	8	10	12	15	18	21	24	27	30	SEM	*P*-value[a]
Dry matter, g/kg															
Control	454.0	443.2	438.6	433.3	440.4	442.0	436.3	421.5	449.9	433.5	423.9	454.2	428.2	10.79	F, L-D
Enogen	492.4	495.8	492.6	460.3	468.5	482.3	501.8	457.8	464.6	469.9	480.6	475.8	453.2		
Butyrate, mM															
Control	2.76	2.31	2.61	2.58	3.27	2.44	1.63	1.68	2.47	2.37	1.75	2.01	2.39	0.549	F, D
Enogen	3.55	4.89	3.81	3.63	4.86	3.91	2.22	1.79	4.12	4.77	3.65	3.38	3.26		
Isobutyrate, mM															
Control	1.68	1.60	1.52	0.94	1.18	0.66	0.22	0.17	0.20	1.15	0.00	0.40	0.58	0.266	LQ-D
Enogen	2.19	1.31	0.97	0.96	0.72	0.50	0.00	0.00	0.59	0.69	0.33	0.31	0.30		
Valerate, mM															
Control	4.05	3.13	0.28	0.44	0.40	0.43	0.27	0.27	0.52	1.08	0.86	0.35	0.26	0.293	LQ-D, L-I
Enogen	3.75	0.79	0.55	0.56	0.68	0.88	0.89	0.63	1.31	0.39	0.37	0.58	1.11		
Isovalerate, mM															
Control	0.57	0.11	0.54	0.27	0.16	0.24	0.37	0.60	0.51	1.02	0.48	0.76	0.63	0.134	F, L-D
Enogen	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.30	0.35	0.27	0.36	0.59		
Caproate, mM															
Control	0.61	4.31	2.34	1.40	0.86	0.75	0.23	0.29	0.28	0.26	0.09	1.46	2.05	0.676	LQ-D, I
Enogen	3.64	0.63	1.56	1.17	0.61	0.77	0.40	0.29	1.06	1.02	0.42	0.55	0.45		
Isocaproate, mM															
Control	0.59	0.26	0.00	0.00	0.16	0.15	0.21	0.05	0.12	0.09	0.10	0.35	0.32	0.125	NS
Enogen	0.23	0.00	0.00	0.05	0.13	0.09	0.00	0.05	0.32	0.06	0.00	0.27	0.00		
Heptanoate, mM															
Control	0.67	0.43	0.48	0.19	0.19	0.37	0.26	0.23	0.36	0.48	0.09	0.26	0.38	0.165	LQ-D, LQ-I
Enogen	2.43	1.12	0.23	0.21	0.23	0.21	0.10	0.04	0.13	0.38	0.38	0.27	0.36		

[a] F = Effect of forage type, $P<0.05$; D = Effect of days of ensiling, $P<0.05$; I = Effect of forage type by day interaction, $P<0.05$; L = Linear effect of days, $P<0.05$; Q = Quadratic effect of days, $P<0.05$; NS = Not significant, $P>0.05$.

[b] Forage type.

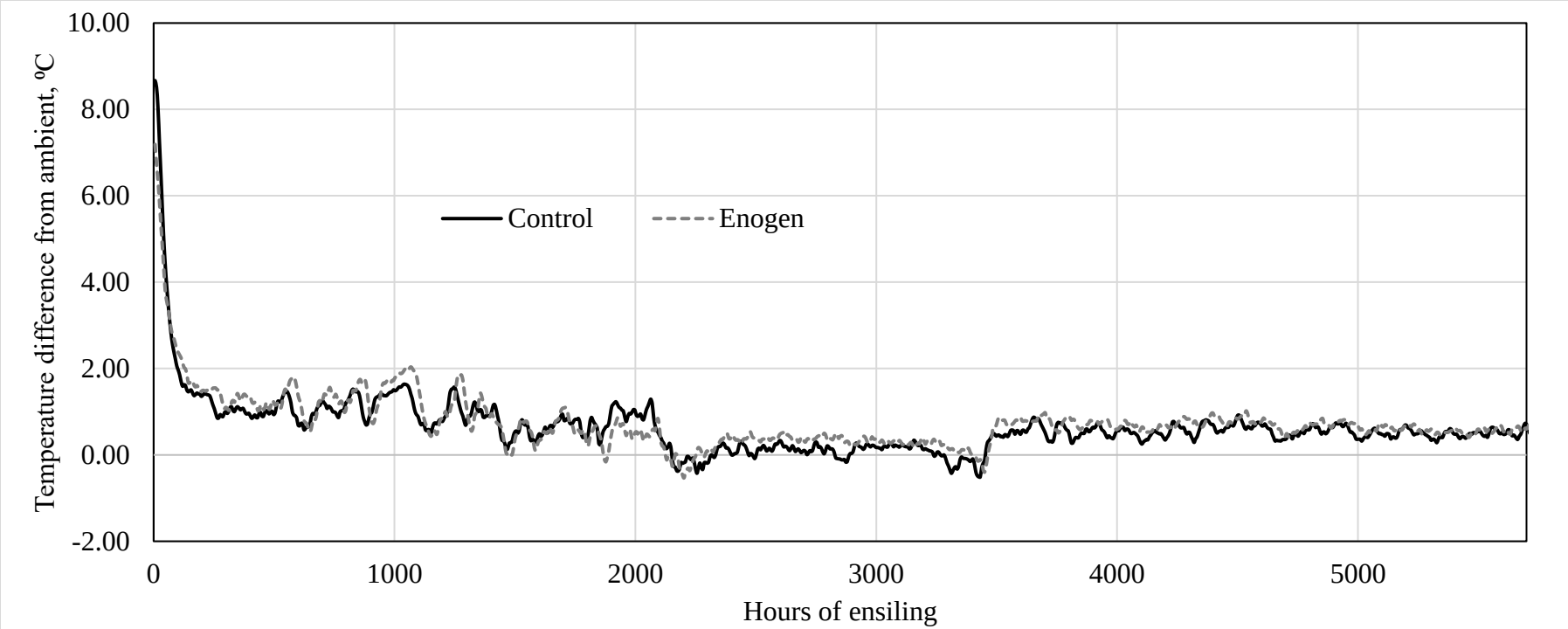

Figure 2.1. The effect of a high-amylase corn on temperatures in experimental silos throughout 240 d of ensiling in experiment 1.

Temperature recording probes were placed in the geometric center of experimental silos (4 silos per treatment) and a temperature probe was placed in an empty silo to record ambient temperature. Effect of forage type, hour, and forage type by hour; $P<0.01$; SEM=0.365.

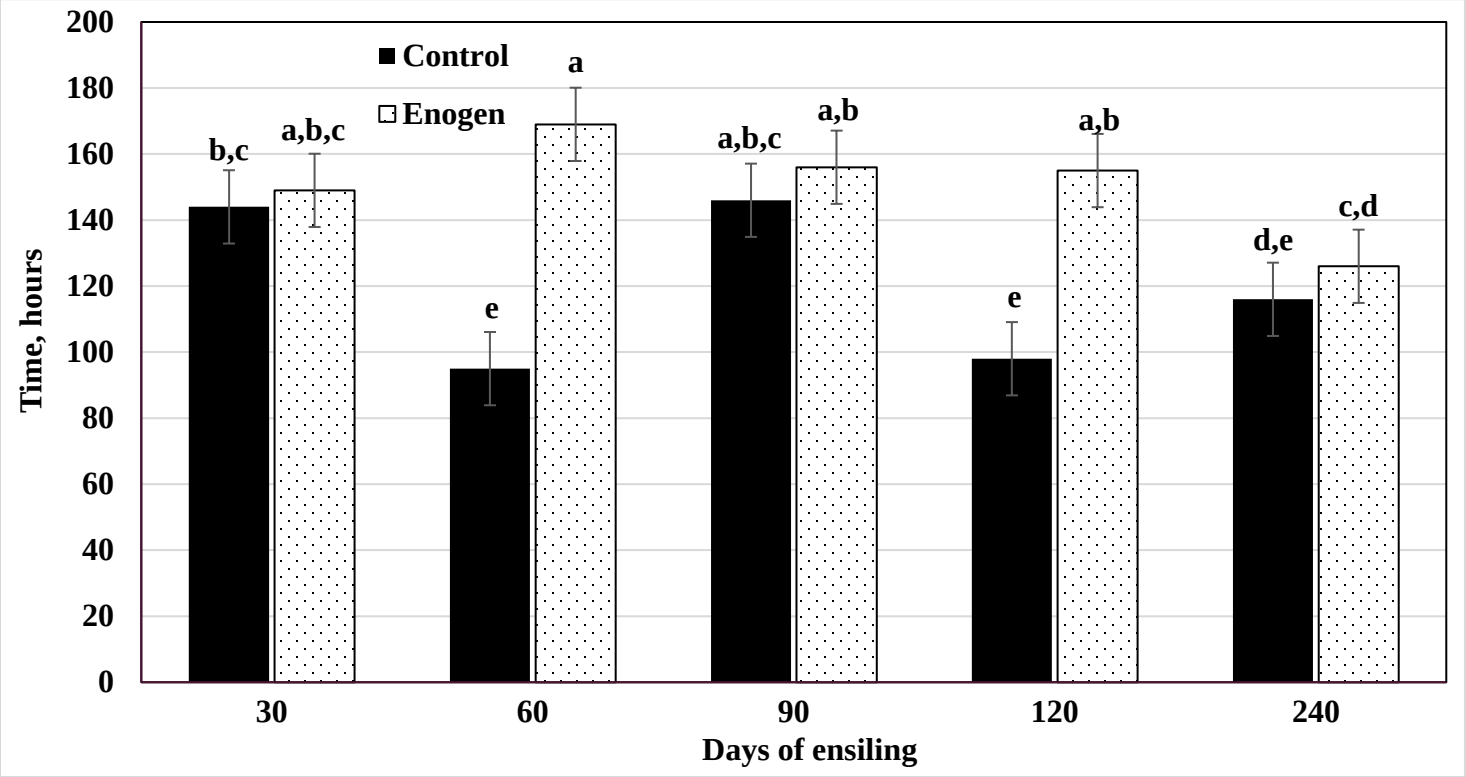

Figure 2.2. Effect of a high-amylase corn hybrid on silage temperatures following exposure to air in experiment 1.

Bars represent time in hours to reach 2ºC difference from ambient temperature threshold (4 experimental silos per treatment for each ensiling period). Error bars represent standard error of means. Effect of forage type, day and forage by day, $P<0.01$. Linear effect of day, $P<0.01$. ^{a,b,c,d,e} Bars without a common superscript differ, $P<0.05$.

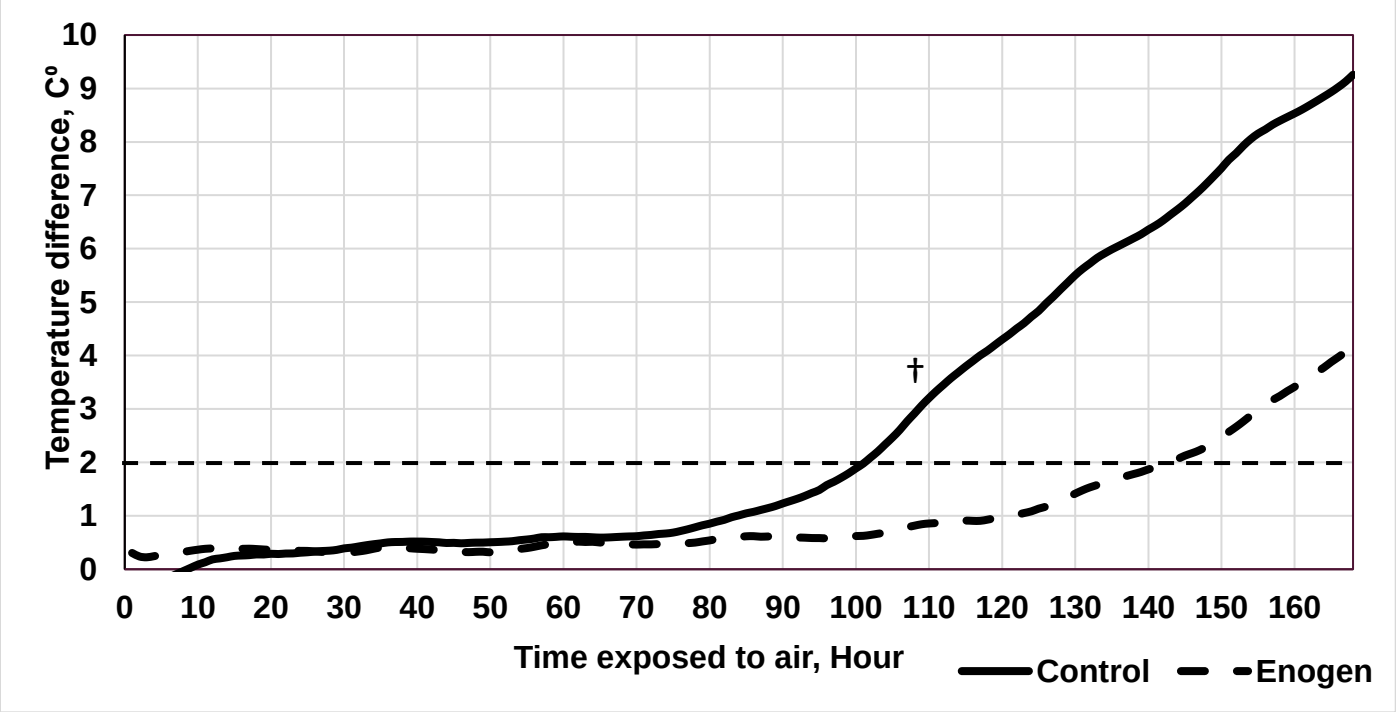

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Figure 2.3. The effect of a high amylase corn hybrid on silage exposed to air for 14 d in insulated containers in experiment 1.

Temperature difference was determined by placing temperature recording probes in the geometric center of silage mass (4 samples per treatment for each ensiling period) and a recording probe was place in an empty container to record ambient temperature. Dashed line represents 2ºC threshold set to determine aerobic stability. Effect of forage type, $P<0.05$; Effect of hour, $P<0.01$; Forage type by hour; $P<0.01$; SEM=0.55. † Indicates point in time of significant difference between forage types ($P<0.05$).

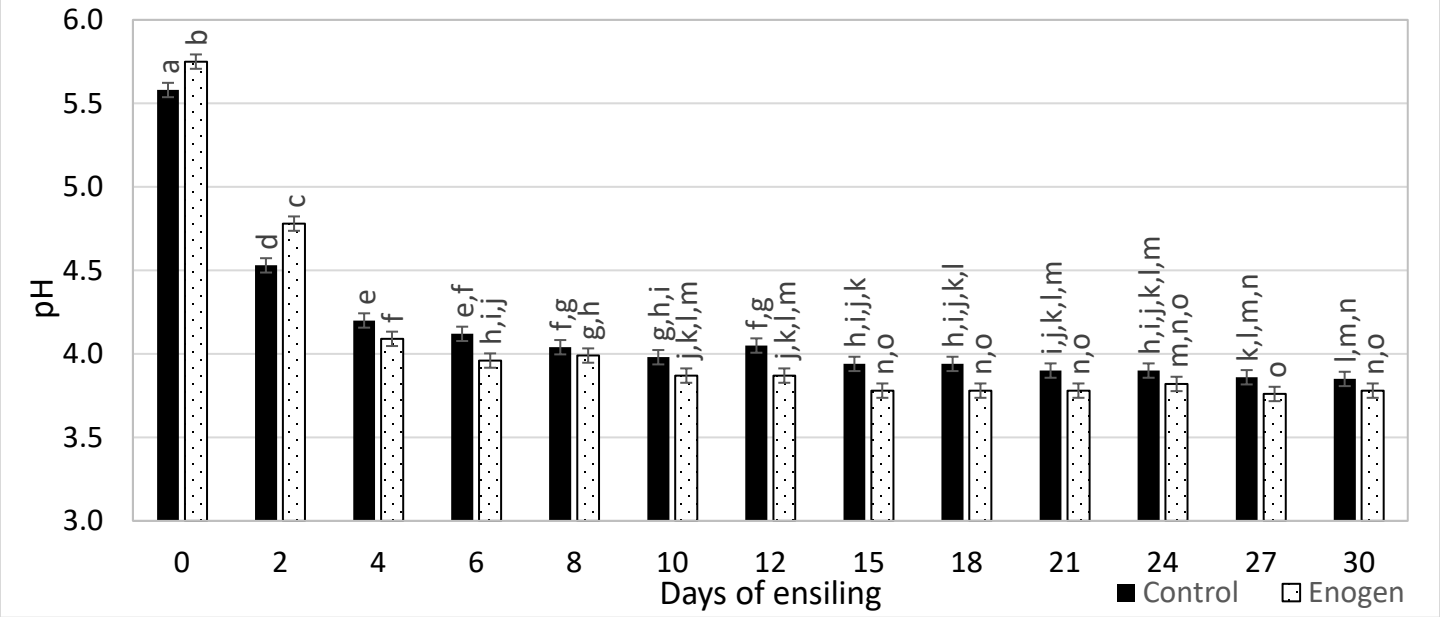

Figure 2.4. The effect of a high-amylase corn hybrid on pH in liquid extracted from corn silage throughout 30 d of ensiling in experiment 2.

pH was measured by pressing liquid extract from 4 samples from each treatment for each ensiling period. Effect of forage type, $P<0.01$; Linear and quadratic effect of day, $P<0.01$; Linear and quadratic effect of forage type by day, $P<0.01$. Error bars represent standard error of means. ^{a,b,c,d,e,f,g,h,i,j,k,l,m,n,o}Bars without a common superscript differ, $P<0.05$.

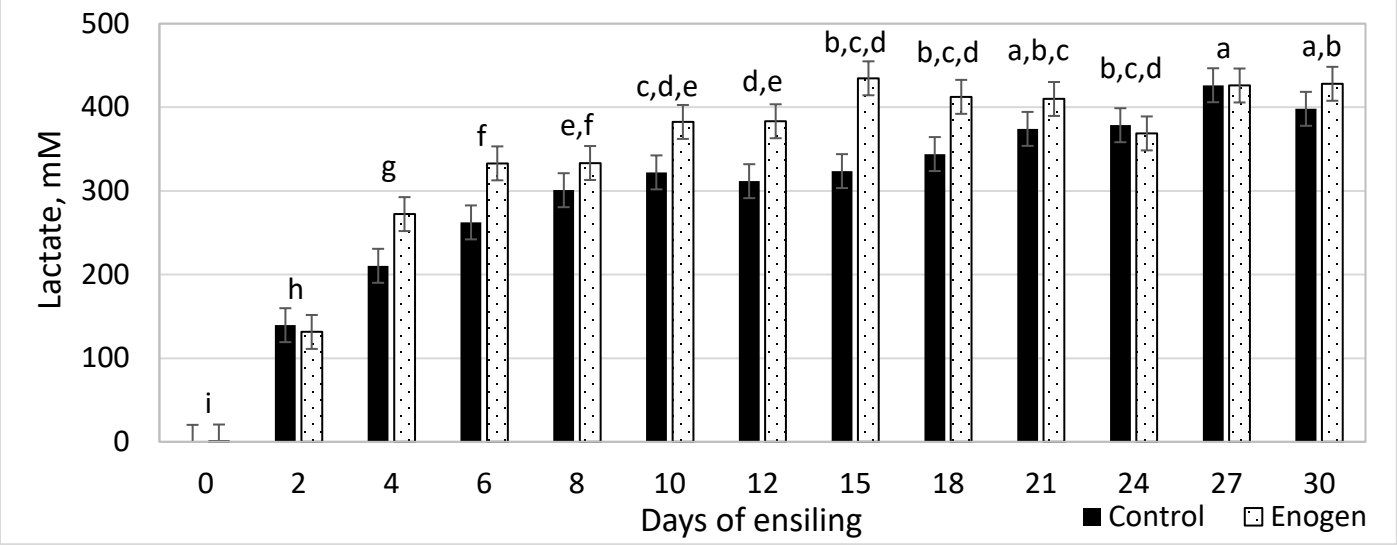

Figure 2.5. Lactate concentrations in liquid extracted of silages throughout 30 d of ensiling in experiment 2.

Lactate concentrations were measured in liquid extracted from silages and analyzed using gas chromatography. Each forage type and ensiling period were replicated in quadruplicate. Effect of forage type, $P<0.01$; Linear and Quadratic effect of day, $P<0.01$; Effect of forage type by day, $P=0.08$. ^{a,b,c,d,e,f,g}Days of ensiling without a common superscript differ, $P<0.05$.

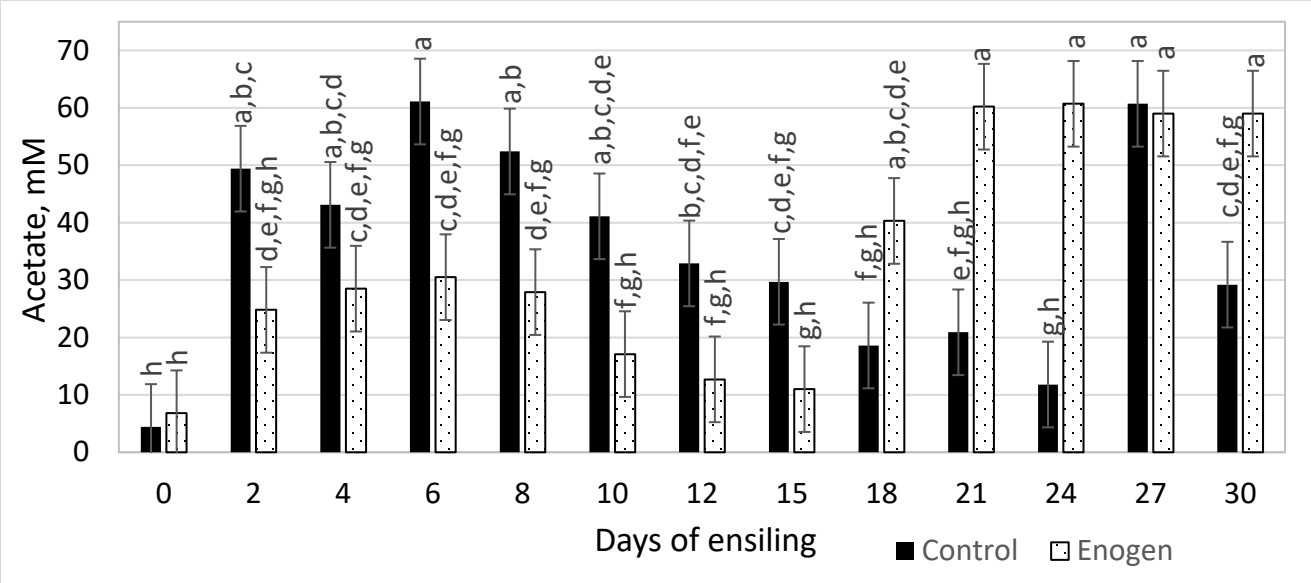

Figure 2.6. Acetate concentrations in liquid extracted from samples throughout 30 d of ensiling in experiment 2.

