FEEDING HIGH LEVELS OF WET CORN GLUTEN FEED TO DAIRY CATTLE

by

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Abstract

Increased pressure for land use and greater demand for cereal grains have substantially increased feed costs for dairy producers. This has forced nutritionists to devise novel diet formulation strategies to help keep feed costs in check. As a result, dairymen are incorporating wet corn gluten feed (WCGF) into diets. Numerous studies have reported production responses to dietary inclusion of WCGF, but few have reported ruminal effects. Therefore an experiment was conducted to monitor production, while simultaneously measuring ruminal fermentation and total-tract digestion in 8 Holstein cows fed 0, 12, 24, and 36% WCGF (DM basis). Results from this study were consistent with recently published papers indicating that increasing dietary levels of WCGF linearly increases milk and milk component production. However, results demonstrate that this increase in production is related to an increase in feed intake, not improved digestibility. In addition to escalating grain prices, recent pressure for land and water use has led to a decrease in the availability of alfalfa. A second experiment was conducted to determine if forage fiber provided by alfalfa hay is necessary to maintain production in diets containing 31% WCGF (DM basis). Eighty primiparous and multiparous Holstein cows were utilized in two 4 × 4 Latin squares to evaluate the effects of feeding alfalfa at 0, 7, 14, and 21% of diet DM. Feeding higher proportions of alfalfa tended to increase ECM yield and decrease BW gain, suggesting that metabolizable energy supply was repartitioned from BW gain to milk production as more alfalfa was included. However, partial budget analysis determined that decreasing alfalfa inclusion rate may improve farm profitability by reducing feed costs and expenses associated with manure handling, despite small losses in productivity. Overall, these research projects suggest that large proportions of WCGF can effectively be fed to dairy cattle without sacrificing milk production, even without the use of alfalfa hay. Therefore, WCGF can be a cost-effective alternative to traditional dietary ingredients.

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CHAPTER 1 - Literature Review

Wet Corn Gluten Feed: Derivation and Composition

Traditionally, dairy producers have utilized grains and forages produced on farm as the primary dietary ingredients of dairy rations. The trend for increased cow numbers per farm is making it infeasible to maintain an integrated production system, forcing dairy producers to purchase feed from off-farm sources. Because traditional feedstuffs can be costly, producers are looking towards co-products to improve their operational profitability. Many co-products are commonly fed to dairy cattle as a means of supplying nutrients and energy for lactation. This review of literature will primarily focus on the use of a corn milling co-product, known as corn gluten feed (CGF).

Ingredients for certain foods and beverages, as well as ethanol fuel, are derived from the processing of grains; in producing these commodities a variety co-products are also created. Because of nutritional attributes, and the price of cereal grains, these co-products play an important role in the livestock feeding industry.

There are two distinctly different grain milling processes that produce alcohol or starch-based products. These two processes are dry milling and wet milling. A majority of refineries are dry milling facilities, which utilize mash distillation. In dry milling, the main objective is to produce alcohol, and as a co-product distillers grains are produced. The other process, wet milling, involves steeping; in this process the refiner is trying to obtain starch for the production of corn sweeteners or ethanol. In wet milling, once the starch is extracted from the kernel, a mixture of corn bran and extractives remain. This remaining portion can be made into CGF.

Weigel et al. (1991) outlined the segregated, complex process of wet milling corn grain. First, a load of corn is delivered to the mill and tested to meet quality standards. Next, as corn is

unloaded into bins, it is thoroughly cleaned via screening and aspiration, getting rid of cobs, dust, chaff, and other foreign material. Material separated during the cleaning process is saved and added back to the co-product feed. From storage, corn is conveyed to large tanks, called steeps, where it is soaked for 30-50 hours in water and 0.1-0.2% sulfur dioxide at ~50°C. During this process, nutrients are extracted from the kernel into the water, creating a liquor. This liquor is then drawn off and used for creating other commodities.

After corn is prepared and steeped, germ is separated. Corn germ is of value because it contains over 80% of the corn's oil. Separators are used to spin this corn germ out of the slurry that was created during the steeping process. The germ is passed through screens and washed to remove any starch from the mixture. Once the remaining starch is removed, oil is extracted from the germ by solvents and agitation.

With the germ removed, an impact mill pulverizes starch particles in the endosperm to release remaining starch and gluten. At this point, bran is separated by screening from the slurry created. Separated bran is used to make the major co-products of the wet milling industry, including wet corn gluten feed (WCGF) or dry corn gluten feed (DCGF). The mill starch, which is the starch/gluten suspension, is then pumped into a centrifuge that will separate the lighter water and gluten from starch. The separated starch is used for a variety of products.

In the United States, 12.1 billion bushels of field corn were produced during 2008, which represents a 12% increase from 2003 (National Agricultural Statistics Service, 2008); of these 12.1 billion bushels, 3.7 billion bushels (≈30%) are destined for the corn milling industry (Seeking, 2008). With so much corn demanded for milling and other processing, corn prices have increased substantially. This price increase has encouraged dairy producers to use milling co-products to help keep feed costs in check.

Because of high fiber in WCGF, it is utilized poorly by monogastrics (Yen et al., 1971; Evvard, 1920), yet it can supply many nutrients to ruminants (NRC, 2001). Studies published when dairy cows were fed high levels of WCGF demonstrate that it can effectively supply nutrients needed for lactation (Armentano and Dentine, 1988; Gunderson et al., 1988; Kononoff et al., 2006), and in some cases increase efficiency of milk production (Boddugari et al., 2001; VanBaale et al., 2001).

Wet CGF primarily consists of gluten, fiber, and soluble nutrients. Studies with beef cattle suggest that WCGF has net energy values similar to corn (Green et al., 1987; Bowman et al., 1988). According to the NRC (2001), WCGF contains approximately 35% neutral detergent fiber (NDF), 2% lignin, and 24% crude protein (CP); however, when balancing a ration, one must recognize variation in nutrient content among batches of WCGF.

To reduce variation between batches, the two major producers of WCGF have developed a modified WCGF marketed under the names Sweet Bran (Cargill, Incorporated, Blair, NE) and Golden Gluten (Archer Daniels Midland Company, Columbus, NE). In producing this feedstuff, each step is controlled in an attempt to reduce product variation. Though nutrient composition among these products is less erratic, shelf life of the feed is limited. To enhance the shelf life and handling characteristics, WCGF can be dried to create dry corn gluten feed (DCGF). Research by Firkins et al. (1985) suggests that WCGF is more digestible than DCGF; but depending on diet type and level of CGF in the diet, the feeding value of the different types of CGF will differ (Green et al., 1987).

Incorporating WCGF into Dairy Cattle Rations

With rising commodity costs, researchers and producers are constantly exploring novel feeding strategies to keep diet related expenses in check. Wet CGF is a relatively inexpensive source of energy ($136.6/ton DM; Penn State Feed Price List, 2009) compared to cracked, rolled, or ground corn ($155/ton DM; average, National Agricultural Statistics Service, 2008). Therefore, if properly incorporated into lactating cow diets, there is tremendous potential to improve farm profitability.

In the early 1980s Staples et al. (1984) published a manuscript reporting the impacts of feeding WCGF at varying levels to lactating dairy cattle. They used 20 multiparous Holstein cows, just past peak lactation, in a 4 × 4 Latin square. Animals consumed one of four TMR that differed in the proportion of WCGF. Wet CGF replaced concentrates at 0, 20, 30, or 40% of ration dry matter (DM); forage level was the same across treatments. Treatments were offered for *ad libitum* intake during each of the 28-d periods. As WCGF was added to the diet these researchers observed significant linear decreases DM intake (DMI; 24.0, 23.3, 22.2, and 21.5 kg/d, respectively) and DM digestibility (74.1, 71.5, 68.9, and 69.3%). Furthermore, the addition of WCGF linearly depressed raw milk yield (30.5, 29.9, 28.1, and 28.1 kg/d), but linearly increased milk fat concentration (2.86, 2.97, 3.15, and 3.21%); therefore, yield of 4% fat corrected milk (FCM) remained unchanged ($P > 0.10$). Milk solids-not-fat (8.71, 8.60, 8.60, and 8.51%) and protein concentration (3.19, 3.14, 3.14, and 3.08%) and yield (0.97, 0.94, 0.88, and 0.87) were depressed linearly by the addition of WCGF to the diet.

The treatments in this trial (Staples et al., 1984) contained 50% forage, but corn silage was the only forage utilized. Macleod et al. (1985) conducted a similar trial with 50% forage, except experimental diets contained 10% hay crop silage in addition to the corn silage. In this trial, treatments compared were control vs. WCGF at either 18.6 or 37.1% of diet DM; WCGF

was substituted for none, half, or all of the soybean meal, and none, one-third, or two-thirds of the cracked corn. Data were collected from 27 primiparous and 30 multiparous Holstein cows in a randomized complete block design. This research group found that feeding WCGF depressed production and intake during the first 4 weeks of feeding. During week 5, this depression of feed intake and milk production eventually disappeared for cows fed the 18%, but not the 37% WCGF diet.

In a second trial, Macleod et al. (1985) used 20 primiparous and 24 multiparous Holstein cows in a randomized complete block design, to compare a control diet to a treatment diet with 26% WCGF (DM basis). Diets contained 33% corn silage and 17% dry hay, and WCGF was substituted for 40% of the soybean meal and 50% of the cracked corn. Cows consuming WCGF produced less milk (26.0 vs. 24.3 kg/d), but had greater milk fat concentrations (3.03 vs. 3.60%). Fat- and solids-corrected milk yield, DMI, and body weight change were similar between treatments ($P > 0.10$).

Feeding WCGF and Optimizing Production

From research previously discussed, it is apparent that large proportions of WCGF can be fed to dairy cattle, but intake and production may be compromised. It is also evident that optimal production is dependent upon the nature of the dietary forage. In 1988, Armentano and Dentine published results from a lactation trial in which they fed varying amounts of WCGF, but the diets were based on several fermented forages with a significant portion being wilted alfalfa silage.

The trial conducted by Armentano and Dentine included 12 multiparous and 4 primiparous Holstein cows in replicated 4 × 4 Latin squares. Cows received increasing amounts of WCGF, up to 36% of ration DM, in place of a grain mixture. Therefore, 2.6, 5.3, or 7.9 kg of

WCGF replaced 2.4, 4.7, and 7.2 kg of concentrate DM, respectively. Milk production, composition, and DMI were not affected by increased feeding of WCGF.

Other researchers have observed similar results to those reported by Armentano and Dentine (1988). Gunderson et al. (1988) fed WCGF at 0, 10, 20, and 30% of the diet DM. Wet CGF replaced 0, 21.2, 40.2, and 60.1% of the hominy, and 0, 34.2, 71.1, and 100% of the soybean meal in each diet. Researchers reported no significant treatment effects on DMI, milk yield, or milk composition.

It is important to note that diets in previously mentioned research contained large proportions of forage, and WCGF was used primarily in place of concentrates, resulting in higher dietary NDF concentrations as WCGF was added. In 2001, VanBaale et al. published results from two experiments where WCGF replaced a mixture of alfalfa hay, corn silage, and corn grain. Diets were formulated to be isonitrogenous and isocaloric.

In experiment 1, VanBaale et al. monitored production of 32 primiparous Holstein cows in a replicated 2 x 2 Latin square with 28-d periods. Cows were housed in free stalls and fed one of two diets; a control diet containing no WCGF, or a treatment diet that consisted of 20% WCGF (DM basis). Cows fed WCGF consumed 2 kg/d more DM ($P = 0.02$), and had greater yields of milk components. Treatment cows produced more energy corrected milk (ECM) compared to the control cows (35.5 vs. 33.2 kg/d, $P < 0.01$), but efficiency, measured as ECM/DMI, was not different among the two treatments ($P = 0.45$).

VanBaale et al. (2001) conducted a second trial to determine the optimal dietary inclusion rate of WCGF. For this trial, 24 multiparous Holstein cows were utilized to make use of six 4 × 4 Latin squares with 28-d periods. Cows were housed in tie-stalls and fed a TMR twice daily. Wet CGF was incorporated into the diets at 0, 20, 27.5, or 35% DM. When compared to the diet with

0% WCGF, researchers observed an increase in DMI for the 20 and 27.5% WCGF diets, but there was no difference for the diet containing 35% WCGF (26.8, 27.7, 27.9, and 26.5 kg/d for 0, 20, 27.5, and 35%, respectively, $P < 0.05$). Cows fed WCGF produced more ECM (approximately 3 kg/d) than controls, however there were no differences in ECM production in the three diets containing WCGF ($P > 0.10$). Results from this study also showed that cows fed WCGF produced ECM more efficiently than controls (1.41 vs. 1.47, 1.47, and 1.53 kg of ECM/kg of DMI).

Schroeder (2003) carried out a similar trial that evaluated the effects of feeding increasing levels of WCGF. Twenty-four multiparous Holstein cows were monitored in a randomized complete block design for 15 weeks. Cows were stratified by age, DIM, and milk yield, and received a TMR containing 0, 15, 30, or 45% WCGF (DM basis). Diets were formulated to be isonitrogenous and isocaloric by substituting WCGF for portions of corn silage, alfalfa haylage, and barley grain. Treatment diets did not affect DMI ($P = 0.22$); however, among cows assigned to each particular treatment, there was a quadratic effect ($P < 0.07$) on feed intake variance. Cows fed treatments containing 0 and 45% WCGF tended to have more variable feed intakes (variances = 14.7, 6.1, 8.8, and 12.4 kg^2/d, respectively). Milk components were not different among treatments ($P > 0.10$), but there was a linear effect on BW gain ($P = 0.04$). Cows consuming 45% WCGF gained 62.6 kg over the 15-week trial, while cows not receiving WCGF gained only 29.6 kg.

