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CORN GRAIN ENDOSPERM TYPE AND THE BROWN MIDRIB 3 MUTATION IN CORN SILAGE: EFFECTS ON NUTRIENT DIGESTION, FEEDING BEHAVIOR, MILK YIELD, AND RUMINAL FERMENTATION OF LACTATING DAIRY COWS

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By

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ABSTRACT

CORN GRAIN ENDOSPERM TYPE AND THE BROWN MIDRIB 3 MUTATION IN CORN SILAGE: EFFECTS ON NUTRIENT DIGESTION, FEEDING BEHAVIOR, MILK YIELD, AND RUMINAL FERMENTATION OF LACTATING DAIRY COWS

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Interactions of corn silage hybrids varying in fiber lignification and corn grain hybrids varying in endosperm type on nutrient digestion, feeding behavior, milk yield, and ruminal fermentation of lactating dairy cows were investigated. Eight ruminally and duodenally cannulated cows were fed diets containing corn silage (*bm3* or isogenic normal) and corn grain (floury or vitreous endosperm) in a 2 × 2 factorial arrangement of treatments. Floury endosperm grain decreased dry matter intake (DMI) by 1.9 kg/d when in control corn silage diets but did not affect DMI when combined with *bm3* corn silage. Effects on DMI occurred primarily because floury endosperm decreased DM meal size when combined with control corn silage, but increased DM meal size when combined with *bm3* corn silage. DMI was not different between endosperm types combined with *bm3* corn silage because of greater meal frequency for *bm3* corn silage. Main treatment interactions occurred for 3.5 % fat-corrected milk yield (FCM) and reflected treatment effects on DMI. Floury corn grain increased ruminal starch digestibility dramatically compared to vitreous corn grain. Corn grain endosperm type affects site of starch digestion, which can interact with fiber source to affect feeding behavior, feed intake, and milk yield.

For my parents, who taught me the love of learning

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

CHAPTER 4

APPENDIX B

LIST OF ABBREVIATIONS

AA	Amino Acid
ADF	Acid Detergent Fiber
BCS	Body Condition Score
bm3	Brown Midrib 3 Mutation in Corn Silage
BW	Body Weight
CP	Crude Protein
DIM	Days in Milk
DM	Dry Matter
DMI	Dry Matter Intake
FCM	Fat-corrected Milk
GIP	Gastric Inhibitory Peptide
GLP-1	Glucagon-like Peptide 1
iNDF	Indigestible NDF
ME	Metabolizable Energy
MNE	Microbial Nitrogen Efficiency
MUN	Milk Urea Nitrogen
NAN	Non-Ammonia Nitrogen
NANMN	Non-Ammonia, Non-Microbial Nitrogen
NDF	Neutral Detergent Fiber
NE_L	Net Energy of Lactation
NE_M	Net Energy of Maintenance

OM	Organic Matter
pdNDF	Potentially Digestible NDF
TCA	Tricarboxylic Acid
TRDOM	Truly Ruminally Degraded Organic Matter
VFA	Volatile Fatty Acids

INTRODUCTION

Energy intake is an important determinant of milk production, and therefore maximizing energy intake of high producing dairy cows is a primary objective for nutritionists. Cows in peak lactation can produce greater than 50 kg of milk per day, and often consume more than 25 kg of feed per day to support production. However, body reserves are frequently mobilized because the cow cannot consume enough feed in a day to meet the energy demands of milk production. Increasing ruminal fiber digestibility of forage in the diet can potentially increase dry matter intake (**DMI**) by reducing physical limitations to feed intake (Allen, 1996). Another strategy to potentially increase energy intake is to increase the energy density of the diet by adding cereal grains. Ruminal starch degradability of grains varies widely and can influence site of starch digestion and regulation of DMI (Allen, 2000). Understanding how fiber and starch digestibility interact to affect DMI might lead to a better understanding of how feed intake is regulated in high producing dairy cows.

Ruminal digestion characteristics can affect production of microbial protein in the rumen. Microbial protein is the primary source of amino acids to the cow and it is important to maximize microbial protein flow so that amino acid availability does not limit milk production. Interactions of starch and fiber digestibility on DMI might change efficiency of microbial protein production. Rapid ruminal fermentation can decrease efficiency of microbial growth (Russell, 1998), but microbial growth can be limited by energy supply in the rumen (Clark et al., 1992), so moderation of ruminal fermentation is critical for maximal efficiency of microbial protein production. Because starch and fiber

digestibility probably interact to affect both DMI and nutrient digestibility, interactions of starch and fiber digestibility probably also occur to affect microbial growth and fermentation.

The objective of this thesis research was to evaluate interactions of endosperm type of corn grain and the brown midrib mutation in corn silage on site of nutrient digestion, feeding behavior, milk yield, ruminal fermentation, and microbial protein production.

CHAPTER 1

A REVIEW OF THE LITERATURE

SECTION I: IMPORTANCE OF NUTRIENT DIGESTIBILITY

Fiber Digestibility

Energy intake is a primary limitation on productivity of high producing cows. As the genetic potential of dairy cows increases, greater energy intake is required to meet energy demands for milk production. Increasing energy density of the diet helps support greater milk yield, and dietary fiber concentration is often reduced in favor of more energy dense concentrate feeds. However, it has been recommended that to maintain a healthy rumen environment, neutral detergent fiber (**NDF**) should comprise 25% of diet dry matter (**DM**) with at least 19% of NDF coming from forage sources (NRC, 2001). This recommendation, while realizing that ruminants require a certain amount of NDF, does not take into account the wide range of NDF digestibility of various forages. In vitro NDF digestibility can range from less than 20% to greater than 60% over a variety of forages (Allen and Oba, 1996), but diets are generally formulated based upon NDF concentration alone. However, NDF digestibility could have significant impact on animal productivity because DMI and milk yield are positively related to NDF digestibility (Oba and Allen, 1999b).

NDF digestibility is determined by the fraction of NDF that is potentially digestible, the rate of NDF digestion in the rumen, and the rate of passage from the rumen

(Allen and Mertens, 1988). Potentially digestible NDF (**pdNDF**) is a laboratory measure of the absolute extent of NDF digestion by ruminal microorganisms. Increasing proportion of pdNDF and decreasing the indigestible NDF fraction (**iNDF**) could result in greater fiber digestibility. However, in vivo NDF digestibility is also a function of the competing processes of rates of digestion and passage; increasing rate of digestion or decreasing rate of passage from the rumen could also increase NDF digestibility. Predicting NDF digestibility is difficult because rates of digestion and passage can be affected by physical structure of the plant, microbial attachment, enzyme activity, particle size reduction, buoyancy, and ruminal motility (Allen, 1996). The least practical method of increasing digestibility of the pdNDF fraction is by reducing rate of passage from the rumen; lower rate of passage could increase digestibility of pdNDF by increasing ruminal retention time but might depress DMI because of greater ruminal distension.

The most practical methods of increasing NDF digestibility lie primarily with increasing amount and rate of pdNDF digestion in forages. Grasses often have a greater proportion of pdNDF to iNDF than legume forages and higher in vitro NDF digestibility, but rate of digestion of legume pdNDF is faster (Smith et al., 1972) and could increase total amount of NDF digested per day in vivo. Additionally, within a forage type, immature plants generally have higher NDF digestibility than mature plants, because as the plant matures the indigestible NDF fraction increases and rate of NDF digestion decreases (Smith et al., 1972). Replacing early vegetative alfalfa hay with late bud or full bloom alfalfa reduced total tract NDF digestibility and DMI (Llamas-Lamas and Combs, 1990). Another potential method to increase pdNDF digestibility is by the use of genetic mutations in forage crops that reduce iNDF and increase pdNDF fraction of the plant.

The first brown midrib mutation in corn, aptly named *bm1*, was discovered at the University of Minnesota in 1924 (Jorgenson, 1931). Since then, three other mutations have been found in corn, named *bm2*, *bm3*, and *bm4*. The various brown midrib mutations, so named because of accumulation of reddish-brown phenolic lignin monomers in the midrib of the leaf, reduce lignin content of corn because of alterations or deletions of genes coding for enzymes involved in lignin biosynthesis. The gene mutation in *bm3* corn occurs for caffeic acid-O-methyl transferase (Vignols et al., 1995). Lignin is an indigestible polymer in plants that is important to maintain structural integrity of plant tissue. Although lignin comprises little of the total structural carbohydrate system in plants, it has been recognized as the primary component of the cell wall that limits digestion (Jung and Deetz, 1993). Negative effects on digestion occur because lignin forms strong covalent bonds with hemicellulose and may also physically block enzymatic access to digestible structural carbohydrate. Reduced lignin synthesis lessens the amount of crosslinking that occurs among lignin and digestible structural carbohydrates to increase digestibility.

It was not until the 1970's that research on the nutritional aspects of brown midrib hybrids occurred. Of the brown midrib mutations, hybrids containing the *bm3* mutation have been most widely used in nutritional experiments because it consistently reduces lignin content and increases in vitro DM digestibility (Colenbrander et al., 1973). The *bm3* mutation decreased lignin and increased in vitro DM digestibility more than other *bm* hybrids (*bm1*) or normal corn hybrids (Muller et al., 1971). Reduced lignin synthesis and greater in vitro DM digestibility occurred in all parts of the *bm3* corn plant, including the leaf, stem, and ear (Weller et al., 1984). As *bm3* corn plants mature, lignin content

increases similar to normal hybrids, but *bm3* hybrids had consistently lower lignin at all stages of maturity (El-Tekriti et al., 1976). Based on in vitro data, *bm3* corn hybrids had the potential to increase in vivo NDF digestion and possibly performance, but little was known about the effects of feeding *bm3* hybrids to animals.

Early in vivo research with *bm3* hybrids agreed with in vitro results. Muller et al. (1972) performed a classical study investigating the feeding value of a *bm3* corn silage hybrid versus a high yielding commercial hybrid. Two adjoining plots were planted with either *bm3* corn for silage or a normal hybrid, and ears were removed from both plant sections so that differences between hybrids could not be attributed to grain concentration differences. When fed to lambs, *bm3* corn silage improved apparent total tract digestibility of cell wall constituents and cellulose versus the normal corn hybrid. Similar to that study, replacing normal silage with *bm3* silage in diets for lactating cows increased apparent total tract digestibilities of cell-wall constituents and cellulose by 33 and 44%, respectively (Weller and Phipps, 1986).

Perhaps the more important benefit of *bm3* corn silage was the observation that animals often increased feed intake. Brown midrib 3 corn silage increased voluntary DMI by lambs by 29% compared to a normal hybrid (Muller et al., 1972). When fed to cows in early lactation, *bm3* corn silage increased DMI by 11% versus a normal hybrid (Block et al., 1981). However, some studies observed no increase in DMI when *bm3* corn silage replaced a normal hybrid (Frenchick et al., 1976; Keith et al. 1979). Lack of treatment effect on DMI when *bm3* corn silage replaces control corn silage could occur if DMI is not limited by ruminal fill. A more recent study found a relationship between pretrial milk yield and DMI response to *bm3* silage (Oba and Allen, 1999a); cows

producing more milk in the pretrial period increased DMI to a greater extent when fed *bm3* silage than cows yielding less milk. The authors concluded that ruminal fill is more limiting to intake for higher yielding cows, and increasing NDF digestibility of forage by feeding *bm3* corn silage might increase DMI to a greater extent in higher producing cows.

A statistical analysis of the literature found that for cows in early or mid-lactation (41 to 154 days in milk [**DIM**]), a one unit increase in NDF digestibility was associated with a 0.17 kg increase in DMI and 0.25 kg increase in 4% fat corrected milk (**FCM**; Oba and Allen, 1999b). Because the *bm3* mutation in corn silage can potentially increase NDF digestibility and might reduce limitations to DMI by physical fill, more research is needed to determine how high producing animals respond to *bm3* corn silage and how this response might be affected by other dietary factors, such as starch digestibility.

Starch Digestibility

Because energy intake is such a limitation on milk production, increasing the energy density of diets by replacing fibrous forages with starchy grains is common. Diets are generally formulated based on starch concentration alone, but ruminal starch digestibility ranges from 42 to 96% over a variety of grain sources (Nocek and Tamminga, 1991). This wide range of starch digestibility can affect whether starch is digested primarily in the rumen or intestines. Site of starch digestion can considerably influence DMI and microbial protein production (Oba and Allen, 2003 a, c). Therefore, changing starch digestibility can have a direct impact on energy intake and milk productivity.

Starch digestibility can be manipulated by processing and conservation methods. Physical processing such as cracking, grinding, flaking, or rolling generally increases ruminal starch digestibility. This processing disrupts the outer coating of the kernel and makes the starch more accessible to microbial attack. Grinding corn increased ruminal and intestinal starch digestion compared to whole corn in steers (Galyean et al., 1979), and decreasing corn grain size from coarsely to finely ground increased ruminal starch digestibility from 35.2 to 70.1% (Callison et al., 2001). Flaking and rolling substantially disrupts the kernel structure and increases ruminal digestibility versus whole or cracked grains (Firkins et al., 2001).

Physicochemical processing methods also increase starch digestibility of grains. Starch granules consist of multiple starch chains arranged in highly organized and less organized sections, called crystalline and amorphous areas, respectively. These chains of starch are associated by a significant amount of hydrogen bonding, but application of heat and water swells the amorphous and crystalline regions of the starch bonds and breaks down interconnecting hydrogen bonds (Rooney and Plugfelder, 1986). This process, known as gelatinization, increases area available for microbial penetration and enzyme access. Steam treatments are often combined with physical processing such as flaking to increase ruminal and total tract starch digestibility. Steam flaking increased total tract starch digestibility by approximately 19 units compared to dry rolled (Plascencia and Zinn, 1996). Similarly, replacement of dry rolled corn with steam flaked corn linearly increased ruminal and total tract starch digestibility (Crocker et al., 1998).

Ensiling grains can increase starch digestibility because the ensiling process partially ferments the grain and weakens the physical structure of the cereal. Conserving

grains as high moisture grain applies both hydration and ensiling processes to increase starch digestibility. High moisture grains are readily fermentable and are a common feed source used to increase energy density of dairy cow rations. High moisture corn increased ruminal starch digestibility by 19 units (Knowlton et al., 1998a) and 24 units (Oba and Allen, 2003b) in high starch diets for lactating cows.

Physical properties of grains can play a substantial role in starch digestibility. When cereal grains are ground, ruminal starch digestibility of wheat, oats, and barley is greater than corn, which is greater than sorghum (Herrera-Saldana et al., 1990). Replacing corn grain with barley in the diet of lactating cows linearly increased ruminal and total tract starch digestibility (Overton et al., 1995). Apparent total tract starch digestibility was greater for steam flaked corn than steam flaked sorghum (Santos et al., 1999). However, even within a single grain source, ruminal starch digestibility can range widely; ruminally degraded starch ranged from 51 to 93% for corn grain (Nocek and Tamminga, 1991).

Wide variation of ruminal starch digestibility within a grain source is because of the physical and chemical structure of the grain kernel. Cereal kernels are composed of three distinct parts; the germ, the outer pericarp, and the endosperm, which comprises most of the total kernel area (Kotarski, 1992). Physical processing disrupts the pericarp and exposes starch granules to digestion, but a protein matrix within the endosperm surrounds starch granules and slows digestion. Starch granules in vitreous endosperm are imbedded in an insoluble protein matrix, whereas granules in floury endosperm are more loosely associated with a soluble protein matrix (Kotarski, 1992). The protein matrix of vitreous endosperm is composed primarily of zein proteins, whereas the more digestible

protein matrix of floury endosperm is comprised of glutelin proteins (Philippeau et al., 2000). Zein proteins are very resistant to ruminal degradation, but glutelin proteins are much more digestible (Romagnolo et al., 1994). Much of the variability of starch digestibility within a grain type is because of the physical and chemical structure of this protein matrix.

Many hybrids of corn grain are commercially available in the U. S., but effects of chemical structure of these hybrids on starch digestibility have been largely overlooked. Vitreousness can range widely across hybrids; 6 commercially available hybrids grown in 3 states varied in vitreousness from 4 to 62% (Allen et al., 2003). Within a hybrid, vitreousness increases as grain matures (Philippeau and Michalet-Doreau, 1997). The potential impact that vitreousness can have on digestibility is tremendous; indeed, averaged across hybrids, vitreousness explained 86% of the variation in ruminal starch degradability (Philippeau and Michalet-Doreau, 1997). In steers, replacing dent corn with a more vitreous flint hybrid decreased ruminal starch digestibility 43% and increased small intestinal starch digestibility (as a percent of starch intake) by 98% (Philippeau et al., 1999a). Although these results indicate that vitreousness of corn grain can have a significant impact on starch digestion, to date only two studies have been published investigating the effects of corn grain endosperm type on in vivo performance. Furthermore, both of these studies were conducted in steers; no information is available on how corn grain endosperm type affects productivity and nutrient digestibility in high producing dairy cows.

SECTION II: INTERACTIONS OF FIBER AND STARCH DIGESTIBILITY

Fiber and starch digestibility could play an important role in energy intake and productivity of dairy cows. The *bm3* mutation in corn silage increases fiber digestibility considerably, and endosperm type of corn grain can have significant effects on starch digestibility. Interactions of endosperm type of corn grain and the *bm3* mutation in corn silage could affect site of nutrient digestion, voluntary DMI, and microbial protein production.

SITE OF NUTRIENT DIGESTION

The rumen has evolved to digest fibrous feeds, and this ability to utilize fiber is a distinct evolutionary advantage, but the disadvantage of this system is the long retention time required for fiber digestion. As the genetic potential of dairy cattle increases, higher milk yields require greater DMI, but voluntary DMI is negatively associated with retention time in the rumen. Thus, a strategy used in diets for high producing dairy cows is to increase nutrient digestibility and energy density. Greater DMI increases rate of nutrient passage from the rumen, which can reduce ruminal nutrient digestibility and shift some digestion postruminally in high producing dairy cows. Additionally, greater ruminal digestibility of fiber and starch might interact to shift site of nutrient digestion from the rumen to other sites in the gastrointestinal tract.

Increasing ruminal starch fermentation can affect the ability of ruminal microorganisms to grow and to digest fiber, as discussed in detail in a later section. Replacing beet pulp with high moisture corn linearly reduced ruminal pdNDF

digestibility from 67.3 to 46.1%. (Voelker and Allen, 2003a). While greater ruminal starch digestion is associated with reduced ruminal fiber digestibility in multiple studies (McCarthy et al., 1989; Crocker et al., 1998; Callison et al., 2001), it is not always associated with reduced total tract NDF digestibility (Crocker et al., 1998; Callison et al., 2001). In the experiment of Crocker et al. (1998), postruminal NDF fermentation linearly increased with greater ruminal starch digestibility but total tract NDF digestibility was not affected. Replacing dry corn with high moisture corn reduced ruminal NDF digestibility, and shifted so much NDF digestion postruminally that hindgut fermentation of NDF contributed 53% of total tract NDF digestion (Oba and Allen, 2003b). However, total tract digestion of NDF was not different between diets containing dry or high moisture corn. This indicates that ruminal starch digestibility can have significant effects on site of fiber digestion without reducing total tract fiber digestibility.

Additionally, greater fiber digestibility of *bm3* corn silage could potentially shift site of starch digestion from the rumen to the intestines. Replacing normal corn silage with *bm3* silage decreased ruminal starch digestibility by 10% but increased postruminal starch digestibility 13 % (Oba and Allen, 2000c). The authors suggested that greater DMI of *bm3* silage might have increased passage rate of starch from the rumen and shifted site of starch digestion to the intestines. Another experiment found that ruminal starch digestibility was 36% lower for *bm3* versus normal corn silages, but differences in total tract starch digestibility were small, indicating compensatory postruminal starch digestion (Greenfield et al., 2001). Greater DMI could explain greater rate of starch passage from the rumen in the Oba and Allen (2000c) experiment, but no differences in DMI were observed for the Greenfield et al. (2001) experiment. Greater starch passage

in diets containing *bm3* corn silage could be the result of other factors; for example, the ruminal fiber mat formed by *bm3* corn silage fiber might be less effective at retaining corn grain particles. Feeding *bm3* silage can affect site of starch digestion, which could play an important role in production response to *bm3* silage.

