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EFFECT OF IN VITRO STARCH DIGESTIBILITY, PROCESSING METHOD, AND NITROGEN SUPPLEMENTATION ON SITE AND EXTENT OF NUTRIENT DIGESTION IN HOLSTEIN STEERS FED A HIGH GRAIN DIET

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By

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ABSTRACT

EFFECT OF IN VITRO STARCH DIGESTIBILITY, PROCESSING METHOD, AND NITROGEN SUPPLEMENTATION ON SITE AND EXTENT OF NUTRIENT DIGESTION IN HOLSTEIN STEERS FED A HIGH GRAIN DIET

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Corn hybrids with different genetics result in corn that has different kernel characteristics. The physical structure and chemical composition of cereal starches, along with the interactions between starch and protein concentration can alter the digestibility of grain for livestock. Recent research (Philippeau et al., 1999; Macken et al., 2003) suggests that grain source (i.e., wheat vs. corn) and corn endosperm type (i.e., flour vs. flint) can increase both ruminal starch degradability and postruminal starch digestion. Degradable intake protein requirements for an animal increase with greater ruminal starch degradability (NRC, 1996) and could be altered based on the extent of ruminal fermentation. Collectively, two experiments were conducted to determine the interaction between corn grain endosperm type/ in vitro starch digestibility (IVSD) and processing method on site and extent of nutrient digestibility and subsequent effects of N concentration on nutrient digestibility. In the first experiment, eight ruminally, duodenally, and ileally cannulated Holstein steers were used in a replicated 4 x 4 Latin square design experiment with a 2 x 2 factorial arrangement of treatments. Treatments were corn grain with high or low IVSD (HIGH vs. LOW) as either dry-rolled or high moisture corn (DRC vs. HMC). When expressed as a percentage of duodenal flow, 21.5% less starch was digested in the small intestine of steers consuming diets containing DRC. Likewise, an 8% decline in apparent total tract starch digestibility was

documented when feeding DRC. Total molar concentration of volatile fatty acid tended to be greater for HIGH diets. Diets containing HMC resulted in greater amounts of microbial nitrogen flow to the duodenum, greater apparent post-ruminal nitrogen digestibility, and greater microbial efficiency. Both processing method and IVSD of corn hybrids appear to impact site and extent of starch digestibility and nitrogen metabolism.

In the second experiment, the interaction between IVSD of two corn hybrids and nitrogen concentration on site of nutrient digestion and nitrogen metabolism in Holstein steers consuming a high-grain diet was investigated. Four Holstein steers (initial BW = 500 ± 21 kg) were used in separate 4 x 4 Latin square design experiments with a 2 x 2 factorial arrangement of treatments. Treatments were IVSD of corn grain (HIGH and LOW) and nitrogen concentration (HNIT and LNIT). A significant interaction of treatments was present for total tract starch digestibility. Both HIGH and LNIT treatments resulted in greater molar concentration of propionate. Corn grain with high IVSD resulted in numerically greater microbial efficiency when compared to LOW corn grain.

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LIST OF ABBREVIATIONS AND SYMBOLS

A:P	acetate to propionate ratio
ADF	acid detergent fiber
ADG	average daily gain
BCP	bacterial crude protein
BW	body weight
CP	crude protein
DIP	degradable intake protein
DM	dry matter
DMI	dry matter intake
DRC	dry-rolled corn
HMC	high moisture corn
MCP	microbial protein
MNE	microbial nitrogen efficiency
MP	metabolizable protein
NDF	neutral detergent fiber
OM	organic matter
TRDOM	truly ruminally degraded organic matter
UIP	undegradable intake protein
VFA	volatile fatty acid
SFC	steam flaked corn

CHAPTER 1

REVIEW OF LITERATURE

1.1 INTRODUCTION

Corn is the world's most widely utilized feed concentrate, and in the United States, comprises roughly 80% of the feed grain consumed by livestock (USDA, 1992). Grain quality is best defined as a measure of the suitability of a grain for its intended use. The highest quality corn for finishing cattle would be the corn that results in the greatest amount of weight gain relative to the amount of corn consumed. Corn hybrids with different genotypes, or grown under diverse environmental conditions, result in corn that has different kernel characteristics. These unique kernel characteristics result in corn that can be designed for specific end uses.

High-grain diets consumed by feedlot cattle contain high concentrations of starch. Typically, processed corn supplies much of the starch found in feedlot rations. Starch in the corn kernel is fermented to volatile fatty acids (VFA; primarily propionate, acetate, and butyrate) in the rumen, then absorbed serving as the main source of energy for the ruminant animal. Propionate is the primary precursor for synthesis of glucose by the liver. Theoretically, the conversion of dietary starch energy into net energy utilized by various body tissues (i.e., portal-drained viscera, etc.) is greater if assimilation occurs via intestinal glucose absorption rather than ruminal fermentation and subsequent volatile fatty acid absorption (Harmon and McCloud, 2001). Ruminal fermentation results in

energetic losses due to methane formation, heat of fermentation, and lower efficiency for use of absorbed substrates. However, limits to small intestinal starch digestion by cattle have been reported (Krehbiel et al., 1996) and Huntington (1997) estimated the threshold to be less than 1.5 kg starch/d in concentrate-adapted animals. Therefore, maximal energetic efficiency of growing-finishing cattle necessitates a high extent of ruminal starch fermentation (Owens et al., 1997), while maximizing starch digestibility in the small intestine for that which escapes ruminal fermentation. For the benefits of the increased efficiency of intestinal starch digestion to be realized, carbohydrate assimilation must occur in the small intestine because large intestinal fermentation is less efficient due to energetic losses attributed to fermentation of substrate in the large intestine (Harmon and McLeod, 2001).

Nitrogen (N) and phosphorus (P) excretion is receiving increased attention from both the beef industry and the Environmental Protection Agency (EPA). Excess environmental N can cause soil build-up, water contamination via leaching, or increased ammonia emissions (Sutton and Beede, 2003). Ammonia is a major concern because it can be dispersed via acid rain, contaminating the ground and water where it falls. Generally, diets that are excessive in protein or have a greater degree of hind gut fermentation result in increased protein excretion and thereby, increased amounts of environmental N.

Recent research (Philippeau et al., 1999; Macken et al., 2003) suggests that both grain source (i.e., wheat vs. corn), as well as corn vitreousness can influence ruminal starch degradability. Little research is available that examines the effects of kernel vitreousness and grain moisture on extent of ruminal, duodenal, and ileal starch

digestibility. With more available information regarding interactions between site of starch digestion and metabolizable N intake, nutritionists could more accurately formulate diets to maximize feed conversion efficiency while simultaneously limiting environmental N pollution.

Improving the feed conversion efficiency of feedlot cattle is of utmost importance when documenting enterprise profitability. Likewise, one of the more expensive nutrients to add to feedlot rations is N. Corn grain with greater vitreousness has been shown to undergo a shift in site of starch digestion from the rumen to the lower gastrointestinal tract (Philippeau et al., 1997). Site of starch digestion and the fermentation profiles associated with such can also have profound effects on an animal's N requirement. Therefore, the interaction between corn grain endosperm type and N supply could affect site of nutrient digestion, microbial protein production, and environmental N losses.

The objectives of the current experiments were to determine the effect of corn vitreousness and processing method on site and extent of starch digestion, digestion kinetics, and nutrient utilization. Based on site of starch digestion and ruminal fermentation profiles, investigation of differing degradable intake protein concentrations were also evaluated. Collectively, these results should yield a clearer understanding of the interaction between site of starch digestion and animal N needs, while also providing important data pertaining to the effect of site of digestion on bovine N and P balance.

1.2 PHYSICAL CHARACTERISTICS OF CORN HYBRIDS

The corn (*Zea mays*) kernel is classified botanically as a caryopsis, which means it has a dry, indehiscent, single-seeded fruit of which the mature ovary wall (pericarp) does not separate naturally from the seed. The corn kernel contains four major structural components: germ, endosperm, pericarp, and the tip cap. These basic structural components of the corn kernel are depicted in Diagram 1.1. The germ is comprised of the embryo (precursor tissues for leaves, stem, and root), and scutellum (absorbs nutrients from the endosperm during germination). The germ contains precise genetic information, enzymes, a large proportion of the kernel's essential amino acids, and assorted vitamins and minerals (most notably phosphorus). The endosperm is made up of elongated cells packed with starch granules embedded into a continuous protein matrix within the individual cells (Wolf et al., 1952). Generally, the endosperm constitutes about 82 to 84% of the dry weight of the kernel and is 86 to 89% starch by weight (Earle et al., 1946). The endosperm contains about 98% of the starch of the kernel, as well as about 74% of the total protein (Earle et al., 1946). The starchy endosperm can be of two types, floury (soft, less vitreous) and flinty (hard, more vitreous), where vitreousness represents the proportion of horny endosperm of the kernel. Many different corn hybrids are commercially available and differ largely in their chemical composition and structure. From a cattle feeding perspective flinty, floury, dent, waxy, and high-oil corns have been evaluated. Flinty corn has a rounded crown and the hardest kernels due to the presence of a large and continuous volume of vitreous endosperm. In contrast, floury corn has a rounded or flat crown, but contains less vitreous endosperm that is more readily digested. This type of corn is also more extensively damaged during handling and artificial drying.

Fine pieces of corn that are produced (fines) after such handling, combined with the formation of stress cracks result in an increased susceptibility to insect and mold damage due to reduced airflow during storage.

Dent corn is the most commercially used corn type in the United States. Dent corn has a depressed crown that forms as the maturing kernel dehydrates. This "dent" is formed as the rigidity of the cylinder of vitreous endosperm prevents the central core of floury endosperm from shrinking uniformly during drying causing an indentation on the crown (Watson, 1987). Dent corns of the United States were developed in precolonial North America from natural hybridization between northern flint and southern flour corns (Watson, 1987). Because corn was developed as a hybrid cross, it can exhibit vast differences in vitreousness induced by environmental and heritable influences (Hamilton et al., 1951). Specifically, environmental factors such as moisture differences between fields, temperature, and soil N supply and uptake effect physical characteristics between corn hybrids. The average chemical composition of yellow dent corn is illustrated in Table 1.1.

Table 1-1. Chemical composition of cracked yellow dent corn grain [a]

Protein, %	Fat, %	NDF, %	ADF, %	Calcium, %	Phosphorus, %
9.8 ± 1.08	4.06 ± 0.64	10.8 ± 3.57	3.3 ± 1.83	0.03 ± 0.07	0.32 ± 0.04

[a]NRC (1996)

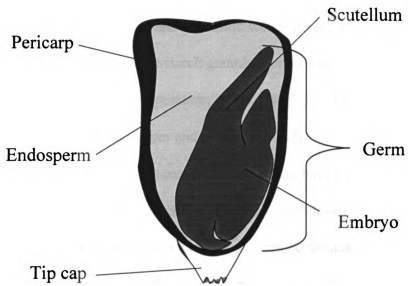
Floury endosperm is characterized by large starch granules loosely associated within a protein matrix (Dado, 1999), resembling basketballs held in a large mesh bag

(Owens and Zinn, 2004). Floury endosperm is opaque to transmitted light. Duvick (1961) reported this was due to minute air pockets between starch granules. Air pocket formation between starch granules in floury endosperm is a result of the protein matrix tearing during kernel drying. After processing, the cells of floury endosperm are completely disrupted, releasing free starch granules due to incomplete closure by the protein matrix (Watson, 1987). Consequently, starch availability in floury endosperm is more susceptible to processing techniques (Huntington, 1997).

Alternatively, the cell walls of more vitreous endosperm are broken after drying, but little release of free starch granules occurs due to the strength of the surrounding protein matrix implying a tightly compacted endosperm where starch granules are embedded in a thick protein matrix (Watson, 1987). Corn hybrids with this type of physical characteristic tend to be more resistant to disease and also produce heavier field test weights relative to less vitreous corn hybrids (Owens, personal communication). Not surprisingly, these two attributes have been important selection criteria for a vast majority of seed corn producers.

The pericarp is the outermost membrane structure of the corn kernel. The pericarp is resistant to water, while also protecting the kernel from insect susceptibility and fungal infection. The pericarp makes up 3 to 6% of the corn kernel dry weight, while containing nearly half of the NDF of the kernel (Owens and Zinn, 2004). The tip cap is the only kernel component not covered by the pericarp, and is the attachment site to the cob.

Diagram 1.1. Structural components of corn kernel



1.2.1 Corn Starch Characteristics

Starch granules are 5 to 30 μm in diameter and are embedded in the protein matrix of the kernel. Starch granules are made up of two glucan polymers, amylose and amylopectin. Amylose is an essentially linear molecule of glucose units linked by α-1,4 bonds while amylopectin is a branched molecule composed of linear regions of α-1,4-linked glucose units with α-1,6-linked branch chains every 20 to 25 glucose residues (Marshall and Whelan, 1974). The ratio of amylose to amylopectin is often utilized as an indicator of grain digestibility. While it is generally accepted that amylose makes up about 25 to 30% of the starch in corn while amylopectin constitutes 70 to 75% (Boyer

and Shannon, 1987), Allen (1991) documented that corn starch can range from nearly 100% amylose to nearly 100% amylopectin due to genetic and environmental differences.

As previously mentioned, the physical properties of starch granules are important in determining their biological and economic value relative to the corn kernel. Some of these important features include starch granule morphology, amylose content, crystallinity, gelatinization temperature, and digestibility. These factors are all altered by different endosperm types (Boyer and Shannon, 1987).

Corn maturity and moisture concentration can have a profound affect on endosperm starch and protein fractions. During kernel development, the cells in the central crown region of the endosperm begin starch accumulation first, with the lower endosperm cells initiating starch synthesis and accumulation much later (Boyer et al., 1977). Creech (1965) documented that with maturity, the hard starch layer and milk line move toward the cob and kernel sugar content declines while starch increases. As corn matures, sugars are translocated from the stover to the ear and these sugars are converted to starch. The accumulated starch is hard above the milk line, and soft below the line. Thus, it is more difficult to fracture a mature kernel because of hard starch accumulation. Likewise, as kernel maturity increases, the amylose content increases resulting in decreased digestibility (Owens and Zinn, 2004). Similarly, Philippeau and Michalet-Doreau (1997) documented that while vitreousness differed between dent and flint genotypes by 11.8%, immature grain had 28.2% less vitreousness when compared to more mature grains.

1.2.2 Grain Processing

Many different dry- and wet-milling techniques are used to amplify starch utilization. Physical processing increases the rate of ruminal starch digestion by breaking the outer coat of the kernel to increase access sites for ruminal microorganisms and enzymes. The application of heat, moisture, and pressure (high moisture or steam-flaked grains) also alters ruminal fermentation; increasing starch digestion by disrupting the protein matrix surrounding starch granules and gelatinizing starch (destroying its crystalline structure; Kotarski et al., 1992). Thus, the main function of grain processing is to improve the efficiency of feedlot cattle production by enhancing starch availability (Owens et al., 1997). Galyean (1996), in a survey of six feedlot consultants responsible for the nutritional programs of 3.6 million cattle, reported that all corn was processed before feeding, most commonly by steam flaking, followed by dry-rolling and high-moisture storage. Huntington (1997) summarized data from 14 trials published on the influence of corn processing on starch digestibility. Ruminal starch digestibility for dry-rolled, high moisture, and steam-flaked corn was 76.2, 89.9, and 84.8% of intake, respectively. Postruminally, 68.9, 67.8, and 92.6% of starch entering the duodenum was digested, while total tract starch digestibilities were 92.2, 95.3, and 98.9% of intake, respectively.