More recently, Kononoff et al. (2006) demonstrated that WCGF can replace portions of concentrates and forage while increasing performance. These investigators divided 36 primiparous and 40 multiparous Holstein cows into three groups at dry-off; two groups were fed a control dry cow TMR with no WCGF and one group received 30% WCGF as a part of the dry-

cow TMR. At parturition one control grouped remained a control; therefore this group never received WCGF, while the other two groups received a TMR containing 38% WCGF. Diets were formulated to keep concentration of CP similar, and to ensure that the concentrations of NDF and energy met or exceeded NRC (2001) recommendations. Researchers also made an attempt to keep the concentration of ruminally undegradable protein similar.

Production, DMI, body condition score (BCS), and health were monitored throughout the entire lactation. Dry matter intake was greater for cows consuming WCGF (21.2 vs. 25.4 and 23.8 kg/d for control, WCGF during lactation only, and WCGF during dry period + lactation; $P < 0.01$). Cows fed the TMR containing WCGF had reduced milk fat concentration (4.15 vs. 3.94 and 3.74%, $P < 0.01$). Wet CGF increased overall milk yield by approximately 4 kg/d (31.1 vs. 35.0 and 34.7 kg/d, $P = 0.03$). This increase in total milk allowed milk fat yield to stay similar between treatments ($P = 0.14$). Feeding WCGF also increased milk protein yield by 0.13 kg/d (1.00 vs. 1.15 and 1.10; $P = 0.03$). The increased milk and milk component yield was likely a result of the higher DMI, explaining why efficiency, measured as milk/DMI, was similar for both treatments ($P = 0.39$).

Feeding Maximum Proportions of WCGF

It is evident that WCGF can be used, in place of other feed ingredients, to supply nutrients and energy for lactation. Because WCGF is low in lignin and high in digestible fiber, it can supply substantial amounts of ruminally-fermentable organic matter with more constant acid production in comparison to high starch concentrates (Stock et al, 2000; Fellner and Belyea, 1991). Wet CGF can also replace portions of forage fiber if the physical characteristics of the ration remain sufficient to stimulate rumination (Allen and Grant, 2000). This thought leads one

to question the proportion of *high cost* dietary ingredients that can be replaced by inclusion of WCGF.

Boddugari et al. (2001) conducted a series of three experiments that examined the maximal amounts of concentrate and forage that could be replaced with WCGF. Results from their three experiments indicate that WCGF has potential to effectively replace all of the concentrate and up to 45% of the forage in properly formulated lactating cow diets.

In experiment 1, 16 Holstein cows were assigned to one of four diets in a replicated 4 × 4 Latin square design with 28-d periods. The four treatment diets contained 54.3% forage (alfalfa silage: corn silage, 1:1 DM basis) with WCGF replacing 0, 50, 75, or 100% of the concentrate portion (DM basis). Soybean meal and corn grain were the concentrates replaced in these diets. Replacing 50% and 100% of the concentrates with WCGF resulted in lower DMI ($P < 0.10$), yet milk and milk fat production remained similar across all four treatments ($P > 0.10$). Diets containing WCGF, in place of concentrates, resulted in greater efficiency of 4% FCM production (1.15 vs 1.32, 1.28, and 1.32 for 0, 50, 75, and 100%, respectively; $P < 0.10$).

The second experiment conducted by Boddugari et al. (2001) made use of the same design with 16 Holstein cows. In this experiment, the 100% concentrate replacement diet from experiment 1 was used as the control diet. The three treatment diets were formulated by replacing the forage portion of the control diet with 15, 30, or 45% WCGF. Replacing forage with WCGF led to greater milk production (29.2 vs. 30.4, 31.4, and 31.1 kg/d for control, 15, 30, and 45%, respectively; $P < 0.10$), while DMI remained unaffected. Replacing forage resulted in lower milk fat concentration (3.70, 3.52, 3.50, and 3.21; $P < 0.10$) but the increased milk yield resulted in similar milk fat yield across treatments. Efficiency of 4% FCM production was not

affected by treatment. Cows fed treatments with 30 and 45% replacement rates spent less time ruminating, however ruminal pH was unaffected by treatment.

Reviewing the findings of the first two experiments by Boddugari et al. (2001) suggests that substituting WCGF for 50% of the concentrates and 30% of forage could be optimal. If these optimal replacement rates are achieved, in this type of ration, WCGF will make up 40% of total ration DM. Using this information, this same group of researchers conducted a third trial that tested this level of WCGF against a control diet that did not contain WCGF. Treatment diets were formulated to contain similar CP (18.2%) and RUP (6.3%). In this third trial, 30 Holstein cows were used. Each cow was randomly assigned to a treatment from parturition through 63 DIM (9 weeks). Results from this trial demonstrated that feeding cows 40% WCGF resulted in a 21% greater efficiency of FCM production compared to the control diet.

Feeding WCGF to Growing Replacement Heifers

The time that WCGF has to be stored is directly related to the amount of WCGF that is being fed to the whole herd. Because WCGF is a highly perishable feed resource, rapid utilization can minimize storage time and related waste. The small herd size of many Midwestern dairies dictates that rapid consumption of WCGF be achieved by incorporating large amounts into the cows' diet or by feeding it to other animals on the farm.

In a component feeding system, Armentano and Dentine (1988) supplemented 2 groups of 10 growing dairy heifers with WCGF at a rate of 2.15 kg of DM/ head daily. Performance of these heifers was compared to 2 groups of 10 that were supplemented a control grain mix at 2.00 kg of DM/head daily. The reason for offering the treatment groups 2.15 kg and the control

groups 2.00 kg was not reported, but doing so kept heifer diets energetically similar. Each group received oat silage for *ad libitum* intake for the first 5 weeks of the trial; thereafter corn silage was fed and limited to 4.6 kg of DM/heifer daily. Heifers were grouped to achieve roughly equal pen weights. No treatment differences ($P > 0.10$) were observed in weight gain, age at first calving, 305 d milk yield, or 305 d milk fat production.

Feeding WCGF as the primary dietary component for growing Holstein heifers has also been evaluated (Bernard et al., 1989; Jaster et al., 1984). Heifers consuming these diets were shown to be very efficient and had high weight gains (1.1 to 1.2 kg/d) compared to heifers receiving diets consisting primarily of grass hay, oatlage, alfalfa haylage, or sorghum-soybean silage. Weight gains of heifers fed high WCGF diets were slightly above the current NRC recommendations (1.0 kg/d); therefore, heifers fed these diets could become fat, subsequent milk production may be reduced, and longevity decreased (NRC, 2001). These studies indicate that WCGF should not be fed as the sole feed to dairy heifers, however combining WCGF with a low quality roughage may lead to desirable heifer performance.

Advantages of WCGF

With high grain prices, feeding cereal grains may decrease farm profitability; luckily the nutritional profile of WCGF allows producers to effectively replace these high cost ingredients while maintaining production. Wet CGF is a highly digestible source of fiber and energy for dairy cows. In addition, the CP content of WCGF is nearly three times that of corn grain (Schroeder, 2003; NRC, 2001). If nutritionists can decrease the amount of supplemental protein required, overall feed costs may be reduced.

Wet CGF contains a large portion of RDP (Kononoff, 2007). Depending on processing procedure WCGF will be around 23.8% CP, with 78% of that being degradable in the rumen (NRC, 2001). This ruminally degradable protein fraction is beneficial to the rumen because it provides a mixture of peptides, free amino acids, and ammonia for microbial growth and synthesis of microbial protein (NRC, 2001).

The risks of acidosis or related digestive disorders are of major concern when feeding feedstuffs that are rapidly degraded in the rumen. Though WCGF is rapidly fermented, it contains a relatively low concentration of starch and other rapidly fermentable carbohydrates; thus WCGF is able to supply energy without substantially increasing rumen acid load (Wickersham et al., 2004). If rations are formulated properly, feeding high levels will rarely lead to digestive disorders.

Wet CGF is readily available in the Midwest United States and can easily be incorporated into dairy cattle rations (Kononoff, 2006; Wickersham et al., 2004). Dairies located near a wet corn milling plant have the benefit of feeding WCGF. However, if WCGF needs to be hauled a considerable distance, economics dictate a necessity to dry this feed yielding DCGF. Dry CGF will be discussed more in subsequent pages.

Limitations of WCGF

Though WCGF has several favorable nutritional characteristics, the handling aspects of this feed introduce challenges for processing plants and dairy producers. As previously mentioned, it is not always economically feasible to feed WCGF on farms a considerable distance from the source. Furthermore, the shelf life of WCGF is considerably limited by

environmental conditions. During periods of hot weather, WCGF will develop mold and spoil in a matter of days (Droppo et al., 1985). To make WCGF easier to handle, and increase its shelf life, it should be dried; however, drying WCGF requires energy and can produce air quality problems (Armentano and Dentine, 1988). Drying CGF will also change the feed's nutritional profile, as fiber will become less digestible and protein will become less degradable (Firkins et al., 1985; Green et al., 1987).

Alternatively, the shelf life of WCGF can be maintained by ensiling. Quality of WCGF was sustained as determined by pH, temperature, and organic acid concentrations of subsurface samples taken daily when WCGF was ensiled in a plastic silo bag (Jaster et al., 1984). However, University of Wisconsin extension publications have noted that ensiling WCGF alone in an upright silo is infeasible, mainly because of problems associated with blower pipes getting plugged. A potential fix is to blend WCGF with some other inexpensive forage and ensile that mixture (Hoffman, 2002).

Consistency of WCGF with regard to nutrient composition is also of potential concern when formulating diets to contain this product. The nutrient composition of WCGF has been shown to be variable among batches (23.8 ± 5.7% CP [mean ± standard deviation] and 35.5 ± 6.8% NDF; NRC, 2001). In a Canadian study, Droppo et al. (1985) tested the DM and nutrient composition of 14 truck loads that had been delivered from a single starch plant 150 km away. Four samples were randomly pulled from each load for analysis. While the range of DM values was wide (40-48%), the variability was less than that observed for many nutrients. The researchers were more concerned about the variability of protein and mineral content between loads; when analyzed, the coefficients of variation ranged from 12 to 35%. Other studies have noted variability from sole suppliers as well (Bernard et al., 1991; Fleck et al., 1989). The

variation in nutrient content likely reflects differences in sources of corn, or differences in the processing technique for that particular batch (Bernard et al., 1991).

Although it is possible to chemically balance a diet to include large amounts of WCGF, physical characteristics of the TMR may limit how much WCGF can be fed. The physical properties of WCGF provide little effective fiber. Effective fiber is the portion of the diet that is believed to stimulate rumination, chewing activity, and saliva secretion, all which help maintain healthy rumen function and normal pH levels (Allen, 1997; Kononoff, 2005). The physically effective NDF (peNDF) content of WCGF is around 11% of NDF based on milk composition, rumination time, and ruminal pH of lactating cows (Allen and Grant, 2000). If a TMR is lacking effective fiber, ruminal pH will drop and milk fat production could be depressed (Grant et al., 1990).

Not only do the small particles of WCGF lack peNDF, but they also have a high specific gravity. Specific gravity will account for variation in digestive tract retention time of similar sized particles (Kaske and Engelhardt, 1990). Having a high specific gravity allows feed particles to more easily escape ruminal entrapment, explaining why passage rate is generally higher for diets with greater proportions of heavy particles, in particular WCGF (Hristov et al., 2003). Allen and Grant (2000) exposed WCGF to ruminal fermentation for 3 h and determined the greatest proportion of particles to be at a specific gravity > 1.4. They also demonstrated that cows fed WCGF with 40% alfalfa silage consumed more DM and had quicker passage rates compared to cows fed a TMR with 66% alfalfa silage or 20% alfalfa silage plus 19% alfalfa hay (all on a DM basis; Allen and Grant, 2000).

Fellner and Belyea (1991) also reported on passage rate in a digestion trial using 4 cannulated, non-lactating Holstein cows in a 4 × 4 Latin square design. Treatment diets

contained DCGF at 0, 20, 40, or 60% of diet DM with DCGF replacing portions of corn silage, chopped alfalfa, soybean meal, and a grain mix. Results from their study showed that feeding 60% DCGF increased intake ($P < 0.05$) and decreased DM digestibility ($P < 0.10$). The authors (Fellner and Belyea) calculated the net energy values for each treatment; relative to book values these calculated values were low. Using this evidence, the authors stated that high levels of DCGF caused a depression in digestibility. This is no surprise because as DMI increased, retention time would have decreased; therefore, extent of digestion would have declined. Surprisingly, ruminal pH was not affected by treatment, but concentration of ruminal acetate was lower for cows consuming 60% DCGF ($P < 0.10$). Milk production data was not reported.

Wet CGF contains almost three times the amount of CP as corn. It is known that feeding excess protein can result in nitrogen waste because only a portion of the available nitrogen is used by microbes or absorbed by the host. Feeding protein above recommended levels results in urinary nitrogen excretion (Flis and Wattiaux, 2005). Urinary nitrogen excretion can be of environmental concern because of its relationship to ammonia volatilization and the potential movement of N to surface or groundwater (Nennich et al., 2006). To reduce nitrogen excretion nutritionists should formulate rations for efficient nitrogen utilization by balancing RDP with ruminally available energy.

Conclusion

Commonly, field nutritionists formulate rations on a least-cost basis. Given the nutritional characteristics of WCGF, feeding it to dairy cattle can be a low cost method of providing energy and nourishment. As a whole, defining the optimum amount of WCGF to include in a TMR is complex because of interactions with other feed ingredients. Diets

formulated to complement the characteristics of WCGF, rather than a single substitution of ingredients (either forage or grain), will increase the likelihood of optimizing its use in lactation diets (VanBaale et al., 2001). Some published work indicates that incorporating WCGF into dairy cattle rations might increase milk fat yield (Macleod, 1985; VanBaale, 2001), potentially because of higher fiber content and lower starch in diets including WCGF. Feeding high levels of WCGF primarily in place of forage has been shown to increase ECM yield; however, this increase in milk yield correlates with an increase in DMI (Kononoff et al., 2006).