Acetate concentrations were measured in liquid extracted from silages and analyzed using gas chromatography. Each forage type and ensiling period were replicated in quadruplicate. Linear effect of day, $P<0.01$; Linear effect of forage type by day, $P<0.01$. ^{a,b,c,d,e,f,g,h}Bars without a common superscript differ, $P<0.05$.

Chapter 3 - Gelatinization characteristics of a high-amylase corn hybrid for steam-flaked corn

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Abstract

Two experiments were conducted to investigate the processing characteristics of Enogen Corn (EC) compared to commodity corn (CON). In Experiment 1, gelatinization and retrogradation of EC were characterized by weighing ground corn samples into differential scanning calorimeter (DSC) pans with addition of deionized water to achieve moisture levels of 20, 30, 40, 50, 60, 70, or 80 % in a 2 × 7 factorial treatment design analyzed in quintuplicate. Pans were heated from 20 ºC to 120 ºC at 10 ºC/ min to observe gelatinization peaks of endotherms. No interactions were observed between grain type and moisture level. Moisture level linearly decreased onset, peak, and endset of gelatinization temperatures ($P < 0.05$). There was a tendency for endothermic enthalpy to be less for EC compared to CON (5.64 vs. 6.27 J/g, respectively; $P < 0.10$). Pans utilized for gelatinization were stored at 4 ºC for 7 d and reanalyzed for retrogradation characteristics. The percentage of starch that was retrograded was decreased with EC at 70 and 80 % moisture compared to other treatments ($P < 0.05$). Experiment 2 consisted of a one-way treatment design with factors of grain type (CON or EC). Grains were weighed (1 kg) into glass jars in duplicate with 50 % w/w water addition. Grains were allowed to temper for 24 h and then were steamed at atmospheric pressure for 25 min. Following steaming, grains were flaked to a common bulk density of 360 g/L. Moisture assimilation was measured prior to steaming and EC absorbed approximately 22 % more water than CON ($P < 0.05$), which tended to decrease dry matter (DM) of EC ($P < 0.10$). Starch availability was measured immediately after flaking and EC was observed to have greater starch availability compared to CON (90.3 vs. 60.6 % of DM, respectively; $P < 0.05$). Increasing moisture content of grains decreases cooking temperatures required to achieve gelatinization. Enogen corn is less

susceptible to retrogradation compared to CON and when flaked at high-moistures levels EC has greater starch availability compared to non-Enogen corn.

Keywords: Enogen corn, gelatinization, steam-flaked corn

Abbreviations: Control corn, CON; dry matter, DM; Enogen Corn, EC; steam-flaked corn, SFC;

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Introduction

Increasing gelatinization or availability of starch has been shown to improve digestibility of grain (Zinn, 1990b). Steam flaking is currently the only method commonly utilized in feedlot operations to gelatinize starch. Several processing variables have been investigated to improve starch gelatinization (Sindt et al., 2006; Zinn, 1990a, 1990b). Reinhardt et al. (1997) evaluated flake density of steam-flaked sorghum and observed that decreasing flake density increased starch gelatinization; however, decreasing flake density decreased the processing throughput and increased power consumption.

Research evaluating the processing of a high-amylase corn hybrid (EC) is limited. Truelock et al. (2020) evaluated processing parameters of pelleted EC and observed that cooled EC pellets had greater starch solubles compared to pellets produced with conventional non-EC corn. Steam flaked EC tended to have greater starch availability than a non-EC corn flaked to a lesser flake density than EC in a study conducted by Horton et al. (2020).

Manufacturing feed containing EC using the processing parameters typically applied to conventional corn increases the starch availability, and thus requires further investigation into the gelatinization characteristic of EC. Therefore, our objective was to evaluate gelatinization characteristics of EC and alternative processing methods to maximize starch availability of EC.

Materials and methods

Two experiments were conducted to evaluate gelatinization characteristics and the impact of addition of high levels of moisture to steam flaked EC.

Experiment 1

Experiment 1 consisted of comparing EC to an isoline control corn to evaluate gelatinization peak, onset of peak, and endothermic enthalpy of the different grains. The experiment was designed as a 2 × 7 factorial treatment arrangement in a randomized complete block design. Factors include both grain hybrid (EC versus CON) and seven levels of moisture (20, 30, 40, 50, 60, 70, or 80 % moisture). Each corn type and moisture level were analyzed in quintuplicate with one replicate analyzed per day. Each day represented a block and the order of treatments was randomized within day.

A sample of each grain type was ground in a cyclone grinder mill (Model 3010-030; UDY, Fort Collins, CO) equipped with a 1 mm screen. Dry matter was determined in duplicate by placing samples in a 105 ºC forced-air oven for 24 h. Ground samples were analyzed for characterization of grain, and gelatinization and retrogradation properties. Analysis of gelatinization characteristics of grain hybrids was performed by differential scanning calorimeter (DSC-60; Shimadzu, Kyoto, Japan). Approximately 10 mg of ground samples were weighed into DSC pans. Deionized water was added to pans by pipette to achieve treatment moisture levels and pans were then sealed. Pans were allowed to equilibrate for 24 h prior to analysis. Samples were heated in the differential scanning calorimeter from 20 ºC to 140 ºC at a rate of 10ºC/min. Endothermic profile was analyzed using TA-60WS thermal analyzer software (Version 2.30; Shimadzu, Kyoto, Japan). Endothermic enthalpy was determined as area under the peak divided by dry weight of the sample.

Retrogradation properties of both grains also were evaluated using DSC. Sample pans that were gelatinized were placed in storage at 4 ºC for 7 d. Storage at this temperature was selected to optimize nucleation of gelatinized starches to increase retrogradation, as this temperature is near the glass transition temperature of cooked cereal starches. The same heating profile used for gelatinizing the starches was used for determining retrogradation properties of both grains at each moisture level. Measurement of endothermic enthalpy was used to evaluate recrystallization of gelatinized starch. The percentage of retrograded starch was determined by dividing the endothermic enthalpy of retrograded starch by endothermic enthalpy of gelatinized starch.

Statistical analysis

Data from experiment 1 were analyzed utilizing the PROC MIXED procedure in SAS (Version 9.4; SAS Inst. Inc., Cary, NC). Gelatinization temperatures, enthalpy, and retrogradation data from experiment 1 were analyzed with moisture, grain type, and the moisture by grain type interaction in the model. Replication was utilized as a random effect. Linear and quadratic contrasts were utilized to examine effects of moisture. Significance was determined at $P < 0.05$, and tendency for significance at $P < 0.10$.

Experiment 2

Experiment 2 was conducted as a 1-way treatment arrangement with two treatments. Treatments include grain type (EC or conventional grain). Order of processing of grains was randomly assigned prior to start of the experiment. Aliquots of EC and CON were obtained, and sub-samples were placed into a 105°C oven for 24 h to determine DM. Grains were weighed (1 kg) into 4 L glass containers in duplicate and 500 g of deionized water were added (50 % w/w). Containers were placed on a motorized table that rotated the containers at 30 rpm, mixing the

corn and water for 24 h. Corn was then placed into perforated stainless-steel baskets and excess water was drained into containers. Excess water was weighed to determine moisture assimilation by the corn. Baskets were inserted into a steam table equipped with 12 individual chambers and steamed at atmospheric pressure for 25 min. Following cooking, grains were passed through a roller mill (R&R Machine Works, Dalhart, TX) and flaked to a common bulk density of 360 g/L. Starch availability was determined by incubating 25 g of flaked grain in 100 mL of a 2.5 % amyloglucosidase solution at 55 ºC for 15 min as described by Sindt (2004). Filtered solution was then viewed through a hand-held refractometer and percent solubles were recorded.

Statistical analysis

Data were analyzed using the MIXED models procedure of SAS (Version 9.4; SAS Inst. Inc., Cary, NC). Starch availability, moisture absorption, and DM from experiment 2 were analyzed with grain type in the model. Similar to the previous analysis, replicate was utilized as a random effect. Significance of main effect was determined at $P < 0.05$, and tendency for significance at $P < 0.10$.

Results

Experiment 1

The results of gelatinization characteristics from experiment 1 are shown in Table 3.1. Moisture levels of 50, 60, 70, or 80 % for each hybrid are presented. This was due to lack of gelatinization peaks at lower moisture levels. Similarly, retrogradation data are not presented for moisture levels below 50 % due to no gelatinization peaks for those levels.

There was no grain type by moisture interactions observed for any measured characteristics; however, a tendency for a grain type by moisture interaction was observed with endothermic enthalpy ($P < 0.10$). Enogen corn at 50 % moisture had the lowest gelatinization

enthalpy. Onset of gelatinization peak linearly decreased as percent moisture increased ($P < 0.05$). Similarly, peak gelatinization temperature and end of gelatinization peak decreased linearly as the moisture addition increased ($P < 0.05$). Moreover, there was a tendency for an effect of grain type on enthalpy of gelatinization with EC having lower enthalpy than CON (5.64 vs. 6.27 J/g, respectively; $P < 0.10$).

Data from retrogradation analyses are presented in Table 3.2. An interaction was observed with change in enthalpy ($P < 0.05$) following storage of gelatinized starch at 4ºC. Enogen corn at 80 % moisture had the lowest retrogradation enthalpy of 0.0 J/g due to no retrogradation peak being observed ($P < 0.05$) but was similar to EC with 70 % moisture. Linear and quadratic contrasts were not determined for onset, peak, and end temperatures from data observed in the retrogradation analyses due lack of observations from EC at 80 % moisture. No peaks were observed at this moisture level with this grain type for any of the replications. The percentage of gelatinized starch that retrograded are shown in Figure 3.1. Moisture by grain type interaction was observed with EC 70 and 80 % having the lowest retrogradation percentage compared to other moisture levels and grain type.

Experiment 2

Following experiment 1, an experiment was conducted to evaluate starch availability of EC flaked at high moisture. Initial moisture content of CON and EC corn samples were 8.97 and 8.97 %, respectively. Dry matter and water absorbed are shown in Table 3.3. After 24 hours of mixing, EC absorbed more water compared to CON ($P < 0.05$). Consequently, DM tended to be less for EC compared to CON ($P = 0.07$). Starch solubles for steam flaked EC were greater than CON (Figure 3.2; $P < 0.05$).

Discussion

Experiment 1

The purpose of the first experiment was to evaluate gelatinization and retrogradation characteristics of EC compared to CON at increasing levels of moisture. It is well established that water content alters the gelatinization characteristics of starch (Ratnayake and Jackson, 2006). In this experiment, increasing the moisture level decreased gelatinization temperatures linearly. This is consistent with a study by Chiotelli et al. (2000), in which the authors evaluated gelatinization of wheat starch at 30, 37.5, 45, and 60 % w/w water and observed a decrease in peak gelatinization temperatures and an increase in enthalpy as moisture increased. Atlay and Gunasekaran (2006) determined that at least 21 % moisture is required for gelatinization of isolated corn starch. In our experiment we did not observe gelatinization curves below 50% moisture. It is likely that analyzing ground whole kernels potentially interferes with gelatinization curves at low moisture levels, due to protein and lipid content of the kernels or absorption of water by other components of the kernel. Similar observations have been reported when using DSC to determine the percentage of gelatinization in mixed rations following pelleting (Zhu et al., 2016). Additionally, gelatinization enthalpies observed in this experiment were lower than reported enthalpies of corn starch by Ratnayake and Jackson (2006), which would suggest potential non-starch reactions are interfering with the DSC analysis.

Gelatinization enthalpies tended to be lower for EC compared to CON. During gelatinization of starch, disruption of granular structures is largely associated with the swelling and breakdown of amylopectin (Ratnayake and Jackson, 2009). This is further shown when evaluating viscosity changes while heating waxy versus normal corn starch, with waxy corn starch having substantially greater cooked viscosity compared to non-waxy starches (Hsieh et al.,

2019). Cheng et al. (2022) evaluated gelatinization characteristics of β-amylase treated wheat starch and observed treatment with an amylolytic enzyme decreased gelatinization enthalpy. This may be due to decreasing the amylose to amylopectin ratio and increasing short-chained branches, which would reduce swelling.

Retrogradation may impact enzymatic degradation of starch and ruminal *in situ* dry matter disappearance (Trotta et al., 2021). Retrogradation as a percentage of gelatinization for CON was similar for all moisture levels, but decreased as moisture levels increased with EC. Zhou et al. (2011) observed retrogradation was greatest with water content of 50 % and decreased at 80 %. The impact of EC on retrogradation is likely attributable to the amylase content of the corn. Morgan et al. (1997) observed a decrease in staling (retrogradation) of bread following treatment with amylase, an effect attributed to decreasing glucose chain length. Shi and Seib (1992) observed that retrogradation is directly proportional to length of glucose chains with longer chains (14- 24 d.p.) being more susceptible to retrogradation. It is apparent that EC, in conjunction with high moisture levels tends to modify gelatinization and limits retrogradation.

Experiment 2

As previously discussed, moisture improves swelling and gelatinization of starch. Due to results from experiment 1, we conducted a second experiment to evaluate flaking of EC at typically high moisture levels compared to commercial flaking systems. Schwandt et al. (2016) surveyed 17 feedlots and determined steamed corn moisture prior to flaking averaged 21.8 %. In this experiment, we added 500 g of water to 1 kg of grain and measured moisture absorption following 24 h of tempering. Enogen corn absorbed more water, which translated into lower final DM of the EC flakes and increased starch solubles. Increasing moisture content has been observed to improve gelatinization of starch; especially when water is present in excess

(Ratnayake and Jackson, 2006). However, at the relatively low levels of moisture obtained in this experiment, there may be little effect of moisture on starch availability. Sindt et al. (2006) compared steam flaking conventional corn at 18 and 36 % moisture and observed an approximate 4% improvement on starch availability for the higher moisture SFC. In this experiment moisture of EC was approximately 7 % greater compared to CON; however, EC improved the starch solubles by 50 %. Commonly, starch availability is correlated to digestibility and serves as a measure of gelatinization (Zinn et al., 2002). The large improvement in starch solubles of EC is similar to results by Horton et al. (2018) and Truelock et al. (2020). Both researchers reported higher starch availability or starch solubles following processing of EC. Enogen corn offers the opportunity to achieve similar starch availability to steam-flaked commodity corn with fewer processing inputs.

Conclusion

Gelatinization, retrogradation, and effects of increased moisture addition were evaluated with high-amylase and conventional corn. Gelatinization temperatures were reduced with increased moisture levels and processing EC tends to require less energy than processing CON. Enogen corn is less susceptible to retrogradation following cooking, which could impact digestibility when fed to cattle. Steam flaking EC at high -moisture levels greatly improves starch availability, but implementation would require substantial modification of current flaking systems in commercial feedlots.

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Table 3.1. Effects of high-amylase corn on gelatinization characteristics measured by differential scanning calorimetry.

	Control[1]				Enogen[1]					
Item[3]	50	60	70	80	50	60	70	80	SEM	*P*-value[2]
$T_{O,}$ ºC	67.86	67.34	67.27	66.78	67.84	66.66	66.79	66.51	0.348	L-M
$T_{P,}$ ºC	77.79	74.26	73.16	73.23	77.29	74.69	73.45	73.13	1.040	LQ-M
$T_{E,}$ ºC	83.84	83.05	79.20	78.56	84.85	82.12	79.82	79.34	1.391	L-M
$\Delta_{Enthalpy}$, J/g	5.31^{b}	7.74^{a}	6.39^{b}	5.65^{b}	3.96^{c}	6.07^{b}	6.41^{b}	6.13^{b}	0.698	g, LQ-M, i

[1] Final moisture content of ground corn samples (5 samples for each moisture by grain type).

[2] G = Effect of grain type, $P < 0.05$; g = Tendency for effect of grain type, $P < 0.10$; M = Effect of moisture level, $P < 0.05$; m = Tendency for effect of moisture, $P < 0.10$; I = Effect of grain type by moisture interaction, $P < 0.05$; i = Tendency for effect of grain type by moisture interaction, $P < 0.10$; L = Linear effect of moisture, $P < 0.05$; Q = Quadratic effect of moisture, $P < 0.05$; NS = Not significant, $P > 0.05$.

[3] T_E = Temperature for end of gelatinization peak ; T_O = Temperature for onset of gelatinization peak; T_P = Temperature for peak gelatinization; $\Delta_{Enthalpy}$ = Endothermic enthalpy of gelatinization.

[a,b,c] Means in a row without a common superscript letter tended to differ, $P < 0.10$.

Table 3.2. Effects of a high-amylase corn hybrid on retrogradation of gelatinized starch measured by differential scanning calorimetry.

	Control[1]				Enogen[1]					
Item[3]	50	60	70	80	50	60	70	80	SEM	*P*-value[2]
$T_{O,}$ ºC	47.75	43.71	43.94	43.85	42.76	42.67	45.73	-	1.630	-
$T_{P,}$ ºC	60.87	56.84	55.65	57.81	58.38	54.34	56.40	-	1.451	M
$T_{E,}$ ºC	67.98	63.58	61.70	62.51	59.22	61.27	57.51	-	1.886	G, m
$\Delta_{Enthalpy}$, J/g	$2.80^{b,c}$	$4.79^{a,b}$	$4.05^{a,b}$	$3.83^{a,b}$	$2.77^{b,c}$	$3.66^{a,b}$	$1.39^{c,d}$	0.00^{d}	0.668	G, LQ-M, L-I

[1] Final moisture content of ground corn (5 samples for each moisture by grain type).

[2] G = Effect of grain type, $P < 0.05$; g = Tendency for effect of grain type, $P < 0.10$; M = Effect of moisture level, $P < 0.05$; m = Tendency for effect of moisture, $P < 0.10$; I = Effect of grain type by moisture interaction, $P<0.05$; i = tendency for effect of grain type by moisture interaction, $P < 0.10$; L = Linear effect of moisture, $P < 0.05$; Q = Quadratic effect of moisture, $P < 0.05$; NS = Not significant, $P > 0.05$.

[3] T_E = Temperature for end of gelatinization peak ; T_O = Temperature for onset of gelatinization peak; T_P = Temperature for peak gelatinization; $\Delta_{Enthalpy}$ = Endothermic enthalpy of gelatinization.

[a,b,c,d] Means in a row without a common superscript letter differed, $P < 0.05$.

Table 3.3. The effect of grain type on moisture absorption after 24-hours.

Item[1]	Control	Enogen	SEM	*P*-value
Water absorbed, %	79.7	97.6	2.23	<0.05
Dry matter,[2] %	66.4	61.7	0.39	0.07

[1] Grain were placed in containers in duplicate and deionized water was added on a 50 % w/w. Containers were placed on a rotating table for 24-h and excess water was weighed to determine water absorbed.

[2] Dry matter was determined by placing flaked grains into 105 °C oven for 24-h.

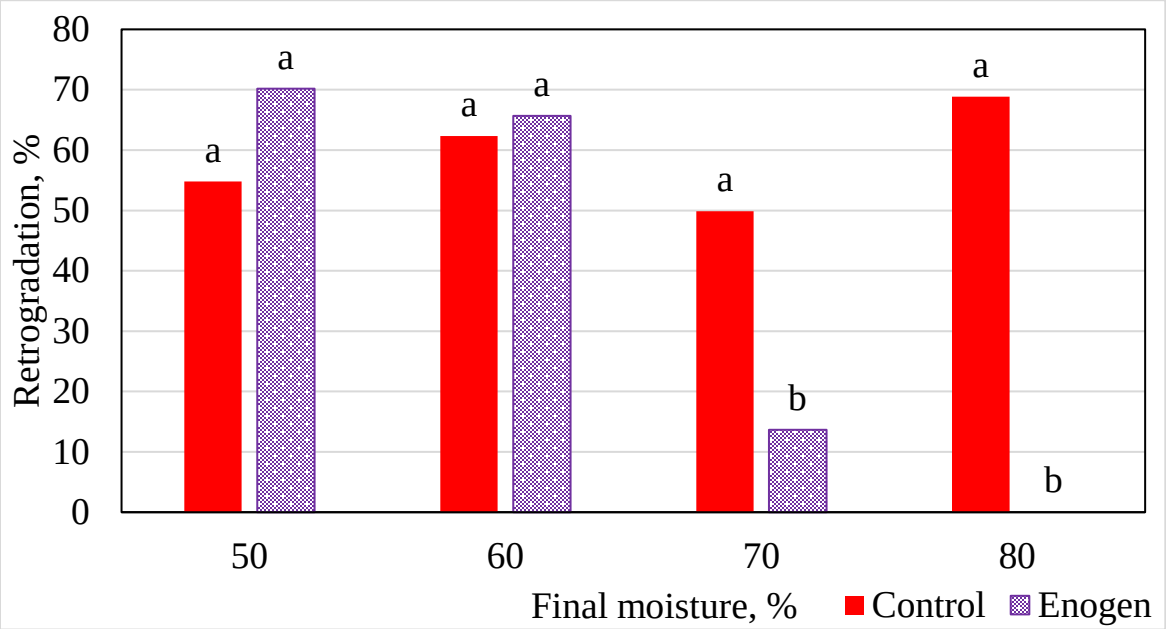

Figure 3.1. Interaction between grain type and final moisture addition (50, 60, 70, or 80%) on percent retrogradation of starch following gelatinization and stored at 4ºC for 7 d.

