2-2011

Effect of Brown Midrib Corn Silage and Dried Distillers Grains Plus Solubles on Lactational Performance and Nitrogen Utilization by Dairy Cows

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THE EFFECT OF FEEDING BROWN MIDRIB CORN SILAGE AND DRIED DISTILLERS GRAINS PLUS SOLUBLES ON LACTATIONAL PERFORMANCE AND NITROGEN UTILIZATION BY LACTATING DAIRY COWS

by

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A THESIS

Presented to the Faculty of
The Graduate College at the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Master of Science

Major: Animal Science

Under the Supervision of Professor Paul J. Kononoff

Lincoln, Nebraska

February, 2011

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University of Nebraska, 2011

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Thirty six Holstein cows, four ruminally cannulated, (mean ± SD, 111 ± 35 DIM; 664 ± 76.5 kg BW) were used in replicated 4×4 Latin squares to investigate the effects of brown midrib (*bm3*) and conventional (DP) corn silages, and the inclusion of dried distillers grains plus solubles (DDGS) on milk production and N utilization. In each 28 d period cows were assigned to one of four treatments: DP plus 0% DDGS (CON); *bm3* plus 0% DDGS (BMR); DP corn silage plus 30% DDGS (DP+DG); and *bm3* plus 30% DDGS (BMR+DG). Dry matter intake was greater ($P < 0.01$) for cows consuming *bm3* (25.8 VS 24.4 ± 0.47 kg), likewise for DDGS (24.3 and 25.9 ± 0.47 kg/d for 0 and 30%). Compared to DP hybrid, NDFD was higher ($P < 0.01$) for *bm3* (32.5 VS 38.1 ± 1.79%). There was a hybrid × DDGS interaction ($P < 0.01$) for total concentration of volatile fatty acids (VFA) and rumen pH as the highly digestible treatment BMR+DG resulted in the highest VFA and the lowest pH. Milk yield was not affected by treatment and averaged 30.6 ± 1.09 kg/d. Milk protein yield (MPY) was greater ($P < 0.01$) for *bm3* and DDGS treatments. There was a hybrid by DDGS interaction ($P = 0.02$) for milk fat yield (MFY) resulting in 1.03, 1.08, 0.84 and 0.78 ± 0.045 kg/d for DP, BMR, DP+DG and BMR+DG. Fat corrected milk (FCM) was affected by DDGS ($P < 0.01$) and averaged 30.0 and 26.4 ± 1.0kg/d (0% and 30% inclusions). Urinary N excretion was similar among treatments; however fecal N was lower ($P = 0.03$) for diets containing *bm3* corn silage which caused lower ($P = 0.02$) manure N. These results indicate that *bm3* corn silage and DDGS

increase DMI, NDFD and MPY; however high inclusion of corn silage with 30% DDGS reduces FCM. Nitrogen excretion was reduced when cows consumed *bm3*

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INTRODUCTION

The continued growth of the corn ethanol industry results in an abundant supply of co-products that may be utilized as feedstuffs for livestock. One of the major co-products derived from the ethanol industry is distillers grains and solubles, wet (WDGS) or dry (DDGS). This feedstuff is characterized by having high content of crude protein (CP), neutral detergent fiber (NDF) and fat or ether extract (EE). Namely, on a dry matter basis CP typically ranges between 26 and 35% (mean 31%), NDF ranges 29 to 38% (mean 34) and EE ranges 9 to 16% (mean 13%) (Dairy One, 2010). This composition represents a valuable supply of nutrients that makes DDGS a feedstuff for dairy rations as it provides CP and energy (NDF and EE) which are key nutrients for milk synthesis. Several studies have shown that DDGS may be effectively included in dairy rations between 20 % (Anderson et al., 2006) and 30% (Janicek et al, 2008). Inclusion of DDGS has resulted in improved feed efficiency (Anderson et al., 2006; Kleinschmit et al., 2006) and milk yield (Janicek et al, 2008).

Stephens and Halpin (2007) indicate that in 2005 over one billion metric tons of forage or forage crops were produced worldwide and that corn silage and alfalfa represent the bulk of this figure. Dairy rations typically contain corn silage as a major component of the ration. According to a feed library containing 100,000 samples (Dairy One, 2010) corn silage contains approximately 8% CP (DM basis), 44% (NDF) and 3.7% lignin (DM basis); however, there is a forage specific corn hybrid that contains a mutation known as *bm3* which causes a 25-40% reduction in the lignin content

(Stepehens and Halpin, 2007). Lignin is a polyphenolic compound that is present in plant cell walls; it confers rigidity to plants and also provides resistance to biodegradation (i.e. microbial enzymatic digestion) possible as a defense mechanism that plants have developed against herbivores (Van Soest, 1994). Considering that lignin is a major factor that limits the availability of plant cell walls materials to ruminants (Van Soest, 1994), a decrease in the amount of this compound in forages results in advantageous nutritional attributes of forage crops. Such advantage is demonstrated by *bm3* corn hybrids which, when fed to dairy cows, typically results in greater DMI (Oba and Allen, 1999) and greater digestibility (Oba and Allen, 2000, Ebling and Kung, 2004).

Corn DDGS is a good source of protein and energy to dairy cows (Schingoethe et al., 2009); however over feeding CP may result in greater N excretion into the environment which nowadays raises concerns about pollution and air quality. Nutrition has typically addressed environmental issues because of the association between feeding practices and nutrient excretion (Powers, 2003). Inclusion of 25% WDGS (DM basis) resulted in lower N excretion in corn silage based diets compared to a control diet with no co-products (Gehman and Kononoff, 2010). Feeding *bm3* corn silage has also resulted in lower N excretion (Weiss and Wyatt, 2006) compared to its isogenetic line without the *bm3* mutation. To the best of our knowledge there has not been an experiment combining DDGS and *bm3* corn silage, thus, the objectives of the present study are 1) to evaluate the effect of DDGS and *bm3* on lactational performance of dairy cows and 2) to measure utilization of N by lactating dairy cows fed the combination of DDGS+*bm3* corn silage.

LITERATURE REVIEW

Rumen Microbial Fermentation

Structural carbohydrates

From an anatomical and biochemical stand point, fiber is not a constituent of the plant cell but rather a term used to describe the insoluble residue prepared from plant material. The composition of this residue depends upon the analytical conditions (i.e. reagents) of the assay used for its determination (Chesson, 1986). A more specific terminology is to name the specific polysaccharides that comprise the plant cell wall that are undigested by mammalian enzymes, namely cellulose and hemicelluloses.

The rumen harbors a wide variety of microbes including bacteria, protozoa, fungi and yeast. These microbes are responsible for digestion of plant material consumed by the host animal. Van Soest (1994) suggests that the rumen's principal function is to digest cellulose. Russell and Hespell (1981) describe three phases in which degradation and fermentation of polysaccharides take place. Firstly when feed enters the rumen, bacteria attach to the feed particles and disassociate carbohydrates polymers from structural plant matrices. Secondly, the polymers are hydrolyzed to small saccharides by extracellular microbial enzymes. Thirdly, an intracellular fermentation of more simple carbohydrates leads to proliferation of the attached bacteria using energy from their substrates (Miron et al., 2001). Further studies of ruminal microbial ecology describe the mechanism by which bacteria attach to the feed particles. Similar to Russell and Hespell (1981), Miron et al. (2001) describe the microbial adhesion process that commences with the movement or transport of non mobile bacteria to the substrate surface followed by initial non-

specific adhesion to unprotected areas on the surface of the substrate which typically involves elements in the bacterial glycocalyx. After this initial process, specific adhesion determines which bacteria ferment a particular substrate. This process is believed to be quite complex and may involve utilization of adhesin proteins, ligand formation, cellulose-binding protein and cellulose-binding domain of enzymes (Miron et al. 2001).

Cellulolytic bacteria

The large microbial population in the rumen is believed to be, approximately 10^{10} to 10^{11} bacterial and 10^{6} protozoal cells/mL, which represents 200 species of bacteria and 20 of protozoa (Russell and Hespell, 1981). Despite the large rumen microbial population, there are some particular species that appear to be dominant. The most important species of cellulolytic bacteria in the rumen are *Ruminococus flavefaciens*, *Ruminococus albus* and *Fibrobacter succinogenes*. Other less prevalent or weaker species are *Butyrivibrio fibrisolvens, Eubacterium cellulosolvens* and several species in the genus *Clostridium* (Dehority, 2003).

Different species of cellulolytic bacteria may be grouped according to their preferred substrate (Table 1). These preferences may overlap for preferred substrates, nonetheless, the hydrolysis of plant cell walls invariably yields seven neutral and two acidic monosaccharides (Chesson and Forsberg, 1988). The basic simple sugars are rhamose, fucose, arabinose, xylose, mannose, galactose and glucose; the acidic monosaccharides are galacturonic acid and glucoronic acid, which can be referred to as simply uronic acid, which is the sum of the two. These monosaccharides are then fermented and utilized by rumen microbes and the final fermentation products that

accumulate within the rumen are acetate, propionate, butyrate, carbon dioxide and methane (Russell and Hespell, 1981).

Nature of cellulose digestion

The nature of bacterial cellulose digestion in the rumen has received little attention compared to the studies involving aerobic cellulolytic fungi (Chesson and Forsberg, 1988). It is possible that rumen cellulolytic bacteria are capable of digesting cellulose in a similar fashion to aerobic fungi as many of the enzymes produced by fungi are also synthesized by the main species of cellulose digesting bacteria. The fungal cellulase system (Wood, 1985) provides insight of how rumen bacteria may carry out cellulose digestion. Recent research supports this idea as the newly described bacterium *Cellulosilyticum ruminicola* metabolizes cellulose through a pathway that applies the fungal mode of cellulase production (Cai et al., 2010).

The aerobic fungal enzymatic system of cellulose digestion involves the excretion of active forms of cellulolytic enzymes and it generally is comprised of three principal enzymes that complement each other to completely hydrolyze cellulose. Table 2 depicts the three main enzymes and their associated action. Briefly, endoglucanase randomly attacks cellulose yielding cello-oligosaccharides. The second enzyme is cellobiohydrolase which hydrolyzes cellulose and cleaves off cellobiose. Finally β-glucosidases hydrolyze the products released by the previous enzymes to glucose. It is important to note that this system is coordinated and the enzymes are orchestrated so that the hydrolytic product of one is the substrate for the next enzyme avoiding substrate competition (i.e. β-glucosidase cannot hydrolyze cellulose). In the rumen environment, bacteria work similarly as there are species capable of digesting only certain

polysaccharides. For example hemicellulose degrading *Butyrivibrio fibrisolvens* and *Prevotella ruminicola* cannot digest cellulose, but degrade xylan and pectin and utilize the soluble sugars as substrates (Dehority, 2003).

Lipid metabolism in the rumen

Current feeding practices in the dairy industry commonly involve feeding grains, vegetable oils or animal fats to increase the energy density of the diet. As with many other nutrients, lipids are extensively transformed by rumen bacteria and as a result the animal absorbs a different profile of fatty acids compared to the original profile in the feed (Van Soest, 1994). Rumen bacteria have restricted utilization of fatty acids for metabolism; nonetheless, they have adapted to deal with these compounds to such extent that the type of fatty acids present in ruminant products is greatly affected by microbial metabolism (Harfoot and Hazlewood, 1988).

The first step in ruminal lipid metabolism is hydrolysis of triglycerides releasing the glycerol backbone and free fatty acids. The glycerol backbone is readily utilized for energy which leads to formation of volatile fatty acids. In the second step, unsaturated fatty acids (UFA) become saturated by microbial action in a process known as biohydrogenation. The position of the double bonds are also altered by rumen microbes and are generally transformed to the *trans* form which is more stable but also harder to hydrogenate which may cause accumulation of *trans* forms relative to *cis* (Van Soest, 1994).

Lipid hydrolysis

The base to link bacterial metabolism of lipids was set by Dawson and Kemp (1969) when they reported rapid hydrolysis of phosphatidylcholine in defaunated sheep.

The ester linkages of lipids entering the rumen are hydrolyzed by enzymatic cleavage. Triglycerides hydrolysis results in free glycerol and free fatty acids; galactose is hydrolyzed off of galactoglycerides which are the major lipids in green leaves. The enzymes involved in these reactions are lipase and phosholipase which have been found to be active in only a small proportion of rumen bacteria (Dehority, 2003). Generally two bacteria, *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica,* are associated with lipid hydrolysis (Dehority, 2003).

The metabolism of lipids by rumen bacteria shows division and affinity for substrates. *Anaerovibrio lipolytica* secrets extracellular lipase (Henderson, 1971) and attacks diglycerides preferably over triglycerides. This bacterium is unable to hydrolyze phospholipids or galactolipids. *Butyrivibrio fibrisolvens* has shown phospholipase activity which is mainly cell-associated and only variable amounts of phosholipase has been found in culture studies upon autolysis (Harfoot and Hazlewood, 1988).

Biohydrogenation

Banks and Hilditch (1931) made important observations that led to further studies of fatty acids profile in ruminant products. They observed that lipids from ruminant tissues had higher concentration of saturated fatty acids than those of non ruminants. Early work with ruminants showed that defaunated sheep were able to hydrogