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**EFFECTS OF ENHANCED *IN VITRO* NEUTRAL
DETERGENT FIBER DIGESTIBILITY OF
FORAGE ON FEED INTAKE AND
PERFORMANCE OF LACTATING COWS**

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EFFECTS OF ENHANCED IN VITRO NEUTRAL DETERGENT FIBER DIGESTIBILITY OF FORAGE ON FEED INTAKE AND PERFORMANCE OF LACTATING COWS

By

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ABSTRACT

Effects of enhanced in vitro neutral detergent fiber digestibility of forage on feed intake and performance of lactating cows

By

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Effects of enhanced in vitro neutral detergent fiber (NDF) digestibility of forage on feed intake, nutrient utilization and performance of high producing dairy cows were evaluated. Brown midrib (bm3) mutation in corn silage increased in vitro NDF digestibility compared to isogenic control. Experimental diets contained either bm3 or control corn silage at similar dietary NDF contents. Cows fed bm3 corn silage increased feed intake, milk production, passage rate for ruminal digesta, post-ruminal starch digestion, and efficiency of microbial nitrogen production. Treatment had no effect on ruminal digesta pool size, chewing activities, and NDF digestibility in the rumen. Bm3 treatment decreased rumen pH, but did not increase fluctuation in rumen pH. In vivo NDF digestibility response to bm3 treatment was negatively related to response in dry matter intake. Beneficial effects of enhanced in vitro NDF digestibility of forage were greater for high producing cows and cows fed high forage diets.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
BCS	Body condition score
BW	Body weight
bm3	Brown midrib 3 mutant for maize
BMR	Brown midrib mutant for sorghum and pearl millet
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
FCM	Fat-corrected milk
FC	Forage to concentrate ratio.
MN	Microbial nitrogen
MEOP	Milk energy output as a percentage of NE_L intake
NDF	Neutral detergent fiber
NAN	Non-ammonia nitrogen
NANMN	Non-ammonia non-microbial nitrogen
OM	Organic matter
SCM	Solid-corrected milk
VFA	Volatile fatty acid

INTRODUCTION

Maximizing DMI is an important management goal for high producing dairy herds. As genetic ability to produce milk increases, nutritional management to meet the energy requirement of dairy cows becomes increasingly important. The amount of energy needed for maintenance and milk production of high producing cows often exceeds the amount of energy that they can consume. Milk yield of high producing dairy cows usually exceeds 50 kg per day at peak lactation. Even though they cannot consume enough feed to meet their energy requirements, cows in early lactation can sustain a high level of milk yield by mobilizing body reserves. Therefore, they are more susceptible to metabolic diseases such as ketosis or fatty liver. Even if high producing cows do not experience a serious metabolic disorder, they may have subclinical disorders that might compromise DMI and milk yield

Digestibility of forage NDF could be a key factor to maximize DMI of high producing cows. Increasing feed intake with adequate fiber supply is a challenge. Dairy cows require effective NDF in diets to maintain rumen function. NRC (1989) recommended that dairy rations contain at least 25% NDF of dietary DM, and more than 75% of dietary NDF come from forage source. However, if dietary NDF content is too high, maximum

DMI is limited by physical fill in the rumen. When physical fill limitation to maximum DMI exists, enhanced ease of NDF hydrolysis may result in rapid disappearance of the NDF fraction from the rumen, reduce physical fill in the rumen, and allow greater voluntary feed intake. Although nutritional importance of NDF is accepted widely, specific effects of NDF degradability are not clearly understood. Forage NDF varies greatly in its digestibility (Nocek and Russell, 1988; Allen and Oba, 1996), which indicates that forage NDF varies in its physical, chemical, and nutritional characteristics. Nutritional importance of enhanced NDF digestibility needs to be evaluated.

A forage with brown midrib mutant consistently increases NDF digestibility (Cherney et al., 1991). A comparison of brown midrib corn silage with isogenic control corn silage may isolate the specific effect of NDF digestibility on animal performance because this comparison minimizes other confounding nutritional and physical characteristics found in forages to be compared. The objective of this series of studies is to evaluate the effects of enhanced NDF digestibility on DMI and animal performance. Specifically, the effects of brown midrib corn silage on DMI, ruminal fermentation, digestion characteristics, and milk production of high producing dairy cows are focused.

CHAPTER 1

A REVIEW OF LITERATURE

Regulation Mechanisms of Voluntary DMI

Understanding regulation mechanisms of voluntary feed intake is essential before any management action is taken to enhance DMI of high producing cows. The mechanisms that regulate DMI have been studied extensively. In general, cows consume feeds to meet their energy requirement. When cows are fed high energy diets which are palatable, digestible and low in fill, feed intake is regulated by the energy density of the ration (Mertens, 1994). According to the theory of Conrad et al. (1964), voluntary feed intake is regulated by either energy density of a diet, or physical fill depending on the digestibility of the diet cows consume. Although this theory simplifies the complex mechanisms that limit DMI of dairy cows, feed intake is regulated by various factors depending upon metabolic status of animals. Integration of several positive and negative stimuli generally contributes to the regulation of voluntary feed intake (Forbes, 1977).

In general, taste, smell, texture and visual appearance influence feed intake. Less palatable feeds often depress DMI. However, psychogenic factors may not enhance voluntary feed intake of cows in early lactation. Nombekela et al. (1994) found that dairy cattle preferred sweet taste compared to the other primary tastes which are sour, salty and bitter. They implied that a sucrose-sweetened diet had the potential to enhance feed consumption. However, in their follow-up study, sucrose supplementation at 1.5% of dietary DM did not enhance voluntary feed intake of dairy cows in early lactation (Nombekela and Murphy, 1995). Although they observed a transient increase in DMI for the sucrose-supplemented diet during first two weeks after parturition, the effect of sweetener did not have a long term (over 12 weeks of experimental period) effect on DMI and milk yield.

The theory that dietary bulk and physical distention of the reticulorumen can limit feed intake (Van Soest, 1994) has been accepted widely. DMI is more likely to be controlled by physical fill when cows are fed high forage diets (Johnson and Combs, 1991; Dado and Allen, 1995). In the study of Dado and Allen, water-filled plastic containers, which equaled 25% of pretrial rumen volume, were placed into the rumen of cows fed 25% of NDF or 35% of NDF in the diet. The inert bulk decreased DMI for cows fed 35% NDF in the ration, but did not affect DMI for cows fed 25% NDF in the ration, indicating physical fill regulates maximum DMI of dairy cows when they are fed high NDF diet. Johnson and Combs (1991) studied the effect of rumen inert bulk on DMI by replacing 25% of reticulorumen contents with a water-filled bladder. They used cows in early lactation which were in slightly positive energy balance. Both DMI and water intake were observed to be depressed due to the rumen inert bulk.

NDF was proposed as a chemical indicator to predict DMI because of its filling characteristics in the rumen (Mertens, 1987). Cell wall constituents ferment slowly and stay in the rumen longer than other feed components. However, dietary NDF content alone cannot predict the filling effect of a diet in the rumen. Filling effect of NDF is often confounded by many other factors; for example, difference in rate of passage may change the filling effects of NDF. Rate of passage is controlled by level of DMI, ambient temperature, particle size, and particle density (NRC, 1987). Grinding and pelleting feeds increases rate of passage (NRC, 1987). In addition, passage from the rumen depends upon the quantity of particles in close proximity to the reticulo-omasal orifice (Allen and Mertens, 1988). Therefore, rate and potential extent of digestion for forage NDF might also affect ruminal retention time of NDF because of their effect on particle density or buoyancy (Allen, 1996). During fermentation, fibrous particles are associated with gases generated by ruminal microbes, which make particles buoyant longer and to be selectively retained in the rumen. Passage from the rumen also depends upon the rate of particle size reduction, which is stimulated by eating chews, ruminating chews as well as microbial degradation (Allen and Mertens, 1988). In addition, NDF itself varies in its degradability in the rumen. Ruminal digestibility of forage NDF has been reported to range from less than 35% to over 75% for various forage types (Nocek and Russell, 1988). Although forage NDF digestibility in vivo varies by animals and feeding conditions, much of the variation could be due to the composition and structural differences of the forages; in vitro NDF digestibility after 30h incubation was reported to vary from less than 30% to 60% for corn silage and alfalfa (Oba and Allen, 1996). Thus, it is impossible that dietary NDF concentration alone measures all these factors that might influence filling effects in the rumen.

Another study observed no effect of rumen inert bulk on DMI (Johnson and Combs, 1992). They inserted a water-filled bladder into the rumen of cows in early and mid-lactation. Then, they offered two diets that differed in forage to concentrate ratio. There was no significant effect of rumen inert bulk on DMI regardless of energy density of diets. It is probably because energy requirement of cows fed both diet groups was well satisfied, and physical fill did not limit feed intake of these cows. Dado and Allen (1995) observed the effect of rumen inert bulk on DMI only for the cows fed a high forage diet. Although physical fill is one of the most important factors which regulate voluntary DMI of high producing dairy cows, the relative effect of fill on DMI greatly depends upon the energy requirement of dairy cows.

Physiological satiety also regulates voluntary DMI of animals. Feeding rapidly fermentable carbohydrates often depressed feed intake (McCarthy et al., 1989; Casper et al., 1990; Oliveira et al, 1993; Aldrich et al., 1993, Overton et al., 1995). Chemoreceptors in the ruminal wall are sensitive to changes in pH, and may regulate feeding behavior of animals (Harding and Leek, 1972). Edward and Poole (1983) reported that the addition of sodium bicarbonate at 2% of dry matter significantly increased feed intake of dairy cows, indicating the possible role of rumen pH in feed intake regulation. Increased serum glucose level and subsequent insulin secretion is the major physiological satiety signals for non-ruminants. Similarly, Deetz and Wangsness (1981) found that intrajugular insulin injection decreased feed intake of sheep. Propionate as well as glucose possibly stimulates insulin secretion for ruminants (De Jong, 1982; Harmon, 1992). Istasse et al. (1987) infused propionate into the rumen approximately 50% of daily propionate supply in two 3-h pulses, and observed concurrent sharp peaks in insulin concentration at the jugular vein. Lactating dairy cows were also observed to respond to high starch diet by elevated plasma insulin concentration (Lee et al., 1990). Propionate alone can directly affect feed intake regulation. Anil and Forbes (1980) demonstrated that the liver has receptors which are sensitive to propionate, and propionate infusion into the portal vein almost completely prevented eating.

It is less likely that a single factor limits maximum feed intake although one factor may sometimes become more dominant than others in regulation of maximum feed intake. Depending upon feeding conditions, both physical fill and physiological satiety can synergistically interact with each other to regulate feed intake. Physical fill limitation to DMI does not necessarily mean that there is no additional capacity in the reticulorumen. Dado and Allen (1995) found a reserve space of more than 16L in the reticulorumen of the dairy cows challenged by both dietary fiber and rumen inert bulk. Mbanya et al. (1993) suggested that DMI be affected by a combined effect of acid production and distention, not by one of the effects alone. They infused acetate, propionate, or both, with or without distention of the rumen by a balloon. Combination of VFA infusion and reticulorumen distention significantly depressed DMI while VFA infusion or distention alone did not. The role of the osmo-receptor or chemo-receptor in the rumen may need to be considered in the relation of distention of the reticulorumen.

Optimizing Rumen Fermentation

Increasing energy density of a diet is another management strategy to improve energy intake of animals. Energy requirements of non-ruminant animals are satisfied relatively easily by supplementing fat, but fat supplementation may lead to nutritional complications for ruminant animals. Palmquist and Jenkins (1980) stated that physical coating of forage particle, reduced cation availability, a toxicity to certain microorganisms were associated with feeding excess fat in a dairy diet. It is generally suggested that fat supplementation should be limited to 5% of DMI, to avoid negative effects on animal performance (Palmquist and Jenkins, 1980; Cant et al., 1993). In addition, fat supplementation may not be financially feasible for some dairy producers. Fat has 2.25 times as much energy as starch does, but fat supplementation can cost two to three times more than cereal grains per calorie.

Non-structural carbohydrates are other major sources of energy for dairy cows. However, starch cannot be fed beyond the buffering capacity of the rumen because starch ferments very quickly in the rumen, and rapid fermentation acid production depresses feed intake by lowering rumen pH and by destroying a stable rumen eco-system. Previous research (McCarthy et al., 1989; Casper et al., 1990; Oliveira et al, 1993; Aldrich et al., 1993, Overton et al., 1995) indicated the negative relation between rapidly fermentable non-structural carbohydrates and DMI (Knowlton et al., 1996). When excess amount of non-structural carbohydrates are available in the rumen, lactate production may increase due to rapid growth of starch fermenting microorganisms. In the rumen of high producing dairy cows compared to that of steers, ruminal microorganisms grow much more rapidly due to faster liquid dilution rate. *Streptococcus bovis* produces lactate with a rapid turnover of ruminal digesta, although this organism ferments starch to formate, acetate, and ethanol at slower growth rate (Russell et al., 1981; Russell and Allen, 1983). Due to the activity of rapidly growing starch fermenters, lactate accumulates in rumen ecosystem and lowers ruminal pH. Lactate producers are more tolerant to lower rumen pH than cellulolytic microorganisms, and they dominate in the rumen by producing more lactate and making ruminal pH even lower (Slyter, 1976). Excess ruminal lactate concentration results in rumen acidosis and depression of DMI. The diet of high producing dairy cows cannot largely rely on non-structural carbohydrates as an energy source.

Optimum amount of starch fermentation in the rumen depends upon the buffering capacity of the rumen because fermentation acid production needs to be balanced with its removal to maintain stable rumen environment (Allen, 1997). Dairy cows require physically effective NDF in diets to maintain rumen function. Physically effective fiber should be distinguished from effective fiber. Effective fiber is determined by the difference in milk fat content when a non-forage source NDF replaces forage NDF in a dairy diet, whereas physically effective fiber is determined by its stimulatory effect on chewing (Mertens, 1997). The contribution of dietary NDF to buffering capacity in the rumen can be evaluated by chewing time because rate of saliva flow is higher during chewing than resting (Cassida and Stokes, 1986). Saliva contains bicarbonate and phosphate ions which buffer the fermentation acids in the rumen (Bailey and Balch, 1961). Since the chemical composition of saliva is relatively constant (Erdman, 1988), buffering capacity of the rumen is mainly determined by the quantity of saliva flow or chewing time. On the other hand, effective fiber does not directly measure the effect of dietary NDF on rumen fermentation. Milk fat content may be confounded by metabolic status of animals because a variable amount of milk fat is derived from mobilized body fat, especially in early lactation (Mooney and Allen, 1997). Physically effective fiber more accurately measures the contribution of dietary NDF to buffering capacity in the rumen.

Dairy rations should contain at least 25% NDF, and more than 75% of the dietary NDF should come from forage sources (NRC, 1989). Dietary forage NDF concentration is more related to rumen pH than dietary NDF concentration (Allen, 1997). Particle length of dietary fiber is closely related to chewing activities (Santini et al., 1983). Finely ground forage may be less effective at stimulating saliva flow into the rumen. The effect of non-forage source NDF on total chewing time varies depending on the particle size of a forage to be compared with (Mooney and Allen, 1997). However, physical effectiveness of non-forage source NDF is generally less than that of forage NDF because of smaller particle size and faster passage rate (Grant, 1997). Adequate amount of forage NDF is needed to maintain rumen function.

The relationship between forage fermentability and physical effectiveness of fiber is not clearly understood. Beauchemin (1991) and Nelson and Satter (1992) reported decreased chewing activities for diets containing alfalfa harvested at early maturity than at late maturity. Grant et al. (1995) found that total chewing time per kg NDF intake was lower for brown midrib sorghum silage which is higher in NDF digestibility than normal sorghum silage. On the other hand, Robinson and McQueen (1997) reported no treatment effects of forage NDF fermentability on chewing activity.

Effects of Brown Midrib Mutant Forages on Animal Performance

Digestibility of forage NDF could be a key factor to maximize DMI of high producing cows. Increasing feed intake with adequate fiber supply is a challenge. If dietary NDF is not sufficient, decreased chewing activity results in lower ruminal pH, leading to decreased cellulolytic bacteria activities and increased risk of rumen acidosis. If dietary NDF content is high, DMI may be limited by physical fill in the rumen. Both situations depress voluntary dry matter intake. When physical fill limitation to maximum DMI exists, enhanced ease of NDF hydrolysis may stimulate rapid disappearance of the NDF fraction from the rumen, reduce physical fill in the rumen, and allow greater voluntary feed intake (Dado and Allen, 1995). Grant et al. (1995) and Dado and Allen (1996) fed silages with similar NDF and CP content but different NDF digestibility to lactating dairy cows, and found significant increases in DMI and milk production for diets with higher NDF digestibility.

Potential extent of NDF digestion and rate of digestion are partly determined by the lignin content of a forage since lignin is indigestible (Van Soest, 1994). The effect of NDF digestibility on voluntary DMI can be studied by using brown midrib mutant forages. Brown midrib mutant forages are significantly lower in lignin content and higher in NDF digestibility than normal control forages (Cherney et al., 1991). The effect of brown midrib 3 (bm3) corn silage on the livestock performance has been studied extensively since the early 1970's. One experiment with outstanding performance difference was reported by Muller et al. (1972). They compare an F2 population of maize which segregated for bm3 with the F2 population of phenotypically normal maize and a commercial hybrid. The ears were removed before ensiling so that different level of grain yield would not affect the results. NDF concentration was about 60% for each silage. Eighteen crossbred ram lambs were randomly assigned to the diets of bm3, the normal counterpart, and another commercial hybrid. Lignin concentrations of silage were 4.5%, 6.8%, and 6.9% for bmr3, normal, and another commercial hybrid, respectively. They reported that 29% greater ($P<.01$) voluntary DMI for bm3 corn silage than for normal silage, when fed to lambs ad libitum. The apparent digestibility of DM ($P<.10$) and NDF ($P<.05$) were higher for bm3 corn silage. They concluded that the less lignified cell wall in the bm3 corn silage resulted in faster and higher extent of ruminal digestion which is associated with greater dry mater intake.

The effects of bm3 corn silage on performance of lactating dairy cows were evaluated (Table 1). Frenchick et al. (1976) reported that bm3 corn silage increased milk yield by 0.66 kg/d. Keith et al. (1979) found .9 kg and 1.0 kg more FCM yield when cows were fed bm3 silage at 75:25 and 60:40 forage to concentrate ratio, respectively. Cows fed bm3 corn silage increased BW gain. Rook et al. (1977) found that cows fed the diet with bm3 corn silage gained 40 kg more BW than cows fed the control diet during the experimental period from the 6th week to 13th week after calving. Absorbed nitrogen and retained tissue nitrogen tended to be higher for cows fed bm3 corn silage. Similarly, Sommerfeldt et al. (1979) reported that cows fed bm3 corn silage gained 58.4 g/d while cows fed control corn silage lost 47.3 g/d.

Similar research has been conducted for sorghum and pearl millet with brown midrib (BMR) mutant (Table 2, 3). The most pronounced BMR effect on lactating dairy cows was recently observed by Grant et al (1995). They compared a BMR sorghum silage with another normal sorghum hybrid, and reported a 24% greater dry matter intake and a 46% more FCM yield for cows fed BMR sorghum silage. Total volatile fatty acid concentration in the rumen was higher, and the rate of forage passage was faster for cows fed BMR sorghum than normal sorghum. Although a sorghum silage is generally cosidered to be nutritionally inferior to alfalfa or corn silage (Lance et al., 1964), BMR sorghum hybrids were shown to be as competitive as those forages (Grant et al., 1995; Lusk et al., 1984). Cherney et al. (1990) reported BMR mutation in pearl millet also improved digestibility, DMI, and ADG of castrated ram. The effect of BMR pearl millet on dry matter intake and weight change was more specifically observed for the second cuttings. They also observed that regrowth of pearl millet BMR was more palatable to grazing lambs than the control; lambs spent 2.6 times more time on grazing BMR plots.

The effects of brown midrib forages on animal performance were not consistent in these previous experiments although general animal responses were positive. Dry matter intake was reported to be higher for animals fed brown midrib mutant plants (Muller et al., 1972; Rook et al., 1977; Block et al., 1981; Cherney et al., 1990; Grant et al., 1995); however, other research reported that DMI was not different between brown midrib forages and control forages (Frenchick et al., 1976; Keith et al., 1979; Sommerfeldt et al., 1979; Wedig et al., 1987). Rook et al. (1977) and Block et al. (1981) used early lactation cows, and observed greater DMI for cows fed bm3 corn silage. High producing dairy cows in early lactation usually experience negative energy balance, and physical fill more dominantly limits voluntary feed intake, especially when fed high NDF diet (Dado and Allen, 1995). Enhanced NDF digestibility can stimulate rapid rumen turnover, allowing greater feed intake. On the other hand, maximum DMI of heifers and steers (Wedig et al., 1987) or mid to late lactation cows (Kieth et al., 1979; Frenchick et al., 1976) may not be limited by physical fill, but more likely by chemostatic factors, therefore more digestible fiber might not stimulate voluntary DMI. The most pronounced effects of brown midrib forages were observed by Muller et al. (1972) and Grant et al. (1995). Muller et al (1972) fed 60% NDF diet, removing ears from corn silage in order to detect more accurate effect of bm3 treatment. Likewise, Grant et al. (1995) fed mid lactation cows at relatively higher dietary NDF (40% of dietary DM).

When forage is supplemented with other feeds, the effect of brown midrib forages might be masked. Frenchick et al. (1976) did not observe significant DMI difference between bm3 corn silage and control, and attributed their results to the fact that forage made up only half of the diet in their experiment. When heifers were offered bm3 corn silage with corn grain at 0%, 1%, and 2% of body weight, DMI was significantly higher only when fed without corn grain (Kieth et al., 1981). Feeding more starch in diets might change rumen environment and diminish the effect of enhanced NDF digestibility. Source of starch and processing methods can affect ruminal fiber digestion.

Responses to brown midrib treatments in apparent total tract NDF digestibility also varied among experiments. Greater total tract NDF digestibility was reported for brown midrib treatments (Muller et al., 1972; Sommerfeldt et al., 1979; Stallings et al., 1982; Weller and Phipps, 1986; Fritz et al., 1988; Cherney et al., 1990; Grant et al., 1995). However, some experiments found that total tract digestibility was not improved by brown midrib forages (Rook et al., 1977; Lusk et al., 1984; Wedig et al., 1988). Similar total tract digestibility within comparisons was attributed to greater DMI for the bm3 treatment (Rook et al., 1977). Total tract digestibility declines as feed intake increases (Tyrrell and Moe, 1975). Greater DMI for brown midrib treatments can be associated with faster rate of digesta passage, diminishing potential digestibility. Wedig et al. (1986) implied that compensatory postruminal digestion might make it more difficult to see the positive effect of BMR treatment on apparent total tract digestibility.

