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**PELLETED BEET PULP SUBSTITUTED FOR HIGH-MOISTURE CORN:
EFFECTS ON FEED INTAKE, RUMINAL FERMENTATION, DIGESTION, AND
MILK PRODUCTION OF DAIRY COWS**

By

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ABSTRACT

PELLETED BEET PULP SUBSTITUTED FOR HIGH-MOISTURE CORN: EFFECTS ON FEED INTAKE, RUMINAL FERMENTATION, DIGESTION, AND MILK PRODUCTION OF DAIRY COWS

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Grain is often substituted for forage in diets for high-producing dairy cows to increase feed intake, diet fermentability, and milk yield, but greater dietary starch concentration can also reduce performance. Beet pulp contains 65-75% insoluble fiber and pectin, so it is frequently included in low-forage diets to moderate ruminal fermentation. To investigate the range of inclusion rates for beet pulp, low-forage diets in which pelleted beet pulp replaced high-moisture corn grain at 0%, 6%, 12%, and 24% of diet dry matter were fed to eight ruminally and duodenally cannulated dairy cows. Substituting beet pulp for high-moisture corn decreased dry matter intake, but milk yield was unaffected and milk fat yield was slightly improved for low inclusion rates. Ruminal pH was unexpectedly similar among treatments. Substituting beet pulp for high-moisture corn improved fiber digestion and dramatically shifted the dominant site of starch digestion from the rumen to the intestines without reducing whole tract starch digestibility. However, efficiency of microbial protein production was not different among treatments. The results of this experiment suggest that substituting beet pulp at low inclusion rates can improve diet digestibility and might improve milk fat yield in high-producing dairy cows.

For Howard and Mary Voelker, who showed me how to learn and to live.

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LIST OF ABBREVIATIONS

ADF	Acid Detergent Fiber
BCS	Body Condition Score
BP	Beet Pulp
BW	Body Weight
CP	Crude Protein
DM	Dry Matter
DMI	Dry Matter Intake
F:C	Forage-to-Concentrate Ratio
FCM	Fat-corrected Milk
HMC	High-Moisture Corn
INDF	Indigestible NDF
k_{abs}	Absorption Rate
k_d	Digestion Rate
k_p	Passage Rate
MN	Microbial Nitrogen
MNE	Microbial Nitrogen Efficiency
MY	Milk Yield
NAN	Non-Ammonia Nitrogen
NANMN	Non-Ammonia, Non-Microbial Nitrogen
NDF	Neutral Detergent Fiber
NE_L	Net Energy of Lactation

NE_M	Net Energy of Maintenance
NFFS	Non-Forage Fiber Source
OM	Organic Matter
pdNDF	Potentially Digestible NDF
SD	Standard Deviation
TCT	Total Chewing Time
TMR	Total Mixed Ration
TRDOM	Truly Ruminally Degraded Organic Matter
VFA	Volatile Fatty Acids

INTRODUCTION

Grain is often used to increase the energy intake and milk yield of dairy cows. Substituting grain for forage can increase feed intake by reducing the filling effect of a diet (Allen, 2000) and often increases energy intake. In some cases, adding grain can also increase ruminal microbial protein production. Greater energy intake and microbial protein production increase the ability of cows to attain their maximum genetic potential for milk production. Increasing milk yield per cow reduces the amount of energy used for maintenance per kilogram of milk produced, thereby increasing the efficiency of milk production.

However, increasing dietary starch concentration while decreasing neutral detergent fiber (**NDF**) from forage can have several negative effects. Feeding less forage NDF reduces chewing (Allen, 1997). Fiber digestibility is reduced when dietary starch concentration is increased, leading to altered volatile fatty acid production in the rumen (Grant and Mertens, 1992). Propionate production is increased, which can depress dry matter intake (**DMI**; Anil and Forbes, 1980) and potentially reduce milk yield or milk fat concentration. Excessive fermentation of starch can decrease the efficiency of microbial protein production because of energy spilling (Strobel and Russell, 1986). Total tract digestibility of starch and of total nutrients can be reduced if increasing starch intake leads to drastic increases in passage rate. While lowering forage intake and increasing grain intake has the potential to increase energy intake and milk yield, this strategy can also have the opposite effect.

There are several alternative strategies for increasing dietary energy content while reducing the risk of negative side effects. One such strategy is to add carbohydrate sources that are more rapidly and extensively fermented than forage NDF and that mimic some of its beneficial effects, but do not bring the same negative effects as starch fermentation. Many non-forage fiber sources fit that description, and beet pulp has been studied in this role because of its high concentrations of rapidly-fermented soluble and insoluble fiber (Mansfield et al., 1994; Swain and Armentano, 1994; O'Mara et al., 1997; Clark and Armentano, 1997). However, the effects of substituting beet pulp for grain on ruminal digestion kinetics and microbial protein production have not been investigated in dairy cattle; most studies have focused on feed intake, chewing response, and milk production.

It might be possible to partially replace grain with beet pulp in energy-dense diets to moderate the negative effects of starch while avoiding the negative effects of a higher forage NDF content. Therefore, the objective of this research was to evaluate the effects of increasing substitution of beet pulp for high-moisture corn on feed intake and chewing behavior, ruminal fermentation, nutrient digestion, and milk production.

CHAPTER 1

A REVIEW OF LITERATURE

Beet Pulp

Beet pulp is a byproduct of the extraction of sucrose from the sugar beet (*Beta vulgaris*). The sugar beet industry originated in Europe in the early 1800s but was not successful in the United States until around 1900, and it has been limited to north-central and western states (Bichsel, 1988). The sugar industry is dominated by cane sugar, most of which is imported, but maintenance of the capacity to produce sugar domestically has traditionally been a priority, especially in time of war (Winner, 1993). In Michigan, nearly all cultivation of sugar beets takes place in the lowlands around Saginaw. In 2000, the state of Michigan ranked fourth in the nation in sugar beet production with 166,000 acres and over 3 million tons of beets (MDA, 2002).

Sugar beets grown in the northern states are generally harvested between September and November and stored in piles to permit more extended operation of processing plants. (In California, beets must be processed immediately due to warmer air temperatures, and part of the crop is left in the dry ground for spring harvest.) A sugar beet weighs about 1-2.5 kg at harvest and contains approximately 25% dry matter (Bichsel 1988). Of that dry matter, 74% is sucrose, 3% is ash, 18% is soluble organic non-sugars (such as carbohydrates, organic acids, protein, amino acids, amides, betaine, and saponins), and 5% is

insoluble organic non-sugars (hemicellulose, cellulose, pectin, and lignin; Tjebbes 1988). To extract the sucrose, the beets are cut into “cossettes” approximately 5 mm² and 5-7.5 cm long. There are several different methods for extracting sucrose, but the process generally uses hot water (which disrupts cell walls) and counter-current diffusion in several steps, along with pressing. Approximately 98% of the sucrose is removed, producing a juice containing approximately 12% sugar, which is purified by precipitation and filtering. The resulting solution is reduced to a concentrate, and the sucrose is crystallized (Bechsel 1988).

Byproducts of beet sugar extraction include lime sludge from the precipitation of impurities, which is sometimes used as a soil improver (Tjebbes 1988); beet pulp, which is sold wet or dried for numerous uses (to be described further); and molasses from the sugar crystallization, which is either added back to the pulp before drying or used to produce citric acid, antibiotics, yeast, and other biological and chemical commodities (Bechsel 1998).

One metric ton of sugar beets will produce approximately 149 kg of sugar and 50 kg of pulp (Tjebbes 1988). The chemical composition of the pulp varies depending on location, year, and processing methods, but dried, unmolassed beet pulp used in several feeding experiments with ruminants has contained, on average (% DM): 6% ash, 23.6% ADF, 46.0% NDF, 11.7% CP, 1.5% ether extract, and 4.5% lignin (Table 1). Beet pulp also contains a significant concentration of gums, specifically pectic substances, an amorphous class of polysaccharides which comprises approximately 25% of dried beet pulp (Thibault

et al., 1991). The usefulness of these pectic substances for adding texture and fiber to human food products has been widely known, and the fermentation of pectin has been studied in nonruminants (including humans) and in ruminants.

Pectin

Pectic substances are a class of polysaccharides that are partially, and variably, soluble in water, and are especially known for their gelling properties in the presence of sugar or acid. (Therefore, the chemistry of pectin is the subject of much industrial, nutritional, and medical study.) Pectin is found in greatest concentrations in the middle lamella of plant cells, and the pectin concentration decreases from the exterior to the interior of the cell wall (Mauseth, 1988).

Pectin adheres plant cells to each other and strengthens the cell wall. The chemical composition and molecular weight of pectin vary among sources, but the defining structural element of pectin is D-galacturonic acid, joined by α -1,4 linkages (Van Buren, 1991). Galacturonic acid may be present as both the acid (COOH) and the methyl ester (COOCH₃). The length of the polymers is affected by species type and by growing and storage conditions, and other neutral sugars and organic acids are present in varying proportions. Rhamnose units are inserted at irregular intervals between the galacturonic acid units; these introduce a kink into the structure and often are linked to arabinan, galactan, or arabinogalactan side chains (Van Buren, 1991). Beet pulp pectin contains high concentrations of these neutral sugars relative to other pectins from other common sources such as apple and citrus pulp. This, in combination with low

molecular weights and a high degree of acetylation, may prevent the alignment required for gelation, so that beet pulp pectin is not as valuable as other pectins for use as a gel (Van Buren, 1991).

Among common components of dairy rations, beet pulp has the highest concentration of pectin, approximately 25% of DM (Thibault et al., 1991). Citrus pulp also has a large concentration of pectin but also has a large concentration of soluble sugars. Legume forages such as alfalfa and clover contain 5 to 10% pectic substances (Gaillard, 1962), whereas grasses contain 2 to 4% pectin (Waite and Gorrod, 1959). Grains contain very little pectin (Kertesz, 1951).

Fermentation of Pectin

Some of the first characterizations of microbial fermentation of pectin were published by Bryant and coworkers in the 1950s and 1960s. They identified *Butyrivibrio fibrisolvens*, *Prevotella* (formerly *Bacteroides*) *ruminicola*, *Lachnospira multiparus*, *Succinivibrio dextrinosolvens*, and *Fibrobacter* (formerly *Bacteroides*) *succinogenes* as pectin-fermenting species (Bryant and Doetsch, 1954; Bryant and Small, 1956a; Bryant and Small, 1956b; Bryant et al., 1958). Dehority (1969) reported that *B. fibrisolvens*, *P. ruminicola*, and *L. multiparus* grew using purified pectin as the sole added energy source. In the same experiment, *L. multiparus* did not grow on purified galacturonic acid, and the authors suggest that the species has an enzyme system that carries out *trans*-elimination to cleave the pectin chains (pectinglycosidase), or enzymes that cleave the methyl ester bond (pectinesterase). Other species that can utilize

galacturonic acid monomers have a different pectinglycosidase, which directly hydrolyzes the α -1,4 bonds, perhaps in addition to other pectin-degrading enzymes (Dehority, 1969).

Fermentation of pectin by *L. multiparus* may be further limited by its inability to degrade cellulose and hemicellulose in order to gain access to pectin (Osborne and Dehority, 1989). These researchers also found that the cellulolytic *Fibrobacter succinogenes* and hemicellulolytic *P. ruminicola* have very limited ability to ferment or utilize purified pectin alone or in mixed culture but are able to degrade and utilize forage pectin synergistically in mixed culture. Therefore, tests of pectin fermentation by isolated bacteria have limited application to the rumen environment.

Efforts have been made to characterize the growth of pectinolytic bacteria in response to feeding, diet composition, and ruminal pH. Leedle and coworkers (1982) reported that, on a high-forage diet, pectinolytic bacteria had the lowest population 2 hr after feeding, and the highest population from 8-12 h after feeding. This is likely caused by a reduction in pH shortly after feeding, when fermentation of readily-available starch and sugars rapidly produces volatile fatty acids (VFA). The pectinolytic and cellulolytic populations responded similarly after feeding, but pectin degraders recovered more quickly than the cellulolytic population. Like cellulose, pectin fermentation is sensitive to low pH. Marounek and coworkers (1985) found that when pectin was the sole carbohydrate, rate of total VFA production and final concentration *in vitro* was greater when pH was 6.5-7.0 than when pH was 5.7-6.4. The sensitivity of pectin degraders to low pH

has also been demonstrated by *in vitro* fermentations at pH 6.7 or pH 6.0 (Strobel and Russell, 1986). Reducing pH from 6.7 to 6.0 decreased pectin utilization by 53%, while the utilization of starch and sucrose was not affected by pH. Bacterial protein production on pectin was also reduced (by 69%) at lower pH, due in part to greater energy spilling (Strobel and Russell, 1986).

Fermentation of pectin is carried out through more than one chemical pathway. Methyl groups can be hydrolyzed by pectin esterase (pectase) to yield pectic acid and methanol, and polygalacturonase (pectinase or polygalacturonidase) catalyzes the hydrolysis of 1,4-glycosidic linkages in pectin to form galacturonic acid (Leng, 1970). Several strains of *B. fibrisolvens* have shown higher esterase activity when grown on pectin compared with glucose, indicating that one mechanism to obtain energy from pectin is by hydrolyzing the ester bonds on methylated pectin chains (Hespell and O'Bryan-Shah, 1988). Leng (1970) suggested, and others have agreed (Fahey and Berger, 1988; Van Soest, 1994), that polygalacturonide is cleaved by polygalacturonase into galacturonic acid monomers. Galacturonic acid is converted to pyruvate through the pentose phosphate pathway and glycolysis, then further metabolized to VFA, especially acetate (Leng, 1970; Van Soest, 1994). An alternate, modified Entner-Doudoroff pathway has been proposed for pectin degradation by *B. fibrisolvens* and *P. ruminicola*, in which galacturonic acid monomers are not formed, but pyruvate is still the end product (Marounek and Duskova, 1999). Acetate was the dominant VFA product from pectin fermentation, and the experiment demonstrated that the two species produced more acetate when grown on pectin

than when grown on D-glucose (Marounek and Duskova, 1999). The dominance of acetate as a product of pectin fermentation is well established (Marounek et al., 1985; Strobel and Russel, 1986; Leedle and Hespell, 1983). Pectin fermentation also produces much less lactate than does the fermentation of starch or glucose (Marounek et al., 1985; Strobel and Russell, 1986; Leedle and Hespell, 1983).

The fermentation rate of pectin is generally intermediate between the rates of fermentation of soluble carbohydrates, such as starch and sugars, and insoluble carbohydrates such as cellulose and hemicellulose. In *in vitro* experiments utilizing rumen inocula from goats fed hay and concentrates, rate of VFA production with pectin as the substrate was more rapid than with hemicellulose at both high and low ends of the typical range of ruminal pH (Marounek et al., 1985). VFA production was more rapid for pectin than for starch in a higher pH range (above 6.5) and was slower for pectin than for starch at a lower pH range (below 6.5; Marounek et al., 1985). Pectin is fermented more rapidly than insoluble fiber components, but like cellulose and hemicellulose, pectin fermentation is inhibited at low pH. Therefore, pectin can increase the rate of carbohydrate fermentation; replacing starch with pectin may also attenuate the postfeeding pH decline, which can improve digestion of cellulose and hemicellulose.

Because the peak in pectin fermentation is delayed for several hours after feeding; because pectin fermenters slow their production of VFA as pH declines; and because pectin fermentation produces very little lactate, adding feeds with

high pectin contents (such as beet pulp) may provide more rapidly and more thoroughly-fermented carbohydrate in diets for dairy cattle without negatively affecting the digestion of cellulose and hemicellulose.

Fermentation of Beet Pulp *In Vitro*

Among the feeds commonly included in diets for dairy cows, beet pulp has one of the highest pectin contents. This, and the rapid and extensive digestion of its insoluble fiber components (Torrent et al., 1994; Bhatti and Firkins, 1995), have made beet pulp attractive as an alternative to grain and forages for increasing diet digestibility. Several comparisons have been made of the *in vitro* and *in situ* fermentation of beet pulp, other byproducts, forages, and grains (Chester-Jones et al., 1991; Sunvold et al., 1995; Sanson, 1993; Bach et al., 1999; Mansfield et al., 1994). However, only a few directly compare grains and beet pulp.

One group (Mansfield et al., 1994) studied production and intake in dairy cattle fed diets with 30% corn or 15% corn plus 15% beet pulp, then used 10-day continuous culture to estimate characteristics of digestion and fermentation. Digestibility of DM, OM, NDF, ADF, and non-structural carbohydrates (**NSC**) was not affected by treatment. Acetate concentration was greater, and butyrate and branched-chain VFA concentrations were lower, for the beet pulp diet than for the high-corn diet. Total VFA and propionate concentrations were not different between treatments. Fluid ammonia concentration and effluent ammonia flow were greater for the all-corn diet.

Chester-Jones and coworkers (1991) replaced rolled corn with dried beet pulp at 0%, 15%, and 30% of diet DM using eight-day continuous culture *in vitro* experiments. The experiment also compared two protein sources, and interactions between carbohydrate and protein sources complicate interpretation of beet pulp effects. No effects of beet pulp concentration were found for digestibility of DM, OM, NDF, or NSC, perhaps due to differences in effects of protein sources. Replacing the high-starch corn with high-fiber beet pulp increased the concentration of acetate and decreased butyrate and isobutyrate content, but did not affect propionate concentration. These results are similar to the work by Mansfield et al. (1994). Carbohydrate source had no effect on bacterial N flow or g bacterial N per kg truly-digested DM. When soybean meal was the primary protein source, substitution of beet pulp for corn decreased both ammonia N concentration in the fluid and effluent ammonia N flow (Chester-Jones et al., 1991), possibly because some amylolytic bacteria are highly proteolytic (Russell et al., 1981).

A third experiment compared, among other feeds, molassed beet pulp and cracked corn as supplements to forage, in eight-day continuous culture (Bach et al., 1999). Because the beet pulp contained molasses, it probably contained more sugars than would unmolassed beet pulp. In this experiment, true digestibility of DM, OM, and NSC were greater for corn than for beet pulp, as might be expected, but NDF digestibility was not affected. The absence of an effect on NDF digestibility might be because there was no difference in pH between the two treatments. Total VFA concentration was higher for corn,

reflecting the greater digestibility of DM and NSC on that treatment. However, concentrations of individual VFA, such as acetate and propionate, and acetate-to-propionate ratio were not different between the treatments, although a beet pulp treatment would be expected to result in greater acetate and lower propionate production. Lactate concentration was not reported. Carbohydrate source did not affect fluid ammonia-N concentration, bacterial N flow, or g bacterial N/ kg OM truly digested.

A primary limitation of using continuous culture and other *in vitro* methods for comparing fermentation of carbohydrates is manipulation of pH. Using buffers to maintain a specific pH range or allowing fermentation to progress without some simulation of the extrinsic factors affecting pH reduces the possibility of determining any effects of carbohydrate source that would be produced in an actual rumen environment. Specifically, the effects of carbohydrate type on VFA production, OM and NDF digestion, and N metabolism, which are affected *in vivo* by pH, may not be accurately reproduced under *in vitro* conditions.

Beet Pulp Substituted for Grain in Dairy Cattle Rations

Four previous experiments have examined the substitution of dried beet pulp for grain in which the treatments were fed *ad libitum* to lactating dairy cattle as a total-mixed ration (**TMR**) with a fixed forage-to-concentrate ratio (**F:C**; beet pulp included in concentrate; Mansfield et al., 1994; Swain and Armentano, 1994; Clark and Armentano, 1997; O'Mara et al., 1997). These studies provide the

most reliable conclusions comparing the effects of beet pulp and grain in dairy cows; eleven other experiments compared grain and beet pulp under less controlled feeding conditions (Bhattacharya and Lubbadah, 1971; Huhtanen, 1987; Sutton et al., 1987; Beauchemin et al., 1991; Sievert and Shaver, 1993; Van Vuuren et al., 1993; Friggens et al., 1995; Petit and Tremblay, 1995a,b; Moorby et al., 1996; Dewhurst et al., 1999; and Keady et al., 1999). Responses such as DMI and milk production from these eleven experiments will be summarized when useful.

The experiment by Mansfield and coworkers (1994) utilized 46 Holstein cows, starting in early lactation, to measure intake, milk yield and milk composition in a 2 x 2 factorial arrangement of treatments. Carbohydrate source (ground corn alone or partially replaced by beet pulp) was one set of treatments, and protein source (soybean meal or meat and bone meal) was the second set of treatments. The corn diets contained 28% or 32% corn (depending on protein source); the beet pulp diets each contained 15% beet pulp substituted for the corn.

Work by Swain and Armentano (1994) utilized 21 Holstein cows in midlactation in an incomplete block design with seven treatments and three periods. Treatments of interest were a basal diet containing 58% finely ground corn, and a diet containing 16% beet pulp and 41% ground corn. The researchers measured DMI, milk production and milk fatty acid composition, ruminal VFA, and chewing behavior, with the main objective of quantifying fiber effectiveness.

O'Mara and coworkers (1997) compared milk production, blood metabolite, ruminal fermentation characteristics, and total tract digestion effects of four diets, two of which contained 20% ground corn with 11% beet pulp, or no corn with 30% beet pulp. Production was measured over the course of 56 days using 18 cows per treatment (15 in early lactation and 3 in midlactation), and rumen and digestion characteristics were measured using four cows, starting in early lactation, in a Latin square design. These four cows were fitted with ruminal cannulas, and infused Yb was used to measure total tract digestibility.

Work by Clark and Armentano (1997) utilized 16 cows in midlactation in a replicated 4x4 Latin square design to study the influence of particle size on the effectiveness of beet pulp; the two diets of interest were the basal diet, containing 45% rolled high-moisture corn and no beet pulp, and the whole-beet-pulp diet, containing 30% corn and 16% whole, dried beet pulp. Measurements included milk production, DMI, ruminal VFA, and chewing activity.

None of the experiments described above specified whether the dried beet pulp was fed in the form of "shreds" (dried without further physical processing) or pellets. It is possible that pelleting would alter the likelihood of sorting, chewing behavior response, rumen fill effect, or ruminal degradation of beet pulp. The more compact form of pelleted beet pulp is more suited to storage and shipping.

In these four experiments, the F:C and the rates of corn and beet pulp substitution varied widely. The goal of Swain and Armentano (1994) and of Clark and Armentano (1997) was to test the "effectiveness" of fiber from non-forage sources through, among other measures, milk fat concentration. Therefore their

F:C were low. In the first experiment, the F:C was 32:68, and control and beet pulp treatments contained 58% and 41% corn grain, respectively (Swain and Armentano, 1994). In the second experiment, the F:C was 38:62, and control and beet pulp treatments contained 45% and 30% corn grain, respectively (Clark and Armentano, 1997). By contrast, the F:C for Mansfield and coworkers (1994) was 53:47; because they were directly comparing corn and beet pulp for normal production rations, their diets started with a more moderate inclusion rate of corn (30%) in the corn diets and equal amounts of corn and beet pulp (15% for each) in the test diets. O'Mara and coworkers (1997) fed grass-silage based diets with a F:C of 60:40 and a different substitution method, starting with a control diet containing 30% beet pulp and no "starch source" (corn or other high-starch grain; all diets contained soybean meal), then replacing two-thirds of the beet pulp (20% of diet DM) with corn grain. The variety in concentrations of forage fiber, non-forage fiber, and starch in the diets used in these four experiments certainly contributed to differences in relative response to beet pulp and corn grain between experiments.

Beet Pulp and the Regulation of Feed Intake

Daily feed intake is determined by meal size and meal frequency (Allen, 2000). The amount of feed consumed at one meal is limited by signals of satiety, including distension, hypertonicity of rumen fluid, and the absorption of metabolic fuels from the rumen and intestines (Allen, 2000). Less attention has been paid

to the mechanisms of hunger in ruminants and the causes of meal initiation, which determine meal frequency.

Physical regulation of intake, described as gut (or rumen) “filling effect,” was first described as the “ballast” of digesta (Lehman, 1941). In dairy cattle whose intake is limited by fill, the rumen is not actually filled to capacity (Dado and Allen, 1995), but signals of distension are integrated with other signals of hunger and satiety (Forbes, 1995). The extent to which distension limits intake would differ between cows with varying milk yields and in different reproductive status, and between diets with different contents of energy and other nutrients and with different dry matter contents and physical forms (Allen, 1996).

The contribution of ruminal distention to satiety results from both weight and volume of digesta. Schettini and coworkers (1999) increased volume of ruminal contents by inserting 50 or 100 tennis balls, and changed the weight added with the volume by using balls with different specific gravity (1.1 or 1.3), for steers fed low-quality orchardgrass. Dry-matter intake was reduced 112 g per kg inert weight and 157 g per liter inert volume added (Schettini et al., 1999). Beet pulp might increase ruminal digesta weight and volume by increasing digesta moisture content, because water contributes to the volume and especially to the weight of ruminal digesta. When diet moisture content was increased by soaking in water, DMI was not reduced (Robinson et al., 1990). However, research with pigs found that maximum potential DMI could be predicted using the water-holding capacity (**WHC**; ml H₂O/g insoluble DM) of

feeds (Kyriazakis and Emmans, 1995) measured by centrifugation or filtration.

Similar research has not been conducted with ruminants.

Beet pulp and corn meal WHC were measured in one experiment by centrifugation according to the American Association of Cereal Chemists and by simple filtration (Ramanzin et al., 1994). The WHC of beet pulp was 6.44 and 5.97 ml H₂O/g insoluble DM, and the WHC of corn meal was 1.25 and 1.27 ml H₂O/g insoluble DM, for the centrifugation and filtration methods, respectively (Ramanzin et al., 1994). For reference, the WHC of alfalfa meal was 4.25 and 3.63 ml H₂O/g insoluble DM for the two methods. Although the WHC of beet pulp has been reported differently relative to forages and other non-forage fiber sources (NFFS; Bhatti and Firkins, 1995), this comparison of beet pulp and corn meal (Ramanzin et al., 1994) suggests that substituting beet pulp for corn grain would increase the water-holding capacity, and therefore the weight and possibly the volume, of ruminal digesta independent of its effects on ruminal DM or NDF pool. This could contribute to the limitation of DMI by distention.

Allen (2000) suggested that “when DMI is regulated by distention in the reticulorumen, substitution of NFFS ... for grain might decrease DMI because of differences in their relative filling effects.” The review combined 33 comparisons of DMI between grain and a variety of NFFS such as soy hulls, corn distillers grains, whole cottonseed, and beet pulp, and found that only 8 of 33 reported increased DMI for NFFS compared to grain, 2 of 33 had decreased DMI; overall, there was no relationship between DMI and the percent of NFFS in the diet (Allen, 2000). Given the wide variation in physical form, NDF content, and NDF

digestibility and passage rate among high-fiber byproducts, it is not surprising that their effects on DMI vary greatly. In addition, ruminal fill probably was not the first limiting factor of DMI in all of these studies (Allen, 2000). As will be discussed later, diet energy content probably limited intake on high-grain diets.

The distension effect of beet pulp is partly a result of its NDF content, rate and extent of NDF digestion, and NDF passage rate. Ruminal fill is often described using NDF pool rather than DM pool (Mertens and Ely, 1979; Bywater, 1984; Mertens, 1994). Most research attempting to describe the effects of fill on intake compares forages to grains, or different forages with varying NDF digestibility. Some of the generalizations about the effects of NDF on ruminal fill resulting from forage research may not apply to NDF from NFFS. In a summary of many studies, DMI decreased with increased NDF when diet NDF was altered by changing the forage-to-concentrate ratio (Allen, 2000). Beet pulp NDF has a shorter initial lag time than alfalfa and is more rapidly digestible than orchardgrass and corn silage (Bhatti and Firkins, 1995; Nocek and Russell, 1988), so slow ruminal NDF digestion (relative to ruminal starch digestion) might be less important in the limitation of intake by fill on beet pulp than it is for forages.

Passage rate of feed NDF from the ruminoreticulum also contributes to its filling effect. Passage rate is limited by digesta particle size and density (Lechner-Doll et al., 1991). Particle size is decreased by mastication and by chemical breakdown; although beet pulp is likely reduced in size as quickly as

grains, the initial particle size of pelleted beet pulp is larger than the particle size of grain, especially of processed grain.

Density is increased by hydration and by increased concentration of indigestible material, and it is decreased by the gas produced during fermentation (Allen, 1996; Bailoni et al., 1998). Functional specific gravity and volume of associated gas were similar for finely ground corn meal and beet pulp and for their insoluble fractions both before and after soaking (Ramanzin et al., 1994). However, this comparison does not account for original particle size and its potential effect on density and gas retention. Beet pulp fermented *in vitro* had a lower functional specific gravity than grain byproducts after 4, 8, and 27 hours of *in vitro* fermentation (Bhatti and Firkins, 1995). Therefore, beet pulp particles are probably more likely to remain suspended in the rumen mat than are grain particles. Lower density probably also leads to a slower passage rate for beet pulp fiber than for fiber from grain. Therefore, beet pulp might increase the limitation of intake by physical distention compared to grain because of longer retention time in the rumen.

In addition to physical regulation of intake, chemical aspects of ruminal fermentation and intestinal absorption contribute to the sensations of hunger and satiety. The digestion of structural and nonstructural carbohydrates can take place in both the rumen and the intestines, with varying efficiency and perhaps with different effects on intake. As most metabolites from fermentation and intestinal digestion pass through the hepatic portal vein, the liver is responsible for many of the metabolic signals of hunger and satiety (Forbes, 1995).

The primary volatile fatty acids produced from the fermentation of starch, cellulose, hemicellulose, and pectin are propionate, acetate, and butyrate. Of these, only propionate is produced in great enough quantity *and* metabolized sufficiently in the liver to affect DMI (Allen, 2000). While acetate is often found in greater concentration in rumen fluid, it is not metabolized to any great extent in the liver (Reynolds, 1995). Ruminal infusion of propionate (compared to acetate) decreased meal size and tended to increase intermeal interval, resulting in decreased DMI (Choi and Allen, 1999). Substituting beet pulp for corn dilutes dietary starch; this would likely reduce propionate production and might increase DMI. Replacing starch with NDF and pectin would probably reduce the rate of VFA production in general and would increase the proportion of acetate produced.

Starch not fermented in the rumen is usually digested and absorbed as glucose in the small intestine of dairy cattle. However, in ruminants, much of the absorbed glucose is probably utilized by the viscera, as there is little net glucose absorption across the portal-drained viscera (Reynolds, 1995). The effect of postruminal glucose absorption on intake in ruminants has not been investigated thoroughly, and various studies have produced conflicting results (Allen, 2000). Because the amount of intestinally absorbed glucose reaching the liver is usually insignificant compared to glucose produced via gluconeogenesis (Weekes, 1991), it is unlikely that glucose absorbed in the small intestine has a significant direct effect on satiety. However, increased absorption of glucose into intestinal tissue might reduce the demand for and uptake of circulating glucose by

intestinal tissue. This should increase the amount of circulating glucose, which could signal satiety. In addition, dietary starch absorbed by the intestines would be converted to lactate in intestinal tissue; and circulating lactate could be metabolized in the liver, thus signaling satiety. However, the effects of starch digestion in the small intestine on intake have not been well examined.

It is not known how the substitution of beet pulp for corn affects the passage rate and ruminal digestibility of starch. If adding beet pulp increases retention time and ruminal starch digestion, increased propionate production could reduce intake. If beet pulp increases ruminal starch passage rate and decreases ruminal starch fermentation, the reduction of propionate absorption might increase intake, and little evidence exists for an effect on intake of increased intestinal glucose absorption.

Responses of DMI to substituting beet pulp for corn grain have varied. Two experiments (O'Mara et al., 1997; Clark and Armentano, 1997) reported greater DMI for the beet pulp diet, and one (Mansfield et al., 1994) reported greater DMI for the corn diet. Adding beet pulp to a high-grain TMR did not affect DMI in one experiment (Swain and Armentano, 1994), and none of the eleven experiments comparing grain and beet pulp under other feeding conditions reported effects on DMI. The differences in DMI response might be explained, in part, by dietary characteristics.

O'Mara and coworkers (1997) measured intake for treatment groups only and therefore did not analyze this response statistically, but DMI was numerically 1.5 kg/d greater for animals fed the beet pulp diet than the corn diet. This is a

surprising result on such a high-forage diet with additional non-forage fiber, where fill might be expected to be the dominant factor limiting intake and the addition of more rapidly fermentable corn grain might be expected to permit greater intake. The depression of intake with the addition of corn grain to a grass-silage diet might be the result of additive effects of fill and propionate (Mbanya et al, 1993). Ruminal distension and propionate treatments which did not reduce intake when administered alone, depressed intake when administered together (Mbanya et al., 1993). Plasma β -hydroxybutyrate was 10.5 mg/L higher on the corn diet than on the beet pulp diet (O'Mara et al., 1997), which might indicate a greater hepatic pool of acetyl-CoA available for use in the TCA cycle, increasing the hypophagic effects of propionate (Oba, 2002). However, beet pulp did not affect rumen fluid propionate concentration collected via ruminal cannula throughout the day (O'Mara et al., 1997). Although VFA concentration is not necessarily indicative of VFA production or absorption, it is not clear that intake was limited by propionate on the corn diet. Nonetheless, the additive satiety effect of fill and metabolic fuels is the most reasonable explanation for decreased DMI on a high-forage diet with added grain. In addition, milk yield was low for these cows, averaging approximately 20 kg/d, so propionate (or other metabolites) would be more likely to be limiting to feed intake for these cows than in high-producing cows with greater energy requirements.

When beet pulp was substituted for corn in a diet containing 38% forage and 62% concentrate, resulting in a diet containing 16% beet pulp and 29% corn grain, DMI increased by 0.4 kg/d (Clark and Armentano, 1997). Rumen fluid

propionate concentration decreased with the addition of beet pulp, so reduced propionate metabolism in the liver could have allowed greater DMI. However, samples were collected only once per period via stomach tube, and this sampling technique and frequency is less reliable than sampling from several sites in the rumen throughout the day. Reducing dietary starch concentration or changing the site of starch digestion by adding beet pulp could also change the amount of starch absorbed as glucose and converted to lactate in the small intestine; and a decrease in circulating glucose or lactate might reduce satiety.

A similar experiment measured no effect on DMI (Swain and Armentano, 1994). Cows in this experiment were also producing about 10 kg/d less milk, and cows with lower feed intake and milk production might be expected to respond less dramatically to dietary changes. The diets that resulted in no DMI effect of beet pulp contained slightly less forage (32% versus 38% of DM) and more corn grain for both corn and beet pulp diets than diets fed by Clark and Armentano (1997). Ruminal propionate was measured by Swain and Armentano (1994), once per period via stomach tube, but was unaffected by treatment. That experiment might have failed to improve DMI with added beet pulp because the ratio of beet pulp to corn was lower, so the amount of beet pulp may have been insufficient to reduce the negative effects of corn grain.

By contrast, substituting 15% beet pulp for corn in a diet containing 53% forage and 47% concentrate reduced intake by 1.3 kg (Mansfield et al., 1994). When sufficient fiber is already being consumed, the substitution of high-fiber beet pulp for corn likely increases the filling effect of the diet and thus reduces

intake. Forage characteristics also may have affected response to beet pulp substitution. The forage component of this diet included corn silage, alfalfa hay, and alfalfa pellets (Mansfield et al., 1994), while Swain and Armentano (1994) and Clark and Armentano (1997) fed only alfalfa silage as forage. Dry alfalfa hay may have had a larger particle size or lower digestibility than alfalfa silage, which could have increased the filling effect of the hay-based diet in addition to the effect of higher forage content. The addition of beet pulp to that diet probably enhanced its filling effect. Cows in this experiment (Mansfield et al., 1994) had moderate milk yields (32 kg/d), so DMI was probably limited by fill.

Responses in DMI to beet pulp substituted for corn grain have not been consistent. While all four experiments described here replaced corn with beet pulp as approximately 15-20% of diet DM, the response probably was affected by the amount and type of forage fed, and possibly by the ratio of beet pulp to corn grain. Intake would likely respond differently to higher or lower rates of beet pulp inclusion in the diet. The response of DMI to beet pulp is probably dominated by fill effect when added to a high-fill (high-forage) diet or when fed to high-producing cows, and DMI response is probably dominated by a reduction of metabolic fuel absorption when beet pulp is added to a high-concentrate diet or fed to low-producing cows. However, fill and metabolic satiety signals are not mutually exclusive and can be additive (Mbanya et al., 1993).

Effects of Beet Pulp on Chewing Behavior

Chewing, especially rumination, is essential for the reduction of digesta particle size and to stimulate the flow of saliva and the buffers it contains into the rumen. Mastication contributes especially to the ruminal digestion of NDF by increasing surface area available for bacterial attachment. Saliva buffers are essential to the prevention of low ruminal pH (especially below 6.0), which inhibits the activity of fibrolytic bacteria (Russell and Wilson, 1996). Because beet pulp can be a significant source of NDF, its effectiveness in stimulating chewing has been compared to forages and concentrates. The ability of a feed to stimulate chewing, salivation, and ruminal motility is implicated in milk fat synthesis, ruminal pH, and fiber digestibility. Animal responses such as chewing time, ruminal pH, acetate:propionate, yields of milk and milk fat, and milk fat content, measured in 31 to 111 experiments (depending on variable), have been used to estimate fiber effectiveness (Armentano and Pereira, 1997). When various non-forage fiber sources were used to increase dietary NDF concentration, responses were similar to, but not always equal to, those induced by adding NDF by adding forage (Armentano and Pereira, 1997). However, adding NFFS reduced ruminal pH, implying that ruminal health and NDF digestibility were not improved despite increased chewing. Using data from Swain and Armentano (1994), Mertens (1997) determined that beet pulp's effectiveness was similar to that of other NFFS, assigning it a pef (physical effectiveness) value of 0.44, where the pef of long grass hay is the standard (1.00).

Both Swain and Armentano (1994) and Clark and Armentano (1997) compared chewing behavior for beet pulp and corn treatments. In the first experiment, tendencies ($P \leq 0.10$) were detected for greater time spent in total chewing (eating + ruminating) and in time chewing per kg DMI for cows fed beet pulp compared to corn (Swain and Armentano, 1994). Although increased milk fat content was also reported for cows fed the beet pulp diet, chewing response and milk fat percent were not correlated across NFFS types, so a direct relationship between chewing effectiveness and milk fat synthesis cannot be assumed. In the second experiment (Clark and Armentano, 1997), beet pulp and corn had similar effects on time spent ruminating or eating. Treatment also did not affect milk fat yield or content. Taken together, these studies suggest that beet pulp is not consistently a reliable source of “effective” fiber by the definition of increasing chewing time or milk fat synthesis.

Beet Pulp and Microbial Efficiency

Microbial protein is the most significant source of amino acids for the lactating dairy cow, not only because of the quantity of protein produced in the rumen and digested in the small intestine, but also because microbial protein is highly digestible and its amino acid profile resembles ruminants' amino acid requirements (O'Connor et al., 1993). The efficiency with which dietary energy and protein are used to produce microbial protein in the rumen varies greatly. Microbial efficiency (**MNE**), defined as g of microbial N passing to the duodenum per kg true ruminally degraded OM (**TRDOM**), ranges from approximately 10 to

50 g/kg TRDOM (Clark et al., 1992). Microbial efficiency might be affected by carbohydrate availability, ruminal pH, passage rate, and the availability of ruminally degradable protein (Firkins, 1996). To the extent that the substitution of beet pulp for high-moisture corn affects these factors, it may affect MNE.

The availability of carbohydrates can be increased by increasing dietary concentrations of nonstructural carbohydrates, increasing the inherent digestibility of structural and nonstructural carbohydrates by plant genetics and preservation method, and reducing forage and grain particle size (within limits). However, the effect of diet fermentability on MNE is not consistent (Overton et al., 1995; Plascencia and Zinn, 1996; Crocker et al., 1998; Callison et al., 2001). Increasing diet fermentability can increase microbial protein production (Hoover and Stokes, 1991), but increasing fermentability can also lead to lower ruminal pH and to greater energy spilling by microbes (Strobel and Russell, 1986). Increasing rate of ruminal starch digestion by changing corn grain conservation method has decreased microbial efficiency (Oba and Allen, 2002b). Substituting beet pulp for highly fermentable corn grain in a high-grain diet might increase MNE if a similar digestibility of carbohydrates were maintained while slowing fermentation to a rate within the ability of microbes to utilize energy for protein synthesis and growth.

Increasing the rate of fermentation can also lead to lower ruminal pH, which has been implicated in the reduction of microbial efficiency, especially as a result of greater energy spilling (Strobel and Russell, 1986). However, most research linking low pH and reduced microbial efficiency has been conducted *in*

vitro (Firkins, 1996), and low pH has not always reduced MNE *in vitro* (Calsamiglia et al., 2002). Calsamiglia and coworkers (2002) also measured the effect of pH fluctuation on microbial efficiency. They reduced *in vitro* pH twice per day from 6.4 to 5.7, or cycled pH between 6.4 and 5.7 every four hours, to imitate fluctuations in pH that might result from meals consumed throughout the day or from a separate grain meal (Calsamiglia et al., 2002). Fluctuations in culture pH did not affect microbial efficiency.

Very little evidence exists that *in vivo* MNE is actually affected by ruminal pH, though it is possible that MNE is reduced at very low pH. Therefore, if beet pulp does increase daily average ruminal pH, that effect alone might not improve microbial efficiency. Only one experiment substituting beet pulp for corn in diets fed to dairy cattle measured ruminal pH; pH was unaffected by treatment, possibly because of the high dietary forage content (O'Mara et al., 1997). Microbial protein production and ruminal pH have not been measured while substituting beet pulp for corn grain.

Increased microbial efficiency has been demonstrated with increasing passage rate of digesta (Clark and Davis, 1983; Firkins, 1996; Oba and Allen, 2000; Oba and Allen, 2002b). This is probably the result of decreased lysis and protozoal predation (Wells and Russell, 1996). Digesta passage rate can be increased by greater DMI and decreased ambient temperature (Kennedy and Milligan, 1978); liquid dilution rate can be increased by greater saliva flow (Owens and Goetsch, 1986) and dietary mineral salts (Rogers et al., 1979). However, MNE is not consistently correlated with liquid passage rate, perhaps

because liquid passage rate is typically high in lactating dairy total; therefore, any increase in liquid passage rate might have little effect on overall microbial passage (Owens and Goetsch, 1986).

The effects of various non-forage fiber sources on passage rate have varied and depend on DMI, NFFS type, particle size, and marker system (Firkins, 1997). Substituting beet pulp for corn grain might decrease passage rate of solids if beet pulp contributes at all to rumen mat formation. Grain particles, beet pulp particles, and forage particles likely form pools with different digestion and passage kinetics within the rumen, so their relative responses to beet pulp and corn might differ. The separate passage rates of NDF and INDF from forage, beet pulp, and other concentrates cannot be accurately measured. If beet pulp increases the passage rate of one or more particulate fraction, MNE might be improved. Due to its high water-holding capacity, beet pulp might reduce liquid dilution rate; however, the potential for an effect on microbial efficiency is less likely because of the inconsistent relationship between liquid dilution rate and microbial efficiency (Owens and Goetsch, 1986).

The efficiency of microbial protein production is also limited by the availability of ammonia, amino acids, and peptides. Hoover (1986) summarized a number of studies which demonstrated that maximal fermentation or microbial production can occur at a wide range of ruminal ammonia concentrations (1 to 76 mg/dl). Therefore, factors other than ruminal ammonia concentration certainly affect microbial efficiency. A deficiency of amino acids or peptides can also reduce microbial efficiency (VanKessel and Russell, 1996); amylolytic

microorganisms are more dependent on amino acids and peptides (Russell et al., 1983), while fibrolytic bacteria are capable of using only ammonia N (Bryant, 1973). Therefore, substituting high-fiber beet pulp for high-starch corn might allow more extensive utilization of ammonia N and thus more efficient microbial efficiency. If, however, diets are formulated with sufficient protein and rumen-degraded protein, then amino acids and peptides probably would not limit growth of starch degraders, and N availability probably would not affect microbial efficiency differently when beet pulp was substituted in part for corn grain.

When beet pulp and corn grain were compared by *in vitro* fermentation, N metabolism responses were inconsistent (Mansfield et al., 1994; Chester-Jones et al., 1991; Bach et al., 1999). One experiment (Mansfield et al., 1994) reported that fluid ammonia concentration and effluent ammonia flow were greater on the corn diet. This implies that more protein was being degraded to ammonia on the corn diet than on the beet pulp diet, or that N was being integrated into bacterial protein more efficiently on the beet pulp diet. However, there was no effect of treatment on dietary CP degradation or on microbial N efficiency (Mansfield et al., 1994). Chester-Jones and coworkers (1991) and Bach and coworkers (1999) found that carbohydrate source had no effect on bacterial N flow or g bacterial N per kg truly digested DM.

When beet pulp was substituted for corn grain at 20% of diet DM for lactating cows, daily mean concentration of ammonia was not affected (O'Mara et al., 1997). Ruminal ammonia concentration is the combined result of ammonia production, ammonia absorption, and microbial ammonia utilization, so the failure

to detect differences in ammonia concentration does not rule out differences in the effect of N availability on microbial protein production. Microbial efficiency has not been compared for diets containing beet pulp or corn grain in lactating dairy cattle.

Effects of Beet Pulp on Milk Production

Three of the four experiments in which corn was replaced with beet pulp (Mansfield et al., 1994; Swain and Armentano, 1994; Clark and Armentano, 1997) reported no effect of treatment on milk yield (**MY**), and those that reported fat-corrected milk (**FCM**) yield found no effect of diet (Mansfield et al., 1994; Clark and Armentano, 1997). Among eleven other experiments in which beet pulp replaced corn under various feeding systems, only two reported increased MY or FCM yield for beet pulp diets; beet pulp decreased MY in one experiment and had no effect in others.

Only one experiment in which beet pulp was substituted for corn in a TMR (O'Mara et al., 1997) found a 1.4-kg/d decrease in raw milk yield when beet pulp was substituted for corn. This was despite a 1.5 kg/d increase in DMI. There were no effects of diet on whole-tract DM or OM digestibility. Ruminal fermentation of the added corn could lead to greater propionate absorption from the rumen. Propionate is a precursor to glucose, which is used to produce lactose, a primary determinant of milk volume (Linzell and Peaker, 1971). Propionate concentration in the rumen was not different between treatments (O'Mara et al., 1997), but quantities of propionate produced and absorbed could

have been greater on the corn diet if the rate of propionate absorption or passage also increased. Finally, the cows used in this experiment were producing only about 20 kg of milk per day (O'Mara et al., 1997). Individual production potential affects response to changes in diet (Voelker et al., 2002), so the effects of replacement of corn with beet pulp in these cows may be different than in high-producing cows.

Milk fat yield or content might often be expected to be greater when cows are fed diets with beet pulp substituted for corn, though multiple, and separate, mechanisms likely exist for the often-observed "milk fat depression." Some diet carbohydrate characteristics that are associated with milk fat depression are small forage particle size, insufficient fiber content, and high grain content (Emery, 1988). When Armentano and Pereira (1997) summarized several studies involving various NFFS, increasing dietary NDF concentration by adding NFFS increased milk fat content, although NFFS NDF contributed less to the prediction of milk percent than did forage NDF in a separate data set. However, the increase in milk fat percent with added NFFS might have been due to decreased milk yield and unchanged milk fat yield with added NFFS (Armentano and Pereira, 1997).

When substituting beet pulp for corn resulted in lower milk yield (O'Mara et al., 1997), cows fed beet pulp also produced 0.07 kg/d less milk fat, so the milk fat percent was not affected by diet. Therefore, the lower milk fat yield was the result of lower milk yield, not the result of greater fat content; nor was there evidence of "milk fat depression" for cows fed the corn treatment. Effects of diet

on milk fat in two other experiments were in the opposite direction. The studies of Swain and Armentano (1994) and Clark and Armentano (1997) were specifically designed to determine the effects of substituting beet pulp for corn grain on milk fat. Swain and Armentano (1994) reported 0.10 kg/d more milk fat and an increase of 0.32 percentage units for milk fat concentration in cows fed the beet pulp treatment compared to the corn treatment. Clark and Armentano (1997), feeding diets with slightly more forage and 11-13 percentage units less corn than Swain and Armentano (1994), reported no treatment effects on milk fat. Mansfield and coworkers (1994), feeding diets with more moderate amounts of corn, reported a 0.18 percentage-unit increase in milk fat content when beet pulp was substituted for half of the corn. Among the ten non-TMR studies that reported milk fat content, three measured greater milk fat content for beet pulp treatments, one for grain treatment, and six reported no effect. Therefore, the response of milk fat synthesis to substitution of beet pulp for corn grain is not consistent, even taking into account other diet characteristics.

Interestingly, milk protein yield or percent was affected by diet in three of the four TMR experiments, though the results vary by experiment. Swain and Armentano (1994), who reported no effect on milk yield and greater milk fat synthesis on the beet pulp diet, also reported no effect of diet on milk protein. O'Mara and coworkers (1997) measured 0.12 percentage units greater protein content in milk from cows fed the beet pulp diet. This may have been caused by differences in milk volume, since milk protein yield was similar and cows fed corn produced 1.4 kg/d more milk, possibly due to greater propionate availability and

lactose synthesis (Linzell and Peaker, 1971). A tendency ($P = 0.07$) for slightly greater milk protein content (0.06 percentage units) on the high-corn diet was the only milk production response reported by Clark and Armentano (1997) between the corn and whole beet pulp diets. Finally, Mansfield and coworkers (1994), who found no difference in milk yield and a greater milk fat content when beet pulp replaced half the corn, reported greater protein content (0.11 percentage units) and yield (0.05 kg/d) for the corn diet.

When higher milk protein concentration occurred in cows fed diets containing greater amounts of corn, the response may have been the result of increased insulin secretion (McGuire et al., 1995) due to greater ruminal propionate production and absorption with added dietary starch. A hyperinsulinemic-euglycemic clamp (McGuire et al., 1995) or simple insulin infusion (Molento et al., 2002) caused a modest increase in milk protein yield and content. This might be a direct effect of insulin on amino acid uptake and an effect of increased supply of amino acids from nonmammary tissues, and it might also be an indirect effect mediated through IGF-I (Mackle et al., 1999).

Diet differences across the experiments, such as forage concentration, type, and quality, and protein source, as well as differences in milk yield and stage of lactation, probably contributed to the variation in milk protein responses to treatments. However, it is clear from these experiments that substituting a highly digestible fiber source for a rapidly-fermentable starch source can affect milk protein content and yield.

Effects of Beet Pulp on Energy Partitioning

O'Mara et al. (1997) reported that blood glucose was not affected by substituting beet pulp for corn, which suggests that cows were in similar energy status across treatments, although DMI was numerically greater for BP. Authors did not discuss energy partitioning responses. However, cows fed the beet pulp diet had positive body weight change, while cows fed the corn diet had slightly negative body weight change. Body weight could have been affected by gut fill, or feeding beet pulp may have shifted the partitioning of energy away from milk production and into body reserves, since cows fed beet pulp also had lower average milk yield. Plasma insulin concentration was not measured. Plasma β -hydroxybutyrate was 10.5 mg/L lower on the beet pulp diet than on the corn diet, consistent with lower metabolism of adipose tissue for the beet pulp diet. However, plasma concentration of non-esterified fatty acids, which are created by adipose mobilization, was not affected by treatment. Regardless of the mechanism, replacing corn with beet pulp probably altered energy partitioning, reducing milk yield and possibly increasing body tissue gain. Again, it should be noted that milk yield for these cows averaged ~20 kg/d, so their response to added beet pulp might differ from the responses of high-producing cows.

Summary

Beet pulp in diets for dairy cattle can supply soluble and insoluble fiber that is fermented more rapidly than forage NDF but less rapidly than starch. Substituting beet pulp for corn grain in a high-concentrate diet can yield a slight

increase in intake, while substituting beet pulp for corn grain in a diet with a moderate proportion of concentrate often reduces intake. This is likely a result of greater filling effects of beet pulp for high-forage diets; for a low-forage diet with low rates of beet pulp inclusion, metabolic fuel absorption probably limits DMI more than ruminal distention. Metabolic fuel absorption would be determined by effects of beet pulp on site of starch digestion, and those effects are unknown.

Adding beet pulp to a diet with a low forage-to-concentrate ratio might stimulate additional chewing under some circumstances, but beet pulp is not a reliable source of “effective” fiber by the definition of increasing chewing time or milk fat synthesis. Very little is known about the effect of beet pulp on ruminal pH, especially *in vivo*, but removing starch and adding NDF and pectin might increase ruminal pH. Due to the rapid rate of digestion of beet pulp NDF, and perhaps due to effects on pH, beet pulp should improve total dietary NDF digestion when substituted for corn grain. Effects of beet pulp on microbial protein efficiency have not been studied in lactating cows; the response is not likely to depend on ruminal pH or N availability but would probably be affected by particle passage rate response and might be affected by liquid passage rate response.

Beet pulp substituted for grain has not had a consistent effect on milk yield; because it is highly dependent on DMI, milk yield might be increased with added beet pulp if the dilution of starch permitted greater intake. Adding beet pulp to high-grain diets has increased milk fat concentration on several occasions, but not in the majority of experiments comparing beet pulp and grain.

Finally, beet pulp may alter the partitioning of energy when substituted for grain, although that response has not been previously studied.