Retrogradation was determined by dividing enthalpy following retrogradation scan divided by enthalpy of gelatinization scan using differential scanning calorimeter (5 samples per treatment for each moisture level). Effect of grain type, $P < 0.05$; Effect of moisture, $P < 0.05$; Interaction of moisture by grain type, $P < 0.05$; Linear effect of moisture, $P < 0.05$; Quadratic effect of moisture, $P = 0.93$; Linear effect of grain type by moisture, $P < 0.01$; Quadratic effect of grain type by moisture, $P = 0.43$. SEM = 9.78. [a,b] Bars without a common superscript differ, $P < 0.05$.

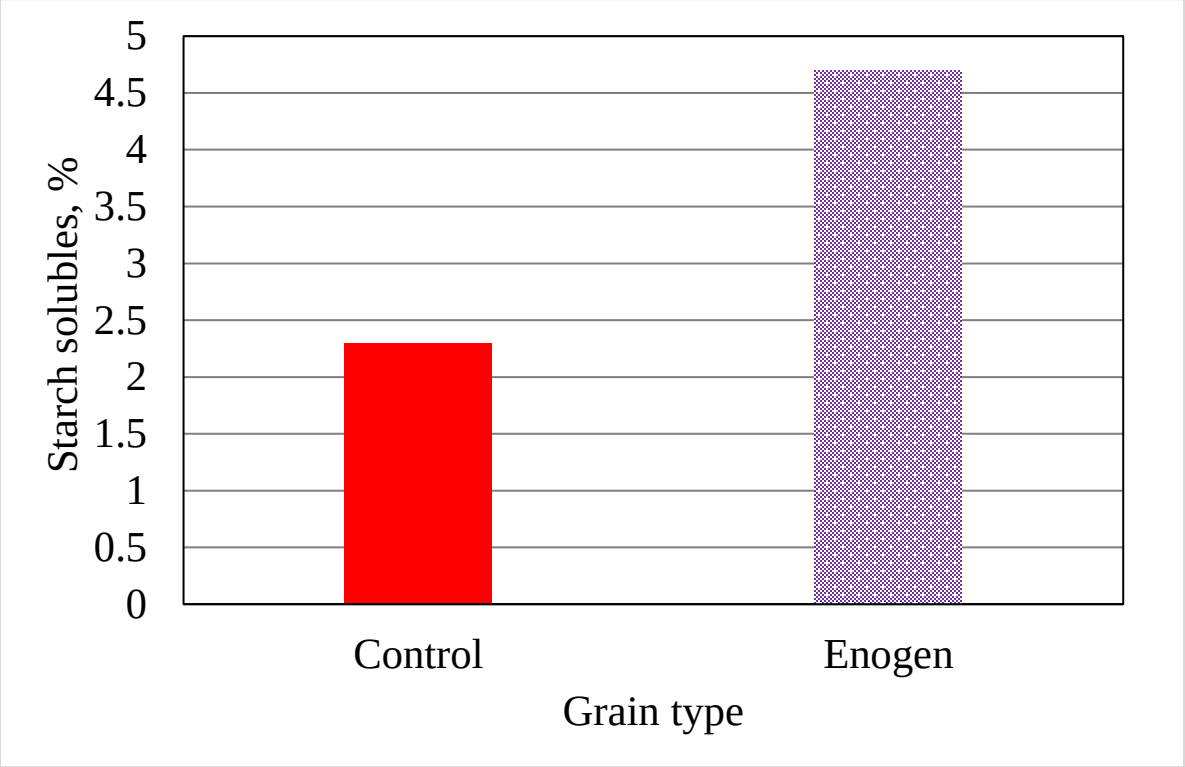

Figure 3.2. The effect of grain type on starch solubles of high-moisture steam-flaked corn.

Solubles were measured using a hand-held refractometer following incubation in an amyloglucosidase solution at 55 °C for 15 min (Sindt et al., 2004). Effect of grain type, $P < 0.05$. SEM = 0.96.

Chapter 4 - Feedlot performance and carcass characteristics of steers fed diets containing steam-flaked corn and corn silage from a high-amylase corn hybrid

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Abstract

Enogen Corn (EC; Syngenta Seeds, LLC) is genetically modified to express high concentrations of α-amylase. Our objective was to evaluate EC as corn silage and as steam-flaked corn in finishing diets fed to steers in comparison to common commodity corn (CON) and a corn hybrid commonly used for silage (CON). A 2 × 2 factorial experiment was conducted with steers (n=960; 388 ± 7.4 kg initial body weight), with factors consisting of silage source and grain source. Steers were blocked within receiving period and by initial body weight, assigned randomly within block to treatments. Steers were housed in 48-pens with 15 (24 pens) or 25 (24 pens) cattle per pen, and harvested after 138, 152, or 166 d. Grains were steam-flaked to densities of 360 or 386 g/L for CON and EC, respectively. Finishing diets (dry basis) consisted of 8 % corn silage, 2 % alfalfa hay, 74.5 % flaked corn, 12 % Sweet Bran, and supplement. Incidence of liver abscesses and carcass weights were recorded at harvest, and longissimus muscle area, 12th-rib subcutaneous fat thickness, marbling score, and USDA yield and quality grades were determined after 36 h of refrigeration. Additionally, strip loins were retained from 2 steers from each pen. Loins were wet aged and fabricated into steaks for determination of color shelf life and long chain fatty acid analysis. There were no interactions between grain source and silage source ($P > 0.05$) for feedlot performance. Cattle fed diets containing EC silage consumed less dry matter ($P < 0.01$) and efficiency of gain was improved by approximately 5.3 % ($P < 0.01$) compared to cattle fed Control silage. Average daily gain and dry matter intake were unaffected by grain source, but cattle fed EC grain were less efficient ($P = 0.02$) compared to cattle fed CON grain. Hot carcass weights were greater for cattle fed the combination of EC silage and CON grain compared to other treatments ($P < 0.05$), but liver abscess incidence and

other carcass measurements were unaffected by grain or silage source. In this experiment, feeding EC as corn silage, but not as grain, improved feedlot performance of steers.

Keywords: corn silage, steam-flaked corn, Enogen

Abbreviations: Body weight, BW; Control, CON; dry matter, DM; dry matter intake, DMI; Enogen Corn, EC; gain to feed, G:F; gas chromatography, GC; Kansas State University Beef Cattle Research Center, BCRC; long chain fatty acids, LCFA; steam-flaked corn, SFC; volatile fatty acids, VFA.

Introduction

The ethanol industry utilizes exogenous alpha-amylase in the process to increase rate of starch fermentation by yeast for ethanol production. Enogen Corn (Enogen Corn, Syngenta Seeds LLC) hybrid was developed to express high concentrations of alpha amylase in the kernel, thus making it possible to reduce use of exogenous enzymes. Interest in EC as a feed ingredient for ruminants is driven by observations of diminished intestinal starch degrading enzyme capacity (Harmon et al., 2004).

Several studies have been conducted utilizing EC in finishing diets as steam-flaked, dry-rolled, or high-moisture corn with varied results (Horton et al., 2020; Volk et al., 2021). Jolly-Breithaupt et al. (2019) fed EC as dry-rolled corn in finishing diets to beef cattle and observed improved feed efficiency compared to conventional corn. Similarly, steam flaking EC has been shown to improve feed efficiency in finishing heifers (Horton et al., 2020). Both of these studies utilized EC corn as the primary starch source in the diets. Rusche et al. (2020) utilized EC silage at a rate of approximately 12% (DM basis) in finishing diets with dry-rolled conventional corn grain and observed no difference in performance or carcass characteristics.

Improving digestion of starch is of considerable interest for feedlot cattle. Although, EC as corn silage has been observed performance of feedlot cattle with dry-rolled corn. Steam-flaked corn is more commonly utilized in finishing diets and improves post ruminal digestion of starch compared to dry rolling corn by separating starch/protein matrix that limits starch digestion in the small intestine (Samuelson et al., 2016; Zinn et al., 2002; Zinn et al., 1995). Enogen corn utilized as corn silage in conjunction with SFC may impact performance in finishing diets. Thus, the objectives of this study were to evaluate effects of EC as SFC and/or silage in finishing diets on feedlot performance, carcass characteristics, and meat quality.

Materials and methods

This study was conducted from April 2018 to September 2018 at the Kansas State University BCRC, Manhattan, KS and protocols and procedures utilized in this study were approved by the Kansas State University Institutional Animal Care and Use Committee.

Experimental design

A 2 × 2 factorial experiment was conducted as a randomized complete block design utilizing factors of EC as SFC and silage compared to CON corn as SFC, and a commercial hybrid commonly used for silage. Crossbred steers (n=960; 388 ± 7.4 kg initial BW) were utilized for this study. Steers were received at the BCRC between March 24th 2018, and April 7th 2018, and were blocked by weight within receiving group. Steers were randomly assigned within block to treatment. Treatment factors consisted of silage type (CON or EC) and grain type (CON or EC). Steers were housed in 24 dirt surfaced pens with 15 animals per pen and 24 dirt surfaced pens with 25 animals per pen for a total of 48 pens. Due to the range of weights received in this group of cattle, it was determined initially that steers would be harvested in three separate groups.

Initial processing and handling

Steers were received on multiple days at the BCRC and were given *ad libitum* access to ground alfalfa hay and water upon arrival. Initial processing of steers included an oral drench for internal parasites (Safeguard; Merck, Kenilworth, NJ), vaccination with Bovishield Gold-5 (Zoetis, Parsippany, NJ) and Ultrabac 7 (Zoetis), an individual identification tag, and individual BWs were recorded. Steers were implanted with Component TE-200 (Elanco Animal Health, Greenfield, IN) on d 1 and received a pour-on for external parasites (Standguard; Elanco Animal Health). Additionally, steers were re-implanted (Component TE-200, Elanco Animal Health) with the first group re-implanted on d 62, second group on d 78, and third group on d 93 of the study. In addition to re-implanting, cattle were re-treated for external parasites (Standguard; Elanco Animal Health).

Ingredients and diet preparation

Enogen corn grain for steam-flaking was sourced from study sponsor and CON grain was a commodity corn sourced locally. Grains were flaked daily. Both grains were conditioned by addition of water and a surfactant (SarTec; Anoka, MN) prior to steaming. Conditioned grain was flaked utilizing an R & R Machine Works steam-flaker (46 x 91 cm corrugated rolls; Dalhart, TX). Enogen corn was flaked to 386 g/L bulk density, while CON corn was flaked to a density of 360 g/L. Differences in flake density was determined from a previous study conducted at the BCRC to produce similar starch availabilities of both grains (Horton et al., 2020). Starch availabilities were measured daily using an enzymatic procedure described by Sindt et al. (2000). Approximately, 200 g of each flaked grains were retained for determination of DM by overnight drying in a 105ºC forced air oven.

Corn hybrids were planted for silage in the spring 2017 near the BCRC. Both varieties were harvested in August 2017 utilizing a commercial harvester equipped with a kernel processor (Model 7980; John Deere, Moline, IL). Whole chopped corn plant was transported to the BCRC using open topped trucks (~7,000 kg/load) and packed into agriculture bags for storage (Hitec Bag, Plastika Kritis; Iraklion, Greece). During filling of the silo bags, markers were placed on the bags to indicate every other load packed into the silo. Following approximately 6 months of ensiling, the storage bags were sampled at previously described indicators, and approximately, 1 kg of silage was retained for analysis of DM, pH, NDF, ADF, and starch.

Rations were prepared daily based on visual assessment of bunks each morning and orts were collected as needed. Steers were fed rations *ad libitum* once daily and were transitioned to a finishing diet over 20 d utilizing 4 step-up diets. Initial diets (on DM basis) contained 50% roughage and each subsequent step decreased roughage level by 10% every 5 d until final diet contained only 10% roughage (2% alfalfa and 8% silage) on 21 d. Table 1. shows the final diet on a DM basis. Dry matter content of silages was monitored daily by placing samples in 105ºC forced air oven for 24 h and rations were updated as-needed to reflect current DM of corn silage. Bunks were top dressed for the final 35 d prior to slaughter with 227 g per head daily of ground corn premix containing ractopamine hydrochloride (Optaflexx; Elanco Animal Health) to deliver 400 mg per animal daily of ractopamine. Additionally, each ingredient was sampled weekly and dried in a 55ºC oven for 48 h. Weekly samples were composited monthly and shipped for analyses for crude protein, starch, ADF, starch, calcium, phosphorus, and potassium contents (SDK Laboratories; Hutchinson, KS).

Silage samples retained were processed prior to the start of the trial to account for changes in DM during feed out. A portion of the fresh samples were pressed using a manual press to collect exudate for determination of pH utilizing a portable pH meter (Thermo Scientific Orion 3-star portable pH meter, Waltham, MA). Dry matter was determined by weighing the sample before and after lyophilizing (Genesis Model 35EL; SP Scientific, Warminster, PA). Samples were ground through a 1-mm screen after lyophilizing and then utilized for nutrient analyses. Ash was determined by placing approximately 1 gram of sample in a muffle oven and heated to 450ºC for 16 h. Neutral detergent fiber and sequential ADF were determined using the Ankom filter bag technique (Ankom Technologies, Macedon, NY). Starch content was determined using an enzymatic method as by Richard et al. (1995) and Saldana et al. (1989). Samples were weighed into 50 mL test tubes with 25 mL of a 0.1M acetate buffer solution prior to addition of a heat stable alpha-amylase (FAA α-amylase enzyme; Ankom Technologies, Macedon, NY). Samples were incubated in a 95ºC water bath for 30 min and then allowed to cool. A glucoamylase solution (Validase GA; Valley Research, South Bend, IN) was then added and tubes were incubated in a 60ºC water bath overnight. Samples were centrifuged at 1000 x g for 10 min and supernatant was collected. Supernatant was analyzed for glucose concentrations to determine total starch by a colorimetric assay kit (AutoKit Glucose; ThermoFischer Scientific, Rockford, IL) in a 96 well plate and absorbance was read at 505 nm using a plate reader. Starch concentrations were calculated from glucose concentrations divided by the DM weight of the sample analyzed.

Final body weights and carcass data collection

As previously described, steers were harvested in 3 groups after 138, 152, and 166 d on feed. Final weight of each pen was measured on a pen-scale before shipping and final weights

were multiplied by 0.96 (4% shrink) as these values are reported as shrunk final BW. Steers were transported approximately 450 km to a commercial abattoir in Holcomb, KS. Animal identification, HCW, and liver abscess prevalence and severity were collected after slaughter. Liver abscess prevalence and severity data were collected utilizing the Elanco scoring system (Liver Abscess Technical Information AI 6288; Elanco Animal Health: 0=no abscess; A^-=one or two small abscesses or inactive scars; A=one or two large abscesses or several small abscesses; A^+=multiple large abscesses). Additionally, carcass data, such as, *longissimus* muscle area, 12^{th}-rib subcutaneous fat, marbling score, USDA Yield Grade, and USDA Quality grade were collected after carcasses were refrigerated for approximately 36 h.

Collection of loins, simulated retail display, and analyses of LCFA

In addition to collecting carcass data, two black-hided steers were randomly selected from each pen for collection of *m. longissimus dorsi* muscle from one side of the carcass for determination of LCFA and to evaluate color stability during a simulated retail display. Loins were removed at the commercial abattoir and vacuum sealed for transportation to the Kansas State University Meats Laboratory. The loins were weighed upon arrival and then wet aged for 21 d. After wet aging strip loins were re-weighed to calculate purge loss as [(initial weigh-final weight)/initial weight] x 100. A meat pH meter (model HI99163; Hanna Instruments, Smithfield, RI) was used to determine pH in the geometric center of each loin. Subsequently, two 2.54-cm steaks were fabricated from the anterior portion of each loin. The first steak was diced and vacuum sealed for analysis of LCFA concentrations. The second steak from each loin was placed on 17S polystyrene foam tray with an absorbent pad (UZ-40; Paper Pak Industries, Washington, GA) and wrapped with PVC film (HIYG Gold Stretch Meat; Berry Plastics Corporation, Evansville, IN). Packaged steaks were placed under fluorescent light in coffin style cases (DMF

8; Tyler Refrigeration Corporation, Niles, MI) for 10 d. Display cases were defrosted twice daily. Steaks were rotated every 12 h to allow for differences in lighting and temperature within the case. Each steak was scanned three times daily with a HunterLab Miniscan (Illuminant A, 2.54-cm aperture, Hunter Lab Associates Laboratory, Reston VA) for determination of L*, a* and b*.

Diced steak samples from each loin were then frozen in liquid nitrogen and ground in a commercial blender (Waring Commercial; Hartford, CT) prior to being lyophilized (Genesis Model 35EL; SP Scientific, Warminster, PA). Long chain fatty acids were extracted from the samples utilizing the method developed by Sukhija and Palmquist (1988). Extracts obtained from samples were analyzed as fatty acid methyl esters using gas chromatography (Agilent Technologies, Santa Clara, CA) with an SP-2500 capillary column (100m length x 0.25 mm diameter x 0.2µm film thickness; Supelco, Bellefonte, PA). Hydrogen was utilized as the carrier gas at 35 mL/min with injection size of 1 µL and 100:1 split. The temperature gradient consisted of initial temperature set to 140ºC for 5 min, then increased 8ºC/min to 180ºC, increased 4ºC/min to 210ºC, and then increased 20ºC/min to a final temperature of 250ºC followed by a 7-min hold. An FID detector set to 250ºC was utilized to compare the extracted samples to a commercial standard containing 37 LCFA (Supelco 37 FAME; Supelco, Bellefonte, PA).

Statistical analyses

The MIXED procedure of SAS (Version 9.4; SAS Inst., Cary, NC) was used to analyze final shrunk BW, ADG, DMI, and G:F. The model utilized fixed effects of silage type, grain source, and the interaction of silage by grain source. Random effect of the model was block. The GLIMMIX procedure was utilized for analysis of categorical data using the parameters outlined previously for feedlot performance. The MIXED procedure was utilized for analysis of LCFA and color data from during retail display. For analyses of color data, repeated measures were

utilized with day of display as the repeated measure and steak as the subject. Compound symmetry was used as the covariance structure. The slice function was utilized for analyzing L*, a*, and b* data to determine differences between treatments each day.

Results

Animals removed from the EC silage/EC grain treatment group: 2 classified as bullers, 2 due to chronic respiratory disease, 2 due to physical injury and 2 were found deceased in the pen and subsequently diagnosed as having respiratory disease. Seven animals were removed from the EC silage and CON grain treatment group: 6 were found deceased and during necropsy determined to have had respiratory disease and another succumbed to heat stress. Three animals were removed from the CON silage and EC grain group: 1 found deceased and later diagnosed with respiratory disease, 1 animal was removed due to chronic respiratory disease, and 1 deceased due to heat stress. Five animals were removed from the CON silage and CON grain treatment group: 2 were found deceased and during necropsy determined to have respiratory disease and 3 were removed due to physical injury.

Silage and grain characteristics

Silage storage bags were sampled prior to initiation of the feeding study and silage samples were collected weekly and composited monthly for analysis. Nutrient composition of the samples taken prior to and during the study are shown in Table 4.2. Enogen corn silage contained greater DM than CON silage (356.7 vs. 269.6 g/kg of DM; $P<0.01$), and CON silage contained less starch (27.9 vs. 307.3 g/kg of DM, $P<0.01$), greater ADF (327.8 vs. 222.5 g/kg of DM, $P<0.01$) and NDF (574.7 vs. 382.1 g/kg of DM, $P<0.01$) compared to EC silage, suggesting CON silage was of poor quality.

Table 4.3 illustrates characteristics and composition of steam-flaked grains utilized in finishing diets. As with silages, composition of grains differed. Dry matter of steam-flaked grains did not differ (P=0.60), although starch availability measure by refractive index showed an increase in available starch with EC grain (P<0.05). Acid detergent fiber, ether extract, phosphorous, and potassium were greater for EC grain than CON grain in this experiment (P<0.05).

Feedlot performance

There were no interactions observed between silage and grain type for measures of feedlot performance (P>0.05). Steers consuming CON steam-flaked corn and EC silage tended to have greater ADG compared to other treatments (P=0.06). Similarly, final shrunk BW tended to be greater for steers consuming CON steam-flaked corn and EC silage (P=0.07). Silage produced from Enogen corn significantly impacted DMI and G:F (P<0.01) as shown in Table 4.4. Dry matter intake was reduced for EC silage (9.99 kg/d) compared to CON silage (10.12 kg/d; P<0.01), which contributed to improved G:F for steers fed EC silage compared to CON silage (0.180 vs. 0.171; P<0.01). Additionally, corn type utilized for steam flaking affected G:F with steers fed EC having a lower G:F compared to CON corn (0.173 vs. 0.178; P<0.05).

Carcass characteristics

An interaction was observed between silage and grain type for HCW. Steers fed CON steam-flaked corn in combination with EC silage had greater HCW compared to other treatments (P<0.01). Hot carcass weight also was affected by main effect of grain type as shown in Table 4.5. Steers consuming CON steam-flaked corn were observed to have greater HCW compared to steers consuming EC steam-flaked corn (406 vs 401 kg; P<0.01). A tendency was observed for steers consuming EC corn as steam-flaked corn a greater percentage of abscessed livers in the

mild category compared to cattle fed CON as steam-flaked corn (11.9 vs 8.2%; *P*=0.06). Main effects of grain or silage type did not affect prevalence of abscessed livers in cattle fed finishing diets (*P*>0.05). Additionally, steers fed EC grain as steam-flaked corn tended to have a greater percentage of carcasses grade Prime compared to steers fed CON grain. (*P*=0.08). Enogen corn fed as either silage or steam-flaked corn did not affect other carcass traits evaluated in this experiment (*P*>0.10).