In a more recent study, Macken et al. (2003) reported that starch digestibility was significantly greater for less vitreous corn grains harvested as either dry rolled (18% moisture) or high moisture (29% moisture) corn when compared to corn grain with greater vitreousness. When fed as dry-rolled corn, steers consuming less vitreous corn grain had improved ADG and feed efficiency. These results suggest that feeding less

vitreous corn grain, regardless of grain processing technique, results in greater ruminal starch fermentation and pre-ileal starch digestibility.

Wet processing methods are used to increase surface area of starch. Corn grain that is to be steam flaked is harvested as dry corn. However, the whole corn kernels are placed into a steam chamber and moisture content is increased to approximately 18%. Afterwards, the kernels are passed through rollers to produce a "flake", which results in a completely disrupted endosperm structure of the corn kernel. Use of corn grain with less vitreousness in the flaking process is not generally practiced as a higher proportion of fines, more fragile flakes, and a reduced flaking rate is typically witnessed (Owens and Zinn, 2004). Greater amounts of fines post-flaking can hasten the development of metabolic disorders.

High-moisture grains vary widely in moisture content and can be processed in many different ways before storage and feeding (Owens et al., 1997). In order to maximize ruminal starch degradability and feed efficiency, high moisture corn grain must have adequate moisture content (26 to 31% moisture) and a sufficient duration of fermentation (Owens and Zinn, 2004). For storage in bunker silos, or other oxygen-limiting structures, grain is typically harvested at higher moisture content (i.e., 26% moisture or above) and rolled or ground to permit thorough packing. Regressions of daily gain and body weight-adjusted metabolizable energy (ME) against the percentage of moisture of high-moisture grain fed in all forms revealed that both ADG and ME should be optimized between 30 and 31% moisture (Owens et al., 1997).

Length of the ensilment and particle size can also affect feeding value. In three separate experiments, Stock et al. (1991) documented that yearling steers consuming

coarse ground HMC gained faster and were more efficient than steers fed whole HMC. Further, as length of storage increased, *in vitro* rate of starch digestion and soluble N content increased, while grain pH decreased (Stock et al., 1991). Benton et al. (2004) also reported that *in situ* starch disappearance corresponded with increased N solubility as the length of storage increased. Knowlton et al. (1998) documented that little starch digestion occurred in the small intestine of lactating dairy cows fed diets consisting of coarsely rolled corn, while a moderate increase was witnessed when the same corn was ground (13% of abomasal flow prior to the ileum). Interestingly, when the same corn hybrid was included in the diet as either rolled or ground high moisture corn, 64% and 59% disappeared in the small intestine, respectively. Particle size has been shown to limit starch digestion in the small intestine, as larger particle sizes are more likely to pass into the large intestine (Rust, 1983).

1.3 SITE OF STARCH DIGESTION

Research has clearly documented that starch digestion can be shifted to various portions of the gastrointestinal tract based on physical characteristics of the corn kernels. The major end product from starch digestion at each site along the gastrointestinal tract varies. Ruminal degradation of carbohydrates produces VFA, while increasing energy loss due to the formation of methane and heat losses associated with fermentation. Digestion in the small intestine is thought to be more energetically efficient (Harmon and McCloud, 2001). Digestion that occurs in the large intestine results in similar energetic losses as ruminal degradation, plus energy and N that is contained in bacteria is excreted in the feces. Understanding how the physical and chemical characteristics of corn kernels affect digestibility is discussed below.

1.3.1 Ruminal Starch Degradability

Structure of the kernel endosperm can range from 0% to 100% vitreous due to genetics. A study involving steers fitted with both ruminal and duodenal fistulas indicated a shift in the site of starch digestion from the rumen to the small intestine when corn with mostly vitreous endosperm replaced corn with little vitreous endosperm (Philippeau et al., 1999). Likewise, corn with greater vitreousness had a higher percentage of starch intake digested in the hindgut, relative to steers consuming corn grain with less vitreousness (Philippeau et al., 1999). Waxy varieties of corn or sorghum contain only amylopectin, and have a faster rate of digestion than nonwaxy varieties (Huntington, 1997). Philippeau and Michalet-Doreau (1997) observed that amount of vitreous endosperm in corn grain explained 86% of the variation in ruminal starch degradability; as vitreousness increased, starch degradability linearly decreased. Less vitreous corn varieties were observed to have higher ruminal and small intestinal starch digestibility than more vitreous corn varieties (Philippeau and Michalet-Doreau, 1997).

Starch in cereal grain can be completely digested during passage through the total digestive tract. However, the rate and extent of ruminal starch digestibility, rate and extent of VFA production, and post-ruminal starch flow vary in response to grain type (Owens et al., 1986). Digestibility of corn grain *in vivo* can range from 51 to 93% and is dependent on a variety of factors (Nocek and Tamminga, 1991). Likewise, different processing (i.e. rolling, grinding, or steam flaking) and conservation methods (i.e. dry or high-moisture) can influence both total tract digestibility of grains as well as site of digestion (Firkins et al., 2001).

Although ruminal starch digestion can be manipulated, its effect on feed intake varies. Site of starch digestion can change the availability of metabolites to the animal. Ruminally degraded starch is fermented to VFA (primarily propionate and acetate) and contributes to production of microbial cells. Increased degradation of starch in the rumen generally increases ME yield from starch because of limited potential for starch digestibility in the small intestine (Kotarski et al., 1992; Huntington, 1997). However, rapid fermentation of starch to VFA in the rumen may overwhelm the buffering and absorptive capacity of the rumen, leading to reductions in rumen pH that may decrease dry matter intake (DMI; McCarthy et al., 1989). Owens et al. (1997) reported that more extensive grain processing reduced average daily gain (ADG) slightly. This reduction was attributed largely to reduced DMI. Reduced DMI of rapidly fermented grain sources and extensively processed grain has been attributed to excessive rates of acid production in the rumen. This accumulation of acids in the rumen leads to subclinical acidosis, which increases day-to-day variation in DMI (Stock et al., 1995). Alternatively, Allen (2000) proposed that propionate decreases DMI by stimulating oxidative metabolism in the liver. Other studies (Herrera-Saldena and Huber, 1989; Oliveira et al., 1995) have shown no changes in DMI as a result of increased ruminal degradation of starch.

1.3.2 Intestinal Starch Digestibility

While it is clear that increased total tract starch digestion improves performance (Nocek and Tamminga, 1991), the optimal amount of postruminal starch digestion is unclear. Much of the available research compiled measures the site of starch digestion relative to different grain sources (i.e., corn, grain sorghum, wheat, etc.), which is confounded by protein content of the diet and fiber concentration.

Starch can bypass the rumen and be enzymatically digested and absorbed as glucose. Intestinal absorption of glucose is theoretically more efficient than ruminal fermentation to VFA (Harmon and McLeod, 2001). Digestibility of starch that passes the rumen intact varies significantly. Owens et al. (1986) calculated that starch digestibility in the small intestine accounts for 80% of the post-ruminal starch digestion, and starch digestion in the small intestine increases as starch flow increases. Site of post-ruminal starch digestion is important because end products of small intestinal and large intestinal starch digestion differ.

Starch is digested in the small intestine by α-amylase secreted by the pancreas, which hydrolyzes amylose and amylopectin into limit dextrins and oligosaccharides (2-3 glucose units; Harmon, 1993). The process is completed by oligosaccharidases that are located on the brush border membrane of intestinal microvilli (Huntington, 1997). Ruminants rely heavily on maltase and isomaltase activity for the production of glucose (Huntington, 1997); however, the capacity of the small intestine for starch digestion appears to be limited by supply of pancreatic amylase, rather than capacity for glucose absorpton (Kreikemeier et al., 1991). Pancreatic amylase secretion is a function of energy intake (Harmon and Taylor, 2005).

The structure of starch entering the small intestine may be more important than the amount of starch when determining starch digestibility. Kreikemeier et al. (1991) infused graded levels of three different carbohydrates (i.e., glucose, raw corn starch, or corn dextrin) into the abomasum of steers and measured starch flow at the ileum. Within any infusion concentration, the disappearance of infused dextrin anterior to the ileum was always greater than the disappearance of raw corn starch (81% vs. 58%). These

differences may be attributed to the fact that corn dextrin is a heat and acid treated derivative of corn starch. Small intestinal enzymes may have limited ability to digest starch that is still surrounded by the protein matrix and still maintains a crystalline structure.

Research has also shown that glucose absorption from the small intestine decreases from the duodenum to the ileum (Krehbiel et al., 1996). Regardless, glucose that is assimilated in the small intestine is either transported into and utilized by intestinal enterocytes or transported to the portal circulation as lactate or glucose (Allen, 2000). Earlier research completed by Rust (1983) indicated that some glucose absorbed from the small intestine is deposited in omasal adipose tissue.

1.4 METABOLIZABLE PROTEIN SUPPLY

Metabolizable protein (MP) must be present in the diet in sufficient amounts and is accounted for by two separate fractions, degradable intake protein (DIP) and undegradable intake protein (UIP). Degradable intake protein is ruminally degraded and supplies peptides, amino acids, and other growth factors for microbial fermentation and growth (Owens and Bergen, 1983). Russell et al. (1992) indicated that insufficient DIP in the diet may reduce energy yield from carbohydrate fermentation, thereby lowering VFA production and energetic efficiency of the diet. According to the NRC (1996), increasing ruminally available starch should increase the synthesis and efficiency of production of bacterial crude protein (BCP) in the rumen and therefore, increase the DIP requirement. This assumption is based primarily on results obtained from Shain et al. (1998) and Cooper et al. (2001), who concluded that finishing diets using high-moisture corn (HMC) require more DIP than dry-rolled corn (DRC) diets because of increased

ruminal starch degradability. Undegradable intake protein is the fraction of MP that escapes ruminal degradation and is equivalent to the MP requirement for the animal minus the MP supplied from BCP (NRC, 1996). Undegradable intake protein is not digested in the rumen and is partially utilized by the small intestine. Approximately one-half of the protein digested in the small intestine is microbial protein (Owens and Bergen, 1983).

The type of protein being supplemented has been shown to greatly impact starch digestion, MCP production, and microbial N efficiency (MNE) for ruminant animals. Microbial efficiency is defined as gram of microbial N reaching the duodenum per kilogram of truly ruminally degraded organic matter (TRDOM). Microbial efficiency can be influenced by passage rate, where a faster rate of passage is positively correlated to microbial efficiency (Oba and Allen, 2000). Conversely, MNE can be reduced when peptides or amino acids are not supplied in sufficient amounts (Van Kessel and Russell, 1996). Ruminal bacteria responsible for fermentation of non-structural carbohydrates grow faster and more efficiently when incorporating amino acids and peptides into microbial protein (Russell and Sniffen, 1984). Likewise, ruminal microorganisms fermenting non-structural carbohydrate can obtain approximately two thirds of their N from amino acids or peptides (Russell et al., 1983). Ruminal fiber digesting bacteria derive all of their N requirements from ammonia, while several species have peptide requirements (Bryant, 1973). Soybean meal and urea are two commonly used sources of protein in finishing diets. Urea is exclusively degraded in the rumen and is used extensively in finishing diets because of its low cost and ease of diet incorporation. Urea supplementation has been shown to increase dietary energy utilization, but does not

directly contribute to the MP supply. Supplemental urea is necessary for organic matter (OM) and starch digestion to be increased, however, flows of total N and microbial N to the duodenum remain unchanged (Milton et al., 1997). Therefore, the use of urea is limited to the amount that provides sufficient ruminal ammonia to maximize MCP production and/or OM digestion. In contrast, soybean meal contains a degradable protein fraction that supplies ammonia, amino acids, and peptides to rumen microbes, as well as an escape fraction that increases MP reaching the small intestine (Milton and Brandt, 1994). In a feeding study to compare urea and soybean meal, Milton and Brandt (1994) found that steers receiving soybean meal had greater feed efficiency. This result was likely due to increased MP supply and/or improvements in fermentation from the provision of ruminally degradable amino acids. Improvements in carcass weight and *longissimus dorsi* area, with little increase in carcass fat, indicate that soybean meal increased total supply of peptides and amino acids available to the animal for protein deposition (Milton and Brandt, 1994). In a subsequent metabolism trial by Milton et al. (1997), total tract starch digestion, duodenal microbial N flow, and MNE were greater in steers supplemented with soybean meal compared to urea, leading to the conclusion that soybean meal supplementation increased MP supply and dietary energy utilization for steers consuming dry-rolled, corn-based diets compared to urea supplemented diets. Alternatively, Gill et al. (1979) reported that urea supplementation was superior to soybean meal for rate of gain and feed efficiency with dry corn, while soybean meal was superior to urea for HMC-based diets. Shain et al. (1998) documented improved ADG and feed efficiency when diets were supplemented with urea. If digestion is limited by available ruminal ammonia for synthesis of MCP, higher solubility of nutrients in HMC

or N should prove beneficial. Conversely, when ruminal fermentation occurs faster than the ATP produced can be utilized by the microbial population, MNE can be reduced when ATP is used for non-growth functions in energy spilling reactions (Russell, 1998).

Available data suggests that high-moisture and steam-flaked corn-based diets require 50% more DIP due to increased potential for MCP production (Klopfenstein and Erickson, 2002). Relative to its' total MP content, high-moisture corn has lower inherent UIP available as a result of increased N solubility, indicating the need for UIP supplementation (Klopfenstein and Erickson, 2002). This conclusion is apparent when evaluating data comparing different protein sources in high-moisture corn based diets. Metabolizable protein requirements for growing feedlot steers are changed throughout the feeding period due to changes in intake, body weight, and composition of gain. It is generally assumed that an animal's DIP requirement increases due to increased intake as body weight increases. Theoretically, the requirement for UIP decreases as body weight increases due to both a larger supply of MCP via increased DIP utilization and the requirement for amino acids are reduced later in the growth curve.

1.5 EVIRONMENTAL NITROGEN AND PHOSPHORUS

Concentrated animal agriculture may affect air quality as well as water quality. After excretion, the organic substrate in solid and liquid animal waste is subject to microbial conversion to cellular biomass and gases, including ammonia. Nitrogen contamination of ground and surface water, combined with air pollution from ammonia emissions, are major environmental concerns. Nitrogen can enter the farm in feed, fertilizer, and legume fixation and may be exported in product (milk, meat, cash crop) or may be lost to the environment (via direct deposition in surface water, leaching, runoff,

ammonia volatilization, or denitrification; Kohn et al., 1997). Nitrogen contamination of surface water can result in algae blooms. These blooms shade aquatic vegetation, thereby reducing photosynthetic activity. Likewise, decomposition of algae consumes dissolved oxygen in the water (eutrophication), impairing its usefulness for recreation, drinking, fishing, and industry uses (Sims et al., 1998).

The amount of surplus N available for conversion to ammonia gas is a function of the dietary N concentration, ration composition and feed efficiency (Auvermann, 2002). Nitrogen digestibility for beef cattle consuming a high concentrate diet is approximately 65% of the feed-borne N (Auverman, 2002). Therefore, when cattle are consuming 11.3 kg/animal/d DM, total N excreted by feedlot cattle consuming a diet containing 13.5% CP diet is 0.24 kg N/animal/d. While ammonia emission for domesticated animals is estimated to be 21.95×10^6 metric tons of N/year or 40% of total N emission, beef cattle operations are responsible for an estimated 8.74×10^6 metric tons of N/year or 16% of the total (Asman, 2002). James et al. (1999) found that decreasing N intake of Holstein heifers by 14% resulted in a 28.1% reduction in ammonia loss from mixed feces and urine. Significant decreases in concentrations of urinary urea N and total N and in proportions of N excreted in urine were also observed (James et al., 1999). Frank and Swensson (2002) found that manure from dairy cows fed low protein diets emitted significantly less ammonia than manure from cows fed high protein diets. Likewise, Marini and Van Amburgh (2003) observed linear decreases in excretion of both urea N and total urinary N in heifers fed diets with reduced N content.

Phosphorus (P) is also a current issue with regard to environmental implications of confinement animal feeding operations (CAFO). Phosphorus also enters the farm via

feed and/or fertilizer. Roughly 30 to 50% of feed P is captured in meat and milk, and 50 to 70% is excreted in manure and urine (Knowlton et al., 2001; Knowlton and Herbein, 2002). Unlike N, P does not volatilize from the pen surface, so whatever is excreted is typically spread over land used for crop production via manure or lost in surface runoff. If manure P application is greater than crop P uptake, P accumulates in the soil and can run off into surface water, causing eutrophication and algae blooms.

Recent data from P feeding trials have demonstrated that P concentration in feces is directly related to P levels in diets. Therefore, P surpluses and potential environmental losses can be reduced through diet manipulation. Dou et al. (2002), through data compiled from three dairy feeding trials, documented that lower P in diets resulted in lower P content in feces. Supplemental P in finishing beef cattle diets appears to be unnecessary (Erickson et al., 1999; Erickson et al., 2000) as the P requirement is met by corn grain in the diet (0.32% P; DM basis; NRC, 1996). Thus the amount of P contributed from corn grain in typical feedlot rations is sufficient to meet requirements. Phytate-P is degraded by ruminal microbes that render the P available to ruminant animals. On average, 95% or more of the P bound to phytate is released to a useable form during ruminal fermentation (Morse et al., 1992). Removing supplemental P from feedlot diets fed to yearling and calf-fed animals resulted in reduced P intakes (51% and 41%, respectively; Erickson et al., 2000). Likewise, reduced dietary P caused a 59% and 38% reduction in manure P concentration for yearling and calf-fed animals.

In summary, corn hybrids with different genetics result in corn that has different kernel characteristics. The physical structure and chemical composition of cereal starches, along with the interactions between starch and protein content can alter the

digestibility of grain for livestock. Recent research (Philippeau et al., 1999; Macken et al., 2003) suggests that grain source (i.e., wheat vs. corn) and corn endosperm type (i.e., floury vs. flinty) can increase both ruminal starch degradability and postruminal starch digestion. Additionally, degradable intake protein requirements for an animal increase with greater ruminal starch degradability (NRC, 1996) and could be altered based on the extent of ruminal fermentation. While corn processing methods have been shown to influence protein requirement (Cooper et al., 2002), little research has been conducted to determine the specific effects of corn grain endosperm type and degradable intake protein (DIP) fractions on N utilization and retention in growing steers with varying rates of ruminal starch degradation. Collectively, two experiments were conducted to determine the interaction between corn grain endosperm type (IVSD) with processing method and dietary N concentration on site and extent of nutrient digestibility in Holstein steers consuming a high-grain diet.

CHAPTER 2

EFFECT OF IN VITRO STARCH DIGESTION AND PROCESSING METHOD OF CORN ON SITE AND EXTENT OF NUTRIENT DIGESTION AND RUMINAL METABOLISM IN HOLSTEIN STEERS FED A HIGH-GRAIN DIET

ABSTRACT

This experiment was conducted to evaluate the interaction between in vitro starch digestion and processing method on site of nutrient digestion in Holstein steers consuming a high-grain diet. Ten ruminally, duodenally, and ileally cannulated Holstein steers (initial BW = 200 ± 21 kg) were used in a replicated 4 x 4 Latin square design experiment with a 2 x 2 factorial arrangement of treatments. Treatments were in vitro starch digestion (high and low) and conservation method (high moisture and dry-rolled). Interaction of treatments was not detected ($P > 0.15$) for any measure of digestibility or ruminal metabolism. When expressed as a percentage of duodenal flow, 21.5% less starch was digested in the small intestine of steers consuming diets containing dry-rolled corn, with an 8% decline in apparent total-tract starch digestibility. Total molar proportion of volatile fatty acid tended to be greater ($P = 0.10$) for diets containing corn hybrids with high in vitro starch digestion. Relative to nitrogen digestibility, diets containing high moisture corn resulted in a greater amount of microbial nitrogen flow to the duodenum and greater microbial efficiency ($P < 0.05$). Both processing method of corn and ruminal starch degradation of corn hybrids appear to have a profound impact on site and extent of starch digestibility and nitrogen metabolism.

2.1 INTRODUCTION

Corn hybrids with different genotypes or grown under diverse environmental conditions, result in corn that has different kernel characteristics. The physical structure and chemical composition of cereal starches, along with the interactions between starch and grain protein content can alter the digestibility of grain for livestock. Research (Philippeau et al., 1999; Macken et al., 2003) suggests that grain source (i.e., wheat vs. corn) and corn endosperm type (i.e., floury vs. flinty) can increase both ruminal starch degradability and postruminal starch digestion. Steam-flaking of more vitreous corn grain has been shown to increase total tract digestibility when compared to diets containing dry-rolled corn (Corona et al., 2006).

Theoretically, the conversion of dietary starch to energy utilized by various body tissues is greater if assimilation occurs via intestinal glucose absorption rather than ruminal fermentation and subsequent volatile fatty acid absorption (Harmon and McCloud, 2001). Fermentation in the rumen and large intestine results in energetic losses due to methane formation, heat of fermentation, and lower efficiency for use of absorbed substrates. However, ruminal degradation of carbohydrate allows for VFA formation and subsequent absorption which is critical to meet the animal's energetic requirement. Therefore, maximal energetic efficiency of finishing cattle necessitates a high extent of ruminal starch fermentation, while maximizing starch digestibility in the small intestine and limiting digestion in the large intestine (Owens et al., 1997).

We hypothesized that corn grain with high in vitro starch digestibility (IVSD) would be more ruminally fermentable than corn grain with low IVSD. Also, corn grain with high IVSD that bypasses ruminal degradation may have greater small intestinal

digestibility. Likewise, high moisture processing of corn would result in both an increase in ruminal starch degradability and the percentage of starch digested in the small intestine when compared to dry-rolled corn. Consequently, greater amounts of starch might be digested in the small intestine for steers consuming diets containing dry-rolled corn due to greater ruminal starch passage. The objective of this experiment was to evaluate the potential interaction between high and low IVSD and conservation method (high moisture ensiled vs. dry rolled) on site of nutrient digestion in Holstein steers.

2.2 MATERIALS AND METHODS

2.2.1 Corn Grain Planting, Harvest, and Processing

Two corn hybrids were obtained from Pioneer Hi-Bred international to represent floury (33R77) and flinty (34B97) endosperm types (Owens, personal communication). While high moisture corn grain containing floury endosperm did not differ in vitreousness compared to high moisture corn grain containing flinty endosperm (45.6% vs. 41.8%; $P = 0.17$), IVSD was significantly greater for 33R77 than 34B97 when evaluated as either HMC or DRC ($P < 0.01$). Therefore, instead of vitreousness, treatments are represented as corn grain with low IVSD (**LOW**) or high IVSD (**HIGH**).

Two corn hybrids representing high (Pioneer 33R77; HIGH) and low (34B97; LOW) IVSD (Pioneer Hi-Bred International, Des Moines, IA) were planted in a 15-acre field located on the Michigan State University farm (East Lansing, MI) on May 8, 2003. These specific hybrids were chosen based on chemical composition differences determined and recommended by Pioneer Hi-Bred International. The field was divided in half, with each half containing one experimental hybrid. Each half was monitored extensively for DM throughout the summer. For high moisture corn conservation, half of

LOW was harvested on September 15, 2003 at a DM of 66.3% and half of HIGH was harvested on September 23, 2003 at a DM of 67.3%. The remaining corn for each variety was harvested as dry corn at 81% DM on December 12, 2003 and commercially dried at 15°C to 87% DM (Michigan Crop Improvement Association and Foundation Seed, Okemos, MI). High moisture corn (HMC) was coarsely ground at the Michigan State University feed mill prior to storage in two separate Ag-Bags for ensiling (Miller-St. Nazianz, St. Nazianz, WI). Dry corn was coarsely rolled (DRC) prior to feeding. Corn was planted at a rate to provide a population for both varieties of 28,000 plants per acre. The average yield for LOW was 130 bushel per acre, while HIGH yielded 162 bushel per acre. Nutrient compositions and physical characteristics of the corn grain treatments used in the experiment are shown in Table 2.1.

2.2.2 Experimental Design and Data Collection

All surgical and animal care procedures described herein were approved by the All-University Committee for Animal Use and Care of Michigan State University (#11/03-145-00).

Eight Holstein steers were utilized in a replicated 4 x 4 Latin square design, with two extra steers. The squares were balanced for carryover effects so that each dietary treatment followed the other dietary treatments an equal number of times. Within each square, four steers were randomly assigned to one of four experimental treatments. A 2 x 2 factorial arrangement of treatments was utilized within each square. Experimental treatments consisted of two corn hybrids (HIGH and LOW) and two corn processing methods (HMC and DRC). Steers were vaccinated and treated for both internal and external parasites prior to surgical cannulation procedures.

Steers (four months old; BW = 267 kg) were transported to the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University where they were fitted with ruminal, duodenal, and ileal cannulas according to procedures outlined by Streeter et al. (1992). Steers were retained overnight for observation and then transported to the Michigan State University Purebred Beef Center. Steers were monitored and surgical sites cleaned twice daily over a four week period to prevent infection. If infection was determined to be present, steers were treated with penicillin and monitored by a consulting veterinarian. After recovery, steers were moved to the Beef Cattle Teaching and Research Center.

Experimental diets contained either DRC or HMC treatment hybrids, corn silage, soybean meal, vitamins and minerals, and limestone. The diets were formulated to contain 13% crude protein, 2.1 Mcal/kg NEm, and 1.4 Mcal/kg NEg (NRC, 1996). Supplemental limestone was added to balance calcium concentration for each diet due to high levels of phosphorus associated with the experimental treatments. Ingredient and nutrient compositions of the experimental diets are presented in Table 2.2 and 2.3. In order to keep diets isoenergetic, 5.6% more corn silage was added to the diets of steers consuming HMC treatments.

High moisture or dry rolled corn, corn silage, soybean meal, vitamins, minerals, and limestone were weighed and mixed daily. Steers were fed once daily at 0800 h at 110% of expected intake. Amount of feed delivered and orts were recorded daily. Dietary feed samples (0.5 kg) and individual steer orts (10% of orts on a wet weight basis) were obtained daily during the collection period, frozen and composited within

each collection period. Diet components were evaluated after each period for DM and diets were re-balanced accordingly.

Experimental periods were 21 d in length: 12 d allotted for diet adaptation, 4 d for total fecal and urine collection, and 5 d for ruminal, intestinal, and fecal digesta sampling. Steers were adapted to metabolism stalls and fecal collection bags utilized throughout the experiment by placing steers in metabolism stalls for a period of 10 d. After this time, DMI of steers was equivalent to previously recorded intake. Steers were housed in covered pens bedded with wood chips to preserve intestinal cannula integrity and prevent lameness. Two steers, one from each square consuming the same treatment, were housed in a pen for use in the experiment if needed. During adaptation, steers were fed 110% of previously recorded intake. After treatment adaptation, steers were placed into metabolism stalls for the remainder of the period for digesta sampling. Steers were weighed at the beginning of the trial and at the end of each period. Data from two steers, receiving identical treatments, were removed prior to the second period due to duodenal and ileal cannula failure. These steers were replaced with two alternate Holstein steers that had been previously adapted to similar diets and to metabolism stalls for the remainder of the trial.

Total urine and feces collection was conducted during the first 4 d of each sampling period. Steers were fitted with a harness and fecal collection bag. Fecal bags were removed at 0700 h, weighed, and a 500 g subsample obtained daily. Samples were immediately frozen at -20°C until nutrient analyses were conducted. Urine collection bags were placed at the end of each metabolism stall to collect all urine excreted by each steer. Urine collection bags were acidified with 50 mL concentrated 6N sulfuric acid as a

preservative for ammonia and emptied into individual plastic containers at 0730 h, weighed, volume determined, and a 100 mL subsample obtained daily.

Chromic oxide (Cr_2O_3) was used as a marker of passage to estimate nutrient digestibility in the rumen, small intestine, large intestine and total tract. Gelatin capsules (1.5 oz; Torpac Inc., Philadelphia, PA) were filled with 5 g Cr_2O_3 and dosed through the rumen cannula at 0730, 1530, and 2330 h (total of 15 g Cr_2O_3 per d) from d 14 to 20 of experimental period. A priming dose of Cr_2O_3 was administered on d 14 at three times the regular dose.

Determination of ruminal, small intestinal, and total tract digestibility was accomplished via collection of duodenal and ileal digesta, and feces collected every 9 h starting at 1200 h on d 16 and continuing until 0300 h on d 18. This sampling time reflects every 3 h time point across a 24 h time interval to account for diurnal variation. One 500 mL mixed rumen sample was obtained during sampling for later purine analysis. Ruminal contents from six different sites were combined and strained to obtain ruminal fluid. One 100 mL liquid subsample was obtained for VFA analysis. Ruminal pH was determined using a calibrated pH probe (model 230A, ATI Orion, Boston, MA) at each sampling time. After collection, each sample was immediately frozen at -20°C until further analysis. Effect of experimental treatments on relative rate of VFA absorption and rate of liquid passage was measured on d 20 using a pulse dose of valeric acid and cobalt EDTA (Allen et al., 2000). Solutions (634 ml, pH 6.0) containing valeric acid (1.84 moles) and Co-EDTA (5 g) were pulse-dosed 2 h post-feeding. Ruminal fluid samples were collected every 30 min during the initial 12 h, every 1 h from 12 to 16 h time points, every 2 h from 16 to 20 h time points, and once at 24 h. Samples were

frozen immediately after collection until later analysis for cobalt and valerate concentration.