Further study of the effects of substituting beet pulp for grain would provide valuable information for using grain and a common non-forage fiber source to improve the efficiency of feed utilization in diets for dairy cattle. It would also provide a large pool of data, collected under identical conditions, which could help clarify basic principles of feed intake, milk production, digestion, and microbial efficiency in ruminants.

Table 1. Chemical composition of dried, unmolassed beet pulp.^a

Component	Mean	SD	N
DM, %	90.5	2.0	7
--- % of DM ---			
OM	93.4	2.9	9
Ash	6.0	2.4	8
ADF	23.6	3.8	9
NDF	46.0	9.4	10
CP	11.7	3.8	14
EE	1.5	1.2	9
Lignin	4.5	1.1	6

^a Sources: Bhattacharya and Sleiman, 1971; Welch and Smith, 1971; Mansfield et al., 1994; Ramanzin et al., 1994; Swain and Armentano, 1994; Torrent et al., 1994; Petit and Tremblay, 1995a; Petit and Tremblay, 1995b; Clark and Armentano, 1997; O'Mara et al., 1997; Witt et al., 1999; Bhatti and Firkins, 1995.

CHAPTER 2

Pelleted beet pulp substituted for high-moisture corn: Effects on feed intake, chewing behavior, and milk production of lactating dairy cows

ABSTRACT

The effects of increasing concentrations of dried, pelleted beet pulp substituted for high-moisture corn on intake, milk production, and chewing behavior were evaluated using 8 ruminally and duodenally cannulated multiparous Holstein cows in a duplicated 4 x 4 Latin square design with 21-d periods. Cows were 79 ± 17 (mean $\pm$ SD) DIM at the beginning of the experiment. Experimental diets with 40% forage (corn silage and alfalfa silage) and 60% concentrate contained 0%, 6.1%, 12.1%, or 24.3% beet pulp substituted for high-moisture corn on a DM basis. Diet concentrations of NDF and starch were 24.3% and 34.6% (0% beet pulp), 26.2% and 30.5% (6% beet pulp), 28.0% and 26.5% (12% beet pulp), and 31.6% and 18.4% (24% beet pulp), respectively. Increasing beet pulp in the diet caused a linear decrease in DMI ($P < 0.05$). Time spent eating per day ($P < 0.05$) and per kg DMI ($P < 0.01$) increased, and sorting against NDF tended to increase ($P = 0.07$), with added beet pulp. Substituting beet pulp for corn caused a quadratic response in milk fat yield ($P = 0.03$), with the highest yield for the 6% beet pulp treatment. A tendency was detected for a similar quadratic response in 3.5% FCM yield ($P =$

0.07). Greater plasma insulin concentration ($P < 0.01$) may have resulted in greater gain of body condition ($P = 0.06$) for cows fed diets with higher starch concentration. Partial substitution of pelleted beet pulp for high-moisture corn decreased intake but also may have permitted greater fat-corrected milk yield.

INTRODUCTION

Grain is often substituted for forage in diets for high-producing cows in an effort to increase intake and milk yield. Reducing dietary neutral detergent fiber (NDF) concentration by decreasing the forage to concentrate ratio usually increases dry matter intake (DMI), probably by reducing the filling effect of the diet (Allen, 2000). However, increasing dietary starch can also negatively affect feed intake and milk production. Feeding less forage NDF reduces chewing (Allen, 1997), which can reduce flow of saliva and its buffers into the rumen. This could possibly reduce ruminal pH, fiber digestion and milk fat concentration. Propionate production increases with greater dietary starch content and probably depresses dry matter intake (Anil and Forbes, 1980), which can limit milk synthesis.

Adding non-forage NDF to low-forage diets might reduce the negative effects of increased starch fermentation without increasing the filling effect of the diet to the same extent as forage NDF. The responses of DMI to various non-forage fiber sources substituted for grain are not consistent (Allen, 2000). Beet pulp contains approximately 40% NDF and is unique in its high concentration of

neutral-detergent soluble fiber, especially pectic substances. Previous experiments have reported a variety of responses in DMI to the substitution of dried beet pulp for corn grain. Of four experiments which substituted beet pulp for grain in a total-mixed ration, two reported increased DMI (Clark and Armentano, 1997; O'Mara et al., 1997), one reported decreased DMI (Mansfield et al., 1994), and one measured no effect of beet pulp on DMI (Swain and Armentano, 1994). Among the same experiments, three reported no effects of treatment on milk yield (Mansfield et al., 1994; Swain and Armentano, 1994; Clark and Armentano, 1997), and one reported decreased milk yield when beet pulp was substituted for corn grain (O'Mara et al., 1997). Fat-corrected milk yield, when reported, was not affected by treatment. In these experiments, beet pulp comprised at least 15% of diet DM. However, the effects of rate of substitution and the mechanisms of intake regulation for diets containing beet pulp have not previously been investigated.

Substituting beet pulp for high-moisture corn in a diet with a low forage content should maximize dry matter intake and 3.5% FCM yield at one or more rates of inclusion. Therefore, this experiment investigated the responses to four concentrations of beet pulp substituted for high-moisture corn (0, 6, 12, and 24% of diet DM) for feed intake, meal patterns, chewing behavior, ruminal nutrient pools, and milk yield and composition.

MATERIALS AND METHODS

Cows and Treatments

Eight multiparous Holstein cows (79 ± 17 DIM; mean $\pm$ SD) from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to a duplicated 4 x 4 Latin square balanced for carryover effects in a dose-response arrangement of treatments. Treatments were diets containing dried, pelleted beet pulp (**BP**) at 0%, 6%, 12%, and 24% substituted for high-moisture corn (**HMC**) on a DM basis. Treatment periods were 21 d with the final 10 d used to collect samples and data. Cows were cannulated ruminally and duodenally prior to calving and assigned randomly to treatment sequence. Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. At the beginning of the experiment, empty body weight (ruminal digesta removed) of cows was 515.9 ± 63.5 kg (mean $\pm$ SD).

Nutrient composition for HMC and BP are shown in Table 1. Experimental diets contained 40% forage (50:50 corn silage: alfalfa silage), high-moisture corn, beet pulp at 0% (**0BP**), 6% (**6BP**), 12% (**12BP**), and 24% (**24BP**) of diet DM, a premixed protein supplement (soybean meal, corn distiller's grains, and blood meal), and a mineral and vitamin mix (Table 2). All diets were formulated for 18% dietary CP and fed as total mixed rations.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed once daily (1100 h) at 110% of expected intake. Amounts of feed offered and orts were weighed for each cow daily during the collection period. Samples of all diet ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily on d 12-19 and combined into one sample per period. Cows were milked twice daily in their stalls during the feeding behavior monitoring phase (d 16-19) and in a milking parlor during the rest of each period. Milk yield was measured, and milk was sampled, at each milking on d 16-19. Empty body weight was measured after evacuation of ruminal digesta on two consecutive days immediately prior to the start of the first period, and on d 20 and d 21 of each period. Body condition score (**BCS**) was determined immediately prior to the start of the first period and on d 21 of each period by three trained investigators blinded to treatments (Wildman et al., 1982; five-point scale where 1 = thin and 5 = fat).

Feeding behavior was monitored from d 16 through d 19 (96 h) of each period by a computerized data acquisition system (Dado and Allen, 1993). Data of chewing activities, feed disappearance, and water consumption were recorded for each cow every 5 sec. When chewing equipment malfunctioned for an individual cow during a 24-h period (1100h to 1100h), chewing behavior was deleted for that cow during that 24-h period. The system successfully collected 83.1% of the total chewing behavior data (average 3.4 d per cow per period). Daily means were calculated for number of meal bouts per day, interval between meals, meal size, eating time, ruminating time, and total chewing time. These

response variables were calculated as daily means then averaged over the four days for each period.

Blood was collected from the coccygeal vein into tubes containing sodium heparin every 9 hours from d 12 - 14, starting at 1400 h on d 12, so that samples represented 3-h intervals of a 24-hour period in order to account for diurnal variation. Blood was centrifuged at 2,000 x g for 15 min immediately after sample collection, and plasma was harvested and frozen at -20°C until analysis.

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

Sample and Statistical Analysis

Diet ingredients and orts were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1mm screen; Authur H. Thomas, Philadelphia, PA). Rumen liquid and solid sub-samples were lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY), ground, and recombined according to the original ratio of solid and liquid DM.

Samples were analyzed for ash, NDF, indigestible NDF (**INDF**), CP, and starch. Ash concentration was determined after 5 h oxidation at 500° C in a muffle furnace. Concentrations of NDF were determined according to Van Soest et al. (1991, method A). Indigestible NDF was estimated as NDF residue after 120-h in vitro fermentation (Goering and Van Soest, 1970). Rumen fluid for the in vitro incubations was collected from a non-pregnant dry cow fed alfalfa hay only. Fraction of potentially digestible NDF (pdNDF) was calculated by difference (1.00 – INDF). Forage samples were analyzed for ADF and sulfuric acid lignin content (Van Soest et al., 1991). Crude protein was analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Glucose concentration was measured using a glucose oxidase method (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), and absorbance was determined with a micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Concentrations of all nutrients except DM were expressed as percentages of DM determined by drying at 105° C in a forced-air oven for more than 8 h.

A commercial kit was used to determine plasma concentration of insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Milk samples were analyzed for fat, true protein, and lactose with infrared spectroscopy by Michigan DHIA (East Lansing).

Energy values were calculated as follows:

$$NE_L \text{ intake} = \text{DMI (kg)} \times (0.0245 \times \text{TDN}(\%)) \text{ (NRC, 1989)}$$

(DM digestibility for calculation of TDN was measured as reported in Chapter 3.)

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

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

NE_M = 0.080 x BW^{0.75} (NRC, 2001); and

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

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

Hunger and satiety ratios were calculated as follows (Forbes, 1995):

Hunger ratio = meal kg DM/ premeal interval

Satiety ratio = meal kg DM/ postmeal interval.

Ratios were calculated for individual meals and averaged for the four days of feeding behavior data collection.

All data were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

where

μ = overall mean,

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

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

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

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

Period by treatment interaction was originally evaluated, but it was removed from the statistical model because it was not significant for response variables of primary interest. Linear and quadratic dose-response effects were evaluated using the same model with diet percent beet pulp (1, 6, 12, and 24%) in place of the fixed effect of treatment. Pearson correlation coefficients were determined between cow-period observations for some parameters. Treatment effects, linear and quadratic responses, and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$.

Data from two cow-periods were excluded from statistical analysis. One cow developed a cecal torsion requiring surgery early in period 3; data and samples were not collected from this cow during period 3, but she recovered sufficiently for her period 4 data to be used. Period 4 data from a second cow were excluded after she showed signs of estrus because her intake and milk yield were outliers (outside the 95% confidence interval for cow-period observations).

RESULTS AND DISCUSSION

Feed Intake

As beet pulp was increasingly substituted for high-moisture corn, dry matter intake decreased ($P < 0.05$; Table 3). Although the relationship detected was linear, DMI was similar for 0BP, 6BP and 12BP, and DMI was numerically lower by approximately 2 kg/d for 24BP. Others have reported varied responses

in DMI to substitution of beet pulp for corn grain. Swain and Armentano (1994) reported no DMI effect. O'Mara and coworkers (1997) did not statistically evaluate DMI but reported a numerical increase in DMI for beet pulp over corn in a high-forage diet. Substituting beet pulp at 16% of DM into a high-corn diet (Clark and Armentano, 1997) increased intake by only 0.4 kg/d, while replacing half of the corn with beet pulp in a higher-forage diet (Mansfield et al., 1994) reduced intake by 1.3 kg/d. The diets fed in the latter experiment are the most similar to diets in the present study and had a DMI response similar to the numerical difference between 24BP and the other three treatments.

Regulation of Feed Intake

Intake can be affected by innumerable variables, such as ruminal fill, meal patterns, metabolic fuels, and ruminal patterns of fermentation and pH. Substituting a high-fiber byproduct for grain may have resulted in the limitation of intake by physical filling effects of the diet, but "fill" is likely a composite of several physical and chemical characteristics of the diet that affect volume and mass of digesta over time (Forbes, 1995). Because volume and mass of wet ruminal contents did not decrease when DMI decreased with increasing dietary BP (Table 3), stimulation of stretch receptors in the rumen wall probably was a predominant factor signaling satiety and reducing DMI with added BP. Water content of ruminal digesta increased linearly as BP was substituted for corn ($P = 0.01$), probably because beet pulp has a higher water-holding capacity than grains and other byproducts, and similar to alfalfa and grass (Ramanzin et al.,

1994; Bailoni et al., 1998). The substitution of beet pulp for high-moisture corn probably increased the water-holding capacity of the digesta and contributed to the tendency for increased digesta wet weight ($P = 0.11$). Neither ruminal DM pool nor ruminal NDF pool was limiting to intake, because DM pool decreased linearly ($P = 0.04$), and NDF pool tended to decrease linearly ($P = 0.11$), as added BP reduced DMI. Therefore, ruminal digesta volume, weight, and water-holding capacity contributed to the limitation of intake by distension when high-moisture corn was replaced with beet pulp.

Feed intake can be increased by reducing intermeal interval or by increasing meal size. Intermeal interval was not affected by treatment, nor was the amount of DM consumed per meal (Table 4). However, the response of hunger ratio (meal size/premeal interval) to beet pulp concentration was quadratic ($P < 0.01$), and was greatest for 12BP and lowest for 24BP, suggesting that hunger had the greatest effect on intake for 12BP and the least effect for 24BP. The quadratic response suggests that hunger eventually was counteracted by other factors limiting intake; cows might eat to minimize total discomfort resulting from both hunger and satiety signals (Forbes, 2000). Satiety ratio was unaffected by treatment ($P > 0.15$), but since hunger and satiety signals are integrated to regulate feed intake (Forbes, 1995), response of hunger ratio or satiety ratio alone cannot describe the mechanisms of intake regulation.

Across cow-period observations, DMI was not related to the number of meals per day, meal length, intermeal interval, or NDF consumed per meal (Table 7); but as meal size increased, DMI also increased (Figure 1a; $R = 0.39$, P

= 0.04). Therefore, the ability to consume a larger amount of feed at one meal, before satiety signals such as absorption of metabolic fuels or ruminal fill overcame hunger, increased the amount of feed consumed in the entire day. Average consumption of NDF per meal increased ($P < 0.001$) with added beet pulp (Table 4), so increasing NDF consumption per meal may have slowed feed intake. However, as will be discussed further, eating rate and time probably did not actually limit daily DMI. While dried, pelleted beet pulp might be expected to physically affect eating behavior, eating chew rate was unaffected by treatment (Table 4).

Eating time per day and per kg DMI increased linearly with added BP ($P = 0.04$, $P < 0.01$, respectively), so rate of feed intake was slowed (Table 5). Others have reported no effect of beet pulp substitution for corn on time eating per day or per kg DMI (Clark and Armentano, 1997). Across cow-period observations, greater time spent in total chewing per kg DMI was associated with decreased DMI (Table 7). As diet NDF intake increased with added BP in the present experiment, time eating per kg NDF decreased ($P < 0.01$). Since forage content was equal across treatments, physical or chemical effects of the fiber in beet pulp apparently had a negative effect on rate of feed intake.

Due to the physical form of pelleted beet pulp, it is easily sorted. While sorting against beet pulp was not measured, cows tended to increasingly sort against dietary NDF ($P = 0.07$) as the concentration of BP increased in the diet (Table 5). In addition, sorting behavior (orts %NDF – feed %NDF) displayed a quadratic relationship to FCM yield (Figure 2; $R = 0.51$, $P < 0.05$), with the

greatest sorting occurring at ~40 kg/d FCM, slightly above the median of 37 kg/d. Therefore, sorting against NDF may have slowed intake; and while sorting behavior increased with production up to 40 kg/d, the highest milk yield was not associated with the highest degree of sorting.

Although replacing high-moisture corn with beet pulp tended to increase sorting and slowed rate of feed intake, time spent eating and in total chewing behavior did not limit DMI. If eating time or total chewing time were limiting to DMI, then those responses should demonstrate a quadratic increase as DMI was reduced with increasing dietary beet pulp concentration, eventually reaching a plateau. However, no quadratic responses were detected for time spent eating, ruminating, or in total chewing (Table 5), and time spent eating or in total chewing was not related to DMI across cow-period observations (Table 7). Therefore, eating and chewing time were not primary factors limiting feed intake.

Intake might be regulated by the metabolism of propionate in the liver (Allen, 2000), and ruminal propionate production is usually increased by greater starch fermentation. The amount of starch truly digested in the rumen decreased from 3.8 kg/d to 0.7 kg/d (Chapter 3), and ruminal molar percent of propionate in total VFA decreased quadratically (Chapter 4) as starch intake was reduced by approximately half (Table 3) and as true ruminal starch digestibility decreased from 46.5% to only 16.9% (Chapter 3) with added BP. Therefore, it is not likely that propionate metabolism in the liver caused the reduction in DMI with added BP, because propionate metabolism probably decreased with added BP. In addition, across cow-period observations, DMI was not related to total valerate

concentration, molar percent of propionate in total VFA, or acetate:propionate (Table 8), and intake tended to be positively correlated with VFA absorption rate ($R = 0.31$, $P < 0.10$). Therefore, ruminal VFA concentration and absorption rate did not negatively affect intake.

Ruminal pH might be implicated in the control of feed intake, particularly in satiety. Ruminal pH was measured over 96 h and is reported in Chapter 4. Substituting BP for HMC decreased DMI and resulted in a range of individual mean daily pH values from 5.58 to 6.56, but pH was not related to treatment (Chapter 4), and mean pH and DMI were not correlated (Figure 1b). Intake was not correlated with daily range, maximum, or minimum pH, but DMI was negatively related to daily standard deviation (Figure 1c; $R = -0.41$, $P = 0.03$) and variance ($R = -0.42$, $P = 0.03$) of ruminal pH. The direction of cause and effect is not established by these results. Increased intake might be associated with reduced pH variation if cows consumed more frequent, smaller meals and thus increased intake while reducing ruminal pH variation. However, increased DMI was associated with greater meal size, not smaller meal size (Figure 1a), and DMI was not related to intermeal interval, so it is unlikely that the consumption of more frequent, smaller meals resulted in increased DMI and decreased ruminal pH variation. Rather, it is more likely that increased pH fluctuations resulted in feed intake depression. Increases in both DMI and daily pH range were associated with the higher-starch diets (Table 3; and in Chapter 4), so the negative correlation between DMI and ruminal pH variation is independent of the treatment effects.

The limitation of intake by ruminal distention was closely related to effects detected among treatment means as beet pulp was substituted for high-moisture corn. By contrast, the relationships between pH variation or VFA absorption and DMI occurred independently from treatment effects for the individual variables.

Milk Production and Nutrient Partitioning

Milk yield was not affected by diet ($P > 0.50$; Table 9), but increasing BP tended to have a quadratic effect ($P = 0.07$) on 3.5% FCM yield and had a quadratic effect on milk fat yield ($P = 0.03$). Yields of FCM and milk fat were highest for 6BP and lowest for 24BP. These effects correspond with non-significant quadratic patterns of raw milk yield and milk fat content in response to increasing BP and decreasing HMC, and they may reflect a slight response in milk lactose and fat synthesis to added beet pulp at the lower concentrations. Lower milk yield for 24BP is likely because of decreased DMI. Three of four other experiments comparing corn grain with beet pulp in TMRs reported no effect of treatment on raw milk yield (Mansfield et al., 1994; Swain and Armentano, 1994; Clark and Armentano, 1997), and those that reported fat-corrected milk yield found no effect of diet (Mansfield et al., 1994; Clark and Armentano, 1997). Only one experiment (O'Mara et al., 1997) found a 1.4-kg/d decrease in raw milk yield when beet pulp was substituted for corn in a grass-silage-based diet. Milk protein and lactose yields and concentrations were not affected in the present experiment by substituting beet pulp for corn in the present experiment (Table 9). The reduction in DMI with increasing BP was

greater than the decrease in milk yield, so the efficiency of milk production (kg FCM/kg DMI) tended to increase ($P = 0.07$) as HMC decreased and BP increased in the diet.

Beginning with a diet containing 36% HMC and 35% starch, then substituting BP containing 40% NDF for the HMC not only served to test the treatment effects of two common feed ingredients; it also provided a large pool of data including variables such as intake, production, energy status, and feeding behavior, with reasonably wide ranges, under identical conditions. Relationships among these variables across cow-period means help both to interpret treatment effects and to clarify basic scientific principles of intake, milk production, and feeding behavior in ruminants. Among cow-period observations, increasing ruminal pH (Chapter 4) was associated with greater yield of FCM ($R = 0.43$, $P = 0.03$) and tended to be associated with greater fat yield, but pH did not affect milk fat content (Figure 3).

Although intake and milk production were influenced by diet, measures of energy intake, energy partitioning to milk and output in milk, and energy balance were not affected (Table 10); nor were changes in body weight different among treatments ($P > 0.45$). While partitioning of absorbed energy to milk (milk NE_L as a percent of NE_L intake) was not affected by adding beet pulp, a tendency ($P = 0.06$) was detected for a slightly more negative change in body condition score; as BP replaced corn in the diets, cows likely mobilized nutrients from body reserves for milk production, rather than adding condition as they did for 0BP and 6BP.

Plasma Insulin

Plasma insulin responds to energy intake, especially glucose and its precursors, and it both regulates and is regulated by energy utilization in various tissues, including liver, muscle, adipose, and mammary tissue. As cows consumed more BP and less HMC, both plasma insulin concentration and the standard deviation of insulin concentration over 24 h decreased (Table 10). Differences were detected between treatments for both mean ($P < 0.01$) and standard deviation ($P = 0.02$), and the effects were linear and negative as BP increased ($P < 0.001$, $P < 0.01$, respectively). Plasma insulin was not related to change in BCS or to percent of NE_L intake partitioned to milk (Table 11).

Insulin secretion can be affected by intake, ruminal fermentation products, and absorption of metabolites in the whole tract. Because both DMI and plasma insulin decreased with added BP, lower feed intake might have resulted in lower insulin, although NE_L intake was not affected by treatment (Table 10). Insulin concentration and DMI were correlated across treatments as well (Table 11; $R = 0.41$, $P = 0.03$). Starch consumed per meal decreased (Table 3) as BP increased, so a smaller amount of starch from high-moisture corn was undergoing rapid fermentation during and immediately after each meal. This could have resulted in lower plasma insulin concentrations and less fluctuation in insulin concentrations (Table 10), with the resulting decrease in energy partitioned to adipose tissue indicated by the tendency for decreased BCS.

Site of starch digestion can affect insulin secretion by altering the form of absorbed fuels. In ruminants, propionate reaching the liver stimulates insulin secretion (Reynolds, 1995). As will be reported in Chapter 4, decreasing the amount of starch in the diet resulted in decreased propionate concentration and increased acetate:propionate in rumen fluid. Thus, lower insulin secretion for the higher beet pulp diets could be a result of lower propionate production. However, insulin concentration was not correlated with rumen fluid propionate concentration or molar percent of total VFA (data not shown). Although absolute rates of production and removal were not measured, the amount and proportion of starch truly digested in the rumen was not related to insulin concentration, either (data not shown), so the insulin response probably cannot be explained by increased propionate absorption alone.

Insulin secretion is also stimulated by increased plasma glucose concentration; although there is little net glucose absorption across the portal-drained viscera in cattle (Reynolds, 1995), plasma glucose concentration may be increased by greater gluconeogenesis, by increased glucose absorption and utilization in small intestine cells, and by greater availability of glucose-sparing fuels such as acetate, β -hydroxybutyrate, and lactate. Most of the glucose absorbed in the ruminant small intestine probably is either utilized within intestinal cells, thus sparing circulating glucose, or absorbed into blood as lactate, which the liver can convert to glucose. Although plasma insulin concentration decreased with added BP, amount of starch digested postruminally (kg/d) was not different among treatments (Chapter 3); and among cow-period

observations, amount or proportion of starch digested postruminally was not related to insulin concentration, so intestinal glucose or lactate absorption alone probably did not contribute to the insulin effect. Only the amount of starch consumed or digested in the whole tract was related to plasma insulin ($R = 0.51$, $P < 0.01$; and $R = 0.52$, $P < 0.01$, respectively), and adding ruminal digestibility of starch to the model did not improve the prediction of insulin concentration from starch intake. Therefore, although replacing HMC with BP shifted starch digestion from the rumen to the intestines, the reduced plasma insulin concentration with added BP probably was caused by the reduction in the total amount of starch consumed and digested, regardless of the form in which it was absorbed.

SUMMARY

Substitution of beet pulp for high-moisture corn resulted in decreased DMI, and ruminal distention probably limited DMI with added beet pulp. Mean and minimum ruminal pH did not affect DMI, but, independent of treatment effects, increased variation in pH might have decreased DMI. Replacing high-moisture corn with beet pulp caused a quadratic response for milk fat yield and the tendency for a quadratic response for FCM yield. Partial substitution of pelleted beet pulp for high-moisture corn decreased intake due to filling effects but also permitted similar or greater fat-corrected milk yield.

Table 1. Nutrient composition of high-moisture corn and dried, pelleted beet pulp.

Nutrient	High-moisture corn	Beet pulp
DM, % as fed	71.5	84.9
	-----% of DM-----	
NDF	10.0	39.9
CP	8.3	8.9
Indigestible NDF	3.8	8.0
Starch	70.5	3.9
Ether extract	4.7	0.7
Ash	1.0	7.8

Table 2. Ingredient and nutrient composition of experimental diets.

	0% BP	6% BP	12% BP	24% BP
Ingredients	-----% of DM-----			
Corn silage	20.1	20.1	20.1	20.1
Alfalfa silage	19.9	19.9	19.9	19.9
Protein mix	19.5	19.5	19.5	19.5
Mineral vitamin mix	4.8	4.8	4.8	4.8
Dried, pelleted beet pulp	0	6.1	12.1	24.3
High-moisture corn	35.6	29.5	23.5	11.4
Nutrient				
DM	50.2	50.5	50.8	51.6
Starch	34.6	30.5	26.5	18.4
NDF	24.3	26.2	28.0	31.6
Indigestible NDF	9.4	9.6	9.8	10.2
Forage NDF	17.1	17.1	17.1	17.1
CP	18.0	18.0	18.0	18.1
% Starch from high-moisture corn	72.7	68.3	62.6	43.7
% NDF from forage	70.2	65.3	61.1	54.1
% NDF from beet pulp	0.0	9.3	17.3	30.7

Table 3. Effects of substitution of pelleted beet pulp for high-moisture corn on nutrient intake and ruminal pool.

	Treatment LS Means					<i>P</i>		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
Intake, kg/d								
DM	23.7	23.8	23.9	21.8	0.7	0.11	<0.05	0.15
OM	22.3	22.4	22.4	20.2	0.7	0.06	0.50	0.15
NDF	5.7	6.2	6.6	6.9	0.2	<0.01	0.01	0.15
Starch	8.2	7.3	6.4	4.0	0.2	<0.01	<0.01	0.21
Ruminal wet contents, kg	77.7	76.7	78.9	80.7	2.2	0.36	0.11	0.58
Ruminal contents volume, L	96.1	92.8	95.5	96.0	3.0	0.64	0.49	0.43
Ruminal contents, %DM	14.2	13.9	13.4	13.2	0.4	0.12	0.01	0.57
Ruminal pool, kg								
DM	11.0	10.4	10.6	9.9	0.5	0.19	0.04	0.74
OM	10.1	9.5	9.7	9.0	0.5	0.14	0.03	0.85
NDF	5.5	5.0	5.2	4.9	0.3	0.19	0.11	0.62
Potentially digestible NDF	2.4	2.1	2.3	2.1	0.2	0.30	0.17	0.61
Indigestible NDF	3.1	2.9	2.8	2.8	0.2	0.42	0.13	0.21
Starch	1.24	1.03	0.87	0.63	0.08	<0.01	<0.01	0.36

Table 4. Effects of substitution of pelleted beet pulp for high-moisture corn on meal patterns and water consumption.

	Treatment LS Means					P		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
Meals/d	9.7	9.5	9.3	9.0	0.4	0.55	0.13	0.93
Meal length, min/meal	37.9	36.6	37.6	40.3	2.9	0.66	0.64	0.45
Meal size, kg								
DM	2.5	2.3	2.4	2.5	0.1	0.70	0.44	0.35
NDF	0.61	0.61	0.68	0.79	0.04	<0.01	<0.01	0.38
INDF	0.26	0.26	0.27	0.29	0.02	0.22	0.06	0.38
pdNDF	0.34	0.36	0.41	0.50	0.02	<0.01	<0.01	0.39
Starch	0.87	0.72	0.63	0.47	0.04	<0.01	<0.01	0.25
Intermeal interval, min	100	100	103	106	4	0.56	0.16	0.94
Eating chew rate, chews/min	73.4	73.5	74.6	74.7	2.8	0.65	0.63	0.86
Hunger ratio ¹ , kg/min	0.16	0.19	0.20	0.14	0.02	0.03	0.01	<0.01
Satiety ratio ¹ , kg/min	0.27	0.21	0.25	0.23	0.04	0.17	0.22	0.30
Ruminating bouts /d	12.6	12.6	12.6	12.6	0.5	1.00	1.00	0.97
Ruminating bout length, min	36.6	37.6	38.9	38.6	2.4	0.66	0.33	0.48
Interval between ruminating bouts, min	70.4	71.2	67.6	67.5	2.7	0.45	0.18	0.95
Ruminating chew rate, chews/min	60.9	62.3	62.7	60.9	1.9	0.39	0.11	0.09
Drinking time, min/d	17	17	18	17	2	0.72	0.30	0.26
Water drunk, L/d	91	98	92	90	4	0.19	0.52	0.32
Drinking bouts /d	11	12	11	11	1	0.14	0.35	0.24

¹ Hunger ratio = meal kg DM/ premeal interval;
Satiety ratio = meal kg DM/ postmeal interval (Forbes, 1995).

Table 5. Effects of substitution of pelleted beet pulp for high-moisture corn on chewing time.

	Treatment LS Means					P		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
Eating time, min								
/d	298	297	302	318	13	0.21	0.04	0.44
/bout	34.0	33.0	33.7	36.6	2.7	0.65	0.65	0.45
/kg of DMI	12.6	12.9	12.9	14.2	0.6	0.05	<0.01	0.38
/kg of NDF intake	52.1	49.6	47.1	46.4	2.4	0.04	<0.01	0.27
/kg of pdNDF intake	93.1	83.6	78.8	74.0	4.0	<0.01	<0.01	0.07
/kg of forage NDF intake	74.1	73.2	74.7	83.1	3.0	0.02	<0.01	0.11
Feed NDF% - orts NDF%	1.0	1.6	3.8	2.2	2.0	0.15	0.07	0.12
Ruminating time, min								
/d	472	467	483	499	24	0.61	0.15	0.73
/bout	35.9	37.1	38.4	38.1	2.5	0.65	0.31	0.45
/kg of DMI	21.0	19.6	20.3	22.4	1.0	0.05	0.07	0.02
/kg of NDF intake	82.7	75.8	73.6	74.6	4.6	0.18	0.05	0.12
/kg of pdNDF intake	148	130	124	119	8	0.01	0.01	0.10
/kg of forage NDF intake	123	115	119	132	6	0.04	0.07	0.02
/kg ruminal DM pool	44.4	46.0	46.8	51.3	3.4	0.31	0.05	0.78
/kg ruminal NDF pool	89.2	98.0	93.3	104	8	0.28	0.13	0.94
Total chewing time, min								
/d	784	792	805	833	25	0.50	0.12	0.89
/bout	35.1	35.3	36.0	38.0	1.8	0.64	0.06	0.77
/kg of DMI	33.6	32.5	34.0	37.4	1.1	0.01	0.29	0.04
/kg of NDF intake	135	125	123	124	6	0.22	0.07	0.15
/kg of pdNDF intake	242	211	206	196	10	<0.01	<0.01	0.05
/kg of forage NDF intake	192	190	199	220	8	0.07	0.01	0.22
/kg ruminal DM pool	73.5	74.5	77.6	83.0	4.7	0.30	0.04	0.78
/kg ruminal NDF pool	148	159	154	168	11	0.36	0.12	0.87

Table 6. Effects of substitution of pelleted beet pulp for high-moisture corn on chewing activity.

	Treatment LS Means					<i>P</i>		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
Eating chews								
/d	22344	23068	23156	24054	1607	0.40	0.09	0.85
/bout	2543	2510	2556	2783	248	0.68	0.81	0.58
/kg of DMI	945	964	980	1118	63	0.01	<0.01	0.29
/kg of NDF intake	3899	3714	3553	3544	232	0.12	0.03	0.26
/kg of pdNDF intake	6962	6395	5992	5627	397	<0.01	<0.01	0.19
/kg of forage NDF intake	5544	5645	5710	6357	348	0.03	<0.01	0.34
Ruminating chews								
/d	28177	28190	28425	31144	1548	0.44	0.13	0.44
/bout	2242	2331	2420	2387	210	0.75	0.28	0.30
/kg of DMI	1225	1188	1200	1313	86	0.61	0.44	0.26
/kg of NDF intake	5053	4606	4418	4731	309	0.30	0.06	0.08
/kg of pdNDF intake	8291	7382	6892	7241	464	0.08	0.02	0.07
/kg of forage NDF intake	9035	7913	7448	7512	534	0.06	0.06	0.03
/kg ruminal DM pool	2627	2765	2757	3156	245	0.34	0.07	0.69
/kg ruminal NDF pool	5456	6141	5644	6431	591	0.37	0.69	0.96
Total chews								
/d	50695	50426	49642	57011	2389	0.19	0.05	0.27
/kg of DMI	2155	2132	2200	2712	136	0.01	0.49	0.08
/kg of NDF intake	8934	8207	8033	8558	481	0.31	0.07	0.08
/kg of pdNDF intake	15985	14124	13592	13548	837	0.05	0.02	0.07
/kg of forage NDF intake	12559	12412	12820	15442	776	0.03	0.53	0.10
/kg ruminal DM pool	4720	4869	5039	5677	410	0.18	0.02	0.68
/kg ruminal NDF pool	9752	10766	10423	11457	950	0.31	0.09	0.72

Table 7. Pearson correlation coefficients between DMI and eating or chewing behavior.

	DMI, kg/d
Meals/d	0.07
Meal length, min	0.09
Intermeal interval, min	-0.31
Meal DM, kg/meal	0.39 ¹
Meal NDF, kg/meal	-0.01
Hunger ratio ² , kg/min	0.36
Time eating, min/d	0.20
Time eating /kg DMI	-0.19
Time eating /kg forage NDF intake	-0.39 ¹
Total chewing time (TCT), min/d	-0.01
TCT / kg DMI	-0.58 ¹
TCT / kg forage NDF intake	-0.70 ¹

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

² Calculated for each meal: kg DMI / premeal interval (Forbes, 1995).

Table 8. Pearson correlation coefficients between DMI and characteristics of ruminal fermentation and pH.

	DMI, kg/d
Total ruminal [VFA], mM	-0.23
% propionate, mol / 100mol VFA	-0.22
Acetate:Propionate	0.18
Valerate absorption rate, %/h	0.31 ²
Ruminal pH mean	0.06
Ruminal pH standard deviation	-0.41 ¹
Ruminal pH minimum	0.27
Ruminal pH range	-0.30
Ruminal pH variance	0.42 ¹

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

² Tendency for significant correlation ($P < 0.10$).

Table 9. Effects of substitution of pelleted beet pulp for high-moisture corn on milk production.

	Treatment LS Means				SE	Trt	<i>P</i>	
	0% BP	6% BP	12% BP	24% BP			L	Q
Yield, kg/d								
Milk	36.4	36.6	35.9	35.4	1.2	0.58	0.95	0.74
3.5% FCM ¹	37.4	38.4	38.0	36.8	1.2	0.20	0.14	0.07
Milk fat	1.34	1.40	1.39	1.33	0.07	0.14	0.04	0.03
Milk protein	1.13	1.15	1.15	1.09	0.03	0.18	0.29	0.11
Milk lactose	1.80	1.82	1.78	1.75	0.07	0.57	0.99	0.71
Milk composition, %								
Fat	3.72	3.84	3.90	3.81	0.22	0.46	0.12	0.16
Protein	3.21	3.21	3.22	3.10	0.11	0.41	0.59	0.32
Lactose	4.95	4.96	4.95	4.94	0.02	0.75	0.66	0.53
kg 3.5%FCM/kg DMI	1.59	1.62	1.60	1.70	0.05	0.26	0.07	0.52

¹ 3.5% fat-corrected milk.

Table 10. Effects of substitution of pelleted beet pulp for high-moisture corn on energy balance and plasma insulin.

	Treatment LS Means					<i>P</i>		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
NE _L intake ¹ , Mcal/d	38.9	38.2	40.0	36.5	1.2	0.21	0.43	0.24
Milk NE _L ² , Mcal/kg	0.72	0.73	0.74	0.72	0.02	0.50	0.13	0.13
Milk NE _L , %NE _L intake	67.4	69.0	66.5	70.7	1.8	0.27	0.67	0.42
BW change, kg /21d	-8.0	6.7	-11.6	-1.5	15.8	0.49	0.50	0.45
BCS change, /21d	0.06	0.11	-0.04	-0.05	0.06	0.15	0.06	0.93
NE _L balance ³ , Mcal/d	3.2	1.8	4.1	1.1	1.1	0.16	0.59	0.39
NE _{Maint} , %NE _L intake	24.0	25.1	23.6	26.2	0.8	0.03	0.06	0.30
Mean plasma insulin ⁴ , μIU/ml	13.0	13.2	11.6	8.9	1.2	<0.01	<0.01	0.26
Plasma insulin standard deviation ⁴ , μIU/ml	4.9	4.7	3.8	2.9	0.5	0.02	<0.01	0.96

¹ NE_{L(intake)} = DMI (kg) x (0.0245 x TDN(%)) (NRC 1989).

² NE_{L(milk)} (Mcal/d) = MY (kg) x (0.0929 x fat% + 0.0563 x true protein% + 0.0395 x lactose%) (NRC, 2001).

³ NE_{L(intake)} – NE_{L(maintenance)} – NE_{L(milk)}, where NE_{L(maintenance)} = 0.080 x BW^{0.75} (NRC 2001).

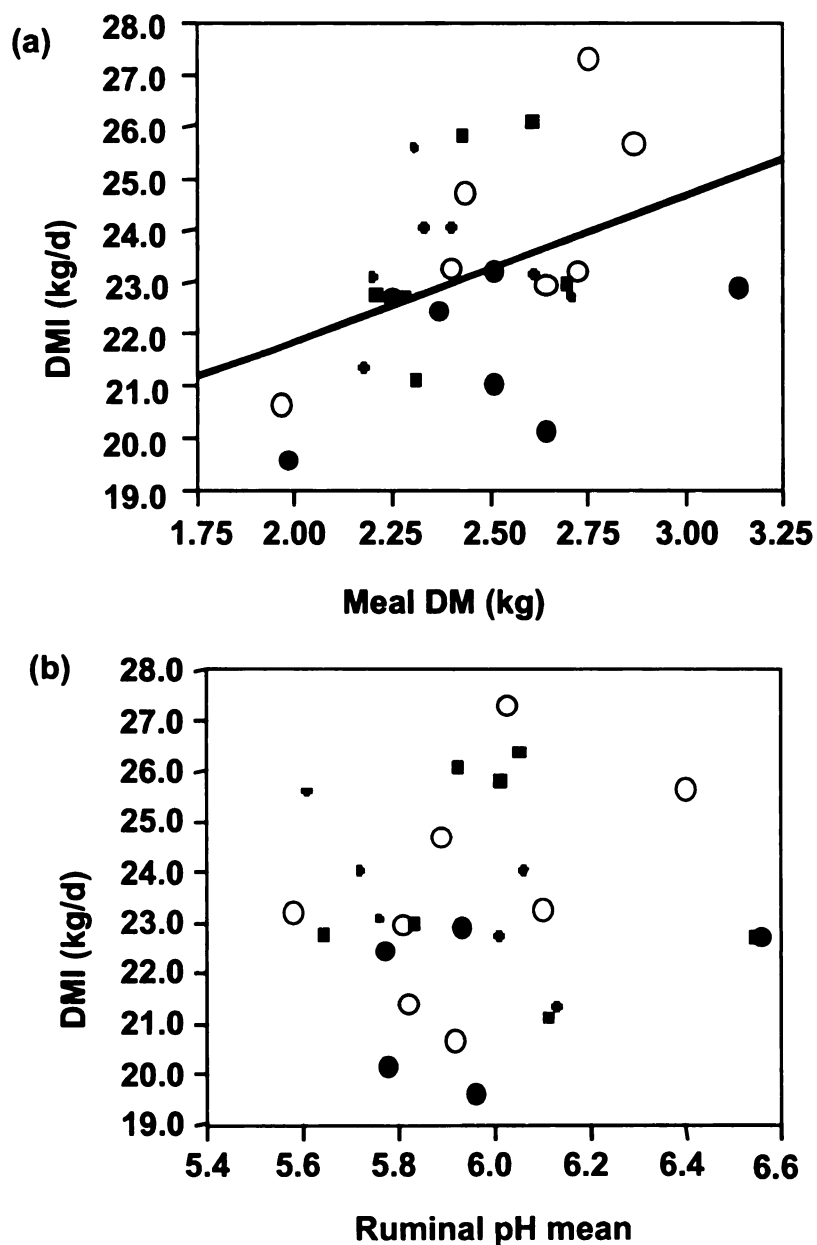
⁴ Calculated using 8 samples collected over 3 d, representing 3-hr intervals of a 24-hr day.

Table 11. Pearson correlation coefficients between plasma insulin and characteristics of intake, production, and energy balance.

	Plasma [insulin], mM
DMI, kg/d	0.41 ¹
Total tract starch digestion, kg/d	0.52 ¹
Milk NE _L , Mcal/kg	0.51 ¹
Milk NE _L , %NE _L intake	-0.23

¹ Correlation is significant (*P* < 0.05).

Figure 1. Relationship between DMI (kg/d) and (a) average kg DMI/meal (DMI = $16.3 + 2.8 \times \text{meal DMI}$; $R = 0.40$; $P < 0.04$); (b) mean ruminal pH ($R = 0.05$; $P > 0.70$); and (c) pH standard deviation (DMI = $27.1 - 11.5 \times \text{pH standard deviation}$; $R = -0.41$; $P < 0.03$). ○ denotes 0% beet pulp, + denotes 6% beet pulp, ■ denotes 12% beet pulp, and ● denotes 24% beet pulp (%diet DM) substituted for high-moisture corn.



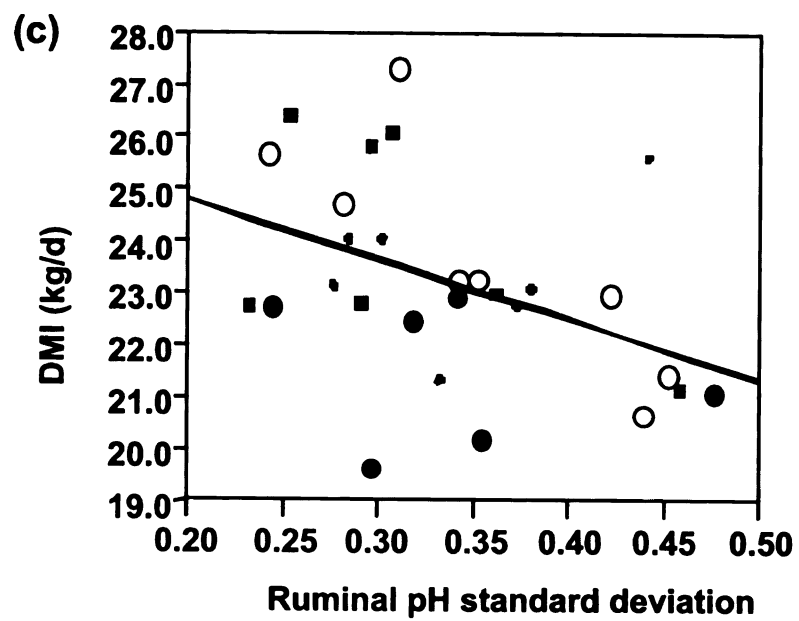


Figure 2. Relationship between sorting against NDF(%NDF in orts - %NDF in feed) and 3.5% FCM yield. Sorting = $-29.6 + 0.9 \times \text{FCM} - 0.2 \times (\text{FCM} - 37.8)^2$; ($R = 0.54$; $P < 0.03$). ○ denotes 0% beet pulp, + denotes 6% beet pulp, ■ denotes 12% beet pulp, and ● denotes 24% beet pulp (%diet DM) substituted for high-moisture corn.

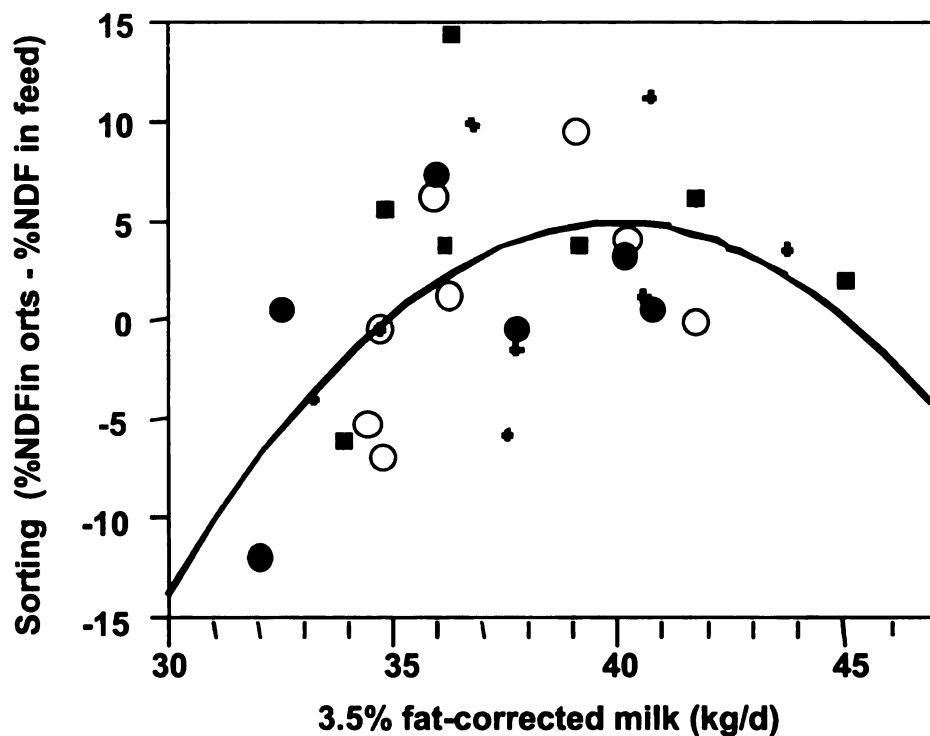
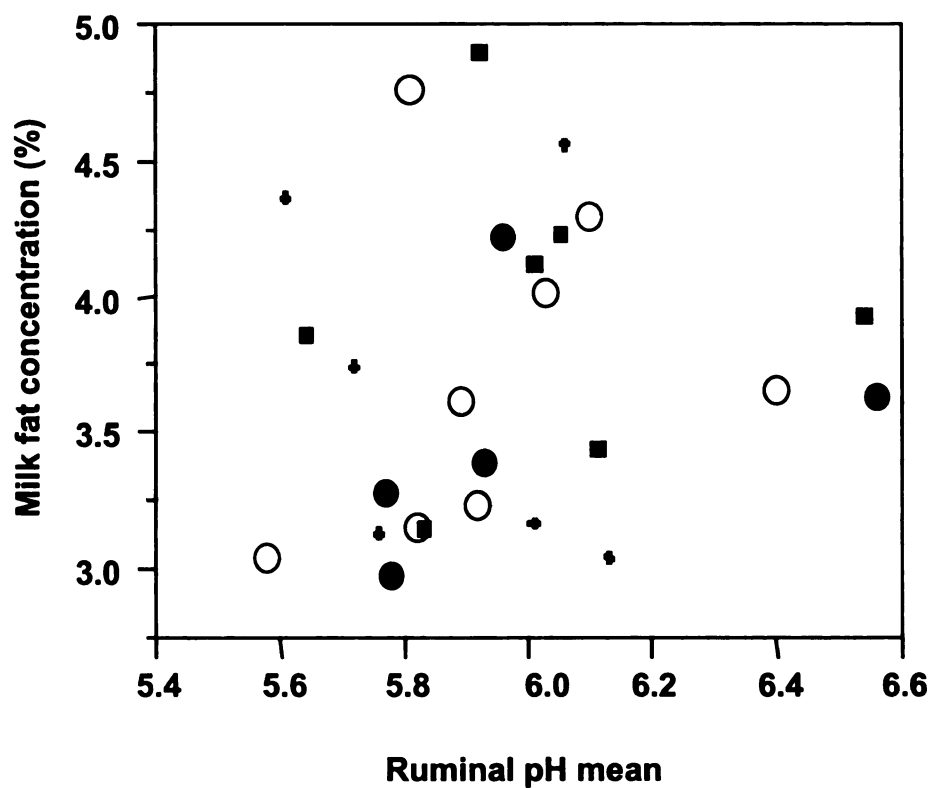


Figure 3. Relationship between milk fat concentration and mean ruminal pH ($R = 0.10$; $P > 0.50$). ○ denotes 0% beet pulp, + denotes 6% beet pulp, ■ denotes 12% beet pulp, and ● denotes 24% beet pulp (%diet DM) substituted for high-moisture corn.



CHAPTER 3

Pelleted beet pulp substituted for high-moisture corn: Effects on digestion and ruminal digestion kinetics in lactating dairy cows

ABSTRACT

The effects of increasing concentrations of dried, pelleted beet pulp substituted for high-moisture corn on digestion and ruminal digestion kinetics were evaluated using 8 ruminally and duodenally cannulated multiparous Holstein cows in a duplicated 4 x 4 Latin square design with 21-d periods. Cows were 79 ± 17 (mean $\pm$ SD) DIM at the beginning of the experiment. Experimental diets with 40% forage (corn silage and alfalfa silage) and 60% concentrate contained 0%, 6.1%, 12.1%, or 24.3% beet pulp substituted for high-moisture corn on a DM basis. Diet concentrations of NDF and starch were 24.3% and 34.6% (0% beet pulp), 26.2% and 30.5% (6% beet pulp), 28.0% and 26.5% (12% beet pulp), and 31.6% and 18.4% (24% beet pulp), respectively. Ruminal DM pool decreased ($P < 0.05$), and NDF turnover rate increased ($P < 0.01$) as beet pulp increased. Potentially digestible NDF was digested more extensively ($P < 0.01$) and at a faster rate ($P < 0.01$) in the rumen with increasing beet pulp, resulting in increased total tract NDF digestibility ($P < 0.01$). Passage rates of potentially digestible NDF and of indigestible NDF were not affected by treatment ($P > 0.20$, $P > 0.10$, respectively). True ruminal digestibility of starch decreased with increasing BP substitution ($P < 0.01$). This was caused by a linear increase

in starch passage rate ($P = 0.01$), possibly because of increasing ruminal fill, and a linear decrease in digestion rate ($P < 0.01$) of starch in the rumen, possibly the result of reduced amylolytic enzyme activity for lower-starch diets. Although true ruminal starch digestibility decreased when more beet pulp was fed ($P < 0.01$), whole tract starch digestibility was not affected ($P > 0.40$) because of compensatory digestion of starch in the intestines. Due to more thorough digestion of fiber in diets containing more beet pulp, total tract digestibility of DM increased ($P < 0.10$), and intake of digestible DM was not affected ($P > 0.40$). Partially replacing high-moisture corn with beet pulp increased fiber digestibility without reducing total tract starch digestibility.

INTRODUCTION

Because starch is usually more completely digested than NDF, replacing forage with grain can increase diet digestibility. However, nutrient digestion can also be reduced if passage rate is drastically increased or if fiber digestibility is severely reduced by increased starch concentration. Depressed fiber digestibility can be caused by several factors: decreased mastication and slower particle size reduction; a reduction in ruminal pH resulting from increased volatile fatty acid (VFA) production and decreased flow of saliva buffers (Strobel and Russell, 1986); and inhibition of fiber digestion independent of effects of mastication or pH (Grant and Mertens, 1992).

When low-forage, high-grain diets are fed, adding carbohydrate sources that are more rapidly and extensively fermented than forage neutral detergent fiber (**NDF**) and mimic some of its beneficial effects, but that do not bring the same negative effects as starch fermentation, can improve the overall digestion and absorption of nutrients. Beet pulp contains approximately 40% NDF and is unique in its high concentration of neutral-detergent soluble fiber, especially pectic substances (~25% of DM). The NDF in beet pulp can be digested more quickly than forage NDF (Bhatti and Firkins, 1995), and pectin is fermented more rapidly than cellulose and hemicellulose (Marounek et al., 1985). Unlike starch, pectin fermentation does not inhibit cellulose and hemicellulose digestion, primarily because pectinolytic bacteria are also inhibited at low pH (Marounek et al., 1985).

Therefore, substituting beet pulp for high-moisture corn grain in a diet with a low forage content should, at some rate of inclusion, improve NDF digestion and therefore increase overall nutrient digestion in the whole tract. Starch digestion kinetics should also be altered, changing the proportion of starch digested in the rumen and intestines, although the direction of that shift cannot be predicted. The objective of this experiment was to measure the effects of substituting beet pulp for high-moisture corn at four concentrations (0, 6, 12, and 24% of diet DM) on ruminal, postruminal, and whole tract digestion of NDF, starch, dry matter and organic matter.