Changing Nutrient Supply

Starch yields different end products depending on site of digestion. As amount of ruminally fermented starch increases, propionate production increases relative to acetate to shift the volatile fatty acid (**VFA**) profile in the rumen. Although propionate is a primary glucose precursor, postruminal starch digestion by amylase yields glucose directly and is theoretically more efficient. In theory, greater postruminal starch digestion could provide more glucose for milk lactose synthesis, but in reality milk yield response to greater postruminal starch digestion is inconsistent.

Several experiments have investigated animal response to postruminal infusions of starch and glucose. Reynolds et al. (2001) found that long term (14 d) infusions of wheat starch into the abomasum of late lactation dairy cows did not affect milk yield but tended to increase energy balance because of a 93% increase in tissue energy retention. Additionally, duodenal glucose infusion increased energy balance of late lactation dairy cows but did not affect milk yield (Lemosquet et al., 1997). In contrast, a cubic increase in milk production was noted for cows in early lactation receiving increasing amounts of corn starch infused into the duodenum (Reynolds et al., 2001). These data show that response to postruminal starch digestion is probably dependent on stage of lactation and

could explain inconsistent effects across the literature for milk yield response to postruminal starch digestion.

Animal response to increased postruminal starch digestion might depend on changes in plasma hormone secretion in response to glucose. In particular, duodenal glucose infusion increased plasma insulin of lactating dairy cows (Lemosquet et al., 1997). Insulin increases glucose uptake by adipose and skeletal muscle (McGrane, 2000), but glucose transporters expressed by the mammary gland are not insulin-sensitive (Zhao et al., 1996), so greater plasma insulin concentrations might not affect glucose uptake at the mammary gland, but could reduce glucose availability if other insulin-sensitive tissues increased glucose uptake. Competition for glucose availability among tissues might explain why postruminal infusions of glucose or starch might not increase milk production but can increase tissue retention (Lemosquet et al., 1997; Reynolds et al., 2001). Why does this effect appear to occur in cows that are later in lactation, while cows in early lactation can respond by increasing milk production? Cows in late pregnancy and early lactation have reduced expression of the insulin-responsive glucose transporter in muscle and adipose tissue, which decreases glucose utilization by peripheral tissues and increases glucose available for milk synthesis (Bell and Bauman, 1997). The lack of response of peripheral tissues to insulin is markedly reduced through early lactation, and recovers as the animal progresses through lactation (Bell and Bauman, 1997).

Greater body weight (**BW**) gain has been observed in several experiments where *bm3* corn silage replaced normal corn silage (Frenchick et al., 1976; Sommerfeldt et al., 1979; Weller and Phipps, 1986), and this effect might occur if *bm3* corn silage shifts site

of starch digestion to the intestines. Perhaps most striking was the experiment conducted by Block et al (1981); feeding cows *bm3* corn silage from weeks 3 to 10 postpartum increased DMI by 2.2 kg per day and although milk yield was numerically increased, the greatest effects of treatment occurred on BW change. Cows consuming *bm3* corn silage gained 10.3 kg over the 8 week period, whereas cows consuming normal corn silage lost 24.6 kg over the same period. Oba and Allen (1999a) found that compared to a control corn silage, *bm3* corn silage increased energy balance of lactating cows by 2.1 Mcal per day. Greater concentrations of metabolizable energy (**ME**) in *bm3* corn silage diets fed ad libitum resulted in more ME partitioned toward tissue energy gain instead of milk energy (Tine et al., 2001). Brown midrib 3 corn silage increases ruminal propionate and shifts a substantial portion of starch digestion to the intestines (Oba and Allen, 2000c), so greater glucose availability in *bm3* corn silage diets might increase plasma insulin concentration and tissue energy retention, but effects of *bm3* corn silage on hormone profiles have not been investigated.

Compensatory Digestion

Although greater passage rate of starch in *bm3* corn silage diets reduces ruminal starch digestibility, *bm3* corn silage reduced total tract starch digestibility by only 1.5 units (Oba and Allen, 2000c) and 1.7 units (Greenfield et al., 2001), and total tract starch digestibility was still greater than 91% in both experiments. This indicates that substantial compensatory digestion occurred in the intestines. While much of this digestion probably occurs in the small intestine, some fermentation probably occurs in the hindgut.

Abomasal infusions of corn starch increased ileal digesta concentrations of glucose and starch (Kreikemeier et al., 1991). Additionally, replacing dent corn grain with a flinty corn grain in the diets of beef steers reduced ruminal starch digestibility, but increased small intestinal and hindgut digestion (Philippeau et al., 1999a). In that study, flint corn increased small intestinal digestibility from 8.9 to 17.6% compared to dent corn, but hindgut fermentation of starch was increased 110% versus dent corn. Infusion of increasing amounts of corn dextrin into the abomasum of steers linearly increased VFA concentration in ileal digesta (Kreikemeier et al., 1991). To some extent, VFA produced from hindgut fermentation can be absorbed in the large intestine and used by the animal as an energy source, but microbial organic matter (**OM**) cannot be digested or absorbed and loss of microbial OM in the feces reduces efficiency of energy utilization by the cow.

Changing site of starch digestion can considerably change the nutrient supply to the animal. Additionally, DMI and microbial protein production can be affected by site and extent of starch and NDF digestion, as discussed in later sections. Because endosperm type of cereal grains is a substantial determinant of starch digestibility, research on effects of endosperm type on site of starch and NDF digestion needs to be conducted. Corn silage with the *bm3* mutation has been shown to interact with site of starch digestion. The interactions of fiber and starch digestibility on site of starch digestion could significantly change nutrient partitioning and DMI, and should demonstrate the importance of nutrient digestibility on performance of dairy cattle.

REGULATION OF INTAKE

Dry matter intake is a primary determinant of milk yield of lactating dairy cows. Given this, understanding how intake is regulated is critical to obtain maximal production. Conrad et al. (1964) proposed that feed intake was determined by physical fill at low digestibility, and as digestibility increased, metabolic factors determined intake. Although overly simplistic, the concept of physical versus metabolic regulators of intake is the basis for many current models of feed intake. In reality, feed intake is probably determined by the integration of a variety of physical, metabolic, and hormonal factors.

Physical Regulation of Intake

Physical regulation of intake can occur when physical fill in the gastrointestinal tract limits feed intake. A significant amount of research has been conducted on factors affecting physical regulation of intake (Allen, 1996; Allen, 2000). The primary cause of physical limitation on intake is long retention time of the fibrous fraction of the diet. Although fiber is crucial to maintaining a healthy rumen environment, digestion of the fibrous feed fraction is slow and can increase ruminal retention time if particles cannot be broken down and passed from the rumen. Limitations to flow from the rumen have been reviewed (Allen, 1996), and include particle size and density, which are closely associated with ruminal digestibility. Ruminal digestion of fibrous feed increases particle fragility and makes particles more susceptible to breakdown during chewing (Chai et al., 1984). Additionally, as ruminal digestion of fiber occurs, particle buoyancy decreases

and particles sink (Sutherland, 1988). With greater rate of NDF digestion, particles are probably broken down faster and sink faster to increase rate of passage from the rumen and decrease the filling effect of the diet. Brown midrib 3 corn silage increases rate of NDF digestion compared to normal corn silage (Ying and Allen, 1995), and because *bm3* corn silage also increases rate of NDF passage from the rumen, *bm3* corn silage might reduce physical limitations to intake and allow greater DMI.

Filling effect of dietary NDF can be increased by greater ruminal starch digestibility. The presence of starch has been demonstrated to reduce rate of in vitro NDF digestion (Grant and Mertens, 1992). Increased ruminal starch digestion may also indirectly impede rate of fiber digestion by reducing ruminal pH. In vitro NDF digestion occurs at a slower rate as pH decreases (Grant and Mertens, 1992), and in the rumen this might increase retention time and could potentially limit DMI. Brown midrib 3 corn silage reduces ruminal pH compared to normal corn silage (Oba and Allen, 2000c), and the authors speculated that *bm3* corn silage reduced ruminal digestion rate of pdNDF because of lower pH in *bm3* silage diets. Although in vitro pdNDF digestibility was greater for *bm3* corn silage compared to isogenic control, reduced pdNDF digestion rate and increased passage rate in *bm3* corn silage diets could explain why *bm3* corn silage does not always increase NDF digestibility in vivo. Greater ruminal starch digestion and lower ruminal pH might reduce rate of pdNDF digestion, and could contribute to physical limitation of intake if it increases ruminal retention time.

Metabolic and Hormonal Regulation of Intake

As physical limitations to feed intake decrease, metabolic and hormonal controls become more limiting to DMI. The liver has been suggested to be one of the primary sites of integration of metabolic and hormonal regulatory factors because of its central anatomical position and because it is the first organ to interact with nutrients absorbed from the gut (Langhans, 1996). Interest in the role of the liver in controlling feed intake has led to many experiments to determine which nutrients exert hypophagic responses. These studies show that metabolites extensively metabolized by the liver have hypophagic effects, whereas those that are not metabolized by the liver do not (Langhans et al., 1985). Langhans et al. (1985) found that hypophagic effects of metabolic fuels occur when they are oxidized in the tricarboxylic acid (**TCA**) cycle in the liver. Oxidation of metabolic fuels could be a primary regulator of feed intake in dairy cattle (Allen, 2000), and because ruminal and postruminal starch digestion produces different metabolic fuels, site and timing of starch digestion could contribute to metabolic regulation of feed intake in ruminants.

Site of Nutrient Supply

Depression of DMI has been observed to occur in some diets containing high proportions of ruminally degraded starch (Allen, 2000). Propionate is a primary end product of starch fermentation by ruminal microorganisms. Infusions of propionate reduced meal size and length to a greater extent than equimolar infusions of sodium chloride or acetate, suggesting that effects of propionate on feeding behavior were due to the substrate rather than effects on osmolality (Choi and Allen, 1999). The liver could be

central to effects of propionate on feeding behavior because hepatic vagotomy reduced hypophagic effects of propionate (Anil and Forbes, 1980). Based on this and other research, Allen (2000) proposed that oxidation of propionate in the liver could regulate feed intake in dairy cattle. Ruminal infusions of propionate linearly reduced 12 h cumulative DMI by reducing meal size (Oba and Allen, 2003e). Increased ruminal starch fermentation increases ruminal propionate concentrations and is probably why increasing amount of ruminally degraded starch can sometimes depress DMI in dairy cows.

If DMI depression can occur because of greater starch fermentation in the rumen, then it might be beneficial to shift site of starch digestion to the intestines to relieve hypophagic effects of propionate. Shifting site of starch digestion to the small intestine allows mammalian amylase to digest starch to glucose. Glucose can be absorbed across the enterocyte into the portal blood, partly metabolized to lactate before entering the portal, or fully oxidized by the enterocyte itself. Because the liver is probably the primary integrator of metabolic satiety signals, glucose or lactate from intestinal starch digestion would have to be absorbed by the liver to directly affect feed intake. Allen (2000) discussed effects of glucose on feed intake, and concluded that glucose is probably not a metabolic regulator of feed intake in ruminants. Partial metabolism of glucose to lactate in the enterocyte can occur, and although 60 to 90% of luminal glucose is metabolized to lactate in rat intestine (Hanson and Parsons, 1976), the extent to which glucose is metabolized to lactate in ruminant intestine is unknown. Infusions of lactate into the jugular or mesenteric veins has inconsistently decreased intake (Baile and Forbes, 1974), and Allen (2000) suggested that inconsistent hypophagic effects of lactate on DMI are probably dependent on hepatic carbon balance and redox state, which affects

lactate removal by the liver (Reynolds, 1995). Additionally, lactate also does not appear to be taken up by the liver in appreciable quantities in the fed state (Reynolds and Maltby, 1994), and so might not directly affect feeding behavior.

However, shifting site of starch digestion to the intestine might cause satiety by hormonal factors. Duodenal glucose infusion increased plasma insulin in dairy cows (Lemosquet et al., 1997). Although insulin probably does not control short-term feed intake (Langhans, 1996), increased plasma insulin: glucagon ratio might decrease gluconeogenesis and increase oxidation of metabolic fuels in the liver (Watford and Goodridge, 2000) and could induce satiety indirectly. Additionally, gut peptides may affect feed intake; cells in the intestinal tract release glucagon-like peptide 1 (**GLP-1**) and gastric inhibitory peptide (**GIP**) in response to absorbed nutrients. The presence of duodenal carbohydrate increased plasma concentrations of insulin, GIP, and GLP-1 (Lavin et al., 1998). GLP-1 may directly signal satiety, because intravenous GLP-1 administration suppressed energy intake in humans (Flint et al., 1998), or may have effects on feed intake by inducing secretion of insulin. GLP-1 and GIP are both potent insulin secretagogues, and greater than 50% of insulin release after a glucose meal may be mediated by GIP and GLP-1 (Tseng et al., 1999). This research indicates that intestinal starch digestion could affect feed intake by inducing secretion of insulin, GIP, and GLP-1. However, little is known about the effects of gut peptides on intake behavior in ruminants, and little research concerning dietary effects on gut peptide secretion in ruminants exists.

Timing of Nutrient Supply

Although substantial evidence exists that propionate can depress DMI in ruminants (Oba and Allen, 2003e), greater ruminally degraded starch decreased DMI in only 3 of 10 experiments (Allen, 2000). Why does increasing the proportion of ruminally degraded starch decrease DMI in some but not all experiments? Oba and Allen (2003d) proposed that inconsistent hypophagic responses to propionate were due to its fate, which might be determined by temporal supply of propionate relative to its use for gluconeogenesis. They found that hypophagia in response to propionate infusion was positively related to plasma glucose concentration. As blood glucose concentration increased, propionate induced a greater depression in DMI. They speculated that sating effects of propionate might occur if propionate could not be used toward gluconeogenesis and was instead oxidized in the TCA cycle. As glucose demand increases, more propionate might be used for gluconeogenesis and less could be oxidized, thus delaying satiety. If propionate flux is too fast for the capacity of the gluconeogenic enzymes, oxidation of propionate might induce satiety. This effect is purely temporal; increased starch digestion to propionate might not limit DMI if propionate flux to the liver is not more than the capacity of the gluconeogenic enzymes to utilize it for glucose. The concept that propionate can induce satiety in a temporal pattern relating to gluconeogenesis has not been specifically tested, but current research in our laboratory is investigating this proposal further.

DMI is probably the most important limitation to milk production in dairy cows. For this reason, it is important to understand factors affecting feeding behavior. Regulation of feed intake is complicated, and DMI is the determined by integration of

physical, metabolic, and hormonal factors. Additionally, DMI is a function of meal size and frequency, as determined by satiety and hunger, respectively (Allen, 2000), so it is important to understand how regulators of feed intake can affect feeding behavior and not just cumulative DMI. Endosperm type of corn grain can substantially change site of starch digestion, which can profoundly affect nutrient profile absorbed by the animal and possibly feeding behavior. Fiber digestibility can affect feeding behavior via physical regulation, but because *bm3* corn silage can shift site of starch digestion, regulation of DMI could occur by metabolic and hormonal factors as well. Interactions of starch and fiber digestibility might occur to affect ruminal fermentation and DMI, which could potentially influence microbial protein supply.

MICROBIAL PROTEIN SUPPLY

The primary source of nitrogen to ruminants is supplied by flow of ruminal microorganisms to the duodenum. Microbial protein has a high biological value because of its amino acid (AA) profile and high digestibility. To reduce limitations to milk yield by AA availability, it is important to maximize microbial protein flow. DMI and nutrient digestibility can play substantial roles in microbial fermentation and efficiency. Energy availability often limits microbial growth, so increasing grain intake can increase substrate available for microbial protein production (Clark et al., 1992). However, increased ruminal starch digestion can result in reduced pH, depressed fiber digestibility, and reduced microbial growth efficiency.

Effects of Ruminal pH on Fiber Digestion and Microbial Growth

Grain is relatively inexpensive and abundant in the U.S., and is commonly used to increase energy density of diets. However, many nutritionally related diseases (acidosis, bloat, liver abscesses) result from use of grains in ruminant diets. These symptoms are related to reduced ruminal pH, associated with feeding rapidly fermented cereal grain diets. Low ruminal pH has been associated with depression in ruminal NDF digestibility. Feeding a highly fermentable grain source, such as steam rolled barley, increased ruminal starch digestibility but reduced ruminal pH and ruminal NDF digestibility (Overton et al., 1995). Additionally, feeding a more fermentable forage source like *bm3* corn silage can reduce ruminal pH (Oba and Allen, 2000c; Greenfield et al., 2001) and might reduce ruminal NDF digestibility (Oba and Allen, 2000c).

To understand how reduced ruminal pH can decrease fiber digestion, effects of low pH on ruminal cellulolytic bacteria has been studied extensively. Chow and Russell (1992) investigated the response of a predominant cellulolytic bacterium, *Fibrobacter succinogenes*, to reduction in external pH. They noted at pH less than 6.0, *F. succinogenes*, which maintains intracellular pH at approximately 7.0, was unable to grow or utilize glucose. As extracellular pH decreased, the pH gradient across the bacterial membrane increased to the point that *F. succinogenes* was unable to maintain the pH gradient and intracellular pH decreased. *Ruminococcus albus*, another predominant cellulolytic bacteria, allows intracellular pH to decline with environmental pH to avoid increasing the pH gradient across the bacterial membrane (Thurston et al., 1993). This method of coping with low external pH is the primary method of acid-resistant bacteria, but the key difference is that acid-resistant bacteria have intracellular enzymes that are

active at lower pH. For *F. succinogenes* and *R. Albus*, internal enzyme systems are not able to maintain function at lower pH, and growth is inhibited at pH < 6.0 (Chow and Russell, 1992; Thurston et al., 1993).

Although research suggests that cellulolytic bacteria cannot digest cellulose under pH 6.0 (Russell and Wilson, 1996), ruminants consuming high grain diets often have a ruminal pH below 6.0. How does cellulose digestion occur if cellulolytic bacteria cannot grow under environments created by typical dairy cow rations? Fermentative capability of mixed ruminal bacteria continued, albeit at a lower rate, as pH declined below 7, and was not inhibited until pH reached 5.3 (Mouriño et al., 2001). Calsamiglia et al. (2002) investigated the effects of varying pH on nutrient digestibility in a continuous culture system. They kept pH constant at 6.4 or 5.7, or fluctuated pH in cycles of 4 hours at pH 6.4 and 4 hours at pH 5.7, or maintained pH at 6.4 except for two cycles where pH dropped to 5.7 for 1 hour, then slowly recovered over the next 3 hours. True NDF digestibility was depressed only when pH was constantly maintained at 5.7. In practical feeding situations, pH is probably not maintained at a constant level; raw data from Dado and Allen (1995), as presented in Allen (1997), shows significant fluctuation of ruminal pH in a dairy cow fed a 35% NDF diet twice daily. The work by Calsamiglia et al. (2002) indicates that the pH fluctuations that occur in normal dairy cattle rations probably do not inhibit fiber digestibility like some in vitro work might suggest.

Most research investigating mechanics of cellulose digestion has been conducted using pure cultures of ruminal bacteria. However, cocultures of cellulolytic and noncellulolytic strains removed more cellulose in vitro than pure cultures of cellulolytic bacteria (Mouriño et al., 2001). The cellulase enzyme complex of ruminal cellulolytic

bacteria hydrolyzes cellulose into smaller cellodextrin and cellobiose pieces. A cellobiose analog, thiocellobiose, inhibited cellulose utilization by *F. succinogenes*; the authors suggested that cellobiose acted as an inhibitor of the cellulase enzyme complex and that microbial consumption of cellobiose was necessary for cellulase complex activity (Maglione et al., 1997). Noncellulolytic strains, such as *Prevotella ruminicola* and *Selenomonas ruminantium* cannot hydrolyze cellulose but can use cellodextrins (Russell, 1985), and this endproduct removal by noncellulolytic strains may increase cellulase enzyme activity. Cross-feeding activity in the rumen may also explain why in vivo NDF digestibility is not reduced to the extent that in vitro pure culture work often predicts.

Increased ruminal starch digestibility might indirectly affect fiber digestion by reducing ruminal pH, but may also directly reduce fiber digestion. In vitro work examined the effects of buffer pH and addition of corn starch on digestion kinetics of NDF in bromegrass hay, alfalfa hay, and corn silage (Grant and Mertens, 1992). Interestingly, for alfalfa hay, reducing pH from 6.8 to 5.8 reduced rate of NDF digestion only slightly. However, addition of corn starch to the media reduced NDF digestion by 75 to 78% for all pH. For bromegrass hay and corn silage, reducing media pH depressed NDF digestion, but similar to alfalfa hay, addition of corn starch further decreased rate of NDF by 75 to 78%. Presence of starch may decrease rate of NDF digestion because some organisms preferentially utilize starch before cellulose (Hungate, 1966). Depression of fiber digestion in diets containing highly ruminally degradable starch sources might occur because of low ruminal pH, but may be highly related to availability of starch itself.

Efficiency of microbial growth can be reduced by low environmental pH. Microbial growth efficiency is defined as grams of microbial protein flow to the duodenum per kg of truly fermented organic matter (**TRDOM**). Energy availability is often the primary limitation on microbial growth (Clark et al., 1992), so generally any increase in fermented OM would increase microbial growth. However, Clark et al. (1992) found a quadratic relationship between TRDOM (kg per day) and efficiency of microbial protein production. Under conditions of energy limitation, increasing amount of TRDOM improves microbial efficiency, but as amount of TRDOM continues to increase microbial efficiency declines. Replacing dry corn with high moisture corn increased ruminal starch fermentation and amount of TRDOM but reduced efficiency of microbial growth, and the authors speculated that a process is known as energy spilling or uncoupling occurred (Oba and Allen, 2003c). Energy spilling is the use of ATP toward non-growth functions, and appears to be a common method of handling excess carbohydrate in ruminal bacteria (Russell, 1998). During periods of energy limitation, bacteria ferment substrate to provide maximal ATP; however, as fermentable substrate increases and energy availability exceeds the ability of the bacteria to use it for growth, ATP is used toward non-growth functions (Russell, 1998). Several processes can contribute to this wasteful energy use. Organisms can switch from VFA to lactate production because lactate generates fewer ATP per mol hexose fermented (Russell, 1998). Rapid increase in heat production by microbial cultures can occur after a pulse dose of glucose, possibly due to increased maintenance energy and futile cycling (Russell, 1986). Membrane ion maintenance is an important component of the maintenance energy of bacteria, and might be used to increase futile cycling. An

inhibitor of the membrane-bound ATPase abolished energy spilling, and a protonophore that increased proton flux across the membrane doubled energy spilling (Russell and Stroebel, 1990). Futile cycles of maintaining proton balance across the microbial membrane might reduce ATP available for growth, and might also indicate why low pH can reduce microbial efficiency.

Effects of Ruminal Turnover Rate on Microbial Growth

Microbial growth efficiency is also affected by turnover rate in the rumen. Feed intake and flow of microbial N to the duodenum are positively related (Clark et al., 1992), so effects of starch and fiber digestibility on DMI are expected to affect microbial protein production. Additionally, increasing rate of passage from the rumen probably has dramatic effects on increasing microbial growth efficiency by decreasing microbial turnover (Wells and Russell, 1996). Feeding *bm3* corn silage increased microbial efficiency, possibly because of increased ruminal passage rate of NDF, despite a reduction of ruminal pH by *bm3* silage (Oba and Allen, 2000c). Microbial efficiency was positively correlated with ruminal passage rate of starch and pdNDF (Voelker and Allen, 2003b), probably from reduced microbial turnover. Microbial turnover in the rumen occurs both by autolysis and predation. Mechanisms of autolysis are not well studied, but might contribute considerably to intraruminal N recycling when ruminal retention times are long. Protozoal predation can have significant effects on microbial efficiency and is often considered the primary cause of bacterial turnover in the rumen. Research with defaunated animals has shown that absence of protozoa increased duodenal microbial N

flow and bacterial efficiency, and reintroduction of protozoa subsequently decreased these variables (Koenig et al., 2000).

Protozoa were not believed to have direct advantage for the host animal because they reduce bacterial efficiency and do not contribute significantly to duodenal N flow because they are selectively retained in the rumen (Williams and Coleman, 1988). However, reduced OM and NDF digestibility in defaunated animals suggests that protozoa probably do contribute to ruminal fermentation (Koenig et al., 2000). A substantial portion of ruminal fiber digestion is due to protozoal degradation (Orpin, 1984). Additionally, protozoa engulf starch particles and digest them more slowly than do ruminal bacteria, thus potentially modulating ruminal pH (Nagaraja et al., 1992). Although the presence of protozoa may reduce bacterial efficiency by predation, the absence of protozoa may reduce bacterial efficiency by low ruminal pH.

Changing starch and fiber digestibility could influence efficiency of microbial protein production and ruminal fermentation. Endosperm type of corn grain can affect ruminal starch digestibility, which could affect ruminal pH, NDF fermentation, and microbial nitrogen efficiency. Additionally, *bm3* corn silage has been demonstrated to reduce ruminal pH but increase rate of passage, so effects of *bm3* corn silage on microbial efficiency are not predictable. Interactions of starch and fiber digestibility on DMI might also influence microbial efficiency by affecting microbial turnover. Research on the interactions of endosperm type of corn grain and the *bm3* mutation in corn silage on microbial efficiency is warranted.

SUMMARY

Fiber digestibility ranges considerably, and the ability to change the extent of lignification and fraction of pdNDF by a single gene mutation (*bm3* mutation in corn silage) provides a model to study NDF digestibility. Starch digestibility is highly related to vitreousness of corn grain endosperm and grain hybrids can range from 100% floury endosperm to highly (> 60%) vitreous endosperm. Individually, fiber and starch digestibility play an important role in the utilization of nutrients for production, but no research has directly examined how starch and fiber digestibility can interact to affect animal productivity.

Corn grain with floury endosperm and *bm3* corn silage might reduce ruminal pH, NDF digestibility, microbial fermentation, and microbial growth efficiency, although greater passage of *bm3* corn silage might reduce negative effects of pH on microbial growth efficiency. Reduced ruminal NDF digestibility from rapid starch fermentation might shift more NDF digestion to the hindgut, but might reduce total tract NDF digestibility if hindgut fermentation cannot compensate. Additionally, interactions of fiber and starch digestibility are expected to affect DMI; greater ruminal starch fermentation might reduce DMI by oxidation of metabolic fuels in the liver, but *bm3* corn silage might reduce filling effects of the diet and increase DMI.

Shifting site of starch digestion to the small intestines could occur by feeding corn grain with vitreous endosperm. Although the evidence is scant, *bm3* could affect starch digestion by increasing ruminal passage rate and shifting site of starch digestion to the intestines. Additionally, if *bm3* silage shifts site of starch digestion to the intestines,

hypophagic effects of propionate from ruminal starch fermentation might be removed, but digestion of starch in the small intestine could also potentially limit intake by other metabolic or hormonal factors. Greater glucose absorption in the small intestine might increase milk yield or tissue retention, or might not affect either. Greater rate of NDF passage for *bm3* silage might increase microbial efficiency by decreasing microbial turnover in the rumen, but greater hindgut fermentation could reduce microbial yield.

There is sufficient evidence to suggest that *bm3* corn silage and endosperm type of corn grain might interact to affect digestion and productivity of lactating dairy cows. We hypothesized that corn grain with floury endosperm is more ruminally fermentable than vitreous corn grain, and will decrease rate of digestion of pdNDF to a greater extent for *bm3* corn silage compared to control silage. Greater ruminal starch fermentation of floury corn grain would have greater effect at reducing meal size and possibly DMI when combined with *bm3* corn silage than control silage. Finally, corn grain with floury endosperm reduces microbial efficiency because of more rapid starch digestion, and might interact with corn silage type because *bm3* silage reduces microbial efficiency by low ruminal pH but increases microbial efficiency by increasing rate of ruminal passage. Therefore, the objective of this thesis was to evaluate the interactions of corn grain endosperm type and the brown midrib 3 mutation in corn silage on site of nutrient digestion, feeding behavior, milk production and energy balance, ruminal fermentation, and microbial protein efficiency in high producing dairy cows.

CHAPTER 2

Interactions of corn grain endosperm type and brown midrib 3 corn silage on ruminal kinetics and site of digestion in lactating dairy cows

ABSTRACT

Interactions of endosperm type of corn grain and the brown midrib 3 mutation in corn silage on ruminal kinetics and site of nutrient digestion of lactating dairy cows were evaluated. Eight ruminally and duodenally cannulated cows (72 ± 8 DIM; mean ± SD) were used in a duplicated 4 × 4 Latin square design experiment with a 2 × 2 factorial arrangement of treatments. Treatments were corn grain endosperm type, floury or vitreous, and corn silage type, *bm3* or isogenic normal. Diets contained 26% neutral detergent fiber (**NDF**) and 30% starch. Interactions of treatments were not observed for any measure of digestibility, but digestion kinetics of starch and fiber interacted to affect digestible OM intake by affecting DM intake. Rate of ruminal starch digestion was faster and rate of ruminal starch passage tended to be slower in diets containing corn grain with floury endosperm, resulting in a mean increase for ruminal starch digestibility of 22.1 units. Although compensatory postruminal starch digestion decreased differences among treatments for total tract starch digestibility, starch entering the duodenum was more digestible for grain with floury endosperm compared to vitreous grain, resulting in greater total tract starch digestibility for floury corn grain compared to vitreous corn grain. Fermentation rate of potentially digestible NDF was not affected by either *bm3*

corn silage or greater ruminal starch digestion of floury grain. Brown midrib corn silage increased total tract NDF digestibility versus control by numerically increasing ruminal and postruminal digestibility of NDF. Endosperm type of corn grain greatly influences starch digestion and should be considered when formulating diets.

INTRODUCTION

Ruminants have dietary requirements for forage to maximize production and ruminal health. However, because fiber is digested more slowly and is retained longer in the rumen than other nutrients, it can potentially limit DMI and milk yield. The brown midrib 3 (***bm3***) mutation in corn silage increases potential digestibility of fiber and allows for greater feed intake while providing forage fiber (Oba and Allen, 2000a). Although increased fiber digestibility can reduce the filling effect of the fibrous feeds and might allow greater feed intake, *bm3* corn silage reduces ruminal pH and might reduce ruminal digestibility of potentially digestible NDF (**pdNDF**; Oba and Allen, 2000c). Feed intake could also be altered by manipulating site of starch digestion (Allen, 2000); site of starch digestion can be affected by grain conservation method (Oba and Allen, 2003b) and method of processing (Callison et al., 2001). Additionally, corn silage with the *bm3* mutation reduced ruminal starch digestibility compared to the isogenic corn silage without the *bm3* mutation (Oba and Allen, 2000c). The authors attributed reduced ruminal starch digestibility in *bm3* corn silage diets to greater passage rate of starch from the rumen. Endosperm type of corn grain also can affect site of starch digestion; flint corn, which has a higher proportion of vitreous endosperm, was digested more slowly

and to a lesser extent than dent corn in the rumen of beef steers (Philippeau et al., 1999a). Endosperm type of corn grain varies widely across commercially available hybrids, but is not considered when formulating diets for lactating dairy cows.

Controversy exists as to the benefits of ruminal versus postruminal starch digestion. Ruminal starch digestion is needed to provide substrate for microbial growth and propionate as a glucose precursor for milk synthesis, but can reduce ruminal pH and inhibit fiber digestibility if starch fermentation is too rapid. Rapid flux of propionate to the liver might limit DMI if it is oxidized instead of being used for gluconeogenesis (Oba and Allen, 2003e). Shifting starch digestion to the intestines can theoretically provide more glucose to the animal, but infusion studies have suggested that increasing small intestinal glucose absorption may not increase glucose available for milk production (Knowlton et al., 1998b; Arieli et al., 2001). Instead, increased glucose may be used for tissue retention (Reynolds et al., 2001) or may be oxidized to CO_2 (Knowlton et al., 1998b). It is important to understand how chemical and structural aspects of dietary ingredients can affect starch and fiber digestibility and how they interact to change ruminal kinetics and site of nutrient digestion. We hypothesized that corn grain with floury endosperm is more rapidly degraded in the rumen compared to corn grain with vitreous endosperm, and will decrease rate of digestion of pdNDF to a greater extent for *bm3* corn silage compared to control corn silage. Greater rate of passage of pdNDF and indigestible NDF (**iNDF**) from the rumen in diets containing *bm3* corn silage will increase ruminal passage rate of starch. The objective of this experiment was to evaluate interactions of the brown midrib 3 mutation in corn silage and corn grain endosperm type on ruminal digestion kinetics and site of nutrient digestion of lactating dairy cows.

MATERIALS AND METHODS

Cows and Treatments

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (72 ± 8 DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to duplicate 4 × 4 Latin squares balanced for carry-over effects. Cows were randomly assigned to treatment sequence. A 2 × 2 factorial arrangement of treatments was used with main effects of corn grain endosperm type (floury or vitreous) and *bm3* mutation (present or absent). Treatment periods were 21 d, consisting of an 11 d diet adaptation period followed by 10 d of collection. Surgical preparation of ruminally and duodenally cannulated cows was performed after dry-off, approximately 50 d prior to calving. Duodenal cannulas were soft gutter type made of tygon and vinyl tubing (Crocker et al., 1998). For each animal, the duodenum was fistulated distal to the pylorus region prior to the pancreatic duct, and the cannula was placed between 10th and 11th ribs as described by Robinson et al. (1985). Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. At the beginning of the experiment, empty body weight (ruminal digesta removed) of cows was 531.8 ± 43.9 kg (mean ± SD).

Two corn hybrids, 6208FQ and 657 (Cargill Hybrid Seeds, Minneapolis, MN), were planted for silage in Spring of 2001 at the Michigan State University Research Farm. The hybrids are isogenic except that Cargill 657 contains the *bm3* mutation. Cargill 6208FQ corn forage was harvested at 30.6% whole plant dry matter and chopped

to 11 mm theoretical length of cut. Cargill 657 corn forage was harvested at 32.2% whole plant dry matter and chopped to 10 mm theoretical length of cut. The chop lengths of the two hybrids differed in order to achieve a similar particle size distribution as measured using a Penn State Particle Size Separator (Lammers et al., 1996). Both hybrids were ensiled in 12.43 m diameter AgBags® oriented in a west to east direction in order to minimize the effects of wind on silage DM. Nutrient compositions and physical characteristics of the corn silage treatments used in the experiment are shown in Table 1.

Two corn hybrids (1 floury and 1 vitreous) were planted for grain in Spring of 2001 at the Michigan State University Research Farm. The floury and vitreous hybrids, SL53 (Crow's Hybrid Corn Company, Kentland, IN) and Z75W (Wilson Genetics, Harlan, IA) were selected based upon their high and low *in vitro* starch digestibility, respectively. Grains were harvested after field drying to ~20% DM, and were commercially dried at low temperature (< 32 °C) to ~14% DM. The corn was ground and bagged for use in the experiment. Vitreousness (% of endosperm) for floury and vitreous corn grain was 3.0 and 67.2%, respectively (Table 2). Nutrient compositions and physical characteristics of the corn grain treatments used in the experiment are shown in Table 2.

Experimental diets contained dry ground corn treatments (floury or vitreous), corn silage (*bm3* or isogenic normal), alfalfa silage (10% of diet dry matter), whole linted cottonseed (7% of diet dry matter), a protein supplement premix (soybean meal, distillers grains, and blood meal), and a premix of minerals and vitamins. Experimental diets were fed as total mixed rations, and were formulated to contain 27% NDF, 18% crude protein

with adequate metabolizable protein, and minerals and vitamins to requirements. Ingredient and nutrient compositions of the experimental diets are shown in Table 3.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed once daily (1100 h) at 110% of expected intake. Amounts of feed offered and orts were weighed for each cow daily during the collection period. Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily on d 12-18 and combined into two samples per period, representing the digestibility (d 12-14) and feeding behavior (d 15-18) sub-periods.

Chromic oxide was used as a marker to estimate nutrient digestibility in the rumen and in the total tract. Gelatin capsules (1.5 oz., Tropac Inc., Airfield, NJ) containing 5 g of chromic oxide and approximately 4 g of ground spelt hulls were dosed through the ruminal cannula at 0400, 1200, and 2000 h (total of 15 g Cr_2O_3 /d) from 7 to 14 d with a priming dose of 3X on d 7. Duodenal (1,000 g), fecal (500 g), and rumen fluid samples (100 mL) were collected every 9 h from d 12 to d 14 so that 8 samples were taken for each cow each period, representing every 3 h of a 24-hour period to account for diurnal variation. Digesta from 5 sites in the rumen was combined and strained to obtain rumen fluid. All digesta and fecal samples were immediately frozen at -20°C until processing.

Effect of treatment on rate of liquid passage was measured on d 19 using a pulse dose of cobalt EDTA (Allen et al., 2000). Cobalt EDTA was dosed two hours after feeding. Rumen fluid was sampled immediately before dosing and at 0.5, 1, 1.5, 2, 2.5,

3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 h after dosing. Samples were immediately frozen.

Ruminal contents were evacuated manually through the ruminal cannula at 1500 h (4 h after feeding) on d 20 and at 0900 h (2 h before feeding) on d 21 of each period. Total ruminal content mass and volume were determined. During evacuation, 10% aliquots of digesta were separated to allow accurate sampling. Aliquots were squeezed through a nylon screen (1 mm pore size) to separate into primarily solid and liquid phases. Samples were taken from both phases for determination of nutrient pool size, and an additional liquid sample was taken to measure VFA concentration. All samples were frozen immediately at –20°C.

Sample and Statistical Analysis

Diet ingredients and orts were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1mm screen; Authur H. Thomas, Philadelphia, PA). Fecal samples were processed similarly, and dried, ground fecal samples were combined on an equal DM basis into one sample per cow per period. Rumen liquid and solid sub-samples were lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY), ground, and recombined according to the original ratio of solid and liquid DM. Duodenal samples were thawed, combined, and filtered into primarily solid and liquid phases using nylon mesh (1 mm pore size) to minimize sampling errors due to segregation of samples into solid and liquid phases. Both phases were weighed, and sub-samples were taken from each phase. Liquid and solid sub-

samples were lyophilized, ground, and recombined by weight according to the original ratio of solid and liquid DM.

Samples were analyzed for ash, NDF, indigestible NDF (iNDF), crude protein (**CP**), and starch. Ash concentration was determined after 5 h oxidation at 500°C in a muffle furnace. Concentrations of NDF were determined according to Van Soest et al. (1991, method A). Forage samples were analyzed for acid detergent fiber (**ADF**) and sulfuric acid lignin content (Van Soest et al., 1991). Indigestible NDF was estimated as NDF residue after 240-h in vitro fermentation (Goering and Van Soest, 1970). Rumen fluid for the in vitro incubations was collected from a non-pregnant dry cow fed only alfalfa hay. Fraction of potentially digestible NDF (pdNDF) was calculated by difference (1.00 – iNDF). Crude protein was analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration was measured using a glucose oxidase method (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), and absorbance was determined with a micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). 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.

Corn silage particle size was determined on wet silage samples using the Penn State Particle Size Separator (Lammers et al., 1996). Wet corn silage samples were homogenized in distilled water to determine pH and concentrations of major VFA and lactate by HPLC (Waters Corp., Milford, MA) as described by Oba and Allen (2003a). Corn grain was dry sieved (Sieve apertures: 4750, 2360, 1180, 600, 300, 150, 75 μm and bottom pan) using a sieve shaker (model RX-86, W. S. Tyler Inc., Gastonia, NC) for

approximately 20 minutes until the bottom pan weight was constant, and the mean particle size of the corn grain was calculated (ASAE, 1968). For determination of corn grain vitreousness, 10 whole kernels from each hybrid were weighed, and kernels were dissected into germ, pericarp, and vitreous endosperm sections and individually weighed. Total (floury and vitreous) endosperm weight was determined by subtracting germ and pericarp weight from whole kernel weight, and vitreousness was calculated by dividing vitreous endosperm weight by total endosperm weight.

Rumen fluid samples taken for measurement of rate of liquid passage were analyzed for cobalt concentration by flame atomic absorption spectrophotometry according to manufacturers recommendations (SpectrAA 220/FS, Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia). Rate of cobalt disappearance was determined by non-linear regression (JMP® Version 4, SAS Institute, Cary, NC) of its decline in concentration in rumen fluid over time after dosing using a one-pool, first-order model after accounting for background cobalt concentration.

Diet ingredients, duodenal digesta, and feces were analyzed for concentrations of chromium. Samples were digested with phosphoric acid (Williams et al., 1962), and chromium was quantified by flame atomic absorption spectrometry (SpectraAA 220, Varian, Victoria, Australia) according to manufacturer's recommendation. Although we intended to use chromic oxide as an external marker in this study, an apparent liquid phase subsampling problem occurred (Appendix A), and iNDF was chosen as a duodenal flow marker. Subsampling problems would not be expected in fecal samples but iNDF was used as a fecal flow marker for consistency. Duodenal and fecal DM flows calculated using chromic oxide were highly correlated to flows calculated using iNDF as

a marker (r^2 = 0.60 and r^2 =0.79, respectively). Nutrient intake was calculated using the amounts and compositions of feed offered and refused. Duodenal flow of microbial OM was determined as described by Oba and Allen (2003c), and true ruminally degraded OM (**TRDOM**) was calculated by subtracting duodenal flow of non-microbial OM from OM intake. True ruminally degraded starch was calculated by subtracting duodenal flow of non-microbial starch from starch intake. Ruminal pool sizes (kg) of OM, NDF, iNDF, pdNDF, and starch were determined by multiplying the concentration of each component by the ruminal digesta DM mass (kg). Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component (%/h) were calculated using the following equations:

Turnover rate in the rumen (%/h) =

100 x (Intake of component / Ruminal pool of component) / 24

Passage rate from the rumen (%/h) =

100 x (Duodenal flow of component / Ruminal pool of component) / 24; and

Digestion rate in the rumen (%/h) =

Turnover rate in the rumen (%/h) – Passage rate from the rumen (%/h).

All data was analyzed using the fit model procedure of JMP® (Version 4, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + PT_{jk} + e_{ijkl}$$

where

μ = overall mean,

C_i = random effect of cow (j = 1 to 8),

P_j = fixed effect of period (k = 1 to 4),

T_k = fixed effect of treatment (l = 1 to 4),

PT_{jk} =interaction of period and treatment, and

e_{ijkl} = residual

A reduced model without period × treatment interactions was used when this effect was not significant ($P > 0.15$). Orthogonal contrasts were used to determine main effect of corn silage type, main effect of corn grain type, and the interaction of corn silage type and endosperm type of corn grain. Pearson correlation coefficients were determined between cow-period observations for some parameters. Main treatment effects and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$. Interactions between treatments were declared significant at $P < 0.10$, and tendencies were declared at $P < 0.15$.

Data from one cow was removed from all statistical analysis due to clinical mastitis during the first period. This cow was replaced with a spare animal for the remaining three periods. Another cow developed pneumonia during the diet adaptation period of period 2, and was recovering during the first day of the collection period. Data from this cow was omitted from the digestibility sub-period of period 2, but was utilized from the feeding behavior sub-period as she had recovered sufficiently.

RESULTS AND DISCUSSION

DM and OM Digestion

No interactions between main treatment effects were found for any measure of DM or OM digestibility, and replacing control corn silage with *bm3* corn silage did not affect DM or OM digestibility. Total tract DM digestibility was higher for floury versus vitreous corn grain ($P < 0.01$; Table 4). Although floury corn grain increased apparent ruminal OM digestibility 9.5 percentage units versus vitreous grain ($P < 0.04$), slightly higher duodenal flow of microbial OM in vitreous diets reduced the differences between endosperm types so that TRDOM did not differ among treatments (Chapter 4). TRDOM averaged 14.5 kg/d and is among the highest reported in literature, primarily because animals in this experiment averaged 22.6 kg/d OM intake and were producing 40 kg/d milk. Postruminal OM digestibility did not differ among treatments, but increases of ruminal OM digestion in diets with floury endosperm resulted in greater total tract apparent OM digestion ($P < 0.01$).

Site of Starch Digestion

Contrary to our hypothesis, no interactions of main treatment effects occurred to affect starch digestion. Although previous research from our laboratory found that *bm3* corn silage reduced ruminal and total tract starch digestibility (Oba and Allen, 2000c), in the present experiment *bm3* corn silage did not affect ruminal starch digestibility and only tended to reduce total tract starch digestibility compared to control corn silage ($P < 0.08$; Table 5). However, endosperm type of corn grain caused substantial changes in site

of starch digestion. Apparent ruminal digestibility of starch increased from 35.0% to 57.0% when floury endosperm grain replaced vitreous corn grain ($P < 0.01$). A previous experiment showed that apparent ruminal starch digestibility was increased from 34.8 to 60.8 % when dent corn replaced flint corn in diets of steers (Philippeau et al., 1999a). Corn grain with floury endosperm also increased true ruminal starch digestibility compared to vitreous grain ($P < 0.001$), but variation was greater for apparent versus true ruminal starch digestibility because of the greater contribution of microbial starch to duodenal starch in the diet containing corn grain with vitreous endosperm and control corn silage compared to other diets (Chapter 4). As expected, vitreous grain shifted the primary site of starch digestion to the intestines; postruminal starch digestibility as a percent of intake was greater for vitreous versus floury corn grain (56.8 versus 39.3 %; $P < 0.03$). However, as a percent of duodenal starch flow, starch digestibility was lower for vitreous grain compared to floury corn grain ($P < 0.03$). This indicates that corn grain with vitreous endosperm is not only more indigestible than floury grain in the rumen, but the starch that passes out of the rumen is also less digestible. Although apparent ruminal starch digestibility was reduced by 39 % for vitreous grain diets, total tract starch digestibility was only reduced 5 % because of substantial postruminal compensatory digestion.

Ruminal starch digestibility was greater for floury corn grain treatments because starch in floury endosperm was digested at a rate of 21.9 %/h versus 12.9 %/h for vitreous endosperm ($P < 0.01$; Table 7). Philippeau et al. (1999b) reported that dent hybrids (51.4% vitreous) degraded faster than flint (71.8% vitreous) hybrids in situ ($P < 0.0001$). Floury endosperm is associated with a digestible protein matrix and is easily

broken down by ruminal bacteria (Kotarski et al., 1992). In contrast, starch granules in vitreous endosperm are embedded in a protein matrix that can resist enzyme hydrolysis (Rooney and Plugfelder, 1986). Vitreous protein matrix is more resistant to digestion because ruminal bacteria digest zein proteins more slowly than glutelin proteins (Romagnolo et al., 1994), and vitreousness of corn grain is positively correlated with concentration of zein protein and negatively correlated with true glutelin protein concentration in corn grain (Philippeau et al., 2000). Because the protein matrix in floury endosperm is more easily hydrolyzed, greater microbial penetration of the starch granule occurs to increase rate of starch digestion. In this study, vitreousness (% of total endosperm) of corn grain was 3.0 and 67.2 % for floury and vitreous hybrids, respectively (Table 2). Additionally, the corn grains used were selected based on their wide range of in vitro starch degradability, which was 7.7 and 1.8 %/h for the floury and vitreous hybrids, respectively (Table 2). The floury and vitreous endosperm corn grains used in this experiment represent the two extreme endosperm compositions that are commercially available.

Corn grain with vitreous endosperm tended to increase ruminal passage rate of starch (21.2 vs. 16.2 %/h; $P < 0.10$; Table 7). We expected floury corn grain might disperse in the liquid fraction and possibly increase rate of starch passage from the rumen. However, mean ruminal liquid passage rate was approximately 19.9 %/h, and rate of starch passage from the rumen and ruminal liquid passage rate were not correlated across cow period means ($r^2 = 0.03$; $P < 0.86$). Faster ruminal starch passage rate in this experiment might be because of greater density of vitreous corn grain; vitreous flint corn grain was more dense than less vitreous dent corn grain (Philippeau et al., 1999b), and

greater particle density decreased mean ruminal retention time (Lechner-Doll et al., 1991). Faster rate of starch digestion and a tendency for slower rate of ruminal starch passage for floury corn grain versus vitreous grain resulted in a lack of treatment effects on ruminal starch turnover rate.

It is unclear when shifting starch digestion from the rumen to the intestines is beneficial for milk production. Small intestinal digestion of starch to glucose is theoretically more efficient than ruminal fermentation to VFA (Owens et al., 1986), and while glucose availability might increase with greater intestinal starch flow, milk production does not necessarily increase (Knowlton et al., 1998b; Arieli et al., 2001; Reynolds et al., 2001). As greater amounts of concentrate are included in ruminant diets, understanding limits to starch digestion and absorption in the intestines of ruminants has become a question of interest. A linear response in net portal glucose absorption was observed for infusions of glucose into the abomasum, but a quadratic response in net portal glucose absorption was observed for infusions of corn starch in to the abomasum; diminished absorption occurred with greater amounts of corn starch infusion (Kreikemeier et al., 1991). This suggests that limitations to small intestinal starch utilization are from starch digestion and not glucose absorption. Huntington (1997) suggested that limitations to small intestinal starch digestion are because of less secretion of effective pancreatic amylase with increasing duodenal starch flow. However, Oba and Allen (2003b) found that postruminal starch digestibility (% of duodenal flow) increased as duodenal starch flow increased ($r^2 = 0.49$; $P < 0.05$). They suggested that limitations to starch utilization by pancreatic enzyme activity would be expected to decrease intestinal starch digestibility as duodenal flow of starch increased, and concluded that

lower digestibility of starch escaping the rumen was because of differences in the physical and chemical characteristics of the starch particles. In this experiment, relationship between postruminal starch digestibility and duodenal starch flow was not significant, but similar to Oba and Allen (2003b) this relationship was not negative (r^2 = 0.23; $P < 0.20$). Primary limitations to starch digestion in the small intestine may more dependent on characteristics of starch particles escaping ruminal digestion.

Ruminal and postruminal starch digestibility ranges widely for a variety of feedstuffs, but generally intestinal starch digestion can compensate for reduction in ruminal starch digestion so that variation in total tract starch digestibility is relatively small (Huntington, 1997). However, greater intestinal starch flow may decrease efficiency of energy use for productive purposes. Abomasal infusion of increasing amounts of corn starch increased ileal concentrations of starch and glucose (Kreikemeier et al., 1991). Greater starch flow to the intestines might increase large intestinal fermentation of starch to VFA. Some of this energy could be absorbed by the cow, but microbial OM from large intestinal fermentation would be lost in the feces. Additionally, even if increasing starch flow and intestinal digestion results in greater uptake of glucose by the gut, the energy might not be used toward milk production but instead might be oxidized or used toward tissue retention if the animal is in positive energy balance (Lemosquet et al., 1997; Knowlton et al., 1998b; Reynolds et al., 2001). Knowlton et al. (1998b) observed that infusing starch hydrolysate in the abomasum versus the rumen increased blood CO_2 from glucose by 93 % ($P < 0.001$). Duodenal glucose infusions increased energy balance without affecting milk yield because of increased plasma insulin (Lemosquet et al., 1997). The shift of starch digestion from the rumen to the

intestines observed in vitreous corn grain diets tended to increase plasma insulin: glucagon ratio ($P < 0.07$; Chapter 3), which might decrease the rate of gluconeogenesis and increase oxidation of fuels, reducing glucose supply.

Inconsistencies with shifting digestion to the intestines coupled with requirements of microbes for fermentable carbohydrate have led many to conclude that extensive ruminal starch fermentation is often more beneficial than intestinal digestion (Huntington, 1997). Ruminal fermentation of starch can increase ruminal propionate production and theoretically could increase substrate available for gluconeogenesis. However, DMI can also be limited by propionate; Oba and Allen (2003d) speculated that if temporal flux of propionate to the liver is greater than glucose demand, DMI depression could occur by oxidation of propionate and other metabolic fuels in the tricarboxylic acid (**TCA**) cycle. Corn grain with floury endosperm was fermented faster and to a greater extent in the rumen, and depressed DMI when combined with control corn silage possibly because of oxidation of fuels, but did not limit DMI when combined with *bm3* corn silage probably because of a concurrent increase in milk production (Chapter 3). Ruminal fermentation of starch is less likely to benefit production if it reduces DMI.

It is unlikely that optimal site of starch digestion is constant across all diets and animals. Postruminal starch digestion could theoretically increase glucose availability but in reality may decrease gluconeogenesis and increase tissue retention. Ruminal starch digestion can increase glucose precursors but can limit DMI if fuels are oxidized in the liver. Microbial protein production is also integrally tied with starch digestion, and probably often plays an important role in animal response to site of starch digestion. The

response of any given animal is also likely to vary considerably, and will depend on energy balance, stage of lactation, hormone responsiveness, and nutrition. Therefore, the optimum site of starch digestion for lactating dairy cows depends on a variety of factors and response is difficult to predict.

Fiber Digestion

Contrary to our hypothesis, no interactions of main treatment effects were observed for digestibility of NDF, pdNDF (Table 6), or ruminal digestion kinetics of pdNDF (Table 7). Corn grain with floury endosperm and *bm3* corn silage were expected to reduce rate of ruminal pdNDF digestion by reducing ruminal pH, but pdNDF digestion rate was not affected by either floury endosperm grain or *bm3* corn silage (Table 7). Although mean pH was lower for both corn grain with floury endosperm and *bm3* corn silage (Chapter 4), pH might not have been low enough to substantially affect pdNDF digestion. Some in vitro work has suggested that substantial rates of cellulose digestion can occur at pH less than 6.0, and it is not until pH drops below 5.3 that lysis or detachment of cellulolytic bacteria occurs (Mouriño et al., 2001). Additionally, although rate of pdNDF digestion can be inhibited by the presence of starch regardless of pH (Grant and Mertens, 1992), previous work in our laboratory reported no relationship between the digestion rate of pdNDF and amount of ruminally degraded starch (Oba and Allen, 2003b). Similarly, increased rate of starch digestion did not affect NDF digestion rate in situ (Callison et al., 2001). Starch digestion may inhibit fermentation of pdNDF in batch cultures where products are not removed, but constant removal of fermentation products from the rumen may reduce any direct effects of starch on pdNDF fermentation.

Interestingly, ruminal starch digestibility and ruminal pdNDF digestibility were positively correlated across cow period means in this experiment ($r^2 = 0.49$; $P < 0.007$). Extensive interaction among ruminal microorganisms occurs, and it is possible that this positive relationship between starch and fiber digestibility represents microbial synergy and cross-feeding. Mixed cultures of cellulolytic and non-cellulolytic bacteria increased cellulose disappearance in vitro more than pure cultures of three predominant species of ruminal cellulolytic bacteria (Mouriño et al., 2001). Highly fermentable, high starch diets in vivo may not be as detrimental to microbial fiber digestion as in vitro studies indicate.

Unlike a previous experiment from our laboratory (Oba and Allen, 2000c), ruminal passage rate of iNDF was not increased by *bm3* corn silage compared to control (Table 7). Other experiments reported no effect on ruminal passage rate of iNDF for *bm3* corn silage (Tjardes et al., 2000) or brown midrib sorghum (Aydin et al., 1999). Differences among these studies are probably because of treatment effects on DMI and are probably related to the extent that ruminal distension limits intake. In this study, ruminal pool size mirrored treatment effects on DMI (Chapter 3), which indicates that ruminal fill was probably not limiting intake. Additionally, range of DMI in this study was 1.9 kg per day and may not have been large enough to significantly affect iNDF passage.

Consistent with the lack of treatment effects on rates of digestion and passage, no treatment effects were observed for turnover rate of pdNDF or iNDF. Additionally, although in vitro NDF digestibility was 12.6 units higher for *bm3* silage than control silage (Table 1), *bm3* corn silage did not increase ruminal or postruminal pdNDF or NDF digestibility in vivo and only slightly increased total tract NDF digestibility ($P < 0.02$;

Table 6). Similarly, Oba and Allen (2000c) reported *bm3* corn silage did not increase ruminal or total tract NDF digestibility. The authors speculated that lack of treatment effects from *bm3* corn silage occurred because of greater rate of iNDF passage and reduced rate of pdNDF digestion; however, in the present experiment, *bm3* corn silage did not affect either rate of iNDF passage or rate of pdNDF digestion. These results suggest that although in vitro NDF digestibility can be improved by *bm3* corn silage, in vivo response is dependent on other factors.

CONCLUSIONS

Floury endosperm improved total tract DM and OM digestibility because of increased starch digestibility. Contrary to our hypothesis, no interaction of treatments for any measure of starch or fiber digestion occurred. Vitreous corn grain fermented more slowly and passed from the rumen faster, resulting in decreased ruminal starch digestibility. Compensatory postruminal starch digestion in diets containing vitreous corn grain resulted in relatively small differences in total tract starch digestion compared to grain with floury endosperm. Greater ruminal starch digestion in floury grain diets and lower ruminal pH for both floury grain and *bm3* corn silage did not affect ruminal fiber digestion kinetics, and a positive relationship between ruminal starch and pdNDF digestibility could suggest substantial interactions between microbial populations in the rumen. Endosperm type of corn grain can affect digestion kinetics and site of starch digestion.

Table 1. Nutrient compositions and physical characteristics of corn silages used to formulate experimental diets.[1]

	Control	*bm3*[2]	SEM
DM, % (oven dried at 55 °C)	30.5 [b]	31.5 [a]	0.3
	—% of DM—		
NDF	41.2 [a]	38.9 [b]	0.5
ADF	24.4 [a]	22.2 [b]	0.3
Lignin	2.25 [a]	1.22 [b]	0.1
Starch	25.8	26.6	0.5
CP	7.70	8.30	0.3
Ether Extract	3.52	3.01	0.23
Ash	2.96	3.32	0.2
Acetate	1.49 [a]	1.29 [b]	0.04
Propionate	0.12	0.10	0.01
Lactate	5.67 [b]	5.95 [a]	0.07
Ethanol	1.89	1.99	0.2
IV TDMD[3]	79.5 [b]	87.1 [a]	0.26
IV NDFD, % of NDF[4]	54.0 [b]	66.6 [a]	0.69
pH	3.78	3.77	0.01
Particle size (% of DM)[5]			
Top	6.87	6.60	1.3
Middle	69.9	69.0	0.84
Bottom	23.0	24.1	0.85

[1] Nutrient compositions of corn silages sampled each period (n = 8 for each silage) were compared by ANOVA. Means for nutrient composition in the same row followed by different superscript letters differ ($P < 0.05$).

[2] Brown midrib 3 mutant for corn silage.

[3] IV TDMD: in vitro true dry matter digestibility estimated after 30 h of incubation.

[4] IV NDFD: in vitro NDF digestibility estimated after 30 h of incubation.

[5] Determined with Penn State Particle Size Separator (Lammers et al, 1996).

Table 2. Nutrient compositions and physical characteristics of corn grains used to formulate experimental diets.[1]

	Floury	Vitreous	SEM
DM, %	86.8	87.0	0.1
	—% of DM—		
Starch	73.4	72.1	0.80
NDF	8.69 [a]	8.43 [b]	0.02
CP	8.19 [b]	9.07 [a]	0.14
Ether Extract	4.81	5.02	0.15
Ash	1.28	1.24	0.04
IVSD, %/h[2]	7.7	1.8	
Vitreousness, % of endosperm	3.0 [b]	67.2 [a]	1.9
	Percent retained		
Sieve aperture (μm)			
4750	0.1	0.3	0.1
2369	4.5	5.5	0.4
1180	51.7 [b]	66.5 [a]	1.1
600	25.7 [a]	18.5 [b]	1.8
300	11.2	7.5	1.2
150	4.6	1.6	1.3
75	2.0	0.0	1.1
pan	0.1	0.0	0.1
Mean particle size (μm)[3]	1377 [b]	1594 [a]	12.7
Standard deviation particle size	655 [b]	744 [a]	11.2

[1] Nutrient compositions of corn grains sampled each period (n = 4 for each grain) were compared by ANOVA. Means for nutrient composition in the same row followed by different superscript letters differ ($P < 0.05$).

[2] In vitro starch digestibility estimated after 24 h of incubation.

[3] Determined using sieve shaker (model RX-86, W. S. Tyler Inc., Gastonia, NC).