Retail display and long chain fatty acid content

Following harvest, *longissimus dorsi* samples were wet aged for 21-d. Purge loss, initial pH and final pH are presented in Table 4.6. There were no effects of silage or corn type on pH or purge loss (*P*>0.05). Figure 4.1. illustrates changes in lightness/darkness (L* values) throughout the 10-d display period. Grain type affected L* values with steaks produced from cattle fed EC corn having lower L* values compared to CON corn (42.0 vs 43.0; *P*<0.01). The main effect of grain type affected L* values for d-3, 4, 6, and 7 (*P*<0.05). Values for a* were affected by a silage by grain interaction (*P*<0.01) and similar to L* an effect of day (*P*<0.01). Figure 4.2. illustrates a* measurements over the 10-d display period with values being similar until d-10. Steaks from cattle fed EC corn and CON silage differed from steaks produced from CON silage and CON corn on d 9 (25.5 vs 26.8; *P*=0.05), but did not differ from other treatments (*P*>0.10). Values for a* from EC grain and CON silage treatment were lower than a* values from steaks receiving diets containing EC silage and EC grain on d-10 (22.3 vs 25.1, respectively; *P*<0.01), and a* values for EC grain and EC silage treatment was also greater than steaks from steers fed EC silage and CON grain treatment (25.1 vs 23.4, respectively; *P*=0.05). Values for b* were affected similar to a* values, with an effect of day (P<0.01) and a silage x grain interaction

(*P*>0.01). Steaks from steers fed CON silage and EC corn had lower b* values (Figure 4.3) compared to steaks from EC silage and EC corn on d-10 (21.5 vs 22.6, respectively; *P*<0.05).

Tables 4.7, 4.8, and 4.9. present LCFA concentrations of grains, silages, and *longissimus lumborum* samples. Enogen corn utilized for steam flaking had greater unsaturated fatty acids than CON corn (*P*<0.01); such as, C18:1n9c and C18:2n6c. Similarly, silage produced from EC was observed to have greater C18:2n6c compared to CON silage (1,066.2 vs 322.7 µg/g of DM; *P*<0.01). An interaction was observed with C23:0 concentrations in loin samples. Samples analyzed from EC corn and CON silage were greater than those of steaks from the EC corn and EC silage treatment (0.47 vs 0.43 mg/g of wet tissue; *P*<0.05). Total fatty acid concentration did not differ among treatments (*P*>0.05). Grain type affected total polyunsaturated fatty acid and omega-6 concentrations, with steaks from the EC corn treatment had greater concentrations compared to those from the CON corn treatment (2.65 vs 2.25 mg/g of wet tissue; *P*<0.01). Treatments did not affect proportions of saturated and monounsaturated fatty acids (*P*>0.05). Total omega-3 concentrations were affected by silage type with steaks from the EC silage treatments having greater concentrations of omega-3s than steak from CON silage treatment (0.16 vs 0.14 mg/g of wet tissue; *P*<0.04).

Discussion

Recent studies have been conducted recently, evaluating EC as an ingredient in finishing diets, and have yielded varied results. Rusche et al. (2020) evaluated EC as corn silage in finishing diets and observed no effect on performance or carcass characteristics. Volk et al. (2021) analyzed 7 experiments that utilized EC as either dry-rolled or high-moisture corn in finishing diets and concluded that feeding EC improved performance in the absence of distiller's grains. Furthermore, the authors determined a 4% improvement in G:F can be observed with

cattle fed EC as dry-rolled corn in finishing diets containing wet corn gluten feed at 25% of the diet. The authors hypothesized that diets containing greater proportions of corn would present a greater response to EC due to greater starch available in the diet as corn is commonly replaced with byproducts in formulations. In this experiment, wet corn gluten feed (Sweet Bran: Cargill starches, sweeteners, and texturizers; Minneapolis, MN) was included at 12% of the diet (DM basis) and was below the level suggested by Volk et al. (2021) to impact an EC response. The current experiment, comparison of corn type as either silage or grain utilized for steam flaking presented challenges. Differences in starch concentration between grains and silages likely contributed to the outcome of this experiment. Although commonly, starch composition of whole corn can vary from 640 to 780 g/kg of DM and corn silage can vary from 122 to 362 g/kg of DM (Eckhoff and Watson, 2009; Ferreira and Mertens, 2005).

Horton et al. (2020) fed EC as steam-flaked corn and reported improved final BW, ADG, G:F, and HCW, but the authors did not report total starch content of grains. They did report a tendency for increased starch availability following flaking of EC in spite of the increased bulk density of Enogen compared to control. In the current experiment, starch availability differed between corn types also. Enogen corn was flaked to a greater flake weight compared to conventional grain in an effort to produce similar starch availabilities in this study and the Horton et al. (2018) experiment. In the current experiment, starch, ADF, EE, phosphorous, and potassium differed between grain types. It is possible the outcome from this experiment is due to compositional differences of the corn and not necessarily attributable differences in the amylase concentrations of grains. Krieger et al. (1998) demonstrated that formation of starch structures can differ by means of pollination or growing environment. In this current experiment CON was sourced locally, whereas EC corn was obtained from another state.

Silages produced from the two silage sources were compositionally different. Silage produced from EC had greater starch and DM concentrations than CON silage. The forages were planted in adjacent fields; however, growing conditions or hybrid characteristics may have impacted the maturity of the corn at time of harvest. Additionally, fiber concentrations were greater for CON silage due to the absence of well-formed ears. Diets containing CON silage would have greater proportion of roughage compared to steers fed EC silage. Defoor et al. (2002) observed greater roughage level in the diet increased with similar rough source increased DMI. This may be due to cattle attempting to eat at a common energy intake (Forbes, 2003). Diets containing steam flaked CON and EC silage would have the greatest proportion of starch or rapidly fermentable energy of the 4 treatments and inversely would have the least amount of roughage in the diet. Although, there was no effect of grain on DMI, an effect of silage type was observed. Steers consuming EC silage had lower DMI than steers consuming CON silage. Numerically, steers consuming the greatest starch concentration of steam flaked CON and EC silage had the lowest intake and the steers consuming the lowest starch concentration of steam flaked EC and CON silage had the greatest DMI. This could have l altered the expected response to EC and influenced lack of G:F improvement reported by other authors.

Research evaluating effect of EC on meat quality is limited. Steaks collected from steers fed EC grain were lighter in color for 5 d throughout the simulated retail display compared to steaks from steers fed CON grain. Although color is considered the most important attribute consumers evaluate when making purchases (Mancini and Hunt, 2005). Differences observed in this experiment for L* values would likely not affect overall color of steaks due to a* and b* values not being different throughout the first 8-d of simulated retail display. Values for a* and b* values did differ on d-9 and 10, with steaks from the EC grain and CON silage treatments

having lower values. This may be due to the effect of EC on PUFA concentrations in tissues. Steers receiving EC grain treatment would have been fed higher fat content and lipid concentration of diets has been observed to influence fat deposition in tissues (Mello et al., 2012). Furthermore, oxidation of unsaturated fatty acids has been shown to decrease shelf-life of meat (Yang et al., 2002). Enogen corn contained greater amounts of PUFAs and MUFAs compared to CON grain. Finishing diets contained approximately 75% steam-flaked corn and the differences between characterization of grains likely contributed to differences in fatty acid composition of steaks. Similar to the grain, EC silage contained elevated levels of PUFAs. Although it did not impact the PUFA concentration in the steaks. A benefit to increased unsaturated fatty acid is from a human health prospective and reduced cardiovascular issues compared to consuming saturated fatty acids; however, oxidation of lipids can lead to rancid flavor and decrease overall liking by sensory panel (Campo et al., 2006; Eckel et al., 2014). A possible remedy is to supplement vitamin E at greater levels to limit lipid oxidation during retail display (Gobert et al., 2010; Liu et al., 1995). Although, discoloration of steaks from steers fed EC grain only began to occur on d-9 and would likely not be displayed for that length of time in a commercial display.

Conclusion

Feeding EC as corn silage improved feedlot performance, but feeding EC as steam-flaked grain had no effect. In this experiment, differences in grain and silage characteristics utilized were substantial, and outcomes are not necessarily attributable to enzymatic activity of the grains or silage. Feeding EC as grain in finishing rations increased PUFA concentrations in meat and may provide potential health benefits to consumers.

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Table 4.1. Composition and nutrient analysis of finishing diets fed to beef steers[a].

	Control silage		Enogen silage	
Item[b]	Control grain	Enogen grain	CON grain	Enogen grain
Ingredient, % of DM				
Control steam-flaked corn	74.5	-	74.5	-
Enogen steam-flaked corn	-	74.5	-	74.5
Control corn silage	8.0	8.0	-	-
Enogen corn silage	-	-	8.0	8.0
Ground alfalfa	2.0	2.0	2.0	2.0
Sweet Bran®	12.0	12.0	12.0	12.0
Supplement[c]	3.5	3.5	3.5	3.5
Analyzed composition, g/kg of diet DM[d]				
Crude protein	140.4	142. 6	138. 9	141.1
Acid detergent fiber	74.3	83.1	67. 9	75.8
Ether extract	29.6	43.4	30.0	43.9
Calcium	7.1	7.2	7. 1	7.2
Phosphorous	2.9	3.7	2.8	3.7
Potassium	7.5	8.7	6.8	8.0

[a] Diets were top dressed with a premix containing 400 mg/animal/day of ractopamine (Optaflexx, Elanco Animal Health, Greenfield, IN) in a ground corn carrier for 35 d prior to harvest.
[b] DM=dry matter.
[c] Consisted of urea, salt, limestone, trace mineral premix, and potassium chloride, and provided 36.4 mg/kg monensin (Rumensin; Elanco Animal Health).
[d] Analyzed nutrient composition of monthly composites of each ingredient in diets. Weekly samples were dried in a 55C oven for 48 and composited on an equal weight basis (6 samples per ingredient; SDK Labs, Hutchinson, KS)

Table 4.2. Composition of control and Enogen corn silage utilized in finishing diets fed to beef steers.

Item, g/kg of DM[a,b]	Control silage	Enogen silage	SEM	*P*-value
Dry matter	269.6	356.7	2.89	<0.01
Acid detergent fiber	327.8	221.5	4.96	<0.01
Neutral detergent fiber	574.7	382.1	7.63	<0.01
Starch	27.9	307.3	7.14	<0.01
pH	3.93	3.78	0.019	<0.01
Analyzed composition, g/kg of DM[c]				
Crude protein	91.5	72.6	2.39	<0.01
Acid detergent fiber	361.5	270.7	5.20	<0.01
Ether extract	20.4	26.3	1.51	<0.01
Calcium	3.9	3.2	0.30	<0.01
Phosphorous	2.4	1.9	0.12	0.03
Potassium	22.8	12.9	2.37	0.04

[a] DM=dry matter.
[b] Analyzed nutrient composition of samples collected from silage storage bags prior to the start of the study.
[c] Analyzed nutrient composition of monthly composites of each ingredient in diets. Weekly samples were dried in a 55C oven for 48 and composited on an equal weight basis (6 samples per ingredient; SDK Labs, Hutchinson, KS)

Table 4.3. Characteristics and nutrient composition of control and Enogen steam-flaked corn.

Item	Control grain	Enogen grain	SEM	*P*-value
Steam-flaked corn characteristics				
Dry matter, g/kg	788.5	787.7	1.24	0.60
Starch availability, %[1]	52.6	53.9	0.31	<0.05
Analyzed nutrient composition, g/kg of DM[2]				
Crude protein	88.1	91.1	1.62	0.26
Starch	714.8	652.5	1.03	<0.01
Acid detergent fiber	28.4	40.2	1.33	<0.01
Ether extract	32.0	50.5	2.30	<0.01
Calcium	0.02	0.11	0.057	0.27
Phosphorous	1.8	2.9	0.20	<0.01
Potassium	3.1	4.7	0.18	<0.01

[1]Anlayzed by a refractive index.

[2] Analyzed nutrient composition of monthly composites of each ingredient in diets. Weekly samples were dried in a 55C oven for 48 and composited on an equal weight basis (6 samples per ingredient; SDK Labs, Hutchinson, KS)

Table 4.4. Feedlot performance of steers fed finishing diets containing steam-flaked control or Enogen corn steam-flaked in combination with control or Enogen silage for 138, 152, or 166 d.

	Control silage		Enogen silage			*P*-value		
Item[a,b]	Control grain	Enogen grain	Control grain	Enogen grain	SEM	Grain	Silage	Grain x Silage
Initial BW, kg	388	388	388	389	7.4	0.67	0.17	0.67
Final shrunk BW, kg[c]	655	656	664	655	6.4	0.17	0.12	0.07
ADG, kg	1.77	1.78	1.83	1.77	0.027	0.18	0.20	0.06
DMI, kg/d	10.29	10.48	9.95	10.04	0.149	0.15	<0.01	0.65
G:F	0.1720	0.1701	0.1838	0.1764	0.00189	0.02	<0.01	0.15

[a] Experiment was conducted with 960 beef steers in a randomized complete block design, with 12 pens per treatment.
[b] ADG=average daily gain; BW=body weight; DMI=dry matter intake; G:F=gain to feed.
[c] Gross BW was measured prior to loading cattle for transport to abattoir multiplied by 0.96 to account for 4% shrink.

Table 4.5. Carcass characteristics for finishing steers fed diets containing control grain and Enogen corn in combination with control or Enogen corn silage

	Control silage		Enogen silage			*P*-value		
Item[1,2]	Control grain	Enogen grain	Control grain	Enogen grain	SEM	Grain	Silage	Grain x silage
HCW, kg	403[a]	402[a]	408[b]	399[a]	3.6	<0.01	0.65	<0.01
Total liver abscesses, %	18.99	23.47	21.38	24.11	2.959	0.18	0.57	0.74
Liver abscess severity, %								
Mild	7.26	13.51	9.07	10.23	2.084	0.06	0.71	0.19
Moderate	4.69	2.97	4.32	5.96	1.371	0.98	0.33	0.21
Severe	6.90	6.84	7.41	7.76	1.828	0.93	0.67	0.90
12th rib fat thickness, cm	1.49	1.48	1.54	1.48	0.033	0.21	0.31	0.40
Marbling score[3]	485	487	486	485	6.9	0.99	0.99	0.84
USDA yield grade	2.75	2.73	2.78	2.71	0.069	0.42	0.95	0.59
Longissimus muscle area, cm^2	91.75	92.05	92.91	92.06	0.201	0.65	0.34	0.35
Quality Grade								
Prime, %	2.58	5.50	3.47	5.13	1.336	0.08	0.84	0.63
Choice, %	82.20	81.93	82.82	80.08	2.552	0.55	0.81	0.62
Select, %	14.42	11.77	13.33	14.41	2.357	0.72	0.73	0.40
Sub-select, %[4]	0.85	0.84	0.43	0.42	0.519	0.99	0.42	0.99

[1] Experiment was conducted with 960 beef steers in a randomized complete block design, with 24 pens having 25 animals per pen and 24 pens having 15 animals per pen. Steers were fed for 138, 152, or 166 d, with 12 pens per treatment.
[2] HCW=hot carcass weight
[3] 400 to 499= Small degree of marbling
[4] Carcasses graded as USDA Standard, Commercial, Utility, or Cutter.
[a,b] Means in row with different superscripts differ, $P<0.05$.

Table 4.6. Initial and final pH, and purge loss of *Longissimus dorsi* muscles following 21-d of wet aging.

	Control silage		Enogen silage			*P*-value		
Item[1]	Control grain	Enogen grain	Control grain	Enogen grain	SEM	Grain	Silage	Grain x silage
Initial pH	5.81	5.72	5.81	5.75	0.061	0.13	0.78	0.72
Final pH	5.72	5.69	5.70	5.71	0.023	0.55	0.99	0.41
Purge loss, %[2]	2.54	2.43	2.55	2.29	0.240	0.36	0.74	0.69

[1] Experiment was conducted with 960 beef steers in a randomized complete block design, with 24 pens having 25 animals per pen and 24 pens having 15 animals per pen. Steers were fed for 138, 152, or 166 d, with 12 pens per treatment. Two steers were randomly selected from each pen (96 loins, 24 per treatment) for collection of loins. pH was measured following harvest and following 21-d of wet aging in the geometric center of each loin.

[2] Purge loss was determined by initial weight minus weight of loins divided by initial weight.

Table 4.7. Long chain fatty acid composition of monthly composites of Enogen and conventional corn fed to beef steers for 138, 152, or 166 d.

Fatty acids, µg/g of DM[a]	Control grain	Enogen grain	SEM	*P*-value
C6:0	0.00	0.00	-	-
C8:0	0.00	0.00	-	-
C10:0	0.72	0.00	0.324	0.18
C11:0	0.00	0.00	-	-
C12:0	0.00	0.31	0.222	0.36
C14:0	1.38	2.81	0.327	0.33
C14:1	0.00	0.00	-	-
C15:0	0.00	0.00	-	-
C15:1	0.00	0.00	-	-
C16:0	475.44	676.06	25.824	<0.01
C16:1	4.31	6.49	0.337	<0.01
C17:0	3.70	5.54	0.213	<0.01
C17:1	0.00	0.55	0.246	0.17
C18:0	59.23	116.48	5.345	<0.01
C18:1n9c	826.26	1,094.11	52.924	<0.05
C18:1n9t	8.36	4.91	2.671	0.14
C18:2n6c	1,623.07	2,942.72	134.840	<0.01
C18:2n6t	0.00	0.00	-	-
C18:3n6	0.00	0.71	0.320	0.18
C18:3n3	50.43	76.60	3.723	<0.01
C20:0	0.00	0.00	-	-
C20:1n9	8.08	9.64	0.354	<0.05
C20:2	0.00	2.01	0.057	<0.01
C20:3n6	0.00	0.00	-	-
C20:3n3	0.00	0.00	-	-
C20:4n6	1.23	2.01	0.288	0.11
C20:5n3	0.48	1.45	0.477	0.09
C21:0	0.35	0.00	0.245	0.36
C22:0	5.72	7.73	0.275	<0.01
C22:1n9	0.00	0.00	-	-
C22:2	0.00	0.00	-	-
C22:6n3	2.46	2.70	0.550	0.77
C23:0	0.00	0.00	-	-
C24:0	7.59	11.23	0.541	<0.01
C24:1n9	2.31	3.41	0.599	0.25
Total fatty acids	3,081.10	4,967.46	218.61	<0.01

[a] DM=dry matter

Table 4.8. Long chain fatty acid composition of Enogen and control silage fed to beef steers for 138, 152, or 166 d.

Fatty acids, µg/g of DM	Control silage	Enogen silage	SEM	*P*-value
C6:0	0.00	0.00	-	-
C8:0	9.28	4.62	0.298	<0.01
C10:0	51.59	54.21	1.921	0.34
C11:0	4.43	0.26	0.151	<0.01
C12:0	12.47	7.68	0.299	<0.01
C14:0	9.92	7.30	0.246	<0.01
C14:1	1.48	2.49	0.207	<0.01
C15:0	2.62	0.78	0.162	<0.01
C15:1	0.00	0.00	-	-
C16:0	267.40	468.23	7.349	<0.01
C16:1	8.18	4.34	0.483	<0.01
C17:0	8.87	7.89	0.334	0.05
C17:1	0.00	0.11	0.075	0.31
C18:0	34.70	69.60	0.811	<0.01
C18:1n9c	44.94	634.31	9.365	<0.01
C18:1n9t	3.15	2.67	0.104	<0.01
C18:2n6c	322.74	1,066.19	21.919	<0.01
C18:2n6t	0.00	0.00	-	-
C18:3n6	184.39	127.95	19.417	0.05
C18:3n3	239.32	192.72	8.107	<0.01
C20:0	13.84	16.04	0.441	<0.01
C20:1n9	300.20	6.88	36.996	<0.01
C20:2	9.06	4.10	0.617	<0.01
C20:3n6	0.00	0.43	0.168	0.08
C20:3n3	0.13	0.00	0.102	0.36
C20:4n6	12.35	6.76	0.267	<0.01
C20:5n3	0.00	2.58	0.265	<0.01
C21:0	6.68	2.65	0.153	<0.01
C22:0	18.09	14.22	0.401	<0.01
C22:1n9	0.00	1.14	0.230	<0.01
C22:2	0.00	0.10	0.069	0.31
C22:6n3	3.63	1.24	0.206	<0.01
C23:0	0.00	0.00	-	-
C24:0	20.63	17.85	0.414	<0.01
C24:1n9	3.81	8.38	0.574	<0.01
Total fatty acids	1,593.90	2,727.48	50.608	<0.01

[a] DM=dry matter

Table 4.9. Fatty acid composition of longissimus lumborum steaks from steers fed silage and steam-flaked corn containing Enogen corn.