MATERIALS AND METHODS

Treatments and Cows

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (79 ± 17 DIM; mean $\pm$ SD) from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to a duplicated 4 x 4 Latin square balanced for carry over effects in a dose-response arrangement of treatments. Treatment periods were 21 d with the final 10 d used to collect samples and data. Treatments were diets containing dried, pelleted beet pulp substituted for high-moisture corn at 0% (**0BP**), 6% (**6BP**), 12% (**12BP**), and 24% (**24BP**) of diet DM. Cows were cannulated ruminally and duodenally prior to calving. Duodenal cannulas were soft gutter type made of tygon and vinyl tubing (Crocker et al., 1998). 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 surgeries were performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. At the beginning of the experiment, empty body weight (ruminal digesta removed) of cows was 515.9 ± 63.5 kg (mean $\pm$ SD).

Nutrient composition for high-moisture corn and pelleted beet pulp are shown in Table 1. Experimental diets contained 40% forage (50:50 corn silage: alfalfa silage), high-moisture corn, beet pulp at 0-24% of diet DM, a premixed protein supplement (soybean meal, corn distiller's grains, and blood meal), and a

mineral and vitamin mix (Table 2). All diets were formulated for 18% dietary CP concentration and fed as total mixed rations.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls, and fed once daily (1100 h) at 110% of expected intake. Amounts of feed offered and refused were weighed for each cow daily during collection periods. Samples of all dietary ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily on d 12-19 and combined into one sample per period (or per cow-period) before drying.

Chromic oxide was used as a marker to estimate nutrient digestibility in the rumen and in the total tract. Gelatin capsules (1.5 oz., Tropac Inc., Airfield, NJ) containing 5 g of chromic oxide and spelt hulls (Wiley mill, 2 mm screen; Authur H. Thomas, Philadelphia, PA) were dosed through the ruminal cannula at 0300, 1100, and 1900 h (total of 15 g Cr_2O_3 /d) from 7 to 14 d with a priming dose of 3X on d 7. Duodenal samples (1,000 g), fecal samples (500 g), and rumen fluid samples (100 mL) were collected every 9 h from d 12 to d 14 so that 8 samples were taken for each cow each period, representing every 3 h of a 24-hour period to account for diurnal variation. Rumen fluid samples were taken by sampling contents from five different sites in the rumen which were then combined and strained. Fluid pH was immediately recorded. Samples were immediately frozen at -20°C.

Effect of treatment on rate of liquid passage was measured on d 15 using a pulse dose of cobalt EDTA (Allen et al., 2000). Cobalt EDTA was dosed two

hours after feeding on d 19. Rumen fluid was sampled before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 h after dosing. Samples were immediately frozen.

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

Sample and Statistical Analysis

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

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

All dried samples were analyzed for ash, NDF, indigestible NDF (**INDF**), CP, and starch. Ash concentration was determined after 5 h oxidation at 500°C in a muffle furnace. Concentrations of NDF were determined according to Van Soest et al. (1991, method A). Forage samples were analyzed for ADF and sulfuric acid lignin content (Van Soest et al., 1991). Indigestible NDF was estimated as NDF residue after 120-h in vitro fermentation (Goering and Van Soest, 1990). Crude protein was analyzed according to Hach et al. (1985). Starch was hydrolyzed enzymatically (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Glucose concentration was then measured using glucose oxidase (Glucose kit #510; Sigma Chemical Co., St. Louis, MO), and absorbance was determined with a micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Concentrations of all nutrients except for DM were expressed as percentages of DM determined by drying at 105°C in a forced-air oven.

Rumen fluid samples taken to measure rate of liquid passage were analyzed for cobalt concentration by flame atomic absorption spectrophotometry (SpectrAA 220/FS, Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia). Rate of cobalt disappearance was determined by non-linear regression of its decline in concentration in rumen fluid over time after dosing, accounting for background (Allen et al., 2000).

Feeds, duodenal digesta, and feces were analyzed for concentrations of chromium. Samples were digested with phosphoric acid (Williams et al., 1962), and chromium was quantified by flame atomic absorption spectrometry (SpectraAA 220, Varian, Victoria, Australia) according to manufacturer's recommendation. Nutrient intake was calculated using the composition of feed offered and refused. Duodenal flow of microbial OM was determined as described by Oba and Allen (2002b), and true ruminally degraded OM (**TRDOM**) was calculated by subtracting duodenal flow of non-microbial OM from OM intake. Ruminal pool sizes (kg) of OM, NDF, indigestible NDF, and starch were determined by multiplying the concentration of each component by the ruminal digesta DM weight (kg). Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component (%/h) were calculated by the following equations:

Turnover rate in the rumen (%/h) =

$$(\text{Intake of component} / \text{Ruminal pool of component}) / 24 \times 100$$

Passage rate from the rumen (%/h) =

$$(\text{Duodenal flow of component} / \text{Ruminal pool of component}) / 24 \times 100;$$

and

Digestion rate in the rumen (%/h) =

$$\text{Turnover rate in the rumen (\%/h)} - \text{Passage rate from the rumen (\%/h)}.$$

To determine differences between treatments, all data were analyzed using the fit model procedure of JMP® according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

where

μ = overall mean,

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

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

T_k = fixed effect of treatment ($k = 1$ to 4), and

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

Period by treatment interaction was originally evaluated, but it was removed from the statistical model because it was not significant. Linear and quadratic dose-response effects were evaluated using the same model with diet percent beet pulp (0, 6, 12, and 24) in place of the fixed effect of treatment. Pearson correlation coefficients were determined between cow-period observations for some parameters. Treatment effects, linear and quadratic responses, and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$.

Data from two cow-periods were excluded from statistical analysis. One cow developed a cecal torsion requiring surgery early in period 3; data and samples were not collected from this cow during period 3, but she recovered sufficiently for her data to be used in period 4. Period 4 data from a second cow were excluded after she showed signs of estrus because her intake and milk yield were outliers (outside the 95% confidence interval for cow-period observations).

RESULTS AND DISCUSSION

Ruminal NDF Digestion

Turnover rate of NDF (Table 3) increased linearly with added beet pulp ($P < 0.01$). A faster turnover rate can result from increased passage rate, increased digestion rate, or both. Passage rates of potentially digestible NDF (pdNDF) and indigestible NDF were not affected by treatment (Table 3). However, digestion rate of pdNDF more than doubled, from 2.71% to 5.86%/h ($P < 0.0001$), from 0BP to 24BP.

As BP was substituted for HMC as up to 24% of diet DM, the proportion of NDF from forage decreased from approximately 70 to 54% of total NDF, and the proportion of NDF from BP increased from 0 to approximately 31% of total NDF (Table 2). Beet pulp NDF has a shorter lag time and more rapid fermentation rate than most other sources of fiber (Bhatti and Firkins, 1995) partly because it has been previously soaked in hot water (Bichsel, 1988). Therefore, increasing the contribution of beet pulp NDF to total NDF can increase the overall rate of NDF digestion independent of any associative effects of beet pulp NDF on the digestion NDF from other sources.

Substituting the readily-fermented pectin and NDF of beet pulp for corn may also increase the rate of digestion of other dietary fiber through associative effects of both fiber and starch. Adding BP likely increased the population of fibrolytic bacteria by providing excess available substrate for fiber fermenters and increasing fibrolytic enzyme activity in the rumen. Dilution of the concentration of dietary starch would also reduce the negative affects of starch fermentation on

cellulolytic bacteria. Measurements of ruminal pH over 96 h in this study are reported in Chapter 4. As mean and minimum daily pH were not different among treatments, the improvement in fiber digestion with added BP was not caused by increased mean pH. However, reduction of NDF digestion by the addition of starch can occur even when pH is held constant (Grant and Mertens, 1992).

Ruminal pH and NDF Digestion

Increased rate of NDF digestion with added beet pulp was not the result of increased mean pH among treatments. However, correlations among cow-period observations between ruminal pH characteristics and rate of ruminal fiber digestion show that greater daily mean pH resulted in more rapid digestion of potentially digestible NDF (Figure 1; $R = 0.41$ $P < 0.05$), as did greater minimum ruminal pH ($R = 0.55$, $P < 0.01$). Lower mean or minimum ruminal pH and greater variability in pH probably slowed fermentation of NDF because low pH (especially below 6.0) slows growth of fibrolytic bacteria, perhaps due to intracellular accumulation of VFA and anion toxicity (Russell and Wilson, 1996). Although higher pH mean and minimum were associated with more rapid fiber fermentation, it did not improve ruminal fiber digestibility ($P > 0.30$), because higher pH also increased passage rates of pdNDF and INDF from the rumen (Table 4). Slower fiber fermentation at low ruminal pH was independent of treatment effects; slower fiber fermentation when ruminal pH variation increased corresponds with treatment effects on pH and fiber digestion kinetics.

Whole Tract NDF Digestion

As NDF intake and the rate of ruminal NDF digestion increased with beet pulp replacing corn grain, the amount of NDF digested in the rumen also increased ($P < 0.01$; Table 5). Ruminal digestibility of total NDF was not affected ($P > 0.10$), but potentially digestible NDF was more completely digested with added beet pulp ($P < 0.01$). There was no compensatory postruminal NDF digestion, so the diets with the greatest ruminal NDF digestibility also had the greatest total tract NDF digestibility ($P < 0.001$).

Ruminal Starch Digestion Kinetics

Corresponding to diet composition and DMI, starch intake decreased from 8.2 kg/d for 0BP to 4.0 kg/d for 24 BP (Table 6; $P < 0.0001$). Starch turnover rate was not affected by treatment (Table 3; $P > 0.40$) due to opposing changes in ruminal rates of both passage and digestion of starch. As HMC was replaced by BP, passage rate of starch increased linearly ($P = 0.01$) and ruminal starch digestion rate decreased from 11.3%/h for 0BP to 1.9%/h for 24BP ($P < 0.01$). As the dietary concentration of HMC decreased, the proportion of starch from HMC decreased (Table 2), and starch from other sources (such as corn silage) was probably fermented less rapidly due to differences in processing and preservation. Rate of starch fermentation also might have been reduced by lower amylase activity in rumen fluid, because of a smaller population of amylolytic microbes and possibly because of depressed enzyme activity of the population.

Site of Starch Digestion

Large differences in starch intake, rapid passage of starch from the rumen for the high-beet pulp diets, and a sharp decrease in ruminal starch digestion rate resulted in much lower true ruminal starch digestibility ($P < 0.01$; Table 6).

Reducing dietary starch content by 14 percentage units (0% BP to 6% BP) resulted in a 31% decrease in true ruminal starch digestion (from 46.5% to 32.3%). From 0BP to 24BP, dietary starch content was reduced by 48%, the amount of starch truly digested in the rumen decreased 82 % (3.8 kg to 0.7 kg), and the percentage of starch truly digested in the rumen decreased 64 percent (46.5% to 16.9%).

Treatment effects on ruminal digestibility of starch were compensated for by intestinal digestion; the percent of starch consumed that was digested postruminally increased from 45.7% to 77.5% ($P = 0.02$) as dietary starch content decreased from 34.6% to 18.4%, and digestibility of starch passing into the duodenum increased from 78.0% to 85.0% ($P = 0.02$). Because postruminal digestion of starch results in the absorption of glucose, which is more energetically efficient than starch fermentation to VFA (Owens et al., 1986), cows may have obtained energy from starch more efficiently when fed diets containing less starch. Because these cows were not ileally cannulated, the proportions of starch digested and absorbed as glucose in the small intestine or fermented and absorbed as VFA in the large intestine could not be measured. As a result of increased postruminal starch digestion, total tract digestibility of starch was the

same across beet pulp treatments ($P > 0.40$) even though the amount of starch digested in the total tract decreased from 7.2 kg/d to 3.5 kg/d ($P < 0.0001$) as starch intake decreased.

Source of starch can affect ruminal and intestinal starch digestion. The rate and extent of starch digestion might be expected to be greater for rolled HMC than for kernels in corn silage, the second most significant starch source in these diets, because of greater surface area:weight in the rolled HMC. As HMC was increasingly replaced by beet pulp, the percent of dietary starch coming from HMC decreased from 73% to 44% (Table 2). It would be possible that ruminal rate of starch digestion (%/h) decreased as BP increased because corn silage grain was digested more slowly than the rolled HMC, and corn silage kernels comprised a greater proportion of total starch as BP increased in the diets. However, total tract digestibility of starch was similar among treatments (Table 7), so starch reaching the duodenum was as digestible, if not more digestible, for diets in which HMC starch comprised a smaller percentage of total dietary starch. Unfermented corn silage kernels would be more resistant to intestinal digestion than unfermented HMC, so it is not likely that a greater proportion of corn-silage grain was resisting ruminal degradation for high-beet-pulp diets (thus reducing ruminal starch digestion rate) and then being digested in the intestines to the same extent as the starch in low-BP diets. While the ruminal digestion of silage kernels was probably affected by dietary starch concentration, the treatment effects detected were not only the result of changing proportions of starch sources.

If the amount of duodenal enzyme activity limits the amount of starch digested in the small intestine, then extent of digestion should decrease as starch flow increases (Owens et al., 1986). However, across cow-period observations, amount of starch digested in the intestines (kg/d) increased linearly with greater duodenal starch flow ($R = 0.98$, $P < 0.0001$; Figure 2a). Intestinal starch digestibility (% of duodenal flow) also tended to increase as duodenal starch flow increased ($R = 0.34$, $P = 0.07$; Figure 2b). Similar responses in intestinal starch digestion were reported in a study in which treatments were dietary starch concentration and fermentability of corn grain (Oba and Allen, 2002a). Since greater flow was not associated with lower intestinal digestibility, starch digestion in the small intestine was probably limited by physical or chemical characteristics of starch escaping ruminal digestion, rather than by enzyme activity (Oba and Allen, 2002a). However, amylase secretion responds to dietary starch concentration (Owens, 1986), so enzyme quantity could possibly limit starch digestion.

Digestion of DM and OM

Substituting highly fermentable fiber for rapidly fermentable starch radically altered ruminal digestion and passage of starch and fiber, probably through both physical and microbial changes in the rumen environment. This resulted in a tendency for decreased DM apparent ruminal digestion ($P = 0.10$) but did not affect apparent or true OM digestion in the rumen (Table 7). Among cow-period observations, ruminal OM digestibility increased with faster ruminal

starch digestion ($R = 0.71$, $P < 0.0001$) and decreased with faster starch passage from the rumen ($R = -0.69$, $P < 0.0001$). Greater true ruminal starch digestibility also increased true ruminal OM digestibility ($R = 0.83$, $P < 0.0001$). Ruminal fiber digestibility and digestion kinetics were not related to TRDOM ($P > 0.20$). Therefore, ruminal starch digestion, but not NDF digestion, affected digestibility of OM in the rumen.

The tendency for a quadratic effect of dietary beet pulp content on flow of DM to the duodenum was detected, with the highest value for 12% beet pulp; OM flow followed a similar pattern but with the highest value for 6% beet pulp (Table 7). However, because compensatory postruminal starch digestion took place as the concentration of beet pulp increased, quantities of DM and OM apparently digested in the whole tract were not affected by treatment. Because DMI decreased linearly with added beet pulp while the amount of DM disappearance did not change, apparent total tract OM digestibility increased, and DM digestibility tended to increase. Therefore, substituting highly digestible NDF for rapidly fermented starch in a high-concentrate diet did not reduce the amount of apparently absorbed DM, but increased total diet digestibility by increasing NDF digestion.

SUMMARY

Increasing the substitution rate of pelleted beet pulp for high-moisture corn decreased the rate of ruminal starch digestion and increased starch passage rate, shifting the site of starch digestion increasingly to the intestines. Added

beet pulp greatly increased the rate at which potentially digestible NDF was digested in the rumen and also increased total tract NDF digestibility. Partially replacing a highly fermentable starch source with beet pulp was accomplished without reducing apparent nutrient digestibility because adding beet pulp increased fiber digestibility without reducing total tract starch digestibility.

Table 1. Nutrient composition of high-moisture corn and dried, pelleted beet pulp.

Nutrient	High-moisture corn	Beet pulp
DM, % as fed	71.5	84.9
	-----% of DM-----	
NDF	10.0	39.9
CP	8.3	8.9
Indigestible NDF	3.8	8.0
Starch	70.5	3.9
Ether extract	4.7	0.7
Ash	1.0	7.8

Table 2. Ingredient and nutrient composition of experimental diets.

	0% BP	6% BP	12% BP	24% BP
Ingredients	-----% of DM-----			
Corn silage	20.1	20.1	20.1	20.1
Alfalfa silage	19.9	19.9	19.9	19.9
Protein mix	19.5	19.5	19.5	19.5
Mineral vitamin mix	4.8	4.8	4.8	4.8
Dried, pelleted beet pulp	0	6.1	12.1	24.3
High-moisture corn	35.6	29.5	23.5	11.4
Nutrient				
DM	50.2	50.5	50.8	51.6
Starch	34.6	30.5	26.5	18.4
NDF	24.3	26.2	28.0	31.6
Indigestible NDF	9.4	9.6	9.8	10.2
Forage NDF	17.1	17.1	17.1	17.1
CP	18.0	18.0	18.0	18.1
% Starch from high-moisture corn	72.7	68.3	62.6	43.7
% NDF from forage	70.2	65.3	61.1	54.1
% NDF from beet pulp	0.0	9.3	17.3	30.7

Table 3. Effects of substitution of pelleted beet pulp for high-moisture corn on ruminal digestion kinetics.

	Treatment LS Means					<i>P</i>		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
Ruminal turnover rate, %/hr								
DM	9.1	9.8	9.4	9.3	0.6	0.34	0.20	0.20
OM	9.4	10.2	9.6	9.6	0.6	0.25	0.24	0.24
NDF	4.5	5.5	5.2	5.9	0.4	<0.01	<0.01	0.24
pdNDF	5.9	7.6	7.2	8.6	0.6	<0.01	<0.01	0.38
Starch	29.0	29.6	28.3	25.4	2.3	0.49	0.87	0.56
Ruminal passage rate, %/h								
Potentially digestible NDF	3.16	3.29	2.74	2.89	0.36	0.49	0.29	0.76
INDF	3.09	3.78	3.44	3.67	0.28	0.11	0.16	0.26
Starch	15.90	19.31	18.33	23.50	2.99	0.07	0.01	0.96
Liquid ¹	18.6	17.5	16.7	16.6	1.0	0.13	0.03	0.21
Ruminal digestion rate, %/h								
pdNDF	2.71	4.29	4.43	5.86	0.47	<0.01	<0.01	0.23
Starch	11.30	10.36	9.45	1.91	3.06	0.02	<0.01	0.27

¹ Measured using Co-EDTA.

Table 4. Correlation coefficients for fiber digestion kinetics and ruminal pH.

	pdNDF digestion rate	pdNDF passage rate	INDF passage rate
Mean ruminal pH	0.41 ¹	0.34 ³	0.37 ²
Minimum ruminal pH	0.55 ¹	0.40 ²	0.44 ¹

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

² Tendency for significant correlation ($P < 0.10$).

³ $P < 0.12$

Table 5. Effects of substitution of pelleted beet pulp for high-moisture corn on digestion of total NDF and potentially digestible NDF (pdNDF).

	Treatment LS Means					<i>P</i>		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
NDF								
Intake, kg/d	5.7	6.2	6.6	6.9	0.2	<0.01	0.01	0.15
Ruminally digested								
kg/d	2.1	2.1	2.3	2.9	0.2	0.04	<0.01	0.39
%	35.9	34.5	34.5	42.5	3.5	0.32	0.14	0.23
Passage to duodenum, kg/d	3.7	4.1	4.3	4.0	0.3	0.37	0.08	0.11
Postruminally digested								
kg/d	0.68	0.72	0.99	0.88	0.18	0.56	0.33	0.47
% of intake	12.0	11.7	15.3	12.7	3.0	0.81	0.54	0.59
% of duodenal passage	16.7	17.6	24.3	22.7	3.8	0.40	0.17	0.50
Total tract digested								
kg/d	2.5	2.9	3.5	3.9	0.22	<0.01	<0.01	0.13
% of intake	43.4	47.3	53.4	57.2	2.1	<0.01	<0.01	0.31
pdNDF								
Intake, kg/d	3.2	3.6	4.0	4.3	0.1	<0.01	<0.01	0.17
Ruminally digested								
kg/d	1.6	2.0	2.3	2.8	0.1	<0.01	<0.01	0.37
%	46.1	56.8	60.3	67.3	3.4	<0.01	0.02	0.20
Passage to duodenum, kg/d	1.6	1.6	1.6	1.4	0.1	0.51	0.81	0.86
Postruminally digested								
kg/d	0.36	0.45	0.49	0.35	0.11	0.77	0.34	0.30
% of intake	10.9	12.6	12.1	8.0	2.9	0.70	0.57	0.40
% of duodenal passage	22.3	17.6	26.7	24.9	7.6	0.82	0.90	0.99
Total tract digested								
kg/d	1.9	2.4	2.8	3.2	0.1	<0.01	<0.01	0.03
% of intake	60.2	67.0	71.6	75.4	1.7	<0.01	<0.01	0.03

Table 6. Effects of substitution of pelleted beet pulp for high-moisture corn on digestion of starch.

	Treatment LS Means					P		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
Intake, kg/d	8.2	7.3	6.4	4.0	0.2	<0.01	<0.01	0.21
Apparently ruminally digested								
kg/d	3.5	2.1	1.6	0.4	0.6	<0.01	<0.01	0.32
%	42.2	27.9	26.1	9.7	10.0	0.03	<0.01	0.74
Truly ruminally digested								
kg/d	3.8	2.4	1.9	0.7	0.6	<0.01	<0.01	0.32
%	46.5	32.3	31.2	16.9	9.8	0.05	<0.01	0.68
Passage to duodenum, kg/d	4.7	5.2	4.8	3.7	0.6	0.11	0.40	0.14
Apparent postruminal digested								
kg/d	3.7	4.1	4.0	3.1	0.6	0.32	0.27	0.14
% of intake	45.7	57.2	62.6	77.5	9.5	0.02	<0.01	0.77
% of duodenal passage	78.0	77.4	83.2	85.0	2.6	0.02	<0.01	0.96
Apparent total tract digested								
kg/d	7.2	6.2	5.7	3.5	0.2	<0.01	<0.01	0.30
%	88.0	86.2	88.6	87.5	1.6	0.49	0.99	0.97

Table 7. Effects of substitution of pelleted beet pulp for high-moisture corn on digestion of DM and OM.

	Treatment LS Means					P		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
DM								
Intake, kg/d	23.7	23.8	23.9	21.8	0.7	0.11	<0.05	0.15
Apparently ruminally digested								
kg/d	6.4	5.3	3.7	4.7	1.1	0.29	0.09	0.16
%	27.4	22.2	16.2	21.9	4.8	0.38	0.10	0.15
Passage to duodenum, kg/d	17.2	18.6	20.2	17.1	1.5	0.35	<0.10	0.08
Apparent total tract digested								
kg/d	16.1	15.9	16.7	15.4	0.5	0.28	0.35	0.22
%	68.4	66.9	70.6	70.8	1.3	<0.10	0.07	0.87
OM								
Intake, kg/d	22.3	22.4	22.4	20.2	0.7	0.06	0.50	0.15
Apparently ruminally digested								
kg/d	7.0	5.4	5.9	5.7	0.8	0.30	0.21	0.32
%	31.2	23.8	26.7	27.9	3.5	0.34	0.22	0.27
Truly ruminally digested								
kg/d	11.4	9.7	10.2	9.4	0.8	0.15	0.85	0.95
%	50.7	42.9	45.9	46.4	3.6	0.34	0.69	0.60
Passage to duodenum, kg/d	16.2	17.0	16.4	14.5	1.0	0.21	0.16	0.12
Apparent postruminal digested								
kg/d	9.5	9.7	10.0	8.6	0.9	0.69	0.50	0.36
% of intake	38.9	43.8	45.0	42.9	3.8	0.67	0.24	0.29
% of duodenal passage	57.8	58.0	60.7	59.7	2.6	0.77	0.51	0.64
Apparent total tract digested								
kg/d	15.6	15.4	16.0	14.6	0.4	0.19	0.37	0.19
%	69.9	68.7	72.2	72.5	1.3	0.09	0.05	0.90

Figure 1. Relationship between rate of digestion of potentially digestible NDF (%/h) and mean ruminal pH. $\text{pdNDF rate of digestion}_d = -11.1 + 2.5 \text{ mean pH}$ ($R = 0.41$; $P < 0.05$). ○ denotes 0% beet pulp, + denotes 6% beet pulp, ■ denotes 12% beet pulp, and ● denotes 24% beet pulp (%diet DM) substituted for high-moisture corn.