Table 3. Ingredient and nutrient compositions of experimental diets (% of dietary DM).[1]

	Control		*bm3*[2]		
	Floury	Vitreous	Floury	Vitreous	SEM
Ingredient					
Normal corn silage	37.5	37.3	—	—	—
Brown midrib 3 corn silage	—	—	39.6	39.2	—
Floury ground corn	22.7	—	20.7	—	—
Vitreous ground corn	—	23.7	—	23.7	—
Protein mix[3]	17.8	17.0	17.9	17.0	—
Alfalfa silage	9.9	9.9	9.8	9.9	—
Whole linted cottonseed	7.2	7.2	7.1	7.2	—
Vitamin mineral mix[4]	4.9	4.9	4.9	4.9	—
Nutrient Composition					
DM	48.0	48.2	48.0	48.2	0.2
OM	93.9	94.0	93.8	93.8	0.1
Starch	29.7	30.0	29.1	29.4	0.3
NDF	26.0	25.8	25.7	25.5	0.3
ADF	11.9 [a]	11.9 [a]	11.5 [b]	11.5 [b]	0.1
Lignin	1.43 [a]	1.42 [a]	1.06 [b]	1.05 [b]	0.03
Indigestible NDF[5]	8.91 [a]	8.89[a]	7.04 [b]	7.02 [b]	0.2
CP	17.3	17.2	17.5	17.4	0.1
Ether extract	4.71	4.77	4.48	4.54	0.1
Forage NDF	72.0	72.0	72.4	72.4	0.3
Treatment corn grain starch, % of dietary starch	55.7 [a]	56.5 [a]	51.9 [b]	52.9 [b]	0.5
Treatment corn silage starch, % of dietary starch	32.7 [b]	32.2 [b]	36.3 [a]	35.6 [a]	0.5

[1] The actual nutrient composition of the experimental diets was calculated for each period (n = 8 for each treatment) and compared by ANOVA. Means for nutrient composition in the same row followed by different superscript letters differ ($P < 0.05$).

[2] Brown midrib 3 mutant for corn silage.

[3] Protein mix contained 74.1% soybean meal (48%), 22.2% corn distillers grain, and 3.8% blood meal.

[4] Vitamin mineral mix contained 59.9% dry ground corn, 17.3% limestone, 10.7% dicalcium phosphorus, 8.7% trace mineral and salt premix, 2.1% magnesium oxide, 0.4% Vitamin A, 0.4% selenium, 0.3% Vitamin D, and 0.1% Vitamin E.

[5] Indigestible NDF: estimated after 240 h of in vitro ruminal fermentation.

Table 4. Effects of corn grain endosperm type and brown midrib 3 corn silage on digestion of DM and OM.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
DM								
Intake, kg/d	23.6	25.5	25.2	24.9	1.0	0.40	0.18	0.07
Apparent total tract digestion								
kg/d	16.8	17.5	18.1	17.2	0.64	0.30	0.83	0.11
%	73.2	70.4	74.8	71.1	1.3	0.35	0.01	0.73
OM								
Intake, kg/d	21.5	23.4	22.8	22.8	1.0	0.53	0.15	0.14
Digestible OMI, kg/d	17.9	19.5	19.5	19.5	0.8	0.15	0.16	0.16
Apparent ruminal digestion								
kg/d	9.62	7.43	9.13	7.98	1.1	0.98	0.11	0.61
%	44.8	31.4	40.7	35.2	4.4	0.97	0.04	0.36
True ruminal digestion								
kg/d	14.3	14.7	15.0	13.8	1.0	0.92	0.53	0.18
%	66.7	63.1	66.2	60.5	3.3	0.59	0.11	0.71
Passage to duodenum, kg/d	11.9	16.1	13.7	14.8	1.2	0.81	0.04	0.21
Apparent postruminal digestion								
kg/d	6.42	9.31	8.07	8.34	1.0	0.74	0.14	0.21
% of intake	29.7	40.3	35.1	37.0	4.3	0.79	0.13	0.28
% of duodenal passage	53.7	56.2	59.4	56.3	3.0	0.27	0.90	0.29
Apparent total tract digestion								
kg/d	16.0	16.7	17.2	16.3	0.62	0.41	0.85	0.12
%	74.5	71.6	75.8	72.1	1.2	0.45	0.01	0.76

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

Table 5. Effects of corn grain endosperm type and brown midrib 3 corn silage on digestion of starch.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Intake, kg/d	6.90	7.63	7.15	7.28	0.3	0.79	0.04	0.15
Apparent ruminal digestion								
kg/d	4.26	2.28	3.75	3.04	0.7	0.82	0.03	0.27
%	61.5	29.0	52.5	40.9	8.6	0.84	0.01	0.17
True ruminal digestion								
kg/d	4.89	4.04	4.54	3.81	0.56	0.33	0.01	0.84
%	70.8	52.0	63.2	51.3	6.0	0.30	0.001	0.40
Passage to duodenum, kg/d	2.65	5.36	3.40	4.23	0.66	0.76	0.01	0.14
Apparent postruminal digestion								
kg/d	2.43	4.74	3.07	3.57	0.67	0.66	0.03	0.15
% of intake	35.4	63.2	43.1	50.4	8.8	0.73	0.03	0.18
% of duodenal passage	90.9	83.5	90.6	83.6	3.6	0.96	0.01	0.91
Apparent total tract digestion								
kg/d	6.69	7.02	6.82	6.63	0.27	0.47	0.70	0.17
%	96.9	92.1	95.6	91.3	0.78	0.08	<.0001	0.66

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

Table 6. Effects of corn grain endosperm type and brown midrib 3 corn silage on digestion of total NDF and potentially digestible NDF.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
NDF								
Intake, kg/d	5.74	6.21	6.09	6.00	0.3	0.71	0.29	0.13
Apparent ruminal digestion								
kg/d	2.14	2.22	2.45	2.39	0.21	0.21	0.97	0.72
%	37.4	34.8	41.0	40.2	3.3	0.20	0.61	0.79
Passage to duodenum, kg/d	3.60	4.01	3.64	3.62	0.27	0.51	0.45	0.42
Apparent postruminal digestion								
kg/d	0.52	0.57	0.70	0.54	0.18	0.68	0.77	0.57
% of intake	8.95	9.96	11.5	9.60	3.0	0.71	0.88	0.62
% of duodenal passage	14.2	14.2	19.7	14.9	4.2	0.44	0.55	0.54
Apparent total tract digestion								
kg/d	2.66	2.80	3.15	2.90	0.13	0.04	0.69	0.16
%	46.3	45.3	52.4	49.7	2.1	0.02	0.37	0.68
Potentially digestible NDF								
Intake, kg/d	3.75	4.03	4.45	4.40	0.19	0.001	0.38	0.22
Apparent ruminal digestion								
kg/d	2.14	2.22	2.45	2.39	0.21	0.21	0.97	0.72
%	57.1	53.0	55.8	54.8	4.7	0.97	0.60	0.75
Passage to duodenum, kg/d	1.61	1.84	2.00	2.00	0.23	0.25	0.61	0.63
Apparent postruminal digestion								
kg/d	0.52	0.57	0.70	0.54	0.18	0.68	0.77	0.57
% of intake	13.6	15.4	15.6	13.0	4.4	0.95	0.92	0.60
% of duodenal passage	30.8	28.6	36.1	25.1	7.4	0.90	0.33	0.51
Apparent total tract digestion								
kg/d	2.66	2.80	3.15	2.90	0.13	0.04	0.69	0.16
%	70.7	69.0	71.3	67.4	2.8	0.85	0.31	0.68
Indigestible NDF								
Intake, kg/d	1.99	2.18	1.64	1.59	0.10	<.0001	0.23	0.07

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

Table 7. Effects of corn grain endosperm type and brown midrib 3 corn silage on ruminal digestion kinetics.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Ruminal digestion rate, %/h								
pdNDF[4]	3.30	3.00	2.79	3.11	0.38	0.48	0.98	0.30
Starch	24.8	11.9	19.0	13.8	3.6	0.55	0.01	0.25
Ruminal passage rate, %/h								
pdNDF[4]	2.58	2.58	2.37	2.68	0.52	0.92	0.77	0.78
iNDF[5]	3.26	3.01	3.29	3.52	0.27	0.27	0.94	0.34
Starch	16.6	24.0	15.7	18.3	3.1	0.27	0.10	0.43
Liquid[6]	18.0	20.4	20.6	20.6	2.0	0.46	0.53	0.54
Ruminal turnover rate, %/h								
DM	10.3	9.44	9.58	10.3	0.80	0.92	0.93	0.33
OM	9.84	9.11	9.19	9.91	0.80	0.92	0.99	0.35
NDF	4.57	4.32	4.46	4.93	0.44	0.54	0.78	0.39
pdNDF[4]	5.88	5.58	5.16	5.88	0.64	0.72	0.72	0.39
iNDF[5]	3.26	3.01	3.29	3.52	0.27	0.27	0.94	0.34
Starch	41.4	36.3	34.8	31.9	3.6	0.14	0.28	0.76

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.
[4] Potentially digestible NDF.
[5] Indigestible NDF.
[6] Measured using Cobalt-EDTA.

CHAPTER 3

Interactions of corn grain endosperm type and brown midrib 3 corn silage on feeding behavior, milk production, and energy balance of lactating dairy cows

ABSTRACT

Interactions of endosperm type of corn grain and the brown midrib 3 mutation in corn silage on feeding behavior, productivity, energy balance, and plasma metabolites of lactating dairy cows were evaluated. Eight ruminally and duodenally cannulated cows (72 ± 8 DIM; mean ± SD) were used in a duplicated 4 × 4 Latin square design experiment with a 2 × 2 factorial arrangement of treatments. Treatments were corn grain endosperm type, floury or vitreous, and corn silage type, *bm3* or isogenic control. Diets contained 26% NDF and 30% starch. Floury endosperm grain decreased DMI 1.9 kg/d compared to vitreous grain when combined with control corn silage but did not affect DMI when combined with *bm3* corn silage. This interaction of treatments occurred because of changes in meal size; floury endosperm grain decreased meal size in control silage diets but increased meal size in *bm3* corn silage diets. Ruminal pool sizes reflected DMI differences among diets, suggesting that ruminal fill was not the primary limitation on intake. Brown midrib 3 corn silage reduced rumination time per day and number of rumination bouts per day. Floury endosperm grain decreased 3.5% FCM 1.2 kg/d when combined with control silage but increased 3.5% FCM 2.1 kg/d when combined with *bm3* corn silage. Starch and fiber digestibility interact to affect feeding behavior and

milk production; additionally, production response to *bm3* corn silage might depend on the grain source that is fed.

INTRODUCTION

Energy intake often limits milk yield for high-producing dairy cattle, and strategies to maximize energy intake are critical to maximize production. Although diets are often formulated based on nutrient concentration alone, an analysis of the literature determined that one unit increase in NDF digestibility is related to increases in DMI of 0.17 kg/d and 4% FCM of 0.25 kg/d (Oba and Allen, 1999b). The brown midrib 3 (***bm3***) mutation in corn silage decreases lignin content and increases in vitro NDF digestibility of forages (Cherney et al., 1991). Feeding brown midrib hybrids has improved DMI and milk production in dairy cows (Grant et al., 1995; Oba and Allen, 2000a), and increases in DMI are a result of changes in meal patterns (Oba and Allen, 2000a). Brown midrib hybrids can affect DMI by changing meal patterns, but how interactions of *bm3* corn silage and other dietary components affect feeding behavior are unknown.

Variation in starch digestibility is often not considered in diet formulation. Ruminal starch digestibility for a variety of feedstuffs ranged from 42 to 96% (Nocek and Tamminga, 1991). Starch digestibility is dependent on several factors, including grain type, processing methods, and physical characteristics of the grain. Starch granules in vitreous or flinty endosperm are surrounded by an insoluble protein matrix that resists digestion; in contrast, floury or opaque endosperm has a soluble protein matrix that is easily digested by ruminal microorganisms (Kotarski et al., 1992). Grain vitreousness is dependent on both hybrid and maturity (Philippeau and Michalet-Doreau, 1997), but is

negatively (r^2 = -0.86) correlated with in situ ruminal starch disappearance across hybrids (Philippeau et al., 1999b). Flint endosperm decreased ruminal starch digestibility 26 percentage units (34.8 vs. 60.8, $P < 0.001$) compared to a dent genotype when fed to steers (Philippeau et al., 1999a).

Increased ruminal starch degradability has significantly depressed DMI in some studies but not others (Allen, 2000). Feed intake is a function of both meal size and meal frequency, determined by satiety and hunger, respectively (Allen, 2000), so meal patterns might be influenced by ruminal starch digestion. Indeed, diets high in ruminally degraded starch depressed DMI by decreasing meal size (Oba and Allen, 2003a), and linear addition of refined corn starch to the diet linearly decreased meal length but tended to increase the number of meals (Krause et al., 2003). Because endosperm type can dramatically change ruminal starch digestibility, research is needed to specifically examine the effect of corn endosperm type on feeding behavior of lactating dairy cows.

Meal patterns and DMI are affected by both changes in ruminal starch and fiber digestibility. The interactions between varying digestibility of fiber source and endosperm type of corn grain on intake and production of dairy cows have not been investigated. We hypothesized that interactions exist between fiber and starch fermentability of diets. Highly fermentable grain would have greater effect at reducing meal size and possibly DMI when combined with *bm3* corn silage than control. The objective of this experiment was to evaluate effects of the brown midrib 3 mutation in corn silage and corn grain endosperm type on feeding behavior, DMI, productivity, and energy balance of lactating dairy cows.

MATERIALS AND METHODS

Cows and Treatments

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (72 ± 8 DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to duplicate 4 × 4 Latin squares balanced for carry-over effects. Cows were randomly assigned to treatment sequence. A 2 × 2 factorial arrangement of treatments was used with main effects of corn grain endosperm type (floury or vitreous) and *bm3* mutation (present or absent). Treatment periods were 21 d, consisting of an 11 d diet adaptation period followed by 10 d of collection. Surgical preparation of ruminally and duodenally cannulated cows is reported in Chapter 2. At the beginning of the experiment, empty body weight (ruminal digesta removed) of cows was 531.8 ± 43.9 kg (mean ± SD).

Two corn hybrids, 6208FQ and 657 (Cargill Hybrid Seeds, Minneapolis, MN), were planted for silage in Spring of 2001 at the Michigan State University Research Farm. The hybrids are isogenic except that Cargill 657 contains the *bm3* mutation. Harvesting conditions of both silages were reported in Chapter 2. Nutrient compositions and physical characteristics of treatment corn silages used in the experiment are reported in Table 1 of Chapter 2.

Two corn hybrids (1 floury and 1 vitreous) were planted for grain in Spring of 2001 at the Michigan State University Research Farm. The floury and vitreous hybrids, SL53 (Crow's Hybrid Corn Company, Kentland, IN) and Z75W (Wilson Genetics,

Harlan, IA) were selected based upon their high and low *in vitro* starch digestibility, respectively. Harvesting conditions of both corn grains were reported in Chapter 2. Vitreousness (% of endosperm) for floury and vitreous corn grain was 3.0 and 67.2%, respectively (Chapter 2). Nutrient compositions and physical characteristics of the corn grain treatments used in the experiment are shown in Table 2 of Chapter 2.

Experimental diets contained dry ground corn treatments (floury or vitreous), corn silage (*bm3* or isogenic normal), alfalfa silage (10% of diet dry matter), whole linted cottonseed (7% of diet dry matter), a protein supplement premix (soybean meal, distillers grains, and blood meal), and a premix of minerals and vitamins. Experimental diets were fed as total mixed rations, and were formulated to contain 27% NDF, 18% crude protein with adequate metabolizable protein, and minerals and vitamins to requirements. Ingredient and nutrient compositions of the experimental diets are reported in Table 3 of Chapter 2.

Data and Sample Collection

Cows were housed and fed as described previously (Chapter 2). Cows were milked twice daily in their stalls during the feeding behavior monitoring phase (d 15-18) and in a milking parlor during the remainder of each period. Milk yield was measured and milk was sampled at each milking on d 15-18. Empty body weight was measured after evacuation of ruminal digesta on the day immediately prior to the start of the first period, and on d 21 of each period. Body condition score (**BCS**) was determined on the same day by three trained investigators blinded to treatments (Wildman et al., 1982; five-point scale where 1 = thin and 5 = fat).

Feeding behavior was monitored from d 15 through d 18 (96 h) of each period by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, and water consumption were recorded for each cow every 5 sec. When chewing equipment malfunctioned for an individual cow during a 24-h period (1100h to 1100h), chewing behavior was deleted for that cow during that 24-h period. The system successfully collected 79.0% of the total chewing behavior data (average 3.1 d per cow per period).

Blood was collected from a coccygeal vessel into two evacuated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), one containing sodium heparin and another containing potassium oxalate and sodium fluoride. Samples were collected every 9 hours from d 12-14, starting at 1400 h on d 12, so that samples represented 3-h intervals of a 24-hour period in order to account for diurnal variation. Blood was centrifuged at 2,000 x *g* for 15 min immediately after sample collection, and plasma was harvested and frozen at -20°C until analysis.

Ruminal contents were evacuated manually through the ruminal cannula at 1500 h (4 h after feeding) on d 20 and at 0900 h (2 h before feeding) on d 21 of each period. Total ruminal content mass and volume were determined. During evacuation, 10% aliquots of digesta were separated to allow accurate sampling. Aliquots were squeezed through a nylon screen (1 mm pore size) to separate into primarily solid and liquid phases. Samples were taken from both phases for determination of nutrient pool size, and an additional liquid sample was taken to measure VFA concentration. All samples were frozen immediately at –20°C.

Sample and Statistical Analysis

Diet ingredients and orts were dried and ground as described previously (Chapter 2). Rumen liquid and solid sub-samples were lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY), ground, and recombined according to the original ratio of solid and liquid DM. All dried samples were analyzed for DM, ash, NDF, indigestible NDF (**iNDF**), potentially digestible NDF (**pdNDF;** 1- iNDF), CP, and starch as described in Chapter 2.

A commercial kit was used to determine plasma concentration of insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA), pancreatic glucagon (Linco Research, Inc., St. Charles, MO), glucose (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), NEFA (NEFA C-kit; Wako Chemicals USA, Richmond, VA), and β-hydroxybutyrate (BHBA; Procedure No. 2440; Stanbio Laboratory, Boerne, TX). Milk samples were analyzed for fat, true protein, and lactose with infrared spectroscopy and urea nitrogen by chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments, Chaska, MN) by Michigan DHIA (East Lansing).

For analysis of feeding and chewing behavior data, daily means were calculated for number of meal bouts per day, interval between meals, meal size, eating time, ruminating time, and total chewing time. Daily means for each response variable were averaged over the number of successful collection days for each period, and for statistical analysis were weighted according to the number of successful collection days.

Energy values for net energy for lactation (NE_L) and net energy for maintenance (NE_M) were calculated as follows:

NE_L intake = DMI (kg) x (0.0245 x TDN %) (NRC, 1989)

DM digestibility for calculation of TDN % was measured as reported in Chapter 2

Milk NE_L (Mcal/kg) = 0.0929 x (Fat %) + 0.0563 x (True Protein%) + 0.0395 x (Lactose%) (NRC, 2001);

$NE_M = 0.080 \times BW^{0.75}$ (NRC, 2001); and

NE_L balance = NE_L intake – NE_M – NE_L (Mcal/d).

Ruminal pool sizes (kg) of OM, NDF, iNDF, and starch were determined by multiplying the concentration of each component in DM by the ruminal digesta DM weight (kg).

All data was analyzed using the fit model procedure of JMP® (Version 4, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + PT_{jk} + e_{ijkl}$$

where

μ = overall mean,

C_i = random effect of cow (j = 1 to 8),

P_j = fixed effect of period (k = 1 to 4),

T_k = fixed effect of treatment (l = 1 to 4),

PT_{jk} =interaction of period and treatment, and

e_{ijkl} = residual

A reduced model without period × treatment interactions was used when this effect was not significant ($P > 0.15$). Orthogonal contrasts were used to determine main effect of corn silage type, main effect of corn grain type, and the interaction of corn silage

type and endosperm type of corn grain. Pearson correlation coefficients were determined between cow-period observations for some parameters. Main treatment effects and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$. Interactions between treatments were declared significant at $P < 0.10$, and tendencies were declared at $P < 0.15$.

Data from one cow was removed from all statistical analysis due to clinical mastitis during the first period. This cow was replaced with a spare animal for the remaining three periods. Another cow developed pneumonia during the diet adaptation sub-period of period 2, and was recovering during the first day of the collection period. Data from this cow was omitted from the digestibility sub-period of period 2, but was utilized from the feeding behavior sub-period as she had recovered sufficiently.

RESULTS AND DISCUSSION

DMI and Meal Patterns

A significant interaction of main effects was detected for DMI ($P < 0.07$; Table 1). Specifically, endosperm type had no effect on DMI within *bm3* corn silage diets, but within control corn silage diets, floury endosperm grain decreased intake 1.9 kg/d compared to vitreous grain. This interaction of treatments was caused by changes in meal intake patterns; floury endosperm grain decreased DM meal size 0.29 kg compared to vitreous corn when combined with control corn silage, but had the opposite effect, increasing DM meal size 0.20 kg compared to vitreous grain for diets containing *bm3* corn silage. Diets containing vitreous corn grain and *bm3* silage did not reduce DMI

because cows consuming *bm3* silage tended to eat a greater number of meals per day (10.6 vs. 11.7, $P < 0.10$). Control of meal size and frequency can be affected by numerous factors related to physical distension, metabolism of fuels, or hormonal effects. Because diets were low in NDF and ruminal pool size responded in a similar fashion to DMI (Table 3), physical factors were probably not limiting DMI. Therefore, the focus of this discussion will be on metabolic and hormonal factors limiting DMI.

Increased ruminal starch degradability has been shown to decrease DMI in some but not all cases (Allen, 2000). Inconsistent effects of ruminal starch fermentation on DMI might be the result of temporal pattern of fuel supply relative to the rate of utilization for milk yield and body tissues. Oxidation of variety of metabolic fuels in the liver has been shown to depress feed intake (Langhans, 1996). Because a primary fuel utilized by ruminants for gluconeogenesis is propionate, this VFA has garnered significant attention. Infusions of propionate into the rumen linearly decreased DMI and DM meal size in lactating cows, and extent of hypophagia induced by propionate infusion was positively related plasma glucose concentration (Oba and Allen, 2003b). Oba and Allen (2003b) speculated that propionate from rapid ruminal fermentation might be oxidized in the liver, causing satiety in animals that are not able to utilize propionate for gluconeogenesis. As milk production increases, increased glucose demand should result in decreased plasma glucose concentrations. In the present experiment, plasma glucose was also negatively correlated with DM meal size across cow period means ($r^2 = -0.37$; $P < 0.04$). Oxidation of metabolic fuels may explain meal size differences within floury endosperm diets. In diets with floury corn grain, rapid fermentation of starch probably occurred, resulting in greater influx of metabolic fuels (including propionate) during

meals; floury endosperm increased ruminal propionate concentration ($P < 0.001$; Chapter 4). Because milk production (and glucose demand) was not increased in diets containing floury endosperm and control corn silage, these fuels might have been oxidized in the liver and reduced meal size. Conversely, in floury endosperm, *bm3* corn silage diets, milk production and glucose demand were increased, and fuels were possibly directed toward gluconeogenesis, thus reducing oxidation of fuels and prolonging meal size.

Although vitreous corn, when combined with *bm3* silage, reduced DM meal size, meal frequency tended to increase to compensate. Oba and Allen (2000a) reported that cows consuming *bm3* corn silage ate smaller meals more frequently when compared to control corn silage in low (29%) NDF diets, and speculated that more rapid VFA absorption might decrease meal size but increase feeding frequency. Although valerate absorption rate (a measure of VFA absorption rate) was not different among treatments, acetate: propionate ratio was greater in vitreous diets containing control silage versus *bm3* silage (Chapter 4). Infusions of propionate reduced meal size and length to a greater extent than equimolar infusions of acetate or sodium chloride, indicating hypophagic effects of propionate on feeding behavior were because of the substrate and not osmolality (Choi and Allen, 1999). Greater ratio of ruminal acetate to propionate in diets containing vitreous corn grain with control versus *bm3* corn silage might indicate that less propionate was produced, which may have increased meal size. Additionally, insulin: glucagon ratio tended to be higher for vitreous grain ($P < 0.07$; Table 6), primarily because the diet containing vitreous corn grain and *bm3* silage increased plasma insulin and reduced glucagon concentrations compared to other treatments. Higher plasma insulin: glucagon ratio is expected to result in increased oxidation of fuels in the

liver (Watford and Goodridge, 2000), and if more propionate were available in this diet because of the lower ruminal acetate: propionate ratio, increased oxidation of propionate may have resulted in satiety sooner and smaller meal size. It must be stressed that oxidation of metabolic fuels is a temporal effect, and although it may decrease meal size, meal frequency may increase to compensate, as appears to have occurred for vitreous corn grain, *bm3* corn silage diets.

In addition to oxidation of metabolic fuels, gut peptides could have influenced meal patterns because of the significant changes in site of starch digestion (Chapter 2). Intraduodenal glucose infusion strongly elicited increases in plasma insulin, gastric inhibitory polypeptide (**GIP**) and glucagon-like peptide-1 (**GLP-1**) in humans (Lavin et al., 1998), and although GIP and GLP-1 appear to play a role in signaling satiety (Havel, 2001), this has not been definitively established, and little work has been conducted on the role of gut peptides in regulation of DMI for ruminants. Understanding how feed intake is controlled is difficult because many factors act synergistically to control meal size and frequency, and predicting feed intake precisely may never be possible for this reason. Additionally, dietary factors can interact to affect meal size and frequency without changing total DMI, which is why it is important to investigate the effects of diets on feeding behavior rather than just DMI to understand the physiology of intake regulation.

Chewing Activity

Chewing time (total and eating) was similar between endosperm types in control corn silage, but floury endosperm grain tended to increase chewing time (total and eating)

versus vitreous grain in *bm3* diets (interaction $P < 0.12$ and $P < 0.03$, respectively; Table 2). These interactions occurred because of the interaction of main treatment effects for DMI combined with greater chewing for the floury corn grain and control corn silage diet. Greater chewing for the diet containing floury grain and control silage also resulted in treatment effects of endosperm type on chewing (total, eating, and ruminating) per kg DMI. Although rate of starch digestion was highest in this diet (Chapter 2), cause and effect is not apparent. Ruminating time per day was positively related to rate of starch digestion across cow period means ($r^2 = 0.37$; $P < 0.05$). Greater mastication increased starch digestion rate of several cereals (Beauchemin et al., 1994), and rate of starch digestion is faster as corn particle size decreases (Callison et al., 2001). Greatest rate of starch digestion in the diet containing floury endosperm grain and control silage could be related to greater chewing per kg DMI; however, the cause of greater chewing is unknown.

Total ruminating time per day, rumination bout length, and ruminating chews per bout and per day were greater for diets containing control versus *bm3* corn silage (Table 2). These results suggest that *bm3* silage may not be as effective as control corn silage in stimulating rumination. In contrast, Oba and Allen (2000b) reported ruminating time per day was not different between *bm3* and control corn silages, and concluded that *bm3* corn silage was as effective as normal corn silage in stimulating chewing activity. Similar to our study, brown midrib sorghum silage tended to decrease chewing per kg NDF intake when compared to normal sorghum silage (Grant et al., 1995). Enhanced fiber degradability and increased particle fragility of brown midrib hybrids could cause brown midrib silages to be slightly less effective at stimulating chewing than normal corn

silages. Brown midrib 3 corn silage stimulated chewing as well as control in the experiment by Oba and Allen (2000b) but not this experiment, possibly because 30-h in vitro NDF digestibility of the *bm3* hybrid used in this study was 10.7 units higher than the *bm3* hybrid used in the study by Oba and Allen (2000a). Because *bm3* hybrids might not be quite as effective at stimulating rumination as some other forages, it may be advisable to increase forage NDF concentration when formulating diets containing *bm3* hybrids.

Ruminal Nutrient Pools

Significant interactions of main treatment effects were detected for ruminal pools of DM ($P < 0.03$) and OM ($P < 0.03$; Table 3). Similar to DMI, floury endosperm decreased DM and OM pool size when combined with control silage but increased pool sizes when in *bm3* silage diets. Significant interactions of treatments for ruminal pools of NDF ($P < 0.03$), pdNDF ($P < 0.08$), and iNDF ($P < 0.02$) were also detected. These pools reflected DMI differences among diets, but also indicate greater pool sizes of pdNDF and less iNDF in diets containing *bm3* silage. The ability of the pool size to reflect changes in DMI indicates that intake was not likely limited by ruminal fill in these animals. Starch ruminal pool size tended ($P < 0.07$) to be greater for vitreous endosperm versus floury, because ruminal turnover rate of starch was numerically greater for floury versus vitreous corn (Chapter 2). Wet weight, volume, and density of ruminal contents did not differ among treatments.

Milk Yield and Composition

In control corn silage diets, floury corn grain decreased 3.5% FCM 1.2 kg/d, but increased FCM by 2.1 kg/d when combined with *bm3* corn silage ($P < 0.10$; Table 4). A similar trend occurred for both milk yield ($P < 0.22$) and milk fat % ($P < 0.11$). Milk fat % was similar between diets containing floury grain regardless of silage type, but compared to floury grain, vitreous endosperm corn grain tended to increase milk fat % in control diets but decrease milk fat % in *bm3* silage diets. Milk fat % was positively correlated with ruminal acetate: propionate ratio ($r^2 = 0.67$; $P < 0.0001$); as ruminal acetate concentrations increased, greater substrate availability might have increased fatty acid synthesis in the mammary gland. Protein, lactose, and SNF composition of milk were not affected by any treatment. Although a significant main effect of silage ($P < 0.005$) was detected for milk urea nitrogen (**MUN**), differences were small (< 1.5 mg/dl).

Several studies document increased milk production when brown midrib hybrids replace normal silage, probably due to increased DMI (Grant et al., 1995; Oba and Allen, 2000a; Tine et al., 2001). However, there have been inconsistent results regarding ruminal starch degradation and milk production. Plascencia and Zinn (1996) noted a 2.6 kg/d increase in milk yield when steam-flaked corn replaced dry rolled corn, probably because of a 1.1 kg increase in DMI. Replacing corn with steam rolled barley decreased DMI 3.2 kg/d and milk yield by 4.3 kg/d (Overton et al., 1995). In contrast, changing apparent ruminal NSC digestibility 38.2 units had no effect on either DMI or milk production (Callison et al., 2001). Increased ruminal starch digestibility usually decreases milk yield in cases where DMI is depressed. In this experiment, differences in milk production reflect patterns of feeding behavior and DMI. Floury endosperm grain

decreased DMI by decreasing meal size when combined with control silage, but when combined with *bm3* corn silage did not decrease DMI because fuels from rapid ruminal starch fermentation were probably utilized for increased milk yield.

Energy Balance and Plasma Metabolites

An interaction of treatments was detected ($P < 0.08$) for NE_L intake (Table 5); floury corn grain decreased NE_L intake 0.8 Mcal/d in control silage diets but increased it 4.3 Mcal/d in *bm3* silage diets. This interaction reflects the changes in DMI observed but to a greater extent because it accounts for greater digestibility of corn grain with floury endosperm. Milk NE_L (Mcal/kg) was increased for grain with vitreous endosperm versus floury with control silage but decreased when combined with *bm3* corn silage (interaction $P < 0.05$) because of the similar treatment effects on milk fat yield. A tendency for an interaction of treatments was detected for NE_L balance ($P < 0.15$). NE_L balance was similar between endosperm types in control silage diets, but was greater for floury endosperm than vitreous within *bm3* silage diets.

Vitreous endosperm corn grain tended to increase partitioning of NE_L intake toward milk NE_L compared to floury endosperm grain ($P < 0.10$; Table 5). Insulin: glucagon ratio tended to be higher for cows consuming vitreous diets ($P < 0.07$; Table 6) because vitreous endosperm numerically increased plasma insulin concentration. Greater plasma insulin concentration might be expected to increase intake NE_L partitioned toward tissue retention and not milk NE_L, but other factors influence energy partitioning, including substrate availability and metabolic hormone secretion. Additionally, because these samples represent only every 3-hr over a 24-hr day but metabolic hormones act in a

much shorter time frame, these data can still be accurate in representing basal hormonal profiles, but pulsatility of insulin and glucagon plays a significant role in whether glucose is used toward tissue energy retention versus milk production. Because more frequent sampling is required to identify insulin and glucagon peaks and nadirs, this study cannot examine how endosperm type affects pulsatility, but there is evidence that differences between hybrids might exist. More research is needed to examine the interactions of starch and fiber digestibility on hormone pulsatility and energy balance.

CONCLUSIONS

Endosperm hybrid and *bm3* mutation in corn silage interact to affect DMI; floury endosperm grain decreased meal length and size in control silage diets, but increased meal length and size in *bm3* silage diets. Total DMI was not reduced in diets containing vitreous corn grain and *bm3* corn silage because *bm3* silage tended to increase meal frequency per day compared to control corn silage. Ruminal pool sizes reflected treatment effects on DMI, indicating that DMI was probably not limited by physical fill. Differences in feeding behavior could have been caused by a number of regulatory factors including, but not limited to, temporal supply of nutrients, oxidation of metabolic fuels, gut peptides, and metabolic hormone profiles. Changing diet fermentability affects milk production primarily by affecting DMI. Additionally, production responses to *bm3* corn silage are probably dependent on grain source. Starch and fiber fermentability can interact to affect feeding patterns and production, but animal response to changes in diet fermentability is complex and predicting how animals will respond is unlikely.

Table 1. Effects of corn grain endosperm type and brown midrib 3 corn silage on feeding behavior.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
DMI, kg/d	23.6	25.5	25.2	24.9	1.0	0.40	0.18	0.07
Meal bouts, #/d	10.8	10.4	11.2	12.1	0.8	0.10	0.74	0.31
Meal length, min	28.0	28.4	27.0	24.0	1.3	0.02	0.23	0.12
DM meal size, kg	2.18	2.47	2.25	2.05	0.13	0.11	0.67	0.03
Intermeal interval, min	95.5	98.0	90.3	93.1	6.9	0.32	0.59	0.97

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

Table 2. Effects of corn grain endosperm type and brown midrib 3 corn silage on chewing behavior.

	Control		*bm3*			P		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Chewing time, min/d								
Total	755	745	728	680	20.3	0.001	0.02	0.12
Eating	259	258	266	242	6.4	0.36	0.02	0.03
Ruminating	496	486	462	438	20.9	0.002	0.16	0.53
Total Chews								
/ kg DMI	2241	2006	1984	1961	95	0.002	0.005	0.02
/ d	50,796	50,432	48,316	45,649	1446	0.002	0.15	0.26
Eating chews								
/ meal	1907	1958	1828	1650	111	0.08	0.56	0.30
/ kg DMI	880	802	816	801	44	0.14	0.05	0.16
/ d	19,939	19,886	19,966	18,660	782	0.30	0.25	0.28
Ruminating chews								
/ bout	2218	2073	1988	1945	177	0.03	0.23	0.51
/ kg DMI	1361	1204	1168	1160	84	0.006	0.04	0.06
/ d	30,854	30,375	28,344	26,981	1584	0.001	0.25	0.57
Ruminating								
Bouts, #/d	14.3	14.9	14.5	14.3	0.6	0.62	0.63	0.28
Bout length, min	36.1	33.4	32.8	32.0	2.5	0.03	0.11	0.39
Interval, min	65.2	62.1	63.4	62.1	2.9	0.74	0.40	0.74
Water Intake, L/d	94.4	90.5	92.9	92.0	4.2	0.99	0.33	0.53

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

Table 3. Effects of corn grain endosperm type and brown midrib 3 corn silage on ruminal pools.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Wet weight, kg	75.0	83.0	74.9	76.4	3.7	0.27	0.12	0.28
Volume, L	89.1	96.9	87.3	90.0	4.9	0.28	0.20	0.53
Density, kg/L	0.85	0.86	0.84	0.85	0.02	0.58	0.54	0.93
Rumen pool, kg								
DM	10.2	12.3	11.6	10.9	0.68	0.95	0.20	0.02
OM	9.37	11.18	10.62	9.92	0.66	0.99	0.29	0.03
NDF	5.41	6.34	5.95	5.37	0.43	0.49	0.59	0.03
pdNDF[4]	2.75	3.16	3.75	3.44	0.27	0.004	0.78	0.08
iNDF[5]	2.67	3.18	2.20	1.93	0.19	<.0001	0.42	0.02
Starch	0.74	0.95	0.89	0.98	0.08	0.24	0.07	0.42

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.
[4] Potentially digestible NDF.
[5] Indigestible NDF.

Table 4. Effects of corn grain endosperm type and brown midrib 3 corn silage on milk yield and components.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Yield, kg/d								
Raw milk[4]	39.8	40.6	42.5	40.6	2.1	0.21	0.59	0.22
3.5 % FCM	39.7	40.9	42.2	40.1	2.2	0.40	0.62	0.10
Fat	1.40	1.45	1.48	1.40	0.09	0.69	0.69	0.07
Protein	1.18	1.20	1.28	1.19	0.07	0.28	0.44	0.19
Lactose	1.95	1.99	2.09	1.98	0.11	0.22	0.58	0.20
SNF	3.50	3.57	3.78	3.56	0.19	0.23	0.52	0.21
MUN, mg/dl	16.5	16.6	15.0	15.8	0.58	0.005	0.24	0.29
Composition, %								
Fat	3.51	3.62	3.49	3.44	0.20	0.04	0.51	0.11
Protein	2.95	2.95	3.00	2.93	0.05	0.50	0.24	0.28
Lactose	4.88	4.89	4.90	4.88	0.04	0.71	0.78	0.18
SNF	8.79	8.80	8.87	8.77	0.08	0.52	0.30	0.19
3.5% FCM / DMI	1.68	1.61	1.67	1.67	0.05	0.20	0.08	0.11

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.
[4] Milk yield not measured during digestibility and rumen evacuation sub-periods.

Table 5. Effects of corn grain endosperm type and brown midrib 3 corn silage on energy balance.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
BW change, kg/21 d	12.7	12.3	16.9	12.1	4.4	0.62	0.51	0.58
BCS change, /21 d	0.04	0.12	0.14	0.32	0.06		*	
NE_L intake, Mcal/d	42.9	43.7	46.1	41.8	1.6	0.65	0.22	0.08
Milk NE_L								
Mcal/kg	0.68	0.70	0.69	0.68	0.02	0.09	0.87	0.05
Mcal/d	27.3	28.0	29.1	27.5	1.5	0.37	0.54	0.11
NE_L balance, Mcal/d	5.46	5.26	6.79	3.11	1.2	0.73	0.11	0.15
Milk NE_L, % of Intake	63.5	64.4	62.9	68.5	2.6	0.36	0.10	0.22

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.
* Significant ($P < 0.15$) period by treatment interaction.

Table 6. Effects of corn grain endosperm type and brown midrib 3 corn silage on plasma metabolite and hormone concentrations.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Glucose, mg/dl	59.6	58.3	58.8	58.6	0.92	0.62	0.16	0.32
Insulin, μIU/mL	13.6	13.7	12.4	14.7	1.5	0.90	0.24	0.26
Glucagon, pg/ml	120	119	118	115	9.7	0.50	0.54	0.84
Insulin: Glucagon	0.11	0.12	0.10	0.13	0.01	0.75	0.07	0.18
NEFA, μeq/L	57.0	58.9	55.2	67.7	4.3		*	
β-hydroxybutyrate, mg/dl	6.47	6.77	6.46	6.72	0.39	0.91	0.31	0.94

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.
* Significant ($P < 0.15$) period by treatment interaction.

CHAPTER 4

Interactions of corn grain endosperm type and brown midrib 3 corn silage on ruminal fermentation and microbial efficiency in lactating dairy cows

ABSTRACT

Interactions of endosperm type of corn grain and the brown midrib 3 mutation in corn silage on ruminal fermentation and microbial efficiency of lactating dairy cows were evaluated. Eight ruminally and duodenally cannulated cows (72 ± 8 DIM; mean ± SD) were used in a duplicated 4 × 4 Latin square design experiment with a 2 × 2 factorial arrangement of treatments. Treatments were corn grain endosperm type, floury or vitreous, and corn silage type, *bm3* or isogenic normal. Diets contained 26% NDF and 30% starch. Increasing ruminal starch digestibility by replacing vitreous corn grain with floury grain reduced mean and minimum ruminal pH. Brown midrib 3 corn silage reduced mean and minimum ruminal pH and increased total VFA concentration. Ruminal pH was positively associated with rate of valerate absorption. Although floury endosperm reduced acetate: propionate ratio in both control and *bm3* corn silage diets, it had a greater effect on reducing acetate: propionate ratio for control silage compared to *bm3* corn silage. Non-ammonia N (**NAN**) flow to the duodenum did not differ among treatments, and because variability was high, differences were not significant for microbial N and non-ammonia, non-microbial N (**NANMN**) flow. More research is

needed to determine how starch and fiber digestibility interact to affect microbial protein production and efficiency.

