THE EFFECTS OF REDUCING DIETARY PHOSPHORUS AND NITROGEN BY THE ADDITION OF BLUEGRASS STRAW TO THE RATIONS OF EARLY TO MID-LACTATION HOLSTEIN DAIRY COWS

By

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To the Faculty of Washington State University:

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Abstract

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Phosphorus (P) excretion by dairy cows is a growing environmental concern. To examine the impact of partially substituting bluegrass straw for alfalfa hay to reduce dietary P in early to mid-lactation (114 days in milk), 24 dairy cows were used. Cows were fed a control TMR (C) or a TMR in which 10% of alfalfa hay DM was replaced by bluegrass straw (BGS) for 3 wk, diets were switched and cows fed for another 3 wk. Daily feed intake and orts were measured, milk, fecal, and blood samples were collected on d 2, 16, and 37 and analyzed for milk composition, fecal P and nitrogen (N), blood urea N and serum P. In vitro digestibility of feed was determined using an Ankom Daisy^{TM} Incubator. Feed sorting was assessed by comparing particle size distribution of fresh TMR and 24 h refusals. Chewing activity was estimated during two 30-min periods weekly. Inclusion of BGS in the diet reduced the concentration of crude protein and P but ADF and NDF were unaffected. Average dry matter intake (DMI) was higher on the BGS than the C diet ($P<0.05$) whereas total *in vitro* digestibility, feed costs, income-over feed cost, milk fat, milk protein, milk lactose, blood urea N, blood P, fecal P and N, and feeding behavior were unaffected. Feed sorting occurred for both diets, but was unaffected by inclusion of BGS. Milk yield averaged 37 kg for C vs. 35 kg for BGS ($P<0.05$). Yield of fat-corrected milk and

percent solids-not-fat were decreased in cows fed BGS ($P<0.05$). Income from milk was reduced by inclusion of BGS in the diet ($P<0.05$; \$15.56/cow/d for C vs. \$14.62/cow/d for BGS). Feed sorting occurred in both diets but was unaffected by inclusion of BGS. In conclusion, partial substitution of BGS for alfalfa hay in diets of early to mid-lactation cows reduced the %P and N in the diet. Although DMI of cows was increased by adding BGS to the diet, milk yield was reduced. Partial replacement of alfalfa hay with BGS may help reduce dietary P and N and aide in nutrient management.

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LITERATURE REVIEW

INTRODUCTION

The NRC (2001) recommends 0.3 to 0.4 % dietary phosphorus (P) for lactating dairy cows. However P is often overfed by 20 to 25% to ensure that low P intakes do not reduce milk yield (Toor et al., 2005). In addition, many commonly used byproduct feeds have high P concentrations, which creates difficulty in formulating diets that do not exceed dietary P requirements. Ingested P above requirement is excreted in the feces, potentially causing an accumulation of P in soils that can contribute to eutrophication of surface waters (Toor et al., 2005).

Nitrogen (N) excretion by livestock is also a growing environmental concern. Fertilizers and manure applied to soils are the main sources of reactive N in both the United States and Europe (Howarth et al., 2002; van Egmond et al., 2002). Excess N entering the environment contributes to eutrophication of lakes and streams, acidification of soil, and release of ammonia and nitrous oxide into the atmosphere (Schwab et al., 2005). Dairy farm N utilization efficiency is reported to be between 14 and 17% for intensive operations and between 24 and 35% for less intensive operations (Paul et al., 1998). Improving N utilization efficiency centers around minimizing urinary and fecal N output from livestock (Spears et al., 2003). Therefore, it is important to meet but not exceed P and N requirements of dairy cows so that P and N excretion is reduced.

Bluegrass straw (BGS) is a roughage that contains high fiber, low protein and low P. Bluegrass straw is a by-product of the Kentucky bluegrass seed industry. In the USA, this industry is primarily located in the tri-state area of Oregon, Washington and Idaho (Holman and Thill, 2005). After seed harvest, the straw remaining in the field has traditionally been burned. However, field burning is now banned in Washington and restricted in Idaho due to air quality and public health concerns (Holman and Thill, 2005). The harvesting and baling of BGS in an effort to reduce field burning emissions has resulted in the availability of a low P, low N feed to the dairy and beef producers of the Pacific Northwest.

Because BGS is a roughage with low N and P content, and is readily available, it is well suited for incorporation into the rations of dairy cows to reduce the P and N intakes. However, BGS is less digestible and lower in energy content than forages such as alfalfa hay. This raises the concern that incorporation of BGS into the diet will cause a reduction in milk yield of cows because energy needs of lactation will not be met. A further concern is that the high fiber content of BGS will cause cows to sort against the BGS in the TMR, leading to high variability in the nutrient content of the TMR throughout the day. Herd members such as subordinate cows that eat after the dominant cows may consume a higher fiber, lower energy diet whereas more dominant cows may consume a lower fiber, higher energy diet.

FEEDING BEHAVIOR

Introduction

Dairy cattle are social animals that group themselves in a herd structure with a social hierarchy (Albright, 1993). Cattle have a diurnal feeding pattern, eating the majority of their meals between dawn and dusk (DeVries and von Keyserlingk, 2005). Cattle spend between four and six hours per day feeding, dividing feeding bouts into nine to fourteen meals (Botheras, 2008; Grant and Albright, 2001). Dairy cows synchronize their behavior, with the majority of the herd feeding, grazing, and lying simultaneously. Although most commercial dairy herds are intensively group housed, cows still synchronize their behavior (Huzzey et al., 2008). Peak grazing times occur at sunrise and sunset (Albright, 1993), whereas in confinement, peak feeding times center around the time fresh feed is delivered rather than the time of day (DeVries and von Keyserlingk, 2005).

The pattern of feeding is one of several behavioral factors that contribute to nutrient intake by lactating dairy cows. Feeding activity, feed sorting and feed bunk competition also affect dry matter and nutrient intake of cows. Understanding feeding behavior and factors that affect feeding behavior can lead to improvements in dry matter intake and reduce variability in nutrient intake.

Factors Affecting Feeding Behavior

Feed bunk attendance is influenced by stimuli such as delivery of fresh feed, return from milking and to a lesser extent, feed push-up (Huzzey et al., 2008). Delivery of fresh feed is the greatest

feed intake stimulus, closely followed by return from the milking parlor (DeVries et al., 2003). Feed push-up (i.e. when feed that is out of reach of the cows is moved closer so cows can access it) appears to have stimulatory effects on bunk attendance, but these effects are minor and short-lived (DeVries et al., 2003).

The time of feed delivery also affects feeding activity and bunk attendance. Many dairy managers feed twice per day, but some managers feed only once per day to reduce labor costs. DeVries et al. (2005) tested the effects of delivering fresh total mixed ration (TMR) once (1x) per day vs. feed delivery twice (2x) per day on feed sorting, aggression, and feeding time. Daily feeding time increased by 10 minutes when the cows were fed 2x per day compared to 1x per day (DeVries et al., 2005). Cows sorted the TMR regardless of the number of times they were fed. However, feeding more frequently resulted in a more even distribution of fresh feed throughout the day. Fewer displacements (i.e. when a cow moves another cow away from the feed bunk) were recorded for cows fed 2x per day, suggesting that all that cows had better access to the feed (DeVries et al., 2005).

Although feeding twice a day yielded benefits in the study by DeVries et al. (2005), feeding too frequently can yield negative outcomes. Mantysaari et al. (2006) compared feeding once (1x) vs. five (5x) times per day, and found that cows fed 1x had higher dry matter intake (DMI), spent more time lying, and spent more time eating in the evening hours than cows fed 5x per day. The researchers concluded that feeding 5x per day was too frequent and led to more restlessness in the cows. Decreased time spent lying is linked with decreased rumination time and increased incidence of foot problems (Greenough and Vermunt, 1991).

Feed bunk design affects salivation and feed intake (Bolson and Pollard, 2004). Cows feeding in a natural grazing position produce between 17 and 20% more saliva than cows feeding at chest height (McFarlane, 1972). Greater saliva production increases the buffering capacity of the rumen, leading to overall enhancement of digestion. Albright and Stricklin (1989) reported that cows prefer a feeding surface elevated to approximately 11 inches over one at ground level. Feed bunks with smooth surfaces encourage higher DMI than bunks with rough surfaces (Bolson and Pollard, 2004). Rough feeding surfaces tend to trap feed, which is difficult to clean up and feed can become rancid, reducing the palatability of the diet (Bolson and Pollard, 2004).

Ambient daily temperature affects the feeding behavior of dairy cows. If daylight temperature exceeds 25º C, cows reduce their feeding time, especially in the afternoon hours (Taweel et al., 2005). The upper critical temperature at which dairy cattle can maintain a stable internal body temperature is 25 to 26º C (West, 2003). Cows spend more time feeding after dark if mid-day temperatures are high, but total daily feed consumption is depressed (Albright, 1993). Reduction in feed and consequently energy intake leads to reduced milk yield. In Holsteins, Barash et al. (2001) found that for every 1º C increase over 25º C, milk yield was reduced by 0.38 kg/d. Photoperiod positively affects milk production as day length increases from less than 12 hours of light to 17 hours of light per day (Dahl et al., 2000). Barash et al. (2001) reported an increase of 1.16 kg of milk per hour of increased day length. Relative humidity also affects DMI and milk yield. Bianca (1965) found that an increase of humidity from 40% to 90% at 29º C depressed milk yield by 31%.

Feed Sorting and Feed Bunk Competition

Dairy cows are commonly fed a total mixed ration (TMR) and housed in free-stall barns (DeVries et al., 2007). The goal of the TMR is to provide a nutritionally balanced ration that is as homogeneous as possible. However, cows continuously sort their TMR as they eat, pushing less desirable components of the feed away and out of reach (Huzzey et al., 2008). Cows sort for the more palatable concentrate components and against the longer roughage components of the diet (Leonardi and Armentano, 2003; DeVries et al., 2007). Feed bunk competition amplifies the effects of feed sorting. Feed bunk competition occurs when there is not enough space at the bunk for all cows to eat simultaneously. Because cows are highly social animals with a structured dominance hierarchy within the herd, dominant cows feed first when space is limiting, while subordinate cows must wait their turn to feed (DeVries et al., 2005). Thus, dominant cows have greater opportunity to sort for the more palatable ration components whereas subordinate cows have the leftovers. Although the TMR is mixed for optimal nutrition, sorting and competition for feeding space may result in some cows not consuming the intended diet.

Problems Resulting From Feed Sorting and Competition

Sub-Acute Ruminal Acidosis

A common concern regarding feed sorting is that the increased grain intake and lower fiber intake will cause sub-acute ruminal acidosis, or SARA (Krause and Oetzel, 2005). SARA is characterized by ruminal pH fluctuations that drop below 5.3 and then return to a normal pH of 6.8 to 7.0 (Kruse and Oetzel, 2005). Cows with SARA have decreased DMI and reduced milk production, as well as an increased risk of laminitis (Tarlton et al., 2002).

Risk factors for SARA include a low fiber, high concentrate diet, high DMI, and feed sorting. Cows that sort their feed have increased grain intake and decreased fiber intake and, if the pH drop from fermentation of grain in the rumen is severe enough in the rumen, SARA develops (Dohme et al., 2008). The greatest risk period for SARA is the few weeks immediately post-calving, which is a period when DMI rapidly increases and cattle are fed a low fiber, high concentrate diet (Kertz et al., 1991). Selection against the physically effective fiber of the TMR intensifies rapid grain fermentation in the rumen and reduces rumen buffering (Cook et al., 2004; DeVries et al., 2008).

Cow Welfare and Lameness

Nearly 25% of freestall-housed dairy cows exhibit some degree of lameness (Espejo et al., 2006). Factors that contribute to lameness include access to feed, the type of floor surface, changes in metabolism, and the amount of time spent standing vs. lying. Increased feed bunk competition has been linked to increased incidence of foot problems, such as laminitis (Greenough and Vermunt, 1991). When bunk competition occurs, subordinate cows spend more time standing idly, waiting for a chance to feed (Botheras, 2008). Standing and walking on hard, abrasive surfaces such as concrete increases the incidence of laminitis and claw horn lesions (Greenough and Vermunt, 1991). Bergsten (1994) found that sole hemorrhage scores were improved by fitting freestalls with rubber mats.

Environmental factors that affect standing and lying time include ambient temperature and freestall design. As ambient temperature increases, cows spend less time lying and more time standing (Cook et al., 2004b). For maximum use of freestalls, it is important to ensure ease of access, adequate lunge space in front of the neck rail for ease of lying down and standing up, and a comfortable lying surface (Cook et al., 2004b). Wandel et al. (2002) reported increased lying times when freestalls contained cushioned surfaces. Increased standing time is linked with higher incidence of lameness (Cook et al., 2004a). In turn, lame cows have reduced DMI and reduced milk production relative to their non-lame peers (Juarez et al., 2003).

Nutrient Content Variability

As cows sort their feed, the nutritive value of the TMR remaining in the feed bunk decreases as the day progresses (DeVries et al., 2007). DeVries et al. (2005) found that the percent neutral detergent fiber (NDF) in the TMR increased over time following delivery of fresh feed. This indicated that the cows were sorting against the fiber portion of the diet. Because of these changes in the TMR composition throughout the day, the researchers postulated that sorting by dominant cows was detrimental to subordinate cows who did not get a chance to eat immediately following delivery of fresh feed. Also, because the nutritive value of the ration changed with time, the meals that cows ate later in the day did not contain the same quantity of nutrients as meals eaten earlier in the day. Inconsistencies of diet can produce such problems as reduced energy intake leading to reduced milk production as well as variable intake of other nutrients (DeVries et al., 2007).

Even if competition for the fresh TMR is minimal, sorting of the TMR will change the nutrient content of the ration throughout the day (DeVries et al., 2007). Cows consume approximately 9 to 14 meals per day instead of consuming all their feed in one feeding bout (Botheras, 2008). Each time a cow eats a meal, she sorts the TMR; accordingly, as the day goes on, the energy content of the TMR decreases and the fiber content increases.

Managing Feed Sorting

Cows sort a TMR regardless of the forage content, chop length of forage, moisture content and forage quality. However, the amount of sorting can be influenced by these factors. Feeding a TMR comprising 45% forage and 55% concentrate results in more sorting compared to feeding a TMR with 60% forage and 40% concentrate (DeVries et al., 2007). This is counterintuitive, as one might expect the cows to sort more when grain is scarcer, but in fact they sort more (DeVries et al., 2007). When the chop length of the forage is longer, cows increasingly discriminate against the forage components of the diet (Leonardi and Armentano, 2003). Decreasing the dietary dry matter (DM) content from 80% DM to about 60% DM reduces sorting (Leonardi and Armentano, 2005). One factor that may have minimal influence on sorting under typical conditions is forage neutral detergent fiber (NDF) content. For example, Leonardi and Armentano (2003) found that increasing the quality of the alfalfa from 44.5% to 34.5% NDF in the TMR did not affect the cows' sorting behavior.

Managing Bunk Competition

When there is not enough space at the feed bunk for all cows in the pen to feed at the same time, competition occurs, with dominant cows displacing subordinate cows (Botheras, 2008; DeVries

et al., 2004). The industry standard allotted bunk space is approximately 0.5 m per cow although some studies suggesting that 0.2 m of space is sufficient (Collis et al., 1980). DeVries et al. (2004) tested the difference in inter-cow aggression and number of displacements at the bunk when cows were allocated 0.5 m vs. 1.0 m of bunk space. The researchers found a 60% greater bunk attendance at peak feeding, a 14% increase in total daily feeding activity and a decrease in the number of displacements when cows were allotted 1.0 m/cow.

Use of some type of barrier between cows in the feed bunk also reduces competition and increases feed access by subordinate cows (DeVries and von Keyserlingk, 2006). Huzzey et al. (2006) found that use of headlock gates resulted in fewer displacements at the feed bunk than use of a simple post-and-rail barrier. Feed stalls, in which a partition projects into the pen to provide additional protection for subordinate cows, are also effective at reducing bunk competition. DeVries and von Keyserlingk (2006) reported a dramatic decrease in aggressive interactions at the feed bunk when feeding stalls were used when compared to a post-and-rail barrier.

In summary, the feeding behavior of dairy cattle is influenced by several factors including social hierarchy, environmental temperature, feed bunk space, frequency of feed delivery and diet composition. Understanding feeding behavior can aide in improving dairy management and facility design to minimize the influences that lead to feed sorting and feed bunk competition. The end goals are to improve the efficiency of diet utilization, maintain cow health, attain high standards of cow welfare and increase dairy profitability.

PHOSPHORUS

Introduction

Phosphorus (P) is an essential macro-mineral required for most body processes including growth, development, energy metabolism, bone formation, milk production, phospholipid synthesis, and fatty acid transport. The NRC (2001) recommends 0.3 to 0.4 % dietary P for lactating dairy cows, depending on milk production. The requirement for P was determined using a factorial method that takes into account requirements for maintenance, lactation and growth. To ensure that cows are not nutritionally deficient in P, dairy producers often feed 20-25% more P than current NRC requirements (Toor et al., 2005). Excess P is excreted in the feces, causing environmental problems in the form of accumulation of P in soils when manure is applied to soil. Excess soil P leaches into surface waters and can cause eutrophication (Toor et al., 2005). As a result of growing concern about these environmental issues, it is important to understand the true P requirements of dairy cows so that cows are fed as closely as possible to their true P requirement, thereby reducing the amount of P excreted.

Overview of Phosphorus Metabolism

Phosphorus from the diet mixes with P in the saliva as feedstuffs enter the rumen. The rumen microbes use this influx of P to satisfy their own requirements. Phosphorus exits the rumen in the form of soluble inorganic P, in microbial products, and in insoluble, undigested compounds. Although there is movement of P across the rumen wall into the bloodstream, there is very little net P absorption from the rumen. Using labeled radioactive P isotopes to track P movement in the body, Pfeffer et al. (2005) found that from the rumen, P flows through the omasum and

abomasum into duodenum. The primary site of P absorption is the small intestine, where there is an extensive uptake of soluble P by both active transport and passive diffusion (Horst, 1984). Blood inorganic P diffuses into the salivary glands, where it is recycled back into the rumen for microbial use as phosphate salts.

Phosphorus is stored in bone, which constitutes 80-85% of P in the mammalian body. The skeleton of a mature cow weighs about 60 kg and contains approximately 3.8 kg of P (Pfeffer et al., 2005). The animal's body maintains a dynamic pool of P, which is added to by dietary P, bone resorption, and soft tissue storage, and depleted by secretions in milk, saliva, and excretions in urine and feces (Horst, 1984). In a model developed by Hill et al. (2008), if a cow absorbed 82 g/d of P in the small intestine, 53 g/d would be recycled back into the rumen via saliva, 1 g/d would exit in the urine, 25 g/d would be secreted in milk, and 4 g/d would be stored as bone.

Excretion of P differs in ruminants from that of monogastrics because ruminants excrete 98-99% of their P in the feces and very little in the urine (Pfeffer et al., 2005). Excreted P is partially endogenous P and partially undigested P from feedstuffs. As P intake increases, apparent digestibility decreases due to greater P losses in the feces (Ekelund et al., 2005; Wu et al., 2000). Some of the P in feces represents inevitable losses, which are derived from sloughed cells and gastric juices and tend to be independent from salivary P levels (Valk and Beyen, 2003).

Phosphorus in the Rumen

Phosphorus enters the rumen via consumed feed and saliva. Salivary P is endogenous in origin and comes from diffusion of inorganic P from the blood into the salivary glands. Ruminal P

concentration varies over time and is highly correlated with feed intake (Fenner et al., 1968), likely due to fluctuating amounts of P influx. Different feedstuffs vary in level and chemical composition of P. In plants, P is found as part of nucleic acids, phospholipids, and phytate P. The latter form is common in cereal grains and legumes and is largely unavailable for absorption in monogastrics (Harland and Morris, 1995). Some rumen microbes secrete phytase enzymes that hydrolyze the phytate into inorganic P (Reid et al., 1947). Knowlton et al. (2001) reported that in an *in vitro* study, over 90% of phytate P was hydrolyzed in the rumen. As plant P is not tightly associated with the cell wall, the majority of it is available for use by rumen microbes (Emanuele and Staples, 1990).

Saliva

Salivary flow plays an important role in homeostatic control of P (Kebreab et al., 2005). Saliva can provide as much as 50% of the inorganic P entering the rumen (Kincaid and Rodehutscord, 2005). Endogenous P diffuses into the salivary glands from the blood. Salivary P is readily used by rumen microbes and is composed mainly of phosphate salts combined with sodium and potassium. Salivation rate is related to DMI and forage intake (Cassida and Stokes, 1986), and can provide up to 80% of the endogenous flow of P into the lower digestive tract (Valk and Beyen, 2003). Valk et al. (2002) found that low plasma P levels caused by a dietary deficiency of P induced reduced levels of P in saliva. With sufficient P intakes, salivary P varies from 13 to 37 mg/dL whereas plasma P is between 4.5 to 6 mg/dL (Valk et al., 2002). According to Kincaid and Rodehutscord (2005), a decrease in dietary P produces an increase of salivary P as percent of total P entering the rumen. This suggests that the microbial P needs exceed the needs of the

animal, hence explaining the diversion of P away from the body P pool into the saliva and the rumen (Kincaid and Rodehutscord, 2005).

Phosphorus Secretion in Milk

Phosphorus is secreted in milk at a fairly fixed percentage. Myers and Beede (2009) found that milk P averaged 0.081%, regardless of dietary P intake. The greatest determining factor for the dietary P requirement for milk production is milk yield, with high producing cows having the highest P requirement for milk production (Pfeffer et al., 2005). Knowlton et al. (2001) reported milk P values between 0.68 to 0.78 g/kg milk during early to mid lactation. Other researchers reported that the average milk P over a complete lactation ranges from 0.85 to 0.94 g/kg (Valk et al., 2002; Wu et al., 2000). Wu et al. (2000) determined that dietary levels of between 0.38% and 0.40% P were sufficient to support milk yield in high producing cows. However, less than 0.31% P in the diet was insufficient to support maximum milk yield in late lactation cows (Wu et al., 2000). Though total milk yield is affected by P status, milk composition is not affected by P intake (Lopez et al., 2004; Valk and Sebek, 1999). Ekelund et al. (2003) showed that milk yield and milk composition were unaffected by P source, with dietary treatments including monosodium phosphate, rapeseed meal, sunflower seed meal, and wheat middlings. Concentrations of milk fat, milk protein and yield of energy-corrected milk (calculated from milk fat and milk protein) were not affected by dietary P source or dietary P level (Ekelund et al., 2003).

In early lactation, up to 30% of bone P is mobilized (Ekelund et al., 2006), seemingly without affecting the cow as long as P is replaced later in the lactation (Karn, 2001; Wu et al., 2001).

Ekelund et al. (2006) demonstrated that a cow in early lactation demineralizes bone for Ca and P, regardless of nutritional P status. Knowlton and Herbein (2002) postulated that because the body naturally mobilizes P reserves during early lactation, this source of P should be considered when determining dietary P requirements. Valk and Sebek (1999) postulated that cows compensate for a P deficient diet by reducing milk yield and DMI. Milk P levels increase as lactation progresses, possibly due to increased protein levels in the milk (Wu et al., 2001). In milk, calcium phosphate is bound to casein, the main protein found in milk, and stabilizes the casein micelle (Zhang and Aoki, 1996). Because late-lactation cows require P for rebuilding of bone to prepare for the next lactation, dietary P should not be decreased relative to milk production (Wu et al., 2001).

Phosphorus Excretion

Phosphorus in Urine

In dairy cows, excretion of P in the urine is negligible because ruminants do not rely on the kidneys to excrete excess P (Horst, 1984). Urinary losses of P, as a percent of total P excretion, are 4 to 9% for grazing steers and 1 to 4% for pen-housed steers (Karn, 2001). In cattle fed 0.49%, 0.40% and 0.31% dietary P, there was no correlation between urinary P excretion and diet P (Wu et al., 2000). Myers and Beede (2009) found no difference in urinary P output in lactating cows fed varying levels of dietary P. Challa et al. (1989) found that urinary excretion of P is negligible until blood P levels exceed 6 to 9 mg/dL. According to Knowlton et al. (2004), increased P absorption in excess of needs for milk production and tissue requirements results in an increase in renal P excretion.

Phosphorus in Feces

Fecal P is comprised of insoluble and undigested P from feed, inevitable losses from normal metabolic functions, and surplus absorbable P in excess of requirements (Spiekers et al., 1993). Phosphorus bound as plant phytate P is broken down into absorbable inorganic P by many species of rumen microbes, though some phytate escapes from the rumen undigested (Harland and Morris, 1995). Kebreab et al. (2005) found that acid insoluble P (unabsorbable P) comprises 18 to 25% of total fecal P, mostly consisting of indigestible dietary phytate P and microbial P. Sources of inevitable fecal P losses are sloughed cells from the digestive tract, P in digestive juices, and some microbial P (Spiekers et al., 1993). This fraction of fecal P accounts for 30 to 60% of total fecal P (Kebreab et al., 2005). Wu et al. (2000) reported that inevitable fecal P comprised 60% of total fecal P by measuring P intake, fecal P excretion and apparent P digestibility.

The most variable portion of fecal P is soluble, available P excreted as a result of excess supply in the diet. Nearly all P fed in excess of dietary requirements is excreted in the feces (Morse et al., 1992). Wu et al. (2000) found that by reducing dietary P from 0.49 to 0.40%, fecal P was reduced by 23%. Valk et al. (2002) determined that a 34% drop in fecal P occurred when P intake was reduced from 3.3 to 2.8 g of P/kg DM in cows with similar milk yields.

Fecal P increases linearly with increasing P intake (Wu et al., 2001). Ekelund et al. (2005) plotted dietary P versus fecal P excretion and fit a linear regression. They found that for every 1g increase in P intake, fecal P increased by 0.86g, which is higher than the value of 0.64g reported by Wu et al. (2000). Reducing dietary P from 0.49 to 0.40% reduced fecal P by 23% and yielded

a linear regression of 0.86 g P due to decreasing P digestibility with increasing dietary P (Ekelund et al., 2005). Clearly, feeding cows as closely as possible to nutritional P requirement greatly reduces the fraction of soluble P in the feces.