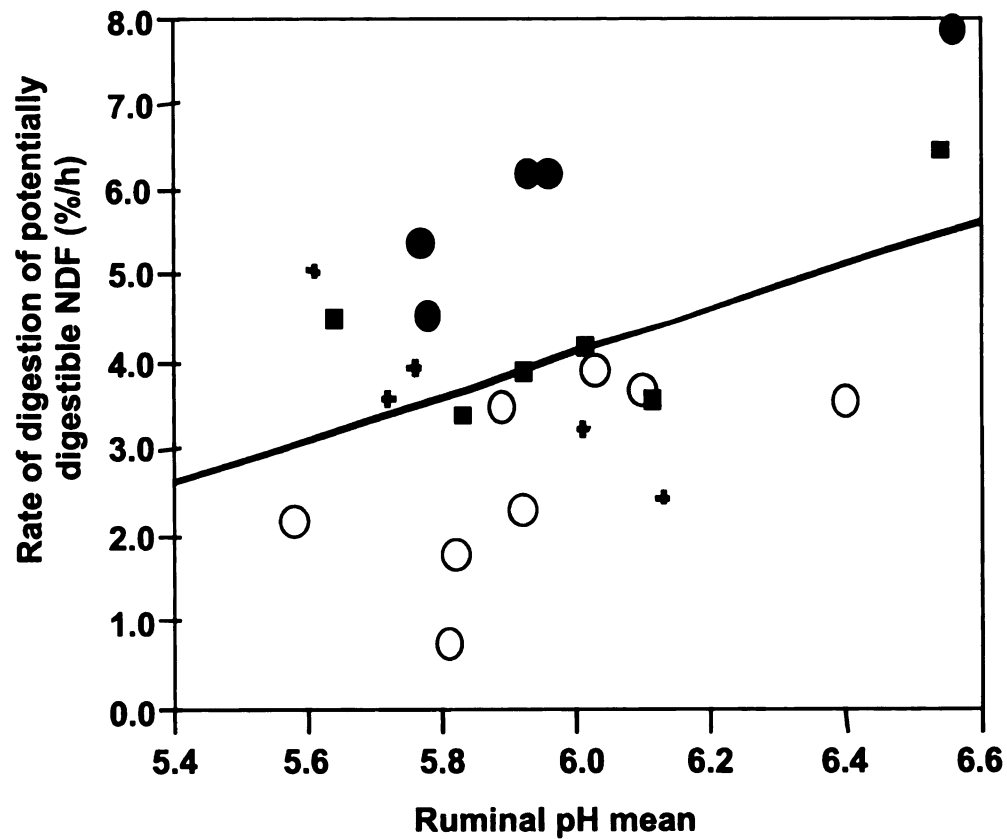
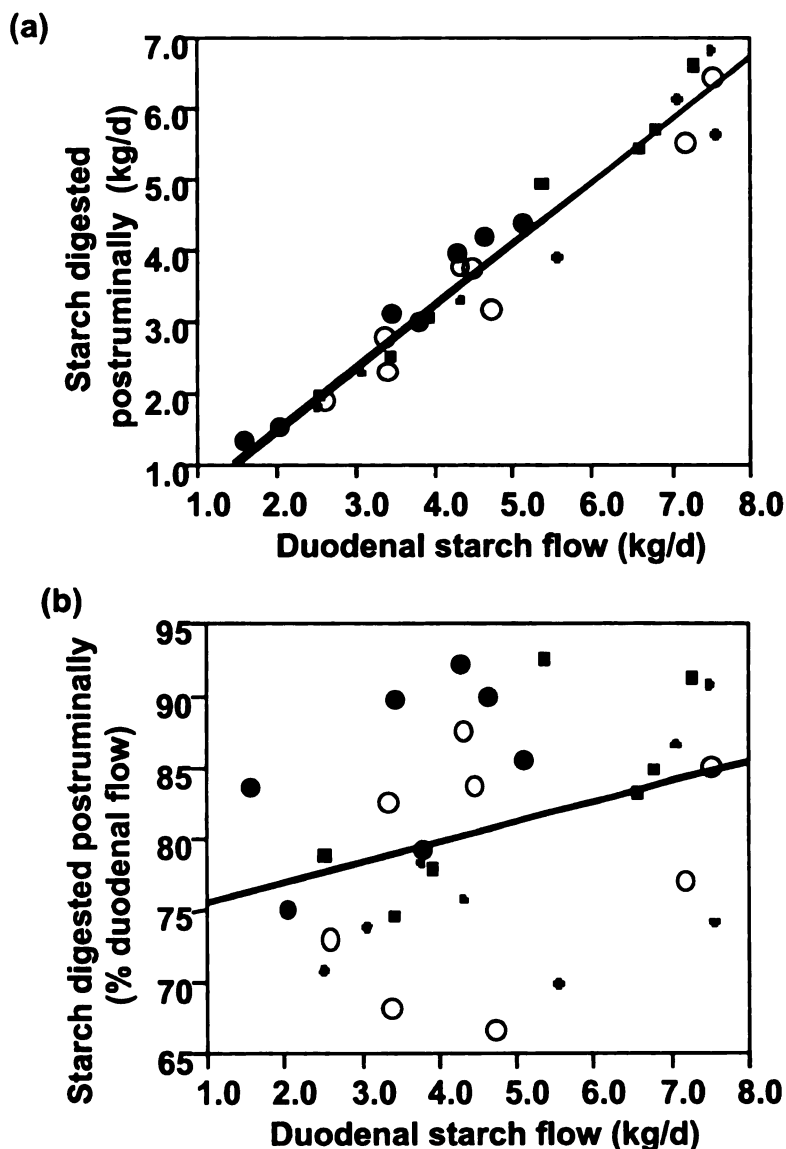


Figure 2. Relationship between (a) starch digested (kg/d) and duodenal starch flow (Starch digested postruminally = $-0.28 + 0.88$ duodenal starch flow; $R = 0.97$, $P < 0.01$), and (b) starch digestibility (% duodenal flow) and duodenal starch flow [Starch digested postruminally (% duodenal flow) = $74.1 + 1.4$ duodenal starch flow; $R = 0.35$, $P < 0.07$]. ○ denotes 0% beet pulp, + denotes 6% beet pulp, ■ denotes 12% beet pulp, and ● denotes 24% beet pulp (%diet DM) substituted for high-moisture corn.



CHAPTER 4

Pelleted beet pulp substituted for high-moisture corn: Effects on ruminal fermentation, pH, and microbial protein efficiency in lactating dairy cows.

ABSTRACT

The effects of increasing concentrations of dried, pelleted beet pulp substituted for high-moisture corn on ruminal fermentation, pH, and microbial efficiency were evaluated using 8 ruminally and duodenally cannulated multiparous Holstein cows in a duplicated 4 x 4 Latin square design with 21-d periods. Cows were 79 ± 17 (mean $\pm$ SD) DIM at the beginning of the experiment. Experimental diets with 40% forage (corn silage and alfalfa silage) and 60% concentrate contained 0%, 6.1%, 12.1%, or 24.3% beet pulp substituted for high-moisture corn on a DM basis. Diet concentrations of NDF and starch were 24.3% and 34.6% (0% beet pulp), 26.2% and 30.5% (6% beet pulp), 28.0% and 26.5% (12% beet pulp), and 31.6% and 18.4% (24% beet pulp), respectively. Substituting beet pulp for corn did not affect daily mean or minimum ruminal pH ($P > 0.15$), but it tended to reduce pH range ($P = 0.07$). Ruminal acetate:propionate responded in a positive exponential (quadratic) relationship to added beet pulp. Rate of valerate absorption from the rumen was not affected by treatment ($P > 0.50$). Substituting beet pulp for corn up to 24% of diet DM did not affect efficiency of ruminal microbial protein production, expressed as microbial N flow to the duodenum as a percent of OM truly digested in the rumen ($P > 0.25$). Microbial efficiency was not correlated to mean pH ($P > 0.90$) or daily minimum pH ($P > 0.90$). While microbial efficiency was

not directly related to concentration of beet pulp fed, it was positively correlated with passage rate of particulate matter, as represented by starch ($R = 0.65$, $P < 0.01$) and INDF ($R = 0.51$, $P = 0.02$), probably due to reduced protein turnover in the rumen.

INTRODUCTION

Achieving optimal temporal patterns of ruminal carbohydrate fermentation is necessary to maximize milk yield and efficiency in dairy cattle, because their primary sources of energy and protein are fermentation products, especially volatile fatty acids (VFA) and microbial protein. Dietary starch concentration is often increased in order to increase diet fermentability, but increasing rate, or even extent, of ruminal fermentation does not necessarily result in optimal fermentation.

Replacing feed ingredients high in cellulose and hemicellulose with ingredients high in starch usually increases ruminal production of VFA and alters the proportions of individual VFA produced. Ruminal infusion of propionate reduces feed intake (Anil and Forbes, 1980) and alters nutrient partitioning and milk production (Reynolds, 1995). Greater VFA production can lead to reduced ruminal pH, which might increase the rate of VFA absorption from the rumen (Dijkstra et al., 1993). Low ruminal pH reduces fiber digestion (Ørskov and Fraser, 1975) and decreases microbial efficiency because of increased energy spilling (Strobel and Russell, 1986). Therefore, optimal ruminal fermentation for

high-concentrate diets can probably be achieved by diluting starch with a non-forage carbohydrate source that is less rapidly fermented, produces less propionate, and does not reduce ruminal pH.

The NDF in beet pulp can be digested more quickly than forage NDF (Bhatti and Firkins, 1995), and pectin is fermented more rapidly than cellulose and hemicellulose, but pectinolytic bacteria are also inhibited at low pH (Marounek et al., 1985). Substituting beet pulp for high-moisture corn in a diet with a moderately low forage content should alter ruminal fermentation and might increase mean or minimum ruminal pH. If beet pulp improves microbial utilization of energy or reduces microbial protein turnover in the rumen, microbial protein efficiency may be improved. The objective of this experiment was to characterize the responses of ruminal fermentation, pH, and microbial protein efficiency to beet pulp substituted for high-moisture corn at 0%, 6%, 12%, and 24% of diet DM.

MATERIALS AND METHODS

Treatments and Cows

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (79 ± 17 DIM; mean $\pm$ SD) from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to a duplicated 4 x 4 Latin square balanced for carry over effects in a dose-response

arrangement of treatments. Treatments were diets containing dried, pelleted beet pulp (**BP**) substituted for high-moisture corn (**HMC**) at 0% (**0BP**), 6% (**6BP**), 12% (**12BP**), and 24% (**24BP**) of diet DM. Treatment periods were 21 d with the final 10 d used to collect samples and data. Cows were cannulated ruminally and duodenally prior to calving. Duodenal cannulas were soft gutter type made of tygon and vinyl tubing (Crocker et al., 1998). 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 surgeries were performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. At the beginning of the experiment, empty body weight (ruminal digesta removed) of cows was 515.9 ± 63.5 kg (mean $\pm$ SD).

Nutrient composition for high-moisture corn and pelleted beet pulp are shown in Table 1. Experimental diets contained 40% forage (50:50 corn silage: alfalfa silage), high-moisture corn, beet pulp at 0 - 24% of diet DM, a premixed protein supplement (soybean meal, corn distiller's grains, and blood meal), and a mineral and vitamin mix (Table 2). All diets were formulated for 18% dietary CP concentration and fed as total mixed rations.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls, fed once daily (1100 h) at 110% of expected intake, and milked twice daily. Amounts of feed offered and refused were weighed for each cow daily during the collection period.

Samples of all dietary ingredients (0.5 kg) and orts from each cow (12.5%) were collected daily and combined into one sample per period.

Chromic oxide was used as a marker to estimate nutrient digestibility in the rumen and in the total tract. Gelatin capsules (1.5 oz. Tropac Inc., Airfield, NJ) containing 5 g of chromic oxide and spelt hulls (Wiley mill, 2 mm screen; Authur H. Thomas, Philadelphia, PA) were dosed through the ruminal cannula at 0300, 1100, and 1900 h (total of 15 g Cr_2O_3 /d) from 7 to 14 d with a priming dose of 3X on d 7. Duodenal samples (1,000 g per a cow) and fecal samples (500 g), and rumen fluid samples for VFA (100 mL) and microbial pellet (350 mL) were collected every 9 h from 12 to 14 d, so that 8 samples were taken for each cow, representing every 3 h of a 24-hour period to account for diurnal variation. Rumen fluid for the microbial pellet was taken from the reticulum, near the reticular-omasal orifice, and strained through a layer of nylon mesh (approximately 1 mm pore size). Rumen fluid samples for VFA were taken by sampling contents from five different sites in the rumen and were then combined and strained. Fluid pH was immediately recorded. Duodenal, fecal, and rumen fluid VFA and microbial pellet samples were immediately frozen at -20°C .

Effect of treatment on rate of liquid passage and relative rate of valerate absorption was measured on d 15 using a pulse dose of valeric acid and cobalt EDTA (Allen et al., 2000). Valeric acid and cobalt EDTA was dosed two hours after feeding on d 19. Rumen fluid was sampled before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 h after dosing. Samples were immediately frozen.

Ruminal pH was monitored from d 16 through d 19 (96 h) of each period by a computerized data acquisition system (Dado and Allen, 1993). Ruminal pH data were recorded for each cow every 5 sec. Electrodes for ruminal pH determination were checked daily and calibrated as needed, and ruminal pH data were deleted for the previous 24 h if readings had changed more than 0.05 unit at pH 7 or pH 4. The system successfully collected 66.1% of the total ruminal pH data (average 2.7 d per cow per period). Daily mean, minimum, maximum, variation range, time below pH 6.0, 5.8, and 5.5, and area below the pH 6.0, 5.8, and 5.5 were calculated. Response variables were averaged over four days for each period.

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

After rumen evacuation, 20 ml of rumen fluid (maintained at 37°C in a water bath) were used to measure consistency in a clean, dry Bostwick consistometer (CSC Scientific Co., Fairfax, Virginia). Distance traveled by the

liquid front (cm) was recorded every 30 s for 300 s, and samples were run in duplicate. Data reported are the distance traveled after 300 s.

Sample and Statistical Analysis

Diet ingredients, orts, and feces were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. Duodenal samples for each cow were thawed, combined by period, and separated into solid and liquid phases for drying. Microbial pellets were obtained from combined rumen fluid samples for each cow by differential centrifugation (Overton et al., 1995). Ruminal digesta, duodenal digesta, and microbial pellets were lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY). All samples were ground with a Wiley mill (1mm screen; Authur H. Thomas, Philadelphia, PA). Dried, ground ruminal digesta and duodenal digesta were recombined according to the ratios of solid and liquid DM in the original whole sample. Samples were analyzed for ash, NDF, indigestible NDF (**INDF**), CP, and starch. Ash concentration was determined after 5 h oxidation at 500°C in a muffle furnace. Concentrations of NDF were determined according to Van Soest et al. (1991, method A). Indigestible NDF was estimated as NDF residue after 120-h in vitro fermentation (Goering and Van Soest, 1990). Forage samples were analyzed for ADF and sulfuric acid lignin content (Van Soest et al., 1991). Crude protein was analyzed according to Hach et al. (1985). Starch was hydrolyzed enzymatically (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Glucose concentration was then measured using glucose oxidase (Glucose kit #510; Sigma Chemical Co., St. Louis, MO),

and absorbance was determined with micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Concentrations of all nutrients except for DM were expressed as percentages of DM determined by drying at 105°C in a forced-air oven.

Feed samples, duodenal digesta, and fecal samples were analyzed for concentrations of Cr. Samples were digested with phosphoric acid (Williams, 1962), and quantified by flame atomic absorption spectrometry (SpectraAA 220, Varian, Victoria, Australia) according to manufacturer's recommendation. Ruminal pool sizes (kg) of OM, NDF, INDF, and starch were determined by multiplying the concentration of each component by the ruminal digesta DM weight (kg). Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component (%/h) were calculated (Oba and Allen, 2002).

Rumen fluid was analyzed for concentration of major VFA and lactate by HPLC (Waters Corp., Milford, MA). Duodenal digesta were analyzed for purines and ammonia to estimate microbial nitrogen flow and non-ammonia non-microbial nitrogen flow to the duodenum. Purine concentration was used as a microbial marker, and purine to microbial N ratio was estimated by analysis of microbial pellets. Total purines were measured by spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 260 nm (Zinn and Owens, 1986). Ammonia concentration was determined for centrifuged duodenal and rumen fluid samples according to Broderick and Kang (1980).

Rumen fluid samples taken to measure rate of valerate absorption were analyzed for valerate concentration by HPLC (Waters Corp., Milford, MA) and cobalt concentration by atomic absorption spectrophotometry (SpectrAA 220/FS, Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia). Rates of valerate and cobalt disappearance were determined by non-linear regression of the decline in their respective concentrations in rumen fluid over time after dosing, accounting for background (Allen et al., 2000).

All data were analyzed using the fit model procedure of JMP® according to the following model:

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

where

μ = overall mean,

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

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

T_k = fixed effect of treatment ($k = 1$ to 4), and

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

Period by treatment interaction was originally evaluated, but it was removed from the statistical model because it was not significant for response variables of primary interest. Linear and quadratic dose-response effects were evaluated using the same model with diet percent beet pulp in place of the fixed effect of treatment. Pearson correlation coefficients were determined between cow-period observations for some parameters. Treatment effects, linear and quadratic

responses, and correlations were declared significant at $P < 0.05$, and tendencies were declared at $P < 0.10$.

Data from two cow-periods were excluded from statistical analysis. One cow developed a cecal torsion requiring surgery early in period 3; data and samples were not collected from this cow during period 3, but she recovered sufficiently for her period 4 data to be used. Period 4 data from a second cow were excluded after she showed signs of estrus because her intake and milk yield were outliers (outside the 95% confidence interval for cow-period observations).

RESULTS AND DISCUSSION

Ruminal pH

A primary hypothesis regarding the substitution of fibrous beet pulp for high-moisture corn was that feeding diets containing high concentrations of rapidly-fermentable grain would result in lower average and nadir ruminal pH values, and that the pectin and insoluble fiber in beet pulp would attenuate this decrease. Diets ranged from 35% starch and 24% NDF in 0BP to 18% starch and 32% NDF in 24BP, but when pH was averaged from continuous collection over four days, no difference existed between treatments (Table 3). Daily minimum pH was not affected by treatment, but daily maximum pH decreased linearly as BP increased ($P = 0.05$), reducing the range of pH ($P = 0.07$). Decreasing range and maximum pH suggest that substituting BP for HMC might have reduced diurnal variation in ruminal pH, but variance and standard deviation

did not decrease. Measures of time and area (time x pH) below pH 6.0, 5.8, and 5.5 were not affected by treatment.

Mean pH was near the pK_a of bicarbonate for all treatments. It is possible that the remaining buffering capacity of the rumen fluid, especially the bicarbonate system, was different between treatments while ruminal pH was similar across treatments (Allen, 1997). Therefore, measurement of rumen fluid buffering capacity would be useful in future studies of effects of carbohydrate source on the rumen environment.

Ruminal Volatile Fatty Acid Concentration and Removal

Total VFA concentration in rumen fluid was similar across treatments (Table 4), but the response of ruminal VFA pool (mol) to increasing BP was quadratic ($P < 0.05$) with the largest VFA pool for cows fed 12BP. Rate of valerate absorption from the rumen, an estimate of VFA absorption, was similar (approximately 40%/h) for all treatments. Ruminal liquid dilution rate was reduced by the substitution of BP for HMC ($P = 0.03$), so VFA escaped more slowly in liquid for diets containing more beet pulp, and a larger proportion of VFA produced may have been absorbed across the rumen wall. Because beet pulp contains high concentrations of pectin, which gelatinizes under the appropriate conditions, rumen fluid consistency was also measured. Rumen fluid consistency was not different among treatments ($P > 0.40$). However, across cow-period measurements, there was a quadratic relationship between liquid passage rate and consistency [passage rate = $0.163 + 0.00128 \times \text{consistency} -$

0.000946 x (consistency – 14.4)²; R = 0.52, P = 0.02]. This suggests that consistency and at least one other factor affected liquid passage rate.

Despite large differences in starch content among diets, lactate concentration was similar for 0BP, 6BP, and 12BP ($P > 0.25$). Another study comparing beet pulp and corn grain also reported no difference in lactate concentration (O'Mara et al., 1997).

As expected, substituting beet pulp for high-moisture corn increased the molar proportion of acetate ($P < 0.0001$) and butyrate ($P < 0.05$) and decreased the molar proportion of propionate ($P < 0.001$). Branched-chain VFAs were lower for 24BP than for the other diets ($P < 0.001$), suggesting that proteolysis was reduced and (or) that incorporation of branched-chain amino acids into microbial protein was increased. Fermentation results of other *in vivo* studies comparing beet pulp and corn in TMRs have varied; the only study detecting fermentation acid effects (Clark and Armentano, 1997) reported results similar to those in this experiment for acetate, propionate, and butyrate. VFA concentrations resulting from comparisons of beet pulp and grain in continuous culture differ from *in vivo* results. Two studies (Mansfield et al., 1994; Chester-Jones et al., 1991) reported greater acetate and lower butyrate concentrations with beet pulp added to corn, and these two and another (Bach et al., 1999) reported no effect on propionate concentration. Differences between *in vitro* and *in vivo* responses may be due to the effect *in vivo* of differential rates of VFA absorption from the rumen (Dijkstra et al., 1993).

Ruminal pH and Volatile Fatty Acids

Across treatments, both slower absorption of VFA and slower liquid passage from the rumen led to higher VFA concentrations in the rumen (Figure 1a,b); VFA concentration was negatively correlated with rate of valerate absorption ($R = -0.70$, $P < 0.0001$) and with liquid passage rate ($R = -0.54$, $P < 0.01$). As VFA concentration increased, ruminal pH decreased ($R = -0.47$, $P = 0.03$) due to greater acid load (Figure 1c). Rate of valerate absorption was unexpectedly slower under lower pH ($R = 0.48$, $P = 0.04$); because VFAs are primarily absorbed in the undissociated state, VFA absorption usually increases under more acidic conditions because a larger proportion of VFA exist in that form (Dijkstra et al., 1993). However, in this case, lower ruminal pH might have decreased rumen motility, resulting in less thorough mixing of ruminal contents and thus a slower rate of VFA absorption. Although rumen motility was not measured, valerate absorption rate increased with greater passage rate of indigestible NDF (Table 5; $R = 0.43$, $P = 0.02$) and tended to increase with greater liquid passage rate (Table 5; $R = 0.33$, $P = 0.08$), which might be indicators of rumen motility. However, passage rate and thus VFA absorption might have been affected by DMI along with or instead of pH, as rate of valerate absorption tended to increase with increasing DMI ($R = 0.31$, $P < 0.10$). Finally, greater utilization of metabolic fuels with higher milk yield might have created a steeper gradient of VFA across the rumen wall, demonstrated by an increase in valerate absorption rate with greater FCM yield ($R = 0.49$, $P < 0.01$).

Ruminal Nitrogen Digestion

Ruminal ammonia concentration responded in a quadratic relationship to dietary BP concentration ($P = 0.04$), with the maximum concentration for 12BP and the lowest concentration for 24BP. Across treatments, ruminal ammonia concentration was not correlated with ruminal pH (Table 7). Ammonia availability probably did not limit microbial protein synthesis for any of the diets, because maximum microbial N production occurs at 5 mg/dl, far below the values in this study, and does not increase at higher ammonia concentrations (Satter and Slyter, 1974). Some amylolytic microbes exhibit extensive proteolysis (Russell et al., 1981), and the additional ammonia N can be used for protein synthesis, absorbed across the rumen wall, or may pass to the duodenum. Microbes which primarily degrade nonstructural carbohydrates obtain approximately two-thirds of their N from amino acids and peptides, not ammonia (Russell et al., 1983), while fibrolytic microbes obtain all N for protein synthesis from ammonia (Bryant, 1973). The rate of incorporation of ammonia into protein for 12BP may not have been sufficient to utilize the ammonia made available by deamination, resulting in a higher ammonia concentration.

Microbial Nitrogen Efficiency

Flow of microbial N in the duodenum decreased linearly ($P = 0.04$) as beet pulp was substituted for corn (Table 6). Mean efficiency of conversion of truly ruminally degraded OM (**TRDOM**) to microbial N (**MNE**) ranged from 36.4 to 41.4 g/kg TRDOM, and these values are within the range of previously published

values of approximately 10 to 50 g/kg TRDOM (Clark et al., 1992). However, MNE was not different among treatments ($P > 0.25$), even though diets varied widely in starch and NDF content. While microbial N flow decreased with added BP, kg TRDOM decreased numerically (not statistically; Chapter 3), so the absence of a MNE treatment effect might have been caused by concurrent decrease in both microbial flow and kg digested.

Ruminal concentrations of amino acids and peptides were not measured in the present study, but their availability could have limited the rate of growth for amylolytic bacteria (Van Kessel and Russell, 1996). However, diets in this experiment were formulated for sufficient RDP; animals were fed 18% dietary crude protein (% of DM) with observed RDP ranging from 55 to 60% (NANMN ranged from 44.6 to 40.2% of N intake). Soybean meal was the primary protein supplement (32-36% of total dietary CP), and soybean meal increases microbial N flow compared to other protein supplements (Clark et al., 1992). Therefore, it is unlikely that amino acid or peptide availability limited microbial growth for the high-starch diets.

Cows absorbed less NAN in the intestines ($P = 0.03$) as NAN flow to the duodenum decreased with increasing BP ($P = 0.02$), but total N absorbed (kg/d) and total tract digestibility of N were not affected by treatment.

Microbial Nitrogen Efficiency and Fermentation

As described above, efficiency of microbial protein production was not affected by drastically altering the carbohydrate source and thus the pattern of

fermentation. Strobel and Russell (1986) suggested that low ruminal pH causes energy spilling and decreases the efficiency with which microbes convert feed energy and nitrogen into protein. However, when data from all cow-periods in this study were pooled, MNE was not associated with mean ruminal pH (Table 7; Figure 2a) or with any other measure of ruminal pH (data not shown). The range of cow-period mean pH values was 5.58 to 6.56, and treatment mean pH was near 6.0 for all treatments. While MNE was not associated with pH for this experiment, such a relationship might exist for a range of pH values below 6.0. Microbial efficiency was not related to ruminal ammonia concentration (Figure 2b; $P > 0.30$) or with VFA concentration (Figure 2c; $P > 0.30$).

Microbial efficiency decreased as ruminal starch digestion rate increased (Figure 2d; $R = -0.61$, $P < 0.01$), suggesting that bacteria spilled energy instead of using the additional energy for greater protein synthesis. Microbial efficiency also decreased as ruminal digestibility of OM increased ($R = -0.70$, $P < 0.0001$); more extensive digestion of OM in the rumen might have been the result of longer retention time, which would increase the opportunity for microbial turnover and therefore reduce MNE. MNE was not related to the rate or extent of ruminal NDF digestion (data not shown).

Microbial Nitrogen Efficiency and Ruminal Passage Kinetics

The dominant factor determining efficiency of microbial protein synthesis was the rate at which microbes escaped the rumen, presumably attached to feed particles. The responses most strongly and positively correlated with MNE

(Table 7) were the ruminal passage rates of particles: starch (Figure 2e; $R = 0.63$, $P < 0.001$), indigestible NDF ($R = 0.45$, $P = 0.02$), and potentially digestible NDF ($R = 0.36$, $P = 0.07$). Microbes associated with particulate digesta may have escaped the rumen more rapidly, reducing microbial protein turnover by reducing both autolysis (Wells and Russell, 1996) and protozoal predation (Wallace and McPherson, 1987). Although microbial N flow increased with greater DMI ($R = 0.51$, $P < 0.01$), microbial efficiency was not related to DMI ($P > 0.60$), so DMI probably did not cause the increased passage rate and subsequent increased microbial efficiency.

Oba and Allen (2002b) have reported similar responses of MNE, or failures of MNE to respond, to pH, fermentation characteristics, and digestion kinetics in the rumen when lactating cows were fed diets quite different from diets in the present study; diets contained ground high-moisture corn or dry ground corn at two dietary starch concentrations. They also found no relationship between MNE and ruminal pH or ammonia concentration, and they reported that MNE decreased as rate of ruminal starch increased. A positive relationship between ruminal starch passage rate and MNE was also demonstrated (Oba and Allen 2002b). The similar responses of MNE in two experiments with very different treatments suggest that, among high-producing cows, the efficiency of microbial protein production is not always affected by ruminal pH or ammonia concentration, that it is reduced at high rates of starch fermentation, and that it is improved by increased particulate passage rate from the rumen.

