STRATEGIC APPROACHES TO DEVELOP OPTIMAL FEEDING PROGRAM OF

BROWN MIDRIB CORN SILAGE TO LACTATING DAIRY COWS IN THE

INTERMOUNTAIN WEST

by

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of requirements for the degree

of

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in

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ABSTRACT

Strategic Approaches to Develop Optimal Feeding Program of Brown Midrib Corn

Silage to Lactating Dairy Cows in the Intermountain West

by

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Utah State University, 2013

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In two lactation studies reported in this dissertation, it was hypothesized that

feeding 35% brown midrib corn silage (**BMRCS**) and 25% alfalfa hay (dry matter basis)

would result in increased dry matter intake (**DMI**) around peak lactation compared with

feeding conventional corn silage (**CCS**), causing longer peak milk production, and that

feeding dairy cows in early lactation a 16% crude protein diet with fair quality alfalfa hay

(**FAH**) in BMR-based diets would maintain milk production, reduce urinary N excretion,

and improve N efficiency compared to those fed high quality alfalfa hay (**HAH**) in CCS-

or BMR-based diets. A third experiment was conducted to assess in situ degradation

kinetics of BMRCS harvested prior to or at maturity. The first lactation study was

performed to determine the long-term effects of feeding BMRCS fed with a high dietary

concentration of good quality alfalfa hay in a high-forage lactation diet on productive

performance of Holstein dairy cows for the first 180 d of lactation. Feeding BMRCS-

based diet did not affect milk production through peak lactation compared with a CCS-

based diet; however, cows fed the BMRCS-based diet maintained heavier body weight through peak lactation and longer peak milk production, which resulted in increased milk yield post peak lactation, leading to greater overall milk production and milk protein yield. A second lactation experiment was performed to investigate if early lactating dairy cows fed with the FAH in BMRCS-based diets would reduce urinary N excretion and improve N efficiency compared to those fed the HAH in CCS- or BMR-based diets. Feeding BMR and HAH had better N utilization by decreasing concentrations of urea in blood, milk, and urine. In addition, feeding BMR-based diets decreased urinary N-to-fecal N ratio, and it was further reduced by feeding the HAH, which can represent an environmental advantage over traditional sources of forages in lactation dairy diets. A third experiment assessed in situ DM and neutral detergent fiber degradation kinetics for two new pre-matured BMR varieties (**pmBMR1** and **pmBMR2**) that can be double-cropped by harvesting at tassel, compared with a sole crop mature BMR (**mBMR**) and CCS harvested at maturity in dry and lactating Holstein dairy cows. The potentially degradable NDF fraction was greater for BMR hybrids compared with CCS with the exception of the pmBMR2, which had the lowest potentially degradable NDF fraction in dry cows. Estimates of ruminal degradability of NDF were greatest for pmBMR1 in both dry and lactating cows. Feeding BMRCS exerted nutritive and environmental benefits when fed with typical Intermountain West lactation dairy diets. Further research is needed to understand interactive aspects of nutrient utilization with other dietary ingredients under different physiological conditions to take full potential benefits of BMRCS.

(193 pages)

PUBLIC ABSTRACT

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The first lactation study was performed to determine the long-term effects of feeding BMRCS fed with a high dietary concentration of good quality alfalfa hay in a high-forage lactation diet on productive performance of Holstein dairy cows for the first 180 d of lactation. Feeding BMRCS-based diet did not affect milk production through peak lactation compared with CCS-based diet; however, cows fed the BMRCS-based diet maintained heavier body weight through peak lactation and longer peak milk production, which resulted in increased milk yield post peak lactation, leading to greater overall milk production and milk protein yield.

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Michael Shane Holt

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LIST OF ABBREVIATIONS

AA = amino acid

ADF = acid detergent fiber

AH = alfalfa hay

AIA = acid-insoluble ash

BMR = brown midrib

BMRCS = brown midrib corn silage

BUN = blood urea nitrogen

BW = body weight

Co = cobalt

CP = crude protein

Cr = chromium

CS = corn silage

CCS = conventional corn silage

DCP = double-cropping

DM = dry matter

DMI = dry matter intake

ECP = endogenous crude protein

ERD = extent of rumen degradation

FAH = fair quality alfalfa hay

FCM = fat corrected milk

HAH = high quality alfalfa hay

iNDF = indigestible neutral detergent fiber

K_d = rate of degradation

LRCpH = Lethbridge research centre ruminal pH measurement system

mBMR = mature brown midrib corn silage Mycogen Seeds

MCP = microbial crude protein

MkN:MaN = milk nitrogen-to-manure nitrogen ratio

MNE = microbial nitrogen efficiency

MP = metabolizable protein

MUN = milk urea nitrogen

N = nitrogen

NAN = non-ammonia nitrogen

NANMN = non-ammonia non-microbial nitrogen

NDF = neutral detergent fiber

NEFA = nonesterified fatty acids

NE_L = net energy lactation

NFC = non fiber carbohydrates

NH_3 = ammonia

NH_3-N = ammonia nitrogen

NPN = non-protein nitrogen

NRC = national research council

OM = organic matter

PD = purine derivatives

pdNDF = potentially digestible neutral detergent fiber

pef = physical effectiveness factor

peNDF = physically effective neutral detergent fiber

pmBMR1= pre-mature brown midrib corn silage MasterGraze^{TM}

pmBMR2 = pre-mature brown midrib corn silage Ray Brothers synthetic BMR84^{TM}

RDP = ruminally degradable protein

RUP = ruminally undegradable protein

SARA = subacute ruminal acidosis

TMR = total mixed ration

TP = true protein

TRDOM = truly ruminally degraded organic matter

UN:FN = urinary nitrogen excretion-to fecal nitrogen excretion ratio

Yb = ytterbium

VFA = volatile fatty acids

CHAPTER 1

INTRODUCTION

Dry matter intake (**DMI**) is a primary determinant of milk yield for lactating dairy cows. Feed intake is determined by many interacting factors, and prediction of feed intake is the "Achilles heel" of diet formulation. Many different dietary characteristics interact with environmental and physiological state of cows. Signals controlling feed intake likely change throughout lactation. Prior to parturition, body reserves are frequently mobilized, because the cow cannot consume enough feed to meet the energy demands toward milk production in early lactation. Control of feed intake is likely dominated by hepatic oxidation of nonesterified fatty acids (**NEFA**) during early lactation, while ruminal distension likely controls feed intake around peak lactation (Allen et al., 2009). Peak milk yield is maximized by feeding low gut fill-diets that are highly fermentable in the rumen. The filling effect of diets is affected most by concentration, digestibility, and fragility of forage neutral detergent fiber (**NDF**). Concentration of forage NDF influences DMI by dairy cows, because forage NDF is filling, and thus DMI decreases as forage NDF increases in the diet. On the other hand, cows consuming sufficient NDF without a sufficient proportion of long particles can exhibit the same metabolic disorders as cows consuming a diet deficient in chemical fiber (Fahey and Berger, 1998). Negative responses due to a lack of long particle fiber include subacute ruminal acidosis, reduced fiber digestion, milk fat depression, displaced abomasums, lameness, and fat cow syndrome (NRC, 2001). Finding an optimal balance between physically effective fiber and readily fermentable carbohydrates is difficult but

crucial for maintaining proper ruminal metabolism (Zebeli et al., 2006; Plaizier et al., 2008) and enhancing productivity.

Chemical and genetic approaches have been employed to improve forage fiber digestibility by decreasing lignin concentration or extent of lignin cross-linking with cell wall carbohydrates. Corn silage (**CS**) with the brown midrib mutation (**BMR**) has been well documented to have higher fiber degradability and will likely increase DMI and milk yield compared with cows fed conventional corn silage (**CCS**; Eastridge, 1999; Gencoglu et al., 2008). The BMR hybrid was developed in 1924 at the University of Minnesota (Jorgensen, 1931). The name "BMR" was attributed to this trait because of how the reddish-brown to tan colored midrib of mutant leaf blades contrasts with the pale green midrib of conventional-type leaf blades. The BMR plants also accumulate reddish-brown to yellow pigment in stalks and roots. Eastridge (1999) reported that on average BMR CS (**BMRCS**) contained 34% less lignin and had 19% higher in vitro NDF degradability than non-BMRCS. Several (Weiss and Wyatt, 2006; Kung et al., 2008; Stone et al., 2012), but not all experiments (Gehman et al., 2008; Castro et al., 2010; Holt et al., 2010) feeding BMRCS, have reported improved lactational performance of dairy cows. Inconsistent effects of BMRCS have been caused by various factors, including cows differing in physiological state and duration of experimentation (Taylor and Allen, 2005; Castro et al., 2010). In a contemporary review of published experiments (n = 11), Gencoglu et al. (2008) reported that cows fed BMRCS averaged 1.2 kg/d higher DMI and 1.7 kg/d more milk than those fed CCS. Only a couple of these studies included alfalfa hay (**AH**) in the diet and those that did fed low concentrations more typical of the Midwestern United States than the Intermountain West.

Although both forages provide the needed fiber components, alfalfa and CS complement each other from a nutritional perspective; CS is high in energy, whereas alfalfa is high in crude protein (**CP**). Great emphasis has been placed on producing high quality AH with an average chemical composition for CP, NDF, and acid detergent fiber (**ADF**) of 21.3, 38.3, and 26.7% DM, respectively (Holt et al., 2010). Feeding high quality AH provides enough CP and forage NDF to support potential milk production by dairy cows; however, ruminal microbes degrade alfalfa protein too rapidly, resulting in excessive excretion of nitrogenous waste by the animal. This process results in inefficient utilization of feed N with high CP degradability of AH in the rumen and limits optimal microbial protein synthesis. It also increases energy cost to convert ruminal ammonia to urea, and elevates N excretion into the environment. Consequently, the full benefit of alfalfa protein is not realized due to its poor utilization by the animal. Excess CP as well as amino acids from cell turnover and enzyme production are deaminated and excreted as urea in urine and milk, while undigested ruminally undegradable protein and metabolic N (sloughed intestinal cells and hind gut fermentation products) are excreted in the feces (Tamminga, 1992). Overall intake of N affects the amount of N excreted via manure, whereas types of carbohydrate and forage have greater impacts on the route (fecal or urinary) of excretion (Weiss et al., 2009). The route and amount of N excretion is of primary environmental concern. Urinary N is more volatile than fecal N and is rapidly converted to ammonia by ureases present in soil and on pen floors (Bussink and Oenema, 1998). Ammonia has been recognized as an important local and regional air pollutant as well as a human health concern. A major source of ammonia is from livestock manure. Ammonia emissions have been estimated to range from 13.1 to 55.5 kg/cow per year

(Pinder et al., 2003), which is between 8 and 34% of total N excreted (assuming a dairy cow in the U.S. excretes an average of 160.6 kg of N in manure per year; Nennich et al., 2006).

Livestock manure is commonly used as an N source for crops. The goal of N management is to efficiently deliver plant-available N to crops through maximizing crop utilization and minimizing losses to the environment. Major losses of N to the environment occur when ammonia gas is lost to the air, or nitrate (**NO$_3$**) is leached to groundwater. Manure that contains mostly ammonium-N contains much plant-available N that is subject to volatilization shortly after manure application. The need for improved agricultural nutrient cycling has become particularly apparent for livestock enterprises where expansion is often limited by the amount of land available for recycling manure and producing high-yielding feed crops. One potential option for simultaneously addressing the need for both increased productivity and decreased NO$_3$-N leaching from agricultural lands is through the introduction of double-cropping systems (Karpenstein-Machan, 2001). In such a system, two crops are harvested in a single year. Production of two crops is possible, because a cool-season crop is harvested in late spring before full maturity, and a warm-season crop is seeded directly afterward. If the cool-season crop is seeded in the fall, it can also serve as a winter cover crop, with the potential to sequester soil N that otherwise would be subject to leaching (Snapp et al., 2005). A general body of evidence indicates that spring N sequestration by winter cover crops can mitigate NO$_3$-N losses from annual cropping systems (Figure 1.1; Heggenstaller et al., 2008). High NO$_3$-N losses from annual crop systems result from a lack of synchronization between soil inorganic N supply and crop N uptake, with high potential for leaching in the spring and

fall, when excess inorganic N is present in soil, but crop growth and N uptake by the plant are minimal or absent (Dinnes et al., 2002). Typically, winter cereal forages in the Intermountain West are harvested at the late vegetative or early boot stage precluding growing corn (a warm season crop), because the remaining growing season is too short (Brown, 2006). However, relatively new varieties of BMRCS have been developed with the intent of harvesting in a pre-mature stage during tasseling. These BMR varieties may be useful in a double-cropping system with a shorter season and for improving fiber degradability by ruminants.

The overall hypothesis in a series of studies reported in this dissertation was that improved ruminally degradable NDF from BMRCS would: 1) increase DMI in lactating dairy cows around peak lactation when DMI is controlled by rumen distension, causing longer peak milk production; and 2) reduce urinary N excretion and improve N efficiency when fed with fair quality AH compared to diets with high quality AH fed with CCS or BMRCS-based diets. In addition, I assessed in situ DM and NDF degradation kinetics for new BMR varieties harvested at tassel compared to a sole crop mature BMRCS and CCS harvested at maturity to evaluate potential nutritive benefits on pre-matured BMR varieties.

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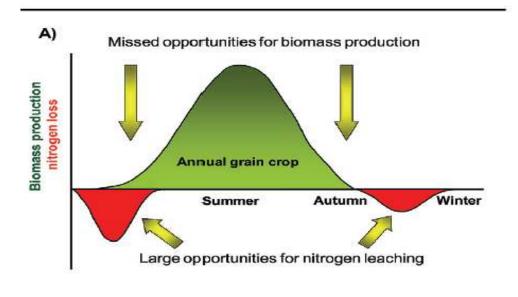
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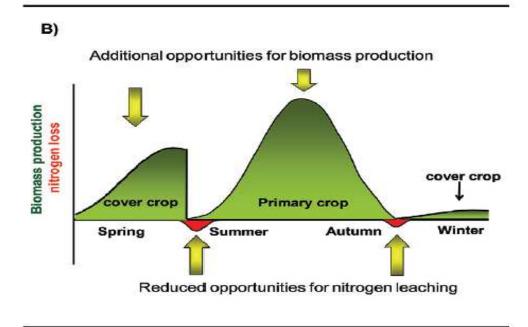
Figure 1.1. Potential for NO_3-N leaching and crop growth in sole and double-crop systems (adapted from Heggenstaller et al. 2008). Representation of the potential seasonal dynamics of NO_3-N leaching and dry matter biomass production (A) in an annual grain cropping system and (B) in a double-cropping system.

CHAPTER 2

REVIEW OF LITERATURE

REGULATION OF DRY MATTER INTAKE

Dry matter intake (**DMI**) is a primary determinant of milk yield for lactating dairy cows. Regulation of feed intake is complex, as it is affected by numerous factors, including physical limitations and changes in endocrine physiology associated with parturition and lactogenesis (Ingvartsen and Andersen, 2000). Around parturition, changes in the concentration of hormones such as insulin, growth hormone, prolactin, estrogen, and progesterone may affect DMI (Allen et al., 2005). Therefore, feed intake is probably determined by the integration of a variety of physical, metabolic, and hormonal factors.

Metabolic and Hormonal Regulation of Intake

Metabolites and nutrients can potentially serve as regulators of feed intake. Mobilization of triglycerides in adipose tissue and the subsequent release of nonesterified fatty acids (**NEFA**) and glycerol along with the production of ketone bodies may act as signals to decrease feed intake in periparturient dairy cattle (Ingvartsen and Andersen, 2000). Perhaps increased concentrations of circulating NEFA in the cow may result in a counterintuitive reduction in feed intake. This reduction in feed intake due to neural signaling in the liver is likely linked to mitochondrial oxidation of NEFA, which provides satiety signals mediated by vagal afferents (Scharrer and Langhans, 1990). The idea that feed intake is controlled by a signal from the liver to the brain stimulated by oxidation of fuels was coined the "Hepatic Oxidation Theory" by Allen et al. (2009). By integrating

the effects of various metabolic fuels on feeding behavior, they developed a conceptual

model by which feed intake may be controlled in ruminants (Figure 2.1). Allen et al.

(2009) reported that the minute to minute fluctuations of oxidized fuels play a greater role

in the feeding behavior than longer periods of time (hours or days) which remain

relatively constant. Hepatic oxidation increases during a meal, resulting in increased

energy status of hepatocytes and decreased rate of hepatic vagal afferents, thus resulting

in satiety. However, following a meal, hepatic oxidation declines, causing decreased

energy status of hepatocytes and increased discharge rate of the hepatic vagus, thus

resulting in hunger (Allen et al., 2009). According to the authors, hepatic oxidation likely

controls feed intake to a greater extent for cows with low nutrient requirements and for

animals in a lipolytic state (e.g., periparturient and stressed animals) than those fed high

forage diets or with very high nutrient requirements such as cows at peak lactation (Allen

et al., 2009). Therefore, as milk yield increases, feed intake control by hepatic oxidation

diminishes, and alternatively control is dominated by distension from gut fill. This

change in the dominant mechanism of intake regulation might occur only 7 to 10 d after

calving for some cows or more than 3 wk for others. The best signs that hepatic oxidation

is less limiting are lower plasma NEFA and ketone concentrations and steadily increasing

feed intake (Allen et al., 2009).

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 devoted to investigating

factors affecting physical regulation of intake (Allen, 1996 and 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 a greater rate of fiber digestion, particles are probably broken down faster, thereby sinking faster, which increases the rate of passage from the rumen and decreases the filling effect of the diet.

FIBER DIGESTIBILITY

Digestibility of neutral detergent fiber (**NDF**) is determined by the fraction of NDF that is potentially digestible, the rate of NDF degradation in the rumen, and the rate of passage from the rumen (Allen and Mertens, 1988). In vitro NDF digestibility can range from less than 20% to greater than 60% over a variety of forages (Allen and Oba, 1996). 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 (**iNDF**) fraction 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 decreasing the 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 the amount and rate of pdNDF digestion in forages. Grasses often have a greater proportion of pdNDF to iNDF and higher in vitro NDF digestibility than legume forages, but rate of digestion of legume pdNDF is faster (Smith et al., 1972) and could increase total amount of NDF digested in vivo. Additionally, within a forage type, immature plants generally have higher NDF digestibility than mature plants, because as the plant matures the iNDF fraction increases, and rate of NDF digestion decreases (Smith et al., 1972). Replacing early vegetative alfalfa hay with late bud or full bloom alfalfa hay decreased 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.

BROWN MIDRIB CORN SILAGE

The first brown midrib (**BMR**) 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 BMR mutations, so named because of accumulation of reddish-brown phenolic lignin monomers in the midrib of the leaf, reduce lignin concentration 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-0-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. Decreased 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 BMR hybrids occurred. Of the BMR mutations, hybrids containing the *bm3* mutation have been most widely used in nutritional experiments, because it consistently lowers lignin concentration 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 or normal corn hybrids (Muller et al., 1971). Decreased 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 *bm1* corn plants mature, lignin concentration 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 animal performance, but little was known about the effects of feeding *bm3* hybrids to animals.

Early in vivo research with BMR hybrids agreed with in vitro results. Muller et al. (1972) performed a classical study investigating the feeding value of BMR corn silage (**BMRCS**) hybrid vs. a high yielding commercial hybrid. Two adjoining plots were planted with either BMR 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, BMRCS improved apparent total tract digestibility of cell wall constituents and cellulose vs. the normal corn hybrid. In a similar study, replacing normal silage with BMRCS 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 a more important benefit of BMRCS was the observation that animals often increased feed intake. Brown midrib corn silage increased voluntary DMI in lambs by 29% compared to a normal hybrid (Muller et al., 1972). When fed to cows in early lactation, BMRCS increased DMI by 11% compared with a normal hybrid (Block et al., 1981). More recent studies have found that periparturient dairy cattle consumed more feed during the last 2-3 wk of gestation when fed BMRCS, likely because of a reduction in ruminal fill. In addition, cows fed BMRCS had higher intakes for the first 3 wk postpartum either because of higher intakes during the prepartum period or because of a reduction in ruminal fill limitations in the postpartum period. The increased intakes led to an increase in milk yield and fat corrected milk (**FCM**) during the 3-wk postpartum period. A carryover effect occurred from the feeding of BMRCS during the periparturient period, resulting in significant increases for milk yield and composition in these cows from wk 4 to 15 of lactation, when all cows were fed a common diet. The results of these

studies indicate the importance of digestible NDF can have in diets of periparturient cows, and the long-term production responses that can occur when intake is increased (Santos et al., 2001; Stone et al., 2012).

However, some studies observed no increase in DMI when BMRCS replaced a normal hybrid (Frenchick et al., 1976; Keith et al., 1979; Holt et al., 2010). Lack of treatment effect on DMI when BMRCS replaced control corn silage could occur if DMI is not limited by ruminal fill. Oba and Allen (1999a) found a relationship between pretrial milk yield and DMI response to BMRCS. Cows producing more milk in the pretrial period increased DMI to a greater extent when fed BMRCS than cows yielding less milk. The authors concluded that ruminal fill is more limiting to intake for higher yielding cows, and thus increasing NDF digestibility of forage by feeding BMRCS 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 d-in-milk (**DIM**)]**,** a one percentage unit increase in NDF digestibility was associated with a 0.17 kg increase in DMI and 0.25 kg increase in 4% FCM (Oba and Allen, 1999b). Jung et al. (2004) reported that in diets containing corn silage (> 40% of the dietary DM), a one percentage unit increase in in vitro NDF degradability of corn silage resulted in a 0.12 kg/d increase in DMI and a 0.14 kg/d increase in 3.5% FCM yield. The BMR mutation in corn silage can potentially increase NDF digestibility and might mitigate limitations to DMI by physical fill.

ENERGY BALANCE AND SITE OF DIGESTION

Greater DMI increases rate of nutrient passage from the rumen, which can limit ruminal nutrient digestibility and result in postruminal digestion 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. Replacing beet pulp with high moisture corn linearly decreased ruminal pdNDF digestibility from 67.3 to 46.1%. (Voelker and Allen, 2003a). While greater ruminal starch digestion is associated with lower ruminal fiber digestibility in multiple studies (McCarthy et al., 1989; Crocker et al., 1998; Callison et al., 2001), it is not always associated with lower total tract NDF digestibility (Crocker et al., 1998; Callison et al., 2001). In the experiment done by Crocker et al. (1998), postruminal NDF digestibility linearly increased with greater ruminal starch digestibility, but total tract NDF digestibility was not affected. Replacing dry corn with high moisture corn lowered 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, 2003a). 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 affecting total tract fiber digestibility.

Controversy exists as to the benefits of ruminal vs. 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 lower ruminal pH and inhibit fiber digestibility if starch fermentation is too rapid. Ruminal starch digestibility ranges from 42 to 96% over a variety of grain sources (Nocek and Tamminga, 1991). Site

of starch digestion can be manipulated by grain conservation (Oba and Allen, 2003a), method of processing (Callison et al., 2001), and endosperm type of corn grain (Philippeau et al., 1999). Apparent ruminal digestibility of starch increased from 35 to 57% when vitreous corn grain was replaced by floury endosperm grain (Taylor and Allen, 2005a). This wide range of starch digestibility can affect whether starch is digested primarily in the rumen or intestines. If ruminal starch degradation is too rapid, flux of propionate to the liver might limit DMI if it is oxidized rather than used for gluconeogenesis (Oba and Allen, 2003b). Shifting starch digestion to the intestines can theoretically provide more glucose to the animal, but infusion experiments have suggested that increasing small intestinal glucose absorption may not increase glucose available for milk production (Knowlton et al., 1998; Areli et al., 2001). Instead, increased glucose may be used for tissue retention (Reynolds et al., 2001).