NITROGEN

Introduction

Nitrogen (N) excretion by livestock is a growing environmental concern. Excess N entering the environment causes eutrophication of lakes and streams, acidification of soil, and release of ammonia and nitrous oxide into the atmosphere (Schwab et al., 2005). It has been reported that fertilizers and manure are the main sources of reactive N in both the United States and Europe (Howarth et al., 2002; van Egmond et al., 2002). In an effort to minimize the environmental impact of livestock operations, the United States Department of Agriculture (USDA) and the Environmental Protection Agency (EPA) have instituted a policy requiring livestock feeding operations to develop a comprehensive nutrient management plan to track influx and efflux of N (Spears et al., 2003). Dairy farm N utilization efficiency has been reported to be between 14 and 17% for intensive operations and between 24 and 35% for less intensive operations (Paul et al., 1998). Methods to improve N utilization efficiency center around minimizing urinary and fecal N output from livestock on farms (Spears et al., 2003).

Nitrogen Metabolism

Nitrogen enters the rumen of cattle as dietary protein, non-protein N (NPN), salivary N, and urea that diffuses across the rumen wall (Walker et al., 2005). Dietary N consists of protein and NPN. Feed NPN is made up of amides, amines, amino acids and nitrate, though urea and ammonium salts can also be added to supplement N intake (Leng and Nolan, 1984). Nitrogen also enters through salivary urea, mucoproteins, and sloughed epithelial cells (Leng and Nolan, 1984).

The bacteria in the rumen catabolize peptides and amino acids to form ammonia, which is used to synthesize microbial protein. Nitrogen exits the rumen in the form of microbial protein and undegraded dietary protein, and by ammonia diffusion across the rumen wall into the blood (Waldo, 1968). In the abomasum, gastric secretions of HCl and pepsin denature and cleave protein into peptides and free amino acids (Skaln and Halevy, 1985). In the duodenum, further secretions of protein digesting enzymes continue to break down protein into constituent parts. The pancreas secretes the endogenous enzymes trypsin, chymotrypsin, and the exogenous enzymes carboxypeptidase A and B (Skaln and Halevy, 1984). Of the N containing compounds entering the duodenum, approximately 65 to 75% are digested in the small intestine (Owens and Zinn, 1988). Absorbed amino acids that are not used for protein synthesis or other vital processes are catabolized into urea and CO_2.

Amino acids absorbed in the small intestine can be incorporated into body tissues and milk protein, or deaminated into ammonia and a carbon skeleton. Ammonia from deamination and ammonia from the rumen are converted into urea in the liver. Urea in blood diffuses across the mammary gland into the milk, contributing to milk N secretion (Hof et al., 1997). Excess urea is excreted from the body in the urine. Urinary N represents loss of absorbed N in excess of requirements (Waldo, 1968). Fecal N loss is composed of undigested microbial and dietary N as well as endogenous losses from digestive juices and sloughed intestinal cells (Strozinski and Chandler, 1972). For total N inputs in a dairy cow, about 20% is secreted in milk, 50% excreted in the urine, 30% excreted in the feces, and 2% retained in body tissues (Hof et al., 1997).

Nitrogen in the Rumen

Much of the protein that enters the rumen is degraded by microbial peptidases and deaminases into amino acids and ammonia (Leng and Nolan, 1984). Microbial proteases are associated with the cell wall of bacteria, so the bacteria must have physical contact with a feed particle for proteolysis to occur (Huber and Kung, 1981). Other N containing compounds such as urea, nucleic acids and nitrates in the rumen are also rapidly converted into ammonia (Leng and Nolan, 1984). Factors affecting dietary protein degradation include the source of protein, rate of passage of ruminal contents, energy intake and feed particle size (Huber and Kung, 1981). Microbes require an adequate supply of N and energy for maximum protein synthesis (Stern and Hoover, 1979). Microbial protein N is derived from ammonia N or preformed amino acids (Stern and Hoover, 1979). Most bacterial species prefer ammonia as an N source for protein synthesis (Conrad and Hibbs, 1968). Protozoa require preformed amino acids and obtain these either from dietary protein or by consuming rumen bacteria (Firkins et al., 2007). Ruminal ammonia concentrations and microbial protein synthesis are heavily influenced by the fermentability and availability of a carbohydrate source. Increasing the readily fermentable carbohydrate source in the diet increases microbial protein production (Lapierre and Lobley, 2001). For every 100g of digested organic matter in the rumen, approximately 16.9 g of microbial protein is synthesized (Stern and Hoover, 1979). When diets contain N in the form of plant protein, efficiency of N conversion into milk and tissue is 32%, whereas efficiency of N conversion of high urea diets ranges between 29 and 32%, indicating that urea is an acceptable source of N for the ruminant (Conrad and Hibbs, 1968).

Ruminal ammonia concentrations are affected by N type (Cross et al., 1974). In diets with crude protein (CP) coming exclusively from soybean meal or urea, rumen ammonia concentrations were 18.7 mg/dL for soybean meal diets versus 14.7 mg/dL for urea diets (Cross et al., 1974). For effective use of NPN, 1 kg of readily fermentable carbohydrate is needed per 100 g of urea, with two thirds of that carbohydrate as starch (Conrad and Hibbs, 1968). Stern et al. (1983) found that an increase in rumen ammonia from 9.6 to 14.4 mg/dL occurred when dietary CP was supplied as corn gluten meal, but total microbial protein synthesis was unchanged. Klusmeyer et al. (1990) reported that an ammonia N concentration of 2 mg of ammonia N/dL of ruminal fluid supported maximal organic matter digestibility. However, decreased feed intake and milk yield was reported for NPN in the diet at 0.45 g NPN/kg body weight (Conrad and Hibbs, 1968).

Ammonia exits the rumen through incorporation into microbial bodies, efflux of ruminal fluid, and absorption across the rumen wall (Leng and Nolan, 1984). Rumen microbes contain approximately 20 to 60% CP on a DM basis (Owens and Zinn, 1988), 50 to 80% of which is synthesized from the rumen ammonia pool (Leng and Nolan, 1984). Absorption of ammonia across the rumen wall is a function of pH, with more alkaline pH associated with greater efflux of ammonia (Leng and Nolan, 1984). Approximately 30% of the total ruminal ammonia flux N is recycled within the rumen when microbial N compounds synthesized from ammonia are broken down into ammonia again (Leng and Nolan, 1984).

Clark et al. (1992) reported passage to the small intestine averaged 265 g/d for total N, 1101 g/d for microbial amino acids, 87 g/d for lysine and 26 g/d for methionine. Non-ammonia N passage to the small intestine increased from 60 to 666 g/d when feed intake increased from 3 to 23 kg/d

(Clark et al., 1992). Rode et al. (1985) reported that passage of bacterial N to the small intestine was maximized in diets containing 38% alfalfa hay and 62% concentrate. The efficiency of microbial protein passage decreased when diets with over 70% concentrate were fed (Clark et al., 1992).

Saliva

Saliva is an important pathway for N recycling in the ruminant (Emery et al., 1960). The N content of saliva varies between 8 and 40 mg/dL (Church, 1988). However, Emery et al. (1960) reported an average salivary urea level of 5.68 mg/dL for cattle fed various ratios of forage to concentrate. Urea diffuses into the salivary glands from the blood, and levels of salivary urea are controlled by the blood urea concentration (Leng and Nolan, 1984). The recycling of N into the rumen via the saliva buffers the rumen against low ammonia concentrations (Firkins et al., 2007).

In sheep, Obara and Shimbayashi (1979) reported no change in the quantity of salivary secretions when 0.1 and 0.2 g urea/kg of body weight was injected into the rumen. Urea doses of 0.3 g urea/kg resulted in a small decrease in salivation, whereas doses of 0.4 and 0.5 g urea/kg severely inhibited salivary secretion (Obara and Shimbayashi, 1979). The highest dose of urea corresponds with the level of NPN found to decrease milk yield and feed intake (Conrad and Hibbs, 1968). Injection of 0.28 mmol/L ammonium salt into the jugular vein inhibited salivation and rumen motility (Obara and Shimbayashi, 1979). The authors proposed that the mechanism for these effects involved the breakdown of urea into ammonia in the rumen. High levels of

ammonia reduce ruminal pH, causing greater efflux of ammonia into the blood, eventually overwhelming the liver's ability to detoxify ammonia into urea (Obara and Shimbayashi, 1979).

Nitrogen in the Blood

Ammonia absorbed into the bloodstream is converted to urea by the liver (Houpt, 1959). Urea equilibrates rapidly with all body tissues, including the saliva and milk and is highly correlated with milk urea nitrogen (MUN) (Broderick and Clayton, 1997). Blood urea nitrogen (BUN) reflects the intake of dietary N, the ratio of dietary CP to digestible organic matter, and protein metabolism in the lower tract (Roseler et al., 1993). High levels of BUN are indicative of inefficient N utilization by the animal (Nousiainen et al., 2004). Lane and Campbell (1966) found that BUN ranged from 2.9 to 22.4 mg/dL in lactating dairy cattle over the course of a year. Likewise, BUN decreases in cows during late gestation, possibly due to an increased need for N by the growing fetus (Lane and Campbell, 1966).

Huntington (1982) reported that portal circulation (i.e. blood flow from the gastrointestinal tract to the liver) ranged from 1.14 to 2.65 L/kg body weight in non-lactating cows and Baird et al. (1980) reported values of 2.53 to 2.57 L/kg body weight in lactating cows. Disappearance of BUN in portal blood was lower than net ammonia absorption, indicating a net N gain of 23.3 g N per day (Huntington, 1982). Urea transfer from the blood to the gastrointestinal tract was 10.3% of daily N intake (Huntington, 1982). Bruckental et al. (1980) reported that BUN loss to the gut was 6.8% of total irreversible urea N loss in lactating dairy cows. Transfer of urea N from blood accounted for 15.6% of the urea N entering into the gut (Mugerwa and Conrad, 1971).

Wang et al. (2007) reported increased BUN and MUN concentrations with increased dietary N. BUN was 8.8 mg/dL and MUN was 9.8 mg/dL for cows fed 11.9% CP, whereas cows fed 15.4% CP had BUN of 15.7 mg/dL and MUN of 19.1 mg/dL (Wang et al. 2007). Roseler et al. (1993) found that BUN was influenced by dietary CP and the amount of both rumen degradable and undegradable CP. Cows fed 12.2% dietary CP had a BUN value of 8.2 mg/dL whereas cows fed 17.6% CP had a BUN value of 20.7 mg/dL (Roseler et al., 1993). Increasing rumen undegradable protein intake from 1.3 kg/d to 1.5 kg/d elevated BUN by 3 mg/dL, and increasing rumen degradable protein from 1.6 to 2.4 kg/d elevated BUN by 8.3 mg/dL (Roseler et al., 1993). Higginbotham et al. (1989) reported that increasing rumen degradable protein by 0.2 kg/d produced an increase of 2.6 mg urea N/dL. Excess N, whether digested in the rumen or small intestine, is excreted via conversion to urea in the liver and, therefore, an excess of either rumen degradable or undegradable protein raises BUN (Roseler et al., 1993). Increased dietary N, regardless of type, increases the amount of urea N circulating in the blood.

Nitrogen Secretion in Milk

Nitrogen is secreted in milk as casein, whey protein, and NPN (DePeters and Cant, 1992). Casein is the major protein in bovine milk and comprises 78% of total milk N (Cerbulis and Farrell, 1975). The whey fraction is made up of protein formed in the mammary gland as well as proteins transferred from the blood (DePeters and Cant, 1992). Whey total N makes up 21.8% of total milk N with whey protein making up 16.9% of total milk N (Cerbulis and Farrell, 1975). The NPN fraction of milk includes urea N, which is measured as MUN and used as a diagnostic tool for N utilization efficiency (Nousiainen et al., 2004). Values of MUN greater than 19 mg/dL

have been associated with decreased fertility in dairy cattle, so MUN may also be a marker of reproductive health of the herd (Butler et al., 1996).

Factors other than diet affect the protein content of the milk. Mastitis results in a decrease in milk casein and an increase in whey (DePeters and Cant, 1992). Higher leukocyte numbers are associated with lower casein and higher whey proteins (Haenlein et al., 1973). As cows progress through lactation, milk N and protein content changes. After calving, casein, NPN and total N rapidly decrease until 5 to 10 weeks of lactation, then slowly increase over the rest of the lactation (DePeters and Cant, 1992). With each parity, milk casein decreases but noncasein N increases, resulting in no net change in total milk protein (Waite et al., 1956).

Because urea equilibrates across the mammary gland, there is a high correlation between BUN and MUN (Hof et al., 1997). Similar to BUN, MUN is influenced by the ratio of dietary CP to energy, increasing as the ratio of protein to energy increases (Oltner and Wiktorsson, 1983). Wang et al. (2007) reported MUN values of 9.8 mg/dL for cows fed diets containing 11.9% CP, and 19.1 mg/dL for cows fed 15.4% CP. Total milk protein also increases with increasing dietary protein, though efficiency of converting feed N into milk N decreases (Wang et al. 2007). Nousiainen et al. (2004) found that dietary CP was the best predictor of MUN, whereas others have found the ratio of protein to energy to be the most important predictor (Kirchgessner et al., 1986; Oltner and Wiktorsson, 1983). Hof et al. (1997) reported MUN values of 10.3 mg/dL when N was fed close to requirement. Roseler et al. (1993) fed lactating dairy cows at NRC (2001) recommendations for rumen degradable and undegradable protein and found that MUN was 11.6 mg/dL. A decrease in dietary CP in the diet from 15.2 to 12.2% lowered the MUN

levels to 5.6 mg/dL whereas an increase in CP to 17.6% increased MUN to 17.8 mg/dL (Roseler et al., 1993). High MUN values indicate inefficient use of dietary N by cows (Roseler et al. 1993), thereby necessitating either a decrease in dietary CP or an increase in readily digestible energy to improve dietary N utilization.

Nitrogen Excretion

Nitrogen in Urine

Urinary N loss represents one of the two routes of N excretion. Urea is approximately 70% of total urinary N and varies under conditions of dehydration (Waldo, 1968). Low protein levels and water restriction decrease urea in the urine, which increases N retention in the body (Waldo, 1968). There is a positive correlation between N intake and N absorption (Kauffman and St-Pierre, 2001). Increased N absorption increases BUN, which in turn increases urinary N excretion (Paul et al., 1998). A 94 g/d increase in dietary N increased urinary N by 74 g N/d (Castillo et al., 2001). Urinary N excretion increased as CP increased both in absolute quantity and in percentage of total N excreted (Marini et al., 2005). Below 400 g N intake per day (13% CP), the main route of excretion was feces whereas, when intake was above 400 g N/d, the urine N increased exponentially (Castillo et al., 2001).

Kauffman and St-Pierre (2001) reported that the renal clearance rate for a 678 kg cow was approximately 1756 L/d, or 2.59 L/kg of body weight. However, Jonker et al. (1998) reported the renal clearance to be 2.10 L/kg of body weight. Increasing dietary CP resulted in decreased N use efficiency (Kauffman and St-Pierre, 2001). There is a linear relationship between MUN and

urinary N excretion (Kauffman and St-Pierre, 2001). Conversion of absorbed N into milk N was 60.3% efficient when dietary CP was 13% but dropped to 42.5% efficiency when dietary CP was increased to 17% and urinary N increased from 93 g/d to 185 g/d (Kauffman and St-Pierre, 2001). Increased dietary CP leads to increased excretion of N in the urine, which in turn leads to increased N output into the environment as ammonia volatilizes from the urine.

MUN is used to estimate urinary N loss via a regression analysis. Nousiainen et al. (2004) reported that urinary N increased 13.4 g/d for every 1 mg/dL increase in MUN. Other studies have indicated somewhat different values; Jonker et al. (2002) and Kauffman and St-Pierre (2001) reported slopes of 12.2 and 17.2, respectively. Increased MUN values indicate a diet that is either too high in CP or deficient in readily fermentable carbohydrate sources (Roseler et al., 1993). MUN can be used to determine N utilization efficiency, giving producers a tool to monitor N excretion into the environment.

Nitrogen in Feces

Fecal N excretion is the second avenue of N loss from the animal. Metabolic fecal N is a portion of total fecal N and is derived from sources within the body such as microbial protein, mucus, sloughed epithelial cells and digestive juices (Strozinski and Chandler, 1972). Digestibility of microbial N ranges from 55 to 74%, and that undigested in the lower tract is voided in the feces (Waldo, 1968). Strozinski and Chandler (1972) reported metabolic fecal N excretion of 4.58 g/kg fecal DM. Dietary N intake is the best predictor of fecal N excretion (Yan et al., 2006). Lactating dairy cows averaging 21.4 kg milk/d excreted 72.2% of N intake in the feces (Yan et al., 2006). Wilkerson et al. (1997) reported a ratio of milk N to intake N of 297 g/kg for cows producing 29

kg milk/day versus 220 g/kg for cows producing 14 kg/d. A linear decrease in fecal N was observed with milk yields of 20, 30 and 40 kg/d resulting in 6.8, 5.8 and 5.3 g N/kg milk, respectively (Wilkerson et al., 1997). The authors concluded that while reducing dietary CP reduces fecal N, increasing milk yield per cow also reduces N excretion on dairies (Wilkerson et al., 1997).

CONCLUSION

As described above, feed intake, efficiency of diet and nutrient utilization, and nutrient excretion into the environment are influenced by factors including feeding behavior, cow herd hierarchy, dietary nutrient levels and efforts to reduce nutrient overfeeding. Minimizing environmental stressors, feed sorting and feed bunk competition leads to overall reduction of nutrient variability of the diet and improvement in feed intake. Incorporating low P and N feeds into the diet reduces dietary levels of P and N, thereby reducing nutrient excretion into the environment. The key to environmentally sustainable, profitable dairy herd management lies in formulating diets that meet but do not exceed cow nutrient requirements at each stage of lactation. The next section of this thesis describes a study in which these principles were taken into account while investigating the impact of incorporating bluegrass straw into the diets of early to mid-lactation Holstein dairy cows.

THE EFFECTS OF REDUCING DIETARY PHOSPHORUS AND NITROGEN BY THE ADDITION OF BLUEGRASS STRAW TO THE RATIONS OF EARLY TO MID-LACTATION HOLSTEIN DAIRY COWS

INTRODUCTION

The NRC (2001) recommends 0.3 to 0.4% dietary phosphorus (P) for lactating dairy cows. However P is often overfed by 20 to 25% to ensure that low P intakes do not reduce milk yield (Toor et al., 2005). In addition, many commonly used byproduct feeds have high P concentrations, which creates difficulty in formulating diets that do not exceed dietary P requirements. Ingested P above requirement is excreted in the feces, potentially causing an accumulation of P in soils that contributes to eutrophication in surface waters (Toor et al., 2005).

Nitrogen (N) excretion by livestock is also a growing environmental concern. Fertilizers and manure applied to soils are the main sources of reactive N in both the United States and Europe (Howarth et al., 2002; van Egmond et al., 2002). Excess N entering the environment causes eutrophication of lakes and streams, acidification of soil, and release of ammonia and nitrous oxide into the atmosphere (Schwab et al., 2005). Dairy farm N utilization efficiency is reported to be only between 14 and 17% for intensive operations and between 24 and 35% for less intensive operations (Paul et al., 1998). Improving N utilization efficiency centers around minimizing urinary and fecal N output from livestock (Spears et al., 2003). Therefore, it is important to meet but not exceed P and N requirements of dairy cows so that P and N excretion is reduced.

Bluegrass straw (BGS) is a roughage that contains high fiber, low protein and low P. Bluegrass straw is a by-product of the Kentucky bluegrass seed industry. In the USA, this industry is primarily located in the tri-state area of Oregon, Washington and Idaho (Holman and Thill, 2005). After the seed harvest, the straw remaining in the field has traditionally been burned. However, field burning is now banned in Washington and restricted in Idaho due to air quality and public health concerns (Holman and Thill, 2005). Harvesting and baling of BGS in an effort to reduce field burning emissions has increased the availability of a low P, low N feed to the dairy and beef producers of the Pacific Northwest.

Because BGS is a roughage with low N and P content and is readily available, it is well suited for incorporation into rations of dairy cows to reduce the P and N intakes. However, BGS is less digestible and lower in energy content than forages such as alfalfa hay (Holman and Thill, 2005). This raises the concern that incorporation of BGS into the diet will reduce milk yield because energy needs of lactation will not be met. Furthermore, cows sort their feed for the more palatable concentrate components and against the longer roughage components (Leonardi and Armentano, 2003). When feed sorting occurs, subordinate cows feeding later in the day consume a higher fiber, lower energy diet than more dominant cows feeding directly after fresh feed delivery (DeVries et al., 2005). Feed sorting may be increased when BGS is incorporated into a total mixed ration (TMR), resulting in high variability in nutrient consumption among cows and across time of day.

To assess the feasibility of feeding BGS to dairy cows, O'Rourke (2007) partially replaced alfalfa hay with BGS in the diet of late lactation Holsteins, averaging 219 days in milk (DIM). Inclusion of 10% BGS in the diet reduced P intake and fecal P excretion without affecting milk yield. This finding suggests that incorporation of BGS into the diet did not decrease the energy content of the ration enough to reduce milk yield, and further suggests that addition of BGS at this level did not cause cows to reject the TMR.

In the current study, we assessed the impact of including 10% BGS in the diet of early to mid-lactation dairy cows. Considering the relatively low P and N content of BGS, we hypothesized that addition of BGS to the TMR would reduce the P and N content of the ration, thereby reducing intakes of P and N and, consequently, reduce fecal P and N excretion. However, given the higher nutrient demands of cows in early to mid-lactation, we predicted that partially replacing alfalfa hay with BGS would reduce milk yield and alter milk composition. Based on the findings of O'Rouke (2007), we predicted that cows would not sort against and reject BGS. Because BGS is cheaper than alfalfa hay, we predicted that addition of BGS to the TMR would lower feed costs, which would compensate for reduced income from milk, resulting in no overall effect on dairy farm economics.

MATERIALS AND METHODS

Subjects, Housing, and Experimental Design

The protocol used in this study was approved by the Institutional Animal Care and Use Committee of Washington State University.

Two groups of 60 Holstein cows were housed in two separate pens of a freestall barn. Pen 1 contained 80 freestalls and had 75 headlock gates and a bunk length of approximately 45 m. Pen 2 contained 56 freestalls, and had 66 headlock gates and a bunk length of approximately 40 m. The headlock gates provided access to approximately 0.6 m of bunk space per gate. Pens were floored entirely in concrete and the freestalls were bedded with sawdust. Feed was delivered on the concrete floor in the alley in front of the headlock gates. For data collection from individual cows, a subset of 12 focal cows per pen was selected based on DIM and milk yield. Selected cows were between 60 and 200 DIM, with an average of 114 DIM, whereas the larger pen groups contained cows across a wider range of stages of lactation. Cows were paired between the two pens based on milk yield so that each subset of 12 cows contained a similar range of milk production.

All cows within a pen were assigned either a control TMR or a TMR containing 10% BGS (dry matter basis). Cows were fed once per day at 0800 hours and milked twice per day at 0900 and 2100 h. Each morning, cows were locked in the headlock gates prior to daily delivery of fresh feed and headlock gates were released approximately 30 to 40 min post feed delivery. This was to allow for cleaning of the pens and herd management. Feed was pushed up periodically

throughout the day, and 24 h refusals were scraped from the feed alley 30 min prior to daily delivery of fresh feed. To allow for ad libitum feed consumption, pen groups were fed at a level producing approximately 10% refusals.

The ingredient composition of the diets is listed in Table 1. Control and BGS diets were mixed immediately prior to feeding. The BGS was chopped in a tub grinder prior to being added to the feed mixer truck.

Table 1: Ingredient composition of control and bluegrass straw (BGS) diets as a percentage of dry matter (DM).

Ingredient	**Control Diet**	**BGS Diet**
	% DM	
Alfalfa haylage	27.3	27.3
Alfalfa hay	25.4	15.4
Bluegrass straw	0	10
Whole cotton seed	6.4	6.4
Concentrate mix[a]	36.4	36.4
DDGS[b]	4.5	4.5
Total	100	100

[a] Ingredients: 43.3% corn; 30.0% ground barley grain; 10.0% peas; 4.5% corn gluten meal; 2.0% sodium bicarbonate; 2.0% Megalac (Church and Dwight Co. Inc., Princeton, N.J.); 1.0% trace mineral premix (97% NaCl, 0.18% Mn, 0.35% Zn, 0.2% Fe, 0.037% Mg, 0.035% Cu, 0.01% I, 0.006% Co, 0.009% Se); 0.5% limestone; 0.4% magnesium oxide; 0.025% vitamin A (30,000 IU/g); 0.05% vitamin D (8810 IU/g); 0.16% vitamin E (500 IU/g); 0.1% 4-Plex (2.58% Zn as Zn Met, 1.43% Mn as Mn Met, 0.16% Cu as Cu Lys, and 0.18% Co as Co glucoheptonate, Zinpro Corp., Eden Prairie, MN.)
[b] DDGS = Distiller's dried grains and solubles

Cows within each pen were fed their respective diet for 3 weeks (Period 1). The diets were then switched between pens and cows were fed the alternate diet for an additional 3 weeks (Period 2). Cows in Pen 1 received the control diet in Period 1 followed by the BGS diet in Period 2, whereas the reverse was true for the cows in Pen 2. Daily feed intake data were collected for the entire pen. Behavioral, fecal, milk and serum data were collected from the subset of 24 focal

cows. In wk 3, one cow suffered from an injured teat and was removed from Pen 1 and placed in a sick pen. This cow's data were eliminated from the entire data set. In wk 6, there was an outbreak of pneumonia at the dairy. One focal cow from Pen 1 was diagnosed and placed in a sick pen. Period 2 data from this cow were discarded. The study was conducted in the months of June and July and, for each day of the study, the high, low, and mean temperatures for the location of Pullman, Washington were obtained from the Weather Underground, Inc. website (www.wunderground.com).

Data Collection

Feeding Behavior

Feeding behavior was observed on d 1, 8, 15, 22, 29 and 36. On observation days, the cows were not handled outside the normal feeding and milking routine. Cows were observed twice per day for 30 min immediately following delivery of fresh feed at approximately 0800 h and again for 30 min between 1600 and 1700 h. Cows were marked with grease-pens for ease of identification during observation. The cow's ID number was written in large numbers on each side of the rump, and a unique mark was placed on the face directly between the eyes for identification in the headlock gates.

Each treatment group was observed separately. Because a different TMR was mixed for each pen, there was a 30 to 45 min time difference in time of feed delivery to each pen. Morning observation began immediately following delivery of fresh feed, so the pen fed first was observed first. The control diet was always fed first, and the BGS diet fed second throughout the

study. During the entire morning observation session, cows were locked in headlock gates. The afternoon observation session occurred when cows were relatively inactive. Cows were not locked up and were able to move freely about the pen. The pen receiving the control diet was also observed first in the afternoon observation sessions.

Instantaneous scan sampling of behavior was performed at 1-min intervals throughout each 30-min observation session. Behaviors were defined as follows: (1) *Present at feed bunk*: Cow's ears are through the headlock gate; (2) *Chewing*: Cow is present at feed bunk, head at any height, jaws are moving rhythmically in a chewing motion. It was assumed that, if a cow was present at the feed bunk and chewing, she was chewing freshly-consumed feed and not ruminating. Intra-observer concordance was established by making 10-min videos of each group of cows at the feed bunk on three separate occasions in the month prior to commencing the study. The observer collected behavioral data twice independently from each video. On average, there was 97% concordance between the two sets of data from each video

The order in which cows were observed was randomized and constant within each observation session, but different across observation sessions. A single observer either stood still in a central location in the central aisle of the barn or walked slowly up and down the center aisle to observe the cows. The observer wore the same clothing for each observation period and moved quietly and calmly. A timer was used to ensure uniformity. When the timer went off, the observer noted the behavior of each cow in sequence on a data sheet with columns for cow ID number, grease-pen marking, each behavior category, and comments. The observer spent no more than 5 seconds

establishing which the behavior the cow was performing. All scans were made by the same observer.

Feed and Fecal Sample Collection

Fresh TMR was sampled each day immediately after fresh TMR delivery to each pen. Grab samples (~150 g) of TMR were obtained at 1 m intervals along the feed alley and combined. Orts were collected in 150 g grab samples from random places in the ort pile after they were removed from the feed alley. Particle size separation was performed on 200 g subsamples of fresh TMR and orts, after which the remainder of the sample was frozen for further analysis. Daily feed intake and feed cost data were collected for each pen using the TMR Tracker Lite ™ computer program.

Fecal samples were obtained by rectal grab sampling on d 2, 16 and 37. Between 100 and 200g of wet feces were collected from each focal cow using a disposable OB glove and brought back to the lab for analysis. Samples were collected between 0800 and 0900 h on sampling days.

Blood and Milk Sample Collection

On d 2, 16 and 37, blood was collected from the coccygeal vein of each focal cow and allowed to clot in the collection tube. Samples were placed on ice and taken back to the lab for serum extraction and analysis.

Milk was collected on d 2, 9, 16, 23, 30 and 37. Samples from the morning and night milking of each cow were combined. One milk subsample from each cow was preserved with bronopol and sent to the Washington State Dairy Herd Information Association (DHIA) testing laboratory for analysis of milk fat, protein, lactose, somatic cell count and solids not fat. A second subsample was untreated and frozen until analysis for milk urea nitrogen (MUN) was performed. Daily milk yield data were collected for the subset of 24 focal cows.

Data Analysis

Behavioral Analysis

Data for morning and afternoon observations were analyzed separately because the cows were locked up in the headlock gates in the morning whereas in the afternoon they were free to choose their activity. For both sessions, the proportion of scans in which each cow was observed to be chewing while present at the feed bunk was calculated. For afternoon sessions, the proportion of scans in which each cow was at the feed bunk out of total scans was also calculated.

Feed and Fecal Analysis

Particle size separation of both fresh TMR and orts was determined using a Penn State Particle Separator (Lammers et al., 1996). Two hundred grams of feed was weighed and placed in the top separator screen. The separator was then shaken back and forth 4 times in each direction for a total of 16 times. The contents of each screen were weighed and recorded.

Feed and fecal samples were dried in a 60°C oven for 48 h to determine DM (AOAC, 2005). Once dry, samples were ground through a 2-mm screen in a Wiley mill (Arthur H. Thomas and Co., Philadelphia, PA). Total DM was determined by drying a subsample of the ground sample in a 100°C oven for 24 h. Feed and fecal samples were analyzed in duplicate for ash, crude protein (CP; AOAC, 1997), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and P (AOAC, 2005).

Crude protein was determined using a Leco FP-528 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI; AOAC, 1997). The N concentration of the sample was converted to CP concentration by multiplying N by 6.25.

Neutral detergent fiber and ADF were determined using an Ankom Fiber Analyzer (Ankom Technology, Macedon, NY; AOAC, 1995). Fiber bags were analyzed for NDF, weighed back and then analyzed for ADF. Post ADF analysis, samples were analyzed for lignin using the protocol for acid detergent lignin (ADL) from Ankom Technology (Ankom, 2005). Sample bags were submerged in 72% H_2SO_4 for 3 h and then rinsed with distilled water until pH > 6.0. Samples were soaked in acetone for 5 min and then removed from acetone and allowed to dry. After all the acetone had evaporated, samples were placed into a 100°C oven for 2 h.

Total *in vitro* digestibility (IVTD) was determined on feed samples using an Ankom Daisy^{TM} Incubator (Ankom Technology, Macedon, NY, USA). A composite sample of each diet was obtained by combining a 100g subsample of each week's ground fresh feed samples. Composite samples were divided among fiber bags and placed in glass jars containing 1600mL of buffer

solution (pH 6.8) pre-warmed to 39°C. Ruminal inoculum was obtained from fistulated Angus beef cows housed at the WSU Beef Center and fed a 100% BGS diet (Mabjeesh et al., 2000). Four hundred milliliters of ruminal inoculum were filtered through cheesecloth into each jar and jars were placed in a Daisy^{TM} Incubator. Jars were continually rotated and kept at 39°C for 48 h. After 48 h the jars were removed from the Daisy^{TM} Incubator and cooled at 4°C for 24 h. Bags were rinsed in tap water, dried in a 100°C oven and weighed to determine total IVTD.

After ashing (AOAC, 2005), samples were assayed for P. Samples were boiled in 5 mL of 3N HCl for 10 min and then diluted to 50 mL in distilled water and stored at 4 ^{o} C. Prior to P analysis, samples were diluted 1 to 4 in deionized water. Phosphorus was measured using a colorimetric assay in a 96 well plate with 25uL of sample and 100uL of vandomolybdate reagent added to each well.

Concentrations of ADL, P and N in the TMR and feces were used to estimate nutrient digestibility. Acid detergent lignin was used as a marker. Phosphorus and N digestibility was calculated using the equation:

Digestibility = (% marker in feed / % marker in feces) * (% nutrient in feces / % nutrient in feed)

The Cornell-Penn-Miner Dairy model (CPM-Dairy) was used to predict microbial protein production, metabolizable protein content, and carbohydrate digestibility of the control and BGS diets.

Serum Analysis

Blood was allowed to clot for approximately 2 h and then centrifuged at 2000 x g for 20 min to obtain serum. Serum was transferred to microcentrifuge tubes and stored in a freezer at -20°C. Blood was thawed at 4°C prior to assay for P, non-esterified fatty acids (NEFA) and blood urea nitrogen (BUN). Serum was deproteinated using a 4:1 ratio of serum to 10% TCA prior to P assay. Serum P concentration was determined using a colorimetric assay (AOAC, 2001). BUN was determined using BioAssay Systems QuantiChrom Urea assay kit (DIUR-500). Serum NEFA was determined using a WAKO HR series NEFA-HR(2) kit (Wako Diagnostics, Richmond, VA).

Milk Composition

Milk samples were analyzed for fat, protein, lactose, somatic cell count and solids not fat by the Washington DHIA Laboratory (Burlington, WA). Fat corrected milk (FCM) was calculated using the equation:

FCM = 0.432 * milk yield (kg) + 16.32 * milk fat (kg; Brog, 1971).

4% energy corrected milk (ECM) was calculated using the equation:

ECM = [(383 * % fat + 242 * % protein + 163.2 * % lactose)/3140] * milk yield (kg; Sjaunja et al. 1990).

To obtain MUN values, milk was deproteinated using a 2:1 ratio of milk to 10% TCA and analyzed using the BioAssay Systems QuantiChrom Urea assay kit (DIUR-500). One milliliter of milk was combined with 0.5mL of 10% TCA and centrifuged at 14,000 rpm for 5 min. Supernatant was transferred to a clean microcentrifuge tube and stored at 4°C. Five microliters of

supernatant and 200uL of reagent were pipetted into a 96-well plate and read at 520 nm for urea content.

Statistical Analysis

The behavioral data were not normally distributed. Data were analyzed both untransformed and ranked, and similarity between results for both analyses indicated reliability of the ranked data analysis (Zar, 1999). Only results from analysis of the ranked data are presented. Changes in proportion of time spent present at the feed bunk (afternoon only), proportion of time spent chewing at the feed bunk (morning and afternoon) for each period, and the effect of order of presenting the two diets to each pen were assessed using PROC MIXED of SAS (v. 9.1; SAS Institute Inc., Cary, NC). The model included pen, time, and pen by time. Cows were the subjects, time in weeks was a repeated measure, and an unstructured covariance structure was used. Mean comparisons were performed on least squared means with Tukey's adjustment for multiple comparisons. No effects of week were found and, therefore, the PROC MIXED analysis was re-run after pooling the data for each 3-wk period. Using pooled data for each cow in each period, Wilcoxon matched pairs tests were performed to compare the difference in the number of scans that each focal cow was present at the feed bunk (afternoon only), and proportion of scans in which each cow was chewing while at the feed bunk (morning and afternoon), when fed the control vs. BGS diet. Because BGS was a novel feed ingredient for the cows, Wilcoxon matched pairs tests were also performed to assess differences in chewing frequency during the first and third week that the cows received each diet.

Statistical analysis on feed, feces, serum, and milk data was performed using PROC GLM of SAS (v. 9.1; SAS Institute Inc., Cary, NC). Serum measurements, fecal data, and milk data from cows were analyzed for the effects of treatment, period and treatment by period interaction using initial values as a covariate to eliminate cow effect. The linear model was:

$Y_{ijk} = u + D_i + P_j + DP_{ij} + Cov_{ijk} + E_{ijk}$

Y_{ijk} = the response of the k^{th} cow in the i^{th} diet and j^{th} period and cov_{ijk} was the initial value

Feed intake, feed costs, income and income over feed cost data were analyzed for the effects of treatment and period. The linear model was:

$Y_{ijk} = u + D_i + P_j + DP_{ij} + E_{ijk}$

Y_{ijk} = the response of the k^{th} observation in the i^{th} diet and j^{th} period

Total *in vitro* digestibility was analyzed for effects of sample and run. The linear model was:

$Y_{ijk} = u + S_i + R_j + SR_{ij} + E_{ijk}$

Y_{ijk} = the response of the k^{th} observation in the i^{th} sample type and j^{th} run

RESULTS

Diets

Inclusion of BGS in the basal TMR reduced dietary P by 8% (from 0.4 to 0.37% DM), and the CP concentration decreased by 8% (from 19.9 to 18.3%; Table 2). The comparatively high fiber content of the BGS did not affect the NDF and ADF concentration in the BGS diet compared to the control diet (Table 2). ADL was also unaffected by inclusion of BGS in the diet (Table 2). Total in vitro digestibility, as measured using the Daisy Incubator, was not affected by inclusion of BGS in the diet (Table 2).

The diet formulations were used in the CPM-Dairy model to obtain estimated differences in microbial protein synthesis and nutrient digestibilities between the two diets. Those estimates were: microbial protein production was reduced by 200 g/d (from 4119 to 3911 g/d); metabolizable protein content was 1545 g/d for control diet and 1467 g/d for the BGS diet; carbohydrate digestibilities of the control and BGS diets were 41.4 and 39.9% of DMI, respectively; NDF fermentability was similar between the diets (10.5 and 10.4% of DMI, for control and BGS respectively); and unavailable carbohydrates comprised 9.9 and 11.9% of total carbohydrates for control and BGS TMR, respectively.

Table 2: Chemical analysis of alfalfa hay and bluegrass straw (BGS) and effect of partial replacement of alfalfa hay with 10% BGS on the chemical composition of the total mixed rations. Means are shown as a % of dry matter.

Chemical composition of alfalfa hay and BGS		
Nutrient	**Alfalfa Hay**	**BGS**
	% DM	
DM	88.7	88.6
CP	22.4	6.9
P	0.33	0.11
ADF	31.9	44.6
NDF	39.8	73.7
IVTD*	77.2^{a}	56.1^{b}
Chemical composition of control and BGS diets		
Nutrient	**Control**	**BGS**
	% DM	
DM	58.1	58.2
CP	19.9^{a}	18.3^{b}
P	0.40^{a}	0.37^{b}
ADF	24.2	24.9
NDF	37.0	40.0
ADL**	6.1	6.4
NE_L, Mcal/kg	1.72	1.63
IVTD	81.2	79.3
* In vitro total digestibility ** Acid detergent lignin ^{a,b} Means in the same row with different superscripts differ ($P<0.05$).		

Feeding Behavior

Inclusion of BGS in the diet had no effect on the proportion of scans that cows spent chewing out of total scans when present at the feed bunk in either the morning (Figure 1) or afternoon (Figure 2) observation sessions. For cows in Pen 2 that were fed BGS in Period 1, there was no difference in morning feeding behavior between week 1, when the BGS diet was novel, and week 3. However, in cows fed BGS during Period 2, there was a difference ($P<0.05$) in chewing frequency during the morning observation session in the first and third week of receiving the BGS diet (Figure 3). Inclusion of BGS in the diet had no effect on proportion of scans that cows spent present at the feeder out of total scans for afternoon observation sessions (Figure 4).

Figure 1: Effect of partial replacement of alfalfa hay with 10% bluegrass straw on time spent chewing while locked in headgates at the feed bunk during morning observation sessions by week. Pen 1 received the control diet and Pen 2 received the BGS diet during Period 1 (wk 1 to 3), and Pen 1 received the BGS diet and Pen 2 received the control diet during Period 2 (wk 4 to 6). (Medians and interquartile ranges.)

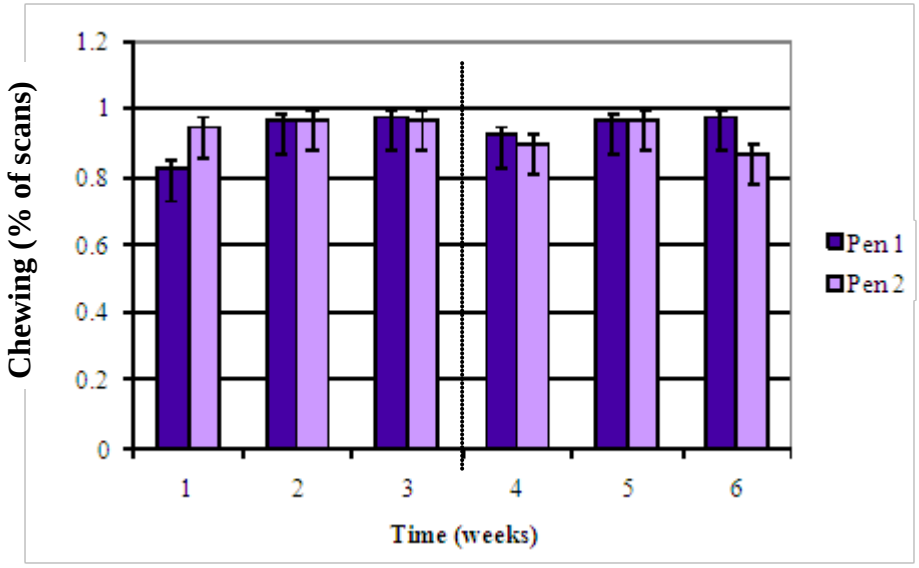


Figure 2: Effect of partial replacement of alfalfa hay with 10% bluegrass straw on time spent chewing when visiting the feed bunk during afternoon observation sessions by week. Pen 1 received the control diet and Pen 2 received the BGS diet during Period 1 (wk 1 to 3), and Pen 1 received the BGS diet and Pen 2 received the control diet during Period 2 (wk 4 to 6). (Medians and interquartile ranges.)

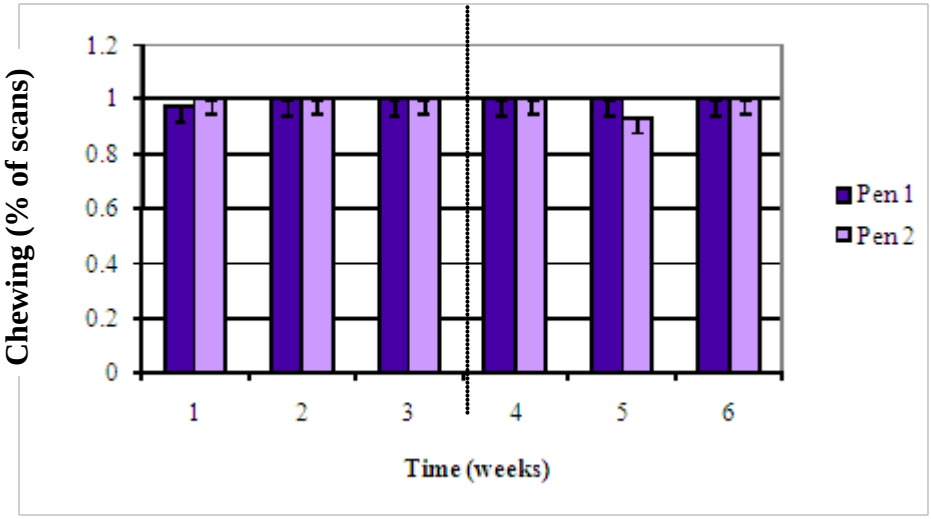


Figure 3: Effect of partial replacement of alfalfa hay with 10% bluegrass straw (BGS) on time spent chewing while present at the feed bunk during the 1st and 3rd weeks of receiving the BGS diet. Morning and afternoon sessions are pooled. (Medians and interquartile ranges.)

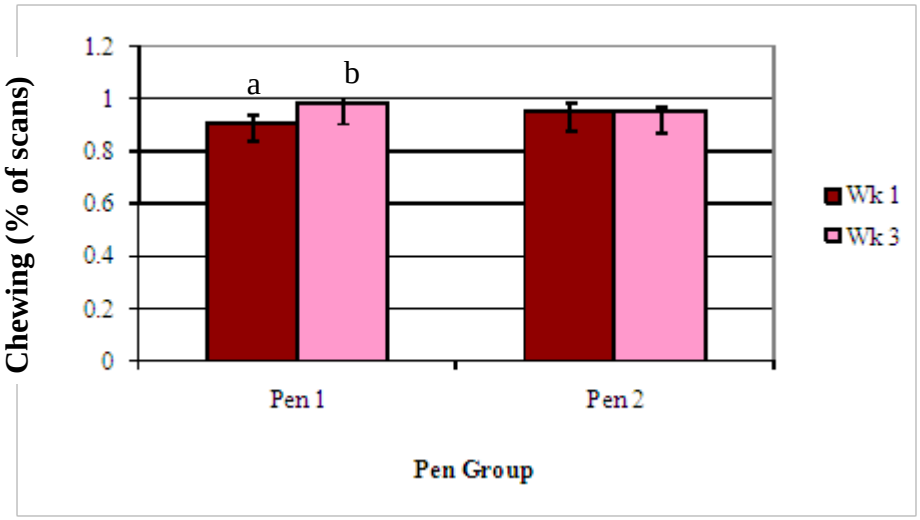
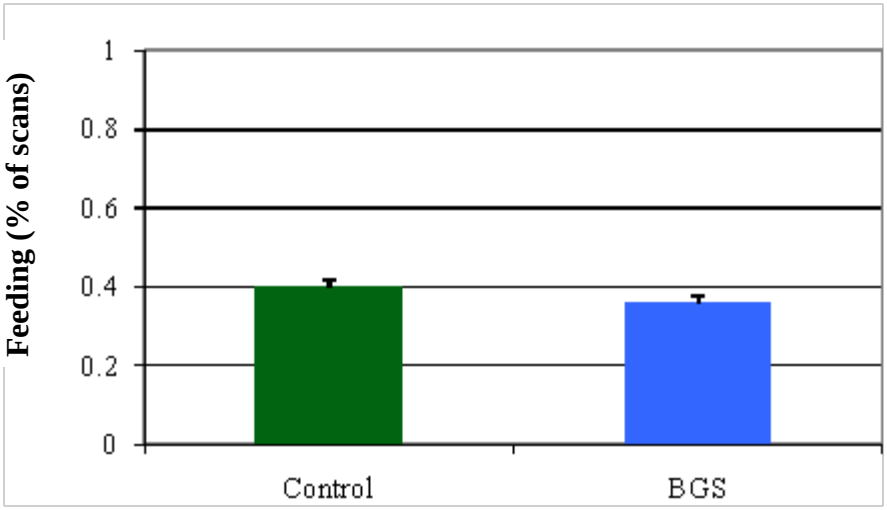


Figure 4: Effect of partial replacement of alfalfa hay with 10% bluegrass straw on time spent at the feed bunk during afternoon observation sessions. (Means and standard errors.)



Feed Sorting

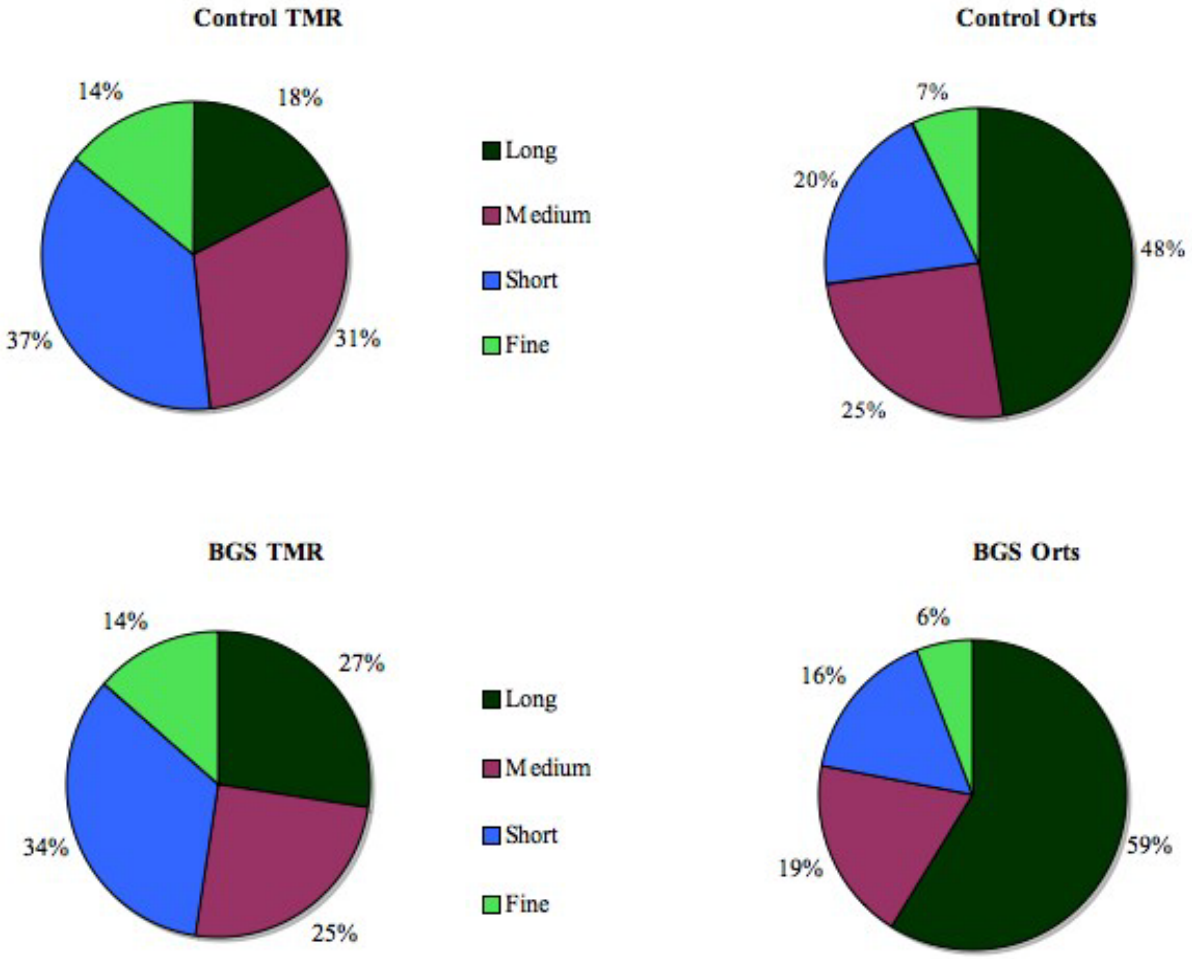
Particle size distribution was affected by the partial replacement of alfalfa hay with BGS in the TMR (Table 3). Inclusion of chopped BGS increased ($P<0.05$) the long particle (>19.0 mm) fraction of the diet and decreased ($P<0.05$) the percentage of particles between 8 and 1.18 mm in length. Sorting of the TMR by cows occurred for both the control and BGS diets. Orts from the BGS diet had a higher percentage of long particles than control orts, and a lower percentage of particles 8 to 19 mm (Figure 5). However, the percentages of long particles in the orts of both TMRs increased by approximately 27 percentage points, thereby indicating similar amounts of sorting against the long particles. Sorting occurred regardless of treatment and degree of sorting was not affected by partial replacement of alfalfa hay with BGS.