Although passage rate of particulate digesta affected MNE, liquid passage rate was unrelated to MNE (Table 7; Figure 2f). Previous experiments have manipulated passage rate *in vitro* and in sheep, especially by increasing liquid dilution rate, and have reported responses in microbial protein flow and production that suggest that increased dilution rate results in greater MNE (Isaacson et al., 1975; Harrison et al., 1976; Kennedy and Milligan, 1978). However, response to liquid passage rate was not separated from the potential concurrent response in particulate passage rate. In addition, the range of dilution rates measured in the present experiment are generally higher than the ranges of rates reported in these other studies. Because liquid passage rate and rumen fluid consistency demonstrated a quadratic relationship, as described earlier, and because fluid consistency was not affected by treatment (Table 4), more than one factor (including consistency) likely affected liquid passage and may have caused particulate and liquid passage to be uncoupled.

Wells and Russell (1996) proposed that the rate of microbial turnover (%/h) decreases geometrically and asymptotically as dilution rate (h^{-1}) increases, and that the extent of the effect depends upon microbial lysis rate. The range of individual dilution rates measured in the present study (10.6-22.9%/h) would fall among and above the top half of dilution rates in the proposed model. Lysis rate was not measured, but assuming that lysis did not occur at the highest rate used in the model (10%/h), an increase of 12%/h in dilution rate would lead to no more than a 5% decrease in microbial turnover, and, it follows, only a small increase in microbial efficiency. More rapid particle passage rate was therefore much more

important than fluid passage rate in improving microbial efficiency. Increasing particulate passage rate removed bacteria from the rumen more quickly, which increased the rate at which microbes escaped the rumen and reduced the extent of microbial lysis, thus increasing microbial efficiency.

SUMMARY

Substituting dried, pelleted beet pulp for high-moisture corn from 0 to 24% of diet DM altered ruminal fermentation but did not affect daily mean or minimum ruminal pH, nor rate of VFA absorption. Among individual observations, ruminal VFA concentration was associated negatively with pH, rate of valerate absorption, and liquid passage rate. Microbial N efficiency was not related to ruminal pH and was not affected by replacing high-moisture corn with beet pulp, but was increased by greater particulate passage rate.

Table 1. Nutrient composition of high-moisture corn and dried, pelleted beet pulp.

Nutrient	High-moisture corn	Beet pulp
DM, % as fed	71.5	84.9
	-----% of DM-----	
NDF	10.0	39.9
CP	8.3	8.9
Indigestible NDF	3.8	8.0
Starch	70.5	3.9
Ether extract	4.7	0.7
Ash	1.0	7.8

Table 2. Ingredient and nutrient composition of experimental diets.

	0% BP	6% BP	12% BP	24% BP
Ingredients	-----% of DM-----			
Corn silage	20.1	20.1	20.1	20.1
Alfalfa silage	19.9	19.9	19.9	19.9
Protein mix	19.5	19.5	19.5	19.5
Mineral vitamin mix	4.8	4.8	4.8	4.8
Dried, pelleted beet pulp	0	6.1	12.1	24.3
High-moisture corn	35.6	29.5	23.5	11.4
Nutrient				
DM	50.2	50.5	50.8	51.6
Starch	34.6	30.5	26.5	18.4
NDF	24.3	26.2	28.0	31.6
Indigestible NDF	9.4	9.6	9.8	10.2
Forage NDF	17.1	17.1	17.1	17.1
CP	18.0	18.0	18.0	18.1
% Starch from high-moisture corn	72.7	68.3	62.6	43.7
% NDF from forage	70.2	65.3	61.1	54.1
% NDF from beet pulp	0.0	9.3	17.3	30.7

Table 3. Effects of substitution of pelleted beet pulp for high-moisture corn on ruminal pH.

	Treatment LS Means					<i>P</i>		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
Daily ruminal pH								
Mean	5.93	5.97	6.02	5.94	0.10	0.53	0.16	0.17
Minimum	5.36	5.39	5.40	5.47	0.12	0.59	0.16	0.95
Maximum	6.56	6.55	6.55	6.43	0.08	0.17	0.05	0.28
Range	1.23	1.19	1.06	1.07	0.09	0.24	0.07	0.50
Variance	0.14	0.12	0.11	0.11	0.02	0.42	0.17	0.34
Standard deviation	0.36	0.34	0.32	0.33	0.03	0.40	0.14	0.26
Time < 6.0	12.9	12.0	12.1	13.8	2.2	0.43	0.26	0.15
Time < 5.8	9.3	8.1	7.4	9.6	2.0	0.43	0.13	0.10
Time < 5.5	4.8	4.1	2.7	3.6	1.4	0.40	0.15	0.24
Area below 6.0	634	565	481	591	149	0.65	0.22	0.24
Area below 5.8	369	325	249	310	102	0.63	0.25	0.32
Area below 5.5	118	109	75	79	42	0.70	0.47	0.66

Table 4. Effects of substitution of pelleted beet pulp for high-moisture corn on ruminal fermentation and VFA removal.

	Treatment LS Means					<i>P</i>		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
Total VFA, mM	138	141	142	142	3	0.35	0.14	0.26
Total ruminal VFA pool, mol	3.99	4.10	4.63	3.83	0.32	0.12	0.07	<0.05
Rate of valerate absorption, %/h	41.3	43.2	37.3	40.0	4.0	0.51	0.57	0.69
Liquid passage rate, %/h	18.6	17.5	16.7	16.6	1.0	0.13	0.03	0.21
Rumen fluid consistency ^a	14.6	14.2	14.0	15.1	1.3	0.84	0.51	0.42
Lactate, mM	0.30	0.31	0.26	0.08	0.16	0.72	0.27	0.64
VFA, mol/100 mol								
Acetate	56.9	59.1	60.2	61.6	1.1	<0.01	<0.01	0.04
Propionate	27.0	24.9	23.0	22.4	1.1	<0.01	<0.01	0.04
Butyrate	11.3	11.5	12.2	12.3	0.4	0.19	0.04	0.60
Branched-chain VFA	2.33	2.34	2.31	1.85	0.09	<0.01	0.28	0.01
Acetate:Propionate	2.19	2.45	2.69	2.80	0.16	<0.01	<0.01	0.04

^aDistance traveled (cm) by front edge of 20 ml rumen liquid in 300 s in Bostwick's consistometer.

Table 5. Pearson correlation coefficients for ruminal VFA concentration and related variables.

	(1)	(2)	(3)	(4)
(1) Ruminal [VFA], mM	...	...	...	...
(2) pH, daily mean	-0.47 ¹	...	...	...
(3) Valerate absorption rate, %/h	-0.70 ¹	0.48 ¹	...	...
(4) Liquid passage rate, %/h	-0.54 ¹	0.15	0.34	...

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

Table 6. Effects of substitution of pelleted beet pulp for high-moisture corn on N digestion.

	Treatment LS Means					<i>P</i>		
	0% BP	6% BP	12% BP	24% BP	SE	Trt	L	Q
N intake, g/d	678	692	697	641	21	0.18	0.23	0.09
Ruminal ammonia, mg/dl	19.6	19.3	21.4	17.8	0.9	0.02	0.09	0.04
Flow to duodenum								
Ammonia N, g/d	19.8	20.6	21.5	19.2	1.5	0.34	0.14	0.10
NAN ¹								
g/d	693	707	691	610	32	0.09	0.02	0.19
% of N intake	102.0	102.5	97.4	95.2	4.2	0.43	0.11	0.92
NANMN ²								
g/d	278	306	287	255	34	0.66	0.42	0.39
% of N intake	40.8	44.6	40.5	40.2	5.1	0.85	0.74	0.69
% duodenal NAN	39.7	43.0	40.9	42.1	4.4	0.90	0.74	0.74
Microbial N								
g/d	415	401	404	352	27	0.22	0.04	0.55
% duodenal NAN	60.3	57.0	59.0	57.9	4.0	0.90	0.73	0.74
g / kg TRDOM ³	36.4	41.4	40.8	39.5	3.3	0.71	0.29	0.33
NAN digested postruminally								
g/d	492	492	485	428	25	0.13	0.03	0.32
% of intake	72.5	71.6	68.7	67.3	3.5	0.58	0.16	0.87
% of duodenal passage	71.2	69.5	70.3	70.2	1.6	0.68	0.64	0.41
N apparently digested in total tract								
g/d	476	477	498	458	15	0.32	0.23	0.15
%	70.5	69.1	71.7	71.5	1.6	0.40	0.31	0.84

¹ Non-ammonia N.

² Non-ammonia non-microbial N.

³ Truly ruminally digested OM.

Table 7. Pearson correlation coefficients for microbial efficiency and related variables.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) Microbial efficiency, g MN ³ /kg TRDOM ⁴	...	...	...	...	...	...	...	...	...	...
(2) Ruminant pH mean	-0.02	...	...	...	...	...	...	...	...	...
(3) Ruminant [ammonia], mg/dl	0.19	-0.13	...	...	...	...	...	...	...	...
(4) Ruminant [VFA], mM	-0.04	-0.55 ¹	0.34 ¹	...	...	...	...	...	...	...
(5) Ruminant starch digestion rate, %/h	-0.61 ¹	-0.21	-0.24	0.11	...	...	...	...	...	...
(6) TRDOM ⁴ , %	-0.70 ¹	0.11	-0.59 ¹	-0.15	0.70 ¹	...	...	...	...	...
(7) Passage rate of starch, %/h	0.65 ¹	0.33	0.34	-0.21	-0.83 ¹	-0.71 ¹	...	...	...	...
(8) Passage rate of pdNDF ⁵ , %/h	0.32 ²	0.44	-0.12	-0.64 ¹	-0.39	-0.27	0.39 ²	...	...	...
(9) Passage rate of INDF ⁶ , %/h	0.51 ¹	0.46 ²	-0.26 ¹	-0.35 ¹	-0.36	-0.17	0.37 ²	0.64 ¹	...	...
(10) Passage rate of liquid ⁷ , %/h	0.20	0.27	0.08	-0.55 ¹	-0.28	-0.25	0.26	0.56 ¹	0.31	...

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

² Tendency for significant correlation ($P < 0.10$).

³ Microbial N.

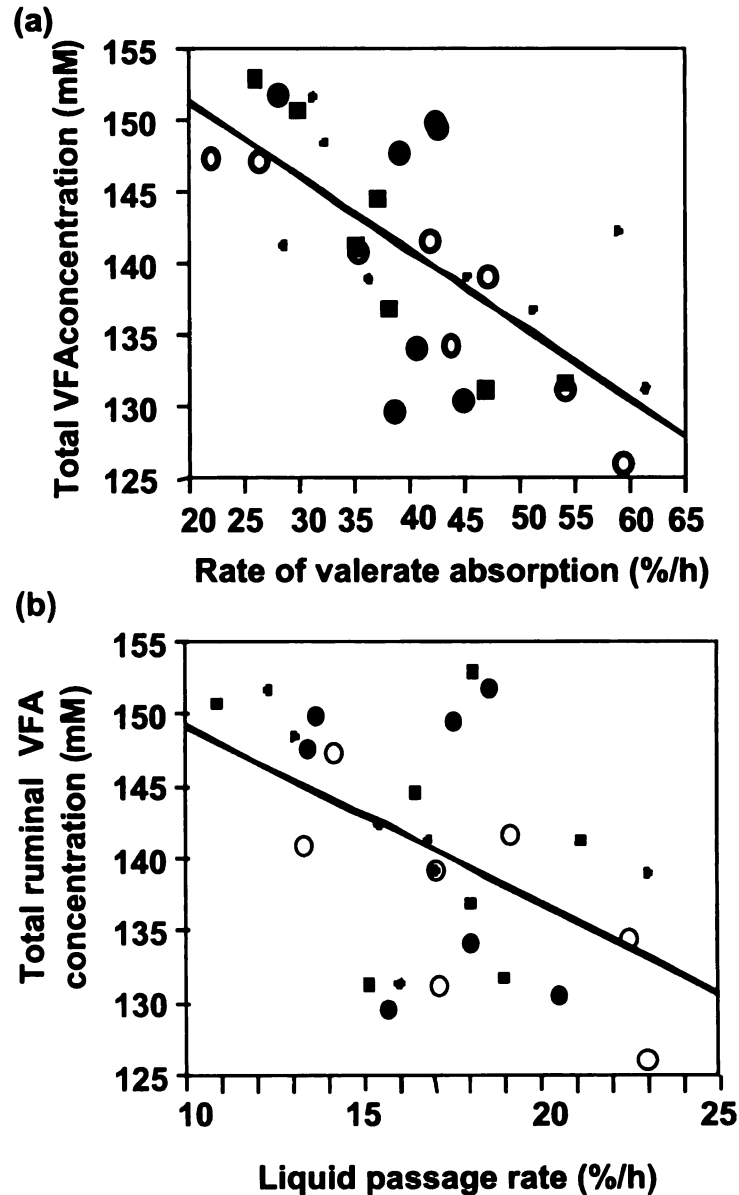
⁴ Truly ruminally digested OM.

⁵ Potentially digestible NDF.

⁶ Indigestible NDF.

⁷ Measured using Co-EDTA.

Figure 1. Relationship between VFA concentration in rumen fluid and (a) rate of valerate absorption ($[VFA] = 162 - 52 \times \text{rate of valerate absorption}$; $R = -0.69$; $P < 0.01$); (b) liquid passage rate ($[VFA] = 162 - 1233 \times \text{liquid passage rate}$; $R = -0.49$; $P < 0.01$) and (c) mean ruminal pH ($[VFA] = 221 - 14 \times \text{mean pH}$; $R = -0.44$; $P < 0.03$). O denotes 0% beet pulp, + denotes 6% beet pulp, ■ denotes 12% beet pulp, and ● denotes 24% beet pulp (%diet DM) substituted for high-moisture corn.



(c)

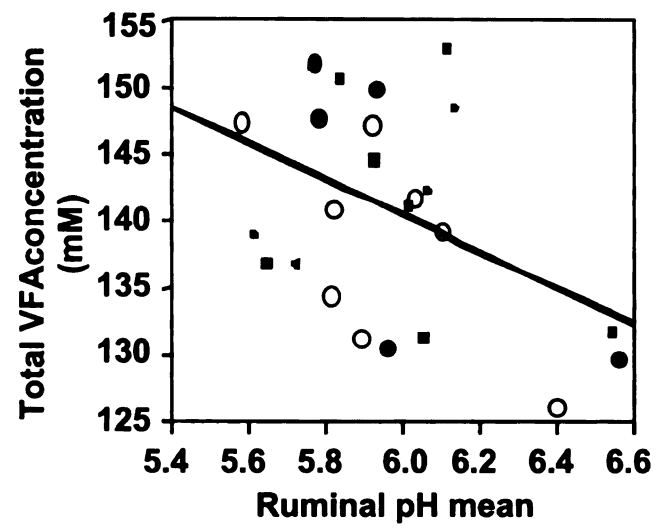
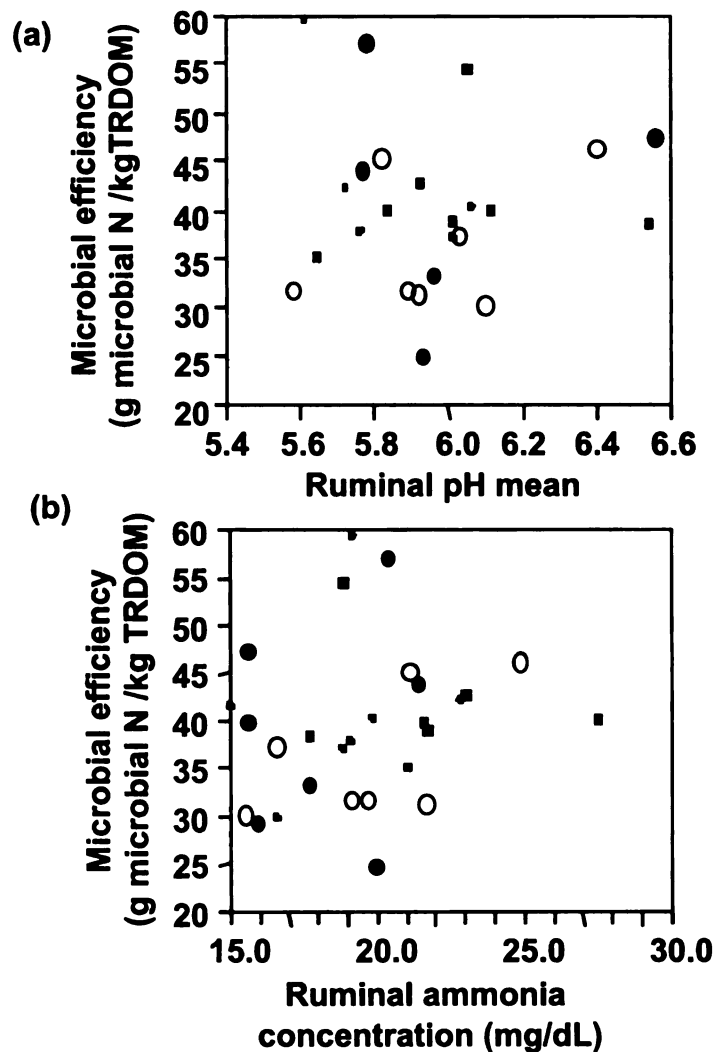
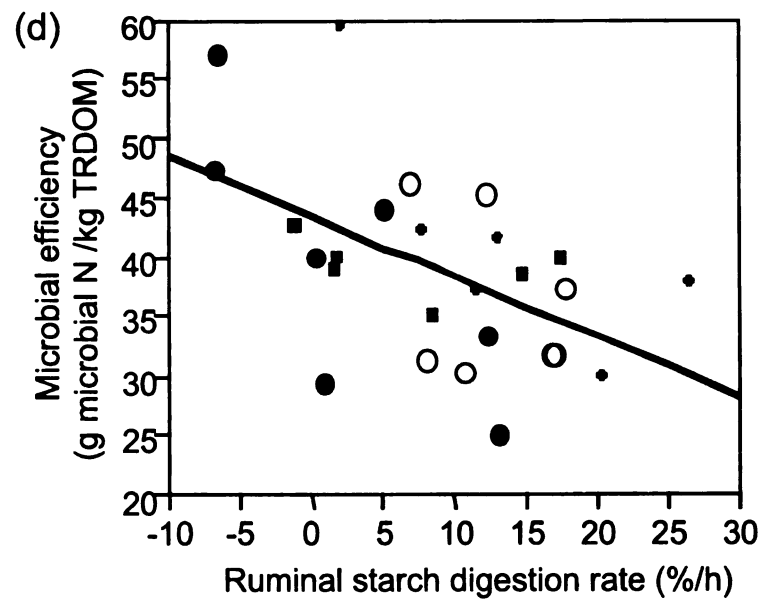
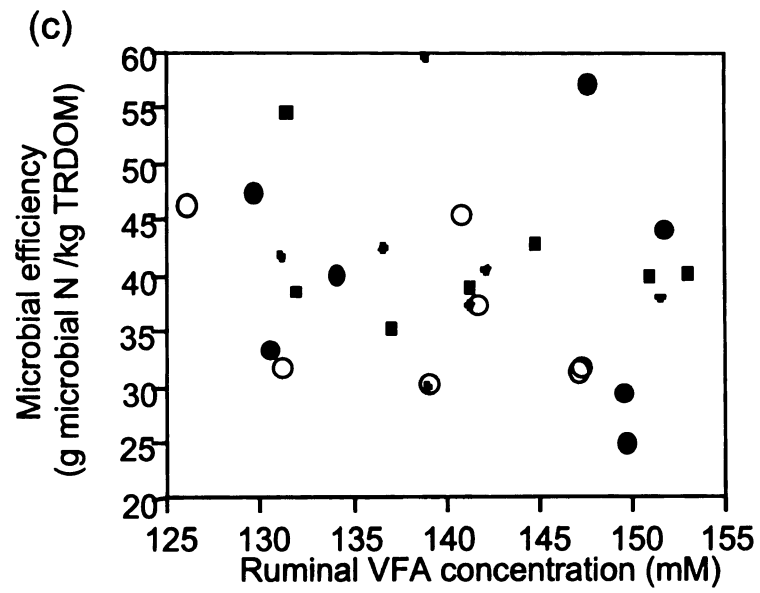
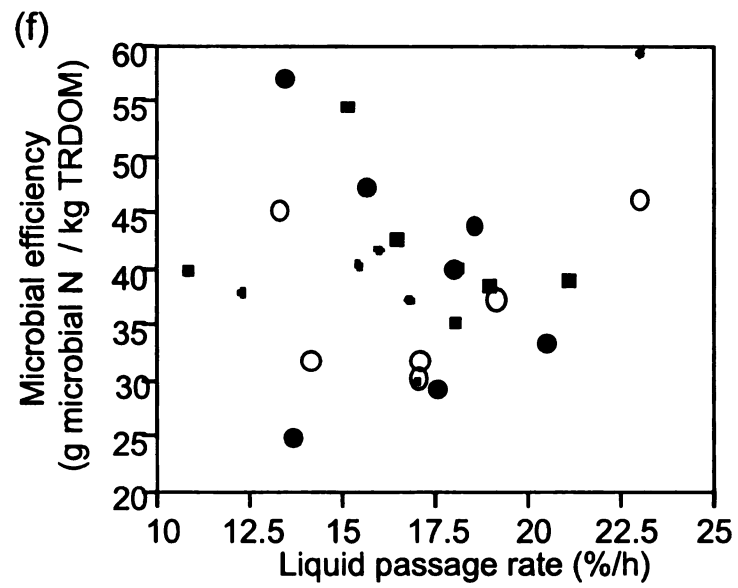
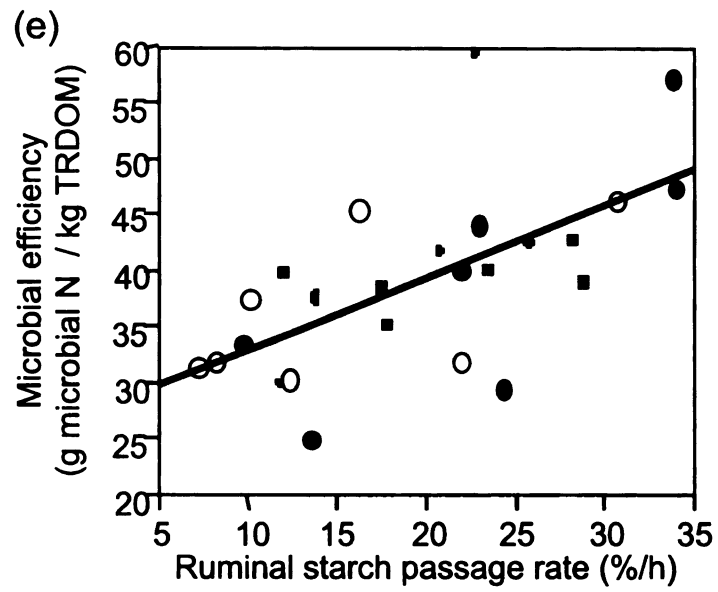


Figure 2. Relationship between microbial N efficiency (g microbial N / kg TRDOM) and (a) 24-h mean ruminal pH ($R = 0.00$; $P > 0.95$); (b) concentration of ammonia in rumen fluid ($R = 0.17$; $P > 0.30$); (c) concentration of VFA in rumen fluid ($R = 0.20$; $P > 0.30$); (d) ruminal starch digestion rate ($MNE = 4.35 - 0.05 \times \text{starch digestion rate}$; $R = -0.52$; $P < 0.01$); (e) starch passage rate ($MNE = 2.65 + 0.06 \times \text{starch passage rate}$; $R = 0.63$; $P < 0.01$); and (f) liquid passage rate ($R = 0.10$; $P > 0.50$). $\circ$ denotes 0% beet pulp, $+$ denotes 6% beet pulp, $\blacksquare$ denotes 12% beet pulp, and $\bullet$ denotes 24% beet pulp (%diet DM) substituted for high-moisture corn.







CHAPTER 5

CONCLUSIONS AND IMPLICATIONS

Substituting dried, pelleted beet pulp for part of the grain in a high-concentrate diet can improve nutrient utilization. Even though substituting beet pulp for high-moisture corn decreased dry matter intake due to greater ruminal fill and increased the amount of time required to consume the diet, energy intake was not reduced. Therefore, milk yield was unaffected, and yield of fat-corrected milk was slightly improved by lower rates of beet pulp substitution.

Rates of fermentation and passage for starch and fiber were affected drastically by diet composition. Substituting rapidly-fermented fiber for rapidly-fermented starch improved the rate and extent of NDF digestion without negatively affecting milk yield. Although this diet change also increased the passage rate of starch from the rumen and reduced ruminal starch digestion, postruminal starch digestion compensated for the decreased ruminal digestion. Because compensatory starch digestion did take place and there was no significant compensatory fiber digestion in the intestines when diets contained more corn, adding a highly digestible fiber source to a high grain diet could potentially result in even greater energy intake. Increasing diet digestibility can also reduce the volume of waste per unit milk produced.

The use of nitrogen sources in feed for microbial protein synthesis was neither improved nor made more efficient by adding beet pulp to a highly fermentable diet. However, the relationship between ruminal particulate passage rate and microbial efficiency was confirmed; and since beet pulp did increase

starch passage rate, it might also improve microbial efficiency in some feeding situations. Increasing the efficiency of nitrogen use might reduce the amount of nitrogen required to maximize absorbable protein and subsequent milk yield. This could reduce the cost of feed because protein supplements are one of the most expensive components of dairy rations, and it may also aid in the reduction of waste nitrogen entering the environment.

Maintaining a “normal” ruminal pH is one of the primary goals in dairy nutrition, yet much study is still required to clarify the factors affecting pH and the mechanisms by which pH affects digestion, health, and production. In this study, ruminal pH did not differ between cows fed a diet containing 24.3% NDF with no beet pulp and cows fed a diet containing 31.6% NDF with the maximum beet pulp inclusion rate (24%). The absence of a pH change could be attributed to equal dietary forage NDF contents, except that adding beet pulp increased chewing, which probably induced greater influx of salivary buffers. Despite abundant support in the literature for a link between pH and microbial efficiency (Strobel and Russell, 1986), we failed to detect a relationship between any measure of daily mean pH or variation in pH and microbial efficiency. Similarly, although increasing fluid pH has previously reduced ruminal VFA absorption rate (Dijkstra et al., 1993), greater daily mean pH was related to greater valerate absorption rate in the present experiment. Therefore, our concepts of the association of pH with measures of digestion and production need to be reevaluated.

In addition to the causes and effects of altered ruminal pH, other questions are raised by the results of this experiment. Beet pulp was added to a diet with one of the lowest possible forage contents and one of the highest possible corn grain contents advisable for the grain, forage, and feeding conditions. However, it is not clear how beet pulp would affect digestion and production at lower or higher dietary contents of forage NDF, nor for grains differing in rate or extent of digestion. The effects on particulate and liquid passage, as well as the relationships between passage rates and other ruminal measurements, suggest that rumen motility was affected by adding beet pulp. Therefore, measurement of the effects of beet pulp on rumen motility would help clarify the relative usefulness of beet pulp as an effective fiber source and would help explain the effects of beet pulp on volatile fatty acid absorption and on particulate and liquid passage rate. Finally, the different effects of particulate and liquid passage rates on microbial efficiency suggest that there might be several strategies for improving microbial efficiency through increased passage rate without reducing the digestibility of the diet.