Some researchers reported increased milk yield (Frenchick et al., 1976; Kieth et al., 1979; Grant et al., 1995), while the others did not (Rook et al., 1977; Sommerfeldt et al., 1979; Block et al., 1981; Stallings et al., 1982). Instead, Rook et al. (1977), Sommerfeldt et al. (1979), and Block et al. (1981) observed that cows fed bm3 corn silage gained more BW.

Block et al. (1981) concluded that energy intake was not limiting milk production in their experiment because extra energy was directed towards BW gain. Data of rumen pH and volatile fatty acid profile did not give clues to why the extra energy from bm3 corn silage was utilized for BW gain (Frenchick et al., 1976; Rook et al., 1977; Sommerfeldt et al., 1979). Milk fat depression was reported when cows were fed bm3 corn silage (Frenchick et al., 1976; Rook et al., 1977; Block et al., 1981). Ruminal acetate to propionate ratio (Block et al., 1981) and rumen pH (Frenchick et al., 1976; Block et al., 1981) were lower for the cows fed bm3 corn silage. However, Grant et al. (1995) observed that milk fat concentration is improved by feeding BMR sorghum silage. Inconsistent energy utilization for bm3 treatments cannot be explained clearly.

Biochemistry of Brown Midrib Mutations

Lignin is amorphous bio-polymer. It plays an important role to maintain structural integrity of plant tissues, but inhibits cell wall degradation in the rumen because of the strong covalent bonds with structural carbohydrates. Lignin synthesis in plants begins with deamination of phenylalanine and tryosine (Cherney et al., 1991). Phenylpropenoic acids are metabolized via hydroxylation and methylation to p-coumaric, ferulic, and synapic acids. Then, those phenolic acids are reduced to their respective aldehydes, and subsequently to p-coumaryl, coniferyl and sinapyl alcohol. Those monomeric alcohols are condensed to polyphenols which are more resistant to degradation by rumen-microorganisms. The metabolized acids, aldehydes, and alcohol are involved in lignin formation. p-Coumaric and ferulic acids are most commonly found in forage that animals consume, and they inhibit the growth of aerobic and anaerobic bacteria (Jung and

Fahey, 1983). In addition, those phenolic monomers especially p-coumaric acid, are cross-linked to structural carbohydrates and decrease the extent of digestion (Burritt et al., 1984). Lignin content is closely related to the ambient temperature, and increases as the physiological maturity of plant proceeds.

Brown midrib mutation modifies the lignin content of plants. Jorgenson (1931) noticed that brown midrib mutation reduces lignin content compared to near isogenic counterpart. Phenotypically, reddish-brown pigmentation is associated with lignified tissues, especially on the leaf midrib. Reduced lignin content possibly contributes to higher forage quality because of more available energy and improvement in digestibility. However, it had not been studied extensively until the 1970's because the trait reduced grain yield, and economical value of corn silage was lower than corn grain. The brown midrib trait had been considered to be less important by plant breeders.

Research conducted since early 1970's showed that the lignin content of a brown midrib forage is significantly lower than its normal counterpart (Cherney et al., 1991). Lignin composition is also influenced by the brown midrib mutation. A brown midrib mutation in sorghum stops aldehyde intermediates being incorporated into the polymer due to the lack of enzymatic activities and results in their accumulation (Bucholtz et al., 1980). Wedig et al. (1989) observed twice as much vanillin in a BMR sorghum as in normal counterpart. Phenolic acid composition in bm3 is also different from normal, lower concentration of p-coumaric acid and similar concentration of ferulic acid has been observed (Fritz et al., 1990; Gerhardt et al., 1994). The lower ratio of p-coumaric acid to ferulic acid in bm3 possibly explains the more extensive ruminal degradation of cell wall components (Hartley and Jones, 1978). Lignin synthesis is associated with deamination of phenylalanine and tyrosine, therefore brown midrib mutations may influence amino acid composition of plants. Gordon and Neudoerffer (1973) reported higher concentration of aspartic acid and lower concentration of phenylalanine and alanine in young bm3 maize (74 days old). Lower concentration of phenylalanine and similar concentration of tyrosine indicated that a brown midrib 1 mutation may block metabolism between phenylalanine and prephenic acid which is a precursor of phenylalanine and tyrosine. Subsequently lignification may be blocked as well.

Cherney et al. (1988) studied the chemical composition of brown midrib mutant and normal counterpart for pearl millet, sorghum, and corn. They used near-isogenic inbreds, near-isogenic hybrids, and chemically induced BMR mutants for the comparisons within sorghum lines, maize lines, and pearl millet lines, respectively. They found lower NDF and lignin concentrations and higher crude protein concentration for brown midrib plants than for normal counterparts of each plant. Fritz et al. (1988), Block et al. (1981), and Keith et al. (1979) reported that the brown midrib forages were higher in crude protein than normal types. Fritz et al. (1988) and Cherney et al. (1990) observed lower NDF concentration for BMR sorghum x sudangrass and pearl millet, respectively. Allen et al. (1997) evaluated 14 different corn hybrids with and without bm3 mutation, and found NDF content was not affected by bm3 mutant and CP content was slightly higher for bm3 corn silage. However, differences in chemical composition between brown midrib mutations and isogenic counterparts have not been consistent for all experiments.

p-Coumaric acid is considered to be more important than ferulic acid in crosslinking of lignin to hemicellulose (Burritt et al., 1984). p-Coumaric acid increased dramatically with plant maturity, while ferulic acid in lignin changed little (Chaves et al., 1982). p-Coumaric acid was found to be higher for normal than for a BMR genotype (Wedig et al., 1989b). The extent of hemicellulosic monosacharide digestion was negatively correlated to the level of p-coumaric acid in plants (Wedig et al., 1989a, b). Ester-bonding occurs between phenolic compounds and uronic acid of hemicellulose for grass (Harkin, 1973), and it inhibits the ruminal degradation of hemicellulose (Van Soest, 1994).

Hemicellulose composition might be altered by brown midrib mutations. The high ratio of xylose to arabinose indicates a more polymerized xylan and less branching with hemicellulosic monosaccharides (Bittner, 1983). This ratio can be a function of forage digestibility since it increases as plants mature (Wedig et al., 1989a). Normal genotypes of sorghum were reported to have higher xylose to arabinose ratio (Cherney et al., 1986; Wedig et al., 1989b). However, difference in hemicellulose modification may be not detectable for immature forages (Wedig et al., 1989a). The difference in hemicellulose composition between brown midrib and normal control has not been studied for maize and pearl millet, and there is insufficient data to say a brown midrib mutation changes hemicellulose composition (Cherney et al., 1991).

Even though NDF digestibility is higher for brown midrib forages, a unit of brown midrib lignin inhibits cell wall digestion more than normal lignin does. A higher degree of cross-linking in the propane side chain of brown midrib lignin was observed (Gordon and Griffith, 1973). The ratio of indigestible residues to ADL was 37% higher for a brown midrib mutant than for normal control, leading Thorstensson et al. (1992) to imply that a unit of brown midrib lignin inhibits NDF digestion more than normal lignin does. Other research reported this ratio was 55% greater for BMR than for normal (Grant et al., 1995). A lower concentration of cinamic acids results in more condensed lignin (Wedig et al., 1989a) and digestibility of brown midrib lignin was reported to be lower (Cherney et al., 1986).

The effect of BMR sorghum on in vitro digestibility was observed as early as four weeks after planting (Hana et al., 1981). Grand et al., (1985) reported that lower lignin content of bm3 corn was detectable for 20 day old corn. However, corn silage is generally harvested close to physiological maturity, so the bm3 effects on digestibility at late maturity are more important. Ramadhan et al. (1976) observed that the effect of bm3 on decreased lignin content and improved digestibility persisted in corn stalk residues even after grain harvest. However, the effect of bm3 gene on improvement in nutritional quality is specific for vegetative portion of a plant. Keith et al. (1980) reported no effect of bm3 grain on performance of growing rats.

There are four distinct brown midrib loci in maize (Kuc' and Nelson, 1964), and they are labeled bm1, bm2, bm3, and bm4. Muller et al., (1971) found that acid detergent permanganate lignin concentration of normal, bm1, bm3, and bm1/bm3 was 6.08%, 5.13%, 4.37%, and 4.60%, respectively. Lechtenberg et al. (1972) compared digestibility of stover from mutant inbred corn plants harvested 35 days after silking. They reported in vitro dry matter disappearance of F2 normal, bm1, bm2, bm3, bm1/bm3, and bm4 was 55.1%, 59.0%, 53.1%, 60.6%, 67.4% and 54.2%, respectively. They concluded that genotype of bm3 was the most promising mutant allele, and most of the following research has focused on bm3 locus of brown midrib maize mutant. On the other hand, numbers associated with the gene symbol for sorghum brown midrib genotypes does not mean that a brown midrib mutant allele is located on a particular locus. In the experiment by Porter et al. (1978), brown midrib mutants of sorghum were chemically induced. Nineteen independently occurring brown midrib mutants were identified and were numerically designated BMR1 to BMR19.

Brown midrib mutations have been identified for maize, sorghum, sudangrass, and pearl millet. This trait can be induced chemically. Porter et al. (1978) soaked sorghum seeds with diethyl sulfate, and induced BMR mutation. However, Cherney et al. (1991) reported that he could not develop a brown midrib mutation for other forage species, and implied that brown midrib trait seems to be occurred only for C4 species. Since the effects of brown midrib mutation is phenotypically expressed by the homozygous recessive, it may not be found in the genomes of polyploid forage species (Vogel and Sleper, 1994).

Agronomic Aspects of Brown Midrib Forages

Although nutritional advantages to animals exist, a brown midrib forage is agronomically inferior to normal genotype due to a less lignified cell wall. Zuber et al. (1977) measured crushing strength and stalk section weight of bm3 and normal corn. They suggested that farmers harvest at near physiological maturity to prevent stalk lodging since their study showed bm3 corn had 17 to 26% lower crushing strength and 8 to 14% lower stalk-section weight than normal control. Brown midrib mutation affects early vigor and growth rate, too. The average linear accumulation rate of total dry matter was 15% higher for normal genotypes. Nicholson et al. (1976) implied that a brown midrib genotype may be more susceptible to *F. moniliforme* and other fungi associated with stalk and ear rots.

Silage yield may be lowered by brown midrib mutation. Brown midrib maize was observed to delay silking by 3 days as average and decrease the ear proportion of total dry matter by 4 to 6% (Weller et al., 1985). The average grain yield, stover yield, and whole plant yield of brown midrib genotypes were 77%, 90%, and 84% of normal genotypes, respectively (Miller et al., 1983). Another research group reported that the bm3 maize yielded 20% lower grain and 17% lower stover than each isogenic normal counterpart on average (Lee and Brebaker, 1984). Recently, Allen et al. (1997) reported bm3 decreased corn silage yield by 12% on average. Miller et al. (1983) as agronomists concluded that normal genotypes offered more potential for silage breeding programs. However, the response to a brown midrib mutant varies greatly by hybrid. Even though a brown midrib mutant generally reduces yield compared to normal control genotype, some hybrids yielded as much as normal counterpart did (Miller et al., 1983). Gentinetta (1989) reported some bm3 hybrids, which contained significantly lower lignin, produced as much grain and stover as normal genotypes did. It might mean that forage breeders can develop a hybrid that is more desirable as an animal feed while maintaining agronomic strength.

Evaluation of specific effects of brown midrib mutation requires two isogenic plants that differ only by the mutation. A brown midrib mutation is incorporated into hybrids by backcross breeding (Allard, 1960; Stoskopf, 1993). Backcross breeding needs a recurrent parent and a donor parent; only the brown midrib mutation should come from a donor parent, and all the other genes must be from a recurrent parent (each hybrid). Therefore, the completion of conversion depends upon number of backcross (BC). The donor parent contributes less to the plant genetics as a backcross breeding program proceeds. The proportion of genes from the donor parent is reduced by one-half following each generation of backcrossing, and expressed by the formula:

$(1 / 2)^{n+1}$ where n is the number of backcrosses.

In the absence of selection, proportion of the donor to the genome is $(1/2)^6 = 0.0156$ in BC5 and $(1/2)^7 = 0.0078$ in BC6 . After plants are backcrossed five or six times, they still have 1.56% or 0.78% of genome from the donor parent, respectively. However, this equation is a simplification for convenience. Depending upon how genes are distributed over the chromosomes, the number of genes from donors left in the plants varies.

Linkage needs to be considered because some genes are located on the same chromosome where the brown midrib mutation is (Stoskopf, 1993). Linkage occurs when a gene is 50 map units or less from an adjacent gene, but genes more than 50 map units apart are not linked and they segregate as if they were on different chromosomes. The probability of eliminating undesirable genes is given by the formula:

$1 - (1 - p)^{\wedge n+1}$ where p is the recombination fraction (p=1/map units)
n is the number of backcrosses.

If genes are 10 units away from bmr, the probability for eliminating those genes after five backcrosses is only about 47% $[\ 1 - (1 - 0.1)^{\wedge 6}\ ]$. Ten to twelve backcrosses may be required to produce true isogenic line which is different from recurrent parent by only one gene (Stoskopf, 1993). Although six backcross breeding with rigid selection is considered to be sufficient to get a near isogenic plant (Allard, 1960), the required number of backcrosses varies depending on species, a trait which is transferred, breeding methods, and how conservative you want to be.

Summary

Maximizing DMI is an important management goal for dairy producers and nutritionists. Physical fill is a factor limiting maximum DMI of high producing dairy cows. Enhanced NDF digestibility may allow more feed intake by rapid turnover of rumen digesta. A brown midrib forage is lower in lignin content and higher in NDF digestibility, and has great a potential in dairy cattle diets despite agronomic defects.

Table 1. Effects of brown midrib 3 (bm3) corn silage on animal performance

	Animal	DMI	Milk Yield	Milk Fat	Body Wt.	F:C (NDF%)
Muller et al.	Lambs	+ 29% ***	---	---	---	(60%)
Frenchick et al.	H - mid lact.	+ 0.91 kg	+ 0.66 kg **	- 0.28% **	+0.11 kg/d **	60:40
Rook et al. (1)	H-early lact.	+ 16% ***	+ 1.6 kg	- 0.29% *	+0.29 kg/d	60:40
(2)	H-early lact.	+ 14% ***	+ 0.8 kg	- 0.02%	+0.76 kg/d ***	85:15
Kieth et al. (1)	H - mid lact.	+ 1.6%	+ 1.3 kg ***	- 0.08%	---	75:25
(2)	H - mid lact.	+ 1.2%	+ 1.6 kg ***	- 0.14%	---	60:40
Sommerfeldt et al.	H-early lact.	- 1.0%	- 0.5 kg	- 0.09%	+1.16 kg/d ***	---
Kieth et al. (1)	Steer	+ 0.47 kg	---	---	+0.08 kg/d *	No Corn
(2)	Steer	+ 0.10 kg	---	---	+0.02 kg/d	2% BW Corn
(3)	Heifer	+ 0.47 kg *	---	---	+0.13 kg/d *	No Corn
(4)	Heifer	- 0.01 kg	---	---	+0.09 kg/d *	1% BW Corn
(5)	Heifer	+ 0.05 kg	---	---	+0.04 kg/d	2% BW Corn
Block et al.	H-early lact.	+ 8.3% **	+ 2.3 kg	- 0.28% **	+0.71 kg/d ***	(36%)
Stallings et al.	H-(109 DIM)	+ 0.5 kg	- 0.4 kg	- 0.14%	+0.08 kg/d	---

* significantly differ ($P < 0.10$)

** significantly differ ($P < 0.05$)

*** significantly differ ($P < 0.01$)

Table 2. Effects of brown midrib 3 (BMR) sorghum silage on animal performance

	Animal	DMI	Milk Yield	Milk Fat	Body Wt.	F:C (NDF%)
Wedig (1)	Steer	+ 0.35 kg	---	---	---	(66%)
(2)	Heifer	+ 0.20 kg	---	---	---	(66%)
Fritz (R x G)	H-non lact.	- 0.20 kg	---	---	---	(75%)
(R x P)	H-non lact.	+ 2.10 kg **	---	---	---	(75%)
Grant et al.	H-mid lact.	+ 21% *	+ 5.7 kg *	+ 0.54% *	---	(40%)

* significantly differ ($P < 0.10$)

** significantly differ ($P < 0.05$)

Table 3. Effects of brown midrib 3 (BMR) pearl millet silage on animal performance

	Animal	DMI	Milk Yield	Milk Fat	Body Wt.	F:C (NDF%)
1st cutting	Sheep	+ 0.3%	---	---	+ 2 g	(66%)
2nd cutting	Sheep	+ 37.8% ***	---	---	+ 172 g **	(66%)

** significantly differ ($P < 0.05$)

*** significantly differ ($P < 0.01$)

CHAPTER 2

EVALUATION OF THE IMPORTANCE OF FORAGE NDF DIGESTIBILITY: EFFECTS ON DRY MATTER INTAKE AND MILK YIELD OF DAIRY COWS

ABSTRACT

Effects of NDF digestibility on performance of dairy cows were evaluated statistically using treatment means for nineteen sets of forage comparisons reported in the literature. All comparisons reported significant differences in NDF digestibility of forages in vitro, in situ, or(and) in vivo. Treatment means were blocked by comparison within an experiment. Statistical model included random effect of block, fixed factorial effect of NDF digestibility (high or low), and dietary NDF level as a covariate. Enhanced NDF digestibility of forage significantly increased DMI and milk production. One unit increase in NDF digestibility in vitro or in situ was associated with 0.15 kg increase in DMI and 0.24 kg increase in FCM(4%). Within this set of comparisons, DMI and FCM (4%) were influenced more by NDF digestibility than by dietary NDF level. The difference in NDF digestibility between the treatments was greater when measured in vitro or in situ than in vivo. NDF digestibility in vitro or in situ might be a better indicator of DMI than NDF digestibility in vivo because forages with high in vitro or in situ NDF digestibility might have shorter rumen retention times, allowing greater DMI at the expense of NDF digestibility in vivo. Digestibility of NDF is an important parameter of forage quality.

(**Key words:** fiber digestibility, intake, fill limitations, forage quality)

Abbreviation key: FC = forage to concentrate ratio.

INTRODUCTION

Dairy cows require effective NDF in diets to maintain rumen function and maximize milk production. Dairy rations should generally contain at least 25% NDF and a large proportion of dietary NDF should come from forage sources (NRC, 1989). Because dietary forage NDF concentration is closely related to chewing activity and is a determinant of rumen pH of dairy cows (Allen, 1997), dairy rations are often formulated to a specific NDF content and(or) forage NDF content. However, NDF content of forage varies widely depending upon species, maturity, and growing environment. Forages are routinely analyzed for NDF content because of its variation as well as its nutritional importance.

Digestibility of NDF may be another important parameter of forage quality because forage NDF varies widely in its degradability in the rumen (Nocek and Russell, 1988; Allen and Oba, 1996) and NDF digestibility possibly influences animal performance. Although dairy cows require forage NDF in diets, feeding excess dietary NDF often limits voluntary feed intake by rumen fill. Mertens (1987) suggested that DMI of dairy cows can be predicted by dietary NDF because of positive relationship between NDF and rumen fill. Therefore, enhanced ease of NDF hydrolysis may stimulate rapid disappearance of the NDF fraction from the rumen, reduce physical fill in the rumen, and allow greater voluntary feed intake (Allen and Oba, 1996). Grant et al. (1995) and Dado and Allen (1996) fed silages with similar NDF and CP content but different NDF digestibility to lactating dairy cows, and found significant increase in DMI and milk production. Several experiments comparing brown midrib mutant corn silage with its isogenic normal control reported enhanced animal performance for cows fed brown midrib corn silage (Frenchick et al., 1976; Rook et al., 1977; Kieth et al., 1979; Sommerfeldt et al., 1979; Block et al., 1981; Stallings et al., 1982). Brown midrib hybrids are consistently higher in in vitro NDF digestibility due to less lignification of plant cell wall (Cherney et al., 1991).

Isolating the specific effect of NDF digestibility on animal performance is difficult. Although many experiments have reported NDF digestibility data, interpretation of results is difficult because of a variety of confounding factors. Experiments comparing forages that differ only in NDF digestibility are rare and it is difficult to confirm that other factors which could potentially affect animal performance did not vary. If two forages are compared at a different forage to concentrate ratio (**FC**), other dietary factors such as ruminal starch digestion might affect DMI and(or) milk production. If the forages are compared at similar FC but different dietary NDF levels, results are confounded by differences in filling effects in the rumen from different NDF concentrations.

Despite technical difficulties for evaluation of NDF digestibility, it is necessary to quantify the effect of NDF digestibility on DMI and milk production because of its potential economic importance. One approach to evaluate the effect of NDF digestibility is a statistical analysis across forage studies. An appropriate statistical model enables confounding factors found in individual experiments to be treated as covariates, then allows development of relevant interpretations about the effect of NDF digestibility on animal performance. The objectives of this analysis are to evaluate and quantify the effect of NDF digestibility on DMI and milk yield of dairy cows based upon the statistical analysis of treatment means reported in the literature.

MATERIALS AND METHODS

A database was developed using treatment means from experiments which reported significant differences in digestibility of NDF among forages either in vivo, in situ, or (and) in vitro. Those experiments which compared legume with grass were excluded from this statistical analysis because of much greater differences in NDF, forage NDF, and (or) starch concentration for diets composed of these forages. In addition, experiments which did not report dietary NDF% and NDF digestibility either in situ or in vitro were excluded from the database. Nineteen sets of forage comparisons (n=38) taken from eight articles in Journal of Dairy Science were utilized for this statistical analysis (Table 1; Llamas-Lamas and Combs, 1990; Arieli and Adin, 1994; Orozco-Hernandez et al., 1994; Ruis et al., 1995; Grant et al., 1995; Dado and Allen, 1996, Sheperd and Kung Jr., 1996; Oba and Allen, 1998).

Llamas-lamas and Combs (1990) evaluated the effect of alfalfa maturity on fiber utilization of mid-lactation (120 DIM) cows. Six cows were fed three diets based on alfalfa hay harvested at early vegetative, late bud, and full bloom maturities. Diets were formulated for 29.4% NDF and 19.3% CP and FC ratios among the treatments varied from 45:55 to 68:32. Arieli and Adin (1994) utilized 168 cows under commercial dairy operation to evaluate the effect of wheat crop maturity on lactational performance. Experimental diets contained either early or late cut wheat silage and diets were formulated to 33% NDF. Orozco-Hernandez (1994) fed 18 mid-lactation cows with two timothy silages which were either wilted or direct-cut and treated with formic acid. The silage treated with formic acid was higher in degradable NDF fraction in situ, and the experimental diets contained approximately 51% NDF. Ruiz et al. (1995) utilized 48 mid-lactation (110 DIM) cows to compare four different forage species: corn silage, dwarf elephant grass, forage sorghum, and bermuda grass. They fed each forage at dietary NDF levels of 31%, 35%, and 39% in a 4 x 3 factorial arrangement of treatments. The dietary CP content and FC ratios were not constant within comparisons. Grant et al. (1995) fed 12 mid-lactation (90 DIM) cows with corn silage, normal sorghum silage, brown midrib sorghum silage, and alfalfa silage at 65% of dietary DM. Except for the alfalfa based diet, each diet contained approximately 40% NDF and 17% CP. Dado and Allen (1996) prepared two alfalfa silages with similar NDF content (approximately 40%) but different in NDF digestibility (38.3% vs. 40.2% after 24h in vitro fermentation). They fed 12 early-lactation (13 DIM) cows with the silages at 83.3% of dietary DM in a crossover design. Dietary NDF content and CP content were approximately 36% and 23.5%, respectively. In the study of Sheperd and Kung (1996), corn silage treated with cellulase enzyme was reported to decrease in vitro NDF digestibility (48.1 vs. 54.0%) after 196 day ensiling. They fed the enzyme treated corn silage and control silage to 19 mid-lactation (80 DIM) cows. The experimental diets contained approximately 36% NDF and 16% CP, and FC ratio was 50:50. Oba and Allen (1998) compared brown midrib corn silage with its isogenic normal control. They fed 32 mid-lactation (89 DIM) cows in a crossover design. The experimental diets contained about 31% NDF and 19.6% CP at 56:44 FC ratio.

Reported treatment means of DMI, milk production, milk composition, BW change per day, rumen pH were collected as continuous response variables, and the data were blocked by comparison within a study to remove variation among the different types of experiments. Digestibility of NDF was a fixed class variable, classified as either high or low, and the statistical model included forage to concentrate (FC) ratio, and the linear and quadratic effects of dietary NDF as covariates. The fit model procedure of JMP® (SAS Inst. Inc., Cary, NC) was used for statistical analysis with the following model:

$$Y_{ijk} = U + B_i + F_j + \text{Ncov} + \text{NNcov} + \text{FCcov} + e_{ijk}$$

where Y_{ijk} = response variable (DMI, milk yield, FCM, BW change, etc)

U = overall mean

B_i = random effect of block (i = 1 to 19)

F_j = fixed factorial effect of fiber digestibility (j = high or low)

Ncov = effect of dietary NDF as a covariate

NNcov = quadratic effect of dietary NDF as a covariate

FCcov = effect of dietary forage to concentrate ratio as a covariate

e_{ijk} = residual, assumed to be normally distributed

RESULTS AND DISCUSSION

DMI and Milk Production

A reduced model without the covariate effect of FC ratio will be discussed since FC ratio was not significant for all response variables. Cows fed diets with more digestible forage NDF increased DMI (22.0 kg vs. 20.9 kg; $P<0.001$). However, for some comparisons in this data set, forages with high NDF digestibility were fed at lower dietary NDF content than their counterpart treatments, so diet NDF content was included as a covariate. The effect of dietary NDF content as a covariate was significant and negative probably because of its effects on rumen fill. However, inclusion of the effect of dietary NDF as a covariate did not negate the effect of NDF digestibility of forage (Table 2); rumen fill is affected by both NDF content of the diet and by forage NDF digestibility.

Diets with more digestible NDF resulted in greater milk yield (28.9 kg vs. 27.3 kg; $P<0.001$) and FCM(4%) yield (26.2 kg vs. 24.5 kg; $P<0.006$). Milk composition was not different between the treatments although milk lactose content tended to be higher ($P<0.11$) for high NDF digestibility treatments. The effect of dietary NDF content as a covariate was significant for both milk production and FCM(4%) production. Milk production increased as dietary NDF content decreased. The quadratic effect of dietary NDF content was significant ($P<0.04$) for FCM(4%) yield, suggesting that either very low or very high dietary NDF content reduced FCM(4%). Energy output in milk may be limited by energy intake when dietary NDF content is high, but also affected by a reduction in milk fat synthesis when dietary NDF level is low. However, inclusion of the effect of dietary NDF as a covariate did not negate the effect of NDF digestibility of forage. These results indicated that forages with high NDF digestibility improved milk production by increasing energy intake.

Maximizing energy intake is essential for maximum milk production. Energy intake can be increased either by increasing energy density of diets or by increasing DMI. Efforts to increase energy density of diets by substituting grain for forage may not always result in increased energy intake because excess fermentation in the rumen depresses voluntary feed intake of animals. Forages with high NDF digestibility allow greater DMI when physical fill limitations to feed intake exist. More rapid hydrolysis of NDF fraction may allow greater DMI by rapid disappearance of NDF fraction from the rumen by increased rate of digestion or (and) passage.

Quantifying the Effect of Enhanced NDF Digestibility

Within this data set, differences in NDF digestibility among forage comparisons determined in vitro or in situ averaged 7.2 units (63.3% vs. 56.1%). However, the average difference in total tract NDF digestibility between high NDF digestibility and low NDF digestibility was only 4.8 units (54.8% vs. 50.0%). Although differences in total tract NDF digestibility in vivo were less than differences measured either in vitro or in situ, they were positively correlated ($P<0.0001$ $R^2=0.72$). Although the reason for smaller differences between treatments for in vivo NDF digestibility is partly because other sources of NDF diluted the forage NDF of interest in the diet, animal factors might also be responsible. NDF digestibility is a function of the potentially digestible fraction and its rate of digestion and rate of passage (Allen and Mertens, 1988). In this statistical analysis, enhanced NDF digestibility was associated with greater DMI, which might result in a higher rate of passage (NRC, 1987), reducing time for microbial degradation of NDF in the rumen. In addition, exposure to acidic conditions in the small intestine and fermentation in the large intestine in vivo might have reduced differences observed for fermentation by rumen microbes in vitro or in situ. NDF digestibility measured in vitro or in situ should more accurately reflect forage quality than in vivo NDF digestibility because in vitro and in situ methods compare forages using a consistent retention time.

Because one of the possible benefits of high NDF digestibility is to enhance DMI, forage NDF digestibility should be evaluated by in vitro or in situ methods which exclude animal factors.

The average difference of 7.2 units of NDF digestibility was associated with 1.1 kg higher DMI resulting in 1.6 kg higher milk yield, and 1.7 kg higher FCM(4%). One unit of enhanced NDF digestibility was positively associated with 0.15 kg of DMI, 0.22 kg milk yield and 0.24 kg of FCM(4%). Theoretical energy balance calculations show that this increase in DMI will support FCM(4%) in excess of this amount: One unit of enhanced NDF digestibility increased DMI by 0.15 kg. Assuming a dietary energy density of 1.70 Mcal NE_L /kg, this increase in DMI is equivalent to 0.26 Mcal. The 0.24 kg of increase in FCM(4%) requires 0.18 Mcal NE_L /day (NRC, 1989), which is about 70% of available extra energy. Although amount of energy from extra NDF digestion in vivo varies depending on levels of DMI (Oba and Allen, 1998), it may contribute to increasing energy intake even more. Energy not accounted for might have been utilized for BW gain. In this statistical analysis, animals fed the diet with forages of higher NDF digestibility tended to gain more BW ($P<0.12$).

CONCLUSION

Enhanced NDF digestibility of forage improves DMI and milk production of dairy cows. NDF digestibility should be measured more routinely to assess forage quality. This information is particularly important to accurately rank forages that might vary in yield and different quality traits. NDF digestibility data from in situ or in vitro is more valuable than in vivo data since it reflects forage quality more accurately by excluding animal factors.

Table 1. Summary of experiments used for statistical analysis.

Source	Comparison	NDF Digestibility	FC ratio	Diet NDF (%, DM)	Forage NDF (%, NDF)	CP (%, DM)	DIM^{h}
Arieli	(A) Early maturity wheat silage	29.4^{a}	34:66	33	54.8	16.5	154
	Late maturity wheat silage	23.7^{a}	36:64	33	57.9	16.5	154
Dado	(B) High NDF digestibility of alfalfa silage	40.2^{b}	83:17	34.9	92.6	23	41
	Low NDF digestibility of alfalfa silage	38.3^{b}	83:17	36.7	92.2	23.9	41
Grant	(C) Brown midrib sorghum silage	65.3^{c}	65:35	40.0	98.2	16.9	146
	Normal sorghum silage	60.8^{c}	65:35	41.6	92.2	16.9	146
	(D) Corn silage	60.6^{d}	65:35	40.1	89.8	17	146
	Normal sorghum silage	43.4^{d}	65:35	41.6	92.2	16.9	146
	(E) Brown midrib sorghum silage	65.3^{c}	65:35	40.0	98.2	16.9	146
	Corn silage	57.1^{c}	65:35	40.1	89.8	17	146
Llamas	(F) Early vegetative alfalfa	64.7^{e}	68:32	31.8	77.2	21.2	152
	Late bud alfalfa	51.7^{e}	53:47	34.6	78.9	19.6	152
	(G) Early vegetative alfalfa	64.7^{e}	68:32	31.8	77.2	21.2	152
	Full bloom alfalfa	41.5^{e}	45:55	30.6	76.0	19.6	152
	(H) Late bud alfalfa	51.7^{e}	53:47	34.6	78.9	19.6	152
	Full bloom alfalfa	41.5^{e}	45:55	30.6	76.0	19.6	152
Oba	(I) Brown midrib corn silage	49.1^{b}	56:44	30.8	71.0	19.6	117
	Normal corn silage	39.4^{b}	56:44	31.6	71.8	19.6	117

Table 1 (continued). Summary of experiments used for statistical analysis.

Source	Comparison	NDF Digestibility	FC ratio	Diet NDF (%, DM)	Forage NDF (%, NDF)	CP (%, DM)	DIM[h]
Orozco	(J) Formic acid treated timothy silage	87.2[e]	81:19	52.2	86.4	NR[g]	142
	Wilted timothy	85.1[e]	80:20	51.4	87.4	NR	142
	(K) Formic acid treated timothy + pellet	87.2[e]	80:20	50.9	89.4	NR	142
	Wilted timothy + pellet	85.1[e]	80:20	50.9	89.6	NR	142
	(L) Formic acid treated timothy + buffer	87.2[e]	79:21	50.4	87.1	NR	142
	Wilted timothy silage + buffer	85.1[e]	76:24	51.9	82.3	NR	142
Ruiz	(M) Corn silage + dwarf elephantgrass (avg)	68.6[f]	40:60	31.4	76.5	18.1	152
	Bermudagrass + forage sorghum (avg)	59.7[f]	36:64	30.9	75.0	18.5	152
	(N) Corn silage + dwarf elephantgrass (avg)	68.6[f]	48:52	35.6	81.2	17.9	152
	Bermudagrass + forage sorghum (avg)	59.7[f]	43:57	35.0	79.2	18.2	152
	(O) Corn silage + dwarf elephantgrass (avg)	68.6[f]	56:44	39.7	84.8	17.5	152
	Bermudagrass + forage sorghum (avg)	59.7[f]	50:50	38.9	83.0	18.2	152
	(P) Bermudagrass	50.6[f]	30:70	30.5	73.3	18.6	152
	Forage sorghum	44.9[f]	41:59	31.2	76.7	18.3	152
	(Q) Bermudagrass	54.1[f]	36:64	34.5	77.7	18.5	152
	Forage sorghum	42.6[f]	49:51	35.5	80.6	17.9	152
	(R) Bermudagrass	55.2[f]	42:58	38.4	81.5	18.6	152
	Forage sorghum	45.5[f]	57:43	39.4	84.5	17.7	152
Sheperd	(S) Corn silage without enzyme treatment	54.0[b]	50:50	36.2	56.4	16.2	122
	Corn silage with enzyme treatment	48.1[b]	50:50	35.9	55.0	15.9	122

Table 1 (continued). Summary of experiments used for statistical analysis.

[a] estimated by in situ assuming 4% /h passage rate

[b] estimated by in vitro digestion

[c] potential extent of ruminal digestion estimated by in situ

[d] potential extent of ruminal digestion estimated by in vitro

[e] potentially digestible NDF estimated by in situ

[f] in vivo total tract digestibility

[g] NR: not reported

[h] DIM: average days in milk of cows at mid-point of experiments

Table 2. Least square means for animal performance by different levels of fiber digestibility (n=38) taking dietary NDF% as a covariate. Calculated from 19 sets of treatment means from 8 articles of Journal of Dairy Science.

	LSM		P-value		R^2
	High FD	**Low FD**	**FD**	**NDF%**	
DMI	22.0	20.9	0.001	0.05	0.96
Milk	28.9	27.3	0.001	0.03	0.99
FCM(4%)	26.2	24.5	0.006	0.08	0.97
Fat%	3.40	3.34	0.33	0.52	0.88
Protein%	2.99	2.97	0.22	0.19	0.99
Lactose%	5.55	5.41	0.11	0.17	0.99
BW(kg/day)	0.36	0.20	0.12	0.16	0.55
Rumen-pH	6.33	6.36	0.71	0.65	0.89
in situ / in vitro NDF digestibility, %	63.3	56.1	-	-	-
in vivo total tract NDF digestibility, %	54.8	50.0	-	-	-

CHAPTER 3

EFFECT OF ENEHANCED IN VITRO NDF DIGESTIBILITY OF CORN SILAGE BY BROWN MIDRIB 3 MUTATION ON DMI AND MILK YIELD OF HIGH PRODUCING DAIRY COWS

ABSTRACT

Effect of enhanced in vitro neutral detergent fiber (NDF) digestibility of corn silage on dry matter intake (DMI) and milk production was evaluated using 32 Holstein cows in a crossover design with 28d periods. At the beginning of experiment, cows were 89 days in milk, and produced 45.6 kg of milk per day. Experimental diets contained (44.6% of dry matter) either brown midrib (bm3) corn silage or an isogenic normal (control) corn silage. NDF digestibility estimated by 30h in vitro fermentation was higher for bm3 corn silage by 9.7 units. NDF and lignin contents were lower for bm3 corn silage by 1.8 units and 0.8 units, respectively. Diets were formulated for 19.6% crude protein, 31% NDF, and 56:44 forage to concentrate ratio. Cows were blocked by days in milk, body weight, milk yield, and body condition score (BCS), and cows within a block were randomly assigned to each diet. DMI, milk production (3.5% fat-corrected milk), solids-corrected milk, and BCS were 2.0 kg, 2.5 kg, 2.6 kg, and 0.09 units / 28d higher for the cows fed bm3 corn silage, respectively. No effect of treatment was observed for milk composition or body weight change. Individual cow milk yield response to bm3 treatment was positively related to pre-trial milk yield and DMI response tended to be positively related to pre-trial milk yield. Enhanced in vitro NDF digestibility was associated with higher energy intake, which resulted in increased milk production and body condition.

(**Key words:** bm3 corn silage, fiber digestibility, intake, fill limitations)

Abbreviation key: BMR = brown midrib mutant for sorghum; **bm3** = brown midrib mutant for corn; **BCS** = body condition score

INTRODUCTION

Maximizing DMI is an important management goal for high producing dairy herds. Cows in early lactation tend to be in negative energy balance, and energy status of animals affects health and reproductive performance as well as milk production. Physical fill is considered to be one of the factors which regulate feed intake of dairy cattle (Allen, 1996). Fibrous fractions of feeds have a greater effect on rumen fill than non-fiber fractions because fiber ferments slowly and is retained in the rumen longer. Mertens (1987) suggested that DMI of dairy cows can be predicted by dietary NDF because of positive relationship between NDF content and bulk density of feeds. However NDF varies in its degradability in the rumen. Ruminal digestibility of forage NDF has been reported to range from less than 35% to over 75% for various forage types (Nocek and Russell, 1988). Although forage NDF digestibility in vivo varies by animals and feeding conditions, much of the variation could be due to the composition and structural differences of the forages; in vitro NDF digestibility after 30h incubation was reported to vary from less than 30% to 60% for corn silage and alfalfa (Allen and Oba, 1996). Enhanced NDF digestibility is speculated to increase DMI when maximum DMI is limited by rumen fill. Faster disappearance of the NDF fraction from the rumen by increased rate of digestion and(or) passage may reduce physical fill in the rumen and allow greater voluntary feed intake (Dado and Allen, 1995).

Evaluation of the effect of NDF digestibility on animal performance is complex because it is difficult to obtain two forages which differ only in NDF digestibility. Most experiments that have had treatment differences for NDF digestibility have had these effects confounded by differences in dietary NDF level, forage to concentrate ratio, protein concentration, and(or) other dietary ingredients. Brown midrib mutants consistently decrease lignin content of forages and increase NDF digestibility (Cherney et al., 1991). The brown midrib 3 gene in corn hybrids was reported to decrease lignin content by 1.1 units and increase 30h in vitro NDF digestibility by 8.4 units compared with isogenic control hybrids with no effect on the NDF content of corn silage (Allen et al., 1997). A comparison of corn silage from a hybrid converted to the brown midrib 3 mutation by backcrossing with its isogenic control hybrid might minimize the confounding of differences in NDF content with a significant difference in NDF digestibility. In the 1970's, corn silage with a brown midrib mutation was extensively studied (Block et al., 1981; Frenchick et al., 1976; Kieth et al., 1979; Rook et al., 1977; Sommerfeldt, et al., 1979; Stallings et al., 1982). However, dietary NDF content or in vitro NDF digestibility was not reported for most studies. Few studies used a total-mixed-ration and concentrates were fed according to milk production. This altered the dietary NDF content and forage to concentrate ratio of experimental diets, making it difficult to draw conclusions about the effects of NDF digestibility on animal performance. In addition, milk production of the cows used in previous experiments was much lower than what is typical for high producing herds presently, which might have affected the results because physical fill might have been less of a limitation to feed intake.

Recently, Grant et al. (1995) offered a sorghum hybrid with the low lignin brown midrib (**BMR**) mutation and a different sorghum hybrid without the mutation in a total-mixed-ration (65% forage, 40% dietary NDF) to mid-lactation dairy cows (90 - 202 DIM). The BMR hybrid had higher NDF digestibility as measured by in situ and in vitro analyses. The BMR sorghum with higher NDF digestibility resulted in a 24% increase in DMI and a 46% increase in FCM. Although cows had relatively low milk yield, they were fed diets with relatively high NDF content. Physical fill might limit DMI of low producing cows if dietary NDF content is high enough. However, the effects of enhanced NDF digestibility on animal performance is of more interest for high producing cows. There have not been any studies to evaluate the effects of enhanced NDF digestibility with high producing cows fed diets with a typical NDF content.

The objective of this experiment was to evaluate the effects of enhanced NDF digestibility on DMI and milk yield of high producing dairy cows consuming the diets with normal NDF content.

MATERIALS AND METHODS

Corn Silage

Two corn silage hybrids were planted in adjacent fields on May 12th, 1995 at the Michigan State University campus farm. Row width was 76cm and plant density was 69,000 plants / ha for both hybrids. The two corn hybrids were Cargill 6727 and the same hybrid that had been converted to the bm3 mutant. Corn was chopped at approximately 30% DM on the 8th and 12th of September for the control hybrid and the bm3 hybrid, respectively. Both hybrids were fermented and stored in adjacent 150 MT concrete bunker silos. During harvest, each load was sub sampled, and the sub sample was packed in 10 x 40 cm PVC experimental mini-silos, and allowed to ferment for 60 d. Brown midrib corn silage was lower in lignin content by 0.8 units and higher in 30h in vitro NDF digestibility by 8.4 units compared to the control corn silage (Table 1).

Cows and Treatments

Thirty-two multiparous Holstein cows (Table 2) in early to mid lactation (89 ±27 days in milk; X ± SD) from the MSU Dairy Cattle Teaching and Research Center were randomly assigned to one of two diets after blocking by parity, BW, DIM, and average milk production. Cows within a block were randomly assigned to adjacent stalls. The diet contained either bm3 corn silage or control corn silage (80% of forage DM), alfalfa silage (ensiled in a bag for this experiment, 20% of forage DM), dried ground corn, high moisture corn, soybean meal, whole linted cottonseed, and premix of minerals and vitamins (Table 3). Diets were formulated for 31% NDF and 19% CP (Table 3). Cows were housed in tie-stalls throughout the experiment except for milking times. The experiment was a cross-over design with two-28 day periods and the final 7 days of each period were used for collection of samples and data.

Sample Collection

Throughout the experiment, cows were fed once daily (1100 h) at 110% of expected intake. Feed offered and refused were weighed for each cow daily during the collection period. Samples of both rations (0.5 kg) and orts (10%) were collected daily during the collection period and composited into one weekly sample per cow. All diet ingredients were sampled separately daily and composited weekly. Cows were milked three times per day in the milking parlor at 0600, 1400, and 2200 h. Milk yield was measured daily during the collection period and averaged over the collection period. Milk was sampled at every milking on days 1, 3, and 6 of each collection period and analyzed for fat, protein, and lactose with infrared spectroscopy by Michigan DHIA (East Lansing, MI). Body weight was measured twice on the two days immediately prior to the start of the first period and on the last two days of each period. Body condition score (**BCS**), on a scale of 1 to 5, (1 = thin to 5 = fat) was determined by three trained investigators one day prior to the start of the first period, and on the last day of each period. Fecal samples were collected from each cow at 0800 and 2000h of days 2 and 7 of each collection period. Response variables were DMI, milk production, milk composition, body weight change, body condition change, and apparent total tract digestibility.

Sample and Statistical Analysis

Ration, orts, silage, and fecal samples were dried in a 55°C forced air oven for 72 hours and analyzed for DM content. All samples were ground with a Wiley mill (1-mm screen; Authur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, ADF, lignin, indigestible NDF, CP, and starch. Ash content was determined after 5h oxidation at 500° C in a muffle furnace. NDF, ADF, and sulfuric acid lignin content were determined sequentially (Van Soest et al., 1991; method A). Crude protein was analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after a NaOH gelatinization step (O'Neil et al., 1993). Indigestible NDF was estimated as NDF residue after 120h in vitro fermentation (Goering and Van Soest, 1970) and used as an internal marker to calculate apparent total tract digestibility (Cochran et al., 1986). Concentrations of all nutrients, except for DM were expressed as percentages of DM determined from drying at 105° C in a forced-air oven.

All data was statistically analyzed using the fit model procedure of JMP® using the following model:

$$Yijk = \mu + Bi + Pj + Tk + eijk$$

where μ = overall mean

B_i = effect of block ($i = 1$ to 16)

P_j = effect of period ($j = 1$ to 2)

T_k = effect of treatment ($k = 1$ to 2)

e_{ijk} = residual, assumed to be normally distributed

The data from four cows in the second period was excluded for the statistical analysis; three cows had severe coliform mastitis and one cow lost function in two quarters after teat injuries.

RESULTS AND DISCUSSION

DMI and Milk Yield

Dry matter intake was higher by 2 kg ($P < 0.0001$) for cows on the bm3 treatment (Table 4). Although both diets were originally formulated for the same NDF content, NDF fraction of bm3 corn silage decreased over the experimental period (Table 2), and dietary NDF level was 0.8 units lower for bm3 treatment diet. However, lower dietary NDF content for the bm3 treatment was not the major factor allowing higher DMI of the cows in this experiment because NDF intake for the bm3 treatment was 0.5 kg higher ($P < 0.005$) than that for normal control. Cows on the bm3 diet were able to consume more NDF because NDF was more readily degraded in the rumen. Since the NDF fraction of bm3 corn silage was more readily hydrolyzed, turnover time of ruminal digesta might have decreased, allowing more space in the rumen and higher feed intake.

Milk yield, FCM(3.5%), and SCM were higher for the bm3 diet by 2.7 kg ($P < 0.002$), 2.5kg ($P < 0.0005$), and 2.6 kg ($P < 0.0001$), respectively. Milk composition was not affected by treatment (Table 5). Production of fat (1.41 kg vs. 1.33; $P < 0.01$), protein (1.23 kg vs. 1.14 kg; $P < 0.0001$), and lactose (2.03 kg vs. 1.87 kg; $P < 0.001$) were significantly higher for the cows fed bm3 silage than those fed control silage. Improved milk production for the cows fed bm3 diet can be attributed to increased DMI because total tract DM digestibility was not different (Table 6). Some researchers reported milk fat depression with feeding bm3 corn silage (Block et al., 1981; Frenchick et al., 1976), but others reported the opposite effect of an increase in milk fat with feeding bm3 corn silage (Grant et al., 1995; Rook et al., 1977). This experiment and others (Kieth et al., 1979; Sommerfeldt et al., 1979; Stallings et al., 1982) found that milk fat concentration did not change significantly. Inconsistent results may be explained by differences in diet formulation or feeding conditions. Enhanced NDF degradability or (and) greater DMI may optimize ruminal fermentation in some cases, but it also may lead to excess fermentation acid production resulting in lower rumen pH and lower milk fat content (Block et al., 1981) depending upon feeding conditions. The effects of enhanced NDF degradability on milk composition should be investigated further.

Energy status of animals was improved by feeding bm3 corn silage (Table 5). Energy balance was estimated as NE_L intake minus NE_L for maintenance and milk production.

Actual total tract DM digestibility was used to calculate NE_L intake (Moe and Tyrrell, 1976) by first converting to expected DM digestibility at 1X maintenance (dividing by 0.92) and converting this value to TDN by adding 4 to adjust for higher calories from digestible dietary fat. This energy balance calculation indicated that cows were in slightly positive energy balance during this experiment and that energy balance was higher for the bm3 treatment. Change in body condition score was positive and 0.09 unit / 28d higher ($P < 0.05$) for the cows fed bm3 diets. Body weight gain was positive and numerically higher for the bm3 treatment, but not statistically significant. Although body weight was measured on two consecutive days each period and averaged, variation was large probably due to the variation of digesta weight in the rumen. BCS might be more appropriate to monitor energy status of animals and to detect the energy balance change in a relatively short period (28d) around a peak lactation. Assuming that one unit of BCS is equivalent to 362 Mcal of NE_L (Crooker and Weber, 1996), the difference in BCS (0.09 unit / 28d) gain was calculated to be 1.2 Mcal NE_L per day, which is equivalent to the energy required to produce 1.7 kg of 3.5% FCM. Since the bm3 treatment improved energy status of animals, evaluation of milk production response only might underestimate the effects of enhanced in vitro NDF digestibility of corn silage on animal productivity. Energy balance in the early lactation is likely to affect health of animals, reproductive performance, and subsequent milk production.

Relationship Between Milk Yield and Milk Yield Response

Animal responses to enhanced in vitro NDF digestibility were generally positive, but animals responded to the bm3 treatment in a different manner depending on production level. The response in DMI (Figure 1) and milk production (Figure 2) to the bm3 treatment (the difference between the bm3 and control treatments within a cow) was plotted against pre-trial milk production (the average milk production of 14 days immediately prior to the beginning of experiment). Variation was relatively large because some cows received the bm3 treatment in the first period (earlier in lactation) and received the control diet in the second period (later in lactation), overestimating the response to the bm3 treatment. Others received the treatments in the opposite order, underestimating the response to the bm3 treatment. However, high producing cows benefited more from enhanced in vitro NDF digestibility of corn silage; higher producing cows had greater increases in DMI ($P < 0.06$), and milk yield ($P < 0.03$) for the bm3 treatment. It is more challenging for high producing cows to meet their energy requirement, and this result indicates that DMI of higher producing cows is limited by rumen fill to a greater extent than lower producing cows. The degree of animal response to the bm3 treatment in DMI and milk yield was highly correlated (r^2=0.78), indicating that improved DMI enhanced milk production.

Nutrient Digestibility

Apparent total tract digestibility of DM, OM, CP, and starch was not significantly different by treatment (Table 6) although total tract digestibility of NDF and ADF were slightly higher for the bm3 treatment by 1.9 units ($P < 0.02$) and 2.9 units ($P < 0.001$), respectively. Enhanced in vitro NDF digestibility contributed little to the increase in diet energy density. Digestibility of NDF estimated by 30h in vitro fermentation was 9.7 units higher for bm3 corn silage (49.1% vs. 39.4%) while the bm3 treatment increased the total tract NDF digestibility by only 1.9 units (32.8% vs. 30.9%). The difference in NDF digestibility estimated by in vitro fermentation may overestimate the difference in total tract NDF digestibility because the in vitro method evaluates NDF digestibility using the same incubation time while actual residence time in the rumen is different (21)

A negative relationship was observed between response in DMI for the bm3 treatment and total tract digestibility of DM ($r^2 = 0.31$, $P < 0.002$; Figure 3) and NDF ($r^2 = 0.41$; $P < 0.0002$; Figure 4). A higher rate of passage associated with increased DMI might reduce NDF digestibility. More rapid partial hydrolysis of bm3 NDF possibly contributed to the reduction of rumen fill, allowing greater DMI. Since enhanced DMI increases rate of passage (NRC, 1987), ruminal retention time of NDF for bm3 corn silage might be shorter than that for control silage NDF. Digestibility of NDF is determined by the potential extent of digestion, rate of digestion, and the time exposed to microbial degradation (Allen and Mertens, 1988). The regression equation for this data set (Figure 4) showed that the advantage of bm3 corn silage for apparent total tract NDF digestibility was eliminated when the bm3 treatment increased DMI by approximately 3kg. Total tract starch digestibility was not affected by change in DMI ($P > 0.36$; Figure 5). In contrast to NDF digestion, starch can be digested extensively in the small intestine as well as in the rumen, compensating for the possible reduction in ruminal digestion from a higher rate of passage. Regardless of the inconsistent effects of enhanced in vitro NDF digestibility on diet energy density, energy intake was increased by the bm3 treatment because of greater DMI (Figure 6).

CONCLUSION

Enhanced NDF digestibility with the brown midrib 3 mutation of corn silage improved DMI, milk production, and BCS of high producing dairy cows. Milk composition was not affected by treatment. Cows producing more milk benefited to a greater extent from high NDF digestibility of corn silage. Higher NDF digestibility in vitro does not necessarily result in increased energy density of diets. The benefits of enhanced NDF digestibility should be investigated further in a whole lactation trial since improvement in energy status at peak lactation might influence persistency of milk yield, animal health, and reproductive performance.

Table 1. Nutrient composition of corn silage used to formulate experimental diets.

	[1]Before study		[2]During study	
	bm3	Control	bm3	Control
DM, %	30.2	33.5	31.7	32.6
NDF (% of DM)	42.0	40.4	38.3	40.1
ADF (% of DM)	21.1	21.0	19.9	21.2
Lignin (% of DM)	1.7	2.5	1.7	2.5
[3]NDFD, %	45.3	36.8	49.1	39.4
CP (% of DM)	8.7	8.4	9.7	9.5
Ash (% of DM)	4.2	3.8	4.5	4.0
Starch (% of DM)	[4]ND	ND	33.1	33.3

[1] Samples were taken each load at harvest and fermented in PVC experimental mini silos for 60 days (November / 1995).

[2] Samples were taken at feed out during the experiment. (May-July / 1996)

[3] NDF digestibility measured by in vitro fermentation for 30 h.

[4] not determined

Table 2. Status of 32 cows at the beginning of experiment.

	Average	**SD**
Parity	2.66	±1.45
Body Wt.	636 kg	±57.0 kg
BCS	2.30	±0.49
DIM	89 d	±27.1 d
Milk Yield	45.6 kg	±6.2 kg

Table 3. Ingredients and nutrient composition of experimental diets (% of dietary DM).

	bm3	**Control**
bm3 corn silage	44.6	-
Control corn silage	-	44.6
Alfalfa silage	11.2	11.2
Dry ground corn	5.6	5.6
High moisture corn	9.3	8.9
Soybean meal	18.8	19.2
Whole linted cottonseed	5.6	5.6
Vitamin & Mineral premix	5.0	5.0
Nutrient composition		
NDF	30.8	31.6
ADF	16.8	17.4
CP	19.6	19.6
Starch	30.3	30.2
[1]NE_L (Mcal / kg)	1.72	1.72
[2]F:C ratio	56:44	56:44

[1] calculated assuming same book values for two corn silage

[2] Forage to concentrate ratio.

Table 4. Effects of enhanced NDF digestibility on feed intake.

	Treatment		*P* - value
	bm3	**Control**	
Intake, kg / d			
DM	25.5	23.5	< 0.0001
NDF	7.7	7.2	< 0.01
Indigestible NDF	3.1	3.4	<0.0001
Starch	7.8	7.1	<0.0001
CP	5.1	4.7	<0.0001

Table 5. Effects of enhanced NDF digestibility on milk production.

	Treatment		**P - value**
	bm3	**Control**	
Production, kg / d			
Milk	41.6	38.9	< 0.01
3.5% FCM	40.9	38.4	< 0.001
4.0% FCM	37.9	35.6	< 0.001
SCM	37.7	35.1	< 0.0001
Fat	1.41	1.33	< 0.01
Protein	1.23	1.14	< 0.0001
Lactose	2.03	1.87	< 0.001
Milk composition, %			
Fat	3.43	3.46	0.89
Protein	2.99	2.95	0.35
Lactose	4.90	4.85	0.88
BW change, kg / d	0.17	0.08	0.45
BCS change /28 d	0.11	0.02	0.05
Energy Balance, Mcal NE_L / d	2.93	1.00	0.02

Table 6. Effects of enhanced NDF digestibility on apparent total tract digestibility (%).

	Treatment		***P* - value**
	bm3	**Control**	
DM	61.5	61.0	0.38
OM	63.0	62.6	0.48
NDF	32.8	30.9	0.02
ADF	34.7	31.8	<0.001
Starch	80.8	83.1	0.18
CP	66.8	67.4	0.45

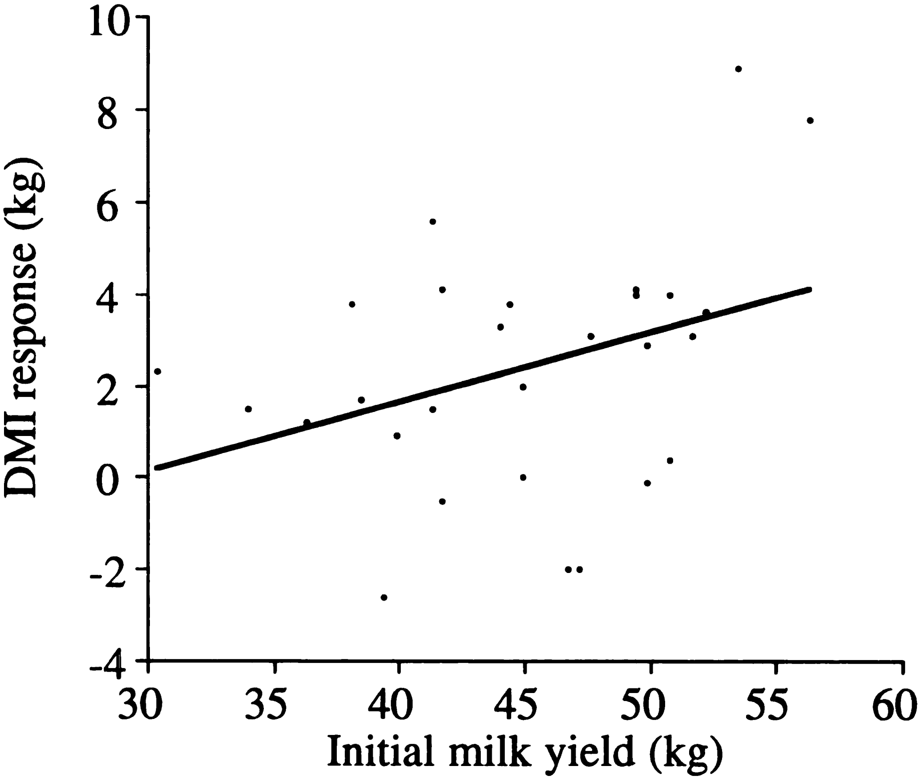

Figure 1. Relationship between average milk yield (kg/d) for 14 d prior to the beginning of the experiment and the response in DMI (kg/d) to the bm3 treatment. DMI response (kg/d) = -4.4 + 0.15 x pre-trial milk yield (kg/d); $r^2 = 0.13$; $P < 0.06$.

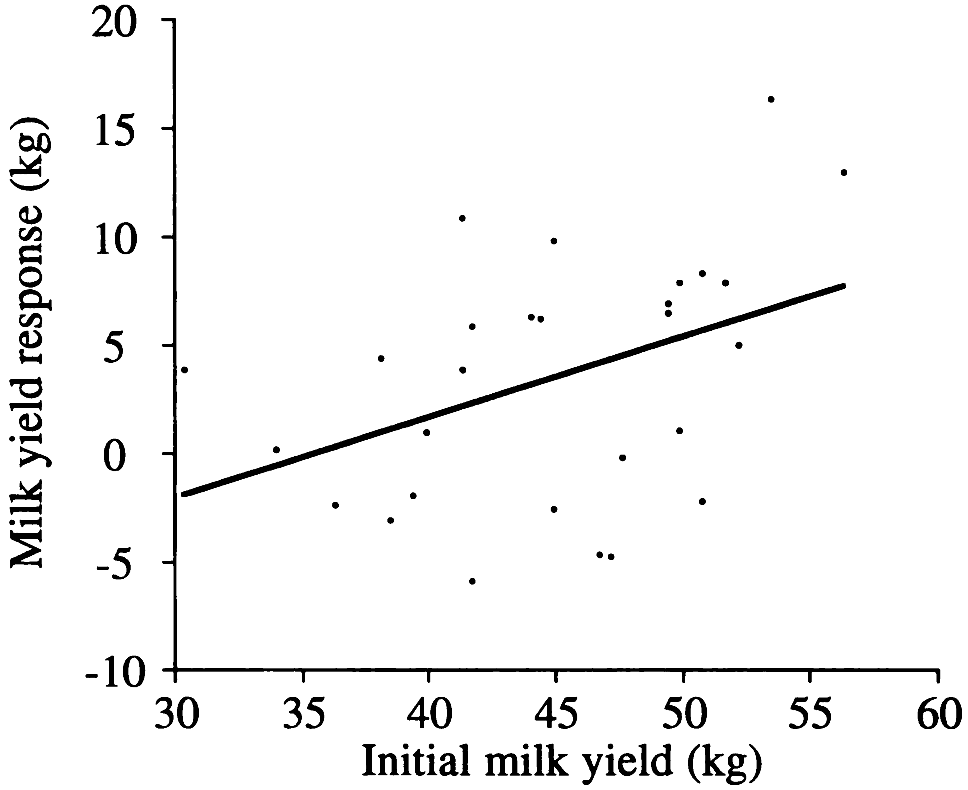

Figure 2. Relationship between average milk yield (kg/d) for 14 d prior to the beginning of the experiment and the response in milk yield (kg/d) to the bm3 treatment. Milk yield response (kg/d) = -13.2 + 0.37 x pre-trial milk yield (kg/d); $r^2 = 0.17$; $P < 0.03$.

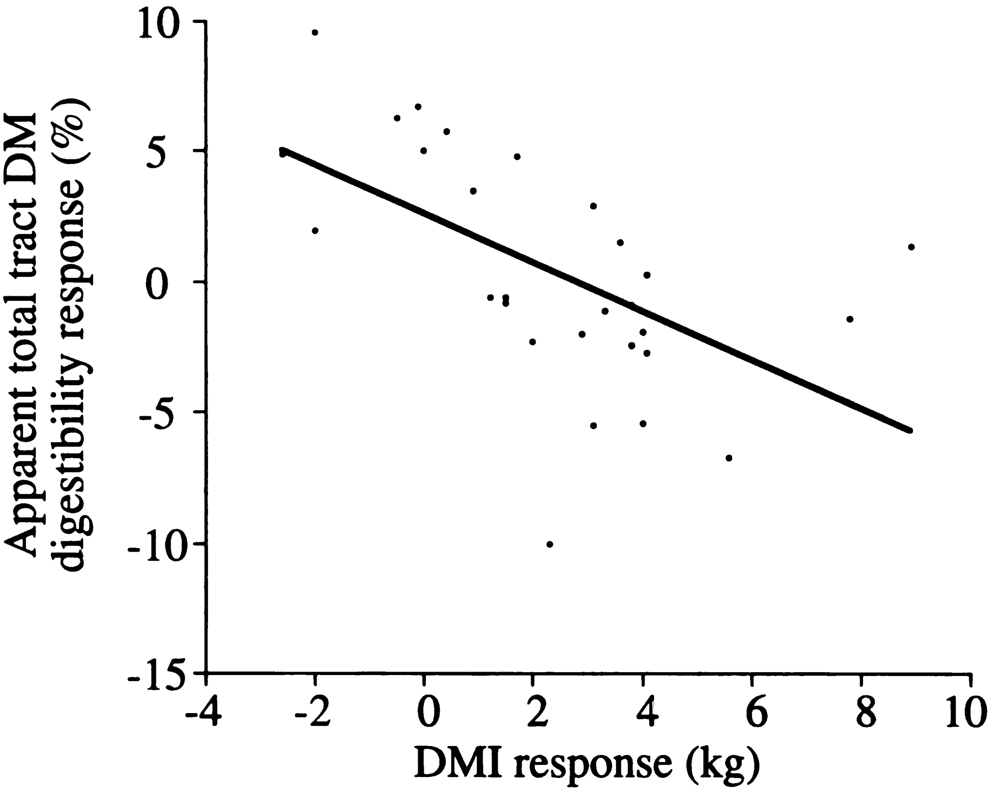

Figure 3. Relationship between responses to bm3 treatment: apparent total tract DM digestibility (%) versus DMI (kg/d). Apparent total tract DM digestibility response (%)= 2.58 – 0.93 x DMI response (kg/d); $r^2 = 0.31$; $P < 0.01$.

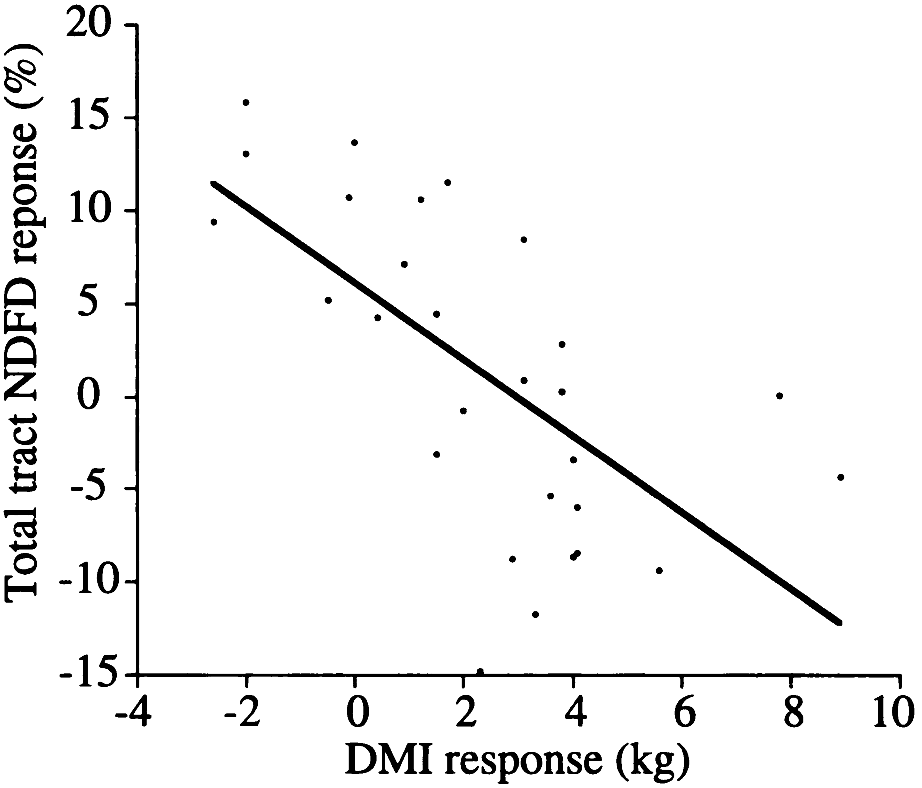

Figure 4. Relationship between responses to bm3 treatment: total tract NDF digestibility (%) versus DMI (kg/d). Total tract NDF digestibility response (%) = 6.07 – 2.05 x DMI response (kg/d); $r^2 = 0.41$; $P < 0.001$.

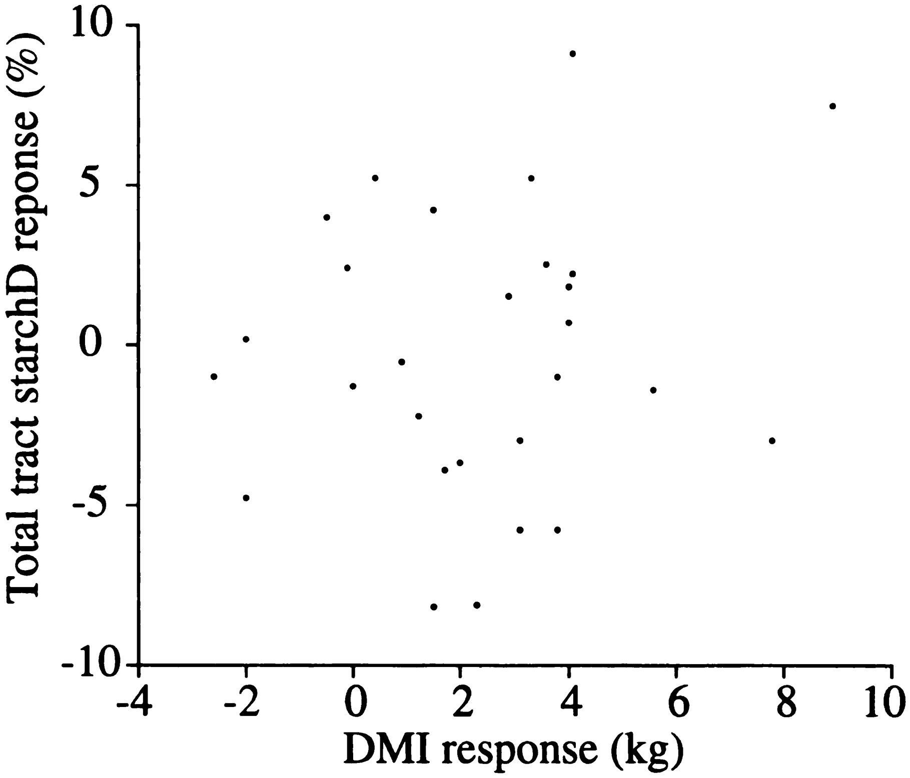

Figure 5. Relationship between responses to bm3 treatment: apparent total tract starch digestibility (%) versus DMI (kg/d). $r^2 = 0.03$; $P > 0.36$.

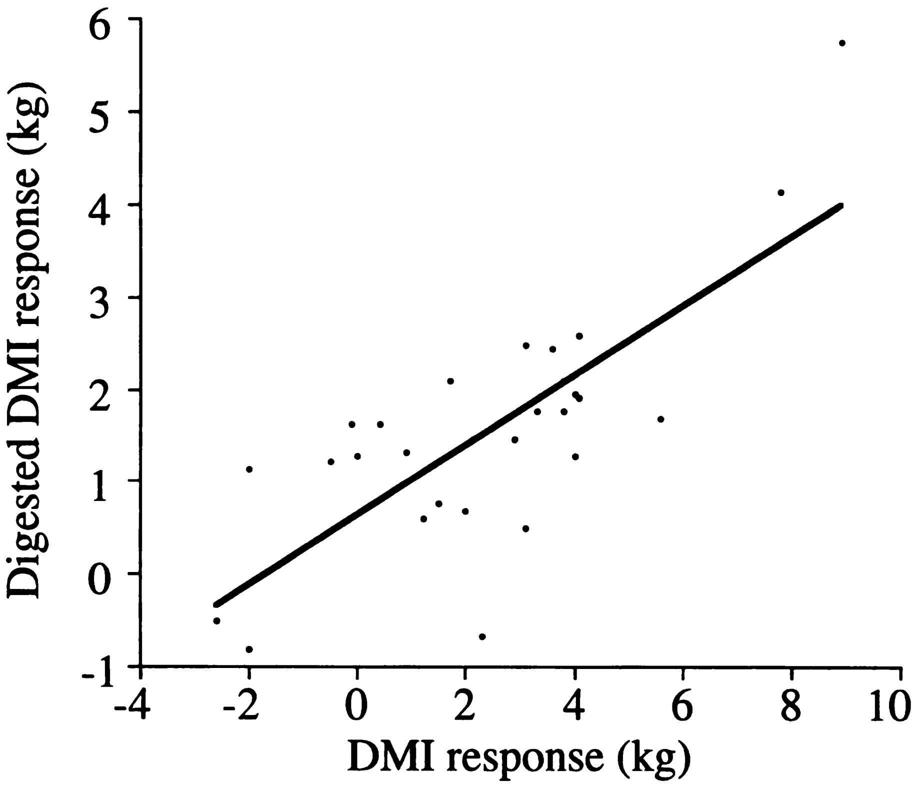

Figure 6. Relationship between responses to bm3 treatment: apparently digested DMI (kg/d) versus DMI (kg/d). Apparently digested DMI response (kg/d) = 0.64 + 0.38 x DMI response (kg/d); $r^2 = 0.58$; $P < 0.0001$.

CHAPTER 4

EFFECTS OF BROWN MIDRIB 3 CORN SILAGE ON FEEDING BEHAVIOR AND RUMINAL DIGESTA KINETICS

ABSTRACT

Effects of brown midrib 3 (bm3) corn silage on meal patterns, chewing activities, and ruminal kinetics of digesta were evaluated using eight multiparous ruminally and duodenally cannulated dairy cows in a duplicated 4 x 4 Latin square design. Experimental diets consisted of either bm3 corn silage or its isogenic normal control at two concentrations (29% and 38%) of dietary neutral detergent fiber (NDF). In vitro NDF digestibility at 30h was higher for bm3 corn silage by 9.4 percentage units. Feeding behavior of animals was monitored continuously for 4 days each period by a computerized data acquisition system. Ruminal digesta was evacuated twice per period to determine the ruminal NDF pool size and its turnover time. Dry matter intake (DMI) increased for bm3 treatments at both dietary NDF contents. However, an interaction between corn silage and dietary NDF content was observed for meal size and interval between meals, indicating that greater DMI for bm3 treatments might be attributed to different DMI regulation mechanisms at each dietary NDF content. There was no effect of corn silage treatments on total chewing time either per day or per kg of NDF intake. Although bm3 diets increased turnover rate of NDF in the rumen, ruminal NDF pool size was maintained because of greater DMI. Enhanced in vitro NDF digestibility of corn silage changed meal patterns and ruminal kinetics of NDF, but did not affect chewing activities of high producing dairy cows.

(**Key words:** brown midrib 3 corn silage, fiber digestibility, feed intake regulation, chewing activity)

Abbreviation key: bm3 = brown midrib mutant for maize

INTRODUCTION

Maximizing DMI is an important management goal for high producing dairy herds. Cows in early lactation tend to be in negative energy balance, and energy status affects productivity and health. Digestibility of NDF is an important quality parameter of forage because it was shown to affect feed intake and milk yield (Oba and Allen, 1998a). Physical fill is one of the factors which limit maximum feed intake (Allen, 1996), and more rapid hydrolysis of NDF fraction in the rumen may allow greater DMI when rumen fill limits maximum feed intake (Oba and Allen, 1998a). Converting a corn silage hybrid to a bm3 mutant is one way to increase NDF digestibility of corn silage (Cherney et al., 1991). Forages with brown midrib mutation increased DMI and milk yield compared with control forages with similar NDF and CP contents (Grant et al., 1995; Oba and Allen, 1998b).

Although it is logically hypothesized that reduced physical fill in the rumen improves DMI more when animals are fed a high NDF than a low NDF diet, research results have not been consistent. Grant et al. (1995) and Dado and Allen (1995) fed high NDF diets (40% and 37%, respectively) and reported that enhanced in vitro NDF digestibility increased DMI and milk yield. However, Robinson and McQueen (1997) reviewed previous experiments which evaluated the effect of ruminal fill on animal performance (Miller et al., 1990; Robinson and McQueen, 1992; Varga et al., 1984) and summarized that the positive effect of forage NDF fermentability might be higher for low NDF diets. Robinson and McQueen (1997) fed forage fiber which differed in its fermentability at three dietary NDF contents, and reported positive effect of enhanced fiber fermentability on DMI and milk yield regardless dietary NDF content. These inconsistent animal responses were explained partly by the difference in energy requirements of animals across the studies; ruminal fill from a high NDF diet may not limit maximum DMI if energy requirement of animals is low, whereas a low NDF diet can limit DMI by fill if energy requirement of animals is not satisfied. However, the effects of forage NDF degradability on the regulation mechanisms of voluntary DMI could be more complex and needs further investigation. Because DMI per day is a function of meal size and meal frequency, evaluation of meal patterns of cows fed forages which differed in in vitro NDF digestibility at different dietary NDF concentration may give clues for relevant interpretation of animal responses to NDF degradability.

Effect of forage NDF fermentability on physical effectiveness of fiber is another area of interest. Dairy cows require a specific amount of effective NDF in diets to stimulate chewing and maintain ruminal functions. Dietary forage NDF concentration and forage NDF particles size are closely related with ruminal pH and chewing activities of dairy cows, respectively (Allen, 1997). However, the relationship between fermentability of forage NDF and chewing activities is not well understood. Beauchemin (1991) and Nelson and Satter (1992) reported decreased chewing activities for diets containing alfalfa harvested at early versus late maturity. Grant et al. (1995) found that total chewing time per kg NDF intake was lower for brown midrib sorghum silage than normal sorghum silage. On the other hand, Robinson and McQueen (1997) reported no treatment effects of forage fiber fermentability on chewing activity. It is generally speculated that enhanced NDF degradation stimulates rapid clearance of NDF from the rumen, resulting in reduced NDF mass in the rumen and reduced chewing activities. However, little data are available to examine the relationships among chewing activities, NDF intake, ruminal NDF pool size, and NDF retention time in the rumen.

The objectives of this experiment were to evaluate the effects of bm3 corn silage on meal pattern and chewing activities of lactating dairy cows fed two concentrations of dietary NDF, and to evaluate their relationship with digestion kinetics in the rumen.

MATERIALS AND METHODS

Corn Silage

Two corn silage hybrids were planted in adjacent fields on May 14th, 1996 at the Michigan State University campus farm. The two corn hybrids were Cargill 6208FQ and Cargill 657 which is the same hybrid that had been converted to the bm3 mutant. Corn plant was chopped at approximately 30% DM on the 10th and 19th of September for the control hybrid and the bm3 hybrid, respectively. Both hybrids were fermented and stored in adjacent 150 MT concrete bunker silos. During harvest, each load was sub sampled, and the sub sample was packed in 10 x 40 cm PVC experimental mini-silos, and allowed to ferment for 30 d. Brown midrib corn silage was lower in lignin content by 0.7 percentage units and higher in 30h in vitro NDF digestibility by 9.4 percentage units compared with the control corn silage (Table 1).

Cows and Treatments

Eight multiparous Holstein cows were assigned to a duplicated 4 X 4 Latin square design balanced for carry over effects. Treatment periods were 21 days with the final 10 days used for collection of samples and data. The experimental treatments were in a 2 x 2 factorial arrangement, and factors evaluated were dietary NDF content (29% and 38%) and bm3 mutation (bm3 and normal control). Cows were cannulated ruminally and duodenally prior to calving, and the experiment started approximately 70 days after calving. The experimental diets contained either bm3 corn silage or normal control corn silage (80% of forage DM) supplemented with alfalfa silage (20% of forage DM), dried ground corn, whole cottonseed, premix of protein supplement (soybean meal, distiller's grains, and blood meal), and premix of minerals and vitamins (Table 2). All diets were formulated for 18% of dietary CP content. Cows were housed and milked in tie-stalls throughout the experiment.

Data and Sample Collection

Throughout the experiment, cows were fed once daily (1200 h) at 110% of expected intake. Feed offered and refused were weighed for each cow daily during the collection period; samples of all diet ingredients (0.5 kg) and orts (10%) from each cow were collected daily and composited into one sample per period. Feeding behavior was monitored from d 12 through d 16 (96 h) of each period by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed consumption, water consumption, and ruminal pH was recorded for each cow every 5 secs. Daily means were calculated for meal bouts, interval between meals, eating chew rate, eating time, eating chews, meal size, ruminating bouts, interval between ruminating bouts, ruminating chew rate, ruminating time, and ruminating chews. The daily means of variables were averaged over the four days. The rumen was evacuated at 1400 h (2 h after feeding) on d 19 and at 1000 h (2 h before feeding) on d 21. Total ruminal content mass and volume were determined. During evacuation, a 10% aliquot of digesta was separated for ease of sub sampling. The aliquot was squeezed through 4 layers of cheese cloth to separate into solid and liquid. Samples were taken from both phases for determination of nutrient pool size.

Chemical Analysis

Diet ingredients, orts, and ruminal digesta samples were dried in a 55°C forced air oven and analyzed for DM content. DM content of ruminal digesta was also determined by toluene distillation (AOAC, 1990). All samples were ground with a Wiley mill (1mm screen; Authur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, ADF, lignin, indigestible NDF, CP, and starch. Ash content was determined after 5h oxidation in a muffle furnace at 500° C. Ash-free NDF, ADF, and sulfuric acid lignin content were determined sequentially (Van Soest et al., 1991; method A). Crude protein was analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after a NaOH gelatinization step (O'Neil et al., 1993). Indigestible NDF was estimated as NDF residue after 120h in vitro fermentation (Goering and Van Soest, 1970). Concentrations of all nutrients, except for DM were expressed as percentages of DM determined by drying at 105° C in a forced-air oven.

The ruminal pool sizes (kg) of DM, OM, NDF, indigestible NDF, and starch were determined by multiplying the concentration of each nutrient by the ruminal digesta weight (kg). Duodenal nutrient flow was estimated by the method described in Chapter 5. Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each nutrients (%/h) were calculated by the following equations;

Turnover rate in the rumen (%/h) =

$$(\text{intake of nutrient} / \text{ruminal pool of nutrient}) / 24 \times 100$$

Passage rate from the rumen (%/h) =

(duodenal flow of nutrient / ruminal pool of nutrient) / 24 x 100

Digestion rate in the rumen (%/h) =

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

Assuming that the indigestible NDF fraction disappears from the rumen only by passage, indigestible NDF intake substituted for duodenal indigestible NDF flow in this calculation. Because of technical difficulty in filtering indigestible NDF residues for duodenal samples, duodenal indigestible NDF flow had been overestimated; the estimated duodenal flow had exceeded intake.

Statistical Analysis

All data were analyzed using the fit model procedure of JMP® (Version 3.2, SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + S_i + C_{j(i)} + P_k + T_l + ST_{il} + e_{ijkl}$$

where

μ = overall mean,

S_i = random effect of square (i = 1 to 2),

$C_{j(i)}$ = random effect of cow within square (j = 1 to 4),

P_k = random effect of period (k = 1 to 4),

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

ST_{il} = interaction of square and treatment,

e_{ijkl} = residual, assumed to be normally distributed.

A reduced model without square × treatment interaction was used when this effect was not significant ($P > .10$). Orthogonal contrasts were used to determine effect of dietary NDF content, effect of corn silage, and interaction of dietary NDF content and corn silage. Treatment effects and their interaction were declared significant at $P < .05$ and $P < .10$, respectively.

RESULTS AND DISCUSSION

DMI and Meal Patterns

Cows fed bm3 corn silage had higher DMI at both dietary NDF contents ($P < 0.05$; Table 3). An interaction of dietary NDF content and corn silage was not detected ($P > 0.54$). This agrees with the observation of Robinson and McQueen (1997) who reported a positive effect of higher forage NDF fermentability on DMI regardless of dietary NDF content. However, a significant interaction between dietary NDF and corn silage was observed for interval between meals ($P < 0.08$; Table 4) and meal size of DM ($P < 0.08$). Observed differences in meal patterns indicate that greater DMI for bm3 treatments might be a result of different DMI regulation mechanisms at each level of dietary NDF content. Meal size was greater (2.2 kg vs. 2.0 kg) and interval between meals was longer (98.8 min vs. 87.8 min) for bm3 treatments compared with control at high NDF content, whereas meal size was smaller (2.2 kg vs. 2.3 kg) and interval between meals was shorter (90.4 min vs. 98.2 min) for bm3 treatments compared with control at low NDF diets.

When cows were fed the dietary treatment with higher NDF, higher DMI for the bm3 treatment was from greater meal size. It was previously noted that physical fill limitation to DMI changed meal patterns of animals (Baumont et al., 1990; Dado and Allen, 1995). Baumont et al. (1990) found that sheep increased frequency of eating bouts when bulk was added to the rumen. Cows challenged by ruminal fill from dietary NDF and ruminal inert bulk increased the percentage of smaller meals and decreased the percentage of larger meals (Dado and Allen, 1995). In these instances, physical fill might have been the dominant factor affecting satiety. When limitation of physical fill to DMI exists, animals might try to maintain DMI by increasing number of eating bouts (Baumont et al., 1990). In our study, greater meal size and longer interval between meals for the bm3 treatments may indicate that bm3 corn silage is less filling in the rumen than the control silage.

When cows were fed the dietary treatment with lower NDF, bm3 treatment increased DMI not because of greater meal size, but because of shorter intervals between meals. Physical fill was less likely to limit their maximum DMI with low NDF diets because the ruminal digesta mass and volume were lower for low NDF diets than high NDF diets (Table 6), indicating that there is additional capacity available in the rumen. The mechanisms, which explain shorter intervals between meals for the bm3 treatment at low NDF diet, are not known and need further investigation. However, it can be attributed partly to lower ruminal pH for the bm3 treatment ($P < 0.01$; Table 6); lower ruminal pH might result in more rapid absorption of volatile fatty acids (VFA) because more VFA are present in the associated form which is readily absorbed through the ruminal wall. It is speculated that rapid absorption of VFA and subsequent metabolite uptake by peripheral tissues makes the cows feel hunger sooner. Elucidating the interaction of ruminal fermentation and physiology of lactating dairy cows may be a fertile area of research.

Ruminal Digesta Kinetics

Ruminal turnover rate of NDF tended to be higher for bm3 treatment ($P < 0.07$; Table 5), indicating that NDF from bm3 corn silage was more readily degraded and passed from the rumen faster, allowing additional capacity in the rumen. However, faster turnover rate of bm3 NDF cannot be attributed to faster rate of digestion. In this experiment, ruminal digestion rate for the potentially digestible NDF fraction was lower ($P < 0.02$) for bm3 treatment possibly due to lower ruminal pH ($P < 0.01$; Table 6). The potential advantage in NDF digestibility for the bm3 corn silage might be diminished because of negative effects of low ruminal pH on fiber digesting bacteria (Hoover, 1986).

Faster rate of ruminal NDF turnover for bm3 treatment was a result of faster rate of passage ($P < 0.04$). Rate of passage is influenced by rate of particle size reduction, particle density, and buoyancy of digesta (Allen, 1996). Particle size reduction is not totally dependent upon microbial fermentation, but occurs primarily as a result of mastication and detrition (Mcleod and Minson, 1988). Illius and Gordon (1991) proposed that rate of particle size reduction in the rumen can be estimated by indigestible NDF content of forage because a lignified cell wall provides plant rigidity. Because in vitro NDF digestibility was higher for bm3 corn silage than control, greater fragility for bm3 NDF might contribute to faster passage rate for the fibrous fraction of ruminal digesta. Stensig and Robinson (1997) showed that passage rate for the digestible NDF fraction and the indigestible NDF fraction can be different because each fraction is not equally distributed in a plant and (or) a diet. In this experiment, passage rate for the digestible NDF fraction was not affected by corn silage treatment possibly because the passage from the rumen is influenced by other factors such as buoyancy of digesta and (or) rate of digestion. However, passage rate for the indigestible NDF fraction was higher for bm3 treatment ($P < 0.0001$), which indicates that fragility of plant cell wall could enhance particle size reduction and subsequent passage from the rumen without microbial degradation. Although diets with bm3 corn silage did not have faster digestion rate of NDF in the rumen, enhanced ease of NDF degradation for bm3 corn silage could contribute to the more rapid clearance of NDF from the rumen.

Cell wall fragility can be estimated by in vitro NDF digestibility to some extent, but the effects of in vitro NDF digestibility on ruminal fill and DMI need to be interpreted with caution, especially for comparisons across different forage species. Hoffman et al. (1998) compared alfalfa silage with perennial ryegrass silage at similar dietary NDF content and forage to concentrate ratio, and found greater DMI for the diet based on alfalfa which was lower in NDF digestibility. In general, legumes differ from grasses in potential extent of digestion or (and) rate of digestion, which possibly affects buoyancy of digesta in the rumen and ruminal fill (Allen, 1996). Waghorn et al. (1989) reported that rate of particle size reduction by mastication is faster for alfalfa than ryegrass. Although particle density and rate of particle size reduction partly account for NDF turnover rate in the rumen, these factors are not estimated directly by in vitro digestion methods.

Chewing Activities

There was no effect of corn silage on ruminating time either per day or per kg of NDF intake (Table 7), or on total chewing time either per day or per kg of NDF intake (Table 8). Although the effectiveness of the more ruminally degradable bm3 NDF at stimulating chewing was similar to control NDF, ruminating time and total chewing time per kg of DMI tended to be lower for bm3 treatments ($P < 0.07$ and $P < 0.11$, respectively). Turnover rate of NDF was higher for bm3 treatments, but the bm3 corn silage did not affect chewing activities possibly because NDF mass in the rumen was maintained due to greater DMI.

In contrast to our observations, it was reported that alfalfa harvested at an earlier maturity decreased chewing activity per kg of alfalfa NDF intake compared with alfalfa harvested at a later maturity (Bucheamin 1991). This implies that forages with higher NDF digestibility might decrease the physical effectiveness at stimulating chewing because NDF digestibility of alfalfa usually decreases with maturity. However, Bucheamin (1991) reported that DMI was lower for cows fed earlier maturity alfalfa hay. Because alfalfa DM disappearance in sacco was faster for the early maturity alfalfa, low DMI could result in reduced NDF mass in the rumen and reduced chewing activity. Robinson and McQueen (1997) found that forage NDF fermentability did not influence chewing activities, and reported that DMI was higher for the diets with more fermentable forage NDF. Nadeau and Allen (1998) showed that NDF digestibility of alfalfa does not affect total chewing time or ruminating time per kg of alfalfa NDF intake, and reported no effect on DMI and ruminal NDF pool size. Within current data set, total chewing time per day were positively related to NDF intake ($P < 0.001$; $r = 0.57$) and ruminal NDF pool size ($P < 0.001$; $r = 0.55$), but not related to ruminal turnover rate of NDF (Table 9). These evidences may lead to the hypothesis that forage NDF degradability does not affect chewing activities as long as enhanced DMI maintains a critical amount of NDF mass in the rumen.

CONCLUSION

Feeding bm3 corn silage increased DMI regardless of dietary NDF content. Meal pattern of animals fed bm3 corn silage was different at each level of dietary NDF, indicating that greater DMI for bm3 treatments might be attributed to different DMI regulation mechanisms at each level of dietary NDF content. Rate of NDF passage from the rumen was faster for bm3 treatments. Chewing activities was not influenced by bm3 corn silage probably because of greater DMI and maintained ruminal NDF pool.

Table 1. Nutrient composition of bm3 corn silage and normal control corn silage.

	bm3	Control
DM% (oven dried at 55°C)	29.9	30.1
DM% (Toluene distillation)	27.8	26.7
-% of DM-		
NDF[1]	41.4	42.9
ADF[2]	20.2	22.4
Lignin[2]	1.3	2.0
Crude Protein	7.5	7.7
Ash[2]	3.6	3.9
Starch[1]	38.3	35.4
IV TDMD[2,3]	83.3	78.2
IV NDFD, % of NDF[2,4]	55.9	46.5

[1] Differ significantly ($P < 0.05$).

[2] Differ significantly ($P < 0.0001$).

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

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

Table 2. Ingredients and nutrient composition of experimental diets (% of dietary DM). [1]

	Low NDF		High NDF	
	bm3	**Control**	**bm3**	**Control**
-Diet Ingredients-				
Normal corn silage	-	32.1	-	50.5
Brown midrib 3 corn silage	35.8	-	55.9	-
Alfalfa silage	8.1	7.7	12.6	12.2
Dry ground corn	26.2	29.2	-	5.4
Cottonseed	3.6	3.7	5.4	5.6
Protein mix[2]	23.3	23.5	23.0	23.2
Vitamin & mineral mix[3]	3.0	3.1	3.0	3.1
-Nutrient Composition-				
DM	45.2^a	45.7^a	35.4^b	35.7^b
NDF	28.7^b	29.1^b	37.5^a	38.4^a
ADF	15.5^b	16.0^b	21.9^a	22.8^a
Lignin	2.0^d	2.4^c	2.9^b	3.4^a
Indigestible NDF[4]	10.5^d	11.8^c	13.4^b	15.6^a
CP	17.7	17.7	18.1	18.0
Starch	37.2^a	37.6^a	26.1^b	26.8^b

[1] Means for nutrient composition in same row followed by different superscript letters differ significantly ($P < 0.05$).

[2] Protein mix contained 80.5% soybean meal, 15.9% distillers grain, and 3.6% blood meal.

[3] Vitamin & mineral mix contains 31.2% dry ground corn, 22.7% limestone, 18.0% dicalcium phosphate, 9.3% sodium bicarbonate, 8.7% salt, 6.3% Magnesium sulfate, 3.0% trace mineral premix, 0.40% vitamin A, 0.35% vitamin D, and 0.09% vitamin E.

[4] Indigestible NDF: estimated after 120h in vitro incubation.

Table 3. Effects of bm3 and control corn silage at two dietary NDF contents on nutrient intake during feeding behavior monitoring period (kg/d).

	Low NDF		**High NDF**			***P* value**		
	bm3	**Control**	**bm3**	**Control**	**SE**	**NDF[1]**	**CS[2]**	**NDF x CS[3]**
DM	23.6	22.8	22.0	20.5	0.5	<0.01	0.05	0.54
OM	21.3	20.6	19.5	18.3	0.5	<0.001	0.06	0.58
NDF	6.6	6.5	8.1	7.7	0.2	<0.0001	0.20	0.45
ADF	3.5	3.5	4.5	4.4	0.1	<0.0001	0.81	0.50
Lignin	0.5	0.5	0.6	0.6	0.02	<0.0001	<0.01	0.88
Indigestible NDF	2.5	2.7	3.0	3.2	0.06	<0.0001	<0.01	0.75
CP	4.0	3.8	3.9	3.6	0.09	0.06	0.02	0.43
Starch	8.7	8.4	5.6	5.4	0.2	<0.0001	0.30	0.88

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.

Table 4. Effects of bm3 and control corn silage at two dietary NDF contents on meal patterns and eating activities.

	Low NDF		**High NDF**				***P* value**	
	bm3	**Control**	**bm3**	**Control**	**SE**	**NDF**[1]	**CS**[2]	**NDF x CS**[3]
Meal bouts (/d)	11.1	10.6	10.3	11.1	0.5	0.96	0.69	0.19
Meal length (min)	29.6	31.4	34.8	29.6	2.1	0.18	0.85	0.29
Intermeal interval (min)	90.4	98.2	98.8	87.8	5.1	0.85	0.76	0.08
Chewing rate (/min)	57.2	52.9	54.6	56.2	1.9	0.86	0.49	0.14
-Eating time (min)-								
/ d	271.1	262.6	299.3	300.6	9.5	<0.01	0.70	0.61
/ bout	26.0	27.0	30.6	28.1	1.9	0.14	0.70	0.35
/ kg DMI	11.9	11.9	13.7	14.8	0.1	<0.001	0.31	0.36
/ kg NDFI	42.3	42.1	37.3	39.2	0.4	0.07	0.70	0.60
-Eating chews-								
/ d	16116	14618	16924	17546	908	0.05	0.64	0.26
/ bout	1551	1514	1716	1648	132	0.27	0.69	0.91
/ kg DMI	705	659	772	849	20	<0.01	0.73	0.18
/ kg NDFI	2538	2344	2099	2258	162	0.12	0.92	0.29
Meal size (kg/d)								
DM	2.2	2.3	2.2	2.0	0.1	0.16	0.31	0.08
NDF	0.6	0.6	0.8	0.7	0.1	<0.001	0.43	0.09
Indigestible NDF	0.2	0.3	0.3	0.3	0.04	<0.01	0.18	0.20

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.

Table 5. Effects of bm3 and control corn silage at two dietary NDF contents on digestion kinetics in the rumen.

	Low NDF		High NDF			P value		
	bm3	Control	bm3	Control	SE	NDF[1]	CS[2]	NDF x CS[3]
-Ruminal turnover rate (%/h)-								
DM	10.62	9.96	9.10	8.38	0.23	<0.0001	<0.01	0.91
OM	11.2	10.4	9.3	8.8	0.26	<0.0001	0.02	0.63
NDF	4.61	4.28	4.94	4.78	0.13	<0.01	0.07	0.51
Digestible NDF	5.50	5.85	6.49	7.24	0.27	<0.001	0.06	0.47
Indigestible NDF	3.73	3.13	3.55	3.27	0.09	0.81	0.0001	0.09
Starch	30.4	29.4	20.6	20.2	1.3	<0.0001	0.58	0.80
-Ruminal passage rate (%/h)-								
NDF	3.55	3.31	3.12	2.82	0.12	<0.01	0.04	0.81
Digestible NDF	3.42	3.56	2.70	2.15	0.21	<0.0001	0.34	0.12
Indigestible NDF	3.73	3.13	3.55	3.27	0.09	0.81	0.0001	0.09
Starch	15.7	13.2	10.0	8.00	0.89	<0.0001	0.02	0.78
-Ruminal digestion rate (%/h)-								
Digestible NDF	2.08	2.29	3.80	5.09	0.28	<0.0001	0.02	0.07
Starch	14.8	16.2	10.6	12.2	0.8	<0.0001	0.08	0.92

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.

Table 6. Effects of bm3 and control corn silage at two dietary NDF contents on ruminal digesta characteristics.

	Low NDF		High NDF			*P* value		
	bm3	Control	bm3	Control	SE	NDF[1]	CS[2]	NDF x CS[3]
Rumen pH	5.62	5.78	5.73	5.90	0.057	0.06	<0.01	0.91
Rumen digesta volume (L)	90.7	90.4	97.7	100.5	2.7	<0.01	0.64	0.57
Density (kg / L)	0.75	0.77	0.76	0.76	0.01	0.82	0.39	0.27
-Digesta composition-								
DM% (55 °C oven)	14.3	14.3	14.2	14.3	0.4	0.66	0.96	0.91
DM% (toluene)	15.7	15.7	14.9	15.1	0.5	0.20	0.91	0.92
NDF (% of oven DM)	65.1	66.9	68.2	66.9	1.1	0.17	0.86	0.16
Starch (% of oven DM)	12.9	12.5	11.4	11.1	0.5	0.002	0.47	0.96
-Rumen mass (kg)-								
Wet digesta	67.9	70.3	74.6	76.5	1.8	<0.01	0.24	0.91
DM	9.7	10.1	10.5	10.8	0.3	0.02	0.29	0.88
OM	8.7	9.1	9.6	9.7	0.3	0.02	0.40	0.62
NDF	6.3	6.7	7.2	7.2	0.2	<0.01	0.30	0.41
ADF	3.1	3.5	3.7	3.9	0.1	<0.01	0.02	0.56
Lignin	0.6	0.7	0.7	0.8	0.02	<0.0001	<0.001	0.47
Indigestible NDF	3.0	3.8	3.7	4.4	0.3	<0.0001	<0.0001	0.68
Starch	1.3	1.3	1.2	1.2	0.1	0.24	0.93	0.85

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.

Table 7. Effects of bm3 and control corn silage at two dietary NDF contents on rumination activities.

	Low NDF		High NDF			*P* value		
	bm3	Control	bm3	Control	SE	NDF[1]	CS[2]	NDF x CS[3]
Ruminating bouts (/ d)	13.1	12.2	12.7	13.4	0.5	0.46	0.78	0.15
Interval between bouts (min)	87.7	79.5	71.0	67.6	6.6	0.04	0.39	0.72
Chewing rate (/ min)	52.0	50.4	55.9	56.7	1.8	<0.01	0.81	0.51
-Ruminating time (min)-								
/ d	394.4	394.9	464.7	495.5	19.9	<0.001	0.44	0.46
/ bout	30.1	33.2	37.1	37.5	1.4	<0.001	0.21	0.33
/ kg DMI	17.0	17.6	21.6	24.3	0.9	<0.0001	0.07	0.27
/ kg NDFI	60.7	62.3	58.5	64.5	2.9	0.99	0.22	0.48
/ kg ruminal DM pool	40.7	40.3	45.1	47.2	2.5	0.04	0.73	0.64
/ kg ruminal NDF pool	63.1	60.4	66.4	71.2	3.6	0.07	0.78	0.32
-Ruminating chews-								
/ d	21511	20739	26729	28339	1627	<0.001	0.80	0.47
/ bout	1624	1715	2108	2148	104	<0.001	0.54	0.81
/ kg DMI	922	916	1232	1371	69	<0.0001	0.34	0.31
/ kg NDFI	3307	3247	3351	3651	232	0.35	0.61	0.45
/ kg ruminal DM pool	2207	2108	2581	2676	181	0.02	0.99	0.60
/ kg ruminal NDF pool	3424	3139	3788	4038	268	0.03	0.95	0.33

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.

Table 8. Effects of bm3 and control corn silage at two dietary NDF contents on total chewing activities.

	Low NDF		High NDF			*P* value		
	bm3	Control	bm3	Control	SE	NDF[1]	CS[2]	NDF x CS[3]
-Total chewing time (min)-								
/ d	666	658	764	796	27	<0.001	0.66	0.46
/ kg DMI	28.7	29.3	35.3	38.8	1.2	<0.0001	0.11	0.26
/ kg NDFI	103.2	104.0	96	103.6	4.3	0.39	0.35	0.45
/ kg ruminal DM pool	68.9	67.2	73.9	75.6	3.4	0.06	0.99	0.61
/ kg ruminal NDF pool	106.5	100.3	108.6	113.9	4.9	0.12	0.92	0.25
-Total chews-								
/ d	37627	35357	43653	45885	2387	<0.01	0.99	0.36
/ kg DMI	1627	1569	2005	2216	102	<0.0001	0.46	0.20
/ kg NDFI	5843	5566	5450	5894	359	0.93	0.82	0.33
/ kg ruminal DM pool	3884	3596	4205	4314	261	0.06	0.74	0.46
/ kg ruminal NDF pool	6003	5354	6173	6497	386	0.11	0.68	0.22

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.

Table 9. Pearson coefficient correlation for chewing activity measurements among 32 observations.

	(1)	(2)	(3)	(4)
(1) Total chewing time, min/d	-	-	-	-
(2) Ruminating time, min/d	0.949[1]	-	-	-
(3) NDFI, kg/d	0.570[1]	0.556[1]	-	-
(4) Ruminal NDF pool, kg	0.551[1]	0.488[1]	0.854[1]	-
(5) Ruminal NDF turnover rate, %/h	-0.020	+0.094	+0.219	-0.284

[1] Correlation is significant ($P < 0.05$).

CHAPTER 5

EFFECTS OF BROWN MIDRIB 3 CORN SILAGE ON NUTRIENT UTILIZATION BY LACTATING COWS

ABSTRACT

Effects of brown midrib 3 (bm3) corn silage on ruminal fermentation, site of digestion, and energy utilization were evaluated using eight multiparous ruminally and duodenally cannulated dairy cows (70 days in lactation) in a duplicated 4 x 4 Latin square design. Experimental diets contained either bm3 corn silage or normal control at two concentrations (29% and 38%) of dietary neutral detergent fiber (NDF). Digestibility of NDF estimated by 30h in vitro fermentation was higher for bm3 corn silage by 9.4 percentage units. Low NDF diets and bm3 treatments increased milk yield. The bm3 treatment depressed milk fat concentration when fed with the low NDF diet. Fat-corrected milk yield (3.5%) tended to be higher for the cows fed bm3 corn silage, whereas daily body weight gain was higher for the cows fed low NDF diets. Both bm3 treatments and low NDF diets lowered rumen pH, but bm3 treatments did not increase fluctuation of rumen pH. Feeding bm3 corn silage increased the energy utilized for milk production, possibly because of consistent nutrient supply from the rumen. Passage rate of ruminal digesta was higher for bm3 treatments, which might contribute to improved microbial efficiency and to shifting site of digestion from the rumen to the intestine. The beneficial effects of bm3 corn silage on animal performance were greater when fed with the high NDF diet.

(**Key words:** brown midrib 3 corn silage, fiber digestibility, rumen pH, energy utilization efficiency)

Abbreviation key: bm3 = brown midrib mutant for maize; **FCM** = fat-corrected milk; **SCM** = solid-corrected milk; **BCS** = body condition score; **NAN** = non-ammonia nitrogen; **MN** = microbial nitrogen; **NANMN** = non-ammonia non-microbial nitrogen; **MEOP** = milk energy output as a percentage of NE_L intake

INTRODUCTION

As a genetic potential of dairy cows for milk production increases, it becomes more challenging to meet the energy requirement of high producing cows. Cows in early lactation tend to be in negative energy balance, and maximizing energy intake in this period is essential for maximum productivity and farm profitability. Enhanced digestibility of forage NDF was reported to increase DMI and milk yield of lactating dairy cows (Oba and Allen, 1998a). Because maximum feed intake of high producing dairy cows is often limited by rumen fill (Allen, 1996), feeding more degradable NDF might allow greater feed intake by rapid turnover of the fibrous fraction in ruminal digesta by increasing rate of digestion, increasing rate of passage, or both (Oba and Allen, 1998a).

Although animal responses to forages with higher NDF digestibility are usually positive, their effects on farm profitability are influenced by many other factors. In general, it is more expensive to obtain a forage with higher NDF digestibility; harvesting a perenial forage at earlier maturity usually sacrifices potential yields. Utilizing forages with a brown midrib mutation is another alternative to obtain enhanced NDF digestibility because it consistently lowers lignin content and enhances NDF digestibility (Cherney et al., 1991; Allen et al., 1997). However, it is reported that the bm3 mutation decreased corn silage yield an average of 12% across hybrids (Allen et al., 1997). Because forages are a significant portion of a dairy ration, their nutritional advantages and economical potential depend upon an appropriate diet formulation to a great extent. Unfortunately, little information is available regarding supplementation of forages with higher NDF digestibility.

Comparison of a bm3 corn silage with its isogenic normal control might provide relevant information about the effects of enhanced in vitro NDF digestibility on animal performance because it minimizes confounding nutritional and physical characteristics of forages with significant difference in NDF digestibility. However, animal responses to bm3 corn silage have not been consistent. Some experiments reported that feeding bm3 corn silage increased milk yield (Frenchick et al., 1976; Kieth et al., 1979; Oba and Allen, 1998), whereas others reported that it increased body fat deposition (Rook et al., 1977; Sommerfeldt et al., 1979; Block et al., 1981; Oba and Allen, 1998). Some studies reported milk fat depression for cows fed a bm3 corn silage (Frenchick et al., 1976;

Block et al., 1981). Despite many production trials, the effects of bm3 corn silage on energy utilization are not well understood. Some studies evaluated fermentation characteristics in the rumen and speculated the relationships among rumen pH, acetate to propionate ratio, and animal performance (Frenchick et al., 1976; Rook et al., 1977; Sommerfeldt et al., 1979; Block et al., 1981). However, none of the previous experiments evaluated the effects of bm3 corn silage on nutrient digestibility in the rumen, microbial efficiency, and site of digestion. Understanding these digestion characteristics is essential to supplement forages with enhanced NDF digestibility in the most effective manner, and to maximize their utilization.

The objective of this experiment was to evaluate the effects of bm3 corn silage on ruminal fermentation, microbial efficiency, site of digestion, and energy utilization by lactating dairy cows fed diets with two dietary NDF concentrations.

MATERIALS AND METHODS

Experimental Designs

Two corn silage hybrids were grown at the Michigan State University campus farm, and ensiled in adjacent 150 MT concrete bunker silos. The two corn hybrids were Cargill 6208FQ and Cargill 657 which is the same hybrid that had been converted to the bm3 mutation. Both hybrids were chopped at approximately 30% DM. Brown midrib corn silage was lower in lignin content by 0.7 percentage units (1.3 vs. 2.0, % of DM) and higher in 30h in vitro NDF digestibility by 9.4 percentage units (55.9 vs. 46.5, % of

NDF) compared with the control corn silage. The experimental treatments were in a 2 x 2 factorial arrangement, and factors evaluated were dietary NDF concentrations (29% and 38%) and bm3 mutation (bm3 and normal control). The experimental diets contained either bm3 corn silage or control corn silage (80% of forage DM) supplemented with alfalfa silage (20% of forage DM), dried ground corn, whole cottonseed, premix of protein supplement (soybean meal, distiller's grain, and blood meal), and premix of minerals and vitamins. The diets were formulated for 18% of dietary CP content. Chemical composition of corn silages and experimental diets were described in Chapter 4.

Eight multiparous Holstein cows were ruminally and duodenally cannulated prior to calving. Duodenal cannulas were soft gutter type made of plastisol. The duodenum was fistulated proximal to the pylorus region and prior to the pancreatic duct and the cannulas were placed between 10th and 11th ribs as described by Robinson et al. (1985). Both ruminal and duodenal surgery were performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Cows were 70d ±7.7d in lactation at the initiation of experiment, and assigned to a duplicated 4 X 4 Latin square balanced for carry over effects. Each period consisted of 21 days and the final 11 days were used for collection of samples and data. Cows were individually housed in tie stalls, milked in their stalls twice daily (0600 h and 1800h), and fed once daily (1200 h) at 110% of expected intake throughout the experiment.

Data and Sample Collection

Rumen pH was monitored from 12 d to 16 d of each period by a computerized data acquisition system (Dado and Allen, 1993), and data was recorded for each cow every 5 seconds. Calibration of pH probes was checked once per day, and adjusted if necessary. When pH differed from the calibration solutions at pH 4 and 7 by more than 0.05 units, the data of last 24 hours was excluded from statistical analysis. Daily means and variation of rumen pH, and the time spent below pH 6.0, 5.8, and 5.5 were determined. The negative effects of low rumen pH were estimated by multiplying the time spent below pH 6.0, 5.8, and 5.5 by the deviation from the pH 6.0, 5.8, and 5.5, respectively (Mackie and Gilchrist, 1979). The rumen pH data were summarized for each day, and the daily means were averaged over the 4 days.

The double marker method was utilized for estimating duodenal flow of nutrients (Faichney, 1980). Chromium was used as a solid phase marker, and dosed as Cr-mordanted wheat straw (30 g/d per cow; approximately 1.2 g Cr / d). Cobalt was used as a liquid phase marker, and infused as Co-EDTA solution (400 ml/d; approximately 0.5 g Co / d). Both Cr-mordanted fiber and Co-EDTA solution were prepared by the method described by Uden et al.(1980). Cr-mordanted fiber was filled in gelatin capsules (1.5 oz, Tropac Inc., Airfield, NJ) and dosed through the rumen cannula twice daily (0730 h and 1930 h). Co-EDTA solution was infused into the rumen for 1 minute out of every 10 minutes from d 11 through d 19 of each period. Both markers were prime-dosed (3x of daily dose) on d 11 of each period. Duodenal digesta samples were collected every 3 hours from d 16 to d 19. The first sample was collected at 1200 h on d 16 and collection time was delayed one hour on each of the following days so that 24 samples were taken for each cow which represented every hour of a 24-hour period to account for diurnal variation. Duodenal digesta was separated to liquid and solid phases by filtering through a polypropylene screen with a 1000 µm aperture size (Fisher Scientific, Pittsburgh, PA) at each sampling time. The digesta retained on the screen and the digesta through the screen were arbitrary defined as solid phase digesta and liquid phase digesta, respectively. Samples were immediately frozen at -20° C, and then lyophilized. Fecal samples were collected every 6 hours concurrently with duodenal digesta sampling. Rumen fluid samples were collected every 3 hours, starting at 1200 h on d 19 and continued for 24 hours for determination of VFA, lactate, and ammonia concentrations. Samples were taken from 5 or 6 different sites within the rumen and strained through four layers of cheesecloth, and frozen at -20° C until analysis.

Feed offered and refused were weighed for each cow daily during each data collection period (d 12 through d 21). During each collection period, samples of all diet ingredients (0.5 kg) and orts (10% of refused feed) from each cow were collected daily and composited into one sample per period. Milk yield and DMI were recorded daily during each collection period and averaged. Milk was sampled at every milking on d 16, d 18, and d 20 of each period and analyzed for fat, protein, and lactose contents with infrared spectroscopy by Michigan DHIA (East Lansing, MI). Rumen empty body weight was measured after evacuating rumen digesta on d –2 and d -1 of the first period, and measured twice on d 20 and d 21 of each period and averaged. Body condition score, on a scale of 1 to 5 (1 = thin, 5 = fat), was assessed by three trained scorers immediately prior to the start of the first period and on d 21 of each period.

Data and Sample Analysis

Diet ingredients, orts, and fecal samples were dried in a forced-air drying oven at 55° C for 72 hours and analyzed for DM content. All samples were ground with a Wiley mill (1mm screen; Authur H. Thomas, Philadelphia, PA). Samples were analyzed for ash, NDF, ADF, lignin, indigestible NDF, CP, and starch. Ash content was determined after 5h oxidation at 500° C in a muffle furnace. Ash-free NDF, ADF, and sulfuric acid lignin content were determined sequentially (Van Soest et al., 1991; method A). Crude protein was analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after a NaOH gelatinization step (O'Neil et al., 1993). Indigestible NDF was estimated as NDF residue after 120h in vitro fermentation (Goering and Van Soest, 1970). Concentrations of all nutrients, except DM were expressed as percentages of DM determined from drying at 105° C in a forced-air drying oven.