2.2.3 Sample analyses

In vitro starch digestibility of composited corn samples obtained at harvest was completed after a 7 h incubation (Goering and Van Soest, 1970). Prior to trial initiation, corn samples obtained at harvest were subsampled, dried in a 55°C forced-air oven for 72 h and ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Starch was analyzed by a one-stage enzymatic method of glucoamylase (Diazyme L-200, Miles, Inc., Elkhart, IN) with a NaOH gelatinization step (Karkalas, 1985); glucose concentration was measured using a glucose oxidase method (glucose kit #510; Sigma Chemical Co., St. Louis, MO), and absorbance was determined with a microplate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Ruminal fluid utilized for in vitro incubations was obtained from a lactating cow consuming a 50% concentrate, 50% forage diet. All assays were performed in duplicate.

Corn kernel particle size was determined for each experimental treatment by dry sieving through 8 sieves (Sieve aperatures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan) using a sieve shaker apparatus (Model RX-86, W.S. Tyler Inc., Gastonia, NC) for 15 min until the bottom pan weight remained constant and mean particle size was calculated (ASAE, 1968). Corn grain vitreousness for both hybrids was determined according to the procedure of Dombrink-Kurtzman & Bietz (1993). Briefly, one hundred grains of each hybrid prior to HMC conservation were randomly selected. Among those 100 grains, 10 groups of 10 grains visually homogeneous in size and shape were formed. From each of those 10 groups, one grain was randomly sampled. A composite sample

was then formed with 10 grains from each hybrid, at each stage. The sample was immersed in distilled H_2O for 5 m, and after that the grains were dried with a paper towel and the pericarp and germ were separated from the endosperm by scalpel dissection. Next, the vitreous endosperm was separated from the farinaceous endosperm with a rotary tool. The weight of vitreous endosperm was determined and expressed as percentage of the total endosperm weight.

Absolute density for experimental corn hybrids was completed using a pycnometer (Ultrapycnometer 1000e, Quantachrome Instruments, Boynton Beach, FL). In general, the typical volume measurement cycle of the pycnometer consists of: opening the venting valves and measuring the ambient pressure, opening the gas input valve to purge the sample cell, followed by closing the vent valves and allowing the pressure to build in the sample cell until the desired target pressure is reached. When the pressure stabilized, the pressure value was saved (Pressure A), the valve to the added volume was opened and once the pressure stabilized the pressure was recorded (Pressure B). The kernel volume was calculated from these two pressure readings. The kernel weight was used in conjunction with the kernel volume to determine density.

Composited diet ingredients and orts were analyzed for DM, ash, NDF, ADF, starch, CP, and P. Samples were dried in a 55°C forced-air oven for 72 h and ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Ash was determined following sample ignition at 500°C for 5 h. Composited samples were analyzed for NDF (Van Soest et al., 1991), ADF (Goering and Van Soest, 1970), CP (Leco, 1988), and starch (Karkalas, 1985), with glucose concentration determined according to the above procedures. All analyses were performed in duplicate and

concentrations of all nutrients, disregarding DM, were expressed as percentages of DM determined after drying at 105°C through a forced-air oven.

Frozen duodenal, ileal, and fecal samples obtained during digesta collection were lyophilized and ground to pass through a 1-mm screen. All digesta samples were analyzed for DM, ash, NDF, ADF, CP, starch and purine concentration. Concentrations of all nutrients, except DM, were expressed as percentages of DM determined by drying at 105°C in a forced-air oven for more than 8 h.

Chromium concentration of digesta samples was evaluated using atomic absorption spectrometry according to the manufacturers' recommendations (Smith-Hieftje 4000, Thermo Jarrell Ash Co., Franklin, MA) following digestion with a phosphoric acid-manganese sulfate solution (Williams et al., 1962). Duodenal digesta flow was calculated according to procedures described in Armentano and Russell (1985).

Rumen samples for purine analysis were prepared following the procedure of Overton et al. (1995). Purines were measured, using a modified procedure of Zinn and Owens, 1986 (Overton et al., 1995), as a bacterial marker. Purine to N ratio was determined and microbial protein production was calculated by dividing purine to N ratio of ruminal microorganisms by the purine percentage of duodenal DM per day (Zinn and Owens, 1986).

Ruminal fluid samples obtained at sampling were thawed and composited by taking two 10 mL aliquots from each time point for each animal yielding two 80-mL subsamples per animal. The first composited ruminal fluid subsample was analyzed for concentrations of VFA, lactate, and NH_3 concentration. The aliquot to be analyzed for ruminal NH_3 concentration was acidified with 2 mL of concentrated sulfuric acid. For

VFA analysis, ruminal samples were centrifuged at 26,000 x G for 30 min. Concentrations of VFA and lactate of supernatant were determined by HPLC (Waters Corp., Milford, MA). Ammonia was measured using the procedure of Broderick and Kang (1980), absorbance was determined using a microplate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA).

The second ruminal fluid subsample was used to measure valerate concentration by HPLC (Waters Corp., Milford, MA) and measurement of cobalt concentration by flame atomic absorption spectrophotometry according to manufacturer's recommendations (SpectrAA 220/FS, Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia).

Nutrient intake was calculated using the amounts and composition of feed offered and refused. Duodenal digesta was analyzed for purines and duodenal flow of microbial N, OM, and starch ratios were estimated by analysis of microbial pellets. Truly ruminally degraded OM was calculated by subtracting duodenal flow of nonmicrobial OM from OM intake. Truly ruminally degraded starch was calculated by subtracting duodenal flow of nonmicrobial starch from starch intake.

2.2.4 Statistical Analysis

Data were analyzed using the linear mixed model procedure of SAS (Version 8e, SAS Institute Inc., Cary, NC) as duplicated (n = 2) 4 x 4 Latin squares using the following model:

$$Y_{ijkl} = \mu + A_i + P_j + Q_k + R_l + QR_{kl} + PQR_{jkl} + e_{ijkl}$$

where μ = overall mean, A_i = random effect of steer (i = 1 to 10), P_j = fixed effect of period (j = 1 to 4), Q_k = fixed effect of corn grain IVSD (k =1 to 2), R_l= fixed effect of

processing method (l = 1 to 2), QR_{kl} = interaction of corn grain IVSD and processing method, PQR_{jkl} = interaction of period, corn grain IVSD and processing method, and e_{ijkl} = residual, assumed to be normally distributed. Orthogonal contrasts were utilized to determine the effect of corn grain IVSD, corn grain processing method, and the interaction of corn grain IVSD and conservation method. Period by treatment interaction was included in the model as two steers consuming the same treatment were removed after the first period and replaced by two alternate steers. Treatment main effects were considered significant at $P < 0.05$, and a tendency declared when $P < 0.10$. Period by treatment interaction was considered significant when $P < 0.05$ and acknowledged in tables with an asterisk rather than probability values. Period by treatment interaction was removed from the model if significance was not detected ($P > 0.05$).

Rates of valerate and cobalt disappearance were determined by nonlinear regression (JMP Version 4, SAS Institute, Inc., Cary, NC) of their decline in concentration in ruminal fluid over time post-dosing using a one-pool, first-order model accounting for background concentrations of each. Rate of concentration decline of valerate and cobalt from the rumen over time were estimated by nonlinear regression. Rate of absorption of valerate was estimated by subtracting rate of concentration decline over time determined for cobalt from that determined for valerate (Allen et al., 2000).

2.3 RESULTS AND DISCUSSION

2.3.1 Ruminal Fermentation

No interactions of main treatment effects were significant for any observed measure of ruminal VFA concentration, pH, or ammonia (Table 2.4). HIGH tended to have greater molar concentrations of total VFA ($P = 0.10$), with numerically greater

proportion of propionate (36.2% vs. 32.7%) and less proportion of butyrate (7.2% vs. 9.0%) than LOW. Previous research has shown that as fermentable grain starch is increased there is greater total VFA concentration, increased production of microbial protein, reduced fiber digestion, decreased ruminal ammonia concentration and decreased acetate:propionate ratios (Huntington et al., 2006). These results demonstrate that HIGH is fermented differently than LOW. Rate of valerate absorption was utilized in this trial as an estimate of VFA absorption across the ruminal epithelium. Voelker and Allen (2003) reported a positive relationship between mean ruminal pH and rate of valerate absorption in dairy cows. Although mean ruminal pH was similar between treatments during this trial, DRC HIGH had a 9% numeric advantage in rate of valerate absorption compared to HMC HIGH. Rate of VFA absorption can be affected by ruminal consistency, blood flow, and rumen motility.

2.3.2 Dry Matter, Organic Matter, and Neutral Detergent Fiber Digestibility

Interaction of treatments was not detected for any measure of DM or OM digestibility (Table 2.5). Likewise, digestion of DM and OM were not affected by corn grain IVSD in this study. However, steers consuming DRC had significantly greater DM and OM intake compared to steers consuming HMC ($P < 0.05$). Dry-rolled corn treatments resulted in greater apparent ruminal degradation of OM ($P < 0.10$) when compared to HMC diets, while no differences between treatments existed when evaluated as a percentage of intake. Steers consuming HMC had significantly greater apparent small intestinal OM digestibility ($P < 0.05$) compared to steers consuming DRC when evaluated as a percentage of intake, with a similar tendency detected for HMC when evaluated as a percentage of duodenal passage ($P < 0.10$). Decreased OMI, coupled with

greater small intestinal OM digestibility resulted in significantly greater ($P < 0.05$) total tract OM digestibility for HMC treatments compared to DRC. These results are similar with those reported by Cooper et al. (2001) who showed a 4% advantage in total tract OM digestibility for steers consuming HMC or steam flaked corn (SFC) relative to DRC.

Treatment effects on neutral detergent fiber (NDF) digestion are presented in Table 2.6. Interaction of treatments was not detected for NDF digestibility. Likewise, no differences were detected for NDF digestibility based on corn grain IVSD. However, treatments containing DRC resulted in greater NDF intake ($P < 0.05$), and significantly greater ruminal, post-ruminal, and total tract NDF digestibility ($P < 0.05$) compared to steers consuming HMC. This result is somewhat surprising as HMC treatments contained greater concentrations of NDF which could promote a better ruminal environment for fiber digestion (Gorocica-Buenfil and Loerch, 2005).

2.3.3 Site of Starch Digestion

Our initial hypothesis was for a potential interaction between corn grain IVSD and processing method to occur. Treatment effects on site of starch digestion are shown in Table 2.7. Corn grain vitreousness reflects the association between starch and protein in the endosperm. In flinty, more vitreous endosperm, the starch granules are surrounded by a more compact and dense protein matrix, thereby limiting accessibility to enzymatic digestion (Kotarski et al., 1992)). In contrast, floury, less vitreous endosperm is comprised of starch granules that are more accessible to rumen bacteria because the granules are less compact and the protein matrix is discontinuous and more soluble (Kotarski et al., 1992). Converse to our initial hypothesis, there were no treatment interactions for any measure of starch digestibility ($P > 0.10$). Likewise, corn grain

IVSD did not affect ($P > 0.10$) site or extent of starch digestion during digesta sampling. Our data obtained during digesta sampling did not corroborate findings from Philippeau et al. (1999) who reported that apparent ruminal starch digestibility increased from 35.0 to 57.0% when a more vitreous corn grain was replaced by corn grain with less vitreousness, nor reported results from Corona et al. (2006) who documented a reduction in post-ruminal and total-tract starch digestibility when vitreousness increased in diets consisting of 73.2% DRC.

A tendency for reduced starch intake was documented for steers consuming HMC compared to DRC ($P < 0.10$). Compared to steers consuming HMC, starch apparently degraded in the rumen was reduced by 10.2% ($P < 0.05$) when evaluated as a percent of intake for steers consuming DRC. Steers consuming DRC tended to have 8.6% less truly ruminally degraded starch than steers consuming HMC treatments ($P < 0.10$). Subsequently, feeding DRC resulted in 10.1% greater starch passage to the duodenum ($P < 0.01$). Even so, no differences between processing methods were documented for apparent small intestinal starch digestibility when expressed as a percent of intake. Contrary to this finding, when small intestinal starch disappearance was expressed as a fraction of duodenal flow, 21.5% more starch was digested in the small intestine of steers consuming HMC ($P < 0.05$) compared to diets containing DRC. The protein matrix associated with HMC is more soluble, allowing more damage to occur from potential ruminal microbial attack, thereby enabling the partially degraded endosperm that passes to the small intestine to undergo heightened enzymatic hydrolysis. Alternatively, factors related to limited time and surface exposure in the small intestine for DRC and the potential limited capacity of gut tissue to produce starch-hydrolyzing enzymes (Owens et

al., 1986; Harmon, 1993) may have also contributed to this result. Steers consuming HMC had 8.3% greater apparent total tract starch digestion when compared to steers consuming DRC (96.7% vs. 88.2%; $P < 0.01$). A primary goal in corn processing is to increase starch availability. These results agree with previous research showing that processing method has a profound effect on site and extent of starch digestion. Huntington (1997) summarized data from 14 research trials and reported average ruminal starch digestibility values of 76% and 90% for DRC and HMC, respectively. Galyean et al. (1976), while not finding significant differences in postruminal starch digestion across processing treatments, documented that HMC was numerically 7% greater than DRC. Cooper et al. (2002) documented a 3% advantage in total tract starch digestibility for HMC and SFC relative to DRC. Likewise, in a review pertaining to the influence of corn grain processing on site and extent of digestion, Owens and Zinn (2005) reported that fine grinding of less vitreous grain will help to maximize starch digestion. Theurer et al. (1999), in a trial involving sorghum grain, reported that steam flaked compared to dry-rolled and reduced steam flaking density resulted in greater ruminal starch degradation. Likewise, Ladely et al. (1995) documented that processing of three different corn hybrids selected on the basis of in-vitro rate of starch digestion (IVRSD) had a more profound impact on IVRSD and cattle performance than did differences among grain hybrids.

Diets containing HMC contained 5.6% more corn silage (DM basis) in an effort to maintain treatment diets isoenergetic. Likewise, a tendency for greater starch intake was shown for DRC diets compared to HMC diets ($P < 0.10$). Increased concentrations of corn silage and decreased starch intake can result in greater total tract digestibility of starch (Turgeon et al., 1983).

2.3.4 Site of Nitrogen Digestion

No differences were detected for N intake between treatments ($P > 0.10$; Table 2.8). Even so, steers consuming HIGH treatments had numerically greater amounts of N truly degraded in the rumen compared to steers consuming corn grain with low IVSD. Russell (1998) reported that when ruminal fermentation is increased, ruminal bacteria can more effectively utilize amino N. Ruminant N requirement is increased due to heightened ruminal fermentation of carbohydrate (NRC, 1996). No differences between treatments for duodenal N flow were detected. However, greater amounts of N reached the ileum of steers consuming DRC ($P < 0.05$), while steers consuming HMC tended to have a 6% advantage in N digested and absorbed in the small intestine compared to steers consuming DRC ($P < 0.10$). Steers consuming HMC had a greater fraction of duodenal N from microbial origin when compared to the DRC treatments ($P < 0.05$; 143.5 vs. 114.6, respectively). Coincidentally, HMC treatments resulted in significantly greater microbial N efficiency (MNE; g microbial N / kg truly fermented OM) compared to DRC ($P < 0.05$). These results document the difference in protein solubility for diets containing HMC; soluble compounds in the rumen are attacked more rapidly and digested more completely than are insoluble compounds due in part to differences in microbial access (Owens and Zinn, 1988). Greater MNE is usually witnessed when greater ruminal fermentation exists, which is corroborated by increased apparent and true ruminal starch digestion in steers consuming HMC in the present study. However, Owens and Goetsche (1988) reported that MNE is greater with less extensive digestion in the rumen (i.e., whole corn vs. DRC) and almost always with forage vs. concentrate diets.

In our study HMC treatments resulted in numerically less truly ruminally fermented OM, but did result in greater microbial nitrogen yield.

Steers consuming HIGH corn hybrids as DRC had the highest concentration of N apparently digested in the large intestine either on a g/d or percentage of duodenal flow basis. These results, combined with N truly degraded in the rumen, document that N escaping ruminal utilization was less digested and absorbed in the small intestine. Even so, no differences were detected between treatments for apparent total tract N digestibility.

2.4 CONCLUSIONS

In the present study, IVSD and processing method of corn grain appear to affect site and extent of nutrient digestion. Feeding corn hybrids with high IVSD resulted in greater ruminal starch degradability. Steers consuming HMC had greater MNE compared to DRC, presumably due to heightened ruminal fermentation of starch. While differences in digestibility due to corn grain IVSD were not evident throughout this trial, diets containing corn grain with high IVSD resulted in greater total VFA concentration and numerically greater proportions of propionate, providing evidence that IVSD does impact ruminal fermentation. Experiments focusing on corn grain with greater differences in vitreousness merit further research attention. However, high moisture conservation of corn grain or rolling corn grain prior to feeding may alleviate potential differences in digestibility of corn grain in high-concentrate feedlot rations due to reduced particle size.

TABLES

Table 2.1. Physical and chemical characteristics of corn grain

	HMC		DRC			*P* =		
	HIGH	LOW	HIGH	LOW	SEM	I[1]	P[2]	I*P[3,5]
DM	64.8	66.9	90.4	90.3	0.01	0.41	< 0.01	0.38
Starch, % DM	73.2	72.5	67.6	65.8	1.6	0.53	< 0.01	0.84
CP, % DM	9.6	9.7	9.7	9.5	0.1	0.69	0.50	0.33
NDF, % DM	3.6	3.7	6.6	6.2	0.7	0.82	< 0.01	0.78
ADF, % DM	1.4[c]	1.3[c]	1.9[b]	2.3[a]	0.1	0.20	< 0.01	0.11
IVSD[4]	74.8[a]	70.1[b]	57.4[c]	43.3[d]	0.7	< 0.01	< 0.01	< 0.01
Absolute density	1.287[bc]	1.291[ab]	1.285[c]	1.306[a]	0.004	0.04	0.18	0.12
Vitreousness, %	45.6	41.8	N/A	N/A	1.8	0.17	*	*
Sieve aperture (μm)	----------% retained ----------							
4750	55.5	44.9	17.0	21.5	4.7	0.53	< 0.01	0.14
2369	38.9	48.8	69.0	67.4	5.0	0.43	< 0.01	0.28
1180	3.7	5.3	9.3	7.0	1.2	0.75	0.02	0.14
600	0.7	0.4	2.3	2.0	0.5	0.60	0.02	0.96
300	0.6	0.4	1.0	1.1	0.3	0.82	0.09	0.67
150	0.8	0.7	0.9	0.6	0.1	0.20	0.99	0.74
75	0.5	0.2	0.3	0.1	0.04	< 0.01	0.01	0.67
Pan	0.01	0.01	0.04	0.01	0.02	0.26	0.34	0.44
Mean part. size (μm)	5408.1[a]	5023.2[a]	3844.1[b]	4064.7[b]	171.8	0.65	< 0.01	0.12

[1]Main effect of in vitro starch digestibility

[2]Main effect of processing method

[3]Interaction between in vitro starch digestibility and processing method

[4]IVSD = In vitro starch digestibility determined after 7 h incubation

[5]Treatment least squares means were separated using the pdiff option of SAS when interaction was less than 0.15. Means within row with unlike superscripts differ ($P < 0.05$)

Table 2.2. Composition of experimental diets (DM basis)

	Processing Method	
Item	Dry rolled	High moisture
Ingredient, % (DM Basis)		
Corn	78.3	72.0
Corn silage	12.6	18.2
Soybean meal	3.8	3.6
Limestone	0.4	1.0
Premix[a]	5.0	5.4
Calculated composition		
Crude protein, %	12.9	12.8
NEm, Mcal/kg	2.1	2.1
NEg, Mcal/kg	1.4	1.5

[a]Contained (DM Basis): 47.7% soybean meal, 22.1% calcium carbonate, 10.0% trace mineral salt, 7.5% urea, 5.6% potassium chloride, 3.3% ground corn, 2.2% dicalcium phosphate, 1.1% selenium 90, 0.16% vitamin A (30,000 IU/g), and 0.28% Rumensin 80

Table 2.3. Nutrient composition of experimental diet (DM basis)

	HMC[1]		DRC[1]		
	HIGH[2]	LOW[2]	HIGH[2]	LOW[2]	SEM
OM	94.8[a]	95.0[a]	95.2[b]	95.3[b]	0.1
Starch	58.8	58.6	56.3	57.9	0.9
CP	13.0	13.1	12.9	13.0	0.1
NDF	6.8[a]	6.8[a]	8.5[b]	7.8[b]	0.4
ADF	3.4	3.3	3.3	3.3	0.1
P	0.5	0.4	0.4	0.5	0.03

[1]High moisture and dry-rolled corn
[2]High and low in vitro starch digestibility
[a,b]Means in the same row followed by unlike superscript letters differ ($P < 0.05$)

Table 2.4. Effects of in vitro starch digestibility and grain processing method on ruminal VFA, pH, and ammonia concentration

	HMC		DRC			*P* =		
Item	HIGH	LOW	HIGH	LOW	SEM	I[1]	P[2]	I*P[3]
Total VFA[4], m*M*	180.7	172.0	184.9	175.1	4.5	0.10	0.50	0.92
VFA, % of total VFA								
Acetate	40.9	42.4	40.5	42.9	1.8	0.21	0.96	0.74
Propionate	35.7	33.4	36.7	32.1	4.1	0.29	0.95	0.72
Butyrate	7.1	9.2	7.2	8.8	1.5	0.13	0.94	0.84
BC VFA[5]	3.16	3.13	2.68	3.89	0.74	0.35	0.82	0.32
Lactate	1.46	1.50	1.94	1.87	0.26	0.94	0.03	0.77
pH	5.57	5.61	5.56	5.57	0.08	0.71	0.74	0.82
NH_3 (mg/100 ml)	1.25	2.34	1.47	1.88	0.42	0.11	0.80	0.45
Val k_{abs}, %/h[6]	44.4	53.0	53.6	48.9	4.4	0.66	0.56	0.15

[1]Main effect of in vitro starch digestibility

[2]Main effect of processing method

[3]Interaction between in vitro starch digestibility and processing method

[4]VFA represented as μmol/ml

[5]Branched-chain volatile fatty acids

[6]Val k_{abs}, h = Rate of valerate absorption (%/h); measured using cobalt-EDTA and valerate

Table 2.5. Effects of in vitro starch digestibility and grain processing method on site of DM and OM digestion

Item	HMC		DRC		SEM	*P* =		
	HIGH	LOW	HIGH	LOW		I[1]	P[2]	I*P[3]
DM								
Intake, kg/d	6.2	5.7	6.9	6.5	0.4	0.23	0.04	0.96
Apparent total tract digestion								
kg/d	4.7	4.4	5.2	4.7	0.3	0.22	0.17	0.78
%	75.9	76.4	74.2	71.9	1.9	0.65	0.13	0.47
OM								
Intake, kg/d	5.9	5.5	6.6	6.3	0.4	0.26	0.03	0.94
Apparent ruminal digestion								
kg/d	2.3	2.1	3.3	2.7	0.4	0.16	0.02	0.58
%	38.9	37.3	49.3	42.0	4.4	0.33	0.11	0.53
True ruminal digestion								
kg/d	4.0	3.6	4.4	3.9	0.4	0.16	0.18	0.80
%	67.1	65.6	68.1	63.3	4.3	0.47	0.88	0.71
Passage to duodenum, kg/d	3.5	3.4	3.3	3.6	0.2	0.80	0.97	0.48
Apparent SI digestion								
kg/d	1.9	1.9	1.4	1.7	0.2	0.57	0.19	0.54
% of intake	34.4	34.8	21.2	26.5	4.4	0.55	0.05	0.63
% of duodenal passage	53.4	55.1	38.0	46.9	5.3	0.37	0.06	0.54
Apparent total tract digestion								
kg/d	4.6	4.3	5.0	4.6	0.3	0.18	0.24	0.81
%	78.3	79.0	75.5	73.3	1.9	0.72	0.05	0.48

[1]Main effect of in vitro starch digestibility

[2]Main effect of processing method

[3]Interaction between in vitro starch digestibility and processing method

Table 2.6. Effects of corn grain endosperm type and grain processing method on NDF digestion

	HMC		DRC			*P* =		
	HIGH	LOW	HIGH	LOW	SEM	I[1]	P[2]	I*P[3]
Intake, kg/d	0.42	0.40	056	0.51	0.04	0.27	0.003	0.63
Apparent ruminal digestion								
kg/d	0.24	0.22	0.45	0.39	0.04	0.34	0.001	0.62
%	57.6	53.3	79.2	76.7	6.4	0.60	0.005	0.89
Passage to duodenum, kg/d	0.18	0.17	0.11	0.12	0.02	0.93	0.01	0.85
Apparent postruminal digestion, kg/d								
% of intake	7.2	13.4	-5.7	-1.5	5.8	0.39	0.04	0.86
% of duodenal passage	9.7	13.9	-29.5	-9.7	10.4	0.27	0.01	0.47
Apparent total tract digestion								
kg/d	0.27	0.27	0.42	0.39	0.04	0.58	0.001	0.70
%	64.6	66.5	73.4	75.0	2.4	0.38	0.001	0.94

[1]Main effect of in vitro starch digestibility

[2]Main effect of processing method

[3]Interaction between in vitro starch digestibility and processing method

Table 2.7. Effects of in vitro starch digestibility and grain processing method on site of starch digestion

	HMC		DRC			*P* =		
	HIGH	LOW	HIGH	LOW	SEM	I[1]	P[2]	I*P[3]
Intake, kg/d	3.6	3.4	3.9	3.9	0.2	0.51	0.06	0.50
Apparent ruminal digestion								
kg/d[4]	3.0	2.7	3.0	2.8	0.2	*	*	*
%	83.4	82.4	76.2	72.0	3.2	0.41	0.01	0.61
True ruminal digestion								
kg/d	3.2	2.9	3.1	3.2	0.2	0.61	0.69	0.51
%	89.5	88.3	80.9	82.5	3.6	0.96	0.09	0.73
Passage to duodenum, kg/d	0.6	0.6	0.9	1.1	0.1	0.45	< 0.01	0.59
Apparent SI digestion								
kg/d	0.4	0.4	0.3	0.4	0.1	0.55	0.96	0.53
% of intake	11.0	10.6	8.6	10.9	2.2	0.69	0.67	0.58
% of duodenal passage	63.2	61.0	34.5	41.9	7.8	0.76	0.02	0.59
Apparent LI digestion								
kg/d	0.1	0.2	0.3	0.2	0.1	0.68	0.16	0.17
% of intake	1.8	4.2	8.0	4.5	2.3	0.83	0.21	0.24
% of duodenal passage	13.2	22.3	28.2	11.0	7.7	0.67	0.84	0.18
Apparent total tract digestion								
kg/d	3.5	3.3	3.5	3.4	0.2	0.44	0.73	0.68
%	96.0	97.3	89.5	86.8	1.5	0.63	< 0.01	0.22

[1]Main effect of in vitro starch digestibility

[2]Main effect of processing method

[3]Interaction between in vitro starch digestibility and processing method

[4]Period by treatment interaction ($P < 0.05$)

Table 2.8. Effects of in vitro starch digestibility and grain processing method on site of nitrogen digestibility

	HMC		DRC			*P* =		
	HIGH	LOW	HIGH	LOW	SEM	I[1]	P[2]	I*P[3]
TRDOM,[5] kg/d	4.0	3.6	4.4	3.9	0.4	0.16	0.18	0.80
N intake, g/d	130.9	116.9	143.0	133.6	8.3	0.12	0.20	0.80
Duodenal N flow								
Total, g/d[4]	194.1	196.5	186.0	187.2	11.2	*	*	*
Microbial, g/d	143.9	143.1	116.6	112.5	10.8	0.83	0.02	0.89
Non-microbial, g/d	50.3	53.5	70.2	74.7	10.7	0.73	0.09	0.95
Ileal N flow, g/d	46.2	44.7	60.1	50.7	4.4	0.21	0.04	0.37
Ruminal true, g/d	80.6	63.4	71.8	58.9	11.0	0.18	0.55	0.85
Ruminal true, % of N intake	61.3	54.9	54.2	46.1	8.1	0.40	0.35	0.92
SI apparent, g/d	143.4	151.8	135.0	131.4	13.1	0.87	0.34	0.69
SI apparent, % of duodenal flow	75.0	77.1	68.6	71.5	3.1	0.40	0.07	0.97
LI apparent, g/d[4]	5.4	3.7	13.8	4.5	3.8	*	*	*
LI apparent, % of duodenal flow[4]	3.0	1.9	7.8	3.0	2.1	*	*	*
TT apparent, g/d	88.3	75.9	94.1	85.6	7.5	0.21	0.34	0.81
TT apparent, %	67.6	63.7	64.7	63.0	2.7	0.34	0.54	0.71
Microbial efficiency, g/kg TRDOM[5]	27.9	29.0	19.6	24.2	2.2	0.23	0.01	0.45

[1]Main effect of in vitro starch digestibility

[2]Main effect of processing method

[3]Interaction between in vitro starch digestibility and processing method

[4]Period by treatment interaction ($P < 0.05$)

[5]TRDOM = Organic matter truly ruminally degraded

CHAPTER 3

EFFECT OF IN VITRO STARCH DIGESTIBILITY OF CORN AND SUPPLEMENTAL NITROGEN CONCENTRATION ON SITE AND EXTENT OF NUTRIENT DIGESTION AND PROTEIN METABOLISM IN HOLSTEIN STEERS FED A HIGH-GRAIN DIET

ABSTRACT

This experiment was conducted to evaluate the interaction between corn grain in vitro starch digestibility (IVSD) and nitrogen concentration on site of nutrient digestion and nitrogen metabolism in steers consuming a high-grain, dry-rolled corn based diet. Eight Holstein steers (initial BW = 500 ± 21 kg; four ruminally and duodenally cannulated) were used in separate 4 x 4 Latin square design experiments with a 2 x 2 factorial arrangement of treatments. Treatments were dry-rolled corn having either high (HIGH) or low (LOW) IVSD and nitrogen (N) concentration (HNIT and LNIT), where HNIT diets contained a greater degradable intake protein fraction as a percentage of crude protein (60.1% vs 48.6%, respectively). A tendency for treatment interaction was documented for ruminal starch degradation ($P < 0.10$). Steers consuming LNIT diets had 0.4 kg/d more starch apparently degraded in the rumen compared to HNIT diets, with LNIT LOW resulting in 0.6 kg/d more ruminal starch degraded than all other treatments. However, no differences were present between treatments when evaluated as a percentage of starch intake. A significant interaction of treatments was present for total tract starch digestion ($P < 0.05$), with LNIT LOW having greater total tract starch degradation than HNIT LOW ($P < 0.05$). Low N concentrations or high IVSD had a 5.6% and 4.2% increase in molar propionate concentration when compared to HNIT or

low IVSD ($P = 0.01$), respectively. Steers consuming LNIT and HIGH had the lowest ruminal pH ($P < 0.05$) indicating greater ruminal fermentation of carbohydrate. High IVSD diets had numerically greater microbial efficiency when compared to LOW. Lower N concentrations may negatively impact total tract N digestibility, while high IVSD can result in improved microbial nitrogen efficiency.