Greater body weight (**BW**) gain has been observed in several experiments where BMRCS replaced normal corn silage (Frenchick et al., 1976; Sommerfeldt et al., 1979; Weller and Phipps, 1986), and this effect might occur if BMRCS shifts site of starch digestion to the intestines. Perhaps most striking was the experiment conducted by Block et al. (1981); in the study feeding cows BMRCS from wk 3 to 10 postpartum increased DMI by 2.2 kg/d and although milk yield numerically increased, the greatest effects of treatment occurred on BW change. Cows consuming BMRCS gained 10.3 kg over the 8-wk period, whereas cows consuming normal corn silage lost 24.6 kg over the same period. Oba and Allen (1999a) found that, compared to control corn silage, BMRCS increased energy balance of lactating cows by 2.1 Mcal/d. Greater concentrations of metabolizable energy in BMRCS diets fed ad libitum resulted in more metabolizable

energy partitioned toward tissue energy gain rather than milk energy (Tine et al., 2001).
In a study by Oba and Allen (2000c), BMRCS increased ruminal propionate and shifted a
substantial portion of starch digestion to the intestines; consequently, greater glucose
availability in BMRCS diets might increase plasma insulin concentration and tissue
energy retention. However, effects of BMRCS on hormone profiles have not been
investigated. Taylor and Allen (2005b) observed that the energy balance was greater for
floury grain than vitreous when fed with BMRCS. Vitreous endosperm corn grain tended
to increase ($P = 0.09$) partitioning of energy toward milk compared with floury
endosperm grain. More research is needed to examine the interactions of starch and fiber
digestibility on hormone pulsatility and energy balance.

Replacing normal corn silage with BMRCS may decrease ruminal starch
digestibility. Oba and Allen (2000a,c) reported that BMRCS decreased ruminal starch
digestibility by 10%, but increased postruminal starch digestibility by 13%. The authors
suggested that greater DMI of BMRCS may have increased the passage rate of starch
from the rumen and shifted the site of starch digestion to the intestines. Another
experiment reported that ruminal starch digestibility was 36% lower for BMRCS
compared with 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 the greater rate of starch passage from the rumen in the study
done by Oba and Allen (2000c), but no differences in DMI were observed by Greenfield
et al. (2001).

In a more recent study, Taylor and Allen (2005a) reported that BMRCS did not
affect ruminal starch digestibility when fed with floury or vitreous corn grain endosperm

types. The vitreous corn grain fermented more slowly and passed from the rumen faster, resulting in decreased ruminal starch digestibility. However, compensatory postruminal starch digestion resulted in relatively small differences in total tract starch digestion compared with floury endosperm grain. Greater ruminal starch digestion in floury endosperm grain diets compared to vitreous grain (57 vs. 35%) did not affect ruminal fiber digestion kinetics of BMRCS. Furthermore, production response to BMRCS is dependent on grain source, because starch and fiber fermentability can interact to affect feeding patterns and productivity. Changing diet fermentability influences milk production primarily by affecting DMI. Feeding floury endosperm grain decreased meal length and size in control silage, but increased meal length and size in BMRCS diets. In addition, total DMI was not decreased in BMRCS diets containing vitreous corn grain, because BMRCS tended to increase ($P = 0.10$) meal frequency/d compared with CCS (Taylor and Allen, 2005b). Greater starch passage in diets containing BMRCS could be the result of other factors; for example, the ruminal fiber mat formed by BMRCS fiber might be less effective at retaining corn grain particles.

RUMEN MAT FORMATION

An extensive stratification of the reticuloruminal contents is typical for grazer ruminants as opposed to browsers (Hofmann and Stewart, 1972; Hofmann, 1989) and serves to optimize formation of fermentation end-products (Tafaj et al., 2004; Clauss et al., 2011). A fluid phase is located in the ventral part of the reticulorumen of cattle, whereas the gas cap is in the dorsal rumen, and a thick-packed mat extends from the dorsal to the central part of the reticulorumen which consists of solid digesta with mainly

large, newly ingested, and buoyant feed particles (Poppi et al., 2001; Tafaj et al., 2004). Rumen mat formation is considered a presupposition, as well as an indicator, of proper rumen function in dairy cows because of its 2 main physiological functions. One is to optimize the ruminal microenvironment, especially ruminal pH, by physical stimulation of rumination, salivation, and ruminal motility (Poppi et al., 2001; Tafaj et al., 2004; Zebeli et al., 2006). This function determines whether fiber inclusion is adequate when feeding highly fermentable diets. The second function of the rumen mat is to promote particle retention, thus allowing for more efficient digestion of fiber in the forestomach (Poppi et al., 2001; Tafaj et al., 2004; Zebeli et al., 2006).

Sutherland (1988) described the rumen mat as a very effective first-stage separator that can modulate the retention time of solid digesta through an increased selective retention ("filter bed" effect) for undigested small feed particles. Through filtration and mechanical entanglement, the rumen mat functions to retain potentially escapable fiber particles, thus increasing the time available for digestion (Zebeli et al., 2006). The formation, maintenance, and consistency of the rumen mat strongly depends on dietary particle size and the specific gravity of particles (Tafaj et al., 2004; Clauss et al., 2011). Rumen mat consistency is a major determinant for the regulation of rumen passage rate of solid digesta. The better the consistency of the rumen mat, the lower the probability of feed particles to escape undegraded to the omasum. Increased escape of potentially degradable feed particles from the rumen negatively affects fiber degradation and feed utilization (Weidner and Grant, 1994).

Negative responses due to lack of long particle fiber include subacute ruminal acidosis (SARA), reduced fiber digestion, milk fat depression, displaced abomasums,

lameness, and fat cow syndrome (NRC, 2001). Including adequate amounts of dietary

physically effective NDF (peNDF) is important for the optimization of ruminal pH when

feeding highly fermentable diets to dairy cows. The peNDF of a feedstuff is the product

of its NDF concentration and the physical effectiveness factor. By definition, the physical

effectiveness factor varies from 0, when NDF is not physically effective (e.g., fiber from

ground concentrates), to 1, when NDF is fully effective (e.g., fiber from coarsely-

chopped hay) in promoting digesta stratification in the rumen, chewing activity, and

rumen buffering (Allen, 1997; Mertens, 1997). The peNDF was estimated by 2

measurement techniques, the NDF concentration of TMR multiplied by amount of DM

retained on a 1.18-mm screen (peNDF>1.18; Kononoff et al., 2003) and NDF

concentration of TMR multiplied by the proportion of DM retained by 19- and 8-mm

Penn State Particle Separator screens (peNDF>8; Lammers et al., 1996). Using a meta-

analytical modeling approach comprising experiments conducted over the last 2 decades,

Zebeli et al. (2010) compared the predictive value of the different peNDF pool sizes for

different response variables. Their data indicated that the prediction capabilities of

peNDF>1.18 and peNDF>8 are similar for some variables such as ruminal pH, and can

be used interchangeably to predict the risk of SARA. However, their effects differed for

the prediction of some other physiological variables such as chewing and rumination

activity and DMI, which were better predicted by peNDF>8 (Zebeli et al., 2010) than by

peNDF>1.18 (Zebeli et al., 2008). This finding is in line with the already stated fact that

longer particles contribute better to rumen mat formation and, the peNDF>8 is a better

predictor of physical fill in the reticulorumen. The main outcome of their modeling

approach was that the effect of peNDF on different response variables showed

breakpoints where a plateau was reached; that is, inclusion of peNDF above the breakpoint did not affect the response variable any further (Figure 2.2). For example, the discovery of similar asymptotic associations between ruminal pH and rumination time with dietary peNDF>8 up to 16.4 to 20.6% in the diet indicated the presence of physiological limits beyond which peNDF>8 could not further improve rumination and rumen buffering in lactating dairy cattle. However, feeding diets with an excess of peNDF>8 was shown to decrease feed intake. Other studies have shown that feeding excessive amounts of peNDF can lower both feed intake and efficiency of feed use (Yang and Beauchemin, 2007; Zebeli et al., 2008). Consequently, the determination of a breakpoint in dietary peNDF is important beyond which no further advantages on ruminal pH response can be expected, particularly in terms of maximization of production responses in high-producing dairy cows. Nutritional strategies for high-yielding dairy cows aim to maximize energy and nutrient intake to support the high nutrient demand and prevent disorders related to energy deficiency, such as ketosis. This challenge becomes particularly relevant during early lactation. For high-producing early lactating cows, a beneficial feeding strategy is to decrease the amount of ruminally degradable starch in the diet, thereby reducing the requirement of peNDF, which, in turn, can increase the DMI by roughly 2 kg/d (Silveira et al., 2007; Lechartier and Peyraud, 2010). This strategy might be a useful alternative for cows with high feed intake and milk production potential to meet their fiber requirements without impairing lactational performance.

Besides considering grain fermentability, the digestibility of forages in the diet is also believed to play a role in the cow's response to dietary peNDF. The rationale behind this relationship is that forages with greater fermentability, such as BMRCS may be

physically more fragile, and thus less effective in stimulating chewing and rumination (Taylor and Allen, 2005c). This potentially could have a negative impact on chewing activity and thus ruminal pH. However, Oba and Allen (2000b) found that rumination time per day was not different between conventional corn silage (CCS) and BMRCS, and furthermore BMRCS was just as effective as CCS in stimulating chewing activity when processed properly. In addition, Zebeli et al. (2010) found that forages with greater fragility had lower degradation rates in the rumen than grains and by-product feeds. With lower degradation rates, rapid VFA accumulation in the rumen was not observed (Zebeli et al., 2010). Previous research in our lab found that BMRCS and CCS had different particle size distribution after processing, with slightly lower peNDF>8 for BMRCS compared with CCS (Holt et al., 2010). In our study, minimum ruminal pH was maintained at least at 5.70, and pH less than 5.8 rarely occurred, signifying that no dietary treatments interfered with ruminal fermentation due to adequate supply of forage NDF and its particle size (Holt et al., 2010). However, milk fat concentration was decreased when feeding BMRCS and was further decreased by feeding non-forage fiber by-products (soyhulls and beet pulp) in the BMRCS-based diet (Holt et al., 2010).

RUMINAL pH

Feeding a more fermentable forage source like BMRCS can lower ruminal pH (Oba and Allen, 2000c; Greenfield et al., 2001) and may reduce ruminal NDF digestibility (Oba and Allen, 2000c). Ruminal pH is lowered when feeding a highly fermentable grain source such as team rolled barley. This causes a decrease in ruminal NDF digestibility; however, ruminal starch digestibility is increased (Overton et al., 1995). Extensive

research has been conducted to understand the effect of low ruminal pH on ruminal

cellulolytic bacteria and the resulting effects of fiber digestion. Chow and Russell (1992)

investigated the response of a predominant cellulolytic bacterium, *Fibrobacter*

succinogenes, to decreasing extracellular pH. They noted at pH levels 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 bacterium, 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). Some studies have reported a decrease in ruminal

pH when cows were fed BMRCS-based diets (Greenfield et al., 2001; Taylor and Allen,

2005a; Gehman et al., 2008). This may have been caused by the increased supply of

fermentable substrate in the rumen due to enhanced NDF digestibility of the BMRCS

(Weiss and Wyatt, 2006). Taylor and Allen (2005c) found that lower ruminal pH for

BMRCS compared with CCS (5.99 vs. 6.22) corresponded with a 3.5m*M* higher total

VFA concentration, suggesting that BMRCS lowered ruminal pH by increasing total

VFA concentration. Other studies reported no effects of feeding BMRCS on total VFA

concentration or ruminal pH (Qiu et al., 2003; Weiss and Wyatt, 2006; Holt et al., 2010).

Gehman et al. (2008) found that the inclusion of monensin in BMRCS-based diets increased ruminal pH by 0.14 units. However, treatments with BMRCS appeared to have negatively affected N digestibility. Reductions in the digestibility of some nutrients when cows consumed BMRCS-based diets may have been caused by increased DMI and possibly increased digestion in the lower gut. The increase in DMI did not affect NDF digestibility, but appeared to have negatively affected N digestibility (Gehman et al., 2008).

NITROGEN METABOLISM AND DIGESTIBILITY

Due to extensive degradation and alteration in the rumen, protein composition and mass absorbed by the animal is different than feed protein consumed by the animal. Nitrogen (**N**) metabolism in the ruminant is a complex pathway involving multiple mechanisms (Figure 2.3: Van Soest, 1994). Feed protein may be categorized as ruminally degradable (**RDP**) or undegradable protein (**RUP**; NRC, 2001). In the rumen, microbes utilize non-protein nitrogen (**NPN**) and true protein from RDP to support growth, and this represents an important source of protein to the animal (Bach et al., 2005). Endogenous protein is another important source of protein to the animal. It originates from sloughing and abrasion of epithelial tissues in the mouth, esophagus, rumen, reticulum, omasum, and abomasum as well as enzymes secreted in the abomasum (Tamminga, 1992). Therefore, metabolizable protein (**MP**) may be defined as protein and amino acids (**AA**) available to the animal for absorption in the small intestine, providing peptides and AA to the ruminant animal. It is comprised of RUP, endogenous protein (**ECP**), and microbial protein (NRC, 2001). Following digestion and absorption in the small intestine, MP

provides the animal with AA to support maintenance, milk production, growth, and pregnancy (NRC, 2001). Nitrogen from MP absorbed in excess of the animal's needs, as well as protein not contributing to MP, is secreted in milk or excreted in feces and urine (Van Soest, 1994).

Microbial protein (**MCP**) consists of protein from rumen bacteria, protozoa, and fungi that pass out of the rumen and enter the small intestine. Bacteria provide most of the MCP available post-ruminally. While protozoa may make up 20 to 70% of the total rumen biomass, protozoa are extensively recycled in the rumen, and therefore may not contribute as much to MCP flow to the duodenum (Jouany, 1996). Ruminally synthesized MCP is an important source of AA for all ruminants, especially those with high nutrient requirements, such as rapidly growing and lactating animals (Bach et al., 2005). Clark et al. (1992), in a review of 152 dietary treatments, estimated MCP composed 34 to 89% of the flow of AA to the duodenum. The AA composition of MCP closely matches the requirements for lactation and growth in ruminants (Table 2.1) and is highly digestible (80%; NRC, 2001).

Rumen microbes are the sole means by which ruminants receive high-quality protein from NPN sources as well as RDP. The two major dietary components required for MCP synthesis in the rumen are RDP and fermentable energy.

Ruminally Available Protein

In order for microbial growth to occur in the rumen, microbes require N to be assimilated into microbial protein. The potentially ruminally degradable pool of protein includes feed protein in addition to endogenous protein from saliva, sloughed epithelial cells, and lysed ruminal microbes (Bach et al., 2005). All enzymatic degradation of

protein in the rumen is of microbial origin (Bach et al., 2005). Nitrogen requirements are generally determined by the function of the microbe itself. Ruminal bacteria which ferment structural carbohydrate, such as cellulose, are believed to utilize ammonia exclusively as an N source. In comparison, bacteria which ferment non-structural carbohydrate, such as starch, are believed to utilize both ammonia and peptides or AA and produce ammonia (Russell et at., 1992). Since protozoa ingest particulate matter instead of attach like bacteria, they are able to utilize bacteria as their primary protein source as well as insoluble feed protein such as soybean or fish meal (Jouany, 1996).

Results from research on ruminal ammonia concentrations required for optimal MCP synthesis are variable. Optimal rumen ammonia concentrations are such that additional ammonia supplied from RDP degradation is not incorporated into MCP and does not result in increased MCP flow, but rather increases rumen ammonia concentration. Satter and Slyter (1974) suggested a concentration of 5 mg/100 mL ammonia as the minimum required to maximize efficiency of MCP synthesis in the rumen based on work with continuous cultures. Studies examining ruminal ammonia concentration requirements in vitro differ from in vivo studies. Reynal and Broderick (2005) observed a linear increase in MCP synthesis with ruminal ammonia increasing up to 12.3 mg/100 mL. Similarly, Boucher et al. (2007) observed optimal MCP synthesis at ruminal ammonia concentrations between 11 and 13 mg/100 mL. Higher ruminal ammonia concentrations were related to decreased MCP synthesis, indicating the optimal ammonia concentration had been met (Boucher et al., 2007). The difference between in vitro and in vivo studies may be due to a more stable ammonia concentration in in vitro fermentative environment allowing for lower ammonia concentrations for optimal MCP synthesis. However, in vivo

measurements under practical conditions (e.g., twice daily feeding, individual meal consumption) may be more useful to determine ruminal ammonia concentration requirements for optimal MCP synthesis in lactating dairy cows.

Amount of available RDP may affect MCP synthesis. Boucher et al. (2007) incrementally added urea to a basal diet that was RDP deficient based on NRC (2001) estimates, resulting in RDP concentrations ranging from 9.2 to 11.6% DM. Microbial protein synthesis responded quadratically to increasing RDP, with rations containing RDP at 10.0 and 10.8% DM resulting in the greatest MCP synthesis. Mabjeesh et al. (1997) also observed a decrease in MCP synthesis with increased RDP (11.7 vs. 10.9% DM). The mechanism causing depressed MCP synthesis at high RDP concentrations is not clear. Reynal and Broderick (2005) fed rations ranging in RDP from 10.6 to 13.2% DM and observed increasing MCP synthesis with increased RDP. The differences between these studies may be due to differences in rations of basal diets, such as source and amount of available carbohydrate and ruminal pH.

Microbial growth and fermentation are dependent on both amount and type of N available in the rumen. Rumen microbes require a mixture of ammonia, peptides, and AA, depending on their fermentation substrate. Because dairy cattle are fed a mixed ration containing both structural and non-structural carbohydrate, a blend of NPN and ruminally available true protein is required to maximize MCP synthesis (Brito et al., 2007). In addition, Brito et al. (2007) observed increased MCP synthesis when rations included soybean meal, cottonseed meal, or canola meal as protein sources as compared with urea. As discussed previously, ration RDP concentrations in a study done by Reynal and Broderick (2005) were higher than those reported by Boucher et al. (2007), but there

was no observed depression of MCP synthesis at higher RDP concentrations. Differences between experiments may have been due to the mixture of RDP sources fed by Reynal and Broderick (2005), including soybean meal, bypass soybean meal, and urea, providing NPN and soluble true protein as compared with urea alone by Boucher et al. (2007). This reinforces the idea that rumen microbes require NPN as well as true protein sources for maximal growth.

Ruminally Available Energy

Ruminally available energy is the most important and limiting factor determining MCP synthesis in lactating dairy cattle. Ruminal microbes depend on carbon skeletons and the availability of ATP for protein synthesis. Microbes are only able to utilize carbohydrates or secondary products of carbohydrate digestion for growth, and microbial yield depends on their growth rate and the fractional degradation of the carbohydrate (Nocek and Tamminga, 1991).

In general, feed intake is a good predictor of MCP synthesis, as increased intake indicates increased substrate availability for microbial fermentation (Broderick, 2003). Clark et al. (1992) summarized 39 experiments and reported a positive relationship between organic matter (**OM**) intake and non-ammonia N (**NAN**) passage to the duodenum. The increased passage of MCP can likely be attributed in part to the increased energy supplied, as OM intake increased. The increased MCP synthesis may also be attributed to reduced nutrient recycling by ruminal microbes. The increase in OM digestion supplies additional nutrients for microbial growth, and the faster rate of growth coupled with faster passage of microbes out of the rumen will reduce recycling of energy and N due to decreased cell lysis (Clark et al., 1992). This will reduce maintenance

requirements for microbes and leave more nutrients available for growth. Van Soest (1994) suggests as intake increases, more feed particles exit the rumen in an earlier stage of digestion with more microbes attached. Thus, microbial recycling is reduced with increased flow of feed OM, resulting in increased MCP flow.

Effects of ruminal degradability of starch on MCP synthesis are variable. Overton et al. (1995) observed that replacing corn with a more rapidly degradable source of starch (i.e., barley) increased MCP yield (1606 vs. 1762 g/d) and proportion of MCP in NAN (46 vs. 56%). Krause et al. (2002) reported an increase in MCP synthesis for rations containing high-moisture corn compared with dry-rolled corn (2,278 vs. 1,969 g/d). In contrast, Sannes et al. (2002) replaced a portion of corn grain with sucrose and observed a reduction in MCP yield (2,091 vs. 1,830 g/d). Yang and Beauchemin (2004) observed increased MCP synthesis with increased forage-to-concentrate ratio, indicating reduced ruminally available energy, and reported no difference between coarse- and flat-rolled barley. Amount of starch included in a ration may have a greater effect on MCP synthesis than degradability. Oba and Allen (2003b) compared 2 types of corn (high-moisture and dry ground) fed at 2 levels (31.6 and 21.2% DM). The amount of truly ruminally degraded OM was higher for high-moisture corn and 31.6% starch treatments, while ruminal ammonia and MCP yield were only affected by starch level. Ruminal ammonia concentrations were reduced for the higher starch rations, indicating more N was captured by the rumen microbes as was evident in the observed increase in MCP yield (2,190 vs. 2,869 g/d). These data indicate that amount rather than degradability of starch may be more influential to MCP synthesis.

Microbial Growth Efficiency

In addition to adequate supplies of energy and N sources, other non-nutritional factors, such as ruminal pH and dilution rate, also play an important role in MCP synthesis. The ultimate goal of proper rumen nutrition is to maximize microbial growth and the amount of RDP that is captured into rumen microbial cells. Maximizing the capture of degradable N not only improves the supply of AA to the small intestine, but also decreases N losses. Microbial growth efficiency is defined as grams of microbial protein flow to the duodenum per kg of truly ruminally degraded OM **(TRDOM).** Energy availability is often the primary limitation on microbial growth (Clark et al., 1992), and so generally any increase in fermented OM would increase microbial growth. However, Clark et al. (1992) found a quadratic relationship between TRDOM (kg/d) and efficiency of MCP 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. The authors speculated that a process known as energy spilling or uncoupling occurred (Oba and Allen, 2003b). 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).

Taylor and Allen (2005c) found reduced microbial N efficiency (**MNE**) when vitreous grain was replaced with floury endosperm corn grain. Greater rate and extent of starch digestion and low ruminal pH for floury endosperm corn grain (Taylor and Allen, 2005b) might have reduced MNE by uncoupling microbial growth from fermentation of

substrate. Low ruminal pH increases maintenance requirements of microbes to maintain ion balance across the cell membrane (Russell, 1998). In their experiment, MNE was negatively correlated with ruminal starch digestibility and positively correlated with mean ruminal pH (Taylor and Allen, 2005b). 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. Oba and Allen (2003a) found that high moisture corn reduced MNE without affecting ruminal pH compared with dry ground corn. This suggests that MNE can be decreased, as ruminal starch digestibility increases independent of effects on ruminal pH.

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 BMRCS increased microbial efficiency, possibly because of increased ruminal passage rate of NDF, despite a reduction of ruminal pH by BMRCS (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 growth 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 to a greater extent by low ruminal pH.

Measuring Microbial Protein

There are a variety of markers used to measure post-ruminal MCP flow. Purines are commonly used to quantify rumen microbial growth. Nucleic acids are composed of purine bases, adenine and guanine, and pyrimidine bases, cytosine, uracil, and thymine. These purine and pyrimidine bases provide the building blocks for DNA and RNA. Using the purine:N ratio of the microbial biomass, purines present in post-ruminal digesta can be used to calculate MCP flow. Various methods have been published outlining isolation and quantification of purines from feedstuffs and digesta, but the most common method was described by Zinn and Owens (1986). There are several assumptions made when a researcher uses purines as a microbial marker. One assumption is that all purines present post-ruminally are of microbial and endogenous origin and little to no dietary nucleic acids are reaching the small intestine. Endogenous purines are end-products of cell

turnover in the rumen and can be accounted for, as they are excreted at a constant rate based on BW, (0.385 mmol/BW$^{0.75}$; Chen and Gomes, 1992). For most feedstuffs, very little nucleic acid is present post-ruminally (Djouvinov et al., 1998). Djouvinov et al. (1998) measured the effective degradability of purines in common feedstuffs, including grass, legume, and cereal forages and protein sources, and found low purine concentration in most feedstuffs, and purines were degraded in the rumen at 80-90%. The exception was fishmeal, which has high purine concentration and low (41%) ruminal degradability, suggesting purines may overestimate MCP synthesis for rations with fishmeal. The assumption that most feed purines are degraded in the rumen has, for the most part, been validated. The presence of feed purine post-ruminally may inflate MCP estimates based on purines, but relative treatment differences should remain true. The other major assumption required for estimating MCP using purines is the ratio of purine:N is constant for a mixed microbial population and the ratio is not affected by dietary treatment. Ruminal microbes are rich in nucleic acids; however, the absolute concentration of purines as a proportion of the total N in ruminal microbes is inconsistent in the literature. Overton et al. (1995) fed TMR with varying ratios of corn to barley to lactating cows and found no effect of treatment on purine:N, averaging 1.22. Clark et al. (1992) summarized data from several studies and found purine:N ratios averaged 0.94 with a range of 0.47 to 1.64. Another common marker to estimate MCP synthesis is urinary purine derivatives (**PD**). When purines of microbial or endogenous origin exit the rumen, they are digested in the small intestine and absorbed as a purine nucleoside, adenine or guanine, and free bases. In cattle, there is high activity of xanthine oxidase in the intestinal mucosa and blood, and all purines are metabolized to PD, allantoin, uric

acid, xanthine, and hypoxanthine (Al-Khalidi and Chaglassian, 1965). Purine derivatives are excreted primarily in urine but can also be excreted in milk. Due to high activity of xanthine oxidase, little xanthine or hypoxanthine is excreted in cattle (Martin-Orue et al., 2000). Without having to account for xanthine or hypoxanthine, allantoin and uric acid compose approximately 85 to 90% and 10 to 15% of urinary PD, respectively (Verbic et al., 1990). While directly measuring purines from duodenal samples is ideal, using urinary PD to estimate MCP has been found to be satisfactory (Vagnoni et al., 1997; Martin-Orue et al., 2000; Moorby et al., 2006). Reynal et al. (2005) reported microbial N flow estimated by omasal purine output to be higher than that estimated by urinary PD; however, there was no marker by treatment interaction, indicating relative differences induced by dietary treatments remained similar. Similar assumptions are required to use PD to calculate MCP flow as using purines; primarily, only microbial and endogenous purines exit the rumen and microbial biomass has an equal purine:N ratio.