Though milk production is important, controlling feed costs while raising replacement heifers is a challenging endeavor. Supplementing WCGF in heifer feeding programs may decrease these feed costs and increase animal performance (Armentano and Dentine, 1988). Such practices would promote more rapid utilization of WCGF, reducing storage loss.

One must also note that researchers have observed dramatic variability between batches of WCGF (Droppo et al., 1985; Bernard et al., 1991). Therefore, field nutritionists must be cautious when feeding high levels of WCGF; furthermore, researchers should design experiments so that inconsistency among batches can be accounted for.

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CHAPTER 2 - Effects of Feeding Increasing Levels of Wet Corn Gluten Feed on Total Tract Digestibility, Ruminal Fermentation, and Rate of Ruminal Fiber Digestion

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ABSTRACT

An experiment was conducted to evaluate the effects of increasing dietary inclusion rates of wet corn gluten feed (WCGF; Sweet Bran, Cargill, Inc.). Four primiparous and four multiparous ruminally cannulated Holstein cows averaging 90 ± 13 days in milk (mean ± SD) were randomly assigned to 1 of 4 sequences in a replicated 4 × 4 Latin square with 28-d periods. Treatments were diets containing 0, 12, 24, and 36% WCGF on a dry matter basis, with alfalfa hay, corn silage, corn grain, soybean meal, expeller soybean meal, and mineral supplements varying across diets to maintain similar nutrient concentrations. Performance, total-tract digestion, and measures of ruminal fermentation were monitored. Linear and quadratic effects of increasing WCGF inclusion rate were assessed using mixed model analysis. Increasing dietary WCGF linearly increased dry matter intake (26.7, 25.9, 29.3, and 29.7 kg/d for 0, 12, 24, and 36% WCGF, respectively) and milk production (36.8, 37.0, 40.1, and 38.9 kg/d). Concentrations of milk components did not differ among treatments; however, protein and lactose yields linearly increased, and fat yield tended to increase linearly when more WCGF was fed. This led to greater production of energy corrected milk (38.2, 38.8, 41.7, and 40.4 kg/d) and solids corrected milk (35.2, 35.7, 38.5, and 37.2 kg/d), but efficiency of production linearly decreased. Increased WCGF in the diet tended to linearly decrease rumen pH (6.18, 6.12, 6.14, and 5.91), possibly because treatments with greater proportions of WCGF had a decreased mean particle size. Ruminal acetate concentration decreased linearly and propionate increased linearly as WCGF inclusion rate increased. Treatments had a quadratic effect on ammonia concentration, with greater concentrations in the 0% and 36% WCGF diets. *In situ* digestibility of soybean hulls showed a significant diet by time interaction, and increasing WCGF linearly decreased *in situ* NDF disappearance at 24 h. No differences were observed in total-tract digestibility of the

nutrients monitored. Change in body condition score increased linearly with WCGF inclusion rate. Results indicate that adding WCGF to dairy rations can increase ECM yield, and this increase appears to be driven, at least in part, by an increase in DMI.

Key words: by-product, dairy cattle, wet corn gluten feed

INTRODUCTION

Increased demand for cereal grains has contributed to rising feed costs. Because many rations are formulated on a least-cost basis, researchers and producers are pressured to devise novel strategies to help keep feed costs in check. Recently, focus has turned to the use of milling co-products, particularly wet corn gluten feed (WCGF). Wet corn gluten feed is a rapidly digested non-forage source of fiber and protein (Firkins, 1997; Boddugari et al., 2001). Feeding WCGF to dairy cattle can be a low cost method of providing energy and nutrients needed for milk production.

Defining the optimum amount of WCGF to incorporate into a TMR is complex because of interactions with other feed ingredients (Allen and Grant, 2000). The production response varies from case to case, and it is highly dependent upon the nature of the other dietary ingredients (Armentano and Dentine, 1988; Schroeder, 2003; Kononoff et al., 2006). Diets formulated to complement the characteristics of WCGF, rather than a single substitution of ingredients, will increase the likelihood of optimizing its use in lactation diets (VanBaale et al., 2001). Physically effective NDF (peNDF) of WCGF was reported as 11% of NDF based on rumination time and ruminal pH; therefore, when evaluating feedstuffs to compliment WCGF, sources of peNDF must be included (Allen and Grant, 2000). When peNDF is provided, rumination is stimulated, leading to longer chewing times, greater saliva production, and normal rumen pH levels (Allen, 1997; Mertens, 1997). If the TMR does not provide adequate peNDF, rumen health may be compromised and milk fat depression can occur (Grant et al., 1990; Nocek, 1997).

Some investigators reported an increase in milk fat when incorporating WCGF into dairy cattle diets (Macleod et al., 1985; VanBaale et al., 2001); this is potentially related to higher fiber

and lower starch content of this feed. Feeding high levels of WCGF primarily in place of forage has been shown to increase ECM yield; this increase in milk yield correlates with an increase in DMI (Kononoff et al., 2006).

Brouk et al. (2006) utilized the known information about the feeding value of WCGF, and associative effects, to formulate four rations with linearly increasing amounts of WCGF. Increasing WCGF in the ration linearly increased milk and milk component production without increasing intake in a pen setting (Brouk et al., 2006). Such a response is suggestive of an increase in diet digestibility, because milk yield per kg of DMI increased with greater WCGF. Also, adding WCGF to the diet could have increased the amount of RDP. Increased RDP may have led to increased rumen fermentation, increasing ruminal digestion rate and microbial protein yield.

Generally, increased production responses to inclusion of non-forage fiber sources are correlated with cows consuming more DM (Armentano and Pereira, 1997). Because the findings of Brouk et al. (2006) were in contrast to the norm, the primary objective of this study was to evaluate the effects of increasing amounts of WCGF on apparent total-tract digestibility in lactating dairy cows, while monitoring performance. Secondarily, our goal was to measure the effects of WCGF on the rumen environment and rate of ruminal fiber digestion of soybean hulls.

MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Design and Treatments

Four primiparous and four multiparous cannulated Holstein cows (90 ± 13 DIM; mean ± SD) from the Kansas State University Dairy Cattle Teaching and Research Facility were randomly assigned to square and sequence within square in a replicated 4 × 4 Latin square design balanced for carryover effects. Animals were randomly assigned to 1 of 8 tie stalls. Treatment periods were 28 d, with the final 8 d used for *in situ* work, data collection, and sample collection. At the beginning of the experiment, BW of cows were to 605 ± 27 kg with BCS of 2.76 ± 0.30.

Cows were offered one of four rations that differed by the amount of WCGF included. Targeted inclusion rates were 0, 12, 24, and 36% WCGF. Chemical composition of the WCGF used in this study is shown in Table 2.1. Experimental diets were essentially the same as those used by Brouk et al. (2006). In formulating diets, the strategy was to develop diets similar in concentrations of CP, NDF, and NFC. As a consequence, diets containing more WCGF had less alfalfa, corn silage, corn grain, and soybean meal.

Data and Sample Collection

Throughout the experiment cows were fed twice daily at 110% of expected intake. On d 25 to 28 of each period, the amount of feed offered and refused was recorded to determine DMI. Samples of all dietary ingredients were collected on d 25 to 28. Grab samples of the TMR and individual dietary ingredients were collected at feeding; representative orts (6.25%) were collected 23.5 h post-feeding. Feed samples were composited into one sample per period. Fecal samples, rumen samples, and rumen pH data were collected every 9 h from d 26 to 28 so that 8 samples were taken from each cow each period, representing every 3 h of a 24-h period to account for diurnal variation. Acid detergent insoluble ash (ADIA) was used as an internal

marker to determine apparent total-tract digestibility of DM and other nutrients (Cochran et al., 1986). *In situ* digestion of soybean hulls was used as an index of diet effects on rate of ruminal fiber digestion on d 21 to 24 of each period.

Cows were milked three times daily in a milking parlor, and milk was sampled and yield recorded for every milking on d 25 to 28 of each period. Body weight and BCS were measured on d 1 of each period and d 28 of the last period. Body condition score was measured on a scale of 1 to 5 by three trained investigators according to Wildman et al. (1982).

Nutrient and Milk Analyses

The Penn State Particle Separator was used to measure particle size for both TMR and orts (Lammers et al., 1996). Diet ingredients and fecal samples were dried in a 55°C forced-air oven for 72 h. Feed ingredients were analyzed for DM concentration, ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and composited by period. Dry fecal samples were ground through a 5-mm screen, then fecal samples from a single cow period were composited on an equal mass basis and ground through a 1-mm screen. Ash concentration was determined after 5 h of oxidation at 500°C in a muffle furnace. Concentration of NDF was determined (Van Soest et al., 1991) using an Ankom Fiber Analyzer (ANKOM Technology, Fairport, NY). Crude protein was determined by oxidation and detection of N_2 (Leco Analyzer, Leco Corp, St. Joseph, MI). Crude fat was determined by ether extract (AOAC, 2000: method 920.9). Starch was determined by alpha-amylase and glucoamylase digestion, followed by colorimetric glucose quantification using a commercial kit (Autokit Glucose; Wako Chemicals USA, Richmond, VA). Concentrations of all nutrients except for DM were expressed

as percentages of DM determined by drying at 105°C in a forced-air oven for more than 8 h. All analyses were performed in duplicate.

Milk samples were analyzed for fat, true protein, and lactose using a B2000 Infrared Analyzer (Bently Instruments, Chaska, MN) by Heart of America DHIA (Manhattan, KS). Energy-corrected milk (ECM; 0.327 x milk yield + 12.86 x fat yield + 7.65 x protein yield; DHI glossary, Dairy Record Management Systems, 2009) and solids-corrected milk (SCM) yield were calculated (Tyrrell and Reid, 1965).

Rate of Ruminal Fiber Digestion

Each of the 8 animals had 16 dacron bags of soybean hulls (0.5 g) placed in the rumen at different time points over a 72-h period starting on d 21. The soybean hulls used in this part of the experiment all came from the same batch and were ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). Dacron bags had dimensions of 5 x 10 cm; pore size was 50 µm (ANKOM Technology, Fairport, NY). Bags were weighted and placed in the rumen, in quadruplicate, at time points that allowed for 12, 24, 48, or 72 h of ruminal digestion. On d 24 all 16 bags were removed from each animal for rinsing. Rinsing was done in warm water (~30°C) using a series 80 wash machine (Kenmore, Hoffman Estates, IL). A wash machine was chosen to rinse the bags to ensure consistant rinsing patterns. After rinsing, bags were dried in a forced-air oven at 55°C for 72 h for determination of DM. Bags were then transferred to an Ankom apparatus to determine NDF residue. Six samples of soybean hulls from the original batch that had not been subjected to ruminal degradation were analyzed for DM and NDF concentration. Dry matter and NDF remaining in bags subjected to *in situ* digestion were

compared to the NDF and DM content of these six samples; DM and NDF disappearance was calculated according to the difference.

Rumen Environment

Grab samples of ruminal digesta were collected from 5 locations throughout the rumen, mixed, and a representative subsample was strained through four layers of cheese cloth to obtain a fluid sample. Rumen fluid pH was measured using a portable pH meter (Orion Research, Boston, MA), and a 20 mL sample was frozen at -20°C until compositing for analyses of VFA and NH_3. Rumen fluid was composited by cow period to yield an 8-mL sample, and 2 mL of 25% metaphosphoric acid was added. Composited samples were frozen, thawed, centrifuged at 30,000 x g for 20 min, and the supernatant was collected for analysis.

Ammonia was determined colorimetrically using an autoanalyzer (Technicon Analyzer II, Technicon Industrial Systems, Buffalo Grove, IL). Ruminal VFA were measured using a gas chromatograph (Model 5890, Hewlett-Packard, Avondale, PA) with a flame ionization detector (compressed air flow was set at 200 mL/min and hydrogen flow was set at 20 mL/min). The chromatograph was fitted with a 1.82 m × 6.35 mm ID glass column, packed with GP 10% SP-1200/1% H_3P0_4 (Supelco #1-1965, Bellefonte, PA). The column was maintained at 130°C, the detector and injector were maintained at 250°C, and carrier gas (N_2) flow was 80 mL/min. Run time was approximately 5 minutes.

Statistical Analysis

In the first two periods of this trial, two cows were documented reaching into neighboring feed bunks. When efficiencies were calculated, distribution analysis showed these cows to be extreme outliers. Intake of these cows was deemed inaccurate, so intake, production, digestibility, and efficiency data of these two cows were omitted from analysis. Intake and efficiency data of the cows that were being stolen from were omitted from analysis as well. Cows changed locations in the tie-stall barn on d 1 of period 3, to allow for one empty stall between each pair of neighboring animals.

Data were analyzed according to the following model using the REML procedure of JMP (version 8.0, SAS Institute, Cary, NC):

$$Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk}$$

where μ = overall mean, T_j = fixed effect of treatment (j= 1 to 4), P_i = random effect of period (i = 1 to 4), C_k = random effect of cow (k = 1 to 8), e_{ijk} = residual error. The fixed effect of time was also included in the model to assess time x treatment interactions when analyzing *in situ* degradation of soybean hulls. Linear and quadratic effects of WCGF inclusion rate were tested. Treatment effects were declared significant at $P < 0.05$. Tendencies for treatment effects were declared at $P < 0.10$.