3.1 INTRODUCTION

Diets consumed by feedlot cattle often contain high concentrations of corn and thereby high concentrations of starch. Starch is fermented in the rumen to volatile fatty acids (VFA) that are then absorbed and serve as the main source of energy for the ruminant animal. Maximal energetic efficiency of finishing cattle necessitates a high extent of ruminal starch fermentation (Owens et al., 1997). Research (Philippeau et al., 1999b; Macken et al., 2003) suggests that corn vitreousness (i.e., floury vs. flinty) can increase ruminal starch degradability and vitreousness of corn grain can be used to predict ruminal starch disappearance (Philippeau et al., 1999a).

Degradable intake protein (**DIP**) is the fraction of dietary protein used by the ruminal microbial population for synthesis of microbial N and is derived from preformed amino acids and non-protein N (**NPN**). Metabolizable protein (**MP**) is the combination of microbial protein, rumen bypass protein, and endogenous protein which can be utilized by the animal. Metabolizable protein requirements increase with greater ruminal starch fermentation (NRC, 1996) and could be altered based on the extent of ruminal fermentation. Increased diet degradability can have a profound impact on the efficiency with which microbes use energy from ruminal fermentation of substrate for growth [defined as grams of duodenal microbial N per kilogram OM truly ruminally degraded

(**TRDOM**)]. Microbial N efficiency (**MNE**) is difficult to predict due to differences in ruminal retention time, pH, and substrate availability. Likewise, MNE can be reduced when peptides or amino acids are not supplied in sufficient amounts (Van Kessel and Russell, 1996). While corn processing methods have been shown to influence protein requirement (Cooper et al., 2002), little research has been conducted to determine the specific effects of corn grain starch availability and DIP fractions on N utilization and retention in growing steers experiencing varying rates of ruminal starch degradation.

We hypothesized that ruminal starch degradability would be increased in diets containing corn grain with floury endosperm and high IVSD. Likewise, higher concentrations of supplemental N would be beneficial to diets containing corn grain with flinty endosperm and low IVSD by causing an increase in microbial N production, MNE, and retained N. Furthermore, low IVSD diets might benefit from less ruminally available N due to less ruminally degradable starch and less ruminal N demand. The objectives of this experiment were to evaluate the effects of corn grain IVSD and supplemental nitrogen concentration on site of nutrient digestion in growing Holstein steers consuming a high grain diet.

3.2 MATERIALS AND METHODS

3.2.1 Treatment Determination

Two corn hybrids were obtained from Pioneer Hi-Bred international to represent floury (33R77) and flinty (34B97) endosperm type (Owens, personal communication) and were utilized in a previous experiment (McPeake et al., 2008). No differences were documented for hybrids processed as high moisture corn (HMC; $P < 0.17$). However, IVSD was 14.1% greater for 33R77 than 34B97 when evaluated as dry rolled corn (DRC;

$P < 0.01$). Therefore, hybrids are represented as corn grain with low IVSD (**LOW**) or high IVSD (**HIGH**).

Agronomic and harvesting conditions were reported in McPeake (2008). Briefly, floury (Pioneer 33R77; HIGH) and flinty (Pioneer 34B97; LOW) corn hybrids were planted in a 15-acre field located on the Michigan State University farm (East Lansing, MI) on May 8, 2003. The field was divided in half, with each half containing one experimental hybrid and harvested as HMC or DRC. Dry corn was coarsely rolled prior to feeding during the trial. Nutrient compositions and physical characteristics of the corn grain treatments used in the experiment are shown in Table 1. Nitrogen treatments were chosen based on crude protein concentration and amount of DIP supplied to the rumen (i.e., **HNIT** vs. **LNIT**), where HNIT supplied 13.3% CP with a DIP balance of -109.7 g/d and LNIT supplied 10.3% CP with a DIP balance of -392.8 g/d. Degradable intake protein balance was estimated using Level 1 of 1996 NRC (NRC, 1996). Additionally, HNIT treatments resulted in daily urea intake of 1.0% DM, whereas LNIT treatments contained no supplemental N derived from urea.

3.2.2 Experimental Design and Data Collection

All animal care procedures described herein were approved by the All-University Committee for Animal Use and Care of Michigan State University (#11/03-145-00).

Four ruminally and duodenally cannulated Holstein steers from a previous metabolism study were used in a 4 x 4 Latin square design. The square was balanced for carryover effects so that each dietary treatment followed the other dietary treatments an equal number of times. Initially, the four steers were randomly assigned to one of four experimental treatments. A 2 x 2 factorial arrangement of treatments was utilized.

Experimental treatments consisted of two corn hybrids with different IVSD and two nitrogen concentrations. Physical and chemical characteristics of corn grain hybrids are presented in Table 3.1, while composition of supplemental protein pellets is displayed in Table 3.2.

Experimental diets contained DRC, cottonseed hulls, and pellets containing both the protein source and vitamin/mineral mix. The diets were formulated to be isocaloric and contained 2.3 mcal/kg NE_m, and 1.4 mcal/kg NE_g (NRC, 1996). Ingredient and nutrient compositions of the experimental diets are presented in Table 3.3.

Dry rolled corn, cottonseed hulls, and pellets were weighed and mixed daily. Steers were fed once daily at 0800 h at 110% of expected intake. Amount of feed delivered and orts were recorded daily. Dietary feed samples (0.5 kg) and individual steer orts (10% of orts on a wet weight basis) were obtained daily during the collection period, frozen and composited within each collection period. Diet components were evaluated after each period for DM and diets were re-balanced accordingly.

Experimental periods were 18 d in length: 14 d allotted for diet adaptation and 4 d for digestion sampling. All steers were housed in a barn equipped with Calan gates and bedded on wood chips to promote cannula integrity. Prior to trial initiation, steers were allowed appropriate time for adaptation to the Calan gate system and for dietary treatments specific to each steer. During treatment adaptation, steers were fed 110% of previously recorded intake.

During digesta sampling, chromic oxide (Cr_2O_3) was used as a marker to estimate nutrient digestibility in the rumen and total tract. Gelatin capsules (1.5 oz; Torpac Inc., Philadelphia, PA) were filled with 5 g Cr_2O_3 and dosed through the rumen cannula at

0730, 1530, and 2330 h (total of 15 g Cr_2O_3 per d) from d 14 to 20. A priming dose of Cr_2O_3 was administered on d 12 at three times the regular dose.

Determination of ruminal and total tract digestibility was accomplished via collection of duodenal digesta and feces every 9 h starting at 1200 h on d 16 and continuing until 0300 h on d 18. This sampling time reflects every 3 h time point across a 24 h time interval to account for diurnal variation. One 500 mL mixed rumen sample was obtained during sampling for later purine analysis. Ruminal contents from six different sites were combined and strained to obtain rumen fluid. Two 100 mL liquid subsamples were obtained for VFA and ruminal NH_3 analyses. Ruminal liquid samples to be analyzed for ruminal NH_3 were acidified at collection with 2 mL concentrated sulfuric acid. Ruminal pH was determined using a calibrated pH probe (model 230A, ATI Orion, Boston, MA) at each sampling time. Each sample after collection was immediately frozen at -20°C until further analysis.

3.2.3 Sample Analyses

Corn kernel particle size for each experimental treatment was dry sieved through 8 sieves (Sieve aperatures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan) using a sieve shaker apparatus (Model RX-86, W.S. Tyler Inc., Gastonia, NC) for 15 min until the bottom pan weight remained constant and mean particle size was calculated (ASAE, 1968). Corn grain vitreousness for both hybrids was determined according to the procedure of Dombrink-Kurtzman & Bietz (1993). Briefly, one hundred grains of each hybrid prior to HMC conservation were randomly selected. Among those 100 grains, 10 groups of 10 grains visually homogeneous in size and shape were formed. From each of those 10 groups, one grain was randomly sampled. A composite sample was then formed

with 10 grains from each hybrid, at each stage. The sample was immersed in distilled H_2O for 5 m, and after that the grains were dried with a paper towel and the pericarp and germ were separated from the endosperm by scalpel dissection. Next, the vitreous endosperm was separated from the farinaceous endosperm with a rotary tool. The weight of vitreous endosperm was determined and expressed as percentage of the total endosperm weight.

Absolute density for experimental corn hybrids was completed using a pycnometer (Ultrapycnometer 1000e, Quantachrome Instruments, Boynton Beach, FL). In general, the typical volume measurement cycle of the pycnometer consists of: opening the venting valves and measuring the ambient pressure, opening the gas input valve to purge the sample cell, followed by closing the vent valves and allowing the pressure to build in the sample cell until the desired target pressure is reached. When the pressure stabilized the pressure value was saved (Pressure A), the valve to the added volume was opened and once the pressure stabilized the pressure was recorded (Pressure B). The kernel volume was calculated from these two pressure readings. The kernel weight was used in conjunction with the kernel volume to determine density

Composited diet ingredients and orts samples were analyzed for DM, ash, NDF, iNDF, ADF, starch, N, and P. Samples were dried in a 55°C forced-air oven for 72 h and ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Ash was determined following sample ignition at 500°C for 5 h. Composited samples were analyzed for NDF (Van Soest et al., 1991), ADF (Goering and Van Soest, 1970), N (Leco, 1988), and starch (Karkalas, 1985). Starch was analyzed by a one-stage enzymatic method of glucoamylase (Diazyme L-200, Miles, Inc., Elkhart, IN) with a

NaOH gelatinization step (Karkalas, 1985); glucose concentration was measured using a glucose oxidase method (glucose kit #510; Sigma Chemical Co., St. Louis, MO), and absorbance was determined with a microplate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Indigestible NDF was estimated as NDF residue after a 240-h in vitro fermentation (Goering and Van Soest, 1970). All analyses were performed in duplicate and concentrations of all nutrients, disregarding DM, were expressed as percentages of DM determined after drying at 105°C through a forced-air oven.

Frozen duodenal and fecal samples obtained during digesta collection were lyophilized and ground with a coffee grinder due to limited dry sample availability. All digesta samples were analyzed for DM, ash, NDF, ADF, N, starch, purine concentration, and iNDF. Concentrations of all nutrients, except DM, were expressed as percentages of DM determined by drying at 105°C in a forced-air oven for more than 8 h.

Chromium concentration of digesta samples was evaluated using atomic absorption spectrometry according to the manufacturers' recommendations (Smith-Hieftje 4000, Thermo Jarrell Ash Co., Franklin, MA) following digestion with a phosphoric acid-manganese sulfate solution (Williams et al., 1962). Duodenal digesta flow was calculated according to procedures described in Armentano and Russell (1985). Indigestible NDF was also quantified in digesta samples as an alternative marker of digestibility.

Ruminal samples for purine analysis were prepared following the procedure of Overton et al. (1995). Purines were measured by the procedures defined by Zinn and Owens (1986) with the modifications of Overton et al. (1995) as a bacterial marker. Microbial protein production was calculated by dividing purine to N ratio of ruminal

microorganisms by the purine percentage of duodenal DM per day (Zinn and Owens, 1986).

Ruminal fluid samples obtained at sampling were thawed and composited by taking two 10 mL aliquots from each time point for each animal yielding two 80 mL subsamples per animal. Composited ruminal fluid was analyzed for concentrations of VFA and lactate, and the alternate subsample for NH_3 concentration. The aliquot to be analyzed for ruminal NH_3 concentration was acidified with 2 mL of concentrated sulfuric acid. For VFA analysis, rumen fluid samples were centrifuged at 26,000 x g for 30 min. Concentrations of VFA and lactate of supernatant were determined by HPLC (Waters Corp., Milford, MA) using a modified procedure developed by Dawson (1994). Ammonia was measured using the procedure of Broderick and Kang (1980), with absorbance being determined using a microplate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA).

3.2.4 Statistical Analysis

Data were analyzed using the linear mixed model procedure of SAS (Version 8e, SAS Institute Inc., Cary, NC) as a 4 x 4 Latin square using the following model:

$$Y_{ijkl} = \mu + A_i + P_j + Q_k + R_l + QR_{kl} + PQR_{jkl} + e_{ijkl}$$

where μ = overall mean, A_i = random effect of steer (i = 1 to 4), P_j = fixed effect of period (j = 1 to 4), Q_k = fixed effect of IVSD (k =1 to 2), R_l= fixed effect of nitrogen concentration (l = 1 to 2), QR_{kl} = interaction of IVSD and nitrogen concentration, PQR_{jkl} = interaction of period, IVSD and nitrogen concentration, and e_{ijkl} = residual, assumed to be normally distributed. Orthogonal contrasts were utilized to determine the effect of

IVSD, nitrogen concentration, and the interaction of IVSD and nitrogen concentration. Main effects of treatment were considered significant at $P < 0.05$, and a tendency declared when $P < 0.10$. Treatment means were separated using the pdiff option of SAS when a tendency for interaction was detected ($P < 0.15$). Period by treatment interaction was removed from the model when significance was not detected ($P > 0.05$).