Site of sample collection used for MCP marker analysis may be based on access to ruminally and/or duodenally fistulated animals as well as the number of animals required to detect differences among treatments. Measuring purines in duodenal samples is ideal but requires duodenally fistulated animals. This not only limits the number of animals that can be used due to availability and time constraints during sampling, but duodenally fistulated animals have a shortened life span and require special care. In comparison, ruminal fistulation is a fairly simple procedure with relatively short recovery period, and animals generally do not require special care, allowing them to be managed the same as an intact animal. A ruminal fistula will allow for access to the omasum, and omasal sampling has been shown to be an alternative to duodenal sampling for studying post-

ruminal flow of nutrients (Ahvenjarvi et al., 2000). Ipharraguerre et al. (2007) reported

similar microbial N flows using purines measured from either omasal or duodenal

samples. It has been suggested the triple marker method (France and Siddons, 1986)

should be used to mathematically reconstitute true digesta due to fractionation of markers

(cobalt (**Co**), ytterbium (**Yb**), chromium (**Cr**), or indigestible NDF) into different digesta

fractions (liquid, small particulate, or large particulate phases). The benefits of omasal

over duodenal sampling for measuring post-ruminal nutrient flow are avoiding effects of

abomasal digestion and secretions and not requiring a duodenally fistulated animal;

however, omasal sampling is more labor intensive and requires the use of multiple

markers, which increases the probability of compounding analytical errors. For example,

Ahvenjarvi et al. (2003) found good agreement between Cr alone and a triple marker

system (Co + Yb + Cr) for estimating flow of OM (r = 0.98) and NDF (r = 0.88) at the

omasal canal, likely due to increased analytical error. The benefits of estimating MCP

synthesis via urinary PD excretion compared with direct measurement of purines post-

ruminally are the ability to use intact animals, increasing sample size, and no need for a

flow marker. Regardless of microbial marker or sampling site, measuring MCP synthesis

remains an imprecise and problematic area in ruminant nutrition.

Ruminally Undegradable Protein

Ruminally undegraded protein is not digested by rumen microbes to be used for

protein synthesis, but does provide a source of AA to the animal contingent on digestion

in the small intestine. The goal of feeding RUP is to complement the AA profile of MCP

in order to maximize N use efficiency as well as to meet AA requirements of the animal

that MCP alone cannot meet (Santos et al., 1998). Ruminally undegradable protein is

assumed to be 100% true protein, and digestibilities vary among feedstuffs, ranging from 50 to 100% digestible (NRC, 2001). The estimates of RUP digestibility assigned to each feedstuff in the NRC (2001) are the approximate mean values as reported in the literature using the mobile bag technique and the three-step procedure according to Calsamiglia and Stern (1995). This procedure approximates ruminal, abomasal, and intestinal digestion. Due to differences in digestibilities of feedstuffs, the conversion of RUP to MP is variable (NRC, 2001).

Flow of RUP to the small intestine is primarily influenced by ration RUP concentration and extent of N digestion in the rumen. Addition of feed sources high in RUP, such as blood meal, corn gluten meal, or treated soybean meal, to rations containing soybean meal have been found to increase flow of non-ammonia, non-microbial N (**NANMN**) flow to the duodenum (Santos et al., 1998). Non-ammonia, non-microbial N measured at the duodenum represents RUP and the endogenous protein fractions of MP. Since endogenous protein is assumed to be dependent on DMI (NRC, 2001) and is not likely affected by protein source in a ration, NANMN can be used as an indicator of effect of diet on RUP flow. Microbial protein synthesis may be reduced when RUP replaces RDP sources due to reduced ruminally available N. Increased supply of NANMN and decreased MCP flow may cancel each other out, resulting in no net change in non-ammonia N (**NAN**; i.e., MP) flow to the duodenum (Santos et al., 1998). At equal N intake of rations containing soybean meal (RUP at approximately 20% DM) or fish meal (RUP at approximately 40% DM), Zerbini et al. (1998) observed reduced MCP flow and increased residual N (total – microbial N) flow at the duodenum for animals consuming fish meal, resulting in similar total N flow to the duodenum.

In addition to N concentration and source, particle size of feed may influence the proportion of RUP and MCP in MP. When the particle size of a ration is reduced, less dietary RUP passes through the rumen and enters the duodenum (Rodriquez-Prado et al., 2004; Yang and Beauchemin, 2006). Processing forages to a smaller particle size increases the rate of rumen N degradation, effectively increasing RDP and decreasing RUP of forage (Yang and Beauchemin, 2004). This allows for a greater contribution by MCP to MP. These studies indicate that N intake, source and amount of CP, and N degradation in the rumen influence RUP flow to the duodenum.

PARTITIONING OF N

Following digestion in the small intestine and utilization for maintenance, lactation, and pregnancy requirements, MP exits the ruminant's body as fecal and urinary N as well as secretion of milk N (Figure 2.3). Fecal N losses can be categorized into 3 groups: undigested RUP, endogenous and metabolic N, and undigested MCP (Tamminga, 1992). The NRC (2001) assumes 80% digestibility for both endogenous protein and MCP. Greater microbial fermentation of both structural (i.e., NDF) and non-structural (i.e., starch) carbohydrates in the large intestine can increase fecal N excretion, thus increasing metabolic N losses in feces (Firkins and Reynolds, 2005). Most feed proteins have a high true digestibility (> 80%; NRC, 2001), and so rations with higher RUP would not be expected to result in higher fecal N when N intake is similar (Davidson et al., 2003). Increased fecal N observed for rations with high RUP may be due to increased DMI stimulated by RUP and not due to excretion of undigested feed protein (Flis and Wattiaux, 2005). Huhtanen et al. (2008) summarized treatment means from 207 lactation

trials and observed fecal N output better associated with DMI than N intake, while the prediction was improved when both were included in the model. Including both DMI and N intake in prediction equations for fecal N excretion is logical, as metabolic and endogenous N, which are major contributors to fecal N, are related to DMI (Van Soest, 1994), while undigested feed N is related to N intake (Huhtanen et al., 2008). Huhtanen et al. (2008) estimated that every 1-kg increase in DMI corresponded to 6.7-g increase in fecal N output.

Urinary N can result from loss of ammonia from the rumen, metabolic losses from the gut, maintenance losses, and inefficient conversion of absorbed AA into milk or body proteins (Tamminga, 1992). Nitrogen has been found to be excreted equally in feces and urine when animals consume low levels of N ($< 400g$ N/d); however, at higher N intake (> 400 g N/d), there is an exponential increase in N excreted in urine (Castillo et al., 2001). Similar to fecal N, Huhtanen et al. (2008) found including both N intake and DMI in a model to predict urinary N excretion improved precision. Researchers have also found a positive correlation between urinary N excretion and milk urea N (**MUN**; Kauffman and St-Pierre, 2001; Nennich et al., 2006). Excess dietary N is converted to urea, which is a soluble compound that will diffuse into various body fluids, such as blood, milk, and urine (Kauffman and St-Pierre, 2001). Nennich et al. (2006) compiled data from 16 individual feeding studies comprised of 372 data points for lactating Holstein cows and found MUN to be an excellent predictor of urinary N excretion. Milk N secretion can be separated into MUN and true protein. As discussed above, MUN represents a waste product of incomplete capture of ammonia in the rumen. High MUN values are associated with rations high in CP, specifically high RDP, which may be

related to high urinary and total N excretion. High MUN values are associated with high levels of circulating urea (DePeters and Ferguson, 1992), which may have negative impacts on reproduction (Butler et al., 1996). While MUN is directly influenced by nutritional factors, the effect of diet on milk protein secretion is more subtle and inconsistent.

Effect of CP Intake on N Excretion

Due to extensive alteration of N from feed in the rumen, dietary CP concentration does not adequately describe the amount or composition of MP supplied to the animal; however, some research examining the effects of N intake on N excretion focused on CP concentration of the ration. Increasing dietary CP concentrations generally corresponds with increased N intake and may often result in increased N excretion from feces, urine, and milk (Groff and Wu, 2005; Olmos Colmenero and Broderick, 2006 a,b). Groff and Wu (2005) fed rations with increasing CP concentrations from 15.0 to 18.8% DM and observed increased fecal, urinary, and MUN N excretion with increasing CP level, with small and varying differences in milk protein yield. Olmos Colmenero and Broderick (2006b) observed similar responses to rations ranging in CP concentration from 13.5 to 19.4% DM, resulting in increased fecal, urinary, and MUN N excretion and a quadratic milk protein yield response, peaking at 16.5% CP. In both studies, DMI was not affected by CP concentration, but N digestibility and intake increased linearly with increased CP, indicating N intake and absorption drove N excretion. Wattiaux and Karg (2004) also observed an increase in urinary N and MUN excretion when RDP and RUP were fed at 105 and 117% of NRC (2001) requirements with no effect on fecal N excretion or milk protein yield. Apparent total tract N digestibility increased with increased CP

concentration, indicating the increased urinary N was likely due to incomplete conversion of absorbed AA into tissue or milk protein as opposed to loss from the rumen due to excess RDP. This study agrees with the assumption that excess N intake is excreted primarily in urine (Castillo et al., 2001). Olmos Colmenero and Broderick (2006b) suggested feeding dairy cattle rations containing 16.5% CP in order to support maximum milk and protein production while minimizing N excretion compared with rations with higher CP concentration.

Effect of Forage Type on N Excretion

Forage type may also affect N partitioning and excretion. Corn and alfalfa silages are forages commonly fed together in rations for dairy cattle. The fermentable starch found in corn silage may complement the RDP found in alfalfa silage in providing a fermentable source of carbohydrate to the rumen microbes, which may decrease ruminal N losses. In addition, corn silage is lower in CP than alfalfa silage (9.0 vs. 20.0%; NRC, 2001), and therefore feeding corn silage by replacing alfalfa silage decreases CP concentration of the ration, which may lower urinary N and total N excretion as discussed above. Brito and Broderick (2006) demonstrated a linear decrease in both fecal and urinary N excretion, as corn silage replaced alfalfa silage. This is likely due to a linear decrease in both DMI and N intake when corn silage replaced alfalfa silage. There was a quadratic response of forage on milk protein yield with the maximum secretion occurring when alfalfa and corn silage were included in the ration at 37 and 13% DM and 24 and 27% DM, respectively. Based on these findings, the authors recommend feeding alfalfa and corn silage in roughly equal proportions (24 and 27% of the ration) in order to capture the positive interactions of these forages on DMI, milk and milk protein yield, and N utilization (Brito

and Broderick, 2006). Wattiaux and Karg (2004) observed that the percentage of consumed N that was excreted via feces decreased and the percentage excreted via urine increased when dairy cows were fed corn silage-based diets compared with the percentages when cows were fed alfalfa-based diets. In isonitrogenous diets, replacing byproduct NDF with starch increased urinary N excretion and decreased fecal excretion of N (Castillo et al., 2001; Hristov and Ropp, 2003). The shift from urine to fecal excretion of N as fiber replaces starch could be caused by hindgut fermentation of the fiber (Gressley and Armentano, 2007) or by lower microbial capture of RDP in the rumen, because fiber is usually less fermentable than starch. Overall intake of N affects the amount of N excreted via manure, whereas types of carbohydrate and forage have greater impacts on the route (fecal or urinary) of excretion.

Limited data suggest that feeding BMRCS may decrease excretion of N, especially via urine (Greenfield et al., 2001; Tine et al., 2001). The increased consumption of rumen fermentable OM by cows fed BMRCS may allow greater conversion of RDP into MCP, thereby decreasing ammonia absorption through the rumen and lowering urinary N excretion. Nitrogen intake has a strong influence on N excretion; therefore, Weiss and Wyatt (2006) conducted a statistical analysis using N intake as a continuous variable and corn silage hybrid as a fixed effect to determine more accurately the effects of feeding BMRCS vs. CCS at a given N intake. Corn silage hybrid did not affect excretion of fecal or urinary N but did affect excretion of N in manure and milk. At a given N intake, cows fed BMRCS excreted 14.8 g/d less manure N and secreted 10.2 g/d more milk N than cows fed CCS. However, the effect of corn silage hybrid on manure N excretion was quantitatively small compared with the effect of N intake. On average, feeding the low

CP diets decreased manure N excretion by approximately 21% compared with feeding the high CP diets, whereas feeding diets with BMRCS decreased manure N excretion by 3.6%. In other experiments in which DMI was similar between cows fed BMRCS and CCS (Greenfield et al., 2001; Tine et al., 2001), excretion of N via manure was decreased between 3.3 and 7.8% when BMRCS was fed. Weiss and Wyatt (2006) reported that feeding BMRCS rather than CCS would have the same effect on manure N excretion as would feeding approximately 22 g/d less dietary N.

Environmental Concerns with N Excretion

Dairy farming is known to contribute to both air and water pollution, primarily due to ammonia emissions from urinary N and nitrates from fecal N in soil and ground water (Tamminga, 1992). Nennich et al. (2005) summarized data from 554 lactating cows from several experiments and estimated the average dairy cow weighing 625 kg, producing 40 kg/d milk with 25 kg/d DMI and 0.68 kg/d N intake would excrete 0.44 kg N/d in manure, approximately equally in urine and feces. About 80% of N consumed in excess of 500 g/d is believed to be excreted in urine (Castillo et al., 2001; Kereab et al., 2001). Urinary N is primarily urea (50 to 90% of N) and is rapidly converted to ammonia by ubiquitous ureases on the pen floor, soil, and manure slurry (Bussink and Oenema, 1998). Ammonia is recognized as an important air, soil, and surface water pollutant, and a major source of ammonia is from livestock manure. Specifically, dairy cows produce more ammonia per animal than other livestock species due to high consumption of high protein rations, large urinary output, and high concentration of urinary N (Nennich et al., 2006). As discussed above, decreasing dietary CP concentration and N intake can not only decrease urinary N, but total N excretion. Identifying factors that affect the gross

efficiency of N use by dairy cows (N output in milk divided by N intake) continues to be a major research area in several areas of the world, because efficiency is relatively low (Weiss and Wyatt, 2006). Research concentrating on decreasing N concentration and intake by dairy cattle while maintaining production is imperative to lowering the environmental impacts of dairy farming.

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Table 2. 1. Essential amino acid profiles of body tissue, milk protein, ruminal bacteria, and common feedstuffs[1]

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
	% of total essential amino acids									
Lean tissue	16.8	6.3	7.1	17.0	16.3	5.1	8.9	9.9	2.5	10.1
Milk protein	7.2	5.5	11.4	19.5	16.0	5.5	10.0	8.9	3.0	13.0
Ruminal bacteria[3]	10.2	4.0	11.5	16.3	15.8	5.2	10.2	11.7	2.7	12.5
Corn silage	6.2	5.7	10.6	27.2	7.9	4.8	12.1	10.1	1.4	14.1
Soybean meal	16.2	6.1	10.1	17.2	13.9	3.2	11.6	8.7	2.8	10.2
Corn	11.5	7.8	8.2	27.9	7.1	5.3	11.5	8.8	1.8	10.0
Blood meal	7.8	11.3	2.2	22.7	15.9	2.1	12.1	7.7	2.8	15.4

[1]NRC, 2001

[2] Arg = Arginine; His = Histidine; Ile = Isoleucine; Lys = Lysiine; Met = Methionine; Phe = Phenylalanine; Thr = Threonine; Trp = Tryptophan; Val = Valine.

[3]Clark et al., 1992
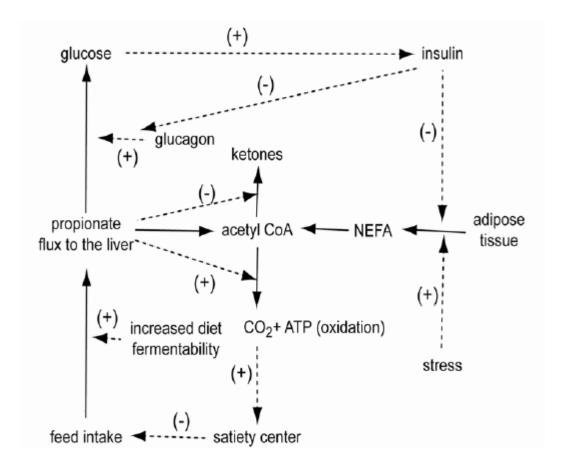
Figure 2.1. Model of control of feed intake by hepatic oxidation theory in ruminants (adapted from Allen et al., 2009). Although propionate is used for gluconeogenesis, there is high flux of carbon from propionate through pyruvate kinase (Steinhour and Bauman, 1988), allowing oxidation depending upon the fate of pyruvate. Oxidation of propionate within a meal increases the energy state of hepatocytes, generating a satiety signal to terminate the meal. Hepatic oxidation of NEFA is limited during meals because increased insulin release inhibits lipolysis in adipose tissue and uptake of NEFA by the liver (Vasilatos and Wangsness, 1980).
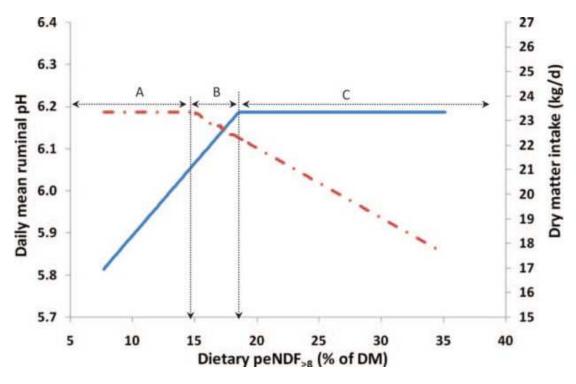
Figure 2.2. Determining Requirements for Physically Effect NDF (adapted from Zebeli et al., 2010). Best-fit broken line models describing the conflicting associations among dietary physically effective fiber (measured inclusive particles > 8 mm; $peNDF_{>8}$) with daily mean ruminal pH (solid line) and DMI (dashed dotted line) in dairy cows. A: This area is typical for high-producing cows in early lactation with very high energy and nutrient demands (> 23.5 kg of DMI/d) at an imminent risk of subacute ruminal acidosis (SARA); the same area also pertains to peNDF-limited diets, and grain fermentability becomes highly important in these diets to mitigate low levels of peNDF. B: Diets falling in this area are potentially limited in peNDF but, due to lower feed intake (< 23.5 kg of DM/d), these cows are at a lower risk of SARA than cows in the (A) area of the graph; however, grain fermentability is still important in these potentially peNDF-limited diets. C: This area is typical for average-producing dairy cows in mid and late lactation consuming < 22 kg of DM/d, which are at no risk of SARA, because these diets are not

limited in peNDF; hence, grain fermentability in the diet does not play a role in

modulating the risk of SARA.
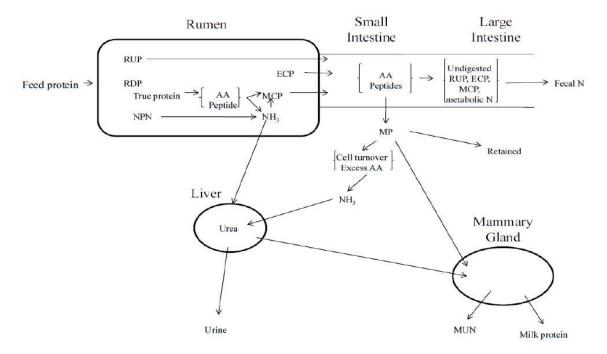
Figure 2.3. Nitrogen metabolism in the ruminant (adapted from Van Soest, 1994)

RUP = ruminally undegradable protein; RDP = ruminally degradable protein; NPN = non-protein nitrogen; AA = amino acid; ECP = endogenous protein; MCP = microbial protein, MP = metabolizable protein; and MUN = milk urea nitrogen.

CHAPTER 3

EFFECTS OF FEEDING BROWN MIDRIB CORN SILAGE WITH A HIGH DIETARY CONCENTRATION OF ALFALFA HAY ON LACTATIONAL PERFORMANCE OF HOLSTEIN DAIRY COWS FOR THE FIRST 180 DAYS OF LACTATION[1]

INTRODUCTION

Anecdotal reports by dairy producers and observations by dairy nutritionists indicate that over the past decade, dairy producers have increased their use of corn silage (**CS**) as a forage source in dairy rations. This has been influenced by the high price of feed, especially corn grain, and the high energy content of CS. Feeding forage levels at 55 to 60% of dietary DM is becoming more common, but lack of energy from concentrates and distention from rumen fill may limit DMI and reduce performance of high producing dairy cows. Intake of DM is critical for dairy cows to achieve high milk production. Therefore, great emphasis has been placed on dietary factors affecting the DMI of lactating dairy cows. Physical fill can be the most dominant mechanism limiting DMI for high yielding cows around peak lactation (Allen, 2000), but it may contribute less in early lactation (Ingvartsen and Andersen, 2000). During the transition period, control of feed intake is likely dominated by hepatic oxidation of NEFA (Allen et al., 2009). At freshening DMI does not meet the energy requirements for maintenance and production

[1] Holt, M. S., J.-S. Eun, C. R. Thacker, A. J. Young, X. Dai, and K. E. Nestor Jr. 2013. Effects of feeding brown midrib corn silage with a high dietary concentration of alfalfa hay on lactational performance of Holstein dairy cows for the first 180 days of lactation. J. Dairy Sci. 96:515–523.

of high producing cows. This results in a negative energy balance accompanied by an increase in the incidence of various metabolic disorders and a reduction in reproductive performance (van Knegsel et al., 2005). Thus, minimizing negative energy balance and maximizing energy intake are among the most critical management aspects associated with feeding dairy cows in early lactation. Finding an optimal balance between physically effective fiber and readily fermentable carbohydrates is difficult but crucial not only for maintaining proper ruminal metabolism (Zebeli et al., 2006; Plaizier et al., 2008), but also for maintaining a stable metabolic health status while enhancing productivity (Ametaj et al., 2010; Zebeli et al., 2011).

Peak milk yield can be maximized by feeding diets with low rumen-fill capacity that are typically highly fermentable. The rumen-filling effect of diets is influenced most by concentration, digestibility, and fragility of forage NDF (Allen and Bradford, 2011). Feeding forages with enhanced digestibility of NDF has been reported to improve DMI and milk yield (Oba and Allen, 1999). Corn silage with the brown midrib mutation has been well documented to have higher fiber degradability and will likely increase DMI and milk yield compared with cows fed conventional corn silage (**CCS**; Eastridge, 1999; Gencoglu et al., 2008). Several (Weiss and Wyatt, 2006; Kung et al., 2008; Stone et al., 2012), but not all experiments (Gehman et al., 2008; Castro et al., 2010; Holt et al., 2010) feeding brown midrib (**BMR)** silage, have reported improved lactational performance of dairy cows. Inconsistent effects of BMR silage have been caused by various factors, including cows differing in physiological state and duration of experiment (Taylor and Allen, 2005a; Castro et al., 2010). Therefore, understanding physiological changes

occurring through lactation and the control of feed intake are critical to diet formulation for BMR silage-based diets.

We hypothesized that feeding 35% BMR silage in a 60% forage diet (DM basis) would result in increased DMI of lactating dairy cows around peak lactation compared with feeding CCS, causing longer peak milk production. The objectives of this study were to evaluate the long-term effects of feeding BMR silage with a good quality alfalfa hay (**AH**) on DMI, productivity, and BW of high producing dairy cows from the onset of lactation through 180 DIM.

MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University. The study was conducted at the Caine Dairy Research Center (Wellsville, UT), Utah State University from February 9, 2011 to October 17, 2011.

Cows and Experimental Diets

Twenty-eight multiparous Holstein cows were used starting at the onset of lactation through 180 DIM. Two dietary treatments were tested in a completely randomized design. Cows were assigned to one of 2 dietary treatments (n = 14) based on previous milk yield and parity. Treatments were based on CCS (62.2% 30 h NDF degradability) or BMR silage (71.4% 30 h NDF degradability) with good quality AH (20.6% CP and 39.9% NDF) as the forage sources (Table 3.1). Treatments were formulated to maintain forage-to-concentrate ratio of 60:40 differing only in the CS hybrids used. Treatments were TMR based on CCS and TMR based on BMR silage (Table 3.2). Diets are typical

of high producing dairy cows in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) with 42% of the forage coming from good quality AH. Rations were formulated based on NRC (2001) recommendations to provide sufficient NE_L, MP, vitamins, and minerals to produce 40 kg/d of milk with 3.5% fat and 3.0% true protein with the inclusion of Rumensin® (Elanco Animal Health, Greenfield, IN).

Two CS hybrids, brown midrib corn hybrid (Mycogen F2F569, Mycogen Seeds, Indianapolis, IN) and conventional corn hybrid (DeKalb DKC61-72, Monsanto Company, St. Louis, MO) were planted during spring 2010 at the Utah State University South Farm (Wellsville, UT). Corn silages were harvested at approximately 30% whole plant DM using a New Holland FP230 pull-type harvester equipped with a mechanical processor (New Holland, PA). The harvested corn crops were treated with silage inoculant (Silage PT®, Nurturite, Twin Falls, ID) at a rate of 112 g/ton of fresh forage to enhance *lactobacillus* fermentation and were ensiled separately in bag silos (Ag/Bag International Ltd., Warrenton, OR).

Cows were housed in individual tie stalls fitted with rubber mattresses, bedded with straw, with free access to water. Cows were fed a TMR for ad libitum intake at 110% of the expected daily intake. All cows were individually fed twice daily at 0830 and 1500 h with approximately 70% and 30% of total daily feed allocation at each feeding, respectively. Feed offered and refused was recorded daily to determine DMI.

Cows were milked twice daily at 0400 and 1600 h. Milk production was recorded daily throughout the experiment. Cows were turned outside to a dry-lot for exercise for at least 1 h daily in the morning after being milked. Milk was sampled twice a month during

the a.m. and p.m. milkings for 2 d. Milk samples were preserved with Broad Spectrum Microtabs II (D & F Control Systems Inc., San Ramon, CA), and were stored at 4°C. Individual milk samples were analyzed for fat, true protein, lactose, and MUN by the Rocky Mountain DHIA Laboratory (Logan, UT) with mid-infrared wave-bands (2 to 15 µm) procedures using an infrared instrument (Bentley 2000; Bentley Instruments, Chaska, MN) calibrated weekly using raw milk standards provided by Eastern Laboratory Services (Fairlawn, OH). An enzymatic procedure was used to determine MUN using a Chemspec 150 instrument (Bentley Instruments, Chaska, MN). Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Milk fat and protein yields were calculated by multiplying milk yield from the respective day by fat and protein concentration of the milk on an individual cow. All cows were weighed on 1, 30, 60, 90, 120, 150, and 180 DIM.

Feed Sampling and Analysis

Corn silage and AH were sampled weekly to determine DM concentration. Diets were adjusted weekly to account for changes in DM concentration. Samples of each CS, AH, and TMR were taken each Monday and frozen immediately. In addition, orts for each treatment diet were sampled each Tuesday and frozen immediately. Frozen samples were thawed and composited by their sample type every month. Composited samples were dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored for subsequent analyses.

Samples of TMR were collected every Monday for particle size analysis using the Penn State Particle Separator as described by Kononoff et al. (2003) equipped with 3 sieves (19, 8, and 1.18 mm) and a pan. Physical effectiveness factor (**pef**) for CS was

calculated as the sum of the proportion of DM retained on 2 sieves, 19 and 8 mm (**pef$_{>8.0}$**; Lammers et al., 1996). The physically effective NDF (**peNDF**) content of the CS was calculated by multiplying NDF concentration of the feed (DM basis) by pef$_{8.0}$ (**peNDF$_{>8.0}$**).

Analytical DM and OM concentration of samples was determined by oven drying at 105°C overnight and by ashing at 550°C, respectively, while N concentration was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

Analysis of Ruminal Fluid

Ruminal fluid samples were obtained using Geishauser probe 4 h after the morning feeding at 30, 60, 90, and 120 DIM. The fluid was collected with a solid, tube-like probe with rows of small holes on the end (Geishauser, 1993). The first 100 mL of ruminal fluid was discharged to avoid contamination from saliva, and then 150 mL was collected for analysis. The pH of the ruminal fluid was measured within 5 min of collecting the samples using a portable pH meter (Oakton pH 6; Oakton Instruments, Vernon Hills, IL). Five milliliters of the ruminal fluid was mixed with 1 mL of 1% sulfuric acid and stored frozen (-40°C) for ammonia-N (**NH$_3$-N**) analysis. Concentration of NH$_3$-N in the ruminal contents was determined as described by Rhine et al. (1998), using a plate reader (MRX[e],

Dynex Technologies, Chantilly, VA). Another 5 mL of the ruminal fluid was collected and added with 1 mL of 25% meta-phosphoric acid, and then stored at -40°C for VFA determination. Ruminal VFA were separated and quantified using a GLC (model 6890 series II; Hewlett Packard Co., Avandale, PA) with a capillary column (30 m × 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was held at 170°C for 4 min, increased to 185°C at a rate of 5°C/min, then increased by 3°C/min to 220°C and held at this temperature for 1 min. The injector and the detector temperatures were 225 and 250°C, respectively, and the carrier gas was helium (Eun and Beauchemin, 2007).

Statistical Analyses

All data were analyzed to characterize cows at two stages of lactation: through peak lactation (1-60 DIM) and post peak lactation (61-180 DIM). This approach was based on the fact that mechanisms regulating voluntary feed intake, mobilization of body fat stores, and milk production differ by stage of lactation (Allen et al., 2009; Allen and Bradford, 2011). Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, 2011) using a model that included type of CS as fixed effect, and cow as a random effect. Covariance structure first-order autoregressive one was used for the repeated measures by day, as it resulted in the lowest values for the Akaike's information criteria and Schwartz's Bayesian criterion. In addition, data for DMI, milk yield, and BW change were averaged at 30 d intervals and analyzed using the same model described above to present overall patterns of the parameters in Figures 3.1, 3.2, and 3.3, respectively. Data for ruminal fermentation parameters (pH, VFA, and NH_3-N) were similarly analyzed; however, due to the lack of differences on the parameters throughout experiment, overall

means were compared between treatments. Least squares means are reported throughout. Treatment effects were declared significant at $P < 0.05$, and differences were considered to indicate a trend toward significance at $P < 0.10$.

RESULTS AND DISCUSSION

Characteristics of CS and Diets

Chemical composition of the forages fed during the experiment is outlined in Table 3.1 Mean concentrations of DM and CP were similar between the CCS and the BMR silage. On average, concentrations of NDF and ADF were slightly higher for the BMR silage than the CCS. This could have been due to the growing season being shorter and colder than normal, forcing silage to be harvested at less than optimal maturity, limiting grain fill, and causing a higher concentration of NDF. In vitro NDF degradability measured after 30 h of incubation was 9.2 percentage units higher for the BMR silage compared with the CCS. Dado and Allen (1995) speculated that a faster disappearance of NDF from the rumen because of increased rate of NDF digestion may reduce distention from gut fill over time and allow greater voluntary feed intake. Increased NDF degradability also increases the energy density of diets and stimulates microbial N production (Oba and Allen, 2000b). Jung et al. (2004) reported that a one percentage unit increase in in vitro NDF degradability of CS resulted in a 0.12 kg/d increase in DMI and a 0.14 kg/d increase in 3.5% FCM yield for diets containing greater than 40% CS (DM basis). Thus, the increase in NDF degradation in BMR silage observed in our study has the potential to substantially improve the productivity of dairy cows fed diets containing BMR silage.

Ingredients and chemical composition of experimental diets are listed in Table 3.2

Although differences existed in NDF concentration between CS hybrids causing the diets

to be slightly higher in NDF for the BMR diet (35.2 and 33.8% for the BMR diet and the

CCS diet, respectively), diets contained similar CP and NE_L (16.5% and 1.55 Mcal/kg on

average, respectively). Physically effective factor and $peNDF_{>8.0}$ were also similar

between treatments (56.9 and 19.6 % average, respectively). The formation, maintenance,

and consistency of the rumen mat strongly depend on dietary peNDF (Zebeli et al.,

2012). However, feeding diets with an excess of $peNDF_{>8.0}$ was shown to decrease feed

intake and feed efficiency (Yang and Beauchemin, 2007; Zebeli et al., 2008). Zebeli et al.

(2012) report that $peNDF_{>8.0}$ is a good predictor of physical fill in the reticulo-rumen and

recommend feeding diets with a $peNDF_{>8.0}$ of 16.4 to 20.6% to improve rumination and

ruminal pH without limiting DMI of lactating dairy cattle. Treatments from our

experiment are within this range.

Intake, Milk Production, and BW

Productive performance is reported in Tables 3.3 and 3.4 for the results through peak

lactation and post peak lactation, respectively. Intake of DM through peak lactation was

not different between dietary treatments. However, DMI post peak lactation tended to

increase by feeding the BMR diet compared with the CCS diet (25.8 vs. 24.7 kg/d; $P =$

0.07). This suggests that ruminal distention from gut fill was not a limiting factor during

the first several weeks of lactation. This can be explained with the hepatic oxidation

theory proposed by Allen et al. (2009) who stated that DMI in the first few weeks of

lactation is controlled primarily by oxidation of fuels in the liver that sends satiety signals

to the brain. As cows move out of a negative energy balance several weeks after

parturition, DMI starts to increase while lipolysis and plasma NEFA concentration decrease, creating less NEFA available for oxidation in the liver, and then feed intake control by hepatic oxidation diminishes (Allen et al., 2009). Therefore, around peak lactation ruminal distension from gut fill becomes the dominant mechanism to control intake (Allen et al., 2009), and feeding diets with increased NDF degradability like BMR silage may allow for greater feed intake (Oba and Allen, 2000a). The BMR silage hybrid has been shown to increase intake in some (Ebling and Kung, 2004; Gehman et al., 2008; Castro et al., 2010), but not all (Taylor and Allen, 2005c; Weiss and Wyatt, 2006; Kung et al., 2008) studies conducted during midlactation. Kung et al. (2008) speculated that the lack of effect on intake with feeding BMR silage may be associated with relatively short experimental periods (mostly less than 4 wk), which may not have been sufficient to cause differences in intake to be expressed when cows are fed with BMR silage. Results of DMI from our experiment averaged at 30-d intervals from the onset of lactation through 180 DIM depicted in Figure 3.1 support the importance of investigating the intake pattern of dairy cows during relatively longer period. In our case, we observed that cows fed the BMR diet increased DMI post peak lactation compared with those fed the CCS diet.

Milk yield was not different between dietary treatments through peak lactation, whereas milk yield post peak lactation increased by feeding BMR diet compared with the CCS diet (41.0 vs. 38.8 kg/d). The increases in DMI of 1.1 kg/d and milk yield of 2.2 kg/d are similar to previous research conducted with BMR silage. In the literature cows fed BMR silage have generally been more productive than those fed CCS. Gencoglu et al. (2008) reported in a contemporary review of published experiments (n = 11) that cows

fed BMR silage averaged 1.2 kg/d higher DMI and 1.7 kg/d more milk than those fed CCS. Tine et al. (2001) reported that BMR silage provided greater amounts of energy due to the increased fiber digestibility when fed to dry cows at maintenance, but the estimated differences in energy values of BMR silage were smaller when fed to lactating cows. Authors suggested that increases in milk production observed when feeding BMR silage may have been primarily driven by increases in DMI related to greater in vitro NDF degradability (Tine et al., 2001). However, not all studies that reported an increase in DMI had an increase in milk yield; some (Frenchick et al., 1976; Block et al., 1981; Gehman et al., 2008) fed a dietary protein concentration less than that recommended by NRC (2001) which may have limited the use of the additional energy intake. Castro et al. (2010) fed a dietary CP averaging 18.8% and observed higher feed intakes for cows fed BMR silage without a significant response in milk yield, but cows may have used the extra intake energy to replenish BW. Like the pattern of DMI, milk yield increased with feeding the BMR diet compared with the CCS diet post peak lactation (Figure 3.2).

Yield of 3.5% FCM was similar between dietary treatments throughout the experiment (41.4 kg/d on average), but milk fat concentration decreased by feeding the BMR diet compared with the CCS diet post peak lactation (3.47 vs. 3.80%). Yield of 3.5% FCM was equal, because yield of milk fat was not affected by CS treatments. This is consistent with previous studies where yield of milk fat was not affected by CS hybrids, but milk fat concentration was reduced for cows fed BMR silage (Qiu et al., 2003; Taylor and Allen, 2005c; Weiss and Wyatt, 2006). Overall milk protein concentration was similar between dietary treatments throughout the experiment (2.96% on average), whereas post peak milk protein yield tended to be higher for the BMR diet

than the CCS diet (1.19 vs.1.13 kg/d; $P = 0.10$). Oba and Allen (1999) suggested that

increased DMI and diet fermentability of BMR silage can enhance microbial protein

yield and flow to the small intestine, hence supplying more MP to the cow. Some studies

that observed an increase in DMI due to feeding BMR silage also reported an increase in

milk protein yield (Oba and Allen, 1999; Qui et al., 2003; Kung et al., 2008). However,

not all reports observing increased DMI did (Gehman et al., 2008; Castro et al., 2010).

Concentration of MUN is used as an indicator of protein nutrition status and efficiency of

N utilization for dairy cows. Although feeding the BMR diet significantly increased

MUN concentration post peak lactation compared with the CCS diet in the current study,

its difference was relatively small (0.8 mg/100 mL). Feeding different CS hybrids did not

affect feed efficiency expressed as 3.5% FCM yield per DMI.

While BW change through peak lactation tended ($P = 0.09$) to be less for cows fed

the BMR diet compared with those fed the CCS diet (-0.22 vs. -0.52 kg/d; Table 3.3),

BW change post peak lactation was not different between the dietary treatments. Other

studies have reported similar numeric increases (0.2 kg/d on average) in BW gain for

cows fed BMR silage (Taylor and Allen, 2005c; Gehman et al., 2008; Castro et al.,

2010). Sommerfeldt et al. (1979) observed increased BW gains (0.1 kg/d) for cows fed

BMR silage in early lactation (42 DIM on average) with no advantage in DMI and milk

yield compared with those fed CCS diet, suggesting that the BMR diet had a slight

advantage in energy that was partitioned toward body tissue during early lactation. In a

study conducted to evaluate the energy balance of dairy cattle fed BMR silage, Tine et al.

(2001) reported an increase in DMI of 2.4 kg/d for cows post peak lactation that resulted

in an extra energy intake of 8.8 Mcal/d. Most of the extra intake energy was partitioned

toward body tissue at 45% with 36% lost as heat and 18% used for milk production. However, energy utilization is affected by several variables; Taylor and Allen (2005c) stated that the capacity of the mammary gland to use nutrients for milk is influenced by hormone secretion and clearance, insulin resistance of tissues, and nutrient demands of various tissues which are all influenced by the stage of lactation and milk production. The pattern of BW change is shown in Figure 3.3, and cows fed the BMR diet resulted in the smallest loss of BW in the first 60 DIM compared with those fed the CCS diet.

Ruminal Fermentation Profiles

Ruminal pH measured at 4 h post feeding was similar between treatments (6.28 on average throughout the study; Table 3.5). Some studies reported a decrease in ruminal pH when BMR silage was fed (Oba and Allen, 2000a; Taylor and Allen, 2005b; Gehman et al., 2008). This may have been caused by the increased supply of fermentable substrate in the rumen due to enhanced NDF digestibility of BMR silage (Weiss and Wyatt, 2006). In our study, AH was fed at the expense of CS which would have increased ruminal pH for both treatments due to the higher buffering capacity of AH compared with CS (Erdman et al., 2011). In our previous study, where high dietary concentrations of AH (25% of DM) were fed with BMR silage, we reported that mean ruminal pH (6.30 on average) were similar between CCS based-diets and BMR silage-based diets with episodes less than 5.8 rarely occurring (Holt et al., 2010). Other studies showed that replacing a portion of CS with alfalfa silage increased ruminal pH (Krause and Combs, 2003; Brito and Broderick, 2006). Despite the increase in DMI due to feeding the BMR diet post peak lactation, dietary treatments did not influence total VFA concentration and their individual molar proportions and NH_3-N concentration throughout the experiment. Although BMR silage

had greater NDF degradability and increased DMI and milk production post peak lactation, no effects on ruminal fermentation characteristics were observed throughout the experiment.

CONCLUSIONS

Feeding BMR silage in high forage diet with a high concentration of good quality AH maintained higher BW after parturition even though DMI was similar through peak lactation. Ruminal distention from gut fill did not appear to be a limiting factor for DMI during the early weeks of lactation. As cows moved into a positive energy balance, the mechanism limiting DMI appeared to change, because DMI and milk production increased for cows fed the BMR diet compared with those fed the CCS diet. Controlling mobilization of body fat stores during transition and maximizing peak milk production are critical to improve animal health and farm profitability. Overall results reported in the current study indicate that feeding BMR silage in high forage diets can have beneficial effects to lessen body fat mobilization in fresh cows without limiting DMI around peak lactation, resulting in longer peak milk production. Further research is needed to examine effects of feeding BMR silage on energy partitioning in transition cows with analysis of NEFA and BHBA to determine physiological effects of BMR silage on body fat mobilization in early lactation and BW gain in later lactation.

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Table 3.1. Chemical composition (means ± SD) of forages (n = 8)

Item, % of DM	Forages[1]		
	CCS	BMR	Alfalfa hay
DM, %	29.2 ± 2.20	30.6 ± 2.90	90.7 ± 1.60
OM	94.6 ± 0.43	93.4 ± 0.46	89.2 ± 1.11
CP	8.62 ± 0.25	8.78 ± 0.31	20.6 ± 0.35
NDF	46.4 ± 2.12	50.7 ± 2.74	39.9 ± 4.34
IVNDFD,[2] %	62.2 ± 2.96	71.4 ± 1.59	ND[3]
ADF	24.9 ± 1.60	27.7 ± 2.27	29.4 ± 3.50
Starch	22.6 ± 0.41	21.7 ± 0.37	ND

[1]CCS = conventional corn silage; BMR = brown midrib corn silage.

[2]IVNDFD = NDF digestibility measured at 30 h of incubation in vitro.

[3]ND = not determined.

Table 3.2. Ingredients and chemical composition (means ± SD) of the experimental diets fed to lactating cows (n = 8)

	Experimental diets[1]	
Item	CCS	BMR
Ingredient, % of DM		
Conventional corn silage	35.1	-
Brown midrib corn silage	-	35.1
Alfalfa hay	24.8	24.8
Corn grain, flaked	19.0	19.0
Corn DDGS[2]	7.8	7.8
Soybean meal, 48% CP	5.6	5.6
Cottonseed, whole	5.5	5.5
Calcium carbonate	1.21	1.21
Salt	0.31	0.31
Urea	0.26	0.26
Magnesium oxide	0.18	0.18
Sodium bicarbonate	0.10	0.10
Vitamin and mineral mix[3]	0.14	0.14
Chemical composition, % of DM		
DM, %	51.6 ± 2.10	52.3 ± 3.80
OM	92.3 ± 0.74	92.2 ± 0.63
CP	16.4 ± 1.19	16.6 ± 0.79
RDP,[4] % of CP	55.7	55.7

RUP,[4] % of CP	44.3	44.3
NDF	33.8 ± 2.85	35.2 ± 3.10
ADF	20.0 ± 2.17	20.9 ± 2.22
NFC[5]	41.7	38.0
NE_L,[4] Mcal/kg	1.56	1.54
Particle size distribution[6]		
$Pef_{>8.0}$	57.0 ± 2.70	56.7 ± 5.03
$peNDF_{>8.0}$	19.3 ± 0.91	19.9 ± 1.77

[1]CCS = conventional corn silage-based TMR; BMR = brown midrib corn silage-based TMR.

[2]DDGS = dried distillers grains with solubles.

[3]Formulated to contain (per kg DM): 226.7 mg of Se (from sodium selenate), 9278.7 mg of Cu (from copper amino acid complex), 40,537.4 mg of Zn (from zinc amino acid complex), 38,653.4 mg of Mn (from manganese amino acid complex), 552.6 mg of Co (from cobalt carbonate), 1,234,585.2 IU of vitamin A, 152,808.1 IU of vitamin D, 3,815.1 IU of vitamin E, and 295 mg of Rumensin[®] (Elanco Animal Health, Greenfield, IN).

[4]Based on tabular value (NRC, 2001).

[5]Non-fibrous carbohydrate = 100 – CP – NDF – ether extract – ash.

[6]Particle size distribution was expressed as % DM retained on sieves using the Penn State Particle Separator (Kononoff et al., 2003). $Pef_{<8.0}$ = physical effectiveness factor determined as the proportion of particles retained on top 2 sieves (Lammers et al., 1996). $peNDF_{>8.0}$ = physically effective NDF determined as NDF concentration (% DM) of diet multiplied by $pef_{>8.0}$.

Table 3.3. Productive performance of Holstein dairy cows fed corn silage-based diets
through peak lactation (1 – 60 DIM)

Item	Diet[1]		SEM	*P*-value
	CCS	BMR		
DMI, kg/d	21.7	21.7	0.34	0.94
DMI, % of BW	3.23	3.36	0.110	0.41
Milk yield, kg/d	42.3	43.1	0.68	0.49
3.5% FCM yield, kg/d	45.6	45.0	1.72	0.80
Milk component, %				
Fat	3.97	3.70	0.124	0.13
Protein	2.91	2.94	0.051	0.76
Lactose	4.85	4.85	0.033	0.87
MUN, mg/100 mL	12.7	13.1	0.28	0.30
Milk component yield, kg/d				
Fat	1.68	1.61	0.078	0.58
Protein	1.22	1.27	0.042	0.46
Lactose	2.06	2.10	0.055	0.56
3.5% FCM yield/DMI	2.14	2.10	0.074	0.69
Mean BW, kg	709	689	17.0	0.40
BW change, kg/d	-0.52	-0.19	0.127	0.09

[1]CCS = conventional corn silage-based TMR; BMR = brown midrib corn silage-based
TMR.

Table 3.4. Productive performance of Holstein dairy cows fed corn silage-based diets post peak lactation (61 – 180 DIM)

	Diet[1]			
Item	CCS	BMR	SEM	*P*-value
DMI, kg/d	24.7	25.8	0.41	0.07
DMI, % of BW	3.48	3.67	0.112	0.27
Milk yield, kg/d	38.8	41.0	0.51	< 0.01
3.5% FCM yield, kg/d	39.0	40.0	0.98	0.46
Milk component, %				
Fat	3.80	3.47	0.085	0.01
Protein	2.97	2.98	0.042	0.86
Lactose	4.93	4.93	0.029	0.27
MUN, mg/100 mL	12.6	13.5	0.29	0.03
Milk component yield, kg/d				
Fat	1.40	1.40	0.045	0.93
Protein	1.13	1.19	0.031	0.10
Lactose	1.86	1.96	0.045	0.14
3.5% FCM yield/DMI	1.58	1.55	0.055	0.89
Mean BW, kg	725	720	14. 9	0.81
BW change, kg/d	0.35	0.42	0.063	0.56

[1]CCS = conventional corn silage-based TMR; BMR = brown midrib corn silage-based TMR.

Table 3.5. Ruminal fermentation characteristics of Holstein dairy cows fed corn silage-based diets from the onset of lactation through 180 DIM.

| | Diet[1] | | | |
Item	CCS	BMR	SEM	*P*-value
Mean pH	6.29	6.27	0.072	0.85
Total VFA, mM	107.2	109.9	2.30	0.42
Individual VFA[2]				
Acetate (A)	61.2	60.9	0.59	0.75
Propionate (P)	25.2	23.3	1.13	0.27
Butyrate	11.5	11.4	0.18	0.88
Valerate	1.78	1.74	0.088	0.74
Isobutyrate	0.84	0.75	0.044	0.18
Isovalerate	1.34	1.32	0.065	0.81
A:P	2.64	2.63	0.098	0.92
NH_3-N, mg/100 mL	8.27	9.03	0.437	0.24

[1]CCS = conventional corn silage-based TMR; BMR = brown midrib corn silage-based TMR.