RESULTS AND DISCUSSION

Diets were formulated to meet or exceed NRC (2001) requirements for a Holstein cow producing 45 kg of milk; ingredient and nutrient analyses are shown in Table 2.2. Results indicate that ingredient compositions of treatments used in this study were similar to treatments used by Brouk et al. (2006). Nutrient composition remained relatively similar across all 4

treatments, with the exception that DM and ash were slightly higher for the diet without WCGF (Table 2.2). The WCGF used in this study was supplied from a single source (Sweet Bran, Cargill, Inc., Blair, NE) and nutrient variability of this product, across the 4 collection periods, was low (Table 2.1).

As the inclusion rate of WCGF increased, DMI increased linearly from 26.7 to 29.7 kg/d ($P < 0.03$; Table 2.3). Because intake is influenced by feed specific gravity (Kaske and Engelhardt, 1990), particle size (Beauchemin and Yang, 2005), and rate of fermentation (Allen, 2000), it is no surprise that DMI increased in diets with greater concentrations of WCGF. Studies done using similar diets indicate higher passage rates in diets with more WCGF and less forage (Allen and Grant, 2000; Boddugari, 2001). A quicker passage will lead to decreased rumen fill per unit of feed intake, which could have stimulated greater feed intake in this study. Other researchers have reported cows consuming WCGF to have higher DMI (VanBaale et al., 2001; Kononoff et al., 2006), but these results are in contrast to studies reporting a decrease in DMI (Staples et al., 1984; Macleod et al., 1985; Boddugari 2001), as well as some who indicate WCGF inclusion does not affect DMI (Armentano and Dentine, 1988; Gunderson et al., 1988; Schroeder 2003). Milk yield increased linearly from 36.8 to 38.9 kg/d as greater proportions of WCGF were offered ($P < 0.007$; Table 2.3); however, concentrations of milk components (3.70 ± 0.11% fat, 3.06 ± 0.08% protein, 5.02 ± 0.03% lactose, mean ± SEM) were not affected ($P > 0.13$; Table 2.4). Because of the increases in total milk, there were greater yields of fat, protein, and lactose as WCGF was added (Table 2.4). The increases in milk and milk component yields from increased WCGF led to greater SCM ($P < 0.01$) and ECM ($P < 0.01$) production (Table 2.3). Similar increases in milk and milk component yields have been reported when WCGF replaced dietary forages (Boddugari et al., 2001; VanBaale et al., 2001; Kononoff et al.,

2006).The total energy being used for productivity, which includes production of milk and changes in BCS, linearly increased as WCGF inclusion rate increased ($P < 0.001$; Figure 1); this is not surprising as DMI was higher for these cows, which likely resulted in greater consumption of energy. Feed efficiency, measured as ECM/DMI, linearly declined as more WCGF was added ($P < 0.007$; Table 2.3). Changes in feed efficiency have not commonly been observed when feeding increasing dietary levels of WCGF (Staples et al., 1984; Armentano and Dentine, 1988; Schroeder, 2003; Kononoff et al., 2006); furthermore, when a change in efficiency has previously been observed it was an increase (Gunderson et al., 1988; Boddugari et al., 2001; VanBaale et al., 2001). Treatments did not affect energy efficiency ($P > 0.33$), measured as total NE for productive use over DMI. Therefore when milk production efficiency dropped in diets with greater WCGF, energy was not lost, but instead partitioned toward BCS.

The dietary level of WCGF had a quadratic effect on MUN. This response does not correspond with treatment effects on dietary CP or milk protein, but it does reflect changes noted in rumen ammonia (Table 2.6). Because MUN is derived from rumen ammonia being absorbed, converted to urea in the liver, and then secreted in milk, one could expect concentrations to coincide.

As WCGF was added to the diet, forage was removed. It has been demonstrated in many accounts that non-forage fiber (WCGF) is more rapidly fermented in the rumen than forage fiber (Armentano and Pereira, 1997; Firkins, 1997). Cows fed more fermentable diets have greater fat deposition due to increased lipogenesis in adipose tissue (Oba and Allen, 2003). This likely explains the linear increase in BCS change as greater proportions of WCGF were fed ($P < 0.02$, Table 2.3). Body weight change was not affected by treatment ($P \geq 0.65$) and did not correspond with changes noted in BCS. With the dietary treatments used in this study, effects on DMI and

expected differences in passage rate from the rumen could have resulted in treatment effects on gut fill, which would complicate interpretation of weight change data. Conversely, Nikkhah et al. (2008) recently demonstrated that BCS provides little information about internal fat stores, and changes in body weight may be a better reflection of total tissue accretion than BCS if gut fill was not altered. However, given the treatments used in this study and the relatively stable body weights of cows, we expect that discrepancies between weight change and BCS change were most likely due to difference in gut fill, and we considered treatment effects on BCS to be meaningful.

Substituting WCGF for portions of corn silage, alfalfa, corn grain, and soybean meal kept dietary NDF values similar (~30%), but the effectiveness of that fiber can be questioned (Armentano and Pereira, 1997). Table 2.5 shows the particle size data of the TMR and orts. All four diets had a relatively small mean particle size. With higher WCGF inclusion, the proportion of TMR particles greater than 8 mm declined. As expected, this increased the proportion of small particles (less than 8mm) for these diets. In all diets, the mean percentage of particles greater than 19 mm was around 3%; this is low, but it is within current recommendations for a lactating cow TMR (Heinrichs and Kononoff, 2002). However, according to these same guidelines, all 4 treatment diets contained an insufficient proportion of particles between 8 and 19mm.

Having an adequate supply of long particles is necessary for healthy rumen function and maintenance of rumen pH (Lammers et al., 1996). Diets lacking long particles are generally more fermentable (Grant et al., 1990), which can lead to greater acid production, explaining the linear drop ($P < 0.001$) in rumen pH as WCGF was added to the diet (Table 2.6). Excessive acid production is often attributed to starch in dairy rations; however, the diet with lowest pH had the lowest starch concentration so they are likely not related in this case. Because pH changes were

likely related to particle size, if the diets with more WCGF had contained a greater proportion of particles longer than 8 mm, rumination could have increased and possibly led to increased chewing activity. Increased chewing activity may have stimulated production of a salivary buffer; therefore rumen pH might not have been affected (Balch, 1971; Kay, 1966). It must also be noted that even though adding WCGF depressed rumen pH, milk fat production was not adversely affected. This suggests the diet with 36% WCGF still provided enough effective fiber to maintain rumen function and promote ruminal biohydrogenation.

Analysis of the orts showed no differences in particle size across the four treatments (Table 2.5). This suggests cows fed the 0% and 12% inclusion rates consumed more long particles. The long particles consumed were most likely from forages, which stimulated rumination and saliva production, explaining the higher pH for these treatments. Comparing particle size data of the orts to the TMR data showed no differences ($P > 0.15$) in the three fractions tested; this suggests that animals did not sort. Because each diet contained a relatively small proportion of long particles, sorting may not have been possible (DeVries et al., 2007), or the moisture level of each diet could have served as a binder preventing sorting (Leonardi and Armentano, 2003).

Measures of ruminal fermentation are presented in Table 2.6. As expected, the lower pH observed as WCGF was added coincided with decreased ruminal acetate and isovalerate concentrations ($P < 0.001$), and increased propionate and valerate concentrations ($P < 0.001$). Differences in diet particle size or ruminal fiber digestibility could have led to these effects (Krause et al., 2003). There were quadratic effects on concentrations of total VFA ($P < 0.09$) and ammonia ($P < 0.01$), where 0 and 36% WCGF-fed cows tended to have higher overall VFA and ammonia concentrations. The differences in concentration might suggest that ruminal absorption

of these components was greater for cows fed 12 and 24% WCGF (Brown et al., 1960; Roffler et al., 1976), or total production was greater for cows fed 0 and 36% WCGF. The dietary inclusion rate of WCGF did not affect butyrate and isobutyrate concentrations. In general, this study shows WCGF significantly affecting the VFA profile, whereas others have observed minimal effects (Firkins et al., 1985; Schroeder, 2003).

Digestibility

Soybean hulls have a highly digestible fiber fraction and minimal associative effects (Weidner and Grant, 1994); therefore, they were used to measure rate of fiber digestion in the rumen. *In situ* digestibility of soybean hulls showed a significant diet by time interaction (Figure 2; $P < 0.001$), and increasing WCGF linearly decreased *in situ* NDF disappearance at 24 h (Table 2.6, $P < 0.01$). A quadratic tendency ($P = 0.051$) was also noted at 24 h, showing the diet with 24% WCGF to have the lowest NDF disappearance of soybean hulls. We speculated that this was because of the effect of diet on rumen pH, but in assessing the relationship between pH and 24 h *in situ* NDF disappearance we found no correlation ($R^2 = 0.0002$). Therefore, it is unclear how increasing WCGF inclusion negatively affected *in situ* digestibility, but pH does not appear to be the primary cause.

Treatment effects on apparent total-tract digestion of DM, OM, NDF, ADF, CP, and starch were assessed (Table 2.7). Statistical analysis showed no significant differences, or tendencies for differences, in apparent digestibility as dietary inclusion rate of WCGF increased. It can be presumed that WCGF is rapidly fermented relative to the forages that were replaced, so it is difficult to speculate as to why differences in digestibility were not observed. It is possible that values provided by our internal marker are inaccurate. According to Thonney et al. (1985), a

concentration of 0.75% ADIA, is needed to confidently measure digestibility in ruminant diets. Although our treatment diets contained moderate levels of plant tissues from corn silage and alfalfa hay, concentrations of ADIA were below 0.75%, ranging from 0.41 to 0.61%. Low concentrations of the internal marker likely resulted in the high standard errors we observed, and statistical contrasts therefore must be interpreted with caution. We must also note that statistical power was weak due to removal of 8 cow periods, making differences more difficult to detect.

The lack of effect on digestibility might also be related to passage rate. It is known that ruminal retention time strongly influences the extent of ruminal degradation (Hristov et al., 2003). The small particle size, and high specific gravity of WCGF, allows these feed particles to escape ruminal entrapment, likely increasing passage rate (Beauchemin and Yang, 2005). Therefore, as forage is replaced with WCGF, diets are likely degraded more rapidly, but extent of digestion may not increase because passage rate increases. Data presented in Table 2.7 represents only total-tract digestion, and kinetics and site of digestion could have differed across treatments.

CONCLUSIONS

Results from this study demonstrate responses to WCGF that are consistent with recently published papers. However, rather than indicating that WCGF improves the rumen environment for fiber-digesting bacteria, production responses to WCGF in this study seem to have been driven by increased DMI. Increased DMI is likely caused by decreased rumen fill due to feeding source of NDF with smaller particles and a more rapid disappearance rate. As a whole, adding WCGF to dairy rations will likely increase ECM yield; however, this increase in production is driven, at least in part, by an increase in DMI.

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Figure 2.1 Total energy partitioned to milk production and BCS change in cows fed increasing levels of WCGF. As WCGF was added, total energy utilization linearly increased ($P < 0.001$). Body condition score loss was assigned an energetic value of 368 Mcal/unit and BCS gain was assigned 459 Mcal/unit (NRC, 2001); milk energy was calculated according to Tyrell and Reid (1965).

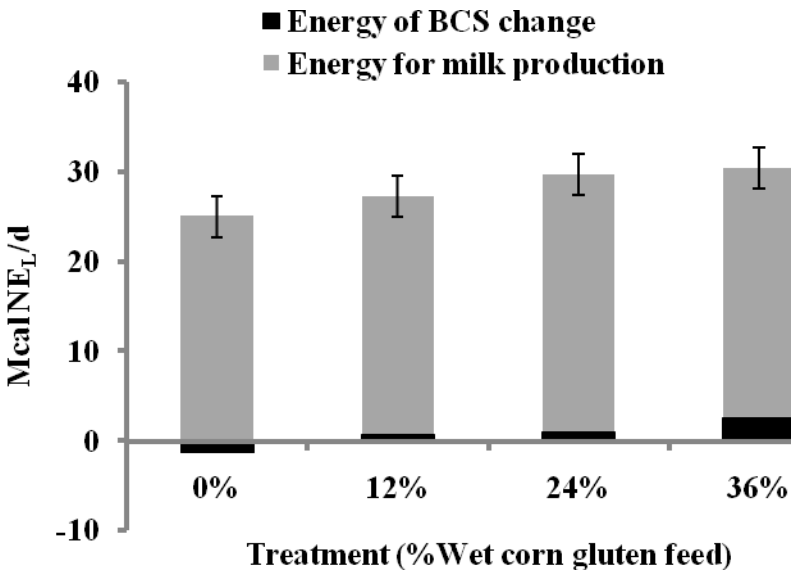

Figure 2.2 ***In situ*** **NDF disappearance of soybean hulls in diets with increasing amounts of WCGF measured over a 72-h period. There was a time x treatment interaction on DM digestibility (*P* < 0.001). Increasing WCGF inclusion rate linearly decreased *in situ* NDF disappearance at 24 hrs (*P* < 0.01); there was also a tendency for a quadratic effect (*P* = 0.051) at this time point driven by decreased *in situ* NDF disappearance of soybean hulls in the diet with 24% WCGF.**

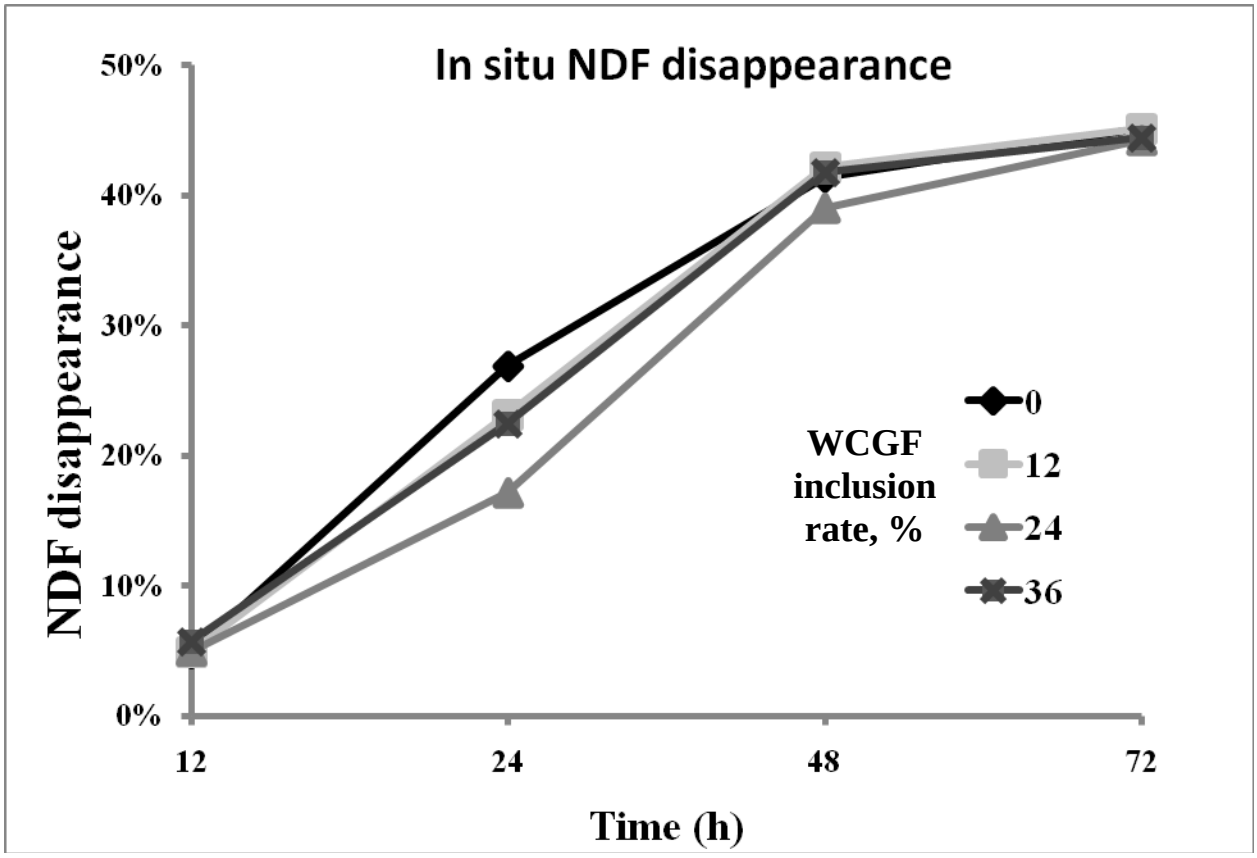

Table 2.1 Nutrient composition of wet corn gluten feed used in experiment[1]

Nutrient	% of DM	Std. Dev.
DM (% as-fed)	56.1	0.9
CP	24.5	0.4
NDF	35.3	1.1
ADF	11.0	0.9
EE	2.3	0.2
Starch	11.2	0.5
Ash	5.8	0.4

[1]Acquired from samples taken on d 25 to 28 of all four periods.

Table 2.2 Ingredient and nutrient composition of dietary treatments

		Treatment[1]			
		0%	12%	24%	36%
%DM	WCGF[2]	0.0	11.4	23.2	33.6
	Corn silage	25.2	25.5	22.1	18.4
	Alfalfa	24.4	24.6	21.2	17.7
	Cottonseed	6.1	6.2	6.2	6.1
	Corn grain	23.5	19.9	17.3	14.6
	Soybean meal	8.6	4.9	2.2	2.2
	Molasses	0.4	0.4	0.4	0.4
	Expeller soybean meal	3.3	3.7	4.0	3.6
	Soybean hulls	5.0	…	…	…
	Limestone	1.00	1.08	1.28	1.36
	Magnesium oxide	0.26	0.24	0.21	0.17
	Micronutrient premix[3]	1.33	1.32	1.33	1.31
Nutrients[4]					
	Dry matter, % as-fed	65.4	60.0	61.3	61.2
	Crude protein	19.3	18.8	19.1	20.1
	RDP[5]	63.5	65.3	63.9	66.6
	Neutral detergent fiber	28.8	28.8	30.4	31.0
	Starch	24.3	27.9	25.5	24.2
	Non-fiber carbohydrates[6]	39.1	40.9	38.6	37.6
	Ether extract	3.4	3.3	3.6	3.6
	Ash	9.4	8.3	8.3	7.7
	Acid detergent insoluble ash	0.61	0.57	0.50	0.41

[1]0%=0% WCGF, 12%=11% WCGF, 24%=23% WCGF, 36%=34% WCGF (DM Basis).
[2]Wet corn gluten feed; Sweet Bran, Cargill, Inc.
[3]Premix consists of 61.0% sodium bicarbonate, 27.3% trace mineral salt, 3.90% 4-plex, 3.90% Se premix, 2.60% Vit E, 1.30% Vit A, and 0.21% Vit D.
[4]Nutrients other than DM expressed as a percent of diet DM.
[5]Calculated according to NRC, 2001.
[6]Calculated as DM – (CP + NDF + EE + ash).

Table 2.3 Effects of treatments on performance of lactating cows

	Treatment[1]					*P*-value	
Item	0%	12%	24%	36%	SEM	Linear	Quadratic
DMI, kg/d	26.7	25.9	29.3	29.7	1.55	0.03	0.55
Milk, kg/d	36.8	37.0	40.1	38.9	2.57	0.007	0.28
SCM, kg/d	35.2	35.7	38.5	37.2	2.54	0.01	0.19
ECM, kg/d	38.2	38.8	41.7	40.4	2.75	0.01	0.19
ECM/DMI	1.44	1.50	1.34	1.29	0.06	0.007	0.20
Body Wt. change, kg/28 d	45.6	14.3	9.2	29.7	17.6	0.65	0.73
BCS change/28 d	-0.02	0.09	0.15	0.25	0.07	0.02	0.92

[1]0%=0% WCGF, 12%=11% WCGF, 24%=23% WCGF, 36%=34% WCGF (DM Basis).

Table 2.4 Effects of treatments on milk components

	Treatment[1]					*P*-value	
	0%	12%	24%	36%	SEM	Linear	Quadratic
Milk fat, %	3.65	3.76	3.72	3.67	0.11	0.93	0.23
Milk protein, %	3.02	3.07	3.05	3.11	0.08	0.13	0.80
Lactose, %	5.02	5.00	5.03	5.01	0.03	0.94	0.96
SCC[2]	40.6	64.1	31.9	50.2	14.8	0.96	0.87
MUN, mg/dL	17.2	16.3	16.3	17.3	0.90	0.83	0.08
Yield, kg/d							
Milk fat	1.37	1.39	1.49	1.44	0.11	0.06	0.21
Milk protein	1.11	1.14	1.21	1.21	0.08	0.01	0.49
Milk lactose	1.85	1.85	2.02	1.95	0.13	0.01	0.32

[1]0%=0% WCGF, 12%=11% WCGF, 24%=23% WCGF, 36%=34% WCGF (DM Basis).

[2]Three outliers removed

Table 2.5 Particle size separation data (% as-fed basis)[1]

		Treatment[2]			
		0%	12%	24%	36%
TMR	> 19 mm	3.85[a]	3.25[ab]	2.97[ab]	2.36[b]
	19 to 8 mm	29.6[a]	29.6[a]	27.2[b]	24.2[c]
	< 8 mm	66.6[a]	67.2[a]	69.9[b]	73.4[c]
Orts[3]	> 19 mm	4.23	5.07	3.85	1.50
	19 to 8 mm[4]	27.4	30.0	30.6	23.7
	< 8 mm[4]	70.6	64.9	65.5	74.7

[1]Measured using a 3 compartment Penn State Particle Size Separator (Lammers et al., 1996).
[2]0%=0% WCGF, 12%=11% WCGF, 24%=23% WCGF, 36%=34% WCGF (DM Basis).
[abc]Means with different superscripts are significantly different by Tukey's HSD ($P < 0.05$).
[3]No significant differences were detected ($P > 0.15$) between the TMR and orts for each fraction across treatment diets.
[4]One outlier removed.

Table 2.6 Effects of treatments on rumen environment

	Treatment[1]					P-value	
(m*M*)	0%	12%	24%	36%	SEM	Linear	Quadratic
Total VFA	168.6	163.8	160.1	165.0	4.5	0.26	0.09
Acetate	97.2	90.6	87.1	84.6	2.4	<0.001	0.15
Propionate	34.4	37.8	36.8	43.1	1.3	<0.001	0.15
Butyrate	25.9	26.0	25.3	26.9	1.1	0.32	0.43
Isobutyrate	2.10	2.11	2.08	2.16	0.08	0.59	0.60
Valerate	4.38	4.76	4.90	5.84	0.26	<0.001	0.02
Isovalerate	3.45	3.36	3.11	2.56	0.31	0.001	0.16
NH_3	16.2	13.1	12.9	15.7	1.2	0.69	0.01
Rumen pH	6.18	6.12	6.14	5.91	0.06	0.001	0.07
24-h *in situ* NDF disappearance, %	26.6	24.8	18.6	22.5	1.00	0.006	0.051

[1]0%=0% WCGF, 12%=11% WCGF, 24%=23% WCGF, 36%=34% WCGF (DM Basis).

Table 2.7 Effects of treatments on total-tract nutrient digestibility

	Treatment[1]					P-value	
Digestibility, %	0%	12%	24%	36%	SEM	Linear	Quadratic
DM	64.5	65.1	72.4	69.7	4.3	0.24	0.72
OM	65.8	66.5	73.3	70.5	4.2	0.27	0.71
NDF	42.4	45.6	58.0	50.9	6.9	0.20	0.43
ADF	42.3	45.9	51.3	45.5	6.7	0.57	0.47
CP	68.7	66.6	74.8	73.9	4.0	0.20	0.90
EE	82.4	78.7	82.8	80.5	2.4	0.88	0.81
Starch	80.3	78.7	82.8	82.4	5.2	0.28	0.77

[1]0%=0% WCGF, 12%=11% WCGF, 24%=23% WCGF, 36%=34% WCGF (DM Basis).

CHAPTER 3 - Technical Note: Simplified Procedure for Quantifying Ruminal Microbe Populations Using Real-time PCR

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ABSTRACT

Diets are directly linked to a ruminant animal's performance through rumen microbial fermentation. Detailed knowledge of microbial populations can be useful when attempting to manipulate fermentation. Currently, there are a variety of molecular techniques employed to quantify ruminal microbiota; however, sample processing requirements for most techniques are complex and time consuming. The objective of this technical note is to outline a simplified procedure for obtaining representative DNA from ruminal digesta, which can be used to analyze relative abundance of microbial populations using real-time PCR.

Key words: rumen, real-time PCR, DNA extraction

TECHNICAL NOTE

Researchers are constantly exploring new ways to improve production of ruminant animals. Many improvements can be made through the manipulation of dietary ingredients. Dietary influences are connected to animal performance through fermentation by ruminal microbiota. Quantifying these microbes can be a challenging proposition because of sampling difficulty, and the morphological and physiological similarities among related species (Weimer et al., 1999). Having a simple method to accurately quantify individual taxa within a rumen microbial community introduces opportunities to gather more information about the rumen. With more information available about ruminal fermentation, nutritionists can formulate rations that will improve health and efficiency of ruminants, as well as reduce methane emissions.

There are a number of molecular techniques employed to quantify bacteria, but each method has limitations. Counting bacteria microscopically can be done, but it is time consuming and obtaining counts on each individual species within the rumen microbial community would be infeasible. Culturing bacteria presents alternative options for counting bacteria; selective media can be used permitting growth of only the species of interest; based on growth rate, population can be estimated. However, keeping anaerobic cells viable *in vitro* can be challenging (Hungate, 1973). Furthermore, culturing methods can be expensive and some require a tremendous degree of manual dexterity. To circumvent the need to grow organisms, hybridization methods exist to probe nucleotide sequences without a need for amplification. Fluoroscence *in situ* hybridization is a probing technique that is widely used for counting microbes in the fields of microbial ecology and clinical diagnostics. However, because of the heterogeneity of rumen digesta, researchers have encountered difficulty enumerating ruminal microbes with this technique (Tepsic and Avgustin, 2001).

Most recently, researchers have utilized real-time PCR as a means of quantifying bacteria. The development and application of this tool is revolutionizing microbiology. Utilization of real-time PCR enables researchers to accurately quantify sequences of DNA specific to individual species. Yet, few reports demonstrate an attempt to quantify the abundance of individual microbial species as a fraction of total ruminal bacteria, possibly because of the labor associated with molecular level analyses.

Real-time PCR is based on an oligonucleotide primer binding to complimentary strands of DNA, then replicating exponentially. The amount of double-stranded DNA is measured through detection of a fluorescent dye that binds to double-stranded DNA. Comparison of populations is based on the proportion of species-specific DNA in the sample. Therefore, obtaining a representative sample for DNA extraction is the most crucial step. Researchers have made progress in this area, but the conventional methods have limitations.

Pioneers of this technique strained rumen digesta to separate large particulate matter, and then the solids portion was discarded (Tajima et al., 2001; Klieve et al., 2003). Utilizing this technique yields a clean, uniformly textured sample of rumen fluid for DNA extraction, but it eliminates the ability to account for microbes that remain attached to digesta particles. Stevenson and Weimer (2007) strained rumen fluid in a similar manner, but they retained the solids portion and processed it to obtain a microbial pellet. Stevenson and Weimer then used 25 ml of ruminal fluid and 25 g of the microbial cell pellet for DNA extraction. This approach works, but it is very labor intensive. In addition, this approach requires rumen solids and fluid to be recombined for DNA isolation; therefore, unless one assumes a homogenous distribution of cells, it is difficult to combine them in the exact ratio that they are found in the rumen.

The objective of our work was to use real-time PCR to similarly quantify bacteria while simplifying the sample preparation process. Our trial utilized ruminal contents from eight ruminally cannulated lactating Holstein cows fed differing diets in a replicated 4 × 4 Latin square with 28-d periods. Cows were housed in tie-stalls and fed one of four diets that differed by the amount of wet corn gluten feed (WCGF) included. Targeted WCGF inclusion rates were 0, 12, 24, and 36% (DM basis), with alfalfa hay, corn silage, corn grain, soybean meal, expeller soybean meal, and mineral supplements varying across diets to maintain similar nutrient concentrations. Diets were fed for *ad libitum* intake. Performance, digestibility, and measures of ruminal fermentation of these cows are reported in chapter 2.

Rumen samples were collected every 9 h from d 26 to 28 so that 8 samples were taken from each cow each period, representing every 3 h of a 24-h period, thus accounting for diurnal variation. In an attempt to obtain a representative sample, we collected digesta and rumen fluid as one sample. This was done to capture the free floating microbes and those adhered to feed particles while maintaining them in similar proportions as in the rumen.

The procedure for sampling ruminal contents entailed obtaining grab samples from 5 locations throughout the dorsal and ventral regions of the rumen. These samples were mixed, and a representative subsample (200 g) was collected. This sample was immediately placed in a freezer set at -20°C. Prior to processing, samples were thawed at room temperature until they became pliant, then composited by cow period. In order to prevent post-sampling fermentation, an effort was made to minimize the amount of time samples sat at room temperature.

Each composited sample was diluted with distilled, deionized water at a 1:1 ratio, and homogenized using a high-speed blender (Waring Commercial, Torrington, CT). A subsample was then obtained from the homogenized mixture and used for microbial DNA isolation using a

commercial kit (ZR Fecal DNA Kit, Zymo Research Corp., Orange, CA). This kit utilized bead-based processing to dissociate microbes from feed particles, followed by a chemical and filter-based isolation procedure of total DNA. Isolated DNA was quantified by spectroscopy (Nanodrop-1000, Nanodrop Technologies Inc., Wilmington, DE), and sample volumes were adjusted to achieve uniform DNA concentrations across all samples.

Quantitative real-time PCR was used to determine relative abundance of bacterial populations using previously validated primers specific for genes encoding 16S ribosomal RNA (Stevenson and Weimer, 2007). Presence of fungi was determined using a primer designed for regions of 18S ribosomal RNA and internal transcribed spacer regions (Denman and McSweeney, 2006). Forward and reverse primer sequences are listed in Table 3.1. Primers were mixed at a 1:1 ratio and each primer was included at a concentration of 200 n*M* or 450 n*M* (Table 3.1). Quantitative real-time PCR was performed in triplicate for each cow period using a commercial mix (Power SYBR Green PCR Master Mix; Applied Biosystems, Foster City, CA). Each well included 4 μL of DNA template in a 20 μL volume. Fluorescence was monitored in real-time using the Applied Biosystems Prism Fast 7500 sequence detection system (Applied Biosystems, Foster City, CA). For all of the primers, amplification consisted of an initial hold for 20 s followed by 40 cycles of 95°C for 3 s and 60°C for 30 s. A normalized reporter (Rn) of 0.2 was set as the common threshold for all genes. The Rn was based on the difference in fluorescence from the base to the peak of each cycle, ROX was used as the dye to measure baseline fluorescence. The number of cycles required to reach the threshold (CT) were recorded for each sample. The CT obtained for each total bacteria sample was used as the reference value for the other species-specific assays. For each sample, relative abundance of each population was calculated ($2^{-\Delta CT}$).

Efficiency of each reaction was evaluated using sample dilution to derive a slope from a 4-point regression curve of the CT values against their log transformed dilution coefficients. Efficiency was calculated according to the equation: $E = -1 + 10^{(-1/slope)}$, where slope refers to the steepness of the line for the diluted samples. Efficiencies of reaction for each taxon are noted in Table 3.2. Efficiencies of reactions ranged from 72.8 to 138.0%; the mean was 98.8%. These primers had been previously validated with high (~100%) efficiencies. In this study, efficiencies were used primarily to assess whether reactions were producing reliable results, rather than to obtain an exact efficiency value for population calculations. Poor efficiencies can be an indication of variation within the DNA sample or represent the presence of some type of inhibitor. Because the observed efficiency values were acceptable, one can infer that pimers and other reagents performed adequately for quantitative analysis to be valid.

Specificity of amplification was assessed by melt curve analysis. Assessment of each melt curve showed no evidence for the presence of multiple amplicons; therefore, we concluded that primers had adequate specificity and final primer inclusion concentrations were satisfactory. In addition, a single peak on melt curves indicated that all fluorescence came from amplicons of interest, with little or no detection of off-target amplicons or primer-dimers.

Based on analysis of efficiencies, melt curves, and CT values, primer concentrations of 200 nmol/L were not sufficient for *Megasphaera elsdenii* or the *Selenomonas ruminantium* group. Increasing primer concentrations to 450 nmol/L resolved issues.

Three of the primers used were genera specific and the other eight targeted individual species. Populations were quantified as a proportion of total bacteria, and statistical assessment of linear and quadratic differences was carried out using mixed model analysis. Samples were deemed outliers and omitted from analysis when studentized residuals were greater than 3 or less

than -3. No more than 6 outliers were removed from any single analysis. Table 3.3 shows relative populations of the genes investigated. Interestingly, dietary treatments had no significant effects on the populations tested, despite differences in rumen pH and VFA concentrations (Table 2.6).

Because dietary factors heavily influence rumen microbial populations, and the four treatment diets were balanced for similar nutrient compositions, it may not be surprising that no major effects on bacterial population were observed. As WCGF inclusion increased, there was a tendency ($P < 0.09$) for decreased proportions of the *Butyrivibrio fibrisolvens* group, a fibrolytic group that is more active in utilizing the intermediates of fiber degradation (oligosaccharides and sugars) than fiber (Haigler and Weimer, 1991; Cotta and Zeltwanger. 1995). Because concentrations of non-fiber carbohydrates were similar across the four diets (Table 2.2), the linear decrease in proportion of *Butyrivibrio fibrisolvens* group in diets with higher levels of WCGF could be related to lower pH observed with these treatments (Table 2.6; Therion et al., 1982). However, the other three species of fibrolytic bacteria, *Prevotella bryantii*, *Prevotella ruminicola*, and *Fibrobacter succinogenes*, monitored in this study were not affected by the inclusion of WCGF. Among fibrolytic bacteria, *Prevotella* species are more acid tolerant than others (Weimer, 1996), and perhaps as a result, relative populations of *Prevotella* were not affected. *Fibrobacter succinogenes* is an acid sensitive organism and its proportion was lower at 36% WCGF inclusion compared to 0% (1.51 vs. 0.92% of total bacteria), but the difference was not significant ($P = 0.34$). Although dietary fiber levels were similar, the amount of ruminally soluble fiber increased with WCGF inclusion rate (Armentano and Pereira, 1997). This increase in degradable fiber could have provided more substrate for microbial growth (Sniffen and Robinson, 1987), but the lower rumen pH observed with these diets created an environment not

conducive to the growth of fiber digesting bacteria (Russell and Dombrowski, 1980). It is possible that combining the two conditions led to no net change of microbial populations. Furthermore, as greater proportions of WCGF were fed, the mean particle size of the TMR decreased. Due to particle size, rate of passage from the rumen may have been too quick for complete utilization by ruminal bacteria, especially the *Butyrivibrio fibrisolvens* group.

Even though only a single effect was observed in response to increasing dietary levels of non-forage fiber, the population counts obtained appear to be accurate and reliable. In general, the mean estimates reported for each taxon coincide respectably to values reported by other researchers who utilized quantitative PCR on rumen samples (Tajima et al., 2001; Stevenson and Weimer, 2007).

The species (Table 3.1) monitored in this study are well characterized, and thought to be essential organisms in the rumen ecosystem, but they represent a minute proportion of all known ruminal microbes (Krause and Russel, 1996; Tajima et al., 1999). Additionally, only a small fraction of all ruminal bacteria have been recovered by cultural methods; therefore, it is not clear whether existing information accurately reflects the inhabitants and growth of key bacterial species in the rumen (Itabashi, 2004). Observations from this study indicate that the eight species quantified accounted for only 10 to 18% of all bacterial 16S ribosomal DNA gene copies (Table 3.3).

The important thing to note is that the described technique worked. Furthermore, this method offers several advantages over traditional approaches to quantifying ruminal microbiota. First, lysis of microbial cells is not a concern because there is no need for culturing or further growth. Consequently, samples do not need to be sustained in an anaerobic environment. More

importantly, this procedure was much simpler and quicker than procedures that involve derivation of a microbial pellet.

Available evidence suggests microorganisms attached to undigested feed particles comprise a major proportion of total ruminal microorganisms (Craig et al., 1987b), however many factors influence the proportion particle-associated microbes (Merry and McAllan, 1983; Craig et al., 1987a). Therefore, it should be most accurate to obtain microbes adherent to feed particles and free floating microbes in the same sample, then extract DNA. The genetic material obtained in this procedure is most likely representative of that combination. As a result, genetic sequences likely amplified in similar proportions to those of organisms present in the rumen.

The procedure described represents a simple technique for quantifying microbial populations. Quantifying rumen microorganisms in conjunction with measures of fermentative end products has tremendous potential to provide nutritionists a better understanding of ruminal fermentation. Through better understanding the physiology of the ruminant animal, management practices can be adapted to improve health and performance of ruminant animals.

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Table 3.1 Primers used for Q-PCR detection of microbial species

Strain tested	*Forward primer* *Reverse primer*	*Concentration nmol/L*	*Reference*[1]
Fungi	GAGGAAGTAAAAGTCGTAACAAGGTTTC CAAATTCACAAAGGGTAGGATGATT	200	(1)
Bacteria	ACTCCTACGGGAGGCAG GACTACCAGGGTATCTAATCC	200	(2)
Prevotella (genus)	GGTTCTGAGAGGAAGGTCCCC TCCTGCACGCTACTTGGCTG	200	(2)
Prevotella bryantii	AGCGCAGGCCGTTTGG GCTTCCTGTGCACTCAAGTCTGAC	200	(2)
Prevotella ruminicola	GAAAGTCGGATTAATGCTCTATGTTG CATCCTATAGCGGTAAACCTTTGG	200	(2)
Butyrivibrio fibrisolvens group	ACCGCATAAGCGCACGGA CGGGTCCATCTTGTACCGATAAAT	200	(2)
Eubacterium ruminantium	CTCCCGAGACTGAGGAAGCTTG GTCCATCTCACACCACCGGA	200	(2)
Fibrobacter succinogenes	GCGGGTAGCAAACAGGATTAGA CCCCCGGACACCCAGTAT	200	(2)
Megasphaera elsdenii	AGATGGGGACAACAGCTGGA CGAAAGCTCCGAAGAGCCT	450	(2)
Selenomonas ruminantium group	CAATAAGCATTCCGCCTGGG TTCACTCAATGTCAAGCCCTGG	450	(2)
Streptococcus bovis group	TTCCTAGAGATAGGAAGTTTCTTCGG ATGATGGCAACTAACAATAGGGGT	200	(2)

[1]1=Denman and McSweeny, 2006; 2=Stevenson and Weimer, 2007.

Table 3.2 Efficiency of PCR reactions obtained using sample dilution

Target taxon	Efficiency[1]
Fibrobacter succinogenes	90.7
Eubacterium ruminantium	88.8
Megasphaera elsdenii	92.5
Prevotella bryantii	90.6
Prevotella ruminicola	75.3
Prevotella (genus)	130.0
Butyrivibrio fibrisolvens group	72.8
Streptococcus bovis group	138.0
Selenomonas ruminantium group	83.7
Total bacteria	125.0
Fungi	99.2

[1]Efficiency was derived from a 4-point regression curve of the CT values against their log transformed dilution coefficients

Table 3.3 Effect of dietary treatment on relative proportions of each taxon

	Treatment[1]					*P*-value		
Target taxon,%	0%	12%	24%	36%	SEM	Linear	Quadratic	Observations
Fibrobacter succinogenes	1.51	1.31	1.74	0.92	0.518	0.34	0.33	26
Eubacterium ruminantium	0.183	0.167	0.201	0.173	0.0407	0.99	0.89	25
Megasphaera elsdenii	0.0154	0.0179	0.0144	0.0302	0.0112	0.38	0.52	26
Prevotella bryantii	0.504	1.04	0.703	1.28	0.373	0.17	0.95	23
Prevotella ruminicola	2.95	4.07	1.14	5.95	1.22	0.49	0.41	24
Prevotella (genus)	49.4	55.3	34.8	49.1	11.7	0.65	0.70	25
Butyrivibrio fibrisolvens group	0.00244	0.00210	0.00199	0.00144	0.000456	0.09	0.78	22
Streptococcus bovis group	0.0823	0.0551	0.0637	0.0441	0.0160	0.22	0.84	26
Selenomonas ruminantium group	6.83	11.9	4.32	7.27	0.447	0.72	0.79	26
Sum of individual species	10.5	18.2	12.3	17.8	6.19	0.58	0.87	25
Fungi	0.267	0.285	0.175	0.174	0.145	0.53	0.95	24

[1]0%=0% WCGF, 12%=11% WCGF, 24%=23% WCGF, 36%=34% WCGF (DM Basis).