Feeds, solid phase duodenal digesta, liquid phase duodenal digesta, and fecal samples were analyzed for concentrations of Co and Cr. Samples were digested with phosphoric acid according to Williams et al. (1962), and read by flame atomic absorption spectrometry (Smith-Hieftje 4000, Thermo Jarrell Ash Co., Franklin, MA). Cobalt concentration of feeds and solid phase duodenal digesta was analyzed by furnace atomic absorption spectrometry by a commercial laboratory (VBSA Inc., Newton, IA). Duodenal flow of nutrients was calculated according to the method of Russell and

Armentano (1985). Passage rates of OM, NDF, and starch were estimated according to the method described in Chapter 4.

Duodenal digesta were analyzed for purines and ammonia to estimate microbial nitrogen flow and non-ammonia non-microbial nitrogen flow to the duodenum. Purines were used as a microbial marker, and purine to microbial N ratio was estimated by analysis of microbial pellets obtained after differential centrifugation of rumen fluid (Overton et al., 1995). Purines were measured by spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 260 nm (Zinn and Owens, 1986). Dried duodenal digesta samples were hydrolyzed for 20 minutes to extract ammonia retained in samples. Rumen fluid samples were centrifuged at 26,000 x g for 30 minutes and supernatants were collected. Ammonia concentration was determined for the duodenal samples and the centrifuged rumen fluid according to Broderick and Kang (1980). The supernatants of centrifuged rumen fluid were analyzed for VFA and lactate by gas chromatography (Perkin Elmer, Norwalk, CT), using pivalic acid as an internal marker.

All data were statistically analyzed using the fit model procedure of JMP® (Version 3.2, SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + S_i + C_{j(i)} + P_k + T_l + ST_{il} + e_{ijkl}$$

where

μ = overall mean,

S_i = random effect of square (i = 1 to 2),

$C_{j(i)}$ = random effect of cow within square (j = 1 to 4),

P_k = random effect of period (k = 1 to 4),

T_l = fixed effected of treatment ($l = 1$ to 4),

e_{ijkl} = residual, assumed to be normally distributed.

A reduced model without square × treatment interaction was used when this effect was not significant ($P > .10$). Orthogonal contrasts were made to determine effect of dietary NDF content, effect of corn silage, and interaction of dietary NDF content and corn silage. Treatment effects and their interaction were declared significant at $P < .05$ and $P < .10$, respectively.

RESULTS

DMI and Animal Performance

DMI was higher for low NDF treatments ($P < 0.001$) and bm3 treatments ($P < 0.02$; Table 1). Energy intake, calculated from digested DMI, was higher for low NDF diets ($P < 0.01$) but was not affected by corn silage treatments. Milk yield, FCM yield, solids-corrected milk yield (SCM) were higher for bm3 treatments regardless of dietary NDF concentration, whereas dietary NDF concentration did not affect milk production. A significant interaction of dietary NDF concentration and corn silage treatments was observed ($P < 0.06$) for milk fat content, indicating that milk fat content was lower for the bm3 treatment when fed with the low NDF diet (3.28% vs. 3.67%), whereas milk fat content was not affected by corn silage treatments when dietary NDF content was high (3.86% vs. 3.90%). Milk fat yield was not affected by either corn silage or dietary NDF content. Brown midrib 3 treatments increased milk lactose content ($P < 0.05$) and did not affect milk protein content, and resulted in significantly higher yield of each component ($P < 0.01$). A significant interaction ($P < 0.10$) for milk solids-non-fat (SNF) content between dietary NDF content and corn silage indicated that the bm3 corn silage tended to increase SNF when fed with the high NDF diet, but had no effect when fed with the low NDF diet. Neither of BW gain or BCS change was affected by bm3 treatments, but low NDF treatment increased BW gain compared to high NDF treatment ($P < 0.01$).

Ruminal Fermentation

Daily mean ruminal pH was lower for bm3 treatments ($P < 0.01$; Table 2) and tended to be lower for low NDF diets ($P < 0.06$). Daily variance and daily range in rumen pH were higher for low NDF diets, but not affected by corn silage treatments. The time spent below pH 6.0 and 5.8 was greater for bm3 treatments, but was not affected by dietary NDF content. Significant interactions between dietary NDF content and corn silage treatments were observed for ruminal concentration of total VFA ($P < 0.07$), lactate ($P < 0.07$), and butyric acid ($P < 0.05$; Table 3). For high NDF diets, total VFA concentrations were 92.6 and 80.4 mM for bm3 and control, respectively. For low NDF diets, total VFA concentrations were 92.8 and 98.3 mM for bm3 and control, respectively. Low NDF diets increased propionate concentration, and decreased acetate concentration and acetate to propionate ratio compared to high NDF diets. Ammonia concentration tended to be lower for bm3 treatments ($P < 0.09$).

Nutrient digestibility

Apparent total tract DM digestibility was not affected by corn silage treatment, but numerically lower for cows fed bm3 corn silage (Table 5). Therefore, amounts of apparently digested DM were not affected by corn silage treatments even though dry matter intake was higher for bm3 treatments ($P < 0.02$). A significant interaction of dietary NDF content by corn silage treatments was observed for true ruminal OM digestion (kg/d; $P < 0.01$). Truly digested OM in the rumen was higher for the bm3 treatment with the high NDF diet, but lower for the bm3 treatment with the low NDF diet compared to the control. However, more OM was digested post-ruminally for bm3 treatments than the control regardless of dietary NDF concentration. Corn silage treatments did not affect NDF digestibility in the rumen, post-rumen, or total tract (Table 5). Ruminal starch digestibility was higher for control treatments ($P < 0.01$), whereas post-ruminal starch digestibility was higher for bm3 treatments ($P < 0.01$; Table 6). Passage rate from the rumen for NDF ($P < 0.04$), indigestible NDF ($P < 0.0001$), and starch ($P < 0.02$) was higher for bm3 treatments (Table 5, 6).

Nitrogen intake and duodenal flow of non-ammonia nitrogen (NAN) were higher ($P < 0.01$; Table 7) for bm3 treatments. Although non-ammonia non-microbial nitrogen (NANMN) was not affected by corn silage treatments, bm3 treatment increased microbial nitrogen (MN) flow at the duodenum ($P < 0.04$). Microbial efficiency estimated by duodenal flow of MN as a percentage of truly digested OM in the rumen was higher for bm3 treatments ($P < 0.01$). Duodenal flow of NAN was also higher for low NDF diets ($P < 0.001$); low NDF diets increased NANMN flow ($P < 0.0001$), but did not increase MN.

Postruminal digestibility of duodenal NAN was higher for low NDF diets and bm3 treatments. Apparent total tract digestibility of nitrogen was not affected by corn silage treatments.

DISCUSSION

Energy Intake and Utilization

Cows fed bm3 corn silage increased yields of milk, FCM, and SCM, which are partly attributed to greater DMI for bm3 treatments. However, bm3 treatments did not significantly increase digested DMI (Table 1). Therefore, enhanced milk production for bm3 treatments might be attributable to factors other than greater digestible energy intake. In this experiment, bm3 treatments lowered rumen pH without increasing its daily variation compared to control treatments (Table 2). Low rumen pH usually decreases methane production in the rumen, which might increase metabolizable energy available for animals. In addition, VFA are more readily absorbed through the rumen wall as rumen pH declines. Because rate of VFA absorption in the rumen is a function of rumen pH and concentration gradient, low rumen pH with less fluctuation might result in greater and more consistent energy supply from the rumen to animals. In contrast to adipose tissue and skeletal muscle, metabolism in the mammary gland is not regulated by insulin (Zhao et al., 1996), and nutrient uptake should occur more consistently. It is speculated that consistent energy supply to animals results in enhanced milk production for bm3 treatments.

Milk energy output as a percentage of NE_L intake (MEOP) was higher for bm3 treatments ($P < 0.05$; Table 1). Energy intake (NE_L) was calculated from digested DMI. Numerically higher energy intake for bm3 treatments may raise a question whether mathematical dilution of maintenance energy output leads to a greater MEOP. However, significant MEOP difference between corn silage treatments was not negated ($P < 0.05$) when MEOP was analyzed using the statistical model with energy intake as a covariate, suggesting that more energy was utilized for milk production when cows were fed bm3 corn silage regardless of amounts of energy intake.

In contrast, low NDF diets increased energy intake ($P < 0.01$) and BW gain ($P < 0.01$), but did not increase SCM yield ($P > 0.26$) compared to high NDF diets (Table 1). It is observed that low NDF diets lowered rumen pH with higher daily variation and greater range in variation compared to high NDF diets (Table 2). Greater fluctuation in rumen pH might indicate inconsistent energy supply to animals, resulting in an increase in energy metabolite utilization by adipose and skeletal muscle tissue and relatively less substrate available for milk production because the mammary gland is not sensitive to insulin (Zhao et al., 1996). The relationship between ruminal fermentation pattern and energy utilization warrants further investigation.

Feeding bm3 corn silage shifted the site of starch digestion from the rumen to post-ruminal digestion (Table 6). Ruminal starch digestion was lower for cows fed bm3 corn silage than control, probably because of faster rate of passage ($P < 0.02$), whereas post-ruminal starch digestion was higher for cows fed bm3 corn silage probably due to compensatory digestion from reduced ruminal digestion. It has been suggested that metabolism of starch through glucose absorbed from the small intestine may be energetically more efficient than metabolism through fermentation to propionic acid in the rumen and subsequent gluconeogenesis in the liver (Black, 1971). Owens et al. (1986) reported ruminal starch digestion was energetically about 70% as efficient as starch digestion in the small intestine for steers. Although the total tract starch digestibility tended to be lower for bm3 treatment in the current study, more starch was digested post-ruminally for bm3 treatments, which might contribute to enhanced milk production (Nocek and Tamminga, 1991). However, greater starch digestion in the small intestine does not necessarily mean greater glucose absorption. Kreikemeier et al. (1991) infused corn starch at 60 g/h into the abomasum of steers, and found that only 38% of corn starch disappearance resulted in net portal glucose absorption, indicating that starch can be fermented to VFA or lactate in the intestines. In addition, ruminants may have limited capacity of starch digestion and absorption in the small intestine (Owens et al., 1986; Nocek and Tamminga, 1991; Harmon, 1992). Although enhanced milk production for bm3 treatments could be attributed partly to increased post-ruminal starch digestion, the relationship between site of digestion and milk production is not understood clearly.

Microbial Efficiency

Duodenal flow of NAN and post-ruminal NAN digestion were higher for low NDF diets and bm3 treatments (Table 7), possibly contributing to greater milk and milk protein yield (Table 1). Low NDF diets increased duodenal NANMN flow compared with high NDF diets (Table 7). Lana et al. (1997) reported that low rumen pH increased amounts of escape protein by decreasing ruminal deamination and ammonia accumulation. However, bm3 treatments increased duodenal MN flow and microbial efficiency, which might be attributed to faster passage rate of ruminal digesta for cows fed bm3 corn silage compared with those fed control corn silage. Greater rate of passage of ruminal digesta might increase flow of attached microorganisms. Ammonia concentration in the rumen tended to be lower for cows fed bm3 corn silage ($P < 0.09$; Table 3), indicating that more ruminal ammonia was incorporated into microbial protein. Although low NDF diets increased passage rate of ruminal digesta, they did not increase duodenal MN flow and microbial efficiency, possibly because lower pH and greater fluctuation in rumen pH inhibited microbial growth.

Milk Composition

Milk fat content was lower when bm3 corn silage was fed with the low NDF diet, compared with the other treatments (Table 1). However, fat yield was not affected by either dietary NDF content or corn silage treatment. The observed lower milk fat content might be explained by relative difference between the rate of milk fat synthesis and the rate of milk lactose synthesis. Milk lactose yield and milk volume were increased for bm3 treatments possibly because of consistent energy supply from the rumen and increased post-ruminal starch digestion. Low milk fat content is commonly associated with reduction in the acetate to propionate ratio in the rumen, but bm3 treatments did not affect acetate to propionate ratio in this experiment. Although milk fat yield was not different among treatments, milk fat was diluted by increased milk yield when cows were fed bm3 corn silage with low dietary NDF. Cows fed bm3 corn silage at low dietary NDF gained more BW, and improved energy status might have limited mobilization of body fat for milk fat synthesis. Milk fat is derived from mobilized body fat, dietary fat, and fatty acids synthesized in the mammary gland. Milk fatty acid composition needs to be evaluated to understand why milk fat content is often depressed when a bm3 corn silage is fed.

Milk lactose content was increased by bm3 treatments, but its mechanism is not well understood. Lactose is synthesized in Golgi vesicles of secretory cells and draws water into the vesicles by osmotic pressure. Therefore, milk lactose content generally does not vary because osmolarity of milk tends to be maintained constant (Holt, 1983). However, mineral elements also exert osmotic pressure in milk. There is negative correlation between lactose content and those of Na and K, and total osmotic pressure of milk is kept constant and equal to that of blood (Holt, 1983). When milk lactose content is increased, it may mean either that mineral content in milk decreases (Linzell, 1967) or that both milk osmolarity and blood osmolarity increase (Wheelock et al., 1965).

CONCLUSION

Brown midrib 3 corn silage increased milk production without a significant increase in digested DMI. Enhanced performance for cows fed bm3 corn silage might be attributed to consistent energy supply from the rumen, faster rate of passage, increased post-ruminal starch digestion, and greater microbial protein production. Microbial efficiency was greater for bm3 treatment. NDF digestion either in the rumen or in total tract was not affected by corn silage treatments. Milk fat content was lowest when cows were fed bm3 corn silage with the low NDF diet. The beneficial effects of bm3 corn silage on animal performance might be greater when fed with a high NDF diet.

Table 1. Effects of bm3 and control corn silage at two dietary NDF contents on DMI and milk production.

	Low NDF		High NDF			*P* value		
	bm3	**Control**	**bm3**	**Control**	**SE**	**NDF[1]**	**CS[2]**	**NDF x CS[3]**
-Intake-								
DMI (kg/d)	24.7	23.9	22.9	21.5	0.4	<0.001	0.02	0.57
Digested DMI (kg/d)	15.9	15.5	14.6	14.0	0.4	<0.01	0.27	0.80
NE_L intake (Mcal NE_L /d)	37.0	36.5	33.9	32.6	0.9	<0.01	0.36	0.68
-Production (kg / d)-								
Milk	36.9	33.5	33.7	30.4	1.0	<0.01	<0.01	0.96
FCM (3.5%)	35.6	34.3	35.8	32.6	1.1	0.51	0.06	0.37
SCM	32.9	31.6	32.7	29.5	1.0	0.26	0.04	0.39
Milk fat	1.22	1.23	1.32	1.20	0.05	0.43	0.29	0.17
Milk protein	1.15	1.05	1.04	0.93	0.03	<0.01	<0.01	0.83
Milk lactose	1.76	1.59	1.61	1.44	0.05	<0.01	<0.01	0.90
-Composition(%)-								
Milk fat	3.28	3.67	3.86	3.90	0.09	<0.001	0.02	0.06
Milk protein	3.10	3.15	3.09	3.06	0.03	0.08	0.77	0.15
Milk lactose	4.75	4.74	4.77	4.71	0.02	0.95	0.05	0.28
Milk SNF	8.56	8.60	8.57	8.48	0.04	0.16	0.40	0.10
BW change (kg / d)	1.10	0.79	0.00	-0.02	0.3	<0.01	0.52	0.58
BCS change (/ 21d)	0.17	0.22	0.10	0.04	0.06	0.07	0.98	0.43
FCM (3.5%) / DMI	1.45	1.43	1.56	1.50	0.04	0.02	0.26	0.68
Milk NE_L (% of NE_L Intake)	66.9	64.0	72.4	68.7	1.6	<0.01	0.05	0.79
Energy balance (NE_L)	+3.0	+3.3	-0.4	+0.6	0.7	<0.01	0.37	0.64

[1] NDF: Effect of dietary NDF content, [2] CS: Effect of corn silage, [3] NDF x CS: Interaction of dietary NDF content and corn silage.

Table 2. Effects of bm3 and control corn silage at two dietary NDF contents on ruminal pH.

	Low NDF		High NDF				*P* value	
	bm3	**Control**	**bm3**	**Control**	**SE**	**NDF**[1]	**CS**[2]	**NDF x CS**[3]
-Rumen pH-								
Mean	5.62	5.78	5.73	5.90	0.057	0.06	<0.01	0.91
Variance	0.126	0.102	0.067	0.061	0.012	<0.001	0.22	0.46
Maximum	6.54	6.60	6.45	6.59	0.060	0.48	0.11	0.49
Minimum	5.03	5.18	5.24	5.38	0.047	<0.001	<0.01	0.96
Range	1.50	1.41	1.21	1.21	0.055	<0.001	0.43	0.43
-Hours-								
< pH 6.0	17.6	15.1	16.8	13.7	1.3	0.39	0.04	0.83
< pH 5.8	15	12.1	12.8	9.6	1.3	0.08	0.03	0.93
< pH 5.5	9.7	6.6	6.5	3.1	1.2	0.01	0.02	0.88
-Area[4]-								
< pH 6.0	1150	868	860	547	117	0.02	0.02	0.90
< pH 5.8	758	541	504	266	98	0.01	0.03	0.91
< pH 5.5	309	206	163	46	63	0.03	0.10	0.91

[1] NDF: Effect of dietary NDF content, [2] CS: Effect of corn silage, [3] NDF x CS: Interaction of dietary NDF content and corn silage.
[4] Area was calculated by the time under a specific rumen pH weighed by the deviation from it.

Table 3. Effects of bm3 and control corn silage at two dietary NDF contents on rumen fermentation.

	Low NDF		High NDF			*P* value		
	bm3	Control	bm3	Control	SE	NDF[1]	CS[2]	NDF x CS[3]
Total VFA (mM)	92.8	98.3	92.6	80.4	4.6	0.06	0.48	0.07
Lactate (mM)	1.2	1.7	1.4	1.3	0.2	0.46	0.27	0.07
-VFA (mol / 100 mol)-								
Acetate	50.1	47.8	51.0	51.0	1.0	0.06	0.27	0.28
Propionate	34.8	35.9	32.7	33.1	0.9	0.01	0.38	0.68
Butyrate	12.7	13.4	13.7	13.0	0.3	0.36	0.91	0.05
branched-chain VFA[4]	2.5	2.9	2.6	2.9	0.4	0.81	0.39	0.85
Acetate: Propionate ratio	1.48	1.34	1.60	1.56	0.08	0.05	0.28	0.52
NH_3 (mg / dl)	11.6	13.4	12.1	13.8	0.9	0.62	0.09	0.96

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.
[4]Branched chain VFA: iso-butyrate and iso-valerate

Table 4. Effects of bm3 and control corn silage at two dietary NDF contents on digestibility of DM and OM.

	Low NDF		High NDF			P value		
	bm3	Control	bm3	Control	SE	NDF[1]	CS[2]	NDF x CS[3]
DM								
Intake (kg / d)	24.7	23.9	22.9	21.5	0.4	<0.001	0.02	0.57
Apparently digested in total tract								
(kg / d)	15.9	15.5	14.6	14.0	0.4	<0.01	0.27	0.80
(%)	67.0	67.9	66.2	68.7	1.3	0.98	0.21	0.54
OM								
Intake (kg / d)	23.3	22.6	21.4	20.2	0.4	<0.0001	0.03	0.60
Apparently digested in the rumen								
(kg / d)	7.6	8.2	7.8	8.1	0.3	0.74	0.12	0.62
(%)	32.7	36.3	36.1	41.0	1.1	<0.01	<0.01	0.57
Truly digested in the rumen								
(kg / d)	14.0	15.2	15.0	14.0	0.4	0.82	0.85	0.01
(%)	60.3	67.7	69.6	69.8	1.4	<0.001	0.02	0.02
Passage to duodenum (kg / d)	15.7	14.4	13.5	12.0	0.4	<0.0001	<0.01	0.84
Apparently digested post-ruminally								
(kg / d)	7.7	6.8	6.1	5.3	0.3	<0.0001	<0.01	0.90
(% of intake)	33.2	30.5	28.8	26.3	1.1	<0.001	0.03	0.94
(% of duodenal passage)	49.2	47.7	45.1	44.7	1.5	0.03	0.56	0.72
Apparently digested in total tract								
(kg / d)	15.3	15.0	14.0	13.4	0.4	<0.001	0.26	0.81
(%)	68.6	69.5	67.9	70.3	1.2	0.96	0.20	0.54

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.

Table 5. Effects of bm3 and control corn silage at two dietary NDF contents on digestibility of NDF.

	Low NDF		High NDF			*P* value		
	bm3	**Control**	**bm3**	**Control**	**SE**	**NDF**[1]	**CS**[2]	**NDF x CS**[3]
NDF								
Intake (kg / d)	6.9	6.8	8.4	8.2	0.2	<0.0001	0.33	0.52
Digested in the rumen								
(kg / d)	1.3	1.2	2.8	2.9	0.1	<0.0001	0.74	0.54
(%)	18.4	18.2	32.4	36.0	1.7	<0.0001	0.32	0.25
Passage to duodenum (kg / d)	5.6	5.6	5.7	5.3	0.2	0.56	0.34	0.40
Passage rate to duodenum (%/h)								
NDF	3.74	3.49	3.36	3.04	0.12	<0.01	0.04	0.86
Digestible NDF	3.42	3.56	2.70	2.15	0.21	<0.0001	0.34	0.12
Indigestible NDF	3.73	3.13	3.55	3.27	0.09	0.81	<0.0001	0.09
Digested post-ruminally								
(kg / d)	0.8	0.8	0.5	0.5	0.2	0.16	0.96	0.82
(% of intake)	11.7	12.2	5.8	6.1	2.5	0.02	0.88	0.99
(% of duodenal passage)	14.0	14.3	7.9	9.7	2.9	0.08	0.72	0.79
Digested in total tract								
(kg / d)	2.0	2.0	3.3	3.3	0.2	<0.0001	0.92	0.79
(%)	30.2	30.3	38.1	42.1	2.4	<0.001	0.41	0.44

[1]NDF: Effect of dietary NDF content, [2]CS: Effect of corn silage, [3]NDF x CS: Interaction of dietary NDF content and corn silage.

Table 6. Effects of bm3 and control corn silage at two dietary NDF contents on digestibility of starch.

	Low NDF		**High NDF**			***P* value**		
	bm3	**Control**	**bm3**	**Control**	**SE**	**NDF**[1]	**CS**[2]	**NDF x CS**[3]
Starch								
Intake (kg / d)	9.2	8.9	5.9	5.7	0.2	<0.0001	0.23	0.81
Digested in the rumen								
(kg / d)	4.5	4.9	3.0	3.4	0.2	<0.0001	0.06	0.99
(%)	49.3	55.0	51.6	60.7	2.0	0.07	<0.01	0.42
Passage to duodenum (kg / d)	4.7	4.0	2.8	2.3	0.2	<0.0001	<0.01	0.84
Passage rate to duodenum (%/h)	15.7	13.2	10.0	8.00	0.89	<0.0001	0.02	0.78
Digested post-ruminally								
(kg / d)	4.0	3.4	2.3	1.9	0.2	<0.0001	<0.01	0.67
(% of intake)	43.7	38.6	39.8	33.0	1.9	0.02	<0.01	0.63
(% of duodenal passage)	85.8	85.7	82.1	83.8	0.7	<0.001	0.29	0.24
Digested in total tract								
(kg / d)	8.5	8.3	5.4	5.3	0.2	<0.0001	0.53	0.67
(%)	93.0	93.6	91.4	93.7	0.4	0.07	<0.001	0.04

[1] NDF: Effect of dietary NDF content, [2] CS: Effect of corn silage, [3] NDF x CS: Interaction of dietary NDF content and corn silage.

Table 7. Effects of bm3 and control corn silage at two dietary NDF contents on N metabolism.

	Low NDF		High NDF			*P* value		
	bm3	**Control**	**bm3**	**Control**	**SE**	**NDF**[1]	**CS**[2]	**NDF x CS**[3]
N Intake (g / d)	698	671	673	625	12.6	<0.01	<0.01	0.43
Passage to duodenum								
NAN[4]								
(g / d)	801	753	742	633	23	<0.001	<0.01	0.21
(% of intake)	114.5	112.8	110.9	101.4	2.7	<0.01	0.05	0.17
NANMN[5]								
(g / d)	281	244	167	176	18	<0.0001	0.44	0.23
(% of intake)	40.0	36.4	25.1	28.7	2.9	<0.001	0.99	0.23
(% of duodenal NAN[4])	34.7	32.0	22.7	27.9	2.7	<0.01	0.66	0.17
Microbial N								
(g / d)	520	509	575	457	29	0.96	0.04	0.08
(% of duodenal NAN[4])	65.3	68.0	77.3	72.2	2.7	<0.01	0.66	0.17
(% of True RDOM[6])	3.74	3.36	3.93	3.25	0.17	0.82	<0.01	0.37
NAN[4] digested post-ruminally								
(g / d)	563	529	533	446	23.7	0.03	0.02	0.28
(% of intake)	80.5	79.5	79.8	72.1	3.4	0.24	0.22	0.34
(% of duodenal passage)	70.2	70.3	71.8	70.0	1.8	0.70	0.64	0.62
N apparently digested in total tract								
(g / d)	460	447	464	439	12	0.89	0.12	0.62
(%)	66.1	66.7	68.9	70.7	1.3	0.02	0.34	0.65

[1] NDF: Effect of dietary NDF content, [2] CS: Effect of corn silage, [3] NDF x CS: Interaction of dietary NDF content and corn silage.
[4] NAN: non-ammonia nitrogen, [5] NANMN: non-ammonia and non-mocrobial nitrogen, [6] RDOM: Ruminally digested organic matter.

CHAPTER 6

GENERAL DISCUSSION AND IMPLICATIONS

Rate of Passage and bm3 Corn Silage

Brown midrib 3 corn silage increased DMI and milk yield, which were partly attributed to increased rate of passage for bm3 treatments. Enhanced DMI and animal performance cannot be attributed to the difference in NDF digestibility in vivo. The second study (Chapter 5) showed that NDF digestibility either in the rumen or in the total tract was not affected by corn silage treatments. Although the first experiment (Chapter 3) showed that total tract NDF digestibility was slightly higher for cows fed bm3 corn silage, cows that responded more to the bm3 treatment with greater DMI decreased total tract NDF digestibility. Enhanced in vitro NDF digestibility does not necessarily increase NDF digestibility in the rumen or in the total tract. Digestibility of NDF is a function of potential extent of digestion, rate of digestion, and rate of passage (Allen and Mertens, 1988). Faster rate of passage is associated with enhanced DMI, and reduces NDF digestibility (NRC, 1987) because ruminal retention time for NDF is shortened. However, differences in ease of NDF hydrolysis and cell wall fragility within a forage species might be estimated by in vitro NDF digestibility. These chemical and physical characteristics of bm3 NDF could contribute to faster rate of passage. Nutritional advantages associated with bm3 treatments were enhanced DMI, improved microbial efficiency, and shifting site of starch digestion from the rumen to the intestines, and all of these could be a result of a faster rate of passage.

Energy Utilization

Although maximum milk production of high producing dairy cows is generally limited by energy intake, enhanced energy intake does not necessarily lead to greater milk energy output. In the second experiment (Chapter 5), cows fed low NDF diets increased energy intake and milk yield, but did not increase SCM yield compared to those fed high NDF diets. On the other hand, digested DMI was not significantly higher for bm3 treatments, but bm3 treatments increased milk yield and SCM yield. It is speculated that fluctuation of rumen pH influences energy utilization of lactating cows. Both low NDF diets and bm3 treatments lowered rumen pH, but daily variation and range of rumen pH were increased by low NDF diets, not by bm3 treatments. Greater daily variation in rumen pH reflects greater variation in fermentable energy supply to the rumen and is likely to result in fluctuation of supply of energy metabolites from the rumen to animals.

An objective of the model discussed below is to demonstrate the importance of fluctuation in serum metabolite flow to determine energy utilization of lactating dairy cows. To simplify the model, it is assumed that milk volume is solely determined by lactose synthesis in the mammary gland due to the osmotic effect of lactose. Rate of milk lactose synthesis (MLS) is a function of rate of glucose uptake (GU) and glucose metabolism efficiency (GME).

$$\text{MLS (g/min)} = \text{GU (g/min)} \times \text{GME} \qquad (1)$$

GME probably varies diurnally. However, incorporating GME in the model is beyond the scope of this discussion, and it is generally agreed that glucose uptake is the limiting step for milk lactose synthesis (Kronfeld, 1982). Therefore, assuming constant GME (= 1.0) for convenience of calculation, equation (1) is modified as:

$$\text{MLS (g/min)} = \text{GU (g/min)} \qquad (2)$$

Because glucose uptake in the mammary gland is not regulated by insulin, glucose uptake should occur more consistently (Zhao et al., 1996). It is reported that rate of glucose uptake in the mammary gland increases linearly when serum glucose concentration increases from 2.2 mM to 3 mM, and becomes constant when the serum glucose concentration exceeds 3.0 mM (Kronfeld, 1982). Because glucose flow to the mammary gland per min (GF) is determined by blood flow rate (BF) and blood glucose concentration (GC), serum glucose content gives only partial information about total flow of glucose to the mammary gland. However, this study (Kronfeld, 1982) showed that a limitation to rate of glucose uptake (GU) exists at a certain point of serum glucose flow. This critical point of GU is arbitrarily set to 1.0 for this discussion. Glucose potentially available (GA) to the mammary gland is a function of GF and uptake efficiency (K).

$$\text{GF (g/min)} = \text{GC (g/L)} \times \text{BF (L/min)} \qquad (3)$$

$$\text{GA (g/min)} = \text{GF (g/min)} \times \text{K} \qquad (4)$$

$$\text{GU (g/min)} = 1.0 \qquad \text{if GA} \geq 1.0 \qquad (5)$$

$$GU\ (g/min) = \min\ \{GA,\ 1.0\} \qquad (6)$$

In contrast to the mammary gland, glucose transport in adipose tissues is stimulated by insulin at higher serum glucose concentrations, then limitation to glucose transport in adipose tissues is less likely.

Although serum glucose concentration is regulated by a homeostatic mechanism, some diurnal variation exists. Four graphs demonstrate several patterns of glucose supply and glucose uptake in the mammary gland for 420 minutes (Figure 1-4). The undulant curve indicates GA, and the area below the GA curve shows total glucose flow that is potentially available to the mammary gland (GT). The line ($y = 1.0$) indicates the potential capacity of mammary gland to transport serum glucose into the cells. The line GU consists of either the GA curve or the line ($y = 1.0$), whichever is lower, indicates the rate of glucose uptake in the mammary gland, and area below this line shows the amount of glucose taken into the mammary gland (GM). Maximum GM occurs by minimizing the period for which GA value is below 1. The area, above the line ($y = 1.0$) but below the line GA, indicates the amount of serum glucose needed to shift towards adipose tissues, and denoted as GF.

$$GT\ (g) = \text{sum}\ \{GA \times \min\} \qquad (7)$$

$$GM\ (g) = \text{sum}\ \{GU \times \min\} \qquad (8)$$

$$GF\ (g) = GT - GM \qquad (9)$$

The comparison between Figure 1 and Figure 2 demonstrates how greater energy intake influences glucose utilization when fluctuation in energy supply is maintained. As total glucose flow (GT) increases from 420g to 450g, both areas GM and GF increases from 407g to 420g and 13g to 30g, respectively. This implies increased milk yield and BW gain. The comparison between Figure 1 and Figure 3 demonstrates how a greater fluctuation in energy supply influences glucose utilization when energy intake is maintained. Total glucose flow (GT) does not change. However, the area GM decreases from 407g to 393g and the area GF increases from 13g to 27g. This implies decreased milk yield and increased BW gain. Comparison between Figure 1 and Figure 4 demonstrates how greater energy intake influences glucose utilization when a fluctuation in energy supply increases. As total glucose flow (GT) increases from 420g to 450g, the area GF increases from 13g to 43g. This implies increased BW gain. In this example, the area GM does not change, implying that greater energy intake can result in no change in milk yield. Although actual regulation mechanisms for glucose utilization could be more complex, this modeling approach shows the importance of fluctuation in the serum glucose flow.

Volatile fatty acids are other energy metabolites for ruminants. Utilization of serum VFA by lactating dairy cows might be explained by the model discussed above. The fluctuation in the blood VFA flow possibly influences their utilization more dramatically because serum concentration of VFA fluctuates greatly. It has been reported that acetate and butyrate can fluctuate diurnally more than 10-fold (Quigley and Heitmann, 1991). Although the mammary gland is insulin resistant, adipose tissue increases acetate utilization with action of insulin (Vernon, 1988). More fluctuation in the serum flow of acetate and butyrate may allow the adipose tissues to uptake more of these metabolites.

Low rumen pH without increased daily variation for bm3 treatment might indicate high and consistent VFA supply from the rumen into the blood circulation. This observation implies that serum energy metabolite level was maintained high without increased fluctuation, which possibly accounts for increased FCM yield for bm3 treatments regardless energy intake level. Low NDF diets in the second experiment increased energy intake and milk yield, but did not improve FCM yield. The low NDF diets lowered rumen pH with greater daily variation and range of rumen pH. Enhanced energy intake increased milk yield; total glucose flow might be high enough to offset the possible loss of mammary glucose uptake resulting from fluctuation of serum glucose flow. However, fluctuation of serum acetate or butyrate concentration is generally greater than that of serum glucose concentration, and it is speculated that a total flow of acetate or butyrate might not compensate the loss of mammary uptake resulting from the fluctuation in flow of these substrates. Accordingly, cows fed the low NDF diets increased BW gain instead of increasing milk energy output. This theory does not negate other possible factors that account for milk fat depression, but indicates that milk fat depression can be partially attributed to the fluctuation of serum flow of substrates. At this point, we do not know that milk fat synthesis is limited more by enzyme activities in the mammary gland or by rate of uptake for milk fat precursors. However, if substrate uptake limits milk fat synthesis to some extent, the potential exists to alter energy utilization of lactating cows by manipulating ruminal fermentation pattern.

Homeostasis and DMI Depression

It has already been discussed that the energy metabolites that exceed uptake capacity of the mammary gland might be shifted towards adipose tissue under the influence of insulin. However, insulin action is sometimes inhibited; in early lactation, all peripheral tissues become insulin resistant so that more nutrients are available for the mammary gland. Insulin resistance in early lactation might be related to the regulation mechanism of DMI. Animals consume feeds to meet their energy requirement. However, it is physiologically more accurate to say that animals consume feeds to maintain homeostasis of the blood or to make up for metabolites used for maintenance, milk production, and body deposition (M. S. Allen, personal communication). Although milk yield generally sets the energy requirement of cows, cows in the early stage of lactation typically consume less than when they produce the same amount of milk later in lactation. Low DMI in early lactation is attributed partially to fill limitation, but metabolic mechanisms might be involved also. The hypothesis that animals consume feeds to maintain homeostasis would explain low DMI in early lactation. Animals can consume more feeds in later stage of lactation probably because rate of serum metabolite uptake is enhanced by insulin. On the other hand, insulin resistance in early lactation suppresses the capacity of peripheral tissues to remove metabolites from the blood pool, and animals need to limit DMI to maintain homeostasis. In other words, animals more seriously need to regulate metabolite inflow to the blood because they have less control on efflux from the blood.

Feeding rapidly fermentable carbohydrate often depresses DMI. The satiety signals such as lower rumen pH, greater serum propionate concentration, or insulin secretion may act to prevent excessive accumulation of serum metabolites and to maintain homeostasis of blood. Fill limitation to maximum DMI demonstrates the concept that physical capacity of the rumen is limited and that restricted digesta flow limits feed intake. Similarly, capacity of the metabolite pool in the blood is physiologically limited, and extremely rapid metabolite inflow or restricted metabolite efflux to peripheral tissue might limit voluntary feed intake. This leads to the theory that ruminal fermentation pattern possibly influences the DMI regulation mechanism of lactating dairy cows (M. S. Allen, personal communication).

Fluctuation of rumen pH could be an important factor to influence DMI and energy utilization of lactating dairy cows. However, it is difficult to isolate specific effects of fluctuation in rumen pH from the effects of low rumen pH; a low NDF diet typically increases fermentation acid production and decreases buffering capacity of rumen digesta simultaneously. It is often observed that low NDF diets decrease DMI, NDF digestion in the rumen, milk fat content, and FCM yield, and these observations have been attributed to low rumen pH. However, they might directly result from greater fluctuation of rumen pH. In the second experiment (Chapter 5), bm3 corn silage treatments lowered rumen pH without negative effects on animal performance. Although daily means of rumen pH and serum metabolite concentration for cows fed a variety of diets are routinely reported in the literature, little data are available for their diurnal variation and variation range. Elucidating the relationship among fermentation pattern in the rumen, serum energy metabolite flow, DMI regulation mechanisms, and energy utilization of lactating dairy cows warrant further investigation.

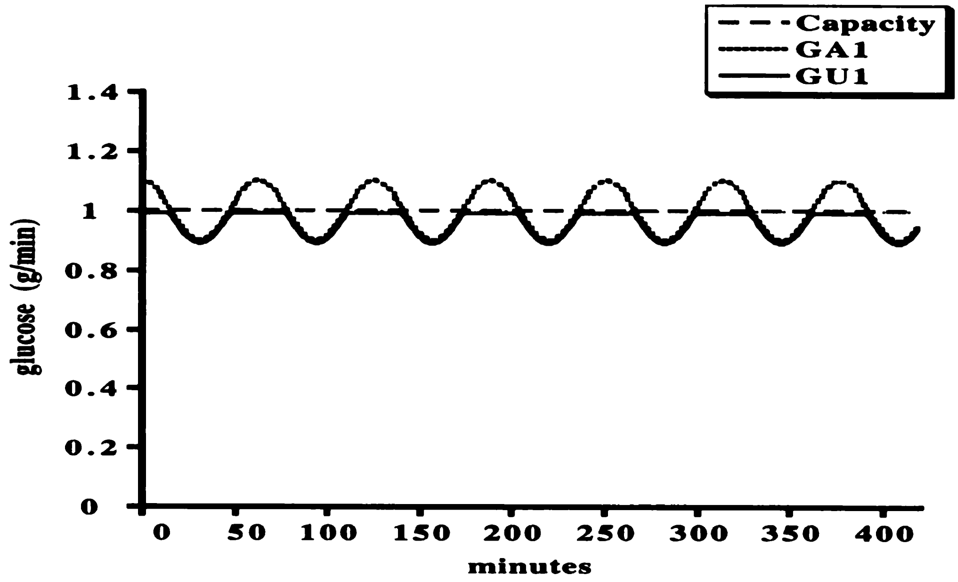

Figure 1. Glucose availability (GA) and utilization by the mammary gland (GU) over time: model 1. Total glucose flow potentially available to the mammary gland (GT) = 420g; Amount of glucose taken into the mammary gland (GM) = 407g; Amount of serum glucose needed to shift towards adipose tissue (GF) = 13g.

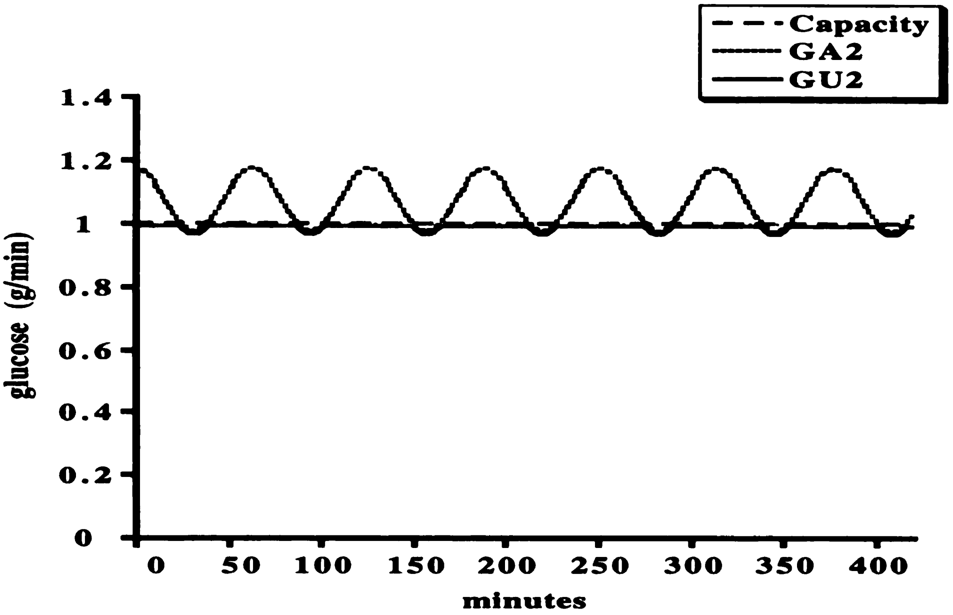

Figure 2. Glucose availability (GA) and utilization by the mammary gland (GU) over time: model 2. Energy intake increases without increasing fluctuation relative to Figure 1. Total glucose flow potentially available to the mammary gland (GT) = 450g; Amount of glucose taken into the mammary gland (GM) = 420g; Amount of serum glucose needed to shift towards adipose tissue (GF) = 30g.

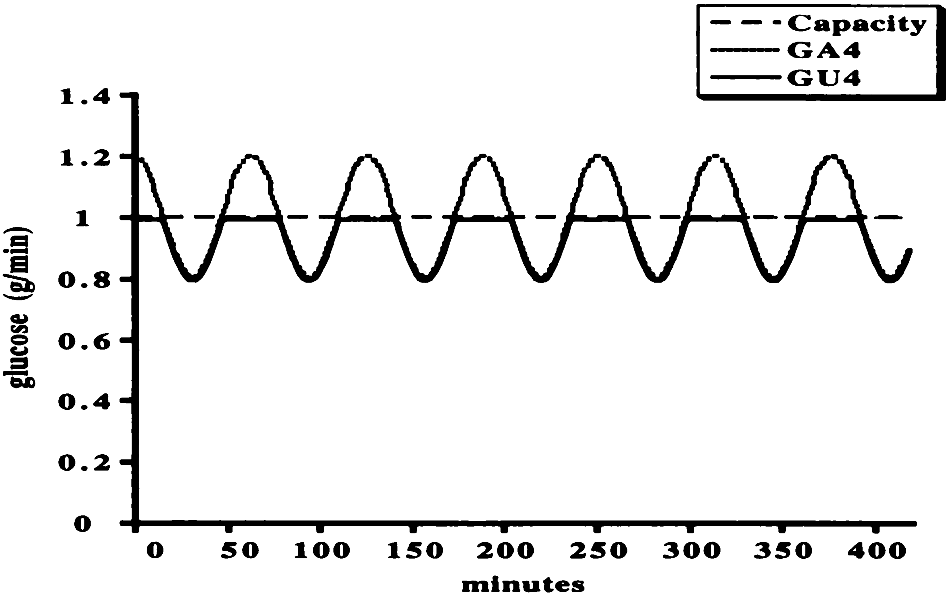

Figure 3. Glucose availability (GA) and utilization by the mammary gland (GU) over time: model 3. Fluctuation increases without change in energy intake relative to Figure 1. Total glucose flow potentially available to the mammary gland (GT) = 420g; Amount of glucose taken into the mammary gland (GM) = 393g; Amount of serum glucose needed to shift towards adipose tissue (GF) = 27g.

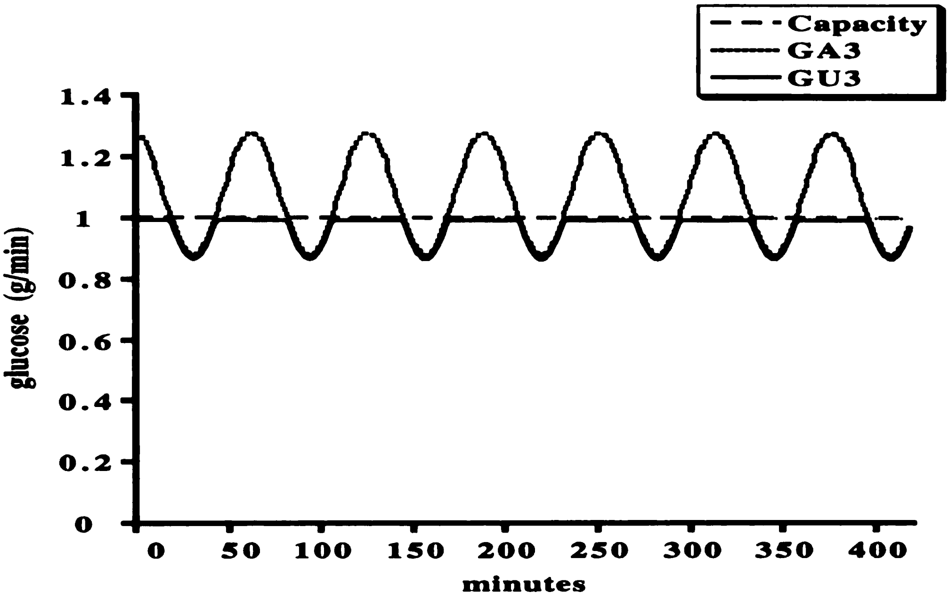

Figure 4. Glucose availability (GA) and utilization by the mammary gland (GU) over time: model 4. Energy intake increases with increased fluctuation relative to Figure 1. Total glucose flow potentially available to the mammary gland (GT) = 450g; Amount of glucose taken into the mammary gland (GM) = 407g; Amount of serum glucose needed to shift towards adipose tissue (GF) = 43g.

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