INTRODUCTION

Maximum milk production is commonly limited in high producing animals by both energy and metabolizable protein supply. Metabolizable protein is derived from microbial protein, feed protein that bypasses ruminal digestion, and endogenous proteins. Of these sources, microbial protein produced in the rumen is the primary source of amino acids to the cow. Microbes are a high quality, highly digestible protein source, yet predicting microbial protein flow to the duodenum is difficult. Increasing diet digestibility has the potential to increase microbial growth, which is often limited by energy availability (Clark et al., 1992). However, diet fermentability can have significant impact on the efficiency by which microbes use energy from ruminally fermented feed toward growth (defined as g microbial N flowing to the duodenum per kg OM truly ruminally fermented [**TRDOM**]). Predicting how diet fermentability will affect microbial N efficiency (**MNE**) is difficult because factors such as pH, retention time, and energy and nitrogen availability all interact to change the efficiency of microbial growth (Clark et al., 1992; Russell, 1998).

Increasing ruminal starch digestibility of high starch diets has inconsistent effects on MNE; greater ruminally degraded starch increased (Plascencia and Zinn, 1996), decreased (Oba and Allen, 2003c), and did not affect MNE (Overton et al., 1995). Microbial efficiency might be increased as ruminal starch digestibility increases if energy availability is limiting microbial growth. However, when fermentation occurs faster than the ATP produced can be used toward cellular growth, MNE can be reduced if ATP is

used toward non-growth functions in energy spilling or uncoupling reactions (Russell, 1998). Energy spilling can occur in situations of low ruminal pH (Stroebel and Russell, 1986) and lack of available N (Van Kessel and Russell, 1996).

Microbial efficiency might be reduced in diets with long ruminal retention times. As retention time increases, microbial autolysis and predation by protozoa are increased and MNE could be reduced (Wells and Russell, 1996). Despite a decrease in ruminal pH, greater MNE for brown midrib 3 (***bm3***) corn silage was attributed to a faster ruminal passage rate, which might have reduced microbial turnover (Oba and Allen, 2000c). Lower ruminal pH might have reduced MNE for *bm3* corn silage compared to control silage when DMI, and presumably rate of passage, were not affected by treatment (Greenfield et al., 2001). Because feeding *bm3* corn silage often reduces ruminal pH, its potential to increase MNE might depend on the extent to which ruminal passage rate is increased relative to the negative effects of low pH on MNE.

We hypothesized that corn grain with floury endosperm reduces MNE because of more rapid starch digestion, and might interact with corn silage type because *bm3* corn silage reduces MNE by low ruminal pH but increases MNE by increasing rate of ruminal passage. The objective of this experiment was to determine interactions of endosperm type of corn grain and the *bm3* mutation in corn silage on ruminal pH, VFA profile, and microbial N efficiency.

MATERIALS AND METHODS

Treatments and Cows

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (72 ± 8 DIM; mean ± SD) from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to duplicate 4 × 4 Latin squares balanced for carry-over effects. Cows were randomly assigned to treatment sequence. A 2 × 2 factorial arrangement of treatments was used with main effects of corn grain endosperm type (floury or vitreous) and *bm3* mutation (present or absent). Treatment periods were 21 d, consisting of an 11 d diet adaptation period followed by 10 d of collection. Surgical preparation of ruminally and duodenally cannulated cows is reported in Chapter 2. At the beginning of the experiment, empty body weight (ruminal digesta removed) of cows was 531.8 ± 43.9 kg (mean ± SD).

Two corn hybrids, 6208FQ and 657 (Cargill Hybrid Seeds, Minneapolis, MN), were planted for silage in Spring of 2001 at the Michigan State University Research Farm. The hybrids are isogenic except that Cargill 657 contains the *bm3* mutation. Harvesting conditions of both silages were reported in Chapter 2. Nutrient compositions and physical characteristics of treatment corn silages used in the experiment are reported in Table 1 of Chapter 2.

Two corn hybrids (1 floury and 1 vitreous) were planted for grain in Spring of 2001 at the Michigan State University Research Farm. The floury and vitreous hybrids, SL53 (Crow's Hybrid Corn Company, Kentland, IN) and Z75W (Wilson Genetics, Harlan, IA) were selected based upon their high and low *in vitro* starch digestibility,

respectively. Harvesting conditions of both corn grains were reported in Chapter 2. Vitreousness (% of endosperm) for floury and vitreous corn grain was 3.0 and 67.2%, respectively (Chapter 2). Nutrient compositions and physical characteristics of the corn grain treatments used in the experiment are shown in Table 2 of Chapter 2.

Experimental diets contained dry ground corn treatments (floury or vitreous), corn silage (*bm3* or isogenic normal), alfalfa silage (10% of diet dry matter), whole linted cottonseed (7% of diet dry matter), a protein supplement premix (soybean meal, distillers grains, and blood meal), and a premix of minerals and vitamins. Experimental diets were fed as total mixed rations, and were formulated to contain 27% NDF, 18% crude protein with adequate metabolizable protein, and minerals and vitamins to requirements. Ingredient and nutrient compositions of the experimental diets are reported in Table 3 of Chapter 2.

Data and Sample Collection

Cows were housed and fed as described previously (Chapter 2). Duodenal (1,000 g), fecal (500 g), and rumen fluid samples (100 mL) were collected every 9 h as previously described (Chapter 2). Digesta from 5 sites in the rumen was combined and strained to obtain rumen fluid for determination of VFA, lactate, and ammonia concentrations. Additional ruminal fluid (400 ml) was collected near the reticulo-omasal orifice at the same time as duodenal and fecal samples for the determination of microbial OM, starch, nitrogen, and purine concentrations. All digesta and fecal samples were immediately frozen at -20°C until processing.

Effect of treatment on relative rate of VFA absorption and rate of liquid passage was measured on d 19 using a pulse dose of valeric acid and cobalt EDTA (Allen et al., 2000). Valeric acid and cobalt EDTA was dosed two hours after feeding. Rumen fluid was sampled immediately before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 h after dosing. Samples were immediately frozen.

Ruminal pH was monitored from d 15 through d 18 (96 h) of each period by a computerized data acquisition system (Dado and Allen, 1993). Ruminal pH data were recorded for each cow every 5 sec. Electrodes for ruminal pH determination were checked daily and calibrated as needed, and ruminal pH data were deleted for the previous 24 h if readings had changed more than ± 0.05 unit at pH 7 or pH 4. The system successfully collected 58.9% of the total ruminal pH data (average 2.4 d per cow per period).

Sample and Statistical Analysis

Processing methods for diet ingredients, orts, digesta, and fecal samples were reported previously (Chapter 2). Microbial pellets were obtained from combined rumen fluid samples for each cow by differential centrifugation (Overton et al., 1995) and were lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY). Microbial pellets were ground with a mortar and pestle and analyzed for ash, CP, and starch as described in Chapter 2.

Rumen fluid was analyzed for concentration of VFA and lactate by HPLC (Waters Corp., Milford, MA) as described by Oba and Allen (2003a). Duodenal digesta was analyzed for purines and ammonia to estimate microbial N flow and non-ammonia

non-microbial N flow to the duodenum. Purine concentration was used as a microbial marker, and purine to microbial N ratio was estimated by analysis of microbial pellets. Total purines were measured by spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 260 nm (Zinn and Owens, 1986). Ammonia concentration was determined for centrifuged duodenal and rumen fluid samples according to Broderick and Kang (1980).

Rumen fluid samples collected to measure rate of valerate absorption were analyzed for valerate concentration by HPLC (Waters Corp., Milford, MA) and for Co concentration by atomic absorption spectrophotometry according to manufacturers specifications (SpectrAA 220/FS, Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia). Rates of valerate and cobalt disappearance were determined by non-linear regression of the decline in their respective concentrations in rumen fluid over time after dosing using a one-pool, first-order model after accounting for background (JMP® Version 4, SAS Institute, Cary, NC).

For analysis of pH data, daily means for pH mean, minimum, maximum, range, standard deviation, time below pH 6.0, 5.8, and 5.5, and area below pH 6.0, 5.8, and 5.5 were calculated. Daily means for each response variable were averaged over the number of successful collection days for each period and for statistical analysis were weighted according to the number of successful collection days.

All data was analyzed using the fit model procedure of JMP® (Version 4, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + T_k + PT_{jk} + e_{ijkl}$$

where

μ = overall mean,

C_i = random effect of cow (j = 1 to 8),

P_j = fixed effect of period (k = 1 to 4),

T_k = fixed effect of treatment (l = 1 to 4),

PT_{jk} =interaction of period and treatment, and

e_{ijkl} = residual

A reduced model without period × treatment interactions was used when this effect was not significant ($P > 0.15$). Orthogonal contrasts were used to determine main effect of corn silage type, main effect of corn grain type, and the interaction of corn silage type and endosperm type of corn grain. Pearson correlation coefficients were determined between cow-period observations for some parameters. Main treatment effects and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$. Interactions between treatments were declared significant at $P < 0.10$, and tendencies were declared at $P < 0.15$.

Data from one cow was removed from all statistical analysis due to clinical mastitis during the first period. This cow was replaced with a spare animal for the remaining three periods. Another cow developed pneumonia during the diet adaptation period of period 2, and was recovering during the first day of the collection period. Data from this cow was omitted from the digestibility sub-period of period 2, but was utilized from the feeding behavior sub-period as she had recovered sufficiently.

RESULTS AND DISCUSSION

Ruminal pH

No interactions of main treatment effects were observed for any measure of ruminal pH. Floury endosperm grain reduced mean and minimum ruminal pH ($P < 0.03$ and $P < 0.02$, respectively; Table 1), but pH maximum, range, and standard deviation were not affected by endosperm type. Floury endosperm grain tended to increase fraction of time spent under pH 6.0 compared to vitreous grain ($P < 0.09$). Greater ruminal starch (and OM) digestibility decreases ruminal pH by increasing fermentation acid production, and although ruminally fermented OM was negatively correlated to ruminal pH for a database of treatment means in the literature (Allen, 1997), effects of fermentable OM on ruminal pH have not been consistent. Replacing ground shelled corn with rolled barley increased ruminal starch digestibility from 41.9 to 74.4% but linearly decreased ruminal pH (Overton et al., 1995). In contrast, replacing dry corn with high moisture corn increased rate and extent of ruminal starch digestion but did not effect any measure of ruminal pH in another study (Oba and Allen, 2003b, c). Ruminal pH is a function of hydrogen ion production from fermentation and removal by absorption, passage, buffering, and neutralization. Ruminal pH decreases only when hydrogen ion production exceeds removal from the rumen and ruminal buffering capacity. Corn grain with floury endosperm increased ruminal starch and OM digestibility (Chapter 2) and hydrogen ion removal was not rapid enough to offset VFA production, resulting in lower ruminal pH.

Brown midrib 3 corn silage reduced mean and minimum ruminal pH compared to its isogenic control corn silage ($P < 0.03$ and $P < 0.04$, respectively). Brown midrib 3

corn silage tended to increase hours under pH 6.0 ($P < 0.09$), but other measures of ruminal pH did not differ between corn silage types. Lower ruminal pH for *bm3* corn silage treatments corresponded with higher total VFA concentration ($P < 0.03$; Table 2). Brown midrib 3 corn silage decreased ruminal pH in several experiments (Block et al., 1981; Oba and Allen, 2000a; Greenfield et al., 2001). Total DMI was not affected by *bm3* corn silage, and although greater ruminally degraded OM might increase fermentation acid production and reduce ruminal pH, TRDOM was not affected by *bm3* corn silage treatment. Brown midrib 3 corn silage did not affect rate of valerate absorption (Table 2) or liquid passage rate (Chapter 2) compared to control silage, so treatment effects on removal of VFA by absorption or passage from the rumen did not occur. Cows fed diets containing *bm3* corn silage spent less time ruminating per day and chewed less during rumination per day than cows consuming control corn silage ($P < 0.002$ and $P < 0.001$; Chapter 3). Chewing stimulates saliva flow, which contains bicarbonate and phosphates that buffer and neutralize fermentation acids produced in the rumen (Allen, 1997). A negative relationship existed between mean ruminal pH and daily rumination time, but silage type also affected this relationship. Mean pH was negatively correlated with both rumination time for control corn silage ($r^2 = -0.55$; $P < 0.03$) and *bm3* corn silage ($r^2 = -0.58$; $P < 0.02$), and although the slopes were not different ($P = 0.59$), the intercept of the regression line was lower for *bm3* than control corn silage ($P = 0.02$). When rumination time is 450 minutes per day, cows consuming *bm3* corn silage had a mean ruminal pH of 5.99 while cows consuming control corn silage had a mean ruminal pH of 6.22. Brown midrib corn silage might reduce ruminal

pH both by stimulating rumination less effectively than control silage and reducing ruminal motility and removal of VFA from the rumen, as discussed below.

Rate of valerate absorption is used as a general measure of VFA absorption across the ruminal epithelium. Mean valerate absorption rate did not differ among treatments and averaged 44 % per h. Because VFA are absorbed across the rumen wall in the undissociated form, valerate absorption rate might be expected to increase as pH decreases. This was demonstrated using the washed rumen technique by Dijkstra et al. (1993); fractional absorption rates of propionate and butyrate increased as pH decreased from 7.2 to 4.5. In contrast, rate of valerate absorption was positively correlated with mean pH across cow period means in the present experiment ($r^2 = 0.53$; $P < 0.003$). A previous study from our laboratory also reported a positive relationship between rate of valerate absorption and mean pH (Voelker and Allen, 2003b). VFA absorption rate is dependent on several factors other than form of VFA, including blood flow, ruminal consistency, surface area, and ruminal motility. In particular, ruminal motility may play a significant role in rate of VFA absorption. Ruminal motility was not measured in this study, because most quantitative measurements of motility interfere with ruminal function. Although no relationship between valerate absorption rate and iNDF passage rate was detected across cow period means in the present experiment, these variables were positively correlated in a previous experiment ($r^2 = 0.43$; $P = 0.02$; Voelker and Allen, 2003b), which may indicate that VFA absorption rate increases as ruminal motility increases. As ruminal motility increases, VFA molecules are expected to be replenished at the rumen wall more rapidly, increasing the concentration gradient across the ruminal

epithelium and rate of VFA absorption. Reduction of ruminal pH may decrease ruminal motility and VFA absorption rate.

Across cow period means, valerate absorption rate was positively correlated with both 3.5% FCM ($r^2 = 0.39$; $P < 0.03$) and the proportion of intake energy used toward milk production (Milk NE_L, % of NE_L intake; $r^2 = 0.59$; $P < 0.001$). Greater partitioning of intake energy toward milk probably occurred because of dilution of maintenance energy with greater milk yield. The positive relationship between VFA absorption rate and milk yield might occur by either greater VFA supply or demand. Greater VFA absorption, perhaps by increased ruminal motility, might increase VFA available for production and milk yield. Conversely, as milk production increases, greater utilization of VFA for production could create a greater concentration gradient across the ruminal epithelium and thus increase VFA absorption rate. Although it is difficult to establish cause and effect between VFA absorption rate and milk production, these data suggest that understanding dynamics of rumen function and VFA absorption might provide practical methods to increase milk yield.

Ruminal Fermentation Acids

No interactions of main treatment effects occurred for concentration or profile of ruminal fermentation acids. Although floury corn grain treatment increased ruminal starch digestibility, there was no effect of endosperm type of corn grain on total VFA concentration. Compared to floury corn grain, vitreous corn grain tended to increase molar proportion of acetate more in diets with control corn silage than *bm3* corn silage (interaction $P < 0.13$; Table 2). Floury grain increased molar proportion of propionate

compared to vitreous corn grain ($P < 0.001$). An interaction of treatment effects occurred for ratio of acetate to propionate. Compared to vitreous grain, floury endosperm reduced acetate: propionate ratio to a greater extent in control corn silage than *bm3* corn silage (interaction $P < 0.07$). Additionally, *bm3* corn silage reduced acetate: propionate compared to control corn silage ($P < 0.05$). Increasing proportion of starch digested in the rumen often decreases the acetate: propionate ratio (Overton et al., 1995; Crocker et al., 1998). Lactate did not differ among treatments. Control silage increased and vitreous grain tended to increase branch chain VFA ($P < 0.007$ and $P < 0.08$; respectively), and although the main effect of silage type is strong, the differences among treatments are probably not enough to be biologically significant.

Nitrogen Flow

Nitrogen intake, ruminal ammonia N, and ammonia flow to the duodenum did not differ among treatments (Table 3). NAN flow to the duodenum was not different among treatments with a mean of 625 g/d and 91.6% of N intake across treatments. These values are high relative to previous reports in the literature, but DMI is also relatively high and NAN flow to the duodenum would increase with DMI. NAN flow was positively correlated with DMI across cow period means ($r^2 = 0.64$; $P < 0.0001$). This increase in NAN flow as DMI increased was not associated with greater passage of feed and endogenous N (NANMN) but rather with greater microbial N flow to the duodenum ($r^2 = 0.55$; $P < 0.002$). A review by Clark et al. (1992) reported the relationship between OM intake and passage of NAN as (NAN = 30.15 [OM intake] – 33.15 [$r^2 = 0.83$]).

Substituting our observed mean OM intake of 22.6 kg/d OM would predict NAN flow to be 648 g/d, which is consistent with mean NAN flow of 625 g/d observed in this study.

NANMN flow to the duodenum was not different among treatments, but the mean of 64.4 g/d seems very low. NANMN averaged only 10.1% of N intake and 11.3% of total duodenal NAN flow. High proteolytic activity in the rumen could decrease NANMN flow to the duodenum. Fulghum and Moore (1963) reported that 38% of isolated ruminal bacteria demonstrated proteolytic activity. Proteolytic species of bacteria in the rumen include *Ruminobacter amylophilus*, *Butyrivibrio fibrisolvens*, and *Prevotella ruminicola* (Wallace and Brammall, 1985). Proteolytic activity appears to be constitutively expressed in isolated ruminal bacteria and differences in proteolytic activity in response to diet is probably more related to changes in size and composition in the microbial population than enzyme activity per se (Wallace and Brammall, 1985). Indeed, as diets increase in cereal grain concentration, proteolytic and amylolytic activity increases because of proliferation of the amylolytic population of bacteria (Hristov et al., 2002). Additionally, although bacteria have a higher proteolytic activity than protozoa (Brock et al., 1982), protozoa can be more important in the fermentation and digestion of less soluble particulate proteins (Hino and Russell, 1987).

Microbial N flow was similar among endosperm types in *bm3* silage diets, but in control silage diets, vitreous corn tended to increase microbial N flow 185 g/d versus floury grain (interaction $P < 0.17$; Table 3). The proportion of duodenal NAN contributed by microbial N averaged 88.7% and is at the high end of the range of 34 to 89% reported in a review by Clark et al. (1992). Greater DMI increases DM passage rate, and because most ruminal microbes are attached to feed particles, greater DMI is

expected to increase microbial flow to the duodenum. In this experiment, microbial N flow tended to be positively correlated with iNDF passage rate ($r^2 = 0.34$; $P < 0.07$) but was not correlated with liquid passage rate ($r^2 = -0.12$; $P < 0.53$) across cow period means. Firkins et al. (1986) reported that microbial flow was highly correlated with fluid dilution rate and speculated that this was because small particles pass largely with the fluid phase, but that experiment was conducted with steers consuming low amounts of finely chopped hay. Similar to our experiment, Voelker and Allen (2003b) found microbial N flow in high producing dairy cows to be more related to passage of iNDF from the rumen rather than liquid passage. In animals consuming fibrous diets at 3 to 4 times maintenance energy, particulate passage rate may become more critical in determining microbial flow.

Microbial efficiency was numerically higher for diets containing vitreous corn grain (41.3 versus 34.5 g/kg TRDOM; $P < 0.12$). Floury endosperm corn grain might have reduced MNE by uncoupling growth from energy supply. Floury corn grain reduced mean and minimum ruminal pH, which increases maintenance requirements of microbes to maintain ion balance across the cell membrane (Russell, 1998). Across cow period means, MNE was negatively correlated with ruminal starch digestibility ($r^2 = -0.47$; $P < 0.01$) and positively correlated with mean ruminal pH ($r^2 = 0.53$; $P < 0.006$). Similar to our study, dent corn decreased microbial efficiency when compared to more vitreous flint corn in steers (Philippeau et al., 1999a). Greater ruminal starch digestion could reduce MNE by increasing substrate availability more than needed for maximum growth rate, increasing maintenance energy from low pH, or increasing cell lysis. MNE was positively related to rate of starch passage from the rumen across cow period means

($r^2 = 0.46$; $P < 0.01$). This relationship has been reported previously (Oba and Allen, 2003c; Voelker and Allen, 2003b), and is probably related to decreased microbial turnover with greater passage from the rumen (Wells and Russell, 1996). Greater MNE in vitreous grain diets could be the result of higher ruminal pH and faster passage rate of starch from the rumen.