CHAPTER 4 - Effects of Alfalfa Hay Inclusion Rate on Productivity of Dairy Cattle Fed Wet Corn Gluten Feed Based Diets[1,2]

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ABSTRACT

An experiment was conducted to evaluate the effects of varying alfalfa inclusion rate in diets containing 31% (dry matter basis) wet corn gluten feed (Sweet Bran, Cargill, Inc.). Eighty primiparous and multiparous Holstein cows averaging 178 ± 90 d in milk (mean ± SD) were randomly assigned to 1 of 4 sequences in a 4 × 4 Latin square design with 28-d periods. Treatments were diets containing 0, 7, 14, or 21% alfalfa on a dry matter basis, with corn silage, corn grain, soybean meal, expeller soybean meal, and mineral supplements varying across diets to maintain uniform nutrient densities. Diets were formulated for similar crude protein, neutral detergent fiber, and non-fiber carbohydrate concentrations. Feed intake, milk production, body weight, and body condition score were monitored, and linear and quadratic effects of increasing alfalfa inclusion rate were assessed using mixed model analysis. As alfalfa inclusion rate increased, dry matter intake tended to increase linearly (26.7, 27.3, 27.4, and 27.5 kg/d for 0, 7, 14, and 21% alfalfa, respectively), and solids-corrected milk (29.9, 30.2, 30.8, and 30.5 kg/d) and energy-corrected milk production (32.9, 33.3, 33.8, and 33.6 kg/d) tended to increase linearly. Body weight gain decreased linearly (22.9, 18.0, 11.2, and 9.5 kg/28 d) with increasing alfalfa inclusion rate. Although increasing the inclusion rate of alfalfa increased the proportion of large particles in diets, treatments had no effect on milk fat yield or concentration. Feeding more alfalfa (up to 21% of dry matter) tended to increase milk yield while decreasing body weight gain, suggesting that metabolizable energy utilization shifted from body weight gain to milk production in these treatments. However, adding alfalfa to the diet had only minor effects on productivity.

Key words: by-product, dairy cattle, alfalfa, wet corn gluten feed

INTRODUCTION

Alfalfa is a cool-season perennial legume that serves as a source of protein and fiber in dairy cattle rations. Dairy nutritionists have traditionally relied heavily on alfalfa; results from a 1995 survey revealed that 62% of dairy cattle in the United States were fed alfalfa (Mowrey and Spain, 1999). Since 1995, however, the number of acres devoted to alfalfa production has declined by nearly 4 million acres (National Agriculture Statistics Service, 2008). Not surprisingly, as the availability of alfalfa has decreased, its cost has increased nearly 50% in the last 20 yr (National Agriculture Statistics Service, 2008). Increased pressure for land use, including greater use of corn in the corn milling industry, has contributed to the loss of alfalfa acres. As a result, nutritionists and producers are reconsidering the role of alfalfa in dairy rations.

Scientific reports as far back as 1933 show that diets utilizing corn silage (CS) as the only forage can support milk and milk fat production equivalent to diets incorporating both alfalfa hay (AH) and CS (Hayden, 1933). Thomas et al. (1970) reported that cows fed CS or AH as the only roughage source produced similar amounts of FCM across 3 lactations. More recent studies have also supported the conclusion that diets can be formulated to support high milk production without the use of AH (Kleinschmit et al., 2007; Kowsar et al., 2008).

Although an increasing amount of corn is being consumed by the corn milling industry, coproducts of this industry provide an opportunity for dairy producers to adopt novel diet formulation strategies to help keep feed costs in check. One such coproduct, produced from the wet milling process, is wet corn gluten feed (WCGF). Wet corn gluten feed is a high-fiber, low-lignin feedstuff that can be easily incorporated into dairy cattle diets (NRC, 2001; Wickersham et al., 2004). Feeding WCGF at up to 36% of ration DM did not affect milk production, composition, or DMI in one study (Armentano and Dentine, 1988). On the other hand, Staples et

al. (1984) showed a decrease in DMI and milk yield but an increase in milk fat percentage when WCGF was fed at high levels. It is important to note that in both studies, diets contained large proportions of forage, and WCGF was used in place of concentrates, resulting in higher dietary NDF concentrations as WCGF was added. In contrast, Kononoff et al. (2006) found that feeding a ration containing 38% WCGF (DM basis) decreased milk fat concentration but increased milk yield, resulting in similar milk fat yields across treatments. In this study, forage decreased from approximately 60% of ration DM in the control, to 38% of DM for the treatment diet (Kononoff et al., 2006).

It is possible to chemically balance a ration that includes large amounts of WCGF, but physical characteristics of the TMR must be accounted for. Although WCGF is relatively high in fiber, the small fiber particles provide little physically effective fiber; physically effective NDF of WCGF was reported as 11% of NDF based on rumination time and ruminal pH of lactating cows (Allen and Grant, 2000). Many investigators have shown that physically effective fiber is necessary for maintaining proper rumen function and preventing milk fat depression (Lammers et al., 1996; Mertens, 1997). In ruminants, physically effective fiber stimulates rumination, which facilitates the secretion of saliva that, in turn, buffers the rumen (Kay, 1966). Because of the mechanical stimulation provided by AH particles, feeding high levels of WCGF without AH could lead to milk fat depression. Therefore, the objective of this study was to evaluate the effects of varying AH inclusion rate, in diets containing 31% WCGF, on milk and milk fat yield and BW gain.

MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Design and Treatments

Forty-one primiparous and 39 multiparous Holstein cows (178 ± 90 DIM; 1.8 ± 0.99 lactations; mean ± SD) from the Kansas State University Dairy Cattle Teaching and Research Facility were randomly assigned to 1 of 8 free stall pens. After initial pen assignments, variation in pen means for DIM, milk yield, and parity were assessed, and the randomization was repeated until the coefficient of variation for all three variables dropped below 25%. Each pen was assigned randomly to one of two 4 × 4 Latin squares balanced for carryover effects. Treatment periods were 28 d, with the final 9 d used to collect samples and data. Recombinant bovine somatotropin (Posilac, Monsanto, St. Louis, MO) was administered on d 1 and 15 of each period. At the beginning of the experiment, BW and BCS of cows were 674 ± 97 kg and 2.92 ± 0.36, respectively.

Cows were offered 1 of 4 rations that differed in amount of AH included. Alfalfa hay inclusion rates were 0, 7, 14, or 21%, primarily replacing CS of similar forage quality (Table 4.1). The strategy for formulating experimental diets was to develop diets with similar concentrations of CP, NDF, and NFC. As a consequence, diets containing more AH had less CS and soybean meal but more corn grain (Table 4.2).

Data and Sample Collection

Throughout the experiment, cows were housed in free stalls and fed twice daily at 110% of expected intake. Amount of feed delivered and refused was recorded on d 19, 20, 21, 26, 27,

and 28 of each period. The TMR and orts were analyzed for DM and particle size was measured. Samples of all dietary ingredients were collected on d 19, 21, 26, and 28 and composited into 1 sample per period. Cows were milked twice daily in a milking parlor; milk was sampled and yield was recorded for every milking on d 21 and 28 of each period. Body weight and BCS were measured on d 1 of each period and d 28 of the last period. Body condition score was measured by 2 trained investigators according to procedures described by Wildman et al. (1982).

Sample Analysis

The Penn State Particle Separator was used to measure particle size for both TMR and orts (Lammers et al., 1996). Diet ingredients were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA). Ash concentration was determined after 5 h of oxidation at 500°C in a muffle furnace. Concentration of NDF was determined (Van Soest et al., 1991; method A) by using an Ankom Fiber Analyzer (ANKOM Technology, Fairport, NY). Crude protein was determined by oxidation and detection of elemental nitrogen (Leco Analyzer, Leco Corp, St. Joseph, MI). Crude fat was determined by ether extract (AOAC, 2000: method 920.9). Starch content was determined by glucoamylase digestion followed by glucose quantification using the glucose oxidase method (Dairy One Forage Testing Laboratory, Ithaca, NY). Concentrations of all nutrients except DM were expressed as percentages of DM determined by drying at 105°C in a forced-air oven for more than 8 h.

Milk samples were composited by day and analyzed for fat, true protein, and lactose with a B2000 Infrared Analyzer (Bently Instruments, Chaska, MN) by Heart of America DHIA (Manhattan, KS). Energy-corrected milk (0.327 × milk yield + 12.86 × fat yield + 7.65 × protein

yield; DHI glossary, Dairy Record Management Systems, 2007) and SCM yield were calculated (Tyrrell and Reid, 1965).

Economic Model Analysis

A breakeven analysis was conducted to determine whether the added milk production from including AH is enough to justify feeding it in this type of ration. Changes in milk income, feed consumed, and feed costs were incorporated in a model to determine the relative difference in AH vs. CS value (DM basis) at different milk:feed cost ratios. Diets compared were the 0% and 21% AH treatments, and production and intake means for these treatments were used in this model. The value of AH was fixed at $250/ton DM ($0.27/kg), and milk value was fixed at $0.20/lb ($0.44/kg), whereas the value of CS and TMR cost varied with the AH price differential and the milk:feed cost ratio, respectively. Addition of 21% AH also allowed the exchange of 5% soybean meal for corn grain, and the cost differential between these commodities was set at $120/ton DM (soybean meal – corn grain, $0.13/kg). Changes to the fixed values had little effect on the results as presented, although the model was somewhat sensitive to the corn grain to soybean meal price differential. To account for this effect of corn and soybean meal prices, we also ran the model using maximum and minimum price differentials for corn and soybean meal from 2003 to 2008 (NATIONAL AGRICULTURAL STATISTICS SERVICE, 2008); $120/ton DM was the mean differential for this time period.

Statistical Analysis

Five cows were removed from the experiment prior to its completion because of various reasons unrelated to treatments, and 2 replacement cows were added during periods 1 and 2. Dry matter intake was divided by the number of cows in each pen to account for missing animals.

Data were analyzed according to the following model by using the REML procedure of JMP (version 6.0, SAS Institute, Cary, NC):

$$Y_{ijk} = \mu + P_i + T_j + N_k + PT_{ij} + e_{ijk}$$

where μ = overall mean, P_i = fixed effect of period (i = 1 to 4), T_j = fixed effect of treatment (j = 1 to 4), N_k = random effect of pen (k = 1 to 8), PT_{ij} = interaction of period and treatment, e_{ijk} = residual error. Linear and quadratic effects of treatment were tested. The interaction term was included primarily to determine if the treatment responses varied by stage of lactation. The model used to analyze milk yield responses also included the random effect of cow nested within pen. Treatment effects were declared significant at $P < 0.05$. Tendencies for treatment effects were declared at $P < 0.10$.

RESULTS AND DISCUSSION

Ration Composition, Feed Intake, and Milk Production

Final analyses showed that nutrient composition of diets remained similar across all 4 treatments, with DM increasing and starch decreasing slightly as more AH was added (Table 4.2). Adding more AH to the ration tended to linearly increase DMI as well as ECM and SCM yield (Table 4.3). Statistical analysis of milk yield showed a significant treatment × period interaction. Cows on this study averaged 290 DIM at the end of the study, and not surprisingly,

milk yield declined during the study; however, this interaction suggested that greater AH inclusion may have supported improved persistence in late lactation. Cows receiving 14 and 21 % AH maintained milk production better through period 4 (means of 28.4, 28.3, 31.1, and 30.9 kg/d for 0%, 7%, 14%, and 21% AH, respectively). However, we are not aware of full-lactation studies demonstrating improved persistence with AH-based diets, and given that period by treatment interactions were not observed for ECM or FCM yields, this result should be interpreted with caution.

Feed efficiency, measured as ECM /DMI, averaged 1.15 ± 0.03 and was similar across treatments. The low feed efficiency in this study was likely due primarily to the fact that relatively late-lactation cows were used. Feed efficiency ratios have often exceeded 1.3 in other studies investigating diets with high inclusion rates of corn gluten feed (Ohajuruka and Palmquist, 1989; Schroeder, 2003; Kononoff et al., 2006).

Fat and protein percentages and yield were not affected by treatment ($P > 0.15$, Table 4.4). Concentrations of milk fat and protein averaged 3.78 ± 0.11 and 3.45 ± 0.07%, respectively, and yield of these components averaged 1.17 ± 0.06 and 1.07 ± 0.03 kg/d, respectively (Table 4.4). We observed a significant linear effect of AH on lactose yield, which was consistent with the tendency for increased SCM yield with higher alfalfa inclusion rates. There was a quadratic effect of AH inclusion rate on MUN ($P = 0.05$), however the range of means was very small and the response did not correspond with treatment effects on dietary CP or milk protein yield; therefore, there is no clear explanation for this observation.

Lack of a significant treatment effect on milk fat yield or concentration suggests that ruminal biohydrogenation was not inhibited in diets with lower AH inclusion rates, which agrees with the results reported by Kleinschmit et al. (2007). In our study, NDF intake was not altered

by treatment ($P > 0.35$, Table 4.3) , but diets with more AH offered greater proportions of particles longer than 19 mm, which would be expected to increase the physical effectiveness of NDF. However, cows sorted against longer particles in the diets with more AH (Table 4.5), which is consistent with previous research (Leonardi and Armentano, 2003; Methu et al., 2001). It is not possible to determine whether sorting occurred because of the difference in TMR moisture or because more long particles were offered. Regardless, the fact that cows sorted against large particles may help explain why milk fat production remained similar across treatments, despite large differences in the physical effectiveness of fiber in the diets as fed.