3.3 RESULTS AND DISCUSSION

3.3.1 Ruminal Fermentation

No interactions ($P > 0.10$) of main treatment effects were observed for ruminal VFA concentration or NH_3 (Table 3.4). However, a tendency for an IVSD by nitrogen concentration interaction was present for ruminal pH. While no differences existed between HIGH and LOW IVSD within the HNIT treatment, steers consuming LNIT HIGH had lower ruminal pH when compared to the LNIT LOW treatment (5.46 vs. 5.72; $P < 0.05$). Ruminal NH_3 was predictably lower ($P < 0.05$) for LNIT compared to HNIT. Satter and Slyter (1974) observed that 5.0 mg/100 mL concentration is necessary to support microbial protein production. The LNIT HIGH treatment had the lowest numeric ruminal ammonia concentration and tended to have the lowest ruminal pH. This may imply a significant role of NH_3 as a buffer in high grain diets. Ammonia has been shown to stimulate Na^+ transport across the ruminal epithelium of concentrate-fed cattle and urea-fed sheep by creating an adaptive metabolism by the rumen mucosa to metabolize greater quantities of NH_3 and/or an increased permeability of the rumen epithelium to the charged ammonium ion (NH^{4+}; Abdoun et al., 2003).

Steers consuming HIGH had decreased proportion of VFA as acetate compared to LOW ($P < 0.05$) with a tendency for greater proportion of VFA as propionate. Steers

consuming LNIT treatments had a greater proportion of total VFA as propionate, with less acetate ($P < 0.05$) compared to HNIT treatments. In a previously conducted trial by McPeake (2008), Pioneer 33R77 was more ruminally degradable which can lead to greater ruminal concentration of propionate (McPeake, 2008). In the present study, HIGH IVSD or LNIT concentrations resulted in reduced proportions of branched-chain VFA concentration ($P < 0.05$) compared to LOW and HNIT treatments. One would expect greater BC VFA with higher nitrogen concentrations as they are metabolites of microbial protein degradation. As expected, ruminal NH_3 concentration was greater for HNIT ($P < 0.01$) compared to LNIT. Higher ruminal NH_3 concentrations can be attributed to increased ruminally available N in the present study.

3.3.2 Digestibility Marker

Comparisons between iNDF and chromium for digestibility of ruminal and total tract OM were completed according to the previously documented procedures. Indigestible NDF provided a more realistic estimate of both ruminal and total tract OM and starch digestibility, as estimates obtained using chromium were unrealistically low. We believe that chromium disassociated from ruminal contents due to high amounts of cottonseed hulls in the diet. Therefore, estimates of digestion were completed using iNDF.

3.3.3 Dry Matter, Organic Matter, and Neutral Detergent Fiber Digestibility

Treatment effects on DM and OM digestibility are presented in Table 3.5. A tendency for treatment interaction was witnessed for DM intake ($P = 0.06$) even though no significant differences existed between treatments. Across all treatments, steers consumed 2% of BW. Steers receiving HNIT HIGH and LNIT LOW had numerically

greater DMI relative to both HNIT LOW and LNIT HIGH treatments (11.0 and 10.8 vs. 9.7 and 9.9 kg/d, respectively). A significant interaction of treatments was documented for apparent total tract DM digestibility ($P < 0.05$). When expressed on a kg/d basis, a greater amount of DM tended to be digested in the total tract for HNIT HIGH and LNIT LOW treatments. However, when expressed as a percentage of intake, no significant difference between IVSD or N concentration was detected in the present trial.

A tendency for interaction between main effects was detected with regards to OMI and followed the same documented trend of DMI. Interestingly, a tendency for interaction between main effects was present for truly ruminally degraded OM. Upon evaluation of simple main effects, steers that consumed the HNIT HIGH treatment tended to experience greater truly ruminally degraded OM (TRDOM) than did steers that consumed the HNIT LOW treatment (8.3 vs. 6.5; $P = 0.12$). Other measures of OM digestibility were similar among treatments in this study.

Treatment effects on site and extent of NDF digestibility are presented in Table 3.6. No differences in NDF intake were realized between treatments. Likewise, no treatment differences were detected for ruminal NDF degradability. However, HIGH HNIT was the lowest numerically for apparent NDF degradability and had significantly more duodenal NDF passage when compared to either N treatment consuming corn grain with low IVSD ($P < 0.05$). A tendency for interaction of treatments was detected for postruminal digestion of NDF. Steers consuming HIGH HNIT diets had significantly greater postruminal digestion of NDF compared to all other treatments ($P < 0.05$) when expressed as either a percentage of DMI or duodenal passage. However, no differences were documented for total tract NDF digestion among treatments.

3.3.4 Site of Starch Digestion

Treatment effects on site of starch digestion are shown in Table 3.7. A tendency for an interaction between treatments was ($P = 0.11$) present for starch intake. A similar tendency for interaction was detected among treatments for starch apparently degraded in the rumen on a kg/d basis, with steers consuming LNIT LOW having significantly greater ruminal starch degradation (kg/d) compared to all other treatments ($P < 0.05$). This result was understandable considering LNIT LOW had the greatest starch intake, but when ruminal starch degradability was evaluated as a percentage of intake no differences between treatments were detected. We hypothesized that LOW would require less ruminal N to maximize ruminal starch degradability compared to HIGH, as we documented a 14% difference for in vitro starch digestibility when comparing these two corn hybrids in a previous study (McPeake, 2008). A tendency for an interaction ($P < 0.10$) was also detected between treatment main effects for starch truly degraded in the rumen with both HIGH HNIT and LOW LNIT being numerically higher than either LOW HNIT or HIGH LNIT. However, no differences were detected when evaluated as a percentage of intake.

No differences were detected between treatments for duodenal starch passage ($P > 0.05$). However, steers consuming HNIT treatments had 17% more starch digested postruminally compared to LNIT ($P < 0.05$) when expressed as a percentage of duodenal starch flow.

Significant interaction of treatments was discovered for apparent total tract starch digestion ($P = 0.03$). Steers consuming LNIT LOW had greater total tract starch digestion compared to HNIT LOW ($P < 0.05$), but no differences were detected for the

HIGH treatments. Vitreousness of corn grain reflects the association between starch and protein in the endosperm. In corn grain containing flinty endosperm the starch granules are surrounded by a more compact and dense protein matrix, thereby limiting accessibility to microbial and enzymatic digestion (Kotarski et al., 1992). In contrast, floury endosperm is comprised of starch granules that are more accessible to rumen bacteria because the granules are less compact and the protein matrix is discontinuous (Kotarski et al., 1992). While most research has documented that corn grain with floury endosperm is more ruminally degradable than more vitreous/flinty hybrids (Philippeau et al., 1999b), Szasz et al. (2002) documented greater ruminal starch degradability in flinty vs. floury HMC treatments. The magnitude of difference in vivo between the two corn hybrids utilized in this trial for ruminal starch degradability does not appear to be great enough to illicit a large enough response to test potential differences in supplemental N level on nutrient digestion. Results from a previous trial in our lab utilizing these same corn hybrids documented that as high moisture corn (HMC), ruminal starch degradability was 10% higher compared to dry-rolled corn (DRC; McPeake, 2008). Total tract starch digestibilities for the current study were within the reported ranges for DRC (Owens, 1997). Therefore, in order to study the importance of N synchrony based on ruminal fermentation characteristics, grain processing (steam-flaked vs. DRC), grain type (sorghum vs. wheat), or corn hybrids with greater vitreousness differences than used in this study may prove to be more appropriate models.

3.3.5 Site of Nitrogen Digestibility

We hypothesized that N concentration would interact with degree of IVSD because supplemental N would be beneficial to diets containing corn hybrids with high IVSD as a result of increased ruminal starch degradability.

A tendency for interaction between treatments was detected for N intake ($P < 0.10$; Table 3.8). By design, N intake was significantly higher for the HNIT treatment during the present trial (202.2 vs. 154.2 g/d; $P < 0.01$), while N intake for all treatments followed the same pattern as did OM intake. Steers consuming HNIT HIGH had significantly greater N intake than did LNIT HIGH or LNIT LOW ($P < 0.05$). Likewise, HNIT LOW resulted in greater N intake than did LNIT HIGH ($P < 0.05$).

While no differences were observed for the amount of total or microbial N flow to the duodenum among treatments ($P > 0.05$), a numeric advantage was present for HNIT HIGH with regards to microbial N flow to the duodenum compared to other treatments. We hypothesized that corn hybrids with low IVSD may undergo less ruminal fermentation of starch, thereby requiring less ruminally degradable N to satisfy the microbial N requirement. This result may illustrate greater ruminal hydrolysis of starch and thereby more extensive utilization of DIP in the HIGH HNIT diet, a premise the most current NRC (1996) is based on.

No differences between treatments were observed for N truly degraded in the rumen. Interestingly, LNIT LOW had numerically the largest value for true ruminal N degradability when evaluated as a percent of N intake. This result is somewhat surprising as we believed that corn hybrids with low IVSD would result in decreased ruminal fermentation of starch. In the present trial, the LNIT treatment supplied 4.9% dietary DIP, while the HNIT treatment supplied 7.9% dietary DIP. Interestingly, it appears as

though the LNIT treatment was able to meet the ruminal N requirement as evidenced by no difference in MNE when compared to the HNIT treatment. Cooper et al. (2002) documented through a 90 animal individually-fed feeding trial that 6.3% DIP was necessary for DRC-based diets, which agrees with current NRC (1996) recommendations. Surprisingly, the LNIT treatment supplied well below the 5.0 mg/100 mL ruminal ammonia level believed necessary to sustain microbial protein production (Satter and Slyter, 1974), but with no detrimental effects on MNE when compared to the HNIT treatment.

A significant interaction of treatments was observed for total tract digestibility of N ($P = 0.03$). While steers consuming the HNIT HIGH treatment had the highest total tract N digestibility on a g/d basis ($P < 0.05$), HNIT LOW was intermediate, and both were higher than either LNIT treatments ($P < 0.05$). When expressed on a percentage basis, both HNIT treatments resulted in an almost 20% advantage in total tract N digestibility when compared to LNIT HIGH.

3.4 CONCLUSIONS

The present study was conducted to determine if site of nutrient digestion would be affected in growing steers based on potential differences in ruminal starch degradability and varying concentrations of supplemental N. No differences between treatments for any measure of starch digestibility were detected when expressed on a percentage basis. Dietary N concentration impacted the amount and extent of N digestibility during this trial. Differences in ruminal starch fermentation that may exist between corn hybrids due to differences in IVSD in this trial were not great enough to warrant differences in supplemental N concentrations. Further research is necessary to

document the need for synchrony of N with ruminal starch fermentation based on other processing methods or grain types. However, corn grain with high IVSD fed in conjunction with greater dietary N concentrations resulted in numerically greater TRDOM, MCP production, and MNE.

TABLES

Table 3.1. Physical and chemical characteristics of corn grain hybrids

Item	HIGH[1]	LOW[1]	SEM	*P*
DM	90.4	90.3	0.01	0.41
	------% of DM-----			
Starch	67.6	65.8	1.6	0.53
CP	9.7	9.5	0.1	0.69
NDF	6.6	6.2	0.7	0.82
ADF	1.9	2.3	0.1	0.20
IVSD[2]	57.4	43.3	0.7	< 0.01
Absolute density (g/cc)	1.285	1.306	0.004	0.01
Vitreousness, %[3]	45.6	41.8	1.8	0.17
Sieve aperture (μm)	----% retained-----			
4750	17.0	21.5	4.7	0.53
2369	69.0	67.4	5.0	0.43
1180	9.3	7.0	1.2	0.75
600	2.3	2.0	0.5	0.60
300	1.0	1.1	0.3	0.82
150	0.9	0.6	0.1	0.20
75	0.32	0.10	0.04	< 0.01
Pan	0.04	0.01	0.02	0.26
Mean particle size (μm)	3844.1	4064.7	171.8	0.65
Standard deviation	1949.0	1843.8	84.2	< 0.01

[1]Corn hybrids having either high or low in vitro starch digestibility

[2]IVSD = In vitro starch digestion determined after 7 h incubation

[3]Vitreousness determined on corn samples preserved as HMC

Table 3.2. Composition of experimental protein/vitamin-mineral pellet (DM basis)

	Supplemental Nitrogen Concentration	
Item	Low	High
Ingredient, % (DM Basis)		
Ground corn	39.8	25.1
Soybean meal, 48% CP	31.8	36.5
Urea	0.0	10.0
Limestone	10.6	10.6
Vitamin-Mineral[a]	17.9	17.9

[a]Contained (DM Basis): 12.0% potassium chloride, 3.0% sodium chloride, 1.8% magnesium oxide, 0.02% selenium, 0.07% vitamin A (30,000 IU/g), 0.001% vitamin E, 0.04% copper sulfate, 0.17% ferrous sulfate, 0.03% manganese sulfate, 0.09% zinc sulfate, 0.0005% iodine, 0.50% Ameribond, and 0.20% Bovatec 91

Table 3.3. Composition of experimental diets (DM basis)

	Supplemental Nitrogen Concentration	
Item	Low	High
Ingredient, % (DM Basis)		
Corn	78.0	78.0
Cottonseed hull	12.0	12.0
Protein pellet[1,2]	10.0	10.0
Calculated Composition		
Crude protein, %	10.3	13.4
DIP, % CP	48.6	60.1
NEm, Mcal/kg	2.3	2.2
NEg, Mcal/kg	1.4	1.3

[1]Low pellet contained: 39.8% ground corn, 31.8% soybean meal, 10.6% limestone, and 17.9% vitamin/mineral premix

[2]High pellet contained: 25.1% ground corn, 36.5% soybean meal, 10.0% urea, 10.6% limestone, and 17.9% vitamin/mineral premix

Table 3.4. Effects of corn grain in vitro starch digestibility and supplemental nitrogen concentration on ruminal VFA, pH, and ammonia concentration

	High N		Low N			*P* =		
Item	HIGH	LOW	HIGH	LOW	SEM	I[1]	N[2]	I*N[3]
Total VFA, mM	191.5	188.8	199.1	183.0	11.5	0.26	0.91	0.41
VFA, % of total VFA								
Acetate	47.6	51.3	42.3	45.6	2.0	0.02	0.002	0.84
Propionate	28.6	25.5	36.4	30.3	3.5	0.09	0.03	0.52
Butyrate	9.6[a]	8.7[ab]	7.6[b]	8.6[ab]	0.6	0.88	0.09	0.13[4]
BC VFA	2.41	2.86	1.68	2.29	0.29	0.01	0.002	0.56
Lactate	1.90	2.01	2.11	2.14	0.25	0.78	0.51	0.89
pH	5.65[ab]	5.62[ab]	5.46[b]	5.72[a]	0.10	0.13	0.48	0.07[4]
NH_3 (mg/100 ml)	6.23	6.28	1.96	3.08	0.70	0.24	< 0.01	0.28

[1]Main effect of in vitro starch digestibility

[2]Main effect of nitrogen concentration

[3]Interaction between in vitro starch digestibility and nitrogen concentration

[4]Treatment least squares means were separated using the pdiff option of SAS when interaction was less than 0.15. Means within row with unlike superscripts differ ($P < 0.05$)

Table 3.5. Effects of corn grain in vitro starch digestibility and supplemental nitrogen concentration on site of DM and OM digestion