NAN postruminally digested (g/d) tended to be less for floury versus vitreous corn grain in control silage diets but greater for floury versus vitreous grain in *bm3* corn silage diets (interaction $P < 0.12$). Amount of NAN digested postruminally reflects differences in DMI that occurred among diets, so postruminal NAN digestibility as a percent of NAN intake did not differ among treatments. Additionally, reductions in NANMN digestion were compensated for by increases in microbial N digestion so that NAN digestibility (% of N intake) did not differ. A significant interaction of treatment effects occurred for postruminal NAN digestibility (% of duodenal flow; interaction $P < 0.05$). Floury endosperm corn grain reduced postruminal NAN digestibility (% of duodenal flow) versus vitreous endosperm in control silage diets but increased postruminal NAN digestibility (% of duodenal flow) versus vitreous grain in *bm3* corn silage diets. Amount of N apparently digested in the total tract did not differ among treatments with a mean of 507 g/d. Vitreous corn grain tended to reduce apparent total tract N digestibility compared to floury endosperm grain (76.0 versus 73.6%; $P < 0.06$). Across cow period means, total tract N digestibility was negatively related with ruminal iNDF passage rate ($r^2 = -0.45$; $P < 0.01$). Greater rate of passage could increase substrate supply to the hindgut and might increase microbial growth and loss in the feces.

Additionally, limitations to N digestion may occur with greater passage rate because of reduced time for enzymatic hydrolysis and digestion.

Partitioning of Duodenal N

Based on analysis of the literature, partitioning of NAN flow into NANMN and microbial N fractions appears to be too low and high, respectively. Clark et al. (1992) determined relationships between OM intake, NANMN flow, and microbial N flow from multiple experiments reporting these variables. Using OM intake as an input into the prediction equations of Clark et al. (1992), NANMN flow in this experiment is predicted to be 301 g/d for cows consuming 22.6 kg/d OM, but our observed value averaged 64.4 g/d (Table 3). Extensive proteolysis by amylolytic bacteria could have reduced the flow of feed N, as discussed previously. However, it is surprising that animals with high DMI and adequate dietary protein (17.4%) would not have considerable NANMN flow to the duodenum. Conversely, microbial N appears to be very high in this study; observed mean microbial N flow was 564 g/d, whereas prediction equations based on OM intake (Clark et al., 1992) predicted microbial N flow to be 354 g/d for cows consuming 22.6 kg/d OM. Few studies exist reporting duodenally cannulated cows with high intakes and milk production, and because microbial N flow is correlated with OM intake ($r^2 = 0.62$; Clark et al., 1992), it is possible that very high microbial N flow occurred in this study.

Although values obtained for NANMN and microbial N flow are within the range reported in the literature (Clark et al., 1992), we do not have complete confidence in partitioning of N between NANMN and microbial N in this experiment. Partitioning duodenal NAN into microbial and non-microbial fractions is problematic.

Overestimation of microbial N to duodenal NAN flow could occur if feed N contamination increased microbial pellet N concentration and N: purine ratio. However, microscopic examination of microbial pellet did not reveal gross contamination by feed particles, and any feed N contamination would have to be exceptionally high in N concentration to significantly affect N: purine ratio, but few feeds have a higher N concentration than microbial protein. Loss of purines in microbial pellet samples but not duodenal digesta samples might overestimate microbial protein contribution to duodenal NAN flow. However, both sample types were lyophilized to minimize effects of heat, and purine analysis was conducted on microbial pellet samples at the same time as the corresponding duodenal digesta sample, so consistent loss of purine in microbial pellet samples but not duodenal samples is unlikely. Additionally, compounds in duodenal samples that absorb at 260 nm will cause overestimation of microbial N concentration in duodenal samples.

CONCLUSIONS

Floury endosperm grain reduced ruminal pH compared to vitreous corn by increasing ruminal fermentation of OM and starch compared to vitreous corn grain. Brown midrib 3 corn silage reduced ruminal pH and increased total VFA concentration compared to control corn silage, probably by reducing amount of neutralization and buffering of fermentation acids. Valerate absorption rate decreases as ruminal pH decreases, and could be the result of reduced ruminal motility. Low ruminal pH for floury endosperm might have reduced MNE by uncoupling energy fermentation from microbial cell

production, while greater passage rate of starch for vitreous grain increased MNE possibly because of reduced microbial turnover in the rumen. However, greater passage of substrate from the rumen in vitreous grain diets may have increased hindgut fermentation and reduced apparent total tract N digestibility.

Table 1. Effects of corn grain endosperm type and brown midrib 3 corn silage on ruminal pH.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Mean	6.01	6.23	5.92	6.01	0.09	0.03	0.03	0.37
Minimum	5.48	5.66	5.36	5.49	0.08	0.04	0.02	0.67
Maximum	6.56	6.74	6.53	6.55	0.07	0.12	0.14	0.22
Range	1.07	1.07	1.15	1.07	0.06	0.45	0.41	0.45
Standard deviation	0.29	0.29	0.32	0.29	0.02	0.42	0.24	0.37
Hours under								
pH 6.0	10.71	7.10	12.75	10.66	2.0	0.09	0.09	0.64
pH 5.8	6.97	4.67	8.01	6.85	1.8	0.27	0.24	0.70
pH 5.5	2.47	1.25	2.68	2.95	1.0	0.28	0.59	0.40
Area under the curve								
pH 6.0	411	257	473	434	114	0.21	0.30	0.54
pH 5.8	200	116	225	226	69	0.25	0.46	0.47
pH 5.5	36	18	41	53	19	0.22	0.85	0.35

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

Table 2. Effects of corn grain endosperm type and brown midrib 3 corn silage on ruminal VFA profiles and concentrations.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Total VFA, mmol	138	136	142	139	2.6	0.03	0.14	0.72
VFA, % of total VFA								
Acetate	53.6	56.3	53.5	54.6	1.2	0.07	0.001	0.13
Propionate	28.3	25.2	28.3	26.7	1.1	0.18	0.001	0.19
Butyrate	12.7	13.4	13.2	13.3	0.5	0.57	0.23	0.35
Branch chain VFA	2.64	2.75	2.31	2.53	0.2	0.007	0.08	0.55
Lactate	0.51	0.46	0.48	0.80	0.3	0.53	0.60	0.48
Acetate: Propionate	1.92	2.29	1.91	2.07	0.1	0.05	0.001	0.07
Rate of valerate absorption, %/h[4]	0.43	0.47	0.41	0.46	0.04	0.67	0.25	0.97

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.
[4] Measured using Co-EDTA and valerate.

Table 3. Effects of corn grain endosperm type and brown midrib 3 corn silage on nitrogen metabolism.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
TRDOM, kg/d[4]	14.3	14.7	15.0	13.8	1.0	0.92	0.53	0.18
N intake, g/d	643	684	699	690	30	0.15	0.43	0.24
Ruminal Ammonia, mg/dl	9.8	10.3	9.4	10.1	0.5	0.46	0.20	0.78
Flow to duodenum								
Ammonia N, g/d	15.8	17.5	16.4	18.6	1.6	0.49	0.12	0.87
NAN[5]								
g/d	555	676	635	633	49	0.68	0.19	0.18
% of N intake	86.5	98.5	90.1	91.2	5.3	0.74	0.23	0.31
NANMN[6]								
g/d	98.6	33.1	70.7	55.3	26	0.95	0.37	0.57
% of N intake	14.7	5.6	11.4	8.6	7.8	0.98	0.37	0.64
% duodenal NAN	16.9	4.5	13.2	10.8	8.4	0.85	0.29	0.47
Microbial N								
g/d	457	642	575	579	74	0.68	0.16	0.17
% duodenal NAN	83.1	95.5	86.8	89.2	8.4	0.85	0.29	0.47
g/kg TRDOM[4]	32.2	42.1	36.8	40.5	4.2	0.72	0.12	0.47
NAN postruminal digestion								
g/d	395	498	470	444	41	0.79	0.34	0.12
% of N intake	61.4	72.6	67.0	64.1	5.2	0.77	0.42	0.17
% of duodenal flow	71.0	73.2	74.3	69.6	1.8	0.94	0.46	0.05
N total tract apparent digestion								
g/d	483	508	534	502	21	0.24	0.86	0.13
%	75.0	74.2	76.9	72.9	1.3	0.79	0.06	0.21

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.
[4] Truly ruminally digested OM.
[5] Non-ammonia N.
[6] Non-ammonia non-microbial N.

CHAPTER 5

CONCLUSIONS AND IMPLICATIONS

Interactions of endosperm type of corn grain and the *bm3* mutation in corn silage can affect feeding behavior and feed intake. Compared to vitreous corn grain, floury endosperm corn decreased DM meal size in control corn silage diets but increased DM meal size in *bm3* corn silage diets. However, DMI was not different among endosperm types in *bm3* corn silage diets because cows consuming *bm3* corn silage with vitreous corn grain increased meal frequency to compensate for reduced DM meal size. These interactions of main effects on feeding behavior may have occurred because of the type and temporal pattern of metabolic fuel supply to the animal. Floury endosperm increased rate and extent of ruminal starch fermentation and molar proportion of propionate in the rumen. Lower DM meal size and DMI for diets containing floury endosperm with control corn silage might have been because the rate of propionate production and absorption exceeded its rate of utilization for gluconeogenesis, resulting in oxidation in the liver and a satiety signal. However, DM meal size and DMI may have been greater for floury endosperm when combined with *bm3* corn silage because greater milk yield increased glucose demand and flux of propionate through gluconeogenesis, delaying satiety.

In diets containing vitreous corn grain, greater DM meal size for control versus *bm3* corn silage might be a result of a greater ratio of acetate to propionate produced in control versus *bm3* corn silage, as indicated by a greater ratio of acetate to propionate

concentrations in the rumen. Less hypophagic effects of acetate versus propionate might reduce satiety signaling in diets containing vitreous corn grain and control corn silage and might act to prolong meal length. Additionally, greater plasma insulin: glucagon ratio in the diet with vitreous corn grain and *bm3* corn silage might have increased oxidation of metabolites in the liver to signal satiety. Numerically higher plasma insulin concentrations might have occurred in vitreous grain diets because greater rate of passage of starch from the rumen effectively shifted the primary site of starch digestion to the intestines. Although digestion of starch to glucose in the small intestine could theoretically increase plasma glucose, greater intestinal starch digestion in vitreous diets did not increase milk yield per se. Additionally, greater passage rate of starch to the large intestine might have increased microbial growth in the hindgut and reduced total tract N digestibility.

Ruminal pH was positively associated with rate of valerate absorption, and suggests that low ruminal pH may reduce ruminal motility and VFA absorption, and could ultimately limit supply of metabolites for milk production. Floury endosperm grain reduced mean ruminal pH, which might have caused the tendency for lower microbial efficiency in floury grain diets by increasing energy spilling. Greater ruminal starch fermentation in diets containing floury endosperm did not reduce ruminal fiber digestibility; on the contrary, ruminal starch and fiber digestibility were positively related. This suggests synergy and cross-feeding between ruminal amylolytic and cellulolytic populations, so high proportions of ruminally degraded starch in diets of lactating cows may not be as detrimental to fiber digestibility as previously indicated with in vitro experiments.

Several important areas of research have been highlighted by results from this experiment. Although ruminally degraded starch is associated with DMI depression in some studies, DMI is not always reduced (Allen, 2000), as occurred in the diet containing floury endosperm grain and *bm3* corn silage. Understanding how DMI is regulated by both metabolic and hormonal factors is important in order to maximize energy intake and milk yield. Additionally, milk yield is determined by supply of metabolites from both the rumen and the intestines, which can change dramatically based on site of nutrient digestion. Although VFA absorption from the rumen can significantly affect substrate supply, little is known about how ruminal motility and pH might affect VFA absorption rate. Finally, understanding how DMI and the ruminal environment interact to affect microbial protein production will allow nutritionists to maximize microbial protein yield and utilization by the animal to reduce environmental N.

This experiment proves that digestion characteristics of starch and fiber can interact to affect feeding behavior and ultimately production. However, animal response to changes in diet fermentability involves understanding interactions between physical, metabolic, and hormonal regulation of feed intake, and predicting how animals will respond is unlikely. Because floury endosperm grain increased DMI and milk yield when combined with *bm3* corn silage but vitreous corn did not, production responses to *bm3* corn silage are probably dependent on grain source. The results from the present experiment suggest that diets should not be formulated based upon starch and NDF concentrations alone, but that consideration of the fermentability of individual ingredients and their interactions is important to maximize DMI and milk yield.

APPENDICES

APPENDIX A

Use of Markers in Assessing Site of Digestion

External and internal markers to estimate duodenal flow are well known for being imprecise. Cr_2O_3 was originally dosed and used in this experiment as an external marker to determine digesta flow. However, the absolute values of DM flow appeared high, even considering that DMI was exceptionally high for surgically prepared animals. Indigestible NDF (iNDF) was consequently used as the final flow marker in this study. It is important to note that DM flow determined using Cr_2O_3 was highly correlated with DM flow using iNDF. Also, because this study is investigating treatments and is not trying to characterize animal response to one particular diet, it is the relative differences among treatments rather than the absolute values of nutrient flows that are important.

Incomplete recovery of Cr_2O_3 will overestimate duodenal DM flow. Sampling digesta before marker concentration reaches steady state will underestimate marker flow and overestimate DM flow (Owens and Hanson, 1992). It is estimated that marker concentration reaches steady state in approximately 5 to 7 d for cattle with constant feed intake (Owens and Hanson, 1992). Additionally, in dairy cattle with high feed intake, time to steady state of Cr_2O_3 outflow would be reached faster than in animals with lower intakes and passage rates. In the present experiment, chromic oxide was dosed for 7 days with a prime dose on the first day, so it is not likely that steady state had not yet been reached. Diurnal variation of marker flow can occur even after steady state has been reached (Corbett et al., 1959). However, frequent (3 times daily) dosing and collection of 8 digesta samples, representing every 3-hr over a 24-hr period, would minimize effects of

diurnal variation on DM flow. Zinn et al. (1980) determined that 8 samples, representing every 6-hr over a 48-hr period was sufficient to account for diurnal variation, and there was no difference between DM flow calculated using Cr_2O_3 as a marker or total collection. Previous research using this method (Oba and Allen, 2003b) has indicated that 8 samples are adequate. Using chromic oxide as a marker has been criticized because it may incompletely mix with ruminal contents when dosed in gelatin capsules (Corbett et al., 1959). However, ground spelt hulls were included in the gelatin capsules with Cr_2O_3 to facilitate dispersal in the rumen. Furthermore, there was no evidence that incomplete mixing occurred because during digesta sampling no Cr_2O_3 was seen in either the rumen or duodenal contents. Incomplete recovery of Cr_2O_3 while subsampling may have occurred during this study. Eight digesta samples are separated into primarily solid and liquid phases using a nylon mesh and are composited into one solid and one liquid sample per cow-period. Mixing and subsampling of solid phase of duodenal contents was performed by hand for several minutes and taking small samples from numerous locations. It is unlikely that settling of Cr_2O_3 would occur in a solid subsample, but the liquid sample, although well mixed, would be expected to exhibit some settling of Cr_2O_3. It is possible that the liquid subsample did not contain a representative amount of marker because of settling to the bottom of the container, and subsequent analysis for Cr in the subsample underestimated actual Cr concentration and overestimated DM flow at the duodenum. Fecal flow was not overestimated to the same degree because fecal samples were composited on a wet weight basis, and also would not expect to exhibit Cr_2O_3 settling because of the more solid nature of the feces. In the future, steps should be taken to prevent differential settling of marker during subsampling.

APPENDIX B

Additional Original Data

Table 1. Effects of corn grain endosperm type and brown midrib 3 corn silage on additional measures of feeding behavior and chewing behavior.

	Control		*bm3*			P		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Eating rate, kg/min	0.36	0.39	0.40	0.39	0.02	0.10	0.38	0.19
Hunger ratio	0.16	0.18	0.18	0.14	0.02	0.59	0.56	0.07
Satiety ratio	0.18	0.20	0.22	0.19	0.02	0.32	0.63	0.07
Eating chews								
/ kg NDF intake	3616	3314	3347	3342	169	0.18	0.10	0.11
/ kg starch intake	3196	2895	3010	2894	190	0.32	0.04	0.33
Ruminating chews								
/ kg NDF intake	5625	4985	4800	4853	375	0.01	0.11	0.06
/ kg starch intake	4906	4319	4308	4176	290	0.02	0.02	0.13
Chew rate, chews/min								
Eating	75.0	75.9	73.6	75.2	1.8	0.49	0.41	0.83
Ruminating	61.6	61.7	60.8	60.7	1.1	0.18	0.98	0.90

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

Table 2. Effects of corn grain endosperm type and brown midrib 3 corn silage on ruminal nutrient concentration and turnover time.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
DM %	13.6	14.6	15.2	14.4	0.42	0.08	0.76	0.02
OM, % of DM	91.5	91.6	91.7	90.9	0.70	0.67	0.53	0.48
NDF, % of DM	53.5	52.1	51.3	49.5	1.31	0.02	0.11	0.86
pdNDF, % of DM[4]	27.1	25.8	32.6	31.3	1.14	<.0001	0.08	0.95
iNDF, % of DM[5]	26.4	26.4	19.2	18.2	0.80	<.0001	0.41	0.43
Starch, % of DM	6.76	7.60	7.48	8.88	0.52	0.05	0.03	0.57
Ruminal turnover time, h								
DM	10.1	11.1	10.7	10.1	0.73	0.79	0.80	0.30
OM	10.6	11.5	11.2	10.5	0.79	0.78	0.88	0.29
NDF	23.0	24.8	23.9	21.5	2.0	0.48	0.85	0.26
pdNDF[4]	18.0	19.0	20.5	18.8	1.8	0.41	0.83	0.37
iNDF[5]	33.2	36.5	32.9	29.6	2.7	0.16	0.99	0.21
Starch	2.58	2.99	2.98	3.32	0.33	0.26	0.24	0.92
iNDF passage to duodenum, kg/d	1.99	2.18	1.64	1.59	0.10	<.0001	0.23	0.07

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.
[4] Potentially digestible NDF.
[5] Indigestible NDF.

Table 3. Effects of corn grain endosperm type and brown midrib 3 corn silage on amount of ruminal VFA and ruminal pH during meal and rumination bouts.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
Fermentation acid, mmol								
Acetate	73.8	76.4	75.7	75.8	1.1	0.37	0.06	0.08
Propionate	39.2	34.4	40.3	37.3	2.1	0.04	0.001	0.32
Butyrate	17.5	18.2	18.6	18.4	0.6	0.21	0.61	0.36
Isobutyrate	1.42	1.42	1.32	1.34	0.1	0.01	0.63	0.81
Valerate	3.03	2.80	3.11	2.94	0.2	0.36	0.11	0.78
Isovalerate	2.23	2.32	1.98	2.18	0.2	0.05	0.15	0.59
Formate	0.02	0.01	0.02	0.01	0.1	0.59	0.40	0.87
Branch chain VFA	3.65	3.74	3.31	3.52	0.3	0.03	0.21	0.63
Lactate	0.70	0.65	0.69	1.10	0.4	0.54	0.62	0.52
Meal initial pH	6.08	6.28	5.98	6.07	0.12	0.12	0.16	0.58
Meal final pH	5.95	6.10	5.89	5.95	0.09	0.09	0.41	0.34
Rumination initial pH	6.00	6.20	5.86	5.97	0.11	0.05	0.09	0.59
Rumination final pH	6.06	6.26	5.99	6.11	0.11	0.13	0.03	0.61

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

Table 4. Effects of corn grain endosperm type and brown midrib 3 corn silage on net energy for maintenance.

	Control		*bm3*			*P*		
	Floury	Vitreous	Floury	Vitreous	SEM	S[1]	G[2]	S × G[3]
NE_M, Mcal/d	10.2	10.3	10.2	10.3	0.22	0.93	0.31	0.80

[1] S = Main effect of corn silage hybrid.
[2] G = Main effect of ground corn grain endosperm type.
[3] Interaction of corn silage hybrid and ground corn grain endosperm type.

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