Production responses to replacement of CS with AH have been inconsistent. This is not surprising, given that different formulation strategies can dramatically influence factors such as particle size, protein degradability, and fiber digestibility when AH is added. Kleinschmit et al. (2007) reported a linear increase in milk yield when AH replaced CS in a diet including 15% (DM) dried distillers grains with solubles. In contrast, Kowsar et al. (2008) reported that when finely-chopped AH partially replaced CS, DMI and milk, protein, and lactose yields all decreased. Diets based on WCGF may provide the ideal setting for removal of AH. Wet corn gluten feed is a source of highly degradable protein (Kononoff et al., 2007), making the loss of RDP from AH less detrimental. Additionally, despite concerns about the lack of physically-effective fiber in such diets, they also tend to contain less starch than typical lactation rations, which may help prevent acidosis-related problems associated with the loss of long-stem AH. Nevertheless, our findings generally agree with those of Kleinschmit et al. (2007), with higher AH inclusion rates tending to increase FCM production.

Energetics

Body condition score was not significantly affected by dietary treatment ($P > 0.57$, Table 4.3). However, cows fed TMR containing more AH gained less BW compared to cows fed less AH ($P = 0.02$). As AH was added to the ration, energy partitioning changed from BW gain to milk production, and total energy for production and gain tended to decrease linearly ($P = 0.06$, Figure 1). Diets with more AH and less CS were not as fermentable (Holden, 1999), which likely decreased ruminal production of propionate. Propionate stimulates insulin secretion both directly and through stimulation of gluconeogenesis, and lactating cows fed more fermentable diets often have higher plasma insulin concentrations (Grant et al., 1990). Decreased fermentability in the high AH treatments may have decreased plasma insulin, resulting in decreased lipogenesis in adipose tissue (Oba and Allen, 2003). However, blood samples were not collected during this study, so this hypothesis cannot be confirmed.

Manure Production

Fecal output was not monitored in this study, but on the basis of previous findings (Weiss et al., 2007), we speculate that manure production was likely influenced. Figure 1 represents the total energy used for production and BW gain of cows consuming each TMR; the tendency for decreased energy yield in the face of increasing DMI strongly suggests that the diets containing more AH were less digestible, which is not surprising given that a dried hay replaced an ensiled forage in these diets (Holden, 1999). Because fecal production is highly dependent on DM digestibility, increased manure production is one likely result of incorporating AH in rations similar to those used in this study.

Economic Analysis

Although feeding greater levels of AH tended to increase ECM production, it also led to greater DMI. The potential economic effects of such a response were evaluated to determine the theoretical value of AH relative to CS. According to the breakeven analysis presented in Figure 2, if the price differential between AH and CS falls below the line at a given milk:feed cost ratio, it is profitable to incorporate AH into this type of ration. However, on the basis of responses to the 0% and 21% alfalfa treatments in this study, adding AH to diets with high WCGF inclusion rates may not be profitable, especially when milk:feed cost ratios are low. This analysis suggests that even with favorable milk:feed cost ratios and expensive soybean meal, AH should demand no more than a $60 premium per ton of DM to be incorporated into similar rations. Additionally, this analysis ignores costs associated with predicted increases in manure output and costs (or benefits) of decreased BW gain when more AH is fed.

CONCLUSIONS

Feeding higher proportions of AH tended to increase ECM yield and decrease BW gain, suggesting that metabolizable energy supply was repartitioned from BW gain to milk production as more AH was included. Nonetheless, decreasing AH inclusion rate may improve farm profitability by reducing feed costs and expenses associated with manure handling, despite small losses in productivity.

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Figure 4.1 Total energy partitioned to milk production and BW gain in cows fed varying levels of AH. As AH was added, total energy utilization tended (*P* = 0.06) to decrease linearly. Body weight gain was assigned an energetic value of 5.975 Mcal/kg (NRC, 2001), and milk energy was calculated according to Tyrell and Reid (1965).

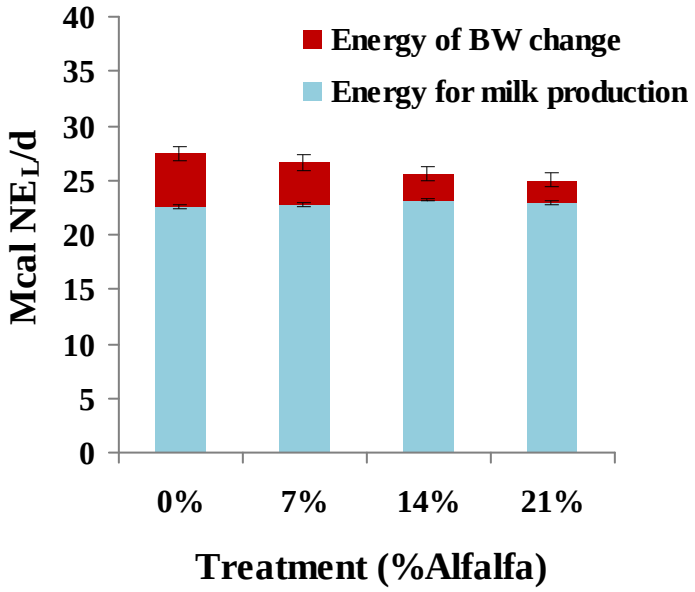

Figure 4.2 Breakeven analysis of AH:CS cost differential. Breakeven analysis was conducted to determine whether the added milk production from including AH is enough to justify feeding it in this type of ration. The line indicates the breakeven additional cost that can be paid for alfalfa compared with CS (per ton of DM) at a given milk:feed cost ratio. Values were calculated by using milk production and DMI data from the 0 and 21% alfalfa diets. The three lines represent the minimum ($80), mean ($120), and maximum ($188) SBM-corn price differential from 2003 to 2008.

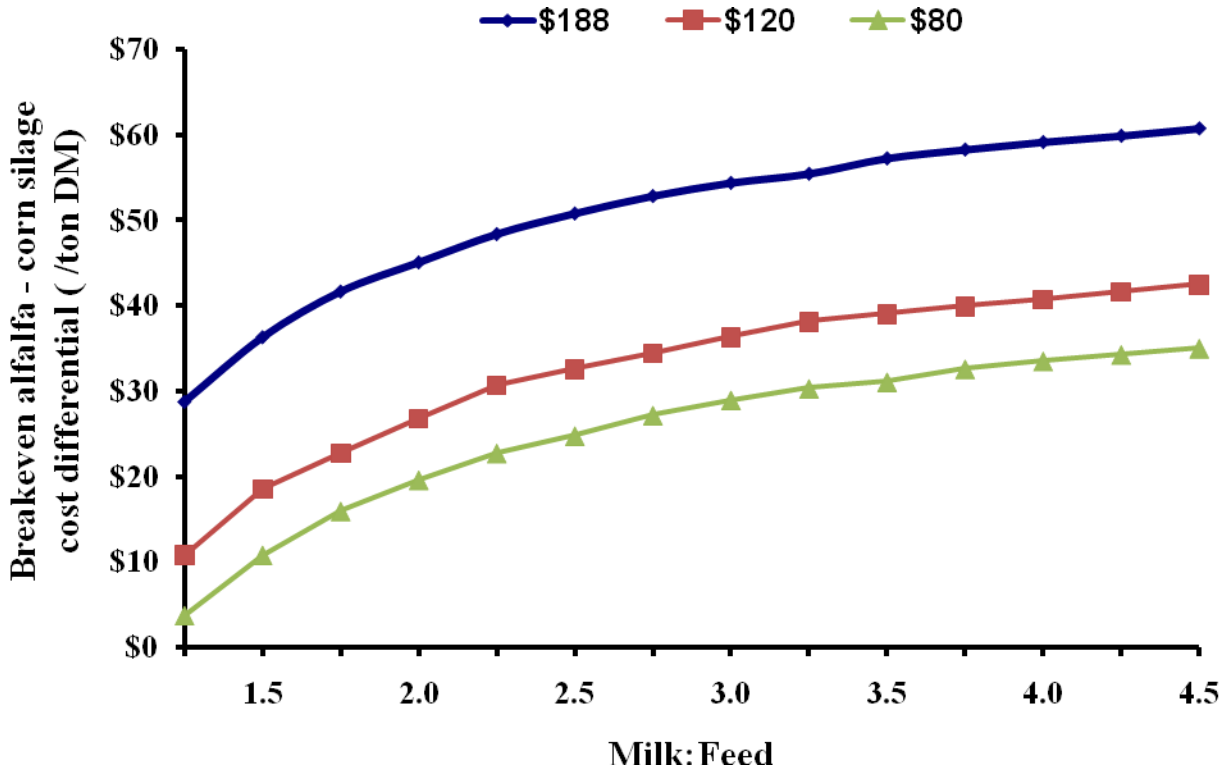

Table 4.1 Composition of CS and AH

	Ingredient	
Nutrient[1]	CS	AH
DM	34.6	86.1
NDF	42.6	40.3
CP	7.6	18.3
Starch	27.7	4.0
EE	2.8	1.1
Ash	5.5	9.3

[1]Nutrients other than DM expressed as a percentage of diet DM.

Table 4.2 Ingredient and nutrient composition of dietary treatments

		Treatment[1]			
		0%	7%	14%	21%
% DM	Corn silage	41.0	33.9	26.7	19.4
	Alfalfa hay	0.0	6.6	13.4	20.2
	WCGF[2]	30.9	31.1	31.4	31.6
	Cottonseed	7.3	7.3	7.4	7.5
	Corn grain	9.7	11.6	13.5	15.6
	Soybean meal	4.9	3.4	1.7	0.0
	Molasses	0.4	0.4	0.4	0.4
	Expeller soybean meal	2.5	2.5	2.6	2.6
	Limestone	1.9	1.7	1.5	1.2
	Micronutrient premix[3]	1.3	1.4	1.3	1.4
Nutrients[4]					
	DM, % as-fed	52.5	55.8	59.5	63.9
	CP	16.5	16.5	16.7	16.7
	NDF	34.6	34.7	34.5	34.7
	Starch	17.7	16.3	16.6	15.8
	NFC[5]	36.0	36.0	36.4	36.5
	Ether extract	3.8	3.7	3.6	3.6
	Ash	9.1	9.0	8.8	8.6

[1]0% = 0% AH, 7% = 6.6% AH, 14% = 13.4% AH, 21% = 20.2% AH (DM Basis).
[2]Wet corn gluten feed; SweetBran, Cargill, Inc.
[3]Premix consists of 48.85% sodium bicarb, 20.92% trace mineral salt, 10.77% magnesium oxide, 9.41% bio-mos, 4.18% 4-plex, 2.72% Se premix, 2.09% Vit E, 0.84% Vit A, and 0.21% Vit D.
[4]Nutrients other than DM expressed as a percentage of diet DM.
[5]Calculated as DM – (CP + NDF + EE + ash).

Table 4.3 Effects of treatments on intake and performance of lactating cows

	Treatment[1]						*P* value	
	0%	7%	14%	21%	SEM	SED	Linear	Quadratic
DMI, kg/d	26.7	27.3	27.4	27.5	1.16	0.38	0.05	0.33
NDF intake, kg/d	9.1	9.2	9.3	9.2	0.18	0.13	0.35	0.92
Milk, kg/d	30.9	31.1	31.7	31.3	1.48	0.26	0.03*	0.16*
SCM, kg/d	29.9	30.2	30.8	30.5	1.36	0.40	0.07	0.30
ECM, kg/d	32.9	33.3	33.8	33.6	1.45	0.43	0.09	0.32
ECM/DMI	1.16	1.14	1.16	1.15	0.03	0.02	0.75	0.88
BW change, kg/28 d	23.0	18.0	11.2	9.5	3.6	5.7	0.02	0.69
BCS change/28 d	0.014	0.031	-0.006	-0.013	0.041	0.065	0.57	0.80

[1]0% = 0% AH, 7% = 6.6% AH, 14% = 13.4% AH, 21% = 20.2% AH (DM Basis).

*Significant treatment by period interaction

Table 4.4 Effects of treatments on milk components

	Treatment[1]						*P* value	
	0%	7%	14%	21%	SEM	SED	Linear	Quadratic
Milk fat, %	3.75	3.81	3.75	3.79	0.11	0.08	0.79	0.83
Milk protein, %	3.47	3.46	3.44	3.44	0.07	0.03	0.38	0.84
Lactose, %	4.77	4.75	4.81	4.76	0.03	0.02	0.64	0.44
SCC, log	2.17	2.19	2.18	2.22	0.06	0.05	0.46	0.80
MUN, mg/dL	12.6	13.0	12.7	12.5	0.48	0.22	0.31	0.05
Yield, kg/d								
Milk fat	1.14	1.17	1.18	1.18	0.06	0.03	0.21	0.44
Milk protein	1.06	1.06	1.08	1.07	0.03	0.01	0.15	0.48
Milk lactose	1.48	1.48	1.54	1.51	0.08	0.02	0.02	0.18

[1]0% = 0% AH, 7% = 6.6% AH, 14% = 13.2% AH, 21% = 20.2% AH (DM Basis).

Table 4.5 Particle size separation (as-fed basis)[1]

		Treatment[2]			
		0%	7%	14%	21%
TMR	> 19 mm	2.2[a]	4.5[a]	9.5[b]	14.1[c]
	19 > 8 mm	39.1[b]	36.6[b]	30.5[a]	26.8[a]
	< 8 mm	58.7	58.9	60.0	59.1
Orts[3]	> 19 mm	2.7[a]	10.5[b]	20.5[c]	24.5[c]
	19 > 8 mm	48.6[c]	39.4[b]	32.1[a]	28.6[a]
	< 8 mm	48.8	50.1	47.3	46.9

[1]Measured with a 3-compartment Penn State Particle Size Separator (Lammers et al., 1996).
[2]0% = 0% AH, 7% = 6.6% AH, 14% = 13.2% AH, 21% = 20.2% AH (DM Basis).
[3]Mean refusal amounts ranged from 3.5 to 4.2 kg/cow daily (approximately 15% of what was offered) and did not vary by treatment ($P > 0.30$).
[abc]Means with different superscripts within a row are significantly different by Tukey's HSD ($P < 0.05$).