Even though forage NDF was low (17% of DM) for all treatments and starch concentration was 35% of DM for the diet containing no beet pulp, ruminal pH was not extremely low and milk fat concentrations for all treatments were above 3.70%, indicating that the diets fed in this experiment were not exceptionally highly fermentable. Beet pulp probably would be of greater benefit, especially in moderating ruminal pH, when more highly fermentable diets are fed, or when a similar diet is fed under less controlled conditions. While the results of

the present experiment suggest that beet pulp can be included as up to 12 percent of diet dry matter without reducing fat-corrected milk yield, the optimal rate of substitution in individual situations should be selected based on nutritional management practices and characteristics of diet, including diet forage-to-concentrate ratio, grain type and fermentability, and forage type, particle size, and quality.

REFERENCES

- Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. *J. Anim. Sci.* 74:3063-3075.
- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80:1447-1462.
- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598-1624.
- Allen, M. S., L. E. Armentano, M. N. Pereira, Y. Ying, and J. Xu. 2000. Method to measure fractional rate of volatile fatty acid absorption from the rumen. *Proc. 2000 Rumen Function Conference, Chicago.* 25:24.
- Anil, M. H., and J. M. Forbes. 1980. Feeding in sheep during intraportal infusions of short-chain fatty acids and the effect of liver denervation. *J. Physiol.* 298:407-414.
- Armentano, L., and M. Periera. 1997. Measuring the effectiveness of fiber by animal response trials. *J. Dairy Sci.* 80:1416-1425.
- Aspinall, G. O. 1970. Polysaccharides. Pergamon Press, Oxford, England.
- Bach, A., I. K. Yoon, M. D. Stern, H. G. Jung, and H. Chester-Jones. 1999. Effects of type of carbohydrate supplementation to lush pasture on microbial fermentation in continuous culture. *J. Dairy Sci.* 82:153-160.
- Bailoni, L., M. Ramanzin, A. Simonetto, N. Oblakov, S. Schiavon, and G. Bittante. 1998. The effect of in vitro fermentation on specific gravity and sedimentation measurements of forage particles. *J. Anim. Sci.* 76:3095-3103.
- Beauchemin, K. A., B. I. Farr, and L. M. Rode. 1991. Enhancement of the effective fiber content of barley-based concentrates fed to dairy cows. *J. Dairy Sci.* 74:3128-3139.
- Bhattacharya, A. N., and F. T. Sleiman. 1971. Beet pulp as a grain replacement for dairy cows and sheep. *J. Dairy Sci.* 54:89-94.
- Bhattacharya, A. N., and W. F. Lubbadah. 1971. Feeding high levels of beet pulp in high concentrate dairy rations. *J. Dairy Sci.* 54:95-99.
- Bhatti, S. A., and J. L. Firkins. 1995. Kinetics of hydration and functional specific gravity of fibrous feed by-products. *J. Anim. Sci.* 73:1449-1458.

- Bichsel, S. E. 1988. An overview of the U. S. beet sugar industry. Pp. 1-8 in M. A. Clarke and M. A. Godshall, eds. Chemistry and Processing of Sugarbeet and Sugarcane. Elsevier, New York.
- Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in rumen fluid and in vitro media. J. Dairy Sci. 63:64-75.
- Bryant, M. P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed. Proc. 32:1809-1813.
- Bryant, M. P., and R. N. Doetsch. 1954. A study of actively cellulolytic rod-shaped bacteria of the bovine rumen. J. Dairy Sci. 37:1176-1183.
- Bryant, M. P., and N. Small. 1956a. The anaerobic monotrichous butyric acid-producing curved rod-shaped bacteria of the rumen. J. Bacteriol. 72:16-21.
- Bryant, M. P. and N. Small. 1956b. Characteristics of two new genera of anaerobic curved rods isolated from the rumen of cattle. J. bacterial. 72:22-26.
- Bryant, M. P., N. Small, C. Bouma, and H. Chu. 1958. *Bacteroides ruminicola* n. sp. And *Succinimonas amylolytica* the new genus and species. Species of succinic acid-producing anaerobic bacteria of the bovine rumen. J. Bacteriol. 76:15-23.
- Bywater, A. C. 1984. A generalised model of feed intake and digestion in lactating cows. Agric. Syst. 13:167-186.
- Callison, S. L., J. L. Firkins, M. L. Eastridge, and B. L. Hull. 2001. Site of nutrient digestion by dairy cows fed corn of different particle size or steam-rolled. J. Dairy Sci. 84:1458-1467.
- Calsamiglia, S., A. Ferret, and M. Devant. 2002. Effects of pH and pH fluctuations on microbial fermentation and nutrient flow from a dual-flow continuous culture system. J. Dairy Sci. 85:574-579.
- Campling, R. C. 1970. Physical regulation of voluntary intake. Pp. 226-234 in A. T. Phillipson, ed. Physiology of Digestion and Metabolism in the Ruminant. Oriel Press, Ltd., Newcastle-upon-Tyne, England.
- Chester-Jones, H., M. D. Stern, H. M. Metwally, J. G. Linn, and D. M. Ziegler. 1991. Effects of dietary protein-energy interrelationships on holstein steer performance and ruminal bacterial fermentation in continuous culture. J. Anim. Sci. 69:4956-4966.

- Choi, B. R., and M. S. Allen. 1999. Intake regulation by volatile fatty acids and physical fill. *S. Afr. J. Anim. Sci* 29(ISRP):40-41 (Abstr.).
- Clark, J. H., and C. L. Davis. 1983. Future improvement fo milk production: potential for nutritional improvement. *J. Anim. Sci.* 57:750-764.
- Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *J. Dairy Sci.* 75:2304-2323.
- Clark, P. W., and L. E. Armentano. 1997. Influence of particle size on the effectiveness of beet pulp fiber. *J. Dairy Sci.* 80:898-904.
- Crocker, L. M., E. J. DePeters, J. G. Fadel, H. Prez-Monti, S. J. Taylor, J. A. Wyckoff, and R. A. Zinn. 1998. Influence of processed corn grain in diets of dairy cows on digestion of nutrients and milk composition. *J. Dairy Sci.* 81:2394-2407.
- Dado, R. G., and M. S. Allen. 1993. Continuous computer acquisition of feed and water intake, chewing reticular motility, and ruminal pH of cattle. *J. Dairy Sci.* 76:1589-1600.
- Dado, R. G., and M. S. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. *J. Dairy Sci.* 78:118-133.
- Dehority, B. A. 1969. Pectin-fermenting bacteria isolated from the bovine rumen. *J. Bacteriol.* 99:189-196.
- De Visser, H., P. L. van der Togt, H. Huisert, and S. Tamminga. 1992. Structural and non-structural carbohydrates in concentrate supplements of silage-based dairy cow rations. 2. Rumen degradation, fermentation and kinetics. *Netherlands J. Agric. Sci.* 40: 431-445.
- De Visser, H., H. Huisert, and R. S. Ketelaar. 1991. Dried beet pulp, pressed beet pulp and maize silage as substitutes for concentrates in dairy cow rations. 2. Feed intake, fermentation pattern and ruminal degradation characteristics. *Netherlands J. Agric. Sci.* 39: 21-30.
- Dewhurst, R. J., K. Aston, W. J. Fisher, R. T. Evans, M. S. Dhanoa, and A. B. McAllan. 1999. Comparison of energy and protein sources offered at low levels in grass-silage-based diets for dairy cows. *Anim. Sci.* 68:789-799.
- Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining, and S. Tamminga. 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as

influenced by volatile fatty acid concentration, pH and rumen liquid volume. *Br. J. Nutr.* 69:385-396.

Emery, R. S. 1988. Milk fat depression and the influence of diet on milk composition. Pp. 289-306 *in* T. H. Herdt, ed. *Metabolic Diseases of Ruminant Livestock*. Vol. 4, no. 2. *Vet. Clin. North Amer. Food Anim. Pract.* W. B. Saunders Company, Philadelphia.

Fahey, G. C., Jr., and L. L. Berger. 1988. Carbohydrate nutrition of ruminants. Pp. 269-297 *in* D. C. Church, ed. *The Ruminant Animal: digestive physiology and nutrition*. Waveland Press, Inc., Prospect Heights, Illinois.

Firkins, J. L. 1996. Maximizing microbial protein synthesis in the rumen. *J. Nutr.* 126:1347S-1354S.

Firkins, J. L. 1997. Effects of feeding nonforage fiber sources on site of fiber digestion. *J. Dairy Sci.* 80:1426-1437.

Forbes, J. M. 1995. *Voluntary Food Intake and Diet Selection in Farm Animals*. CAB International, New York.

Forbes, J. M., and F. D. Provenza. 2000. Integration of learning and metabolic signals into a theory of dietary choice and food intake. Pp. 3-19 *in* P. B. Cronjé, ed. *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*. CAB International, New York.

Friggens, N., G. C. Emmans, S. Roberston, D. G. Chamberlain, C. T. Whittemore, and J. D. Oldham. 1995. The lactational responses of dairy cows to amount of feed and to the source of carbohydrate energy. *J. Dairy Sci.* 78:1734-1744.

Gaillard, B. D. E. 1962. The relationship between cell wall constituents of roughages and the digestibility of the organic matter. *J. Agric. Sci.* 59:369-373.

Goering, H. K., and P. J. Van Soest. 1970. *Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications)*. Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Gordon, G. L. R., and M. W. Phillips. 1992. Extracellular pectin lyase produced by *Neocallimastix* sp. LM1, a rumen anaerobic fungus. *Letters in Appl. Microbiol.* 15:113-115.

Grant, R. J. 1997. Interactions among forages and nonforage fiber sources. *J. Dairy Sci.* 80:1438-1446.

Grant, R. J., and D.R. Mertens. 1992. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. J. Dairy Sci. 75:2762-2768.

Grant, R. J., V. F. Colenbrander, and D. R. Mertens. 1990. Milk fat depression in dairy cows: role of particle size of alfalfa hay. J. Dairy Sci. 73:1823-1833.

Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, and D. L. Palmquist. 1997. Role of insulin in the regulation of milk fat synthesis in dairy cows. J. Dairy Sci. 80:1076-1084.

Grovum, W. L. 1995. Mechanisms explaining the effects of short chain fatty acids on feed intake in ruminants –osmotic pressure, insulin and glucagons. Pp. 173-198 in W. v. Engelhardt, S. Leonhard-Marek, G. Breves, D. Giesecke, eds. Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. Ferdinand Enke Verlag, Stuttgart.

Hach, C. C., B. K. Bowden, A. B. Lopelove, and S. V. Brayton. 1987. More powerful peroxide Kjeldahl digestion method. J. AOAC 70:783-787.

Harrison, D. G., D. E. Beever, D. J. Thomson, and D. F. Osbourn. 1976. Manipulation of fermentation in the rumen. J. Sci. Fd Agric. 26:617-620.

Hespell, R. B., and P. J. O'Bryan-Shah. 1988. Esterase activities in *Butyrivibrio fibrisolvens* strains. Appl. Environ. Microbiol. 54:1917-1922.

Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755-2766.

Hoover, W. H., and S. R. Stokes. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci. 74:3630-3644.

Huhtanen, P. 1987. The effect of dietary inclusion of barley, unmolassed sugar beet pulp and molasses on milk production, digestibility and digesta passage in dairy cows given silage based diet. J. Agric. Sci. Finland. 59:101-120.

Isaacson, H. R., F. C. Hinds, M. P. Bryant, and F. N. Owens. 1975. Efficiency of energy utilization by mixed rumen bacteria in continuous culture. J. Dairy Sci. 58:1645-1659.

JMP® Version 3.2. 1997. SAS Institute Inc., Cary, North Carolina.

Karkalas, J. 1985. An improved enzymatic method for the determination of native and modified starch. J. Sci. Food Agric. 36:1019-1027.

Keady, T. W. J., C. S. Mayne, D. A. Fitzpatrick, and M. Marsden. 1999. The effects of energy source and level of digestible undegradable protein in

concentrates in silage intake and performance of lactating dairy cows offered a range of grass silages. *Anim. Sci.* 68:763-777.

Kennedy, P. M., and L. P. Milligan. 1978. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. *Br. J. Nutr.* 39:105-117.

Kertesz, Z. I. 1951. *The Pectic Substances*. Interscience Publishers, Inc. New York.

Kyriazakis, I., and G. C. Emmans. 1995. The voluntary feed intake of pigs given feeds based on wheat bran, dried citrus pulp and grass meal, in relation to measurements of feed bulk. *Br. J. Nutr.* 73:191-207.

Lechner-Doss, M., M. Kaske, and W. V. Engelhardt. 1991. Factors affecting the mean retention time of particles in the forestomach of ruminants and camelids. Pp. 455-482 *in* T. Tsuda, Y. Sasaki, and R. Kawashima, eds. *Physiological Aspects of Digestion and Metabolism in Ruminants*. Academic Press, Inc. New York.

Leedle, J. A. Z., M. P. Bryant, and R. B. Hespell. 1982. Diurnal variations in bacterial numbers and fluid parameters in ruminal contents of animals fed low- or high-forage diets. *Appl. Environ. Microbiol.* 44:402-412.

Lehman, F. 1941. Die lehre vom ballast. *Z. Tierphysiol. Tierernaehr. Futtermittelkd.* 5:155-173.

Leng, R. A. 1970. Formation and production of volatile fatty acids in the rumen. Pp. 406-421 *in* Phillipson, A. T., ed. *Physiology of Digestion and Metabolism in the Ruminant*. Oriel Press, Newcastle upon Tyne, England.

Linzell, J. L., and M. Peaker. 1971. Mechanism of milk secretion. *Physiol. Rev.* 51:564-597.

Mackie, T. R., D. A. Dwyer, K. L. Ingvarsen, P. Y. Chouinard, J. M. Lynch, D. M. Barband, and D. E. Bauman. 1999. Effects of insulin and amino acids on milk protein concentration and yield from dairy cows. *J. Dairy Sci.* 82:1512-1524.

Mansfield, H. R., M. D. Stern, and E. E. Otterby. 1994. Effects of beet pulp and animal by-products on milk yield and *in vitro* fermentation by rumen microorganisms. *J. Dairy Sci.* 77:205-216.

Marounek, M. S. Bartos, and P. Brezina. 1985. Factors influencing the production of volatile fatty acids from hemicellulose, pectin and starch by mixed culture of rumen microorganisms. *Zeitschrift für tierphysiologie, tierernährung und futtermittelkunde* 53:50-58.

Marounek, M., and D. Duskova. 1999. Metabolism of pectin in rumen bacteria *Butyrivibrio fibrisolvens* and *Prevotella ruminicola*. Letters in Appl. Microbiol. 29:429-433.

Mauseth, J. D. 1988. Plant Anatomy. The Benjamin/Cummings Publishing Company, Inc. Reading, Massachusetts.

Mbanya, J. N., M. H. Anil, and J. M. Forbes. 1993. The voluntary intake of hay and silage by lactating cows in response to ruminal infusion of acetate or propionate, or both, with or without distension of the rumen by a balloon. Br. J. Nutr. 69:713-720.

McDowell, G. H., and E. F. Annison. 1991. Hormonal control of energy and protein metabolism. In T. Tsuda, Y. Sasaki, and R. Kawashima, eds. Physiological aspects of digestion and metabolism in ruminants. Academic Press, Inc., New York.

McGuire, M. A., J. M. Griinari, D. A. Dwyer, and D. E. Bauman. 1995. Role of insulin in the regulation of mammary synthesis of fat and protein. J. Dairy Sci. 78:816-824.

Mertens, D. R. 1994. Regulation of forage intake. Pp. 450-493 in G. C. Fahey, Jr., M. Collins, D. R. Mertens, and L. E. Moser, ed. Forage Quality, Evaluation, and Utilization. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Mertens, D. R., and L. O. Ely. 1979. A dynamic model of fiber digestion and passage in the ruminant for evaluating forage quality. J. Anim. Sci. 49:1085-1095.

Michigan Department of Agriculture. 2002. News releases: Michigan Agricultural Commodities Map. www.mda.state.mi.us/market/commodity/sugar.html

Molento, C. F. M., E. Block, R. I. Cue, and D. Petitclerc. 2002. Effects of insulin, recombinant bovine somatotropin, and their interaction on insulin-like growth factor-I secretion and milk protein production in dairy cows. J. Dairy Sci. 85:738-747.

Moorby, J. M., R. J. Dewhurst, C. Thomas, and S. Marsden. 1996. The influence of dietary energy source and dietary protein level on milk protein concentration from dairy cows. Anim. Sci. 63:1-10.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle, sixth revised edition. National Academy Press, Washington, D. C.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle, seventh revised edition. National Academy Press, Washington, D. C.

Neel, J. P. S., E. C. Prigge, and E. C. Townsend. 1995. Influence of moisture content of forage on ruminal functional specific gravity and passage of digesta. *J. Anim. Sci.* 73:3094-3102.

Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *J. Dairy Sci.* 71:2070-2107.

Oba, M., and M. S. Allen. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. *J. Dairy Sci.* 82:589-596.

Oba, M., and M. S. Allen. 2000. Effect of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two levels of dietary NDF: 33. Digestibility and microbial efficiency. *J. Dairy Sci.* 83:1350-1358.

Oba, M. 2002. Effects of propionate infusion on feeding behavior of lactating dairy cows. PhD Diss., Michigan State University.

Oba, M., and M. S. Allen. 2002a. Effects of corn grain conservation method on ruminal digestion kinetics for lactating dairy cows at two dietary starch concentrations. *J. Dairy Sci.* (in press).

Oba, M., and M. S. Allen. 2002b. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. *J. Dairy Sci.* (in press).

O'Connor, J. D., C. J. Sniffen, D. G. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *J. Anim. Sci.* 71:1298-1311.

O'Mara, F. P., J. J. Murphy, and M. Rath. 1997. The effect of replacing dietary beet pulp with wheat treated with sodium hydroxide, ground wheat, or ground corn in lactating cows. *J. Dairy Sci.* 80:530-540.

Ørskov, E. R., and C. Fraser. 1975. The effects of processing of barley-based supplements on rumen pH, rate of digestion, and voluntary intake of dried grass in sheep. *Br. J. Nutr.* 34:493-500.

- Osborne, J. M., and B. A. Dehority. 1989. Synergism in degradation and utilization of intact forage cellulose, hemicellulose, and pectin by three pure cultures of ruminal bacteria. *Appl. Environ. Microbiol.* 55:2247-2250.
- Overton, T. R., M. R. Cameron, J. P. Elliott, and J. H. Clark, and D. R. Nelson. 1995. Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley. *J. Dairy Sci.* 78:1981-1998.
- Owens, F. N., and Goetsch, A. L. 1986. Digesta passage and microbial protein synthesis. Pp. 196 – 223 in L.P. Milligan, W.L. Grovum, and A. Dobson, eds. *Control of digestion and metabolism in ruminants : proceedings of the Sixth International Symposium on Ruminant Physiology.* Prentice-Hall, Englewood Cliffs, N.J.
- Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. *J. Anim. Sci.* 63:1634-1348
- Petit, H. V., and G. F. Tremblay. 1995a. Ruminal fermentation and digestion in lactating cows fed grass silage with protein and energy supplements. *J. Dairy Sci.* 78:342-352.
- Petit, H. V., and G. F. Tremblay. 1995b. Milk production and intake of lactating cows fed grass silage with protein and energy supplements. *J. Dairy Sci.* 78:353-361.
- Plascencia, A., and R. A. Zinn. 1996. Influence of flake density on the feeding value of steam-processed corn in diets for lactating dairy cows. *J. Anim. Sci.* 74:310-316.
- Ramanzin, M., L. Bailoni, and G. Bittante. 1994. Solubility, water-holding capacity, and specific gravity of different concentrates. *J. Dairy Sci.* 77:774-781.
- Reynolds, C. K. 1995. Quantitative aspects of liver metabolism in ruminants. Pages 351-372 in *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, W. v. Englehardt, S. Leonhard-Marek, G. Breves, D. Geisecke, ed. Ferdinand Enke Verlag, Stuttgart, Germany.
- Robinson, P. H. Cl. J. Sniffen, and D. F. Smith. 1985. Development of a one-piece reentrant cannula for the proximal duodenum of dairy cows. *J. Dairy Sci.* 68:986-995.
- Robinson, P. H., P. L. Burgess, and R. E. McQueen. 1990. Influence of moisture content of mixed rations on feed intake and milk production of dairy cows. *J. Dairy Sci.* 73:2916-2921.

- Rogers, J. A., B. C. Marks, C. L. Davis, J. H. Clark. 1979. Alteration of rumen fermentation in steers by increasing rumen fluid dilution rate with mineral salts. *J. Dairy Sci.* 62:1599-1605.
- Roubouts, F. M., and W. Pilnik. 1980. Pectic enzymes. pp. 227-282 in *Microbial Enzymes and Bioconversions*, A. H. Rose, ed. Academic Press, London.
- Russell, J. B., W. G. Bottje, and M. A. Cotta. 1981. Degradation of protein by mixed cultures of rumen bacteria: identification of *Streptococcus bovis* as an actively proteolytic rumen bacterium. *J. Anim. Sci.* 53:242-252.
- Russell, J. B., C. J. Sniffen, and P. J. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *J. Dairy Sci.* 66:763-775.
- Russell, J. B., and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *J. Dairy Sci.* 79:1503-1509.
- Sanson, D. W. 1993. Effects of increasing levels of corn or beet pulp on utilization of low-quality crested wheatgrass hay by lambs and in vitro dry matter disappearance of forages. *J. Anim. Sci.* 71:1615-1622.
- Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Brit. J. Nutr.* 32:199-208.
- Schettini, M. a., E. C. Prigge, and E. I. Nestor. 1999. Influence of mass and volume of ruminal contents on voluntary intake and digesta passage of a forage diet in steers. *J. Anim. Sci.* 77:1896-1904.
- Shaw, J. C., and W. L. Ensor. 1959. Effect of feeding cod liver oil and unsaturated fatty acids on rumen volatile fatty acids and milk fat content. *J. Dairy Sci.* 42:1238-1240.
- Sievert, S. J., and R. D. Shaver. 1993. Carbohydrate and *Aspergillus oryzae* effects on intake, digestion, and milk production by dairy cows. *J. Dairy Sci.* 76:245-254.
- Stouthamer, A. H., and C. Bettenhausen. Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. *Biochim. Biophys. Acta*, 301:53-70.
- Strobel, H. J., and J. B. Russell. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. *J. Dairy Sci.* 69: 2941-2947.

- Sunvold, G. D., H. S. Hussein, G. C. Fahey, Jr., N. R. Merchen, and G. A. Reinhart. 1995. In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and rumen fluid from cattle. *J. Anim. Sci.* 73:3639-3648.
- Sutton, J. D., J. A. Bines, S. V. Morant, and D. J. Napper. 1987. A comparison of starchy and fibrous concentrates for milk production, energy utilization and hay intake by Friesian cows. *J. Agric. Sci., Camb.* 109:375-386.
- Swain, S. M., and L. E. Armentano. 1994. Quantitative evaluation of fiber from nonforage sources used to replace alfalfa silage. *J. Dairy Sci.* 77:2318-2331.
- Thibault, J. F., F. Guillon, and F. M. Rombouts. 1991. Gelation of sugar beet pectin by oxidative coupling. *In* R. H. Walter, ed. *The Chemistry and Technology of Pectin*, p. 119-133. Academic Press, Inc., New York.
- Tjebbes, J. 1988. Utilization of fiber and other non-sugar products from sugarbeet. pp. 139-145 *in* M. A. Clarke and M. A. Godshall, eds. *Chemistry and Processing of Sugarbeet and Sugarcane*. Elsevier Science Publishers B. V., Amsterdam, the Netherlands.
- Torrent, J., D. E. Johnson, and M. A. Kujawa. 1994. Co-product fiber digestibility: kinetic and in vivo assessment. *J. Anim. Sci.* 72:790-795.
- Van Buren, J. P. 1991. Function of pectin in plant tissue structure and firmness. *In* *The chemistry and technology of pectin*, R. H. Walter, ed., pp. 1-22. Academic Press, New York.
- Van Kessel, J. S., and J. B. Russell. 1996. The effect of amino nitrogen on the energetics of ruminal bacteria and its impact on energy spilling. *J. Dairy Sci.* 79:1237-1243.
- Van Soest, P. J. 1994. *Nutritional Ecology of the Ruminant*, 2nd ed. Cornell University Press, Ithaca.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583-3597.
- Van Vuuren, A. M. C. J. van der Koelen, and J. Vroons-de Bruin. 1993. Ryegrass versus corn starch or beet pulp fiber diet effects on digestion and intestinal amino acids in dairy cows. *J. Dairy Sci.* 76:2692-2700.
- Voelker, J. A., G. M. Burato, and M. S. Allen. 2002. Effects of pretrial milk yield on responses of feed intake, digestion, and production to dietary forage concentration. *J. Dairy Sci.* 85: (in press).

Waite, R., and A. R. N. Gorrod. 1959. The comprehensive analysis of grasses. *J. Sci. Food Agric.* 10:317-326.

Wallace, R. J., and C. A. McPherson. 1987. Factors affecting the rate of breakdown of bacterial protein in rumen fluid. *Br. J. Nutr.* 58:313-323.

Weekes, T. E. C. 1991. Hormonal control of glucose metabolism. Pp. 183-200 *in* T. Tsuda, Y. Sasaki, and R. Kawashima, eds. *Physiological Aspects of Digestion and Metabolism in Ruminants*. Proc. Seventh international symposium on ruminant physiology. Academic Press, Inc., New York.

Weimer, P. J., G. C. Waghorn, C. L. Odt, and D. R. Mertens. 1999. Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. *J. Dairy Sci.* 82:122-134.

Welch, J. G., and A. M. Smith. 1971. Effect of beet pulp and citrus pulp on rumination activity. *J. Anim. Sci.* 33:472-475.

Wells, J. E., and J. B. Russell. 1996. Why do so many ruminal bacteria die and lyse so quickly? *J. Dairy Sci.* 79:1487-1495.

Wildman, E.E., G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt, Jr., and T.N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65:495-501.

Williams, C. H., D. J. David, and O. Iismaa. 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophotometry. *J. Agric. Sci.* 59:381-385.

Winner, C. 1993. History of the crop. Pp. 1-36 *In* D. A. Cooke and R. K. Scott, eds. *The Sugar Beet Crop*. Chapman and Hall, New York.

Witt, M. W., L. A. Sinclair, R. G. Wilkinson, and P. J. Buttery. 1999. The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the production and metabolism of sheep: food characterization and growth and metabolism of ewe lambs given food *ad libitum*. *Anim. Sci.* 69:223-235.

Zinn, R. A. and F. N. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. Anim. Sci.* 66:157-166.

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