	Control silage		Enogen silage			*P*-value		
Fatty acid[1,2]	Control grain	Enogen grain	Control grain	Enogen grain	SEM	Grain	Silage	Grain x silage
C10:0	0.03	0.03	0.03	0.03	0.002	0.17	0.43	0.84
C11:0	0.01	0.02	0.00	0.01	0.003	0.08	0.94	0.36
C12:0	0.03	0.03	0.03	0.03	0.002	0.09	0.26	0.45
C14:0	1.31	1.31	1.35	1.58	0.102	0.22	0.12	0.25
C14:1	0.30	0.30	0.30	0.36	0.031	0.29	0.25	0.33
C15:0	0.23	0.21	0.25	0.29	0.019	0.73	<0.01	0.13
C15:1	0.03	0.03	0.03	0.03	0.001	0.35	0.09	0.85
C16:0	12.04	12.17	12.38	14.14	0.820	0.22	0.13	0.29
C16:1	1.72	1.70	1.73	1.97	0.131	0.34	0.25	0.28
C17:0	0.70	0.63	0.79	0.85	0.057	0.94	<0.01	0.24
C17:1	0.57	0.47	0.61	0.61	0.047	0.29	0.03	0.23
C18:0	6.24	6.49	6.51	7.27	0.419	0.19	0.18	0.52
C18:1n9c	16.94	16.64	17.16	18.93	1.227	0.51	0.26	0.35
C18:1n9t	0.56	0.46	0.43	0.50	0.119	0.92	0.68	0.46
C18:2n6c	1.86	2.45	1.89	2.24	0.084	<0.01	0.91	0.83
C18:2n6t	0.04	0.05	0.04	0.06	0.005	0.04	0.45	0.45
C18:3n6	0.04	0.04	0.04	0.04	0.002	0.69	0.35	0.89
C18:3n3	0.09	0.09	0.09	0.11	0.005	0.02	0.03	0.18
C20:0	0.03	0.04	0.03	0.04	0.002	0.07	0.15	0.28
C20:1n9	0.08	0.07	0.07	0.08	0.007	0.69	0.84	0.32
C20:2	0.04	0.04	0.04	0.05	0.002	0.02	0.77	0.38
C20:3n6	0.11	0.11	0.11	0.10	0.003	0.55	0.21	0.38
C20:5n3	0.04	0.04	0.04	0.04	0.004	0.95	0.32	0.77
C21:0	0.01	0.01	0.01	0.02	0.001	0.11	<0.05	0.20
C22:0	0.01	0.01	0.01	0.01	0.001	0.05	0.99	0.99
C22:6n3	0.01	0.01	0.01	0.01	0.002	0.69	0.89	0.89
C23:0	$0.44^{c,d}$	$0.47^{a,c}$	$0.45^{c,d}$	$0.43^{b,d}$	0.012	0.65	0.10	0.04
C24:0	0.02	0.02	0.02	0.01	0.001	0.84	0.15	0.07
C24:1n9	0.01	0.01	0.01	0.01	0.001	0.24	0.61	0.87
Total SFA	20.30	20.63	21.04	23.85	1.364	0.22	0.12	0.33
Total MUFA	19.07	18.75	19 29	21.37	1.383	0.48	0.25	0.34
Total PUFA	2.34	2.64	2.27	2.66	0.091	<0.01	0.78	0.92
Total Omega-6	1.94	2.34	0.97	2.34	0.088	<0.01	0.87	0.86
Total Omega-3	0.14	0.15	0.15	0.16	0.007	0.16	0.04	0.47
PUFA:SFA	0.12	0.14	0.11	0.12	0.006	0.04	0.02	0.09
Ω-6: Ω -3 ratio	14.51	16.50	13.70	15.08	0.877	0.04	0.18	0.71
Total fatty acids	43.52	43.76	44.46	49.86	2.891	0.29	0.19	0.33

[1] Milligrams per gram of wet sample

[2] SFA=saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA=polyunsaturated fatty acids.

[a,b,c,d] Means within same row with uncommon superscripts differed, $P<0.05$

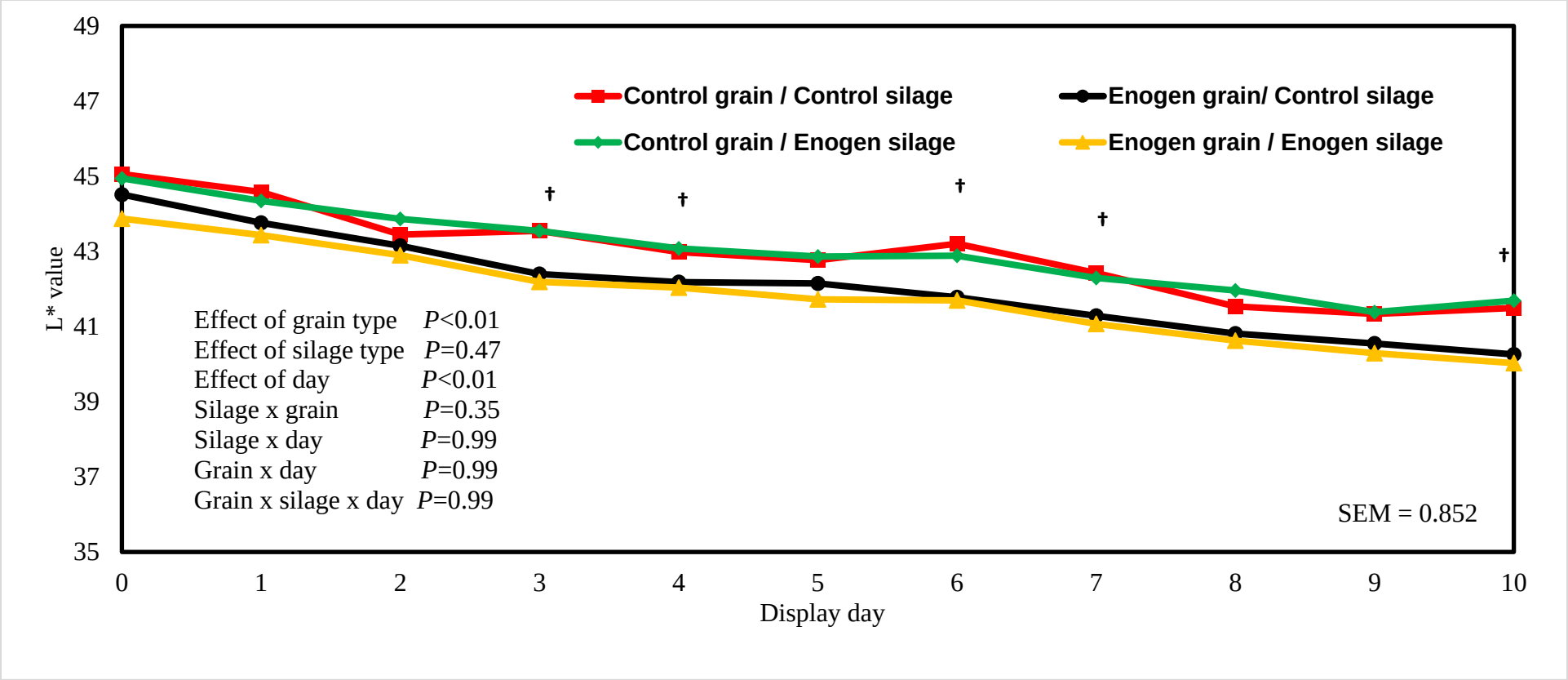

Figure 4.1. *Longissimus lumborum* steaks L* values following retail display from steers fed silage and steam-flaked corn produced from Enogen corn.

Steaks were displayed in a simulated retail display for 10-d (24 steaks per treatment). † Effect of grain type within day differed, $P<0.05$.

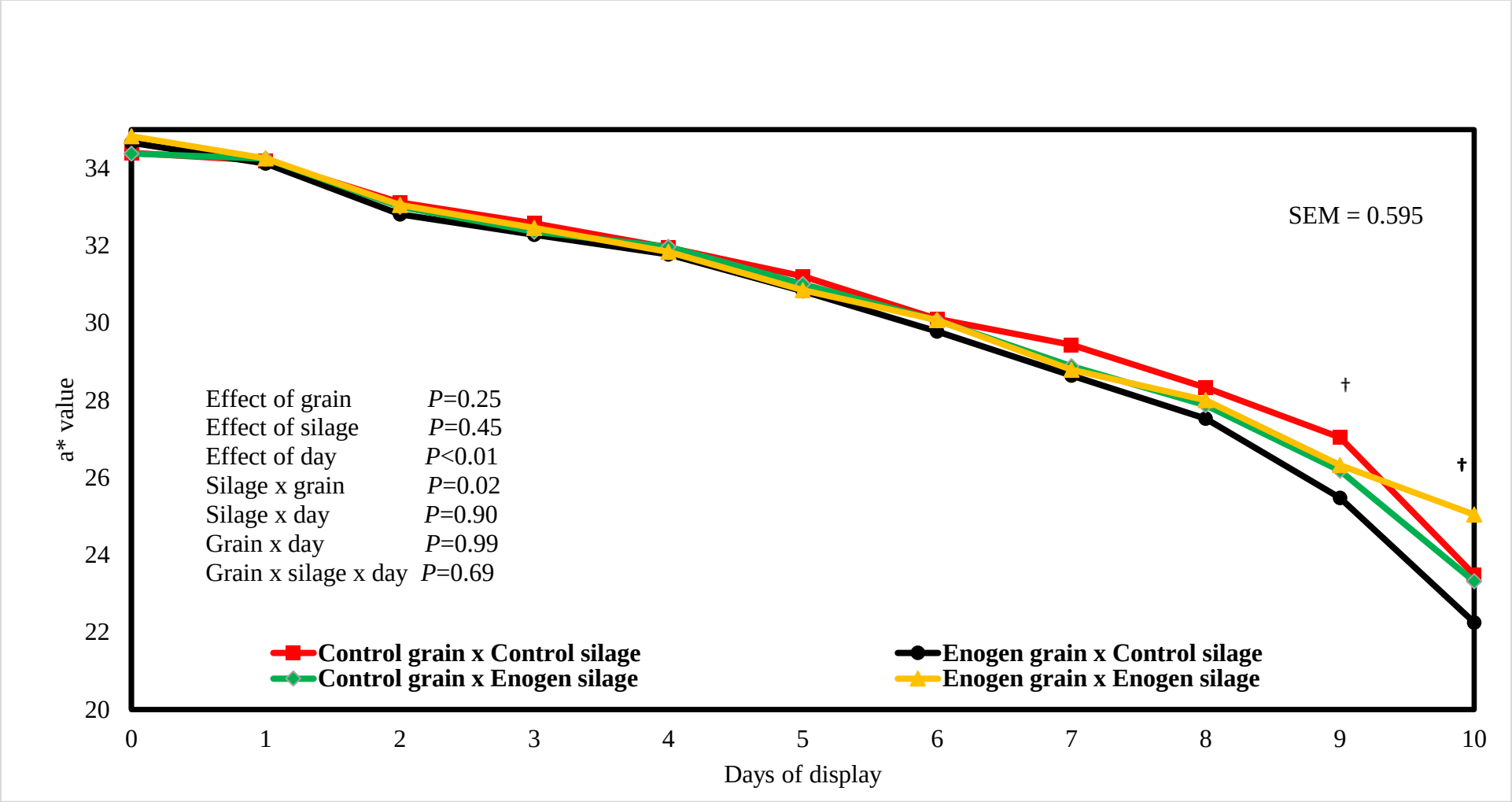

Figure 4.2. *Longissimus lumborum* steaks a* values following retail display from steers fed silage and steam-flaked corn produced from Enogen corn.

Steaks were displayed in a simulated retail display for 10-d (24 steaks per treatment). † Interaction of grain x silage within day, $P<0.05$.

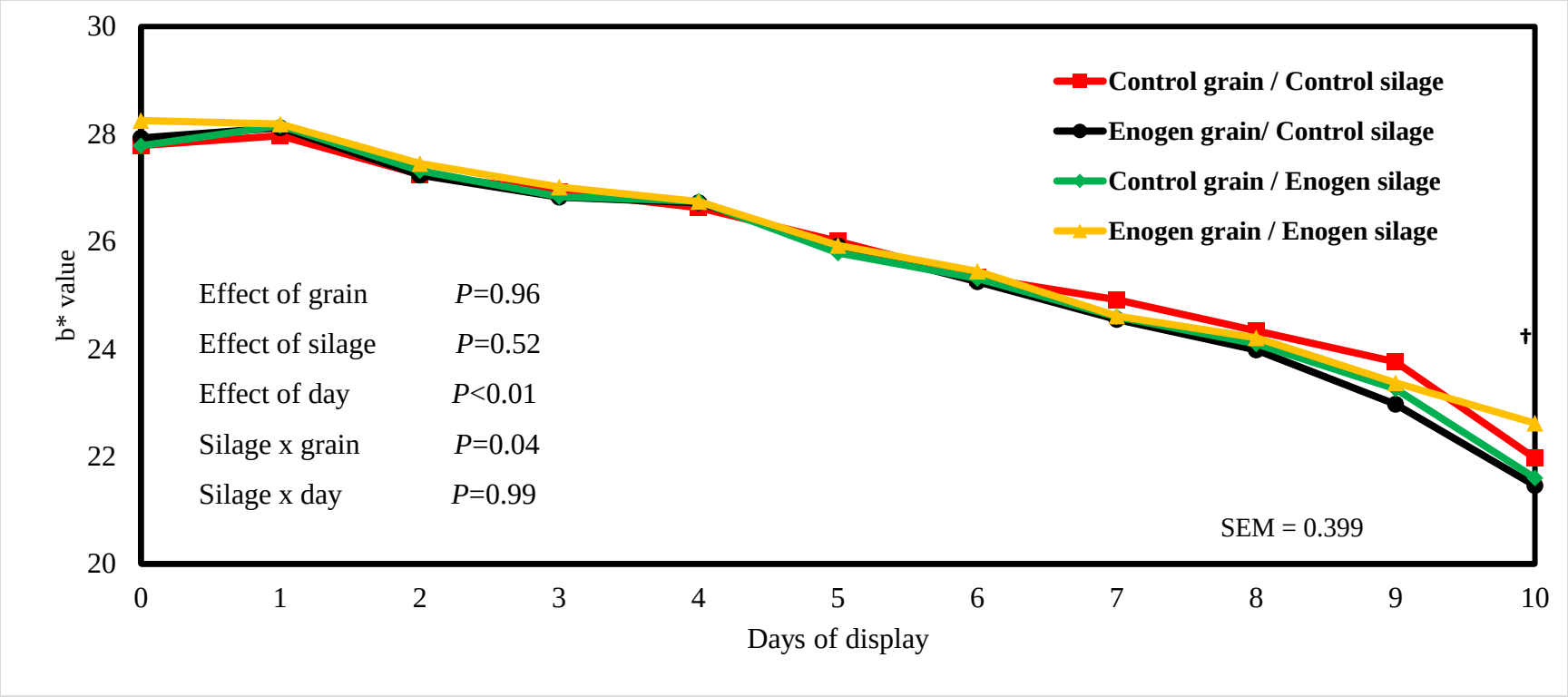

Figure 4.3. *Longissimus lumborum* steaks b* values following retail display from steers fed silage and steam-flaked corn produced from Enogen corn.

Steaks were displayed in a simulate retail display for 10-d (24 steaks per treatment). † Interaction of grain x silage within day, $P<0.05$.

Chapter 5 - The effects of a high-amylase corn hybrid as either steam-flaked corn or combination of high-moisture corn/dry-rolled corn on site and extent of digestion in steers fed finishing diets

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Abstract

Enogen Corn (EC; Syngenta Seeds, LLC) was developed to express high-concentrations of alpha-amylase to facilitate fermentation of starch to ethanol. This genetically modified corn has been investigated as a grain source for finishing diets. Our objective was to evaluate rate of starch fermentation and site and extent of digestion of finishing diets comprised of EC fed as a combination of high-moisture corn (HMC)/dry-rolled corn (DRC) or steam-flaked corn (SFC). Two separate experiments were conducted as replicated crossover designs with two treatments (EC or Control) in 4 periods (15 d). The first experiment utilized 7 Holstein steers equipped with ruminal, duodenal, and ileal cannulas to compare diets comprised of a combination of HMC/DRC as EC or control (CON). Each period consisted of incubating *in situ* bags filled with non-EC in the rumen for 0, 4, 8, 12, and 16 h on d 10. Digesta samples were collected in 8 h intervals on d 11 to 14, increasing by 2 h each day to represent a 24-h post-feeding cycle. Ruminal samples were collected for analysis of pH, ammonia, and volatile fatty acid concentrations (VFA). Corn hybrid did not affect rate of ruminal starch disappearance (P>0.01). Similarly, corn hybrid did not affect percent ruminal or total tract starch digestion (*P*>0.10). Ruminal fluid from steers fed CON contained greater concentrations of acetate compared to those fed EC (80.4 vs 76.5 mM, respectively; *P*=0.05), and inversely, butyrate concentrations tended to be greater for steers consuming EC treatments compared to CON (20.8 vs 19.2 mM, respectively; *P*=0.09). Experiment 2 utilized the same study design, but compared SFC as either EC or CON. Enogen corn was flaked to 390 g/L, while CON was flaked to 360 g/L to produce similar starch availabilities. Similar to the previous experiment, EC did not affect rate of ruminal starch disappearance or total tract digestion of starch (*P*>0.10). Butyrate concentrations were greater in ruminal fluid from steers fed EC compared to those fed CON (23.3 vs 18.7 mM,

respectively; $P<0.01$); however, acetate concentrations were similar between treatments ($P=0.78$). Enogen corn fed as either a combination of HMC/DRC or SFC did not alter digestion of starch but did affect individual VFAs produced in the rumen.

Keywords: Dry-rolled corn, Enogen Corn, high-moisture corn, steam-flaked corn

Abbreviations: Acid detergent fiber, ADF; Control corn, CON; dry matter, DM; dry-rolled corn, DRC; Enogen Corn, EC; high-moisture corn, HMC; organic matter; OM; mean geometric particle size, D_{gw}; standard deviation of particle size, S_{gw}; steam-flaked corn, SFC; neutral detergent fiber, NDF; volatile fatty acids, VFA;

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Introduction

Corn is the primary grain processed and fed in beef cattle finishing diets (Samuelson et al., 2016). Depending on degree of processing, digestibility of starch increases, potentially improving feedlot performance (Owens et al., 1997). A review by Owens et al. (1986) outlined potential limitations to intestinal starch digestion in ruminants, including limitations in secretion of pancreatic alpha-amylase.

Enogen Corn (Syngenta Seeds, LLC) is a corn hybrid developed to reduce necessity for exogenous alpha-amylase in fuel ethanol production from cereal grains, and has been investigated for use as corn source to improve starch utilization in beef cattle. Feeding of EC as SFC improved feedlot performance, in spite of the fact that EC was steam flaked to 390 g/L, while non-EC was flaked to 360 g/L (Horton et al., 2020). Zinn (1990) compared three flake densities in a digestibility experiment with SFC and observed decreasing flake density improved total tract starch digestion. Improved feedlot performance observed by Horton et al. (2020), with

EC flaked to a greater flake density compared to conventional corn is counter intuitive to observations by Zinn (1990).

Jolly-Breithaupt et al. (2016) compared site and extent of digestion of EC to an isoline corn fed as DRC. Enogen Corn numerically improved ruminal and post ruminal starch digestion. Furthermore, total tract digestion of starch was greater for the EC treatments compared to the isoline control (P=0.02). Therefore, we hypothesized that feeding EC as a combination of HMC/DRC or as SFC would alter rate of ruminal starch fermentation and site and extent of starch digestion.

Materials and methods

Two digestibility experiments were approved and conducted as approved by the Kansas State University Institutional Animal Care and Use Committee.

Study design

Both experiments utilized 7 Holstein steers (average BW = 471 kg). Steers were surgically fitted with ruminal, duodenal, and ileal cannulas, and housed in a metabolism barn with slatted floor individual pens equipped with feed bunks and waterers. Both experiments were conducted as replicated cross-over designs with two treatments (CON or EC). Treatments were randomly assigned to animal within a sequence. Each sequence consisted of a cross-over of treatments, with each sequence comprised of two collection periods. Sequence was replicated in duplicate, with each experiment comprised of four 15-d periods.

Experiment 1

Experiment 1 consisted of a combination of Enogen HMC and Enogen DRC compared to a combination of non-Enogen HMC and non-Enogen DRC. Diets for experiment 1 are shown in Table 5.1.

For this experiment, Enogen and an isoline control corn utilized for producing HMC were grown in adjacent fields and harvested at approximately 700 g/kg of DM. Grains were transported approximately 32 km to the Beef Cattle Research Center at Kansas State University, where grains were ground using a commercial grinder equipped with 12.7 mm screen (H1030; Haybuster, Jamestown, ND) and then packed into upright concrete silos for ensiling and storage. Additionally, aliquots of both corn hybrids were received at the Beef Cattle Research Center and transported to a commercial feed mill to be dry rolled to similar particle size (~3500 μm).

High-moisture corn was removed from silos daily to mix into rations and samples were collected. Diets were mixed daily and fed *ad libitum*. On d 4 through 14, 10 g of titanium dioxide was top-dressed onto feed in individual feed bunks and hand-mixed into diets for use as an indigestible marker. Samples of each high-moisture corn type were collected daily, a portion was placed in a 105ºC oven for 24 h to monitor DM content of the ensiled grains and a portion was utilized for particle size analysis with a Ro-Tap (W. S. Tyler; Mentor, OH). High-moisture corn DM was monitored and updated as-needed to maintain formulated DM proportions of HMC in the diet. Additionally, HMC and DRC samples were collected daily and composited within each sampling period for analyses. Particle size was determined for DRC aliquots prior to the start of the study by probing bulk containers utilized for storage. Samples of mixed rations and orts were collected daily and dried in a 55ºC oven for 48 h. Dried samples were ground utilizing a 1-mm screen and composited on a DM basis within each period.

The experiment consisted of four 15-d periods consisting of an initial 10 d for diet adaption and 5 d for sampling. Day 10 was utilized for determining rate of ruminal starch disappearance by incubating *in situ* bags (5 cm x 10 cm; Ankom Technology, Macedon, NY) for 0, 4, 8, 12, and 16 h. Approximately 3 kg of non-EC corn was ground through a 1-mm screen

and utilized as the substrate for *in situ* analyses. Dacron bags were prepared in duplicate for each incubation time with approximately 1 g ground corn on a DM basis. Dacron bags were placed into large mesh bags with weights and placed into the rumen via the ruminal cannula. Dacron bags designated as time point 0 were placed into the rumen for 2 min and then removed. Following incubation, bags were placed into containers with clean cool water, and gently rinsed. Bags were then placed into a 105ºC oven for 48 h to determine disappearance. Data were corrected for 0 h disappearance by subtracting disappearance of weight at h 0 from the initial sample weights at other time points.

The first 4 d of the sampling portion of the period consisted of collection of duodenal, ileal, and fecal material starting at h 0 and collecting at 8-h intervals. Each day, through d 14, collection times advanced 2-h to represent a 24-h post-feeding cycle. Duodenal, ileal, and fecal samples were collected and stored in Whirl-Pak bags (Madison, WI) in a -20ºC freezer for analysis. Additionally, approximately 500 mL of ruminal contents were collected at h 0 and advanced 6-h each day to represent a 24-h post-feeding cycle. Ruminal contents were blended (Commercial Blender CB15; Waring Commercial, Torrington, CT) to separate particle associated bacteria for determination of microbial crude protein synthesis. Blended samples were strained through 8-layers of cheesecloth and filtrate was stored at -20ºC for further analysis. At h 0 on d 15, steers were pulse dosed via the ruminal cannula with a 200-mL solution containing 3 g of Co-EDTA. Ruminal contents were mixed, and samples were collected at h 0, 2, 4, 6, 8, 10, 12, 18, and 24. Each sampling time, ruminal contents were collected, strained through 4-layers of cheesecloth, and pH was measured using a portable pH meter (ThermoScientific Orion 3-star portable pH meter; Waltman, MA). An unacidified 20-mL aliquot of filtrate was retained for

analysis of cobalt and an 8-mL aliquot was retained with the addition of 2 mL of a 25% meta-phosphoric acid solution into vials stored at -20ºC for determining VFA concentrations.

Duodenal and ileal digesta samples were lyophilized (Genesis Model 35EL; SP Scientific, Warminster, PA) and composited on an equal dry weight basis for each animal within each period. Additionally, fecal samples were dried in a 55ºC oven for 48 h, ground through a 1-mm screen and composited on an equal dry weight basis within each animal for each period. Feed, orts, and digesta composites were sent to a commercial laboratory (SDK Labs; Hutchinson, KS) for analysis of nitrogen, starch, and OM. Composited orts and digesta samples were analyzed for TiO_2 as described by Titgemeyer et al. (2001). Approximately 0.3 g of digesta and 0.6 g of orts samples were weighed in duplicate. Samples were ashed overnight and 1 g of sodium sulfate was weighed into each beaker. Samples were then digested using 5 mL of sulfuric acid heated to 280ºC for 15 min. Deionized water was added to each sample to achieve a final sample weight of 50 g. A 250-µL aliquot of each sample was transferred into 96 well plate and 30 µL of 30% hydrogen peroxide was added to each well. Samples and standards were read at 410 nm utilizing a plate reader to determine titanium concentration.

True nitrogen and organic matter digestibility was determined by isolating ruminal bacterial cells and utilizing cytosine as a marker for microbial fermentation. Bacterial cells were isolated from blended ruminal samples by differential centrifugation as described by Cecava et al. (1990). Bacteria cells were lyophilized, as previously described, and composited for each steer within each period. Isolated bacterial cells were analyzed for OM by placing in a 450ºC muffle oven for 12 h and nitrogen was determined using a LECO TruMac N Analyzer (LECO Corporation, Saint Joseph, MI). Bacterial and duodenal cytosine concentrations were determined as described by Milton et al. (1996). Samples were analyzed using an HPLC (1200 Series;

Agilent Technologies, Santa Clara, CA) equipped with a C18 column (4.6 mm x 100 mm, 3.5 µm particle size, Eclipse Plus C18; Agilent Technologies) and utilized an eluent consisting of 20 mM potassium phosphate buffer adjusted to a pH of 7.00, with at a flow rate of 1 mL/min. An internal standard of 5-methyl cytosine was utilized to correct cytosine concentrations in each sample. A ratio of microbial cytosine: nitrogen was determined and utilized to calculate duodenal nitrogen flow from microbial origin by dividing duodenal cytosine concentration by the microbial cytosine:nitrogen ratio. Similarly, true organic matter digestibility was calculated by determining the cytosine:organic matter ratio.

Acidified ruminal fluid samples were analyzed for VFA and ammonia concentrations. Samples were centrifuged and VFA concentrations were determined utilizing gas chromatography equipped with a flame ionization detector (Agilent 7890 GC; Agilent Technologies, Santa Clara, CA.) equipped with a Nukol capillary column (15 m x 0.35 mm x d_f 0.50 µm; Supelco, Bellefonte, PA). Standards were prepared for quantification of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate, isocaproate, and heptanoate. Ammonia concentrations were determined using a method outlined by Broderick and Kang (1980) and wavelength measured using a plate reader (BioTek PowerWave XS plate reader; BioTek, Winooski, VT).

Cobalt concentrations were measured in unacidified ruminal samples by atomic absorption (Perkin Elmer AAnalyst 100; PerkinElmer, Waltham, MA). Samples were centrifuged at 25,000 g for 25 min. Supernatant was transferred into test tubes and samples from h 0, 2, 4, 6, and 8 were diluted to ensure concentrations were within the linear range of the assay.

Experiment 2

Experiment 2 was conducted utilizing the same 7 canulated Holstein steers from the previous experiment. This experiment utilized a similar study design, except treatments consisted of diets containing either EC or CON as SFC (Table 5.2). Corn was steam-flaked daily to bulk densities of 360 g/L for CON corn and 390 g/L for EC as previously determined by Horton et al. (2020) to achieve similar starch availabilities between grain types. Dry matter and particle size was determined daily, as described in experiment 1. Additionally, starch availabilities were determined by heating 25 g of corn flakes in a 100 mL solution containing 2.5%-amyloglucosidase solution for 15 min at 55ºC (Sindt, 2004). The solution was filtered, and percent solubles were determined using a handheld refractometer. Percent solubles were corrected for DM and starch availability was determined using regression equations as described by Sindt (2004). This experiment was conducted similar to the previous experiment with respect to sample collection and sample processing except for collection of ruminal samples for VFA, ammonia, and pH analysis. These samples were collected concurrently, during collection of digesta samples on d 11 through 14. This was done to represent a 24-h post-feeding cycle. Dosing of CoEDTA solution and collection of ruminal fluid occurred on d 15 as previously described in experiment 1.

Statistical analyses

Data from both experiments were analyzed utilizing MIXED procedure in SAS (Version 9.4; SAS Inst. Inc., Cary, NC). Intake, flow, and digestibility data from each experiment were analyzed with sequence, period, and grain type included in the model. Animal within sequence was utilized for the random effect. Ammonia concentration, pH, and VFA from each experiment were analyzed using repeated measures. Heterogeneous compound symmetry covariance

structure was utilized for analyses, with the repeated measure defined as hour and subject as animal. Sequence, period, and grain type was included in the model and animal within sequence was utilized as a random effect.

Results

Experiment 1

Differences in corn types from both HMC and DRC are shown in Table 5.3. Composition of DRC and HMC between grain types differed in several components. Control HMC was greater in DM than EC ($P<0.01$). Starch content of EC HMC was greater than CON (743 vs. 721 g/kg of DM; $P<0.05$) and inversely CP was lower for EC compared to CON (81 vs. 85 g/kg of DM; $P<0.01$) . Similar to HMC, starch content for DRC was approximately 4.8% greater for EC compared to CON (712 vs 680 g/kg of DM; $P<0.05$) and CP was greater for CON than EC (; 93 vs 85 g/kg of DM; $P<0.05$). Particle size did not between HMC grain types ($P=0.90$), but was greater for dry-rolled CON corn compared to dry-rolled EC (3,404 vs 3,137 µm; $P<0.01$) even though both were processed using similar parameters to achieve similar particle size.

Nutrient flow and digestibility from experiment 1 did not differ between treatments. Data from this experiment is expressed in Table 5.4. Numerically, steers consuming the EC treatment consumed more DM, OM, and starch though this did not affect digestibility or flow ($P>0.10$). Starch digestion in the small intestine as a percent of intake did not differ ($P>0.10$), but was numerically greater for EC compared to CON (47.8 vs 21.9 %). Additionally, starch flow from the small intestine was numerically greater for CON than EC (656 vs 433 g/d; $P>0.10$). Intake starch disappearance in the small intestine was numerically greater, with steers fed the EC grain combination having greater starch disappearance than cattle fed CON grain combination (8.5 vs 6.3).

Fluid passage rate was measured by dosing steers with CoEDTA and passage rate were similar between EC and CON treatments (8.62 vs 7.59%/h, respectively; P=0.20). Uncorrected rate of ruminal starch disappearance is illustrated in Figure 5.1A. and corrected for zero-hour starch disappearance is illustrated in Figure 5.1B. An interaction between grain type and hour was not observed (P>0.10). An effect of hour was observed for ruminal starch disappearance (P<0.05), but was not affected by grain type (P>0.10). Ruminal fermentation was evaluated by measuring pH, VFA, and ammonia concentrations at h 0, 2, 4, 6, 8, 12, 18, 24 post-feeding. There were no interactions between grain type and hour for pH, VFA, and ammonia concentrations as illustrated in Figure 5.2 and 5.3. Ammonia and pH were similar between grain types (P>0.10); however, were affected of hour (P<0.05). An effect of grain type was observed for acetate, heptanoate and isocaproate (P<0.05), but no other VFAs. Acetate concentrations were affected by grain type, with steers fed CON treatment observed to have greater acetate concentrations compared to steers fed EC (80.4 vs 76.5 mM; P=0.05). Propionate concentrations were similar between grain types (55.5 vs 55.1 mM; P=0.85); however, a tendency for an effect of grain type was observed, with butyrate concentrations tending to be greater in ruminal fluid of EC fed steers compared CON fed steers (20.8 vs 19.2 mM ; P=0.09). An effect of grain type was observed in steers fed CON grain combination having greater concentrations of isocaproate than cattle fed EC (0.23 vs 0.20 mM; P<0.01) and similarly, heptanoate concentrations in steers fed the CON treatment were greater than EC fed cattle (0.11 vs 0.06 mM; P<0.01).

Experiment 2

Similar analysis was conducted in experiment 2 as was in experiment 1. Although, in this experiment, SFC was fed as the concentrate as either EC or CON. There were no comparisons made between experiment 1 and 2.

Composition of steam-flaked corn types are presented in Table 5.5. Starch concentrations did not differ between hybrids (*P*=0.21), although were numerically lower for EC grain compared to CON grain. Particle size differed between hybrids (P<0.01); however, this was expected, due to being flaked to different bulk densities.

Nutrient flows and digestibility are illustrated in Table 5.6. Nutrient intake did not differ between treatments (*P*>0.10). Flow of nutrients to the duodenum were similar, though numerically the EC treatment had 27.5% greater starch flow to the duodenum than CON (*P*=0.11). Ruminal starch digestibility for steers fed CON treatment was approximately 2.9% greater than that of EC treatment (*P*=0.11). Digestibility and flow of nutrients were similar in the small intestine between grain types (*P*>0.10). Similarly, digestibility in the large intestine was not affected by grain type (*P*>0.10), with the exception of nitrogen disappearance as a percent of entry. Numerically, nitrogen flow was greater from the small intestine for the steers fed CON grain compared to EC (60 vs 62 g/d; *P*=0.57); however, nitrogen flow in feces was numerically lower for CON treatment than EC (49 vs 52 g/d; *P*=0.37). The differences between entry and output of nitrogen in the large intestine tended to be greater for steers fed CON grain compared to receiving EC grains (17.8 vs 8.2 %, *P*=0.09). Total tract digestion of OM, starch, and nitrogen were similar between grain types (*P*>0.10).

Nutrient flows were not affected by grain hybrids; however, ruminal fermentation products differed between grain types. Figures 5.5 and 5.6 illustrate the interaction between grain type and hour for pH, ammonia, VFAs, and acetate:propionate ratio. Interactions between grain type and hour were not observed (P>0.10). Ammonia concentrations differed between hour post-feeding (P<0.01), but did not differ between grain type (4.99 vs 4.59 mM; *P*=0.22). An effect of hour was observed a majority of VFA analyzed (*P*<0.01) and similarly, total VFA concentrations

(P<0.01). Acetate concentrations were not affected by grain type (P=0.78); however, propionate concentrations were affected by grain type, with greater concentrations in ruminal fluid from steers fed CON grain compared to EC grain (54.0 vs. 44.1 mM; P<0.01). Butyrate concentrations in ruminal fluid were affected by grain type, with greater concentrations of butyrate being observed in steers fed EC grain compared to CON (23.3 vs 18.7 mM; P<0.01). Steers consuming the CON grain treatment tended to have greater total VFA concentration than steers fed EC grain (149.5 vs 144.6 mM; P=0.06).

For this experiment, a common cytosine to nitrogen ratio was utilized due to issues with the analysis. Cytosine concentrations were affected by period (P<0.05). Data from period 4 differed from period 2 and 3, but were similar to period 1 (data not shown; P<0.05). This may be due to processing of ruminal fluid during collection, freezing, or differential centrifugation. Cytosine concentrations were not affected by grain type for EC and CON (0.0295 vs 0.0280 mg/g, respectively; P=0.57), thus an average cytosine:nitrogen ratio of 0.288 mg/g was utilized for determining microbial nitrogen flow and microbial efficiency for both treatments. Experiments similar to ours have utilized methodology to accommodate experimental error involved determining microbial synthesis (Cooper et al., 2002; Sindt et al., 2006b).

Discussion

The objective of these experiments was to evaluate the impact of EC on rate of ruminal fermentation, site, and extent of starch digestion. Digestion of starch is of considerable interest due to grain inclusion of beef cattle finishing diets is over 50% on a DM basis (Samuelson et al., 2016). Of the potential sites of digestion in ruminants, small intestinal starch digestion is of greater interest, due to being more energetically efficient than ruminal fermentation; however, ruminants have limited ability to digest starch due to limited pancreatic amylase secretion

(Owens et al., 1986). Horton et al. (2020) fed steam-flaked EC in finishing heifer diets and reported greater ADG, which improved feed efficiency by 5%. Total tract starch digestion with SFC is generally 99% and would mean that site of digestion would have to have been altered by EC grain to improve performance. Jolly-Brieihaupt et al. (2019) observed a similar response when comparing EC to conventional corn in finishing diets as DRC. A digestibility experiment, comparing EC to an isoline control as DRC, observed an improvement in total tract digestibility of starch, OM, and DM (Jolly-Breithaupt et al., 2016). To our knowledge, these experiments are the first to compare digestibility of SFC and a combination of HMC/DRC produced from EC in finishing diets.

Total tract digestibility of starch from experiment 1 was approximately 93 to 94% for both grain types, which was lower than experiment 2 (approximately 99%). This coincides with a review of digestibility experiments by Owens and Zinn (2005) that observed digestibility of SFC is approximately 99%. In the same review, total tract disappearance for DRC and HMC is approximately 89 and 99%, respectively. This data would suggest a 50:50 mixture of DRC and HMC would be between these reported values. In these experiments, total tract digestion was similar between respective treatments. Although, in the case of SFC and HMC it would be challenging to observe differences, due to total tract digestion is approximately 99%. Several feedlot performance experiments have been conducted comparing EC to a non-EC grain type and reported varied results. As mentioned previously, total tract starch digestion of SFC is generally 99% and would mean that site of digestion would have to have be altered by EC grain to improve performance.

In both experiments, inclusion of grain was approximately 80% of the diet on a DM basis. Differences in characteristics of the hybrids may have been a factor that impacted the

results of these experiments. Enogen corn in experiment 1, had starch content compared to control corn. Though not significant, this likely caused greater starch intake of the steers receiving the EC treatment, which altered the quantity of starch flow through the digesta tract. Similarly, EC steam-flaked corn in experiment 2 had numerically lower starch levels compared to CON corn, although these levels are within the range commonly observed in corn grain (Garcı´a-Lara et al., 2019). It can be challenging to differentiate the effect of EC due to the increased alpha-amylase or the characteristics of the corn grain itself. Corona et al. (1990) evaluated digestibility of four corn hybrids provided by the same seed company and determined vitreousness of the corn hybrid can affect digestibility, thus making it challenging to discern differences in individual characteristics between hybrids as multiple characteristics within a grain may affect digestibility.

Ruminal digestibility of starch, protein, or organic matter were similar between grains as both SFC or HMC/DRC combination. Additionally, rate of ruminal starch disappearance was measured using *in situ* technique and were similar between treatments. Although, in these experiments the corn placed in the *in situ* bags was a non-EC type and was used to determine the impact of EC on amylase activity in ruminal fluid. Klingerman et al. (2009) evaluated exogenous amylase activity in ruminal fluid incubated for 0, 3, 6, and 24 h and observed stability of alpha-amylase activity. Although, an *in situ* experiment evaluating proportions of EC with conventional corn observed a linear increase in disappearance as proportion of EC increased (Horton et al., 2020). Similarly, another *in situ* experiment utilized ground corn treated with increasing levels exogenous amylases and observed a linear improvement in disappearance with higher levels of amylase addition (Gutiérrez et al., 2005). Results from our experiments would

suggest that the impact of EC is limited to the grain itself and does not alter enzyme activity of the ruminal environment.

Ruminal digestibility of nutrients may have been similar between grain types, but ruminal fermentation products differed between hybrids. In experiment 1, acetate concentrations in ruminal fluid of steers fed DRC/HMC combination from EC were less compared to CON and tended to have greater butyrate concentrations. Similarly in experiment 2, butyrate concentrations were greater in steers fed steam-flaked EC compared to steam-flaked CON. These results differ from other authors who have performed similar experiments with EC. An experiment evaluating EC as silage on ruminal fermentation of dairy cows observed a decrease in butyrate concentrations in ruminal fluid (Cueva et al., 2021). Jolly-Breithaupt et al. (2016) observed no difference in individual VFA in ruminal fluid in steers consuming EC or non-EC corn as DRC. It is apparent from these experiments, that EC as either HMC/DRC combination or SFC in beef cattle finishing diets altered ruminal fermentation products. This may be due to enzymatic activity during steam flaking or ensiling altering degree of polymerization of starch, thus altering substrate for ruminal fermentation.

As previously discussed, improving small intestinal digestion of starch offers opportunity to improve efficiency in ruminants. Although, not statistically tested, post-ruminal starch digestion between the two experiments was greater for SFC compared to the combination of HMC/DRC. This is likely attributed to grain processing method and not the grain type. Cooper et al. (2002) compared DRC, HMC, and SFC to evaluate ruminal and total tract starch digestion. In this experiment, the authors observed approximately 99% post-ruminal starch digestion from SFC, while HMC or DRC was observed at approximately 85%. In these current experiments, intestinal digestion of starch was similar between grain types with either SFC or HMC/DRC

combination. Flow of starch to the duodenum was numerically greater for EC compared to CON in both experiments and DMI was numerically greater for EC treatment in both experiments, which likely increased starch flow to the duodenum. In the first experiment, with a combination of HMC/DRC, there was numerical increase for starch digestion as a percent of intake for steers consuming the EC treatment compared to CON grain combination. Starch digestion as a percent of entry was not affected by grain type; however, steers fed EC grain as DRC/HMC combination had a numerical two-fold increase in starch disappearance in the small intestine. Jolly-Breithaupt et al. (2016) observed feeding EC as DRC did not impact post-ruminal digestion of starch, but similar to our experiment, EC appeared to numerically improve post-ruminal digestion of DRC/HMC combination. Steam flaking EC did not affect small intestine digestibility of starch. In a review by Huntington et al. (2006), the authors evaluated the relationship between starch entry into the small intestine and starch digested. They concluded that as starch (g/d) flow increased, efficiency of digestion decreases in ruminants. This was not the case in these two experiments, flow of starch to the duodenum was numerically greater for EC in both experiments, but starch digestibility of entry to the small intestine was similar between treatments.

During the preparation of experiment 1, whole corn from both hybrids was dry rolled to achieve similar micron size between grain types to limit potential differences that may affect digestibility. Similar roller mill settings were utilized during processing, but mean geometric particle size of EC was smaller than CON grain by approximately 300 µm. Scott et al. (2003) compared several corn processing methods in two finishing experiments. In one of their experiments, the authors compared DRC (4,619 µm) to finely DRC (3,991 µm) and observed no difference in performance or carcass characteristics; however, did observe a decrease in DMI

when feeding finely ground corn (715 µm). Similarly, Galyean et al. (1979) evaluated ruminal and intestinal digestion of starch from whole corn and corn ground to three different particle sizes (3.18, 4.76, and 7.94 mm). In this experiment, the authors did not observe differences between ground particle sizes, only differences between the effect ground versus whole corn. It would seem unlikely that the difference in particle size associated with the DRC portion of the diet in this experiment would impact the results, but could account for the numerical increase in starch flow to the duodenum.

Particle size of SFC differed between grain types, and starch availabilities tended to differ between SFC grain types in experiment 2. Particle size of the DRC in the previous experiment was approximately 300 µm difference between grain types; however, in this experiment particle size was approximately 800 µm difference between flaked grain types. Average geometric particle size was less for EC grains compared to CON, although flaked to a greater flake density. Enogen corn was flaked to 390 g/L to achieve similar starch availably as CON grain flaked to 360 g/L, due to a correlation between starch availability and its effects on digestibility (Zinn et al., 2002). The difference in bulk density and similarity in starch availabilities demonstrates the effect EC grain has on processing characteristics. Similar to this study, Horton et al. (2020) showed a decrease in particle size with the greater flake density of EC. This is due to more fines associated with flaking EC, which may affect nutrient flow. Sindt et al. (2006) fed heifers SFC (4,667 µm) and SFC that had been excessively handled to break up the flakes to a smaller particle size (3,330 µm), which would have created more fines and observed no difference in feedlot performance. The impact of fines associated with steam-flaked corn on digestibility is unknown, but in this experiment starch flow to the duodenum was numerically greater in steers fed steam-flaked EC compared to CON. Additionally, a numerical increase in ruminal starch

digestibility of steam-flaked CON and a tendency for increased total VFA concentration compared to steam-flaked EC may be attributed to more small starch particles from EC flaked corn bypassing ruminal fermentation.