	High N		Low N			*P* =		
Item	HIGH	LOW	HIGH	LOW	SEM	I[1]	N[2]	I*N[3]
DM								
Intake, kg/d	11.0[a]	9.7[a]	9.9[a]	10.8[a]	0.8	0.67	0.97	0.06[4]
Apparent total tract digestion								
kg/d	8.4[a]	7.7[a]	7.6[a]	8.4[a]	0.5	0.97	0.93	0.05[4]
%	77.4	79.4	73.6	77.4	2.1	0.26	0.25	0.69
OM								
Intake, kg/d	10.3[a]	9.1[a]	9.3[a]	10.1[a]	0.7	0.76	0.99	0.07[4]
Apparent ruminal digestion								
kg/d	4.5	5.1	5.3	6.4	0.9	0.36	0.27	0.78
%	44.3	57.5	55.3	63.4	8.7	0.27	0.38	0.79
True ruminal digestion								
kg/d	8.3[a]	6.5[a]	6.5[a]	7.3[a]	0.9	0.52	0.53	0.12[4]
%	80.9	70.1	72.4	72.6	5.9	0.41	0.64	0.39
Passage to duodenum, kg/d	5.3	3.6	3.7	3.5	0.9	0.26	0.28	0.38
Apparent total tract digestion								
kg/d	7.9	7.3	7.0	7.9	0.6	0.78	0.78	0.16
%	75.9	78.0	72.1	75.9	3.7	0.46	0.47	0.83

[1]Main effect of in vitro starch digestibility

[2]Main effect of nitrogen concentration

[3]Interaction between in vitro starch digestibility endosperm type and nitrogen concentration

[4]Treatment least squares means were separated using the pdiff option of SAS when interaction was less than 0.15. Means within row with unlike superscripts differ ($P < 0.05$)

Table 3.6. Effects of corn grain in vitro starch digestibility and supplemental nitrogen concentration on NDF digestion

	High N		Low N			*P* =		
	HIGH	LOW	HIGH	LOW	SEM	I[1]	N[2]	I*N[3]
Intake, kg/d	1.7	1.6	1.4	1.7	0.2	0.48	0.46	0.17
Apparent ruminal digestion								
kg/d	1.4	1.4	1.5	1.5	0.1	0.67	0.49	0.86
%	85.4	90.9	87.1	87.6	1.9	0.12	0.66	0.19
Passage to duodenum, kg/d	0.33[a]	0.16[ab]	0.21[b]	0.23[b]	0.06	0.07	0.47	0.03[4]
Apparent postruminal digestion								
% of intake	2.6[a]	-3.5[b]	-1.7[b]	-2.6[b]	1.5	0.02	0.19	0.06[4]
% of duodenal passage	10.0[a]	-41.6[b]	-19.0[b]	-22.3[b]	14.4	0.05	0.70	0.08[4]
Apparent total tract digestion								
kg/d	1.4	1.4	1.4	1.4	0.1	0.83	0.75	0.64
%	87.1	87.6	85.3	85.2	1.4	0.89	0.16	0.84

[1]Main effect of in vitro starch digestibility

[2]Main effect of nitrogen concentration

[3]Interaction between in vitro starch digestibility and nitrogen concentration

[4]Treatment least squares means were separated using the pdiff option of SAS when interaction was less than 0.15. Means within row with unlike superscripts differ ($P < 0.05$)

Table 3.7. Effects of corn grain in vitro starch digestibility and supplemental nitrogen concentration on site of starch digestion

	High N		Low N			*P* =		
	HIGH	LOW	HIGH	LOW	SEM	I[1]	N[2]	I*N[3]
Intake, kg/d	5.6[a]	5.1[a]	5.4[a]	5.7[a]	0.4	0.67	0.28	0.11[4]
Apparent ruminal digestion								
kg/d	4.1[b]	4.1[b]	4.2[b]	4.8[a]	0.2	0.11	0.04	0.09[4]
%	79.7	82.1	79.1	84.5	2.9	0.22	0.77	0.63
True ruminal digestion								
kg/d	4.8[a]	4.3[a]	4.3[a]	4.9[a]	0.3	0.95	0.88	0.07[4]
%	86.5	85.0	83.9	85.0	2.3	0.90	0.41	0.39
Passage to duodenum, kg/d	1.1	1.0	1.1	0.9	0.2	0.36	0.78	0.99
Apparent postruminal digestion								
% of intake	13.4	11.5	12.3	7.3	3.0	0.27	0.39	0.60
% of duodenal passage	71.3	63.7	54.3	46.6	7.4	0.29	0.05	0.99
Apparent total tract digestion								
kg/d	5.1[ab]	4.7[b]	4.8[ab]	5.3[a]	0.3	0.74	0.55	0.03[4]
%	92.4	93.6	91.0	91.7	1.6	0.56	0.34	0.89

[1]Main effect of in vitro starch digestibility

[2]Main effect of nitrogen concentration

[3]Interaction between in vitro starch digestibility and nitrogen concentration

[4]Treatment least squares means were separated using the pdiff option of SAS when interaction was less than 0.15. Means within row with unlike superscripts differ ($P < 0.05$)

Table 3.8. Effects of corn grain in vitro starch digestibility and supplemental nitrogen concentration on site of nitrogen digestion

	High N		Low N			*P* =		
	HIGH	LOW	HIGH	LOW	SEM	I[1]	N[2]	I*N[3]
TRDOM,[4] kg/d	8.3[a]	6.5[a]	6.5[a]	7.3[a]	0.9	0.52	0.53	0.12[5]
N intake, g/d	218.8[a]	185.6[ab]	147.7[c]	160.7[bc]	16.1	0.37	<0.01	0.07[5]
Duodenal N flow								
Total, g/d	266.4	209.4	190.5	171.1	49.9	0.48	0.30	0.72
Microbial, g/d	215.0	134.5	138.4	144.4	41.8	0.25	0.30	0.19
Non-microbial, g/d	51.4	74.9	64.1	26.6	42.7	0.84	0.60	0.38
N digestibility								
True ruminal								
g/d	167.4	110.6	87.6	134.1	51.5	0.88	0.43	0.18
% of N intake	71.2	55.4	57.5	81.3	23.5	0.83	0.74	0.31
Total tract digestion								
g/d	156.0[a]	130.9[b]	89.2[c]	103.3[c]	16.1	0.44	<0.01	0.03[5]
% of N intake	72.1[a]	70.3[a]	54.0[b]	63.5[ab]	3.3	0.29	0.01	0.14[5]
Microbial efficiency, g/kg TRDOM	24.4	18.5	20.5	19.1	2.9	0.12	0.44	0.29

[1]Main effect of in vitro starch digestibility

[2]Main effect of nitrogen concentration

[3]Interaction between in vitro starch digestibility and nitrogen concentration

[4]TRDOM = Organic matter truly ruminally degraded

[5]Treatment least squares means were separated using the pdiff option of SAS when interaction was less than 0.15. Means within row with unlike superscripts differ ($P < 0.05$)

CHAPTER 4

FINAL DISCUSSION AND CONCLUSIONS

The current experiments were conducted to determine the potential interactions between in vitro starch degradability (IVSD) and either processing method or supplemental N on site of nutrient digestion. In the first experiment, the interaction between IVSD of two corn hybrids (HIGH vs. LOW) and processing method (HMC vs. DRC) on site of nutrient digestion in Holstein steers consuming a high-grain diet was investigated. Digestibility and ruminal metabolism was not influenced by an interaction between main effect treatments. Feeding HIGH resulted in a tendency for greater molar VFA concentrations. Even so, 21.5% less starch was degraded in the small intestine of steers consuming diets containing DRC with a subsequent decline in apparent total-tract starch digestibility. Likewise, a tendency for greater ruminal ammonia levels was present for diets consisting of LOW. Collectively, feeding LOW corn grain results in reduced ruminal fermentation of starch. Diets containing HMC resulted in greater amounts of microbial nitrogen flow to the duodenum, greater apparent post-ruminal nitrogen digestibility, and greater microbial efficiency.

Previous research has documented that endosperm type can have a profound impact on site and extent of starch digestibility (Philippeau et al., 1999). In research trials utilizing dairy cows, Taylor et al. (2005) documented that corn grain containing floury, less vitreous endosperm resulted in greater ruminal and total tract starch digestibility. The corn hybrids utilized in the present trial were provided by Pioneer

based on agronomic data and research findings conducted across a variety of locations. Ideally, these two hybrids would have been grown in conjunction with other hybrids selected for differences in vitreousness and tested thoroughly prior to trial initiation. However, current experimental treatments represent Pioneer corn hybrids that would be commercially planted based on agronomic value and that have historical differences in vitreousness. Results indicate that vitreousness was not different between the two corn hybrids planted, while IVSD was significantly higher for HIGH vs. LOW as DRC (14.1%; $P < 0.01$). Even so, the effect of corn hybrid did not interact with processing method for any measure of digestibility. Therefore, we believe that the effect of endosperm type may not be as pronounced in feedlot diets that typically contain 70 to 80% corn grain. However, processing method of corn grain hybrids has a profound impact on site and extent of starch digestibility and nitrogen metabolism.

Between trials, in vitro rate of starch digestibility after 7 h was completed for the corn hybrids defined in the first trial. This was completed in order to establish if DRC or HMC would be a more appropriate model for the second trial which was based on potential differences in ruminal starch fermentation. A highly significant interaction of treatments was detected ($P < 0.01$; Figure 4-1). The magnitude of difference (14%) was greater between HIGH and LOW when evaluated as DRC as compared to HMC (4.7%, respectively). Therefore, DRC containing HIGH or LOW corn grain was utilized for the second trial. Dietary fiber was provided in the first trial via corn silage, whereas cottonseed hulls were used exclusively during the second trial.

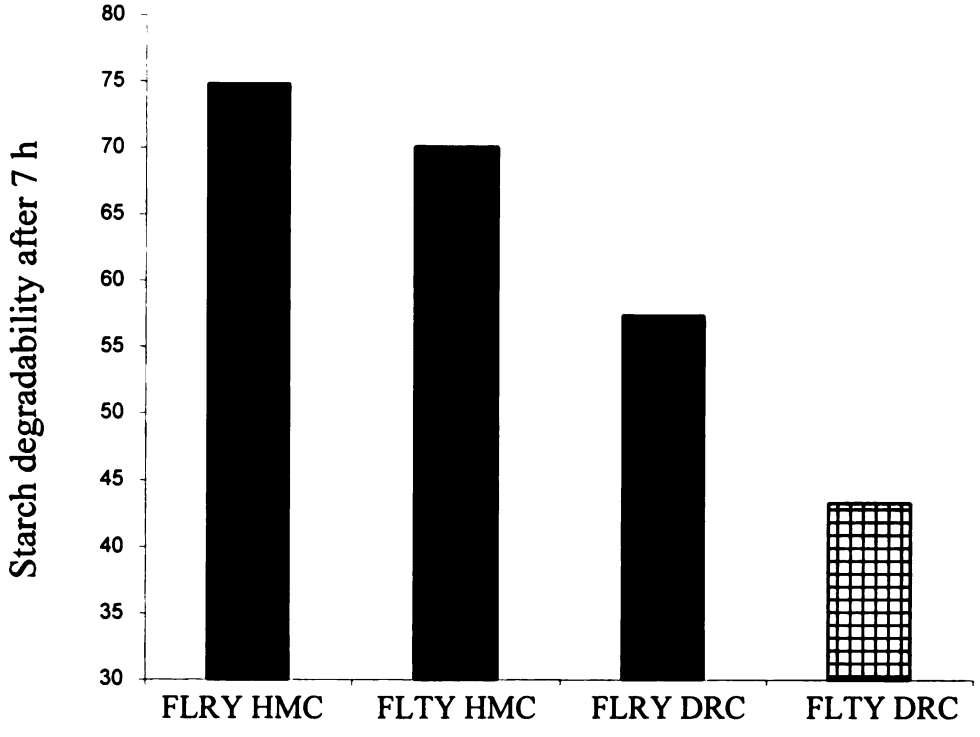


Figure 4-1 In vitro starch degradability for corn grain containing floury and flinty endosperm type processed as HMC or DRC after 7 h.

Evaluation of the interaction between IVSD and nitrogen concentration on site of nutrient digestion and nitrogen metabolism in Holstein steers consuming a high-grain diet was investigated in the second experiment. Treatments were IVSD of corn grain (HIGH and LOW) and N concentration (HNIT and LNIT). Cottonseed hulls were incorporated at a rate of 10% of DMI due to reduced quality of corn silage available and a low, unavailable N content. Noticeable visual differences were apparent when collecting ruminal digesta samples compared to the first trial which utilized corn silage. Ruminal digesta was very watery and loose and may explain why the chromium oxide apparently disassociated from the other ruminal contents, leading to the decision to utilize iNDF as a marker in this trial.

Steers consuming LOW LNIT diets had significantly greater ruminal starch degradability compared to all other treatments and increased total tract starch digestibility compared to steers consuming LOW HNIT ($P < 0.05$). Steers consuming HIGH LNIT had the least total tract N digestibility when evaluated on either a g/d or percentage basis ($P < 0.05$). We hypothesized that steers consuming HIGH corn grain would have greater ruminal starch fermentation, and thereby greater ruminal utilization of dietary N. While diets containing HIGH corn grain did result in greater MNE when compared to LOW, no differences in absorption or retention of N and phosphorus were found between IVSD treatments. Greater microbial efficiency is generally related to increased ruminal fermentation. These results show that corn grain hybrids utilized in the second trial do not require different concentrations of DIP. Although the in vitro data suggested that ruminal fermentation of starch would be much higher for floury endosperm, data collected in vivo did not correspond.

Understanding nutrient synchrony is a very promising concept that may allow beef cattle operations to be more efficient. However, nutrient synchrony requires the ability to quantitatively predict the fate of ruminal protein and carbohydrate. Successful nutrient synchrony requires accounting for the complex interaction between the animal, its environment, the ruminal microbial population, and the diet (Hall and Huntington, 2008).

The research described herein is a small piece of the puzzle that pertains to enhancement of beef cattle efficiency. The current research trials document that IVSD of corn grain could be considered during diet formulation in order to maximize nutritional inputs. Further research is needed to evaluate the effects of grain vitreousness and processing methods on growth performance and end-product merit in feedlot cattle.

FUTURE WORK

Further research is necessary to ascertain whether or not performance differences can be realized in feedlots based on fermentation differences between corn hybrids with different vitreousness. A major obstacle for feedlots will be their ability to source specific corn grain hybrids based on potential differences in ruminal fermentation. While nutrient synchrony is theoretically a sound strategy to reduce excess environmental N and P, nutrient synchrony in general is very hard to predict as available research does not precisely quantify fates of various feedstuffs. This is due in part to our current understanding of ruminant digestion and metabolism. Additional research is needed to elucidate these responses. With current input prices at an all-time high, enhancement of beef cattle efficiency should be of paramount importance within the research community.

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