[2]Expressed as mol/100 mol.
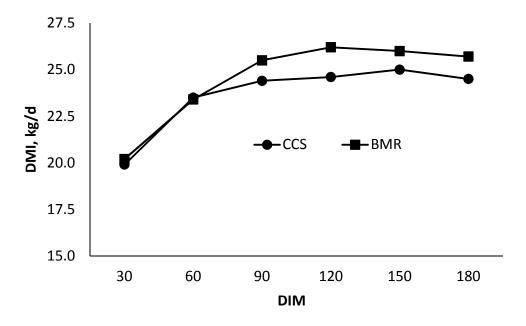
Figure 3.1. Effects of feeding corn silage-based diets on DMI averaged at 30 d intervals

for Holstein dairy cows from the onset of lactation through 180 days-in-milk. Dietary

treatments were conventional corn silage-based TMR (CCS) and brown midrib corn

silage-based TMR (BMR). Each point represents the mean of 14 cows. Over the entire

180-d experiment, LSM for DMI was 23.7 and 24.5 kg/d for the CCS and the BMR,

respectively, whereas effect of dietary treatments was $P = 0.06$ with SEM = 0.28.
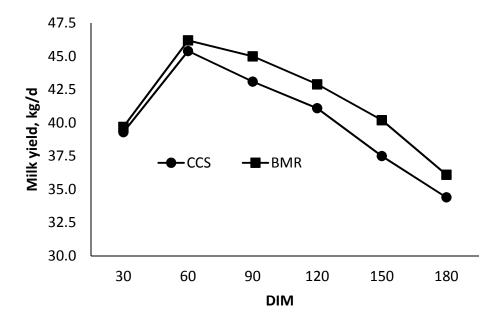
Figure 3.2. Effects of feeding corn silage-based diets on milk yield averaged at 30 d

intervals for Holstein dairy cows from the onset of lactation through 180 days-in-milk.

Dietary treatments were conventional corn silage-based TMR (CCS) and brown midrib

corn silage-based TMR (BMR). Each point represents the mean of 14 cows. Over the

entire 180-d experiment, LSM for milk yield was 40.0 and 41.7 kg/d for the CCS and the

BMR, respectively, whereas effect of dietary treatments was $P < 0.01$ with SEM = 0.42.
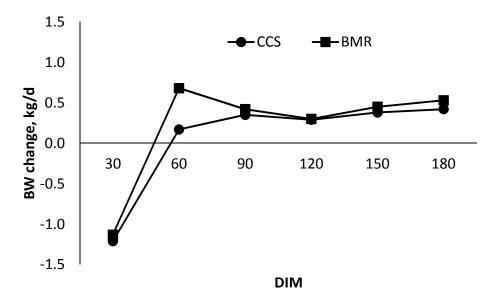
Figure 3.3. Effects of feeding corn silage-based diets on BW change averaged at 30 d intervals for Holstein dairy cows from the onset of lactation through 180 days-in-milk. Dietary treatments were conventional corn silage-based TMR (CCS) and brown midrib corn silage-based TMR (BMR). Each point represents the mean of 14 cows. Over the entire 180-d experiment, LSM for BW change was 0.07 and 0.19 kg/d for the CCS and the BMR, respectively, whereas effect of dietary treatments was $P = 0.24$ with SEM = 0.072.

CHAPTER 4

CORN SILAGE HYBRIDS AND QUALITY OF ALFALFA HAY AFFECT DIETARY NITROGEN UTILIZATION BY EARLY LACTATING DAIRY COWS[1]

INTRODUCTION

A major emphasis has been placed on feeding less dietary CP to high-producing dairy cattle for 2 primary reasons. One is to improve profitability by increasing the efficiency of converting feed N intake to milk N output while maintaining overall milk production. The other reason is that feeding lower CP diets decreases the excretion of N to the environment and consequently lowers ammonia (NH_3) emissions. Olmos Colmenero and Broderick (2006) reported that diets containing 16.5% CP supported maximal production in dairy cows with minimal N excretion to the environment compared with diets with higher CP concentration. It is well established that, as the CP concentration of the diet increases, the proportion of protein degraded in the rumen also increases. Losses of dietary N can be reduced by decreasing protein degradation in the rumen and/or increasing N use by ruminal microorganisms. Microbial protein (**MCP**) synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein (Storm and Ørskov, 1983). The total amount of MCP flowing to the small intestine depends on nutrient availability and efficiency of use of nutrients by ruminal bacteria. When dietary RDP is in

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[1] Holt, M. S., K. Neal, J.-S. Eun, A. J. Young, J. O. Hall, and K. E. Nestor Jr. Corn silage hybrids and quality of alfalfa hay affect dietary nitrogen utilization by early lactating dairy cows. Manuscript submitted and in review process (Manuscript ID #: J. Dairy Sci. 13-6689).

excess of the amount required by ruminal microorganisms, the protein is degraded to

NH_3-N in the rumen, absorbed into the blood, converted to urea in the liver, and excreted

into the urine. Ammonia produced from the activity of naturally occurring enzymes on

urea and other N compounds found in the manure constitutes a major portion of

atmospheric ammonia (NRC, 2002). Potential effects on air quality are a major reason

government regulators are increasingly concerned about excretion of N via manure

(NRC, 2002). Under typical dairy cattle feeding conditions, manipulation of RDP or the

efficiency of N use in the rumen is the most effective strategy to reduce N losses

(Tamminga, 1996).

Various chemical and physical dietary factors can affect ruminal fermentation, and

consequently N efficiency and milk production. Overall intake of N influences the

amount of N excreted via manure, whereas types of carbohydrate and forage have greater

impacts on the route (fecal or urinary) of excretion (Wattiaux and Karg, 2004). The 2

most common forages fed to dairy cows in the United States are alfalfa and corn silage

(**CS**); they complement each other by providing available N and fermentable energy for

MCP synthesis in the rumen, respectively. In general, alfalfa is low in fiber and high in

CP, and degrades rapidly and extensively in the rumen compared to other forages (Martin

et al., 2004). Efficiency of N use in alfalfa silage-based diets are relatively low (Castillo

et al., 2001) because of the high concentration of RDP (Nagel and Broderick, 1992). Due

to the fermentation process, alfalfa silage contains more NPN than alfalfa hay (**AH**) (52

vs. 10%, respectively; Martin et al., 2004). Conversely, CS is rich in starch and, thus,

provides a key source of fermentable energy to the rumen microbial population. Corn

silage made from brown midrib hybrids typically has higher OM degraded in the rumen

and increases flow of microbial N to the small intestines, without affecting total tract apparent digestibility (Oba and Allen, 2000). Thus, the current lactation study was performed to investigate our hypothesis that feeding dairy cows in early lactation a 16% CP diet with fair quality AH (**FAH**) in brown midrib CS (**BMR**)-based diets would maintain milk production, reduce urinary N excretion, and improve N efficiency compared to those fed high quality AH (**HAH**) in conventional CS (**CCS**)- or BMR-based diets. It was envisioned that overall effects of the combinations of main dairy forages could demonstrate significant contribution of forages to nutrient utilization and management and might be used to formulate optimal dairy rations with relatively high forage concentrations to improve environmental performance of lactating dairy cows.

MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University (approved protocol number: 1436). The study was conducted at the Caine Dairy Research Center (Wellsville, UT), Utah State University from February to June, 2011.

Cows and Experimental Design and Diets

Eight multiparous lactating Holstein cows surgically fitted with ruminal cannula were used. Days in milk averaged 23 ± 11.2 at the start of the experiment. Average BW was 639 ± 53.6 kg at the beginning of the experiment and 649 ± 42.2 kg at the end of the experiment.

The design of the experiment was a double 4×4 Latin square. Within each square, cows were randomly assigned to a sequence of 4 diets during each of the four 21-d periods (14 d of treatment adaptation and 7 d of data collection and sampling). A 2×2 factorial arrangement was used; conventional CS (**CCS**) or BMR was combined with FAH (46.7% NDF and 18.4% CP) or HAH (39.2% NDF and 20.7% CP; Table 4.1) to form 4 treatments: CCS with FAH, CCS with HAH, BMR with FAH, and BMR with HAH (Table 4.2). Diets were isonitrogenous across treatments averaging 15.9% CP. Diets are typical of high producing dairy cows in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) with 42% of the forage coming from AH.

Two CS hybrids, brown midrib corn hybrid (Mycogen F2F569, Mycogen Seeds, Indianapolis, IN) and conventional corn hybrid (DeKalb DKC61-72, Monsanto Company, St. Louis, MO) were planted during spring 2010 at the Utah State University South Farm (Wellsville, UT). Corn silages were harvested at approximately 30% whole plant DM using New Holland FP230 pull-type harvester equipped with a mechanical processor (New Holland, PA) treated with a silage inoculant (Silage PT®, Nurturite, Twin Falls, ID) at a rate of 112 g/t of fresh forage to enhance *Lactobacillus* fermentation and were ensiled separately in bag silos (Ag/Bag International Ltd., Warrenton, OR).

Cows were housed in individual tie stalls fitted with rubber mattresses, bedded with straw, and were fed a TMR for ad libitum intake with at least 10% of daily feed refusal. All cows were individually fed twice daily at 0830 and 1500 h with approximately 70% and 30% of total daily feed allocation at each feeding, respectively. Feed offered and

refused was recorded daily, and daily samples were collected to determine DMI. Cows had free access to water.

Cows were milked twice daily at 0400 and 1600 h. Milk production was recorded daily throughout the experiment. Cows were turned outside to a dry-lot for exercise for at least 1 h daily in the morning after being milked. Milk was sampled during the a.m. and p.m. milking on 3 consecutive days (d 16 to d 18) in each period. Milk samples were preserved with Broad Spectrum Microtabs II (D & F Control Systems Inc., San Ramon, CA), and were stored at 4°C. Individual milk samples were analyzed for fat, true protein, lactose, and MUN by the Rocky Mountain DHIA Laboratory (Wellsville, UT) with mid-infrared wave-bands (2 to 15 µm) procedures using an infrared instrument (Bentley 2000; Bentley Instruments, Chaska, MN) calibrated weekly using raw milk standards provided by Eastern Laboratory Services (Fairlawn, OH). An enzymatic procedure was used to determine MUN using a Chemspec 150 instrument (Bentley Instruments, Chaska, MN). Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Milk fat and protein yields were calculated by multiplying milk yield from the respective day by fat and true protein concentration of the milk on an individual cow. To convert milk total protein (**TP**) to milk N, 6.38 was used as the conversion factor (DePeters and Cant, 1992), and total milk N (g/d) was calculated as milk TP/6.38 + MUN, where milk TP and MUN were expressed as g/d.

Feed Sampling and Analysis

Corn silages and AH were sampled weekly to determine DM concentration. Diets were adjusted weekly to account for changes in DM concentration. Samples of the TMR fed and orts for individual cows were collected daily during the data collection period,

dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored for subsequent analyses.

Analytical DM concentration of samples was determined by oven drying at 135°C for 3 h; OM was determined by ashing, and N concentration was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000). The NDF and ADF concentrations were sequentially determined using an ANKOM[200/220] Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

Feed DM and nutrient digestibility was measured during the last week in each period using acid-insoluble ash (**AIA**) as an internal marker (Van Keulen and Young, 1977). Fecal samples (approximately 100 g, wet weight) were collected for each cows from the rectum twice daily (a.m. and p.m.) every 12 hours moving ahead 2 h each day for the 5 d sampling of fecal beginning on d 15. This schedule provided 12 representative samples of feces for each cow. Samples were composited across sampling times for each cow, dried at 60°C for 72 h, ground to pass a 1-mm screen (standard model 4), and stored for chemical analysis. Apparent total tract DM and nutrient digestibilities were calculated from concentrations of AIA and nutrients in diets fed, orts, and feces.

In Vitro NDF Degradation of CS and AH

The Daisy II in vitro fermentation system (ANKOM Technology) was used to examine the NDF degradation of CS and AH used in the lactation trial. Five hundred

milligrams (± 20 mg) of CS or AH was weighed into artificial fiber bags (#F57, ANKOM

Technology), which were then heat-sealed. Ruminal fluid was collected 4 h after the

morning feeding (1100 h) from 2 ruminally cannulated, dry Holstein cows. To prepare

the ruminal fluid, ruminal contents were obtained from various locations within the

rumen and composited. The ruminal contents were placed in sealed containers,

transported to the lab, and strained through a polyester screen (pore size 355 μm; B & S

H Thompson, Ville Mont-Royal, QC, Canada) under a stream of oxygen-free CO_2. Four

hundred milliliters of ruminal fluid (pH of 6.7) were then added to each fermentation jar,

together with 1,600 mL of anaerobic buffer, and fermentation was allowed to continue

for 30 h at 39°C. Bags were removed in quadruplicate (plus one empty bag) at 30 h of

incubation, and then washed under cold tap water until excess water ran clear. Bags were

dried at 55°C for 48 h, and NDF degradation was determined using the same procedure

described in the feed analysis. The experiment was replicated on 2 occasions.

Ruminal pH Measurement

Ruminal pH was continuously measured for 2 consecutive days starting on d 18

using the Lethbridge Research Centre Ruminal pH Measurement System (**LRCpH**;

Dascor, Escondido, CA) as described by Penner et al. (2006). Readings in pH buffers 4

and 7 were recorded prior to placing the LRCpH system in the rumen. Ruminal pH

readings were taken every 30 s and stored by the data logger. After about 48 h of

continuous pH measurement, the LRCpH was removed from the rumen, washed in 39°C

water, and millivolt readings were recorded in pH buffers 4 and 7. The daily ruminal pH

data was averaged for each minute and summarized as minimum pH, mean pH, and

maximum pH. In addition, daily episodes, duration (h/d), and area (pH × min) when

ruminal pH was less than 5.8 were calculated. The threshold 5.8 was assigned because it has been defined by others (Nocek, 1997; Maekawa et al., 2002; Beauchemin and Yang, 2005) to cause ruminal acidosis.

Ruminal Fermentation Characteristics

Ruminal contents were sampled 0, 3, and 6 h after the a.m. feeding on d 20 and 21. Approximately 1 L of ruminal contents was obtained from the anterior dorsal, anterior ventral, medial ventral, posterior dorsal, and posterior ventral locations within the rumen, composited by cow, and strained through a polyester screen (pore size 355 μm). Five milliliters of the filtered ruminal fluid was added to 1 mL of 1% sulfuric acid and samples were retained for NH_3-N determination. Concentration of NH_3-N in the ruminal contents was determined as described by Rhine et al. (1998). Another 5 mL of the filtered ruminal fluid was taken at 3 h after the a.m. feeding and added to 1 mL of 25% of meta-phosphoric acid, and the samples were retained for VFA determination. The VFA were quantified using a gas chromatograph (model 5890, Hewlett-Packard Lab, Palo Alto, CA) with a capillary column (30 m $\times$ 0.32 mm i.d., 1 μm phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was 170°C held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium (Eun and Beauchemin, 2007).

Urine Sampling and Analysis

Spot urine samples were collected for each cow on d 18 to 21 at 0600 and 1800 h.

Samples of urine were acidified to pH < 4.0 by using 4 *M* HCl and frozen (-20°C), and

this solution was later thawed and composited for each cow during each period and

diluted with 39 parts diluent to 1 part urine. Diluent was 0.202% sodium 1-heptane

sulfonic acid and 0.086% ammonium dihydrogen phosphate ($NH_4H_2PO_4$), and the

solution was brought to pH 2.1 with 4 *M* HCl. The ratio of urinary purine derivatives

(**PD**) to creatinine is widely used to estimate the MCP flow to the duodenum (Gonda,

1995; Shingfield and Offer, 1998). Both PD and creatinine were analyzed by using an

HPLC instrument (Waters Corp., Milford, MA) according to the procedures of Shingfield

and Offer (1999). Urinary creatinine was used as a marker to estimate urine volume

(Valadares et al., 1999; Leonardi et al., 2003). In calculating urine volume, we assumed

that creatinine output averaged 28 mg/kg of BW as estimated by Whittet (2004). Previous

investigators have reported similar daily creatinine outputs, ranging from 25 to 30 mg/kg

of BW (McCarthy et al., 1983; Jones et al., 1990). The ratio of the urinary PD allantoin

and uric acid to creatinine was used to estimate the relative differences in MCP

production (Shingfield and Offer, 1998). Based on estimates of urinary excretion of PD,

MCP supply was estimated according to the method of Chen and Gomes (1992).

Statistical Analyses

Data were summarized for each cow by measurement period. All data were

statistically analyzed using the mixed model procedure in SAS (SAS Institute, 2012).

Data for intake, digestibility, milk production, VFA profiles, N utilization, and urinary

metabolites were analyzed with a model that included the effects of type of CS in the diet

(CCS vs. BMR), type of AH (FAH vs. HAH), and the interaction between type of CS and AH. Cow and period were the terms of the random statement.

Data for NH_3-N concentration were analyzed using the model described above except that the fixed effect of time after feeding was included using the repeated option. The covariance structure that resulted in the lowest values for the Akaike's information criteria and Schwartz's Bayesian criterion was used (Littell et al., 1998).

Residual errors were used to test main effects and interactions. Differences were considered significant at $P < 0.05$ and trends were discussed at $0.05 < P < 0.10$. When the interaction between type of CS and AH in the diet was $P < 0.10$, contrasts were used to examine the effects of AH within type of CS using single degree of freedom contrasts. Contrasts were considered significant at $P < 0.05$. Results are reported as least square means.

RESULTS AND DISCUSSION

Characteristics of CS, AH, and Diets

Chemical composition of forages fed during the experiment is outlined in Table 4.1. Mean concentrations of DM and CP were similar between CCS and BMR. On average, concentrations of NDF and ADF were slightly higher for the BMR than the CCS, which could have been due to the growing season being shorter and colder than normal, forcing corn crop for the BMR to be harvested at less than optimal maturity. As expected, in vitro NDF degradability measured after 30 h of in vitro incubation was higher for the BMR compared with the CCS (71.4 vs. 62.2%). Chemical compositions of AH are similar to those of comparable quality reported by Mertens (2002), with concentrations higher for

CP and lower for NDF in HAH vs. FAH (20.7 vs. 18.4% and 39.2 vs. 46.7%, respectively). Additionally, in vitro NDF degradability for AH after 30 h of in vitro incubation was greater for the HAH than the FAH (46.7 vs. 37.9%).

Ingredients and chemical composition of experimental diets are listed in Table 4.2. Concentration of CP in the CCS-based diets was as expected (16.0%), but the BMR-based diets were slightly lower than expected (15.7%), due to the CP of BMR being lower than the original analysis that was used for diet formulation. The concentration of estimated RDP averaged 10.1% DM across all diet treatments. Differences existed in NDF concentration between CS hybrids, causing BMR-based diets to be slightly higher in NDF concentration (38.4 vs. 35.6% for BMR- vs. CCS-based diet, respectively), whereas NE_L was similar among all diets due possibly to higher ruminal degradability for the BMR.

Intake, Digestibility, and Milk Production

Intake of DM averaged 24.1 kg/d across all diets, and was not affected by dietary treatments (Table 4.3). Additionally, intakes of OM and CP were not influenced by source of CS and AH. Due to the higher concentration of NDF in BMR, however, cows fed BMR-based diets had slightly higher intake of NDF compared to those fed CCS-based diets (10.2 vs. 9.17 kg/d). Quality of AH did not impact intake of fiber. No interaction effect between source of CS and AH was found for any measure of intake. We would not expect any effect on DMI in response to feeding different CS in our experiment due to the relatively short experimental periods and early stage of lactation. We have recently reported that, through peak lactation (1–60 DIM), DMI was not different between CCS- and BMR-based diet, whereas DMI post-peak lactation (61–180

DIM) tended to increase ($P = 0.07$) by feeding the BMR-based diet compared with the CCS-based diet (25.8 vs. 24.7 kg/d; see chapter 3). Feeding diets with increased NDF digestibility like BMR may allow for greater feed intake during mid-lactation when ruminal distention from gut fill becomes the dominant mechanism controlling intake, but the effect may be less apparent during early lactation when feed intake is controlled primarily by oxidation of metabolic fuels in the liver (Allen et al., 2009; see chapter 3). In addition, Kung et al. (2008) speculated that experimental periods longer than 3 wk may be needed for sufficient differences in intake to be expressed when cows are fed with BMR. Although HAH is very palatable and consequently can increase feed intake of cows, it failed to affect intake of DM and nutrients in this study.

Total tract digestibilities of DM and OM did not differ in response to CS hybrids (Table 4.3), which agrees well with previous studies conducted with BMR (Taylor and Allen, 2005a; Weiss and Wyatt, 2006; Holt et al., 2010). In general, nutrient digestibilities with BMR-based diets are similar to or slightly higher than diets with CCS hybrids (Weiss and Wyatt, 2006). However, in one experiment, Gehman et al. (2008) reported DM digestibility to be reduced for BMR diets, likely due to the negatively associative effect between intake and digestibility. Total tract digestibilities of DM and OM increased by feeding HAH compared with FAH. High quality AH has a greater concentration of CP and NFC with less fiber than FAH, and NFC in alfalfa is approximately 45% pectin (Martin and Mertens, 2005). Because pectin ferments rapidly and completely, the HAH would have a higher digestibility of DM and OM compared to the FAH. Total tract digestibility of CP was higher for CCS-based diets compared with BMR-based diets. A reduction in CP digestibility when feeding BMR-based diets has

been reported by others (Tine et al., 2001; Gehman et al., 2008; Holt et al., 2010). Greater passage of substrate from the rumen in cows fed BMR-based diets may have increased hindgut fermentation and decreased apparent total tract CP digestibility in these and our studies. Total tract digestibility of CP was not affected by quality of AH. No differences were observed for NDF and ADF digestibilities due to dietary treatments. There was no interaction effect between source of CS and AH on any measure of digestibility.

Milk yield and 3.5% FCM yield averaged 40.7 and 40.4 kg/d, respectively (Table 4.4), and were not influenced by CS hybrid or quality of AH. Similarly, concentration of milk fat, true protein, lactose, and yield of these major milk components were not observed to be different among treatments. In a review of 11 published studies, cows fed BMR have generally been more productive than those fed CCS (Gencoglu et al., 2008); milk yield was 1.7 kg/d greater with a 1.2 kg/d increase in DMI for cows fed BMR, and yield of milk fat was greater for BMR vs. CCS (1.40 vs. 1.36 kg/d), but milk fat concentration tended to be reduced ($P = 0.10$) by 0.08 percentage units and was related to greater milk yield for these cows. The increased milk yield by the cows fed BMR is generally attributed to an increase in DMI. Like our case with no effect on DMI, no difference in milk yield is in agreement with our recent finding that milk yield was not different between CCS- and BMR-based diet through peak lactation (see chapter 3), but milk yield post-peak lactation increased by feeding the BMR-based diet compared with the CCS-based diet (41.0 vs. 38.8 kg/d).

Utilization of N

Nitrogen intake and secretion into milk averaged 623 and 193 g/d, respective, with no differences observed among treatments (Table 4.5). However, BMR-based diets

tended to increase ($P = 0.08$) efficiency of feed N to milk N compared with CCS-based diets (0.33 vs. 0.31) primarily because the BMR-based diets numerically secreted more N into milk with less intake of feed N. Concentration of MUN decreased by feeding BMR-based diets. Others have reported decreased MUN concentration when feeding BMR (Taylor and Allen, 2005b; Kung et al., 2008; Holt et al., 2010). Lower MUN concentration with similar milk protein yield would suggest that cows fed BMR may have been more efficient in converting feed N into milk and body tissue N. Jonker et al. (1998) indicated that MUN is indirectly affected by efficiency of ruminal N fermentation and carbohydrate digestibility either through an increase in milk N secretion, a decrease in N intake, or an increase in fecal N. In our study, feeding HAH further reduced MUN concentration, which may have been affected indirectly by increasing fecal N output. Furthermore, Jonker et al. (1998) stated that the amount of urea secreted into milk is proportional to the concentration of the urea in blood, and this amount is directly proportional to the concentration of urea excreted into the urine of dairy cows. In our study concentrations of MUN, BUN, and urinary urea N all followed the same pattern being reduced by feeding BMR, and these concentrations were further reduced by feeding HAH.

Ruminal NH_3-N concentration was lower for cows fed BMR-based diets than those fed CCS based-diets (Table 4.5), but it was not affected by quality of AH. This is consistent with the pattern observed for BUN and urinary urea N in the BMR-based diets. In the rumen, dietary protein is degraded to NH_3-N, absorbed into the blood, converted to urea in the liver, and excreted in the urine, resulting in a high correlation between these variables. Oba and Allen (2000) reported that ruminal NH_3-N concentration was reduced

when BMR was fed, which they attributed to increased MCP synthesis. Others have reported a similar reduction in ruminal NH_3-N concentration when feeding BMR (Weiss and Wyatt, 2006; Gehman et al., 2008; Holt et al., 2010), implying better ruminal N utilization when BMR is fed.

Most studies have shown that the amount of NH_3 produced from cattle manure in the short term (i.e., days) is strongly correlated with the amount of urinary N in the manure, which is strongly correlated with N intake (James et al., 1999; Cole et al., 2005). In the current study, feeding BMR-based diets with similar N intake reduced urinary N excretion by 25% with a tendency ($P = 0.10$) for less N excreted into manure (Table 4.5). In contrast, fecal N excretions did not differ due to source of CS. Cows fed with HAH in their diets excreted 15% less N into urine, but they excreted more fecal N, resulting in no effect on manure N excretion in response to feeding different quality of AH. From an extensive review of published studies, Castillo et al. (2000) reported that on average 72% of the N consumed by dairy cows was excreted into manure and that there was a linear relationship ($R^2 = 0.93$) between N intake and N excretion into manure. Moreover, for N intake above 400 g/d, the proportion of N excreted into urine increased exponentially, whereas proportional N output into feces and milk declined linearly. Castillo et al. (2001) suggested that a reduction in dietary CP from 19.0 to 15.0% of diet DM would reduce urinary N excretion from 225 to 151 g/d without significantly altering milk production. In our study, approximately 69% of the N consumed was excreted into the manure with N intake and urinary N excretion averaging 623 and 174 g/d, respectively. Greenfield et al. (2001) and Tine et al. (2001) reported a reduction in urinary N excretion when feeding BMR. Weiss and Wyatt (2006) reported that cows fed BMR with similar N intake tended

to excrete ($P = 0.08$) less N via manure and secreted more N into milk. In an experiment by Gehman et al. (2008), BMR-based diets had greater N intake and greater fecal N excretion with no significant difference in milk N, urinary N, and manure N excretion. They suggested that the increase in fecal N excretion for the BMR-based diets was due to increased N intake and decreased N digestibility (Gehman et al., 2008).

Urinary N excretion-to-fecal N excretion ratio (**UN:FN**) and milk N-to-manure N ratio (**MkN:MaN**) are reported in Table 4.5. When less N is found in the urine relative to feces (lower UN:FN), less ammonia loss from manure is expected, because urinary N is more vulnerable to environmental losses than fecal N (Wattiaux and Karg, 2004). Similarly, a higher MkN:MaN is more desirable, because it indicates that less manure N must be managed per unit of milk N produced by the herd. In our study, feeding BMR-based diets decreased the UN:FN, and it was further reduced by feeding HAH. While cows fed the BMR-based diets tended to increase the MkN:MaN ($P = 0.08$), quality of AH did not affect the ratio. The lower ratio of UN:FN with a greater ratio of MkN:MaN for the BMR-based diets indicates that feeding BMR may reduce manure NH_3-N by reducing excretion of urinary N and increasing secretion of milk N per unit of manure N excreted. Feeding the HAH shifted the route of N excretion from urine to feces, which is an effective way of reducing NH_3 volatilization and resultant pollution. However, this benefit was not achieved on excretion of manure N through feeding the HAH. The faster passage rate for the HAH may have increased fermentation in the hindgut, reducing microbial capture of RDP in the rumen and consequently causing an increase in fecal N excretion.

Contrary to our hypothesis, feeding HAH reduced urinary N excretion compared to FAH. However, cows fed the BMR-FAH had the same UN:FN (0.66) as the CCS-HAH, and feeding BMR with FAH or HAH had a better MkN:MaN than CCS-based diets, which supports our hypothesis that feeding BMR with FAH would maintain milk production in early lactation cows while improving N efficiency. It is assumed that quality of legume hay such as AH would affect the metabolic route of N excretion (Weiss et al., 2009); thus, we expect that feeding HAH would reduce the UN:FN compared with feeding FAH even in BMR-based diets. Similar concentration of ruminal NH_3-N between FAH and HAH in BMR diets (7.56 vs. 7.78 mg/100 mL) may have contributed to the similar UN:FN. As there was not a sizable difference in the UN:FN between FAH and HAH under BMR diets (0.66 vs. 0.49; $P = 0.25$), nutritive quality of AH would not impact N utilization in view of environmental performance of dairy cows fed BMR due to enhanced nutrient utilization and its contribution to the overall N utilization by BMR. However, relatively small nutritive differences between FAH and HAH tested in our study should not be discounted on the extrapolation of our result on the UN:FN.

Ruminal Fermentation Characteristics

Cows fed CCS- or BMR-based diets maintained similar ruminal pH and its diurnal patterns (Table 4.6). Feeding HAH increased maximum ruminal pH and duration under pH 5.8 (h/d), but minimum and mean pH and daily episodes lower than pH 5.8 were not affected by any dietary treatment. Ruminal pH decreases only when hydrogen ion production exceeds removal from the rumen and ruminal buffering capacity. Substantially greater rate and extent of ruminal degradation by HAH compared to FAH likely resulted in a greater rate of VFA production with inadequate hydrogen ion removal

and buffering to offset VFA production, resulting in a longer duration of time for ruminal pH lower than 5.8.

Total VFA concentration did not differ due to source of CS, but cows fed with HAH increased the total VFA concentration compared to those fed with FAH (Table 4.6). Source of CS did not influence molar proportion of acetate, but feeding HAH tended to decrease acetate ($P = 0.08$). Molar proportion of propionate was not affected by source of CS, whereas it increased when HAH was fed only in CCS-based diet, resulting in a CS × AH interaction. Cows fed CCS-based diets tended to increase ($P = 0.10$) butyrate proportion. While ratios of acetate-to-propionate and acetate plus butyrate-to-propionate were similar between CCS-based and BMR-based diet, these ratios decreased when cows were fed with HAH only in CCS-based diets, leading to interactions between source of CS and AH. Feeding CCS-based diets increased molar proportion of isobutyrate, whereas cows fed with HAH decreased molar proportion of isovalerate only in CCS-based diet, resulting in a CS × AH interaction. The effect of feeding BMR on total VFA concentration and molar proportions of individual VFA has not been consistent in the literature (Castro et al., 2010). Differences among experiments are likely from interactions with other diet ingredients or cow effects. In our previous research with BMR fed with high dietary concentrations of AH, we reported no differences in VFA concentration and molar proportions of individual VFA (Holt et al., 2010; see chapter 3).

PD and Creatinine Excretion

Effects of dietary treatments on urinary creatinine, PD, ratio of PD-to-creatinine, ratio of allantoin-to-creatinine, and estimated MCP production by PD production according to Chen and Gomes (1992) are reported in Table 4.7. All the measures did not

differ across dietary treatments, except that creatinine production tended to decrease ($P =$ 0.07) when cows were fed BMR-based diets compared to those fed CCS-based diets. Although feeding BMR-based diets decreased ruminal NH_3-N concentration, it did not attribute to MCP production. The ultimate goal of proper nutrient utilization in the rumen is to maximize microbial growth and the amount of RDP that is captured into rumen microbial cells. Maximizing the capture of degradable N not only improves the supply of AA to the small intestine, but also decreases N losses. Oba and Allen (2000) observed that feeding diets containing BMR increased microbial N flow to the duodenum as well as microbial efficiency, possibly due to a faster rate of passage. Their diets for BMR treatments differed from the CCS treatments by containing a higher inclusion of CS and less ground corn than the CCS treatments; however, diet composition was similar for NDF and starch concentrations (Oba and Allen, 2000). In contrast, Gehman et al. (2008) did not observe any difference in PD production or MCP flow with experimental diets that contained more CS and less ground corn in BMR treatments compared with CCS treatments. In the current experiment, CS and flaked corn were fed at the same dietary concentrations for both BMR and CCS treatments. However, diet compositions for BMR-based diets were slightly higher in NDF and approximately 3% lower in NFC concentration compared with CCS-based diets. Given the fact that energy is the most limiting factor in microbial growth (Bach et al., 2005), it is interesting to note that the BMR treatments with relatively low NFC in the diets were able to maintain similar MCP production compared with CCS-based diets. Further research is required to examine effects of AA balanced diets with BMR to optimize MCP production.

CONCLUSIONS

Dairy cows excrete substantially more N into manure than they secrete into milk, which increases milk production costs and environmental N pollution. Optimal dairy feeding programs should consider their effects on crop selection in order to maximize dairy production with reducing environmental impacts. Decreased UN:FN due to feeding BMR and/or quality of AH highlights a great opportunity to improve efficiency of N utilization for dairy production by selecting forage crops that use N more efficiently. Feeding BMR increased MkN:MaN by channeling more dietary N into milk as opposed to manure N excretion. While HAH reduced N excretion into the urine, it only shifted the route from urine to fecal excretion without increasing the ratio of MkN:MaN. Overall data of N utilization in the current study indicate that feeding forages higher in ruminal degradability such as BMR and HAH had better N utilization as evidenced by decreased concentrations of BUN, MUN, and urinary urea N, which can represent an environmental advantage over traditional sources of forages in lactation dairy diets. Due to stage of lactation and design of experiment, however, DMI and milk production were mostly unaffected by diet treatments tested in this study. As improving productive performance of dairy cows is a primary means to optimize dietary N utilization so as to proportionally reduce N emissions to environment, more animal experiments are needed to investigate the possibility that feeding BMR-based diets can take advantage of decreased N excretion to improve productive performance of dairy cows.

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Table 4.1. Chemical composition (means ± SD) of forages (n = 4)

Item, % of DM	Forages[1]			
	CCS	BMR	FAH	HAH
DM, %	28.5 ± 1.96	27.5 ± 1.30	90.4 ± 0.85	93.8 ± 0.86
OM	94.7 ± 0.31	93.3 ± 0.42	89.4 ± 0.70	89.1 ± 0.32
CP	8.40 ± 0.419	8.30 ± 0.369	18.4 ± 0.27	20.7 ± 1.49
NDF	44.8 ± 2.45	52.2 ± 2.60	46.7 ± 3.04	39.2 ± 3.02
IVNDFD,[2] %	62.2 ± 2.96	71.4 ± 1.59	37.9 ± 4.43	46.7 ± 3.99
ADF	25.0 ± 1.36	28.4 ± 1.38	32.6 ± 1.60	28.5 ± 3.09
Starch	25.8 ± 2.37	19.6 ± 3.14	ND	ND

[1]CCS = conventional corn silage; BMR = brown midrib corn silage; FAH = fair quality alfalfa hay; HAH = high quality alfalfa hay.

[2]IVNDFD = in vitro NDF degradability measured at 30 h of incubation.

[3]ND = not determined.

Table 4.2. Ingredients and chemical composition (means ± SD) of the experimental diets fed to lactating cows (n = 4)

| | Experimental diets[1] | | | |
| | CCS | | BMR | |
Item	FAH	HAH	FAH	HAH
Ingredient, % of DM				
Conventional corn silage	35.1	35.1	-	-
Brown midrib corn silage	-	-	35.1	35.1
Alfalfa hay	24.8	24.8	24.8	24.8
Corn grain, flaked	18.4	18.4	18.4	18.4
Corn DDGS[2]	7.80	7.80	7.80	7.80
Cottonseed, whole	5.42	5.42	5.42	5.42
Soybean meal, 48% CP	4.37	2.21	3.79	2.00
Beet pulp, pellets	1.91	4.07	2.49	4.30
Calcium carbonate	1.21	1.21	1.21	1.21
Salt	0.31	0.31	0.31	0.31
Urea	0.26	0.26	0.26	0.26
Magnesium oxide	0.18	0.18	0.18	0.18
Sodium bicarbonate	0.10	0.10	0.10	0.10
Vitamin and mineral mix[3]	0.14	0.14	0.14	0.14
Chemical composition, % of DM				
DM, %	53.2 ± 0.34	53.1 ± 3.00	50.1 ± 3.70	50.3 ± 1.68
OM	92.6 ± 0.58	92.2 ± 0.62	91.9 ± 0.56	91.7 ± 0.35

CP	16.0 ± 0.96	16.0 ± 0.41	15.7 ± 0.68	15.7 ± 0.69
RDP[4]	10.2	10.3	10.0	10.2
RUP[4]	5.80	5.70	5.70	5.50
NDF	36.7 ± 1.65	35.7 ± 2.53	39.2 ± 2.87	38.3 ± 1.84
ADF	20.6 ± 0.86	20.8 ± 1.85	22.6 ± 2.41	22.1 ± 1.37
NFC[5]	37.6	38.7	34.5	35.7
NE_L,[4] Mcal/kg	1.57	1.57	1.56	1.57

[1]CCS–FAH = conventional corn silage (CCS) and fair quality alfalfa hay (FAH) diet; CCS–HAH = CCS and high quality alfalfa hay (HAH) diet; BMR–FAH = brown midrib corn silage (BMR) and FAH diet; and BMR–HAH = BMR and HAH diet.

[2]DDGS = dried distillers grains with solubles.

[3]Formulated to contain (per kg DM): 226.7 mg of Se (from sodium selenite), 9278.7 mg of Cu (from copper amino acid complex), 40,537.4 mg of Zn (from zinc amino acid complex), 38,653.4 mg of Mn (from manganese amino acid complex), 552.6 mg of Co (from cobalt carbonate), 1,234,585.2 IU of vitamin A, 152,808.1 IU of vitamin D, 3,815.1 IU of vitamin E, and 295 mg of Rumensin® (Elanco Animal Health, Greenfield, IN).

[4]Based on tabular value (NRC, 2001).

[5]Nonfiber carbohydrates = 100 – CP – NDF – ether extract – ash.

Table 4.3. Nutrient intake and total tract digestibility of lactating cows fed conventional (CCS) or brown midrib corn silage (BMR) with fair (FAH) or high quality alfalfa hay (HAH)

Item	CCS FAH	CCS HAH	BMR FAH	BMR HAH	SEM	CS	AH	CS × AH
Intake, kg/d								
DM	23.4	24.5	23.9	24.6	1.46	0.70	0.24	0.81
OM	21.7	22.6	21.7	22.6	1.35	0.85	0.27	0.79
CP	3.89	4.05	3.76	3.88	0.231	0.20	0.22	0.85
NDF	9.58	8.76	10.2	10.1	0.596	0.01	0.19	0.32
ADF	5.77	5.29	6.12	6.04	0.371	0.01	0.19	0.33
Digestibility, %								
DM	67.5	73.6	67.3	70.4	1.92	0.29	< 0.01	0.34
OM	70.1	75.6	70.0	72.6	1.78	0.30	0.01	0.34
CP	70.1	72.4	67.4	68.6	1.97	0.05	0.28	0.75
NDF	55.8	58.5	55.9	56.6	2.58	0.61	0.36	0.59
ADF	53.4	56.9	54.5	55.7	2.99	0.97	0.29	0.59

The top of the table has spanning headers: "Diets" spanning CCS and BMR columns, with "CCS" over FAH/HAH and "BMR" over FAH/HAH, and "Significance of effect[1]" spanning CS, AH, and CS × AH.

[1]CS = type of corn silage in the diet (CCS vs. BMR), AH = type of alfalfa hay in the diet (FAH vs. HAH), and CS × AH = interaction between CS and AH.

Table 4.4. Milk production and composition and efficiencies of DM and N use for milk

production of lactating cows fed conventional (CCS) or brown midrib corn silage (BMR)

with fair (FAH) or high quality alfalfa hay (HAH)

| | Diets | | | | | Significance of effect[1] | | |
| | CCS | | BMR | | | | | |
Item	FAH	HAH	FAH	HAH	SEM	CS	AH	CS × AH
Yield, kg/d								
Milk	40.5	40.6	41.0	40.7	2.10	0.73	0.90	0.82
3.5% FCM	40.0	40.4	41.3	39.8	1.87	0.69	0.58	0.36
Milk composition, %								
Fat	3.46	3.42	3.51	3.47	0.213	0.63	0.72	0.97
True protein	2.79	2.79	2.78	2.94	0.103	0.25	0.18	0.17
Lactose	4.86	4.84	4.87	4.83	0.041	0.93	0.31	0.67
Milk component yield, kg/d								
Fat	1.39	1.39	1.44	1.38	0.081	0.53	0.42	0.38
True protein	1.11	1.14	1.14	1.18	0.043	0.24	0.26	0.80
Lactose	1.96	1.99	2.02	1.93	0.093	0.98	0.56	0.27
Efficiency								
Milk yield/DMI	1.77	1.66	1.72	1.65	0.164	0.76	0.17	0.52
3.5% FCM yield/DMI	1.72	1.66	1.74	1.62	0.175	0.31	0.24	0.53

[1]CS = type of corn silage in the diet (CCS vs. BMR), AH = type of alfalfa hay in the diet

(FAH vs. HAH), and CS × AH = interaction between CS and AH.

Table 4.5. Nitrogen utilization of lactating cows fed conventional (CCS) or brown midrib corn silage (BMR) with fair (FAH) or high quality alfalfa hay (HAH)

| | Diets | | | | | Significance of effect[1] | | |
| | CCS | | BMR | | | | | |
Item	FAH	HAH	FAH	HAH	SEM	CS	AH	CS $\times$ AH
N intake, g/d	622	649	601	621	37.4	0.19	0.20	0.83
Milk N, g/d	184	187	186	192	7.6	0.48	0.44	0.84
Milk N:N intake[2]	0.30	0.29	0.32	0.32	0.17	0.08	0.71	0.96
MUN, mg/100 mL	12.9	10.9	9.91	8.42	0.80	<0.01	0.02	0.71
BUN, mg/100 mL	14.1	11.6	10.9	8.99	0.46	<0.01	<0.01	0.56
Urinary urea N, mg/100 mL	727	592	497	415	38.4	<0.01	<0.01	0.29
NH_3-N[3], mg/100 mL	10.8	9.42	7.56	7.78	0.815	<0.01	0.30	0.17
Urinary N excretion,[4] g/d	215	181	162	138	11.3	<0.01	0.02	0.65
Fecal N excretion,[5] g/d	215	273	245	283	36.2	0.32	0.03	0.62
Manure N excretion,[6] g/d	430	454	406	421	24.4	0.10	0.28	0.80
UN:FN[7]	1.10	0.66	0.66	0.49	0.127	0.01	0.01	0.24
MkN:MaN[8]	0.43	0.42	0.49	0.47	0.039	0.08	0.67	0.83

[1]CS = type of corn silage in the diet (CCS vs. BMR), AH = type of alfalfa hay in the diet (FAH vs. HAH), and CS $\times$ AH = interaction between CS and AH.

[2]Efficiency of use of feed N to milk N.

[3]Ruminal ammonia-N.

[4]Predicted using the following equation: $0.026 \times$ MUN, mg/100 mL $\times$ BW, kg (Wattiaux and Karg, 2004).

[5]Predicted using the following equation: N intake, g/d – urinary N excretion, g/d – milk N, g/d.

[6]Manure N, g/d = urinary N excretion, g/d + fecal N excretion, g/d.

[7]UN:FN = urinary N to fecal N ratio, where urinary N and fecal N are expressed in g/d.

[8]MkN:MaN = milk N to manure N ratio, where milk N and manure N are expressed in g/d.

Table 4.6. Ruminal fermentation characteristics of lactating cows fed conventional (CCS) or brown midrib corn silage (BMR) with fair (FAH) or high quality alfalfa hay (HAH)

	Diets					Significance of effect[1]		
	CCS		BMR					
Item	FAH	HAH	FAH	HAH	SEM	CS	AH	CS × AH
Minimum pH	5.74	5.73	5.73	5.67	0.093	0.54	0.63	0.65
Mean pH	6.43	6.50	6.47	6.43	0.058	0.76	0.63	0.20
Maximum pH	6.89	7.03	6.95	7.00	0.424	0.64	0.02	0.24
pH < 5.8								
Daily episodes	6.23	3.38	1.25	4.63	3.333	0.42	0.90	0.19
Duration, h/d	0.22	3.19	0.33	1.94	1.539	0.61	0.05	0.55
Area, pH × min	1.66	0.92	0.92	1.61	1.016	0.97	0.98	0.43
Total VFA, mM	121	129	125	131	2.7	0.26	0.03	0.64
Individual VFA[2]								
Acetate (A)	61.7	59.1	61.4	61.1	1.24	0.30	0.08	0.18
Propionate (P)	21.2[b]	24.8[a]	22.8	23.1	0.80	0.72	< 0.01	< 0.01
Butyrate (B)	12.6	11.6	11.2	11.1	0.55	0.10	0.35	0.36
Valerate	1.54[b]	2.11[a]	1.89	2.06	0.249	0.06	< 0.01	0.02
Isobutyrate	0.87	0.83	0.69	0.71	0.033	< 0.01	0.73	0.21
Isovalerate	1.53[a]	1.18[b]	1.47	1.38	0.117	0.32	< 0.01	0.08
A:P	2.85[a]	2.43[b]	2.72	2.72	0.164	0.25	0.01	0.01
(A+B):P	3.46[a]	2.90[b]	3.22	3.21	0.181	0.57	< 0.01	< 0.01

[a,b]Means in the same row within CCS and BMR subgroups with different superscripts differ based on single degree of freedom contrasts ($P < 0.05$).

[1]CS = type of corn silage in the diet (CCS vs. BMR), AH = type of alfalfa hay in the diet (FAH vs. HAH), and CS $\times$ AH = interaction between CS and AH.

[2]Expressed as mol/100 mol.

Table 4.7. Daily excretion of urinary creatinine, allantoin, uric acid, and estimated ruminal microbial protein production of lactating cows fed conventional (CCS) or brown midrib corn silage (BMR) with fair (FAH) or high quality alfalfa hay (HAH)

Item	Diets					Significance of effect[1]		
	CCS		BMR					
	FAH	HAH	FAH	HAH	SEM	CS	AH	CS × AH
Creatinine, mM	8.62	9.02	8.92	8.86	0.382	0.81	0.58	0.45
Allantoin, mM	11.0	10.4	10.8	9.61	1.08	0.65	0.36	0.76
Uric acid, mM	1.51	1.54	1.59	1.65	0.035	0.01	0.27	0.67
PD,[2] mM	12.5	11.9	12.4	11.3	1.10	0.77	0.39	0.78
PD:creatinine	1.47	1.35	1.41	1.28	0.150	0.55	0.30	0.94
Allantoin:creatinine	1.29	1.18	1.23	1.09	0.141	0.49	0.28	0.90
Creatinine production[3]	160	158	156	158	5.1	0.07	0.92	0.14
PD production[4]	233	212	220	202	21.4	0.54	0.28	0.94
Allantoin production[5]	204	184	192	172	20.7	0.48	0.26	0.99
MCP,[6] g/d	1053	958	997	914	97.2	0.54	0.28	0.94

[1]CS = type of corn silage in the diet (CCS vs. BMR), AH = type of alfalfa hay in the diet (FAH vs. HAH), and CS × AH = interaction between CS and AH.

[2]PD = total purine derivatives (allantoin + uric acid).

[3]Creatinine production, mmol/d = (28 × BW)/113.1 (Janicek et al., 2008).

[4]PD production, mmol/d = [creatinine production × (PD:creatinine)]

[5]Allantoin production, mmol/d = [creatinine production × (allantoin:creatinine)].

[6]Microbial protein production, g/d = ({[PD production − (0.385 × BW$^{0.075}$)]/0.85} × 70 × 6.25)/(0.13 × 0.83 × 1,000) (Janicek et al., 2008).

CHAPTER 5

ASSESSMENT OF IN SITU DEGRADATION KINETICS OF BROWN MIDRIB CORN SILAGE HYBRIDS HARVESTED PRIOR TO OR AT MATURITY

INTRODUCTION

The expansion of livestock enterprises on many farms is often limited by the amount of land available for producing high yielding feed crops for cattle. Double-cropping (**DCP**) land is one means of increasing forage production per acre. In such a system, 2 crops are harvested on the same land in a single year. An example of a DCP system might be to harvest a fall-seeded small grain by early summer, and then plant corn for harvest in the fall. The DCP system increases the amount of time land is used for crop production and can increase potential profit. Although a warm-season DCP may yield smaller returns than a full-season crop, the value of the combined crops makes this a practice more economically competitive in some areas (Brown, 2006). There are also ecological advantages to increasing the amount of time the land is in production. For example, a winter grain crop can act as a cover crop, with the potential to sequester soil N and prevent erosion (Snapp el al., 2005). Heggenstaller et al. (2008) reported that the use of well-adapted DCP systems can lower nitrate-N leaching in the spring and fall relative to present annual cropping systems. In addition, incorporating alternative crops in a DCP system can break pest cycles, thereby reducing the incidence of disease and insect outbreaks (Buntin et al., 2002).

In a traditional scenario where crops are ensiled and fed to ruminants, DCP system provides several advantages, including improved productivity, higher feed quality, and

the associated efficiency in BW gains associated with continuous cycling of nutrients between livestock and land (Heggenstaller et al., 2008). Triticale (*Triticale hexaploide* Lart.) is a cross between wheat (*Triticum*) and rye (*Secale*) that can be used in a DCP system with the potential to be a high yielding silage crop for livestock. It was reported that total triticale/corn DCP systems had the capacity to produce combined DM yields 25% greater than total DM production by conventionally managed, sole-crop corn in north central Iowa (Heggenstaller et al., 2008). Brown (2006) reported that in Idaho triticale produced more DM and removed more P from the soil than wheat or barley when double-cropped with corn silage, but due to the short growing season in the Intermountain West (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) the winter cereal forages had to be harvested in the premature boot stage to accommodate a more timely planting of the corn. Typically, winter cereal forages in the Intermountain West are harvested at the late vegetative or early boot stage precluding growing corn, because the remaining growing season is too short. Harvesting silage corn at tassel is a relatively new technique that may be useful for DCP system with a shorter growing season and for improving fiber degradability by ruminants.

Chemical and genetic approaches have been employed to improve forage fiber digestibility by decreasing lignin concentration or extent of lignin cross-linking with cell wall carbohydrates. Brown midrib (**BMR**) forage genotypes usually contain less lignin and may have altered lignin chemical composition (Bucholtz et al., 1980; Cherney et al., 1991; Vogel and Jung, 2001). Corn breeding efforts have resulted in commercially available BMR hybrids mostly being targeted for silage. The characteristic reddish-brown to tan colored midribs of mutant leaf blades contrasts with the pale green midrib of wild-

type leaf blades. Mutant plants also accumulate reddish-brown to yellow pigment in stalks and roots. This phenotype has been associated with decreased lignin concentration and altered lignin composition compared to wild-type. The BMR corn is generally viewed as being lower yielding than non-BMR corn, but feeding BMR silage has resulted in increased production of dairy cows due to its lower lignin concentration and consequently increased rumen degradability (Gencoglu et al., 2008; Sattler et al., 2010). Similarly, in situ and in vitro digestion studies have shown that BMR forages have a greater extent of NDF degradation than their conventional counterparts (Grant et al., 1995). Relatively new BMR corn hybrids have been introduced as highly rumen degradable corn forage crops that can be double-cropped in areas with shorter growing season by harvesting at tassel. Therefore, the objective of this study was to assess in situ DM and NDF degradation kinetics for these new pre-matured BMR (**pmBMR**) compared to a sole crop BMR (**mBMR**) and conventional corn silage (**CCS**) harvested at maturity. It was hypothesized that in situ DM and NDF degradation would be enhanced in the pmBMR compared to the mBMR and the CCS due mainly to stage of maturity.

MATERIALS AND METHODS

Corn Production, Forage Samples, and Laboratory Analysis

Three BMR hybrids and one CCS hybrid were grown and harvested on private property near Burley, ID. Sole-crop corn hybrids of CCS (Dekalb DKC61-72; Monsanto Co., St. Louis, MO) and mBMR (Mycogen F2F387; Mycogen Seeds, Indianapolis, IN) were planted in farm ground that previously contained corn grown for silage. Sole-crop hybrids were seeded on May 2, 2011 with a planter (DB90, John Deere, Moline, IL) that

delivered approximately 99,010 seeds/ha in 56 cm rows. Approximately 396 ha of each hybrid were harvested at 30% DM on September 15, 2011 using a self-propelled forage harvester (Model 7750, John Deer).

Double-crop pmBMR varieties were the MasterGraze[TM] MC-BMR (**pmBMR1**; Masters Choice Inc., Anna, IL) and the synthetic BMR84[TM] (**pmBMR2**; Ray Brothers Seed Farms, Ironside, OR) planted using the same planter described previously, but delivered approximately 173,267 seeds/ha in 56 cm rows. Double-crop varieties were planted on June 20, 2011 following the harvest of triticale planted in the fall of 2010. Triticale was selected as a winter cover crop due to its high yield potential in Idaho (Brown, 2006). Because we sought to manage for total forage DM production rather than grain yield in the DCP system, corn to be harvested at tassel was planted at elevated densities relative to sole-crop corn. Approximately 198 ha of each DCP variety were harvested 75 d after planting on September 5, 2011. Corn plants were cut at tassel with a self-propelled windrower (Model WR9770, Hesston, Duluth, GA), allowed to wilt for approximately 28 h, and chopped using the same forage harvester used for the CCS and the mBMR. All forages were blown directly from the harvester into a truck with a box-style wagon which had been previously calibrated for weight. Dietary treatments included CCS (control), mBMR, pmBMR1, and pmBMR2.

Yields were determined by dividing the weight of each corn silage hybrid or triticale by the exact acreage from which it was harvested. Sole-crop corn plants harvested at maturity yielded 53.6 and 52.0 Mg/ha, respectively, for the CCS and the mBMR. Double-cropping corn plants harvested at tassel yielded 24.7 and 24.9 Mg/ha, respectively, for the

pmBMR1 and the pmBMR2, whereas triticale harvested at boot stage yielded 26.7 Mg/ha. Total yield for the triticale/corn DCP system was 51.6 Mg/ha.

During harvest, grab-samples were collected and compiled for forage analysis to assess DM concentration, in vitro degradability, and nutrient composition. Harvested samples were immediately frozen and sent to a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) for analysis prior to ensiling. Corn plants were ensiled separately for 90 d in bag silos (Ag/Bag International Ltd., Warrenton, OR) before representative samples were taken for determination of in situ degradation kinetics.

Forage samples were ground to pass through a 1.0-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored for nutritive value determination. Analytical DM and OM concentrations of forage samples were determined by oven drying at 105°C for 3 h and by ashing at 550°C for 5 h, respectively, while N concentration was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) according to AOAC (2000). Neutral detergent fiber and ADF concentrations, both inclusive of residual ash, were determined according to Van Soest et al., 1991), as modified for use with an ANKOM[220] fiber analyzer (ANKOM Technology, Macedon, NY). Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable α-amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO). Samples of 500 mg were weighed in duplicate into nylon bags with a 50 μm pore size and placed into the fiber analyzer for 75 min for NDF analysis, and subsequently for 60 min for ADF analysis. After each procedure, bags were rinsed in acetone.

In Situ Incubation Procedures

Two nonlactating dry and 2 lactating Holstein dairy cows (multiparous) surgically

fitted with ruminal cannula were used to incubate samples for in situ measurements for

ruminal degradation kinetics of DM and NDF. The study was conducted at the Caine

Dairy Research Center (Wellsville, UT), Utah State University. Use of the animals was

approved by the Utah State University Institutional Animal Care and Use Committee.

Cows were housed in group pens and had ad libitum access to both feed and water.

Dry cows were fed a diet containing 51.1% alfalfa hay, 23.4% wheat straw, 21.5% oat

hay, 2.6% CCS, 1.0% wheat midds, and 0.4% vitamin and trace mineral supplement,

whereas the diet for lactating cows contained 47.6% alfalfa hay, 15.3% high moisture

corn, 15.2% CCS, 7.4% corn dried distillers grains with solubles, 5.4% beet pulp, 3.2%

whole cotton seed, 2.5% soybean meal, 0.9% fat supplement (Ener G II^{TM}, Nutri-Tech

Solutions, Yandina, Queensland, Australia), and 2.5% vitamin and trace mineral

supplement (DM basis).

Dacron bags (10 × 20-cm; ANKOM Technology) with an average pore size of 50

μm were filled with 4.0 g of air-dried silage samples ground through a 4.0-mm screen

(standard model 4) to yield an approximate sample DM/surface area of 10 mg DM/cm^2.

In situ incubation was replicated in triplicate. This provided 3 bags/cow and 6 total

bags/silage for each time point. Bags were heat-sealed and placed in mesh bags (43 × 39

cm; 12 in situ bags in a mesh bag) with 3 × 5-mm pores that permitted ruminal fluid to

percolate freely. Three mesh bags in each cow were incubated in the ventral rumen.

Samples were incubated for 0, 4, 8, 16, 24, 48, and 96 h. Upon removal, bags were rinsed

in water to remove ruminal contents on the exterior and frozen until all bags had been

collected. Bags were machine washed for 5 rinse cycles consisting of a 1-min agitation and a 2-min spin. Additional bags were also prepared and machine rinsed without ruminal incubation, thereby creating a 0-h incubation time. After rinsing, residues were dried at 55°C in a forced-air oven for 48 h and weighed to determine residual DM. Dried residues were ground to pass a 1-mm screen and analyzed for NDF degradation kinetics.

Statistical Analysis

In situ ruminal DM and NDF degradation data were fitted to the first order exponential model with discrete lag (Mertens, 1977) using the iterative Marquardt method and the nonlinear regression procedure of SAS (SAS Institute, 2011). All data were analyzed separately by stage of lactation on test cows (dry vs. lactating cows), as we assessed in situ degradation parameters of treatments using dry cows as well as lactating cows independently. For each cow and type of feed, the following model was fitted to the percentage of DM and NDF degradation:

$$R_{(t)} = B \ x \left(e^{-k_d \, (t-L)} \right) + C,$$

where $R_{(t)}$ = indigested total residue at any time t, B = insoluble potentially digestible fraction, k_d = fractional rate of digestion of B, t = time incubated in the rumen in h, L = discrete lag time in h, C = fraction not digested after 96 h of incubation. Effective ruminal degradability (extent of rumen degradation, **ERD**) was calculated using the model of Ørskov and McDonald (1979):

$$ERD = A + \{B \ x \ [k_d \, / (k_d + \ k_p)]\},$$

where k_p = assumed ruminal passage rate of 4.0 %/h for dry cows and 6.0 %/h for lactating cows. The wash fraction *A* was the percentage of substrate washed out of the bag at 0 h.

Data was analyzed in a completely randomized design with the MIXED procedure of SAS (SAS Institute, 2011), and the model included the effect of corn silage hybrids. In addition, pre-planned orthogonal contrasts were tested: 1) CCS vs. BMR (mBMR + pmBMR1 + pmBMR2), 2) mBMR vs. pm BMR (pmBMR1 + pmBMR2), and 3) pmBMR1 vs. pmBMR2. Least square means are reported throughout. Significance was declared at $P < 0.05$ and tendency at $P < 0.10$.

RESULTS AND DISCUSSIOIN

Nutrient Profiles of Silages

Concentrations of CP, NDF, and ADF were higher in the pre-matured BMR silages compared to the CCS and the mBMR, whereas the pre-matured BMR contained lower concentrations of starch and nonfiber carbohydrates (**NFC**; Table 5.1). Nutrient composition between the pmBMR1 and the pmBMR2 was similar. A decline in fiber concentration with increasing maturity can be attributed to the dilution effect created by the increasing content of grain in corn as the crop matures (Coors et al., 1997; Darby and Lauer, 2002). Additionally, CP has been shown to decline with increasing maturity (Johnson and McClure, 1968; Sheperd and Kung, 1996). On silage samples tested in this study, increased starch concentration corresponded to decreased concentrations of CP and fiber. A similar effect may have occurred with the concentration of acid detergent lignin. The pre-mature BMR varieties had similar or higher acid detergent lignin concentration

compared to the CCS, and as expected, the mBMR was 24% lower in acid detergent lignin than the CCS. Decreased lignin concentration in BMR has been the hybrid's most notable nutritive benefit. Less lignin synthesis decreases the amount of cross-linking that occurs among lignin and digestible structural carbohydrates, thus increasing plant digestibility (Casler and Jung, 1999; Vogel and Jung, 2001). The lack of starch in the pmBMR silages could have attributed to the increase in lignin as well as CP and NDF concentrations. Lignin-to-NDF ratio was highest in the CCS (0.063) but was similar between BMR silages (0.046 on average). In addition, in vitro NDF degradability measured at 30 h of incubation was similar across BMR silages and was higher than that of the CCS (66.9 vs. 56.6%). 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).

In Situ Degradation Kinetics of DM and NDF

Kinetics of DM degradation is reported in Table 5.2. The CCS had the greatest wash fraction compared to the BMR hybrids in dry and lactating cows. The DM wash fraction represents the percentage of DM available immediately in the rumen. The high DM fraction in the CCS may have resulted from higher concentrations of water-soluble carbohydrates compared to the BMR hybrids. An accumulation of various solutes, including sugars and starch, is observed in corn plants, as they mature, and consequently this result was expected due to the fact that NFC concentration of the CCS exceeded 47% of the total forage DM.

The potentially degradable DM fraction generally exhibited responses that were mirror-opposites to that observed for the wash fraction. As expected, differences of the

potentially degradable DM fraction between the BMR hybrids and the CCS were greater in dry cows compared to lactating cows (19 vs. 20%, respectively). This is due largely to the differences in the undegradable fraction for the CCS in dry and lactating cows (15.9 and 23.0%, respectively). The potentially degradable DM fraction was highest for the mBMR in dry cows, while in lactating cows this fraction was highest for the pmBMR1. Different fractional rates of particulate passage between dry and lactating cows (assumed to be 4.0 vs. 6.0 %/h) may result in the different responses in the potentially degradable DM fraction between the test cows. In addition, it is likely that the different overall nutrient compositions on BMR corn silage varieties would interact with the different fractional passage rates between the test cows.

As expected, the CCS had the greatest undegradable fraction in lactating cows, but surprisingly, the greatest undegradable fraction in dry cows was found in the pmBMR2. Because of the high wash fraction and rapid degradability of the CCS at the early time points, estimates of ERD of DM were greatest for the CCS in lactating cows followed by the pmBMR1 in dry cows.

All lag times were relatively low (approximately ≤ 1 h) with no distinct patter in dry cows except for the pmBMR1 which took 5.6 h. There was no apparent explanation for the greater lag time of the pmBMR1 in dry cows. In lactating cows, however, lag time for the pmBMR1 was < 1 h, while both the CCS and the mBMR averaged 7.0 h in lag time. Estimates of rate of degradation ($\mathbf{K_d}$) for DM were greater for the BMR hybrids compared to the CCS and were noticeably greater for the pmBMR varieties than the mBMR in dry cows (8.08 vs. 2.97 %/h). Estimates of K_d for DM were much lower in lactating cows compared to dry cows due to increased fractional rate of particulate

passage. In the lactating cows, the BMR hybrids had a lower K_d than the CCS (1.89 vs. 2.80 %/h).

Patterns of DM degradability in dry cows revealed that the pmBMR2 ceased further degradation in the rumen by 48 h of incubation, peaking at 79%, whereas the CCS continued to degrade, reaching 84% by 96 h of incubation (Figure 5.1). This interaction was not seen in lactating cows where the pmBMR2 started lower at early incubation time points and remained lower through 96 h of incubation.

Results for NDF degradation kinetics are reported in Table 5.3. The wash fraction for NDF comprised a relatively small percentage of the total NDF pool, ranging from 5.2 to 15.6% in dry and lactating cows. Theoretically, NDF is insoluble in water (Van Soest, 1982) and therefore should be completely recovered at 0 h in in situ bags. In practice, recovery is rarely complete but is commonly > 90% for small cereal grain forages (Coblentz et al., 2000), and is often unreported (Bargo et al., 2001), or correction procedures are used to set disappearance of NDF to 0% at l h (Hackmann et al., 2010). However, substantial losses of NDF at l h were reported for immature perennial cool-season grasses, such as timothy (*Phleum pretense*; Hoffman et al., 1993) or winter-stockpiled tall fescue (*Festuca arundinacea*; Flores et al., 2007) that have ranged up to 29.4% of the NDF pool. In the present study, the wash fraction was greatest for the pmBMR varieties ranging from 11.9 to 15.5% in dry and lactating cows. This may be attributed to higher concentrations of pectin in the plant cell walls of the pmBMR silages. The polysaccharide components of plant cell walls are cellulose, hemicellulose, and pectin. Cellulose is composed of β-1, 4 linked glucose, whereas hemicellulose and pectin are composed of mixtures of both hexose and pentose sugars with a variety of linkage

types (Hatfield, 1993). Most of the pectin in the cell wall is lost by solubilization during the first step of the detergent system where NDF is isolated. The net result is that NDF significantly underestimates cell wall concentration when high concentrations of pectin exist (Vogel and Jung, 2001).

The potentially degradable NDF fraction comprised large percentages of the NDF pool ranging from 61.0 to 89.8% and 47.7 to 59.4% for dry and lactating cows, respectively. As expected, the BMR hybrids had greater potentially degradable NDF fraction compared to the CCS with the exception of the pmBMR2 which had the lowest potentially degradable NDF fraction in dry cows.

Patterns of NDF degradability in dry cows indicated that degradation of the pmBMR2 rapidly declined after 24 h of incubation (Figure 5.2). As a result, the undegradable NDF fraction was greatest for the pmBMR2 in dry cows as opposed to the CCS in lactating cows. Estimates of ERD of NDF were greater for the pmBMR varieties compared to mature silages (Table 5.3), and were greatest for the pmBMR1 in both dry and lactating cows (58.9 and 38.7%, respectively). The decrease in the ERD of NDF for lactating cows compared to dry cows was expected; however, it is interesting to point out that ERD of NDF was 4.5, 10, and 20% less for the mBMR, the CCS, and the pmBMR varieties, respectively. This can partially be explained by the difference in K_d between the test cows. Estimates of K_d decreased dramatically for the pmBMR varieties, only slightly for the CCS, but increased for the mBMR. Estimates for lag time were < 0.06 h in dry cows, whereas in lactating cows lag times were similar for the CCS and the pmBMR varieties, but nearly double for the mBMR (11.0, 10.8, and 20.7 h, respectively). The effects of lag time on NDF degradability for the mBMR are depicted in Figure 5.2.

Results for true NDF degradability should be interpreted with caution, because correction procedures to calculate degradability of NDF to 0% at l h were not used in our study.

Although dairy cows require forage NDF in diets for maximum productivity, excess dietary NDF often limits voluntary feed intake because of physical fill in the rumen. Enhanced NDF degradability in the rumen may stimulate rapid degradation of NDF from the rumen, reduce physical fill, and allow greater voluntary feed intake (Allen and Oba, 1996). In situ degradation of NDF was greater for all BMR hybrids compared to the CCS. Among BMR hybrids total degradation of NDF was greatest for the pmBMR1 and greater for the mBMR than the pmBMR2, indicating that in situ NDF degradation may have been influenced more by hybrid than stage of maturity.

IMPLICATIONS

Incorporating alternative crops in a DCP system has the potential to improve continuous cycling of nutrients between livestock and land without decreasing forage yields. However, lack of NFC in silage corn harvested at tassel may require additional supplementation of grains to provide nutrient requirements for high producing dairy cows. The lack of starch in the pmBMR silages could have attributed to increased CP, NDF, and lignin concentrations. Nearly half of the DM in the CCS was comprised of water-soluble NFC which was available immediately in the rumen; however, the fraction of undegradable DM for the CCS was greater than the BMR hybrids, and thus the fraction of potentially degradable DM was lower for the CCS compared to the BMR silages. The increased degradability of the pmBMR varieties should produce more VFA and may provide an efficient energy source for dry cows and heifers. In the lactating

cows, however, extent of DM degradation for the pmBMR2 was not as great as the CCS. In addition, the higher concentration of starch in mature corn silages (CCS and mBMR) would provide more energy available for rumen microorganisms, which can increase microbial population and microbial protein synthesis available for the host animal. Feeding the mBMR to high producing cows may allow for less grain to be fed, whereas feeding the pmBMR silage may need to be supplemented with additional energy in high producing cows. Increases in in situ NDF degradability of the BMR hybrids have the potential to substantially improve the productivity of dairy cows fed diets containing relatively high concentrations of forage without negatively influencing feed intake due to increased NDF degradability. However, in situ NDF degradation is insufficient for estimating the nutritional value of such types of forage because of differences in rate of degradability. More research is needed to determine the effects of feeding pmBMR-based diets on NDF digestibility, feed intake, and lactational performance of dairy cows.

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Table 5.1. Chemical composition (means ± SD) of forages (n = 3)

Item, % of DM	Corn silage hybrid[1]			
	CCS	mBMR	pmBMR1	pmBMR2
DM, %	29.8 ± 0.53	28.2 ± 0.93	26.0 ± 1.00	36.8 ± 3.2
OM	94.6 ± 0.21	94.6 ± 0.37	94.7 ± 0.43	94.2 ± 0.33
CP	6.5 ± 0.57	7.8 ± 0.45	11.8 ± 1.20	10.7 ± 1.46
Fat	2.8 ± 0.82	2.9 ± 0.69	2.3 ± 0.52	1.9 ± 0.60
NDF	39.5 ± 0.99	47.1 ± 0.64	56.4 ± 0.75	53.5 ± 0.60
IVNDFD,[2] %	56.6 ± 0.71	67.7 ± 0.71	67.7 ± 0.79	65.2 ± 0.84
ADF	23.0 ± .071	26.1 ± 1.20	34.8 ± 1.11	33.9 ± 1.05
Starch	32.5 ± 1.91	25.8 ± 1.48	1.1 ± 0.42	2.2 ± 0.55
NFC[3]	47.1 ± 1.90	39.2 ± 1.67	17.9 ± 2.99	22.9 ± 2.51
ADL[4]	2.5 ± 0.50	1.9 ± 0.33	2.5 ± 0.57	3.0 ± 0.66

[1]CCS = conventional corn silage; mBMR = brown midrib corn silage harvested at maturity; pmBMR1 = brown midrib corn silage 1 harvested prior to maturity; and pmBMR2 = brown midrib corn silage 2 harvested prior to maturity.

[2]IVNDFD = in vitro NDF degradability measured at 30-h of incubation.

[3]Acid detergent lignin.

[4]Nonfibr carbohydrates = 100 – CP – NDF – ether extract – ash.

Table 5.2. Kinetics of in situ ruminal DM degradation of corn silage hybrids measured in dry and lactating dairy cows

Item	Treatment[1]					Contrast[2]		
	CCS	mBMR	pmBMR1	pmBMR2	SEM	1	2	3
Dry cows[3]								
Wash fraction, %	51.7^a	42.9^b	42.1^c	43.4^b	0.12	< 0.01	0.63	< 0.01
Potentially degradable fraction, %	32.4^d	47.4^a	45.4^b	36.8^c	0.25	< 0.01	< 0.01	< 0.01
Undegradable fraction, %	15.9^c	9.7^c	12.5^c	19.8^a	0.29	< 0.01	< 0.01	< 0.01
Extent of rumen degradation, %	67.2^b	62.9^c	74.0^a	66.5^b	0.37	< 0.01	< 0.01	< 0.01
Lag time, h	1.38^b	0.01	5.65^a	0.80^d	0.195	0.03	< 0.01	< 0.01
K_d,[4] %/h	3.70^c	2.97^d	9.44^a	6.72^b	0.110	< 0.01	< 0.01	< 0.01
Lactating cows[5]								
Wash fraction, %	51.6^a	43.0^b	37.6^c	43.1^b	0.06	< 0.01	< 0.01	< 0.01
Potentially degradable fraction, %	25.4^d	43.2^b	51.7^a	38.7^c	0.68	< 0.01	< 0.01	< 0.01
Undegradable fraction, %	23.0^a	13.8^c	10.7^d	18.2^b	0.62	< 0.01	0.43	< 0.01
Extent of rumen degradation, %	59.5^a	53.6^b	50.8^d	51.6^c	0.16	< 0.01	< 0.01	< 0.01

Lag time, h	7.77^c	6.64^c	0.01	3.54^b	0.443	< 0.01	< 0.01	< 0.01
K$_d$,[4] %/h	2.80^a	1.97^b	2.05^b	1.72^b	0.073	< 0.01	0.15	0.11

[a-d]Within a row, means with different superscript differ ($P < 0.05$).

[1]CCS = conventional corn silage; mBMR = brown midrib corn silage harvested at maturity; pmBMR1 = brown midrib corn silage 1 harvested prior to maturity; and pmBMR2 = brown midrib corn silage 2 harvested prior to maturity.

[2]1 = contrast between CCS vs. BMR (mBMR + pmBMR1 + pmBMR2); 2 = contrast between mBMR vs. pm BMR (pmBMR1 + pmBMR2); and 3 = contrast between brown midrib corn silages harvested prior to maturity (pmBMR1 vs. pmBMR2).

[3]Fractional passage rate of dry cows was assumed to be 4.0%/h.

[4]Rate of DM degradation.

[5]Fractional passage rate of lactating cows was assumed to be 6.0%/h.

Table 5.3. Kinetics of in situ ruminal NDF degradation of corn silage hybrids measured in dry and lactating dairy cows

Item	Treatment[1]					Contrast[2]		
	CCS	mBMR	pmBMR1	pmBMR2	SEM	1	2	3
Dry cows[3]								
Wash fraction, %	8.3[c]	5.2[d]	15.6[a]	11.9[b]	0.13	< 0.01	< 0.01	< 0.01
Potentially degradable fraction, %	66.9[c]	89.8[a]	70.2[b]	61.0[d]	0.30	< 0.01	< 0.01	< 0.01
Undegradable fraction, %	24.9[b]	5.0[d]	14.2[c]	27.1[a]	0.32	< 0.01	< 0.01	< 0.01
Extent of rumen degradation, %	36.2[c]	36.3[c]	58.9[a]	47.0[b]	0.39	< 0.01	< 0.01	< 0.01
Lag time, h	0.03[c]	0.02[d]	0.06[a]	0.05[b]	0.001	< 0.01	< 0.01	< 0.01
K_d,[4] %/h	2.12[d]	2.92[c]	6.49[a]	5.45[b]	0.001	< 0.01	< 0.01	< 0.01
Lactating cows[5]								
Wash fraction, %	7.0[d]	7.8[c]	13.2[a]	12.9[b]	0.11	< 0.01	< 0.01	0.34
Potentially degradable fraction, %	47.7[c]	55.5[b]	59.4[a]	54.3[b]	1.31	< 0.01	0.28	0.10
Undegradable fraction, %	45.3[a]	36.8[b]	27.4[c]	32.8[b]	1.20	< 0.01	< 0.01	0.05
Extent of rumen degradation, %	20.8[d]	31.8[b]	38.7[a]	27.3[c]	0.41	< 0.01	< 0.01	< 0.01

Lag time, h	11.0[bc]	20.7[a]	12.3[b]	9.2[c]	0.70	0.01	< 0.01	0.02
K_d,[4] %/h	2.57[b]	4.67[a]	4.51[a]	2.45[b]	0.001	< 0.01	< 0.01	< 0.01

[a-d]Within a row, means with different superscript differ ($P < 0.05$).

[1]CCS = conventional corn silage; mBMR = brown midrib corn silage harvested at maturity; pmBMR1 = brown midrib corn silage 1 harvested prior to maturity; and pmBMR2 = brown midrib corn silage 2 harvested prior to maturity.

[2]1 = contrast between CCS vs. BMR (mBMR + pmBMR1 + pmBMR2); 2 = contrast between mBMR vs. pm BMR (pmBMR1 + pmBMR2); and 3 = contrast between brown midrib corn silages harvested prior to maturity (pmBMR1 vs. pmBMR2).

[3]Fractional passage rate of dry cows was assumed to be 4.0%/h.

[4]Rate of NDF degradation.

[5]Fractional passage rate of lactating cows was assumed to be 6.0%/h.
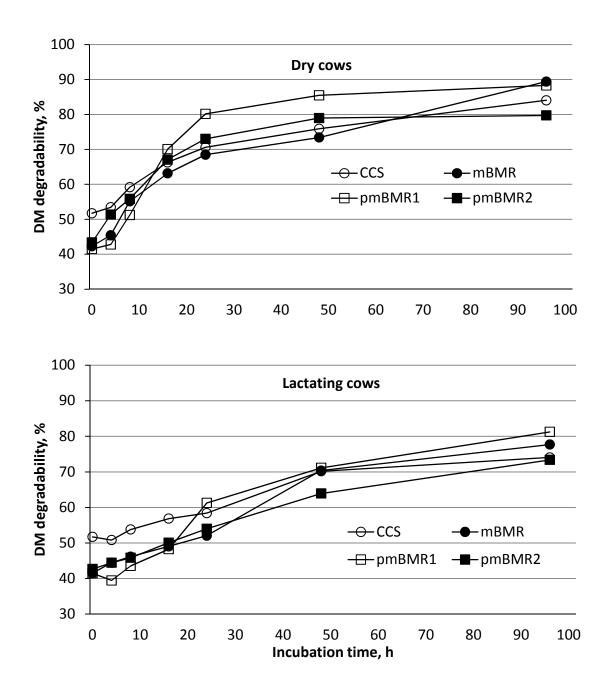
Figure 5.1. In situ degradability of DM in dry and lactating dairy cows measured at 0, 4,

8, 16, 24, 48, and 96 h. CCS = conventional corn silage; mBMR = brown midrib corn

silage harvested at maturity; pmBMR1 = brown midrib corn silage 1 harvested prior to

maturity; and pmBMR2 = brown midrib corn silage 2 harvested prior to maturity. In dry

cows, effect of type of silage, incubation time, and the interaction between type of silage

and incubation time were $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively, with SEM = 0.86. In lactating cows, effect of type of silage, incubation time, and the interaction between type of silage and incubation time were $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively, with SEM = 0.62.
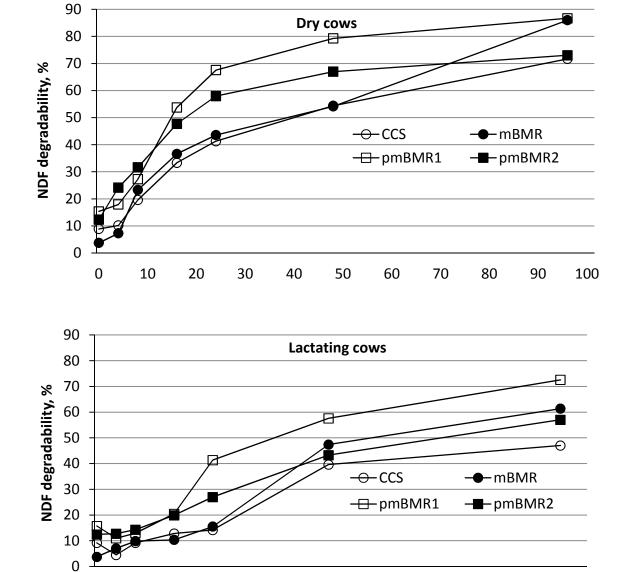
Figure 5.2. In situ degradability of NDF in dry and lactating dairy cows measured at 0, 4,

8, 16, 24, 48, and 96 h. CCS = conventional corn silage; mBMR = brown midrib corn

silage harvested at maturity; pmBMR1 = brown midrib corn silage 1 harvested prior to

maturity; and pmBMR2 = brown midrib corn silage 2 harvested prior to maturity. In dry

cows, effect of type of silage, incubation time, and the interaction between type of silage and incubation time were $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively, with SEM = 1.21. In lactating cows, effect of type of silage, incubation time, and the interaction between type of silage and incubation time were $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively, with SEM = 1.12.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Forages are the foundation up on which good dairy nutritional programs are built. Over the past decade, dairy producers have increased their use of CS as a forage source. This has been influenced by the high price of feed, especially corn grain, and the high energy content of CS. Considerable advances have been made in forage production, and progress is being made relative to quality for two most important forages in dairy rations, CS and alfalfa. Feeding forage levels at 55 to 60% of dietary DM is becoming more common, but lack of energy from concentrates and distention from rumen fill may limit DMI and reduce performance of high producing dairy cows. 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. Degradability of NDF is an important parameter of forage quality, because forage NDF varies widely and influences ruminal metabolism and metabolic health of dairy cows. Alfalfa and CS complement each other by providing available N and fermentable energy for microbial protein synthesis in the rumen. Microbial protein provides the majority of protein supplied to ruminants. However, when dietary protein is in excess of the amount required by ruminal microorganisms, the protein is degraded to ammonia in the rumen, and excess N is excreted in the manure. Major emphasis had been placed on feeding less dietary CP to high producing dairy cow to improve profitability and decrease the excretion of N into the environment. Ammonia-N has been recognized as an air and water pollutant. Urinary

N is more volatile than fecal N, and thus the route and amount of N excretion is of environmental concern.

The expansion of livestock enterprises is often limited by the amount of farmland available for producing forage crops and manure disposal. Double-cropping is one means of increasing forage production per acre while decreasing N losses due to volatilization in early spring and summer. The research presented here has addressed the effects of feeding BMRCS on DMI, lactational performance, and N utilization. In addition, in situ degradation kinetics of BMRCS was investigated using BMR varieties harvested prior to or at maturity.

Nutrient profiles for BMRCS used in these studies averaged higher concentrations of NDF and ADF and lower concentrations of NFC compared to the CCS. This could have been due to the growing season being shorter and colder than normal, forcing silage to be harvested at less than optimal maturity, limiting grain fill, causing a higher NDF and lower NFC concentrations. In the in situ experiment, increased starch concentration corresponded to decreased concentrations of CP and fiber. Chemical compositions for the pmBMR were higher in CP, NDF, and ADF compared to the CCS and the mBMR, whereas the pmBMR contained lower concentrations of starch and NFC. Thus, the CCS had the greatest DM wash fraction, compared to the BMR hybrids; however, potentially degradable DM fraction was highest for the mBMR in dry cows, while in lactating cows this fraction was highest for the pmBMR1. As expected, the CCS had the greatest undegradable fraction in lactating cows, but surprisingly, the greatest undegradable fraction in dry cows was found in the pmBMR2.

The BMR silages had a lower lignin-to-NDF ratio, and hence in vitro NDF degradability measured after 30 h of incubation was higher across all BMR silages compared with the CCS. In the in situ experiment, the NDF wash fraction was greatest for the pmBMR varieties which may be attributed to higher concentrations of pectin in the plant cell walls of the pmBMR CS. As expected, the BMR hybrids had greater potentially degradable NDF fraction compared with the CCS with the exception of the pmBMR2 which had the lowest potentially degradable NDF fraction in dry cows. In situ degradation of NDF was greater for all BMR hybrids compared with the CCS. Among BMR hybrids total degradation of NDF was greatest for the pmBMR1 and greater for the mBMR than the pmBMR2, indicating that in situ NDF degradation may have been influenced more by hybrid than stage of maturity. More research is needed to determine the effects of feeding pmBMR-based diets on NDF digestibility, feed intake, and lactational performance of dairy cows.

Faster disappearance of NDF from the rumen because of increased rate of NDF digestion may reduce distention from gut fill over time and allow greater voluntary feed intake when BMRCS based-diets are fed. However, inconsistent effects of BMRCS on DMI have been caused by various factors, including cows differing in physiological state and duration of experimental periods (mostly less than 4 wk). Results of DMI from the onset of lactation through 180 DIM support the importance of investigating the intake pattern of dairy cows during relatively longer periods. In our case, we observed that cows fed the BMR diet increased DMI post peak lactation compared to those fed the CCS diet. This suggests that ruminal distention from gut fill was not a limiting factor during the first several weeks of lactation, but around peak lactation ruminal distension from gut fill

becomes the dominant mechanism to control feed intake. Due to the relatively short experimental periods and early stage of lactation in the N utilization experiment, no effect was found between source of CS and AH for any measure of intake.

In general, nutrient digestibilities with BMR-based diets are similar to or slightly higher than diets with CCS hybrids. Total tract digestibilities of DM and OM did not differ in response to CS hybrids. However, total tract digestibility of DM and OM increased when feeding the HAH compared with the FAH, because the HAH would have higher concentrations of pectin which ferments rapidly and completely in the rumen. Total tract digestibility of CP was higher for CCS-based diets compared with BMR-based diets. Greater passage of substrate from the rumen in cows fed BMR-based diets may have increased hindgut fermentation and decreased apparent total tract CP digestibility. Total tract digestibility of CP was not affected by quality of AH.

Like the pattern of DMI, milk yield increased with feeding the BMR diet compared with the CCS diet post peak lactation. Milk yield was not different between dietary treatments through peak lactation, whereas milk yield post peak lactation increased by feeding the BMR based-diet. The increases in DMI of 1.1 kg/d resulted in an increased milk yield of 2.2 kg/d increases in milk production observed when feeding BMR silage may have been primarily driven by increases in DMI due to greater NDF digestibility. In addition, increased NDF digestibility may also increase the energy density of diets, as cows fed the BMR diet resulted in the smallest loss of BW in the first 60 DIM compared to those fed the CCS diet, suggesting that the BMR diet had a slight advantage in energy that was partitioned toward body tissue during early lactation.

Throughout this project, we did not find major impacts of feeding BMRCS-based diet on ruminal fermentation. Cows fed CCS- or BMR-based diets maintained similar ruminal pH and its diurnal patterns; however, substantially greater rate and extent of ruminal degradation of the HAH compared with the FAH likely resulted in a greater rate of VFA production with inadequate hydrogen ion removal and buffering to offset VFA production, resulting in a longer duration of time for ruminal pH lower than 5.8. Total VFA concentration did not differ due to source of CS, but cows fed with the HAH increased the total VFA concentration compared to those fed with the FAH. Source of CS did not influence molar proportion of acetate, but feeding the HAH tended to decrease acetate ($P = 0.08$). Molar proportion of propionate was not affected by source of CS, whereas it increased when the HAH was fed only in CCS-based diet, resulting in a CS $\times$ AH interaction. Although feeding BMR-based diets decreased ruminal NH_3-N concentration, it did not attribute to MCP production. Diet compositions for BMR-based diets were slightly higher in NDF and approximately 3% lower in NFC concentration compared with CCS-based diets. Given the fact that energy is the most limiting factor in microbial growth, it is interesting to note that the BMR treatments with relatively low NFC in the diets were able to maintain similar MCP production compared with CCS-based diets.

The BMR-based diets tended to increase ($P = 0.08$) efficiency of feed N to milk N compared with CCS-based diets because the BMR-based diets numerically secreted more N into milk with less intake of feed N. Concentration of MUN decreased by feeding BMR-based diets. Lower MUN concentration with similar milk protein yield would suggest that cows fed BMR may have been more efficient in converting feed N into milk

and body tissue N. Feeding the HAH further reduced MUN concentration, which may

have been affected indirectly by increasing fecal N output. Concentrations of MUN,

BUN, and urinary urea N all followed the same pattern being reduced by feeding BMR,

and these concentrations were further reduced by feeding the HAH. Ruminal NH_3-N

concentration was lower for cows fed BMR-based diets than those fed CCS based-diets,

but was not affected by quality of AH. Feeding BMR-based diets with similar N intake

reduced urinary N excretion by 25% with a tendency for less N excreted into manure ($P =$

0.10). In contrast, fecal N excretions did not differ due to source of CS. Cows fed with

the HAH in their diets excreted 15% less N into urine, but they excreted more fecal N,

resulting in no effect on manure N excretion in response to feeding different quality of

AH. When less N is found in the urine relative to feces (lower UN:FN), less ammonia

loss from manure is expected, because urinary N is more vulnerable to environmental

losses than fecal N. Similarly, a higher MkN:MaN is more desirable, because it indicates

that less manure N must be managed per unit of milk N produced by the herd. Feeding

BMR-based diets decreased the UN:FN, and it was further reduced by feeding the HAH.

While cows fed the BMR-based diets tended to increase the MkN:MaN, quality of AH

did not affect the ratio. The lower ratio of UN:FN with a greater ratio of MkN:MaN for

the BMR-based diets indicates that feeding BMR may reduce manure NH_3-N by reducing

excretion of urinary N and increasing secretion of milk N per unit of manure N excreted.

Feeding the HAH shifted the route of N excretion from urine to feces, which is an

effective way of reducing NH_3 volatilization and resultant pollution. However, this

benefit was not achieved on excretion of manure N through feeding the HAH. The faster

passage rate for the HAH may have increased fermentation in the hindgut, reducing

microbial capture of RDP in the rumen and consequently causing an increase in fecal N excretion. Feeding the HAH reduced urinary N excretion compared with the FAH. However, cows fed the BMR-FAH had the same UN:FN compared to those fed the CCS-HAH, and feeding BMR with the FAH or the HAH had a better MkN:MaN than CCS-based diets, which supported our hypothesis that feeding BMR with the FAH would maintain milk production in early lactating cows while improving N efficiency. Nutritive quality of AH would not impact N utilization in view of environmental performance of dairy cows fed BMR due to enhanced nutrient utilization and its contribution to the overall N utilization by BMR. However, relatively small nutritive differences between the FAH and the HAH tested in our study should not be discounted on the extrapolation of our result on the UN:FN.

In conclusion, these studies demonstrate that feeding BMR silage in a high forage diet with a high concentration of AH can have beneficial effects to lessen body fat mobilization in fresh cows without limiting DMI around peak lactation, resulting in longer peak milk production. In addition, feeding BMR increased MkN:MaN by channeling more dietary N into milk as opposed to manure N excretion, whereas HAH reduced N excretion into the urine, it only shifted the route from urine to fecal excretion without increasing the ratio of MkN:MaN indicate that feeding forages higher in ruminal degradability can represent an environmental advantage over traditional sources of forages in lactation dairy diets. Additional ecological advantages may be achieved by incorporating BMRCS in a DCP system. Increased degradability of NDF for pmBMR1 has the potential to improve ruminal fermentation and energy efficiency in dairy cows. Feeding BMRCS exerted nutritive and environmental benefits when fed with typical

Intermountain West lactation dairy diets. Further research is needed to understand interactive aspects of nutrient utilization with other dietary ingredients under different physiological conditions to take full potential benefits of BMRCS.

APPENDIX

February 28, 2013

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Dr. Nestor,

I am preparing my dissertation in the Animal, Dairy, and Veterinary Sciences Department at Utah State University. I hope to complete my degree in the April of 2013.

I am requesting your permission to include the paper titled: Effects of feeding brown midrib corn silage with a high dietary concentration of alfalfa hay on lactational performance of Holstein dairy cows for the first 180 of lactation, of which you are a coauthor. Your contribution will be acknowledged in a footnote to the chapter title.

Please indicate your approval of this request by signing in the space provided, attaching any other form or instruction necessary to confirm permission. If you have any questions, please call me at the number above.

Thank you,

Michael Shane Holt

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CURRENT FIELDS OF INTEREST:
- Manipulation of ruminal fermentation and its contribution to animal production.
- Enhancement of forage utilization by ruminants.
- Improvement of nutritive value of low-quality forage for ruminants.
- Implementing a nutritional management plan to reduce environmental pollution.
- Analyzing dairy records to implement a nutritional management plan.
- Application of current strategies to improve performance of dairy cattle.

TEACHING INTEREST:
- Principles of Animal Nutrition
- Applied Ruminant Nutrition
- Dairy Cattle Production and Management
- Lactation, Milk, and Nutrition

EDUCATION:

Ph.D., Animal Science with emphasis in Dairy Nutrition, Utah State University. Logan, UT, 2010 - Present; Planned Ph.D. Dissertation Title: **Strategic Approaches to Develop Optimal Feeding Program of Brown Midrib Corn Silage to Lactating Dairy Cows In The Intermountain West**; Advisor: Jong-Su Eun, Ph.D. Expected Date of Completion: May, 2013.

M.S., Dairy Science, Utah State University, Logan, UT, May 2010; Thesis Title: **Effects of Corn Silage Hybrid and Dietary Nonforage Fiber Sources on Feed Intake, Ruminal Fermentation, Digestibility, and Lactational Performance of Holstein Dairy Cows**; Advisor: Allen J. Young, Ph.D; Research Advisor: : Jong-Su Eun, Ph.D.

B.S., Animal Science , Utah State University , Logan UT, May, 2001.

A.A., Equine Science, Collage of Southern Idaho, Twin Falls, ID May, 1997.

PROFESSIONAL EXPERIENCE:

Nutrition Consultant: 2002 – Present, Cargill Animal Nutrition, Rupert, ID.
Manager of Domestic Sales: 2001 – 2002 Mountain Sunrise Feed, Enterprise, UT

ACADEMIC HONORS:

- **Graduate Researcher of the Year Nominee**: Utah State University, Logan, UT; Spring, 2013.
- **Outstanding Alumni**: Collage of Southern Idaho, Twin Falls, ID; 2002.

SPECIAL SKILLS AND TECHNIQUES:

- Fermenter operation for continuous culture system.
- In vitro and in vivo techniques for evaluation of feedstuff.
- GC analyses of long chain fatty acids, volatile fatty acids, and methane.
- Basic techniques for nutrient analysis.
- Handling experimental animals.
- Statistical analysis using the Statistical Analysis System (SAS).
- Computer skill: proficient in using Microsoft Office and feed formulation software.

PROFESSIONAL MEMBERSHIP:

- American Dairy Science Association
- Nevada Cattleman's Association
- Utah Cattleman's Association

PUBLICATIONS:

Refereed Journal Articles

Holt, M. S., K. Neal, J.-S. Eun, A. J. Young, J. O. Hall, and K. E. Nestor Jr. Corn silage hybrids and quality of alfalfa hay affect dietary nitrogen utilization by early lactating dairy cows. Manuscript submitted and in review process (manuscript #: JDS-13-6689).

Holt, M. S., J.-S. Eun*, A. J. Young, X. Dai, and K. E. Nestor. 2013. Effects of feeding brown midrib corn silage with a high dietary concentration of alfalfa hay on lactational performance of Holstein dairy cows for 180 days-in-milk. J. Dairy Sci. 96:515–523.

Dschaak, C. M., C. M. Williams, **M. S. Holt**, J.-S. Eun, A. J. Young, and B. R. Min. 2010. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. J. Dairy Sci. 94:2508–2519.

Holt, M. S., C. M. Williams, C. M. Dschaak, J.-S. Eun, and A. J. Young. 2010. Effects of corn silage hybrids and dietary nonforage fiber sources on feed intake, digestibility, ruminal fermentation, and productive performance of lactating Holstein dairy cows. J. Dairy Sci. 93:5397–5407.

Abstracts in Refereed Conference Proceedings

Holt, M. S., K. Neal, J.-S. Eun, J. E. Creech, A. J. Young, and X. Dai. In situ degradation kinetics of brown midrib corn silage hybrids harvested prior to or at maturity. Abstract submitted to the 2013 ADSA-ASAS Annual Meeting.

McDonald, M. N., **M. S. Holt**, A. J. Young, J.-S. Eun, and K. E. Nestor Jr. Lactational performance and ruminal fermentation profiles of dairy cows fed different corn silage hybrids ensilaged without or with microbial inoculant. Abstract submitted to the 2013 ADSA-ASAS Annual Meeting.

Eun, J.-S., **M. S. Holt**, A. J. Young, and D. R. ZoBell. Nutritional strategies to optimize feeding brown midrib corn silage to dairy and beef cattle. Abstract submitted to the 2013 ADSA-ASAS Annual Meeting.

C. S. Saunders, **M. S. Holt**, J.-S. Eun, D. R. ZoBell, A. J. Young, and K. E. Nestor Jr. Growth performance and ruminal fermentation characteristics of growing beef steers fed brown midrib corn silage-based diet. Abstract submitted to the 2013 Western Section ASAS Annual Meeting.

Holt, M. S., A. J. Young, J.-S. Eun, and K. E. Nestor. 2012. Effects of corn silage hybrids and quality of alfalfa hay on nitrogen metabolism and ruminal fermentation of early lactating dairy cows. J. Dairy Sci. 95 (Suppl. 2):176. (Abstr.)

Holt, M. S., A. J. Young, X. Dai, K. E. Nestor, and J.-S. Eun. 2012. Effects of feeding brown midrib corn silage with a high dietary concentration of alfalfa hay during early and midlactation on milk production of Holstein dairy cows. J. Dairy Sci. 95 (Suppl. 2):608. (Abstr.)

Dschaak, C. M., C. M. Williams, **M. S. Holt**, J.-S. Eun, and A. J. Young. 2010. Effects of condensed tannins supplementation on ruminal fermentation and lactational performance of dairy cows when fed high or low forage diet. J. Dairy Sci. 93(E-Suppl. 1):81. (Abstr.)