The lack of effect of grain type on starch digestion in experiment 1 may be due to the ensiling of the HMC. Hoffman et al. (2011) observed during 240-d of ensiling HMC, the starch/protein matrix is degraded, which improves starch and nitrogen digestibility. In the current experiment, the HMC was ensiled for approximately 180-d and may have allowed starch digestibility of HMC/DRC from either EC or CON to be similar. In experiment 1, a tendency for greater nitrogen digestibility was observed in the small intestine. This may be due to the amylase activity of the EC improving separation of the starch/protein matrix and improving proteolytic enzyme degradation of zein proteins. Nitrogen digestibility in experiment 2 was similar between grain types; however, Zinn (1990) observed decreasing flake density improved digestion of nitrogen in the small intestine. This is attributed to decreasing flake density mechanically separating the starch/protein matrix, which improves starch and nitrogen digestion (Zinn et al., 2002). The flake densities utilized for EC and CON would have an expected difference in starch and nitrogen digestion. Amylase activity in EC grain may provide separation of the starch/protein matrix similar to the mechanical separation observed by decreasing flake density.

From these experiments, it is still unclear how EC has improved performance reported by researchers. Horton et al. (2020) observed a 5% improvement in feed efficiency and hypothesized this may be due to EC altering site of starch digestion. These experiments did not provide data to support that hypothesis. It may be beneficial to re-evaluate EC as SFC or HMC/DRC combination in similar experiments that restrict feed intake. This would allow for

similar nutrient flows to the duodenum and more accurately detect difference in starch or nitrogen digestion in the small intestine.

Conclusions

In these experiments, EC did not affect site and/or extent of starch digestion compared to conventional corn. Feeding EC as HMC/DRC combination appears to numerically improve digestibility of starch that enters the small intestine and improve nitrogen digestion in the small intestine. This may be beneficial in optimizing nitrogen utilization in finishing diets and reduce nitrogen excretion. Steam flaking EC has similar digestibility to a non-EC corn flaked to a lower flake density. Enogen provides an opportunity to increase throughput in commercial flaking operations by decreasing flake density without compromising digestibility.

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Table 5.1. Composition and nutrient analyses of finishing diets comprised of dry-rolled and high-moisture corn combinations in experiment 1.

	Grain type	
Item[a]	Control corn	Enogen corn
Ingredient, % of DM		
Control high-moisture corn	40.5	-
Enogen high-moisture corn	-	40.5
Control dry-rolled corn	39.9	-
Enogen dry-rolled corn	-	38.8
Ground alfalfa	10.0	10.0
Soybean meal	4.0	5.1
Liquid molasses	3.0	3.0
Supplement[b]	2.6	2.6
Analyzed nutrients, g/kg of diet DM[c]		
Dry matter	800.2	771.8
Organic matter	955.8	955.5
Starch	533.8	567.3
Crude protein	133.8	130.7
Acid detergent fiber	78.3	70.4
Neutral detergent fiber	150.1	129.4
Calcium	8.3	8.6
Phosphorous	2.9	3.0

[a] DM=dry matter.

[b] Consisted of limestone, urea, salt, potassium chloride, trace mineral premix, and vitamin premix to provide on a total diet DM basis 36.4 mg/kg monensin (Rumensin; Elanco Animal Health), 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, 2,205 IU/kg vitamin A, 15.5 IU/kg vitamin E, and 287 IU/kg vitamin D.

[c] Analyzed (SDK Labs; Hutchinson, KS) composition of daily samples dried in a 55ºC oven for 48 h and composited on a dry weight basis for each period (4 samples per grain type)

Table 5.2. Composition and nutrient analyses of finishing diets containing steam flaked Enogen corn in experiment 2.

	Grain type	
Item[a]	Control corn	Enogen corn
Ingredient, % of DM		
Control steam-flaked corn	81.7	-
Enogen steam-flaked corn	-	81.7
Triticale haylage	7.0	7.0
Corn steep liquor	7.0	7.0
Supplement[b]	4.3	4.3
Analyzed nutrients, g/kg of diet DM[c]		
Dry matter	718.1	717.6
Organic matter	960.2	958.7
Starch	567.8	561.8
Crude protein	147.1	147.2
Acid detergent fiber	59.1	51.4
Neutral detergent fiber	135.5	141.3
Calcium	4.3	7.3
Phosphorous	5.2	8.4

[a] DM=dry matter.

[b] Consisted of limestone, urea, salt, potassium chloride, trace mineral premix, and vitamin premix to provide on a total diet DM basis 36.4 mg/kg monensin (Rumensin; Elanco Animal Health), 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, 2,205 IU/kg vitamin A, 15.4 IU/kg vitamin E, and 287 IU/kg vitamin D.

[c] Analyzed (SDK Labs; Hutchinson, KS) composition of daily samples dried in a 55ºC oven for 48 h and composited on a dry weight basis for each period (4 samples per grain type)

Table 5.3. Analyzed composition[a] of dry-rolled and high-moisture corn in experiment 1.

	Grain type[b]			
Ingredient[c]	Control corn	Enogen corn	SEM	*P*-value
High-moisture corn				
Dry matter, g/kg	700.0	660.0	0.37	<0.01
Ash, g/kg of DM	13.5	16.4	0.78	<0.05
Starch, g/kg of DM	720.8	743.3	5.82	<0.05
Crude protein, g/kg of DM	85.0	81.0	0.69	<0.01
NDF, g/kg of DM	95.5	70.3	1.88	<0.01
ADF, g/kg of DM	27.3	23.3	1.01	<0.05
Dgw, µm[d]	1874	1886	72.7	0.90
Sgw, µm[e]	3.5	3.3	0.06	0.16
Dry-rolled corn				
Dry matter, g/kg	858.4	842.1	1.84	<0.01
Ash, g/kg of DM	13.8	14.8	1.32	0.62
Starch, g/kg of DM	679.3	712.0	8.31	<0.05
Crude protein, g/kg of DM	93.2	85.0	1.82	<0.05
NDF, g/kg of DM	92.8	94.9	6.77	0.84
ADF, g/kg of DM	38.9	32.5	3.88	0.29
D_{gw}, µm[d]	3,404	3137	65.6	<0.01
S_{gw}, µm[e]	2.0	2.2	0.05	<0.01

[a] Analyzed (SDK Labs; Hutchinson, KS) composition of daily samples dried in a 55ºC oven for 48 h and composited on a dry weight basis for each period (4 samples per treatment)
[b] Control corn is an isoline hybrid of similar genetics without high-amylase expression; Enogen corn express high-concentrations of alpha-amylase.
[c] ADF=acid detergent fiber; D_{gw}=mean geometric particle size; DM=dry matter; NDF= neutral detergent fiber; S_{gw}= standard deviation of particle size.
[d] Mean geometric particle size determined by placing 200 g of grain in sieves and calculating geometric particle size using equations in ASAE S319.4.
[e] Standard deviation of particle size calculated using equations in ASAE S319.4.

Table 5.4. Influence of grain type on nutrient flow and digestibility of finishing diets comprised of high-moisture corn/dry-rolled corn combinations in experiment 1

	Grain type			
Item[a]	Control	Enogen	SEM	*P*-value
No. of observations	14	14		
Intake, g/d				
DM	10,304	10,585	584.2	0.70
OM	9,853	10,115	560.6	0.70
Starch	5,344	6,004	337.0	0.12
Nitrogen	224	217	11.8	0.74
Flow to duodenum, g/d				
Apparent OM	3,898	4,319	546.3	0.56
True OM	2,101	2,604	533.4	0.47
Starch	969	1025	216.0	0.85
Total nitrogen	195	221	22.61	0.40
Microbial nitrogen	156	155	16.9	0.98
Feed nitrogen	40	61	25.2	0.44
Fluid passage rate, %/h	7.69	8.62	0.745	0.20
Ruminal digestibility, %				
Apparent OM	60.3	57.4	4.75	0.62
True OM	77.9	74.7	4.99	0.60
Starch	82.3	83.7	3.03	0.67
Feed N	85.7	76.4	10.56	0.40
Microbial efficiency[b]	20.7	21.8	2.80	0.76
Flow from small intestine, g/d				
OM	2,346	2,079	312.8	0.51
Starch	656	433	152.7	0.13
Nitrogen	74	64	9.8	0.46
Small intestinal digestibility				
OM, % of entry	34.7	49.4	7.82	0.17
OM, % of intake	16.2	22.3	5.28	0.37
Starch, % of entry	21.9	47.8	14.64	0.20
Starch, % of intake	6.3	8.5	3.39	0.62
Nitrogen, % of entry	59.5	69.8	4.97	0.09
Fecal output, g/d				
OM	1,782	1,730	163.9	0.80
Starch	380	359	53.6	0.75
Nitrogen	57	54	5.1	0.55
Large intestinal digestibility				
OM, % of entry	16.6	11.0	7.3	0.55
OM, % of intake	5.6	3.4	2.31	0.49
Starch, % of entry	13.3	6.0	18.5	0.37
Starch, % of intake	4.8	1.5	2.25	0.14
Nitrogen, % of entry	18.4	8.5	7.66	0.31
Total tract digestion, %				
OM	82.1	82.9	1.33	0.60
Starch	93.2	94.2	0.77	0.26
Nitrogen	74.6	74.9	2.09	0.93

[a] DM=dry matter; OM=organic matter.
[b] Microbial N g/kg of OM truly fermented.

Table 5.5. Analyzed composition[a] of steam-flaked corn types in experiment 2.

	Grain type[b]			
Ingredient[c]	Control corn	Enogen corn	SEM	*P*-value
Dry matter, g/kg	795.9	796.7	1.14	0.60
Ash, g/kg of DM	11.4	14.4	0.48	<0.01
Starch, g/kg of DM	711.0	689.0	10.89	0.21
Starch availability, %	52.0	52.8	0.35	0.10
Crude protein, g/kg of DM	87.2	89.7	2.27	0.48
ADF, g/kg of DM	15.3	23.0	1.02	<0.01
NDF, g/kg of DM	73.2	93.0	3.09	<0.01
Calcium, g/kg of DM	0.13	0.13	0.001	0.99
Phosphorus, g/kg of DM	2.3	3.0	0.11	<0.01
Dgw, µm[d]	7,714	6,897	44.84	<0.01
Sgw, µm[e]	1.8	1.9	0.01	<0.01

[a] Analyzed (SDK Labs; Hutchinson, KS) composition of daily samples dried in a 55ºC oven for 48 h and composited on a dry weight basis for each period (4 samples per treatment)
[b] Control corn is commercially available hybrid without high-amylase expression; Enogen corn express high-concentrations of alpha-amylase.
[c] ADF=acid detergent fiber; Dgw=mean geometric particle size; DM=dry matter; NDF= neutral detergent fiber; Sgw= standard deviation of particle size.
[d] Mean geometric particle size determined by placing 200 g of grain in sieves and calculating geometric particle size using equations in ASAE S319.4.
[e] Standard deviation of particle size calculated using equations in ASAE S319.4.

Table 5.6. Influence of grain type on nutrient flow and digestibility of finishing diets comprised of steam-flaked corn in experiment 2.

	Grain type[a]			
Item[b]	Control	Enogen	SEM	*P*-value
No. of observations	14	14		
Intake, g/d				
DM	9,292	9,856	476.4	0.23
OM	8,965	9,530	450.4	0.21
Starch	5,283	5,512	272.1	0.40
Nitrogen	218	232	11.4	0.20
Flow to duodenum, g/d				
Apparent OM	3,136	3,339	325.5	0.35
True OM	851	913	545.5	0.83
Starch	650	829	102.1	0.11
Total nitrogen	201	186	22.9	0.35
Microbial nitrogen[b]	176	175	13.4	0.95
Feed nitrogen	25	11	16.4	0.61
Fluid passage rate, %/h	6.87	7.57	0.554	0.22
Ruminal digestibility, %				
Apparent OM	65.4	65.5	1.97	0.98
True OM	91.6	90.7	5.81	0.76
Starch	87.9	85.4	1.43	0.11
Feed N	80.6	75.1	3.97	0.22
Microbial efficiency[c]	21.5	20.9	1.57	0.27
Flow from small intestine, g/d				
OM	1,487	1,492	128.4	0.95
Starch	255	287	46.4	0.26
Nitrogen	60	58	2.6	0.57
Small intestinal digestibility				
OM, % of entry	51.9	53.2	2.61	0.62
OM, % of intake	18.1	18.9	1.71	0.63
Starch, % of entry	59.9	62.1	4.52	0.68
Starch, % of intake	7.4	9.4	1.10	0.21
Nitrogen, % of entry	67.5	67.1	2.88	0.86
Fecal output, g/d				
OM	1,196	1,247	113.38	0.46
Starch	66	74	14.3	0.34
Nitrogen	49	52	4.3	0.37
Large intestinal digestibility				
OM, % of entry	19.8	15.9	3.62	0.29
OM, % of intake	3.3	2.6	0.68	0.23
Starch, % of entry	75.4	72.9	3.53	0.46
Starch, % of intake	3.5	3.9	0.55	0.36
Nitrogen, % of entry	17.8	8.2	6.25	0.09
Total tract digestion, %				
OM	86.9	86.9	0.80	0.94
Starch	98.8	98.7	0.24	0.33
Nitrogen	77.7	77.5	0.97	0.88

[a] Control grain flaked to 360 g/L and Enogen grain flaked to 390 g/L.
[b] DM=dry matter; OM=organic matter.
[c] Utilized a ratio of 0.0288 for determining cytosine:N ratio.

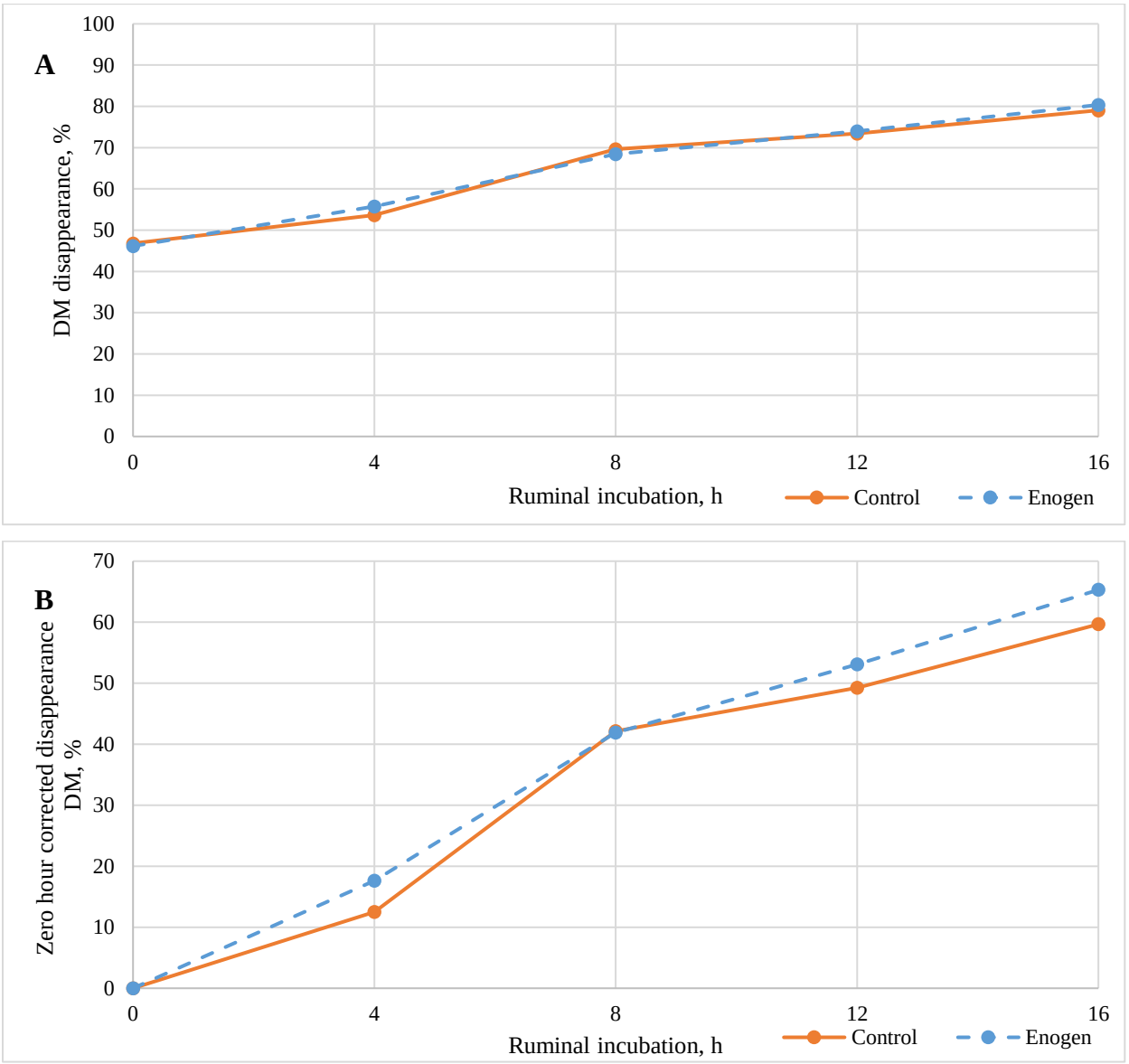

Figure 5.1. The effect of grain type on dry matter disappearance of ground corn in *in situ* bags incubated for 0, 4, 8, 12, or 16 h in steers fed a combination of high-moisture/dry-rolled corn.

A) Impact of ruminal incubation time and grain type on dry matter disappearance of ground non-Enogen corn in *in situ* bags incubated for 0, 4, 8, 12, or 16 h (2 bags per time point) in steers fed either a combination of Enogen dry-rolled and high-moisture corn or control dry-rolled and high-moisture corn. Effect of Hour, $P<0.01$; Effect of grain type, $P=0.90$; Interaction of hour x grain type, $P=0.89$. SEM= 3.35. **B)** Impact of ruminal incubation time and grain type on dry matter disappearance of ground corn in *in situ* bags incubated for 4, 8, 12, or 16 h (2 bags per time point) in steers fed either a combination of Enogen dry-rolled and high-moisture corn or control dry-rolled and high-moisture corn corrected for hour zero disappearance. Effect of Hour, $P<0.01$; Effect of grain type, $P=0.59$; Interaction of hour x grain type, $P=0.80$. SEM= 5.27.

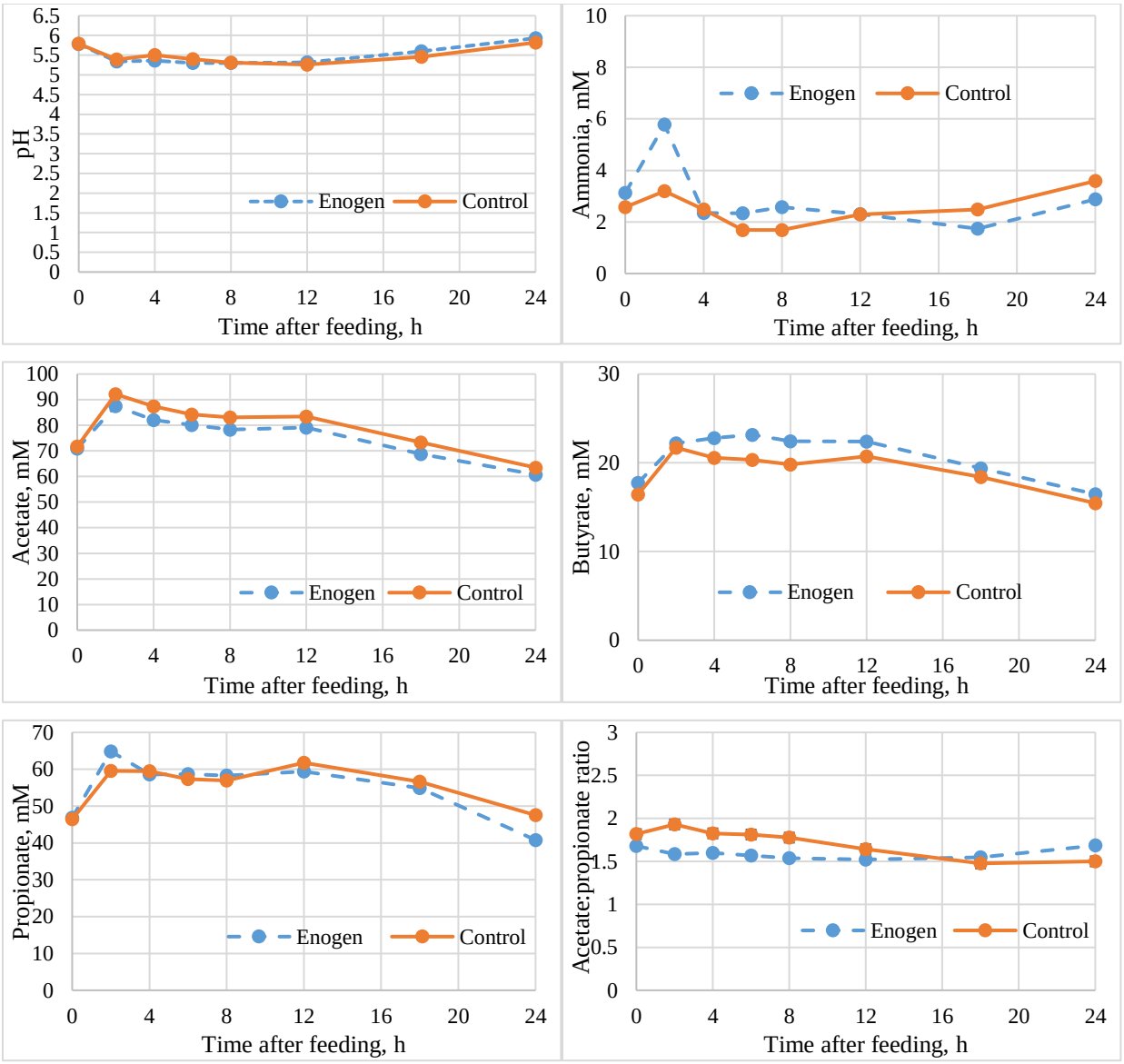

Figure 5.2. Impact of hour post-feeding and grain type on pH, ammonia, acetate, propionate, butyrate and acetate: propionate ratio of ruminal fluid from steers fed either a combination of Enogen dry-rolled and high-moisture corn or conventional dry-rolled and high-moisture corn.

pH: Effect of hour, *P*<0.05; Effect of grain type, *P*=0.97; Interaction of hour x grain type, *P*=0.21. SEM= 0.095. Ammonia: Effect of hour, *P*<0.05; Effect of grain type, *P*=0.24; Interaction of hour x grain type, *P*=0.38. SEM= 1.25. Acetate: Effect of hour, *P*<0.01; Effect of grain type, *P*=0.05; Interaction of hour x grain type, *P*=0.99. SEM= 5.05. Propionate: Effect of hour, *P*<0.01; Effect of grain type, *P*=0.85; Interaction of hour x grain type, *P*=0.94. SEM= 7.42. Butyrate: Effect of hour, *P*<0.01; Effect of grain type, *P*=0.09; Interaction of hour x grain type, *P*=0.99. SEM= 2.71. Acetate:propionate ratio : Effect of hour, *P*=0.83; Effect of grain type, *P*=0.14; Interaction of hour x grain type, *P*=0.79. SEM= 0.26.