Table 3: The distribution of particle size in the fresh TMR and orts from the control and bluegrass straw (BGS) diets. (Least squares means and standard errors.)

Screen Size, mm	**Control**		**BGS**		**SE**
	Fresh TMR, %	Orts, %	Fresh TMR, %	Orts, %	
> 19.0	17.2[a]	43.8[b]	27.1[c]	53.9[d]	1.5
19.0-8.0	30.0[a]	23.1[b]	24.6[c]	17.6[d]	1.5
8.0-1.18	36.7[a]	18.5[b]	33.3[c]	14.6[d]	1.5
< 1.18	14.0[a]	6.6[b]	13.5[c]	5.4[d]	1.5

[a,b,c,d] Means in the same row with different superscripts differ ($p<0.05$).

Figure 5: The distribution of particle size in the fresh TMR and orts from the control and bluegrass straw (BGS) diets. The percent of particles within each size are noted numerically.



Phosphorus

Overall DMI of cows increased by 5% with inclusion of 10% BGS in the diet (Table 4). Therefore, although the % P was lower with BGS diets (Table 4), increased DMI yielded similar intakes of P, as estimated from the % dietary P and average DMI among cows. Phosphorus digestibility was calculated using ADL as an internal marker. Estimated phosphorus digestibility and total fecal P excretion were unaffected by the BGS (Table 4). Serum P concentration was unaffected by inclusion of BGS in the diet, and the serum P values were within the normal range of 4 to 8 mg/dL for lactating dairy cattle with adequate P intakes (Table 4; Morse et al. 1992). Secretion of P in milk was calculated using 0.9g P/kg milk and multiplied by milk yield (NRC, 2001). Overall P balance, calculated using estimated P intake, fecal P excretion and milk P secretion, was unaffected by inclusion of BGS in the diet.

Table 4: Overall dry matter intake (DMI), estimated P intake, fecal P concentration, P digestibility and total P balance of cows fed control and bluegrass straw (BGS) diets. (Least squares means and standard errors.)

Measure	Control	BGS	SE
DMI, kg/d	25.6[a]	27.0[b]	0.4
Estimated P_I, g/d	102.9	99.9	1.9
Fecal P, %	0.69	0.67	0.03
Fecal ADL, %	14.5	14.4	0.4
P digestibility, %	28.3	23.9	2.7
Fecal P,g	75.0	76.0	2.8
Serum P, mg/dL	7.5	7.8	0.3
Milk P,g	33	32	1.1
P balance, g	-5.1	-8.1	2.9

* Value from NRC (2001)
[a,b] Means in the same row with different superscripts differ ($p<0.05$).

Nitrogen

Estimated N intake, calculated using dietary N and DMI, was reduced 5.2% (738.3 vs. 699.6 g/d; P<0.05) by the inclusion of BGS (Table 5). Although estimated N intake was reduced, fecal N concentration was unaffected. Apparent N digestibility of the TMR was reduced by inclusion of BGS. Blood urea N values were highly correlated (r^2 = 0.74) to MUN (Table 5). Although MUN was reduced by inclusion of BGS (P<0.05), BUN concentration was unaffected. Urinary N excretion was not determined.

Table 5: Overall dry matter intake (DMI), estimated N intake, fecal N concentration, BUN, MUN and serum NEFA concentration of cows fed control and bluegrass straw (BGS) diets. (Least squares means and standard errors.)

Measure	Control	BGS	SE
DMI, kg/d	25.6[a]	27.0[b]	0.4
Estimated N_I, g/d	738.3[a]	699.6[b]	11.0
Fecal N, %	3.1	3.0	0.2
Fecal ADL, %	14.5	14.4	0.4
N digestibility, %	59.5[a]	57.2[b]	0.7
BUN, mg/dL	21.3	20.4	0.8
MUN, mg/dL	24.1[a]	20.6[b]	0.5
NEFA, mEq/L	0.2	0.2	0.03

[a,b] Means in the same row with different superscripts differ (p<0.05).

Milk Yield and Milk Components

Inclusion of BGS reduced milk production by 4.6% ($P<0.05$; Table 6). Similarly, 3.5 fat-corrected milk was reduced by 5.6% ($P<0.05$). Milk components of fat, lactose, protein and somatic cell count were unaffected by dietary treatment, although solids-not-fat was reduced in cows fed BGS.

Table 6: Milk yield, 3.5 fat corrected milk (FCM), 4% energy corrected milk (ECM), and milk components of cows fed control and bluegrass straw (BGS) diets. (Least squares means and standard errors.)

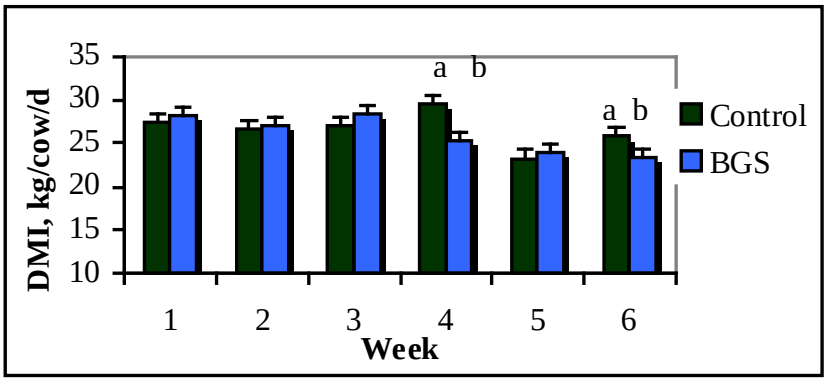
Measure	Control	BGS	SE
Milk yield, kg/d	36.6[a]	34.9[b]	0.5
3.5 FCM, kg/d*	37.7[a]	35.6[b]	0.8
4% ECM**	20.7[a]	19.2[b]	0.5
Milk fat, %	3.6	3.6	0.07
Lactose, %	4.9	4.9	0.01
Protein, %	3.0	3.0	0.02
SNF, %	8.9[a]	8.8[b]	0.02
SCC, # of cells	236.4	199.7	37.3

* 3.5 FCM = 0.432 * milk yield (kg) + 16.32 * milk fat (kg; Brog, 1971).
** 4% ECM = [(383 * % fat + 242 * % protein + 163.2 * % lactose)/3140] * milk yield (kg; Sjaunja et al. 1990).
[a.b] Means in the same row with different superscripts differ ($p<0.05$).

Weekly Dry Matter Intake, Milk Production, and Ambient Temperature

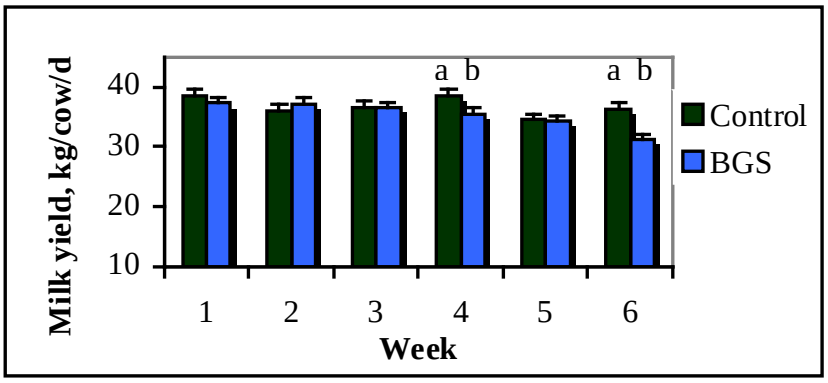
Dry matter intake was lower on the BGS than the control diet during wk 4 and 6 although DMI over the full 6-wk study was higher (P<0.05) on the BGS diet (Figure 6; Table 3). There was a corresponding reduction in milk yield in during wk 4 and 6 (Figure 7).

Figure 6: Effect of partial replacement of alfalfa hay with 10% bluegrass (BGS) straw on average dry matter intake (kg/cow/d) by week. Week 1 dry matter intake was used a covariate for wk 2 to 6. (Least squares means and standard errors.)



[a,b] Columns with different superscripts differ (P<0.05).

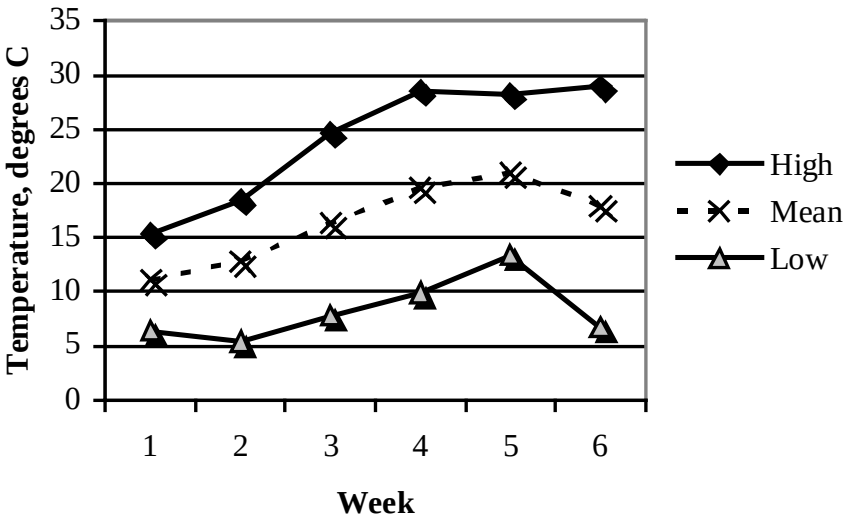
Figure 7: Effect of partial replacement of alfalfa hay with 10% bluegrass (BGS) straw on milk yield (kg/cow/d) by week. Week 1 milk yield was used as a covariate for wk 2 to 6. (Least squares means and standard errors.)



[a,b] Columns with different superscripts differ (P<0.05).

Average daily temperature tended to be higher in Period 2 than in Period 1. There was an overall increase in daily high temperatures throughout the study (Figure 8). During wk 2, there was a snowfall, which contrasted with a heat wave during wk 4 to 6.

Figure 8: Average weekly high, low and mean ambient temperature in °C as recorded from Weather Underground, Inc.



Economics

At the time of the trial, alfalfa hay cost $270/ton, whereas BGS cost $85/ton. Feed costs were unaffected by the partial replacement of alfalfa hay with of BGS in the diet. The $0.24/cow/d reduction in feed cost from incorporation of BGS was not statistically significant. At the time of the study, milk prices were $0.42/kg. Milk yield was reduced in BGS fed cows and consequently income from milk was reduced as well ($P<0.05$). Although income was reduced, the overall income over feed cost ratio was unaffected by inclusion of BGS. The $0.24/cow/d reduction in feed cost compensated for the reduced milk income and therefore the final economic outlook was statistically unaffected by the inclusion of BGS in the diet.

Table 7: Pricing of alfalfa hay, bluegrass straw (BGS) and milk for June and July, 2008. Feed costs, income from milk and income over feed costs for control and BGS diets. (Least squares means for feed costs, income and IOFC.)

Unit Price	**Cost**		
Alfalfa hay, $/ton	270		
BGS, $/ton	85		
Milk price, $/kg	0.42		
Unit Price	**Control**	**BGS**	**SE**
Feed costs, $/cow/d	8.07	7.83	0.35
Income, $/cow/d	15.56[a]	14.62[b]	0.24
IOFC, $/cow/d*	1.95	1.88	0.035
* IOFC = Income over feed cost [a,b] Means in the same row with different superscripts differ ($P<0.05$).			

DISCUSSION

Diet Composition and Dry Matter Intake

Bluegrass straw is much lower than alfalfa hay in CP (22.4 vs. 6.9%) and P (0.33 vs. 0.11%) and higher in NDF (39.8 vs. 73.7%). Therefore, partial replacement of alfalfa hay with 10% BGS reduced dietary P and CP concentrations. Any differences in NDF, ADF, and ADL that may have resulted from inclusion of BGS were too small to be detected in the mixed diets. Although BGS is less digestible that alfalfa hay (Table 2), the relatively low inclusion rate of BGS and similar ADF and ADL content of the diets did not yield a significant difference in IVTD.

The digestibility of the diets in the rumen of a lactating dairy cow may not have been represented by data obtained from the Daisy^{TM} Incubator. Ruminal inoculum was taken from beef cows fed 100% BGS diets, whereas the dairy diets contained approximately 50% concentrate. The microbial population of the ruminal inoculum was undoubtedly predominantly fibrolytic, with a small population of amylolytic microbes. The exposure of the fibrolytic microbial population to diets containing approximately 50% concentrates might have caused a proliferation of the starch-digesting microbes (Emery et al., 1960). The rapid proliferation of starch-digesters and fermentation of starch results in a buildup of lactic acid, which lowers the pH of the rumen and inhibits the action of fiber-digesting microbes (Dohme et al., 2008). The rumen microbial population of the dairy cows used in the study was already adapted to the diet, and no such acidosis effect was likely to have occured.