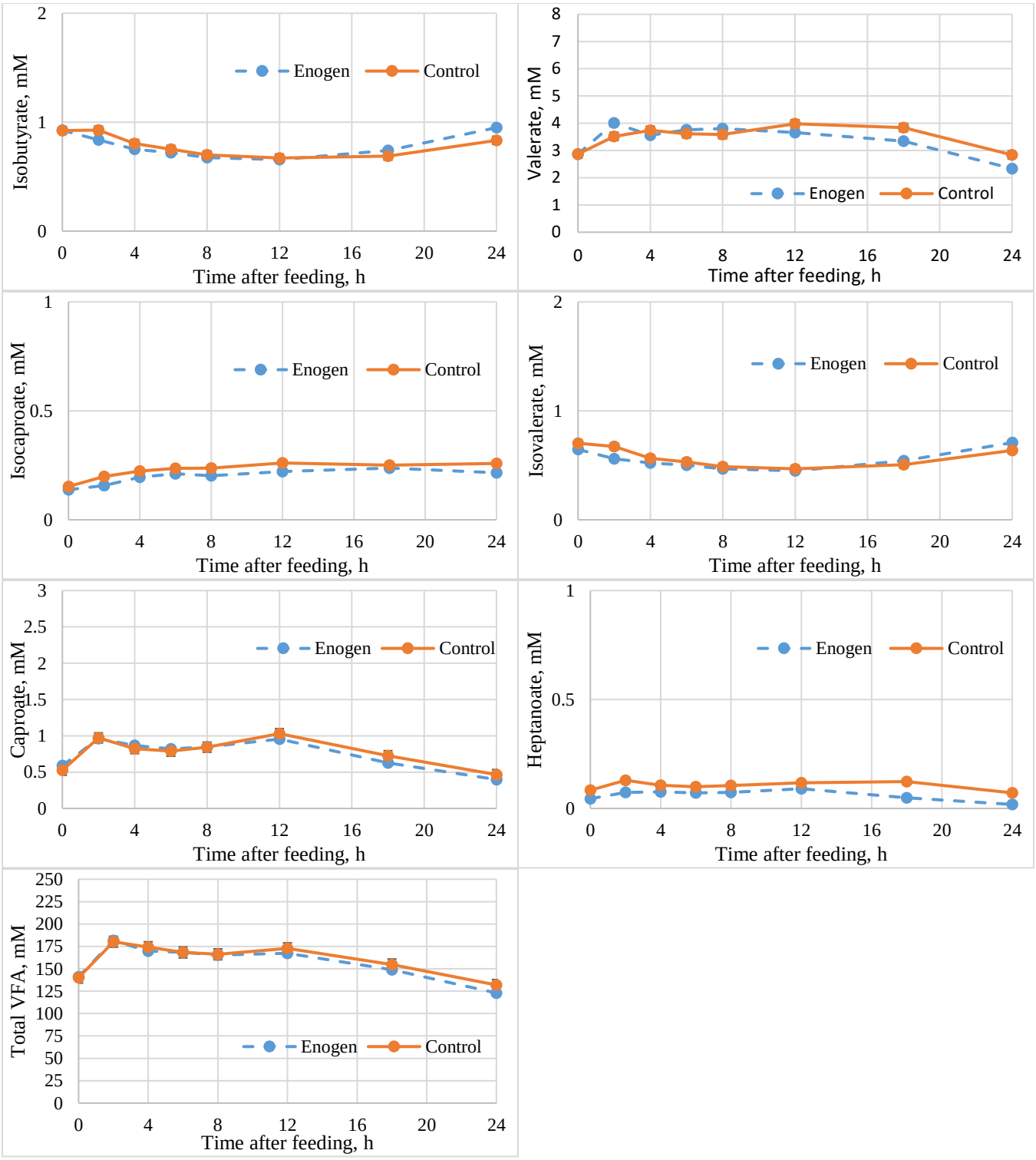

Figure 5.3. Impact of hour post-feeding and grain type on volatile fatty acids and total volatile fatty acids in ruminal fluid from steers fed either a combination of Enogen dry-rolled and high-moisture corn or conventional dry-rolled and high-moisture corn.

Isobutyrate: Effect of hour, *P*<0.01; Effect of grain hybrid, *P*=0.86; Interaction of hour x grain hybrid, *P*=0.87. SEM= 0.09. Isovalerate: Effect of hour, *P*<0.01; Effect of grain hybrid, *P*=0.31; Interaction of hour x grain hybrid, *P*=0.67. SEM= 0.06. Isocaproate: Effect of hour, *P*<0.01; Effect of grain hybrid, *P*<0.01; Interaction of hour x grain hybrid, *P*=0.99. SEM= 0.03. Valerate: Effect of hour, *P*=0.07; Effect of grain hybrid, *P*=0.77; Interaction of hour x grain hybrid, *P*=0.98. SEM= 0.83. Caproate: Effect of hour, *P*<0.01; Effect of grain hybrid, *P*=0.85; Interaction of hour x grain hybrid, *P*=0.99. SEM= 0.21. Heptanoate : Effect of hour, *P*=0.29; Effect of grain hybrid, *P*<0.01; Interaction of hour x grain hybrid, *P*=0.99. SEM= 0.05. Total VFA: Effect of hour, *P*<0.01; Effect of grain hybrid, *P*=0.39; Interaction of hour x grain hybrid, *P*=0.98. SEM= 9.72.

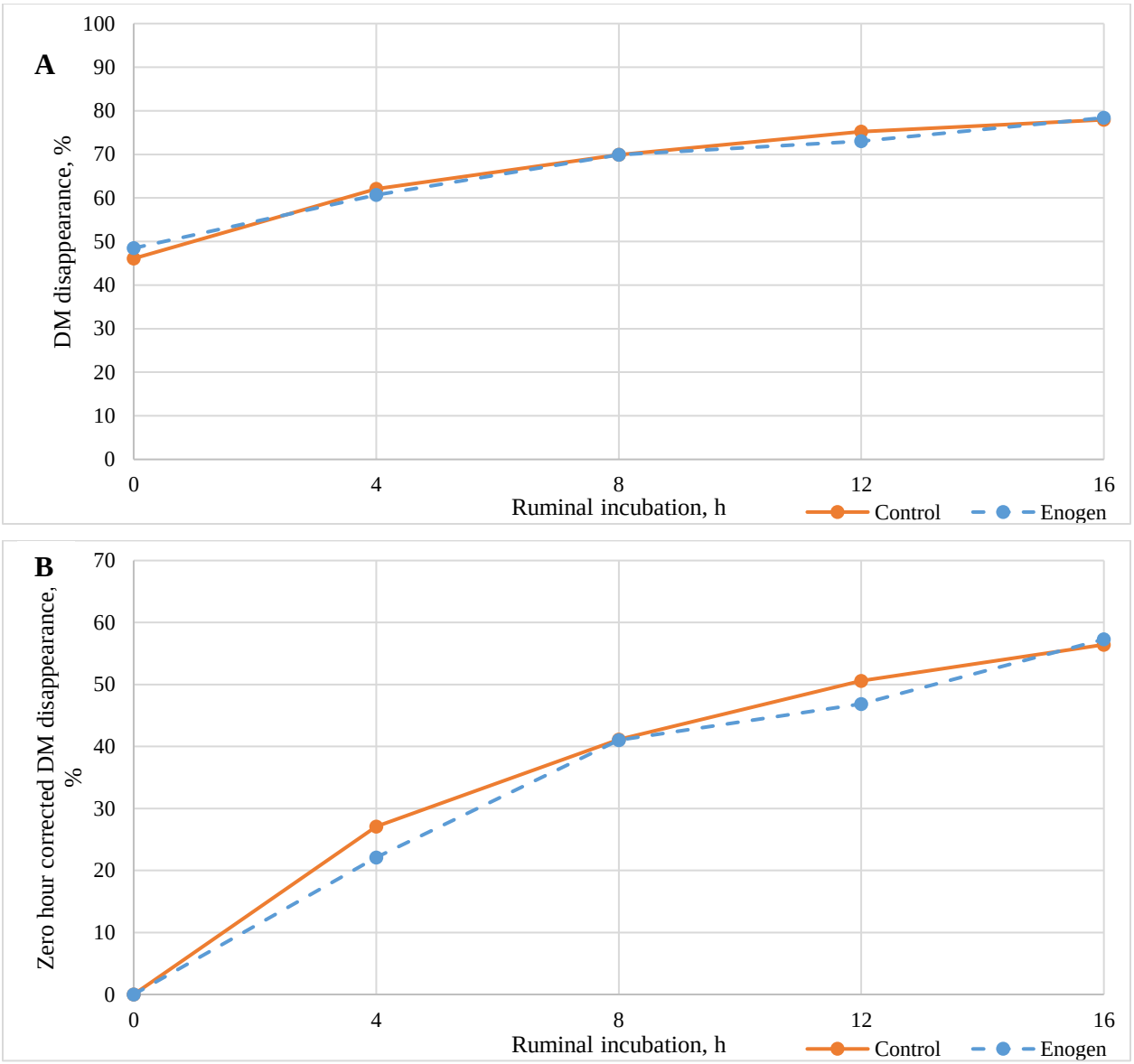

Figure 5.4. The effect of grain type on dry matter disappearance of ground corn in *in situ* bags incubated for 0, 4, 8, 12, or 16 h in steers fed steam-flaked corn.

A) Interaction between ruminal incubation time and grain hybrid on dry matter disappearance of ground non-Enogen corn in *in situ* bags incubated for 0, 4, 8, 12, or 16 h (2 bags per time point) in the rumen of steers fed steam-flaked corn from either Enogen or conventional corn. Effect of hour, $P<0.01$; Effect of grain type, $P=0.95$; Interaction of hour x grain type, $P=0.83$. SEM= 2.92. **B)** Interaction between ruminal incubation time and grain type on dry matter disappearance of ground corn placed in *in situ* bags incubated for 4, 8, 12, or 16 h (2 bags per time point) in steers fed either Enogen steam-flaked corn or conventional steam-flaked corn, then corrected for hour zero disappearance. Effect of hour, $P<0.01$; Effect of grain type, $P=0.74$; Interaction of hour x grain type, $P=0.74$. SEM= 6.39.

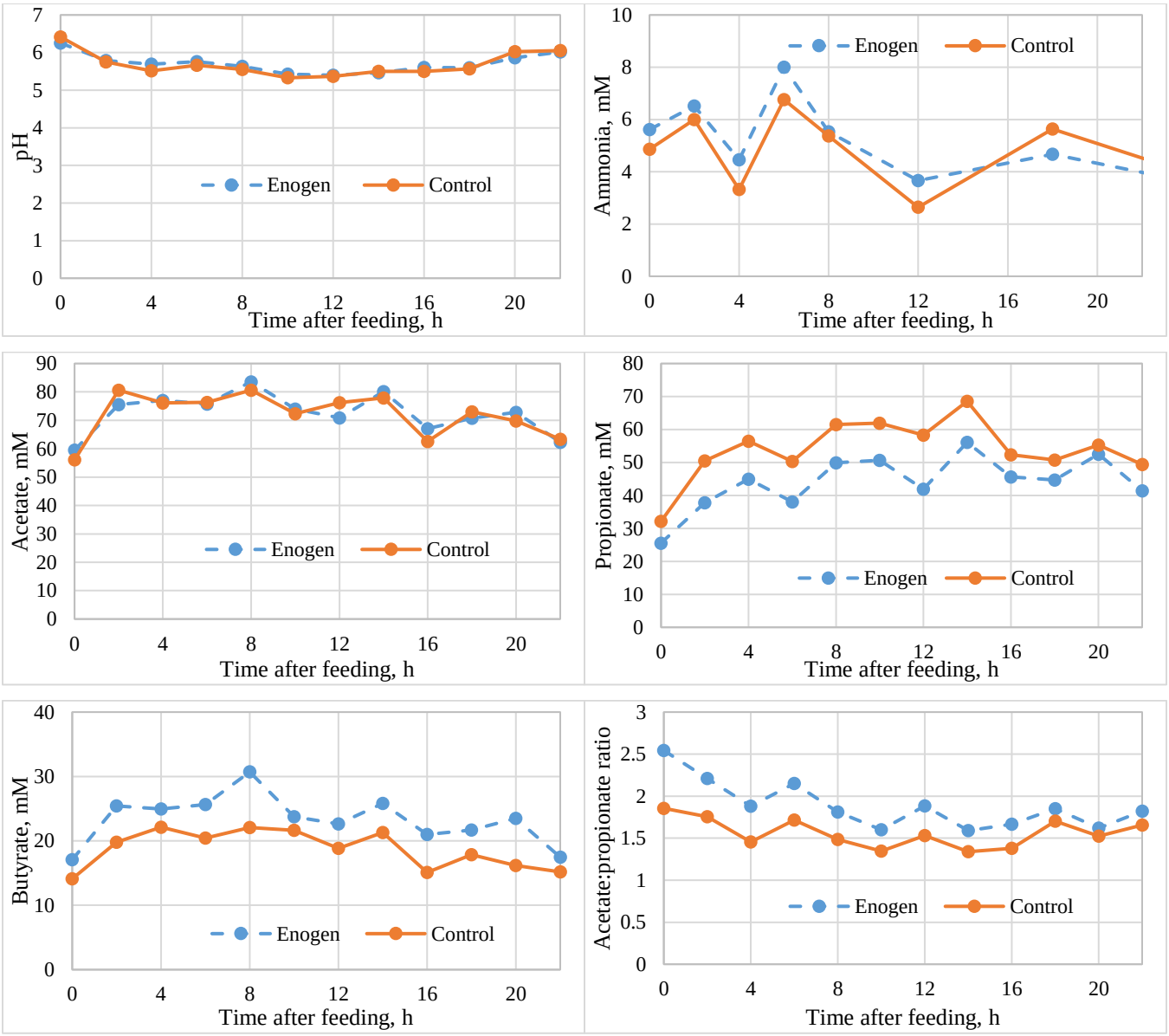

Figure 5.5. Impact of hour post-feeding and grain type on pH, ammonia, acetate, propionate, butyrate and acetate: propionate ratio in ruminal fluid from steers fed either Enogen or conventional corn as steam-flaked corn.

pH: Effect of hour, *P*<0.01; Effect of grain type, *P*=0.66; Interaction of hour x grain type, *P*=0.32. SEM= 0.089. Ammonia: Effect of hour, *P*<0.01; Effect of grain type, *P*=0.22; Interaction of hour x grain type, *P*=0.97. SEM= 1.45. Acetate: Effect of hour, *P*<0.01; Effect of grain type, *P*=0.78; Interaction of hour x grain type, *P*=0.89. SEM= 4.65. Propionate: Effect of hour, *P*<0.01; Effect of grain type, *P*<0.01; Interaction of hour x grain type, *P*=0.98. SEM= 6.70. Butyrate: Effect of hour, *P*<0.01; Effect of grain type, *P*<0.01; Interaction of hour x grain type, *P*=0.83. SEM= 2.57. Acetate: propionate ratio : Effect of hour, *P*<0.01; Effect of grain type, *P*<0.01; Interaction of hour x grain type, *P*=0.93. SEM= 0.25.

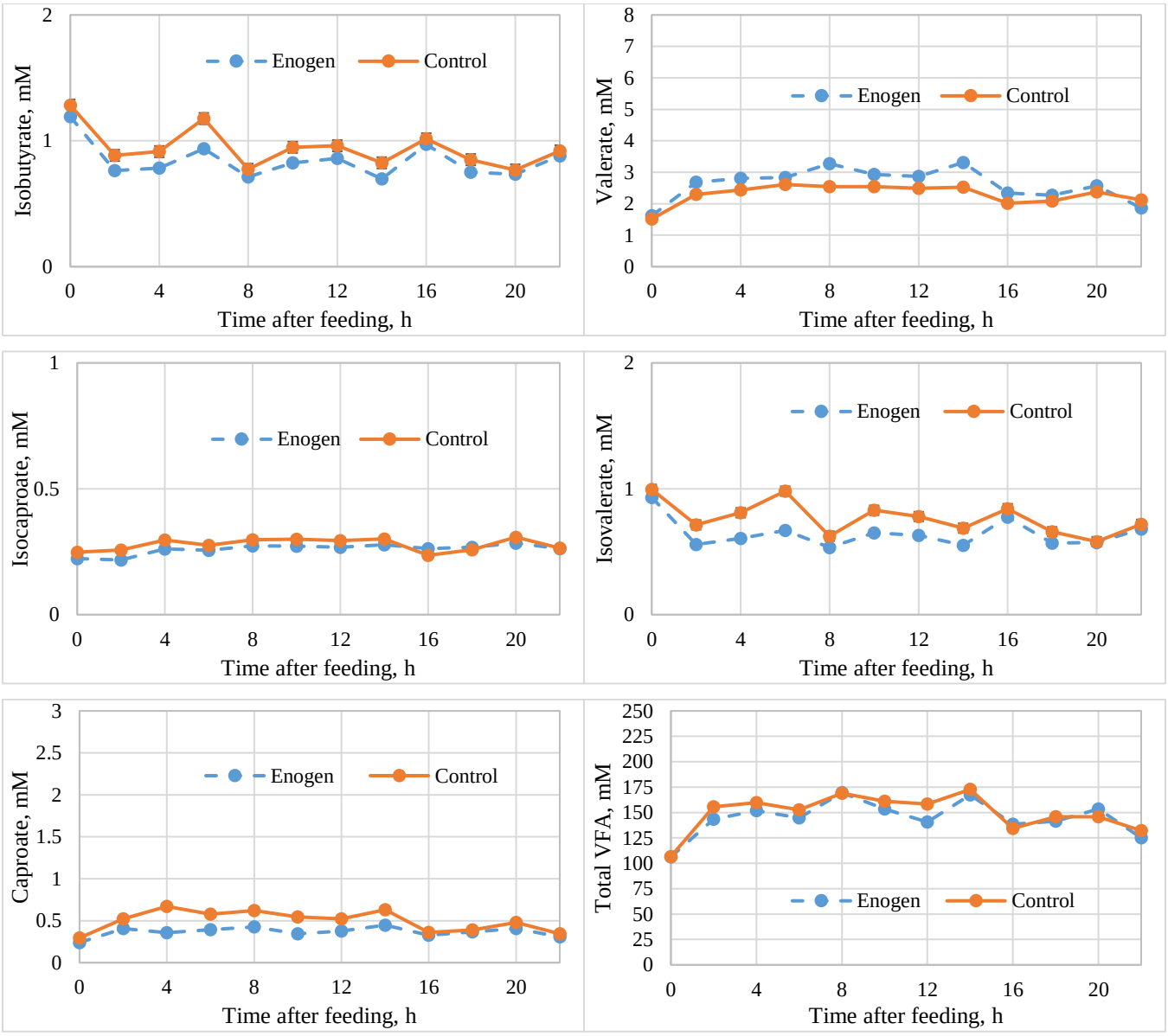

Figure 5.6. Interaction between hour post-feeding and hybrid on volatile fatty acids and total volatile fatty acids measured in ruminal fluid from steers fed either Enogen or conventional corn as steam-flaked corn.

Isobutyrate: Effect of hour, *P*<0.01; Effect of grain type, *P*<0.01; Interaction of hour x grain type, *P*=0.99. SEM= 0.10.
Isovalerate: Effect of hour, *P*<0.01; Effect of grain type, *P*<0.01; Interaction of hour x grain type, *P*=0.90. SEM= 0.10.
Isocaproate: Effect of hour, *P*=0.98; Effect of grain type, *P*=0.39; Interaction of hour x grain type, *P*=0.99. SEM= 0.06.
Valerate: Effect of hour, *P*=0.01; Effect of grain type, *P*<0.01; Interaction of hour x grain type, *P*=0.51. SEM= 0.28.
Caproate: Effect of hour, *P*<0.05; Effect of grain type, *P*<0.01; Interaction of hour x grain type, *P*=0.84. SEM= 0.12.
Total VFA: Effect of hour, *P*<0.01; Effect of grain type, *P*=0.06; Interaction of hour x grain type, *P*=0.83. SEM= 9.37.