Though overall DMI was higher for BGS fed cows ($P<0.05$), weekly DMI was only affected by inclusion of BGS during wk 4 and 6. During these weeks, DMI actually decreased in BGS fed cows. During wk 4 there was an increase in ambient temperature, which could have contributed to the reduction in DMI in BGS fed cows (Figure 5). More heat is produced in the rumen from fermentation of fiber than from concentrate (Fuquay, 1981) and, because BGS is higher in %NDF, it is possible that cows consumed less of the BGS diet to compensate for heat production in the rumen. The decrease in milk production during wk 4 and 6 corresponded with the decrease in DMI.

Feeding Behavior

Overall proportion of scans in which chewing was recorded out of the total scans when cows were present at the feed bunk was not affected by inclusion of BGS in the diet. Time spent chewing was approximated by 1-0 scan sampling at 1-min intervals for each 30-min observation session. The exact number of chews performed during the observation period was not recorded due to the difficulty in simultaneously observing all focal 12 cows within each pen group of 60 cows. Some data points were missing because certain cows failed to enter the headlock gates during morning feeding. During the afternoon observation period, a time of low cow activity at the dairy (with no feed delivery or milking occurring at this time), cows spent more time lying down than eating.

More feeding behavior data could have been obtained by ensuring that all focal cows entered the headlock gates for morning observations as well as by extending the length of the afternoon observation to give cows more opportunity to feed. Ideally, chewing would be measured by use

of an electronic collar on each cow (Kononoff et al. 2002; Penning, 1983; Rutter et al. 1997). Kononoff et al. (2002) found that use of an automatic wireless bite recorder was more accurate than observational scan sampling in recording the amount of time spent eating and ruminating by individually housed dairy cows. Yang and Beauchemin (2006) used halters fitted with piezo disks to measure jaw movement, and reported increased rumination time in cows fed higher %NDF diets over controls.

Although overall frequency of chewing was not affected by addition of BGS, cows fed BGS during Period 2 were observed chewing less frequently on their first day of receiving the BGS diet than in the last week of receiving this diet. This finding could explain the lower DMI and milk yield of BGS-fed cows during wk 4, and may have been related to elevated ambient temperature at this time, but does not appear to have been due to novelty or lower palatability of the BGS given that no similar effect was detected among cows fed BGS in Period 1.

Feed Sorting

Sorting of the TMR by cows is influenced by dietary forage content, chop length of forage, moisture content of the diet and forage quality. Cows sort for the concentrate portion of the diet and discriminate against long fibers. Leonardi and Armentano (2003) reported that feed sorting increased as the chop length of the forage increased. These researchers also found that increasing the quality of the alfalfa (from 44.5% to 34.5% NDF) in the TMR did not affect the feed sorting behavior of cows (Leonardi and Armentano, 2003). In the current study, although the long fiber portion of the diet was increased by the addition of BGS, sorting was unaffected. This could be because the increase in long fibers was not sufficient to cause an increase in feed sorting.

Perhaps the cows did not respond to the reduction of dietary P and CP with a decease in fecal P and N concentration because they did not consume the intended diet. Cows were fed for approximately 10% refusal rate, meaning they did not have to consume all of the long particles of the diet before being supplied with fresh feed. Dairy cows are typically fed for a refusal rate of 5% or greater to avoid empty feed bunks and allow access to feed at all times. If the refusal rate was lowered to less than 5% on the current study, less feed sorting, a greater intake of BGS, and consequently a reduction in fecal N and P concentration, might have been seen.

Cows sorted their feed regardless of dietary treatment, and the fraction of the diet sorted against (the long fraction) contained the lowest %P and CP of the diet components. Inclusion of BGS increased the long particle (>19.0 mm) fraction of the diet. Both control and BGS diets had more long particles than recommended (Heinrichs and Kononoff, 2002). Orts from the BGS diet had a higher percentage of long particles than control, which could indicate greater sorting in cows fed BGS. However, when the percentage of long particles in the orts was compared to the percentage of long particles in the fresh TMR, the long particle fraction increased the same number of percentage points for both diets. The BGS orts contained a higher proportion of long particles than control orts because the BGS diet started out with a higher portion of long particles, indicating that degree of feed sorting was not increased by addition of BGS.

When the length of the forage is longer, cows increasingly discriminate against the long particles in the diet (Leonardi and Armentano, 2003). Although inclusion of BGS did not lead to increased feed sorting, reducing the chop length of the BGS could be a method used to encourage the cows

to consume more of the BGS in the diet. However, chop length of BGS should be no less than two inches, as any finer chop than that results in increased ruminal passage and decrease of physically effective fiber (Yang and Beauchemin, 2007).

Phosphorus Intake and Fecal Excretion

Although % dietary P was reduced by inclusion of BGS, estimated P intake between treatment groups was not affected. Dry matter intake increased in cows fed BGS, which resulted in similar P intakes. Because there was no difference in P intake, fecal excretion of P was not affected by addition of BGS. Serum inorganic P was unaffected by inclusion of BGS, indicating no change in the amount of P absorbed with similar amounts of P intake.

Because fecal P excretion is highly correlated to P intake, similar P intakes resulted in similar levels of fecal P excretion regardless of dietary treatment. Ekelund et al. (2005) reported 23% reduced fecal P excretion when dietary P was reduced from 0.49 to 0.40%. Valk et al. (2002) found that a 34% drop in fecal P occurred when P intake was reduced from 0.33 to 0.28% P DM in cows with similar milk yields. Because there was no treatment effect on P intake, milk P secretion and fecal P excretion, overall P balance was unaffected by inclusion of BGS in the diet. Though BGS is a low P feed, the increase in total feed intake offset the lower % P such that BGS addition to the diet did not affect fecal excretion of P into the environment.

Nitrogen Intake and Fecal Excretion

Estimated daily N intake was reduced by the addition of BGS ($P<0.05$). However, calculated N digestibility of the TMR was reduced by inclusion of BGS ($P<0.05$). This could be caused by

reduced milk production in BGS fed cows, leading to less N leaving in the milk which would show up as a decrease in apparent N absorption. Because BGS has less readily fermentable carbohydrate for microbial protein production and a greater proportion of longer particles than the control diet, increased cud chewing, salivation and thus N recycling to the rumen could account for the decrease in calculated N digestibility (Lapierre and Lobley, 2001; Table 2). Data obtained from the CPM-Dairy model suggested that BGS reduced microbial protein production in the rumen. Microbial protein production depends on amount of N and fermentable carbohydrate supplied to the rumen (Leng and Nolan, 1984). A fibrous feedstuff such as BGS has a low CP content as well as a low energy content, therefore BGS fermentation does not yield the same amount of microbial protein as a higher CP, higher energy feed such as alfalfa hay. Thus incorporation of BGS in the diet reduces the ability of microbes to synthesize microbial protein from the diet. A decrease in microbial protein production can result in an increase of ruminal bypass protein (Roseler et al., 1993). The CPM-Dairy model predicted a 10% increase in unavailable CP with the addition of BGS to the diet. Thus, the decrease in microbial production is indicative of a reduction in N digestibility by incorporating BGS into the diet (Table 4).

Blood urea N was unaffected by inclusion of BGS, and although within the normal range of 8.0 to 22.4 mg/dL, values were high compared to target BUN values between 13 and 17 mg/dL for cows producing 36 kg of milk per day (Lane and Campbell, 1966; Jonker, 1998). High levels of BUN are indicative of inefficient N utilization by the cow, and can be caused by high levels of dietary CP or inadequate amounts of readily digestible carbohydrate (Nousiainen et al., 2004). Data obtained from the CPM-Dairy model suggest that the overall carbohydrate digestibility of the BGS diet was lower and the amount of unavailable carbohydrate was higher than the control

diet. Kauffman and St-Pierre (2001) reported decreased efficiency of N utilization as dietary CP increased. The high BUN levels found in the current study are likely because both control and BGS diets were higher in CP (19.9 and 18.3%, respectively) than the recommended 16.5 to 17.5% of DM (NRC, 2001).

As dietary CP increases, BUN and MUN increase, hence BUN values were highly correlated with MUN values. However, MUN was reduced in cows fed BGS though BUN was unaffected (Table 5). Wang et al. (2007) reported MUN values of 9.8 mg/dL for cows fed diets containing 11.9% CP, and 19.1 mg/dL for cows fed 15.4% CP. Roseler et al. (1993) found that a decrease in dietary CP in the diet from 15.2 to 12.2% lowered the MUN levels to 5.6 mg/dL whereas an increase in CP to 17.6% increased MUN to 17.8 mg/dL. In the current study, a reduction in dietary CP by addition of BGS led to a reduction in MUN values. However fecal N excretion was not reduced. Urinary N excretion was not determined in this study. Yan et al. (2006) reported that lactating dairy cows consuming 486 g N/d and averaging 21.4 kg milk/d excreted 72.2% of N intake in the feces. However, Devant et al. (2000) reported dietary CP at 14 and 17% of the diet DM had no effect on fecal N excretion in dairy heifers. Van Vuuren et al. (1993) reported increased fecal N values with increased levels of indigestible CP in the small intestine. Fecal N was not affected by dietary treatment in the current study and supports the hypothesis that urinary N excretion is more likely to reflect N intake than fecal N excretion.

Fecal N is influenced by milk N secretion (Wilkerson et al., 1997). Wilkerson et al. (1997) reported a decrease in fecal N when milk yield increased, with secretion of N in the milk increasing as milk yield increased. Ideally, dietary CP levels are lower than the diets fed in the

current study. BUN and MUN values were consistent with cows fed high CP diets, indicating that further measures to reduce dietary CP are needed.

Milk Yield and Composition

Previous studies have successfully incorporated low CP, high fiber feeds into lactating dairy cow diets without affecting milk yield and DMI. Wu (2005) reported that milk yield was unaffected in high producing dairy cows (97 DIM, 43 kg/d) when 10% soy hulls were substituted for 6% of the alfalfa hay in the diet. Although soy hulls are a high fiber feed, their small particle size may result in fast ruminal passage and not reduce total DMI of cows. Wu et al. (2003) found that reducing dietary P (0.33 vs. 0.42% DM) and increasing the fiber portion of the diet (48% vs. 58% DM) did not affect DMI but lowered milk yield (34.0 vs. 36.5 kg/d). O'Rourke (2007) reported that milk yield was unaffected by 10% inclusion of BGS in the diet in late lactation cows. However, in the current study (114 DIM) average daily DMI was increased by 1.4 kg/d and milk yield was reduced by 1.7 kg/d by 10% inclusion of BGS. The reduction in energy content of the diet by addition of BGS caused cows to increase DMI to support milk production. Although BGS-fed cows produced less milk than control-fed cows ($P<0.05$), serum NEFA levels were unaffected (Table 5). NEFA levels in cows in greater than 100 DIM are not sensitive to small dietary energy changes and, therefore, any dietary energy deficiency has to be fairly large for NEFA levels to increase (Martin and Sauvant, 2007). As BGS-fed cows did not utilize body fat stores, this finding indicates that incorporation of 10% BGS into the TMR did not result in a weight loss situation.

Valk and Sebek (1999) investigated the effects of a low P diet (0.28% P) in lactating cows and found no effect on milk fat, protein and lactose, though milk yield was reduced. Likewise, Wu and Satter (2000) reported no effect on milk yield and milk composition in cows fed either 0.38 or 0.48% dietary P. In the current study, partial replacement of alfalfa hay with BGS did not affect concentration of milk components of fat, protein, lactose and somatic cell count. However solids-not-fat was reduced in BGS-fed cows.

Economics

Bluegrass straw was a much lower cost feed ingredient than was alfalfa hay (Table 7). Inclusion of 10% BGS in the diet decreased feed costs by $0.24/cow/d, although this value was not statistically significant. Nevertheless, if the lack of statistical significance reflects Type 2 error due to low statistical power, the money saved by addition of BGS could amount to a considerable savings when taken on a whole herd basis. For example, for a 100 cow dairy, feed costs could be reduced by $24 per day and $720 per month by incorporating 10% BGS into the diet. Because milk yield was reduced in BGS-fed cows, income from milk was also reduced (Table 7). However, income over feed cost was unaffected by addition of BGS. The loss in milk income was compensated by the slight reduction in feed costs. Thus, the overall finances of the dairy were not significantly affected by inclusion of BGS in early to mid-lactation rations.

CONCLUSION

Inclusion of 10% BGS in the rations of early to mid-lactation dairy cows reduced the P and N content of the diet. However, because DMI increased in cows fed BGS, P intake was not affected although N intake was reduced. Sorting of the TMR by all cows contributed to intakes of P and N that were higher than expected. The high BUN and MUN values reflect the high dietary CP level and possibly indicate a need for more fermentable carbohydrate in the ration. Although fecal P concentration was not affected, the %P in the diet was reduced by inclusion of BGS, giving producers an option for reducing P content of the ration. Feeding behavior and feed sorting were similar between treatment groups. Milk yield was reduced by inclusion of BGS, but overall IOFC was unaffected. Reduced income from milk was compensated by the reduction in feed costs, indicating that finances were not impacted by inclusion of 10% BGS in the diet. Bluegrass straw has been found acceptable for inclusion in the diets of late lactation cows. The results of this study show that BGS is also an acceptable feedstuff that can be used as a forage extender in the diets of cows in early to mid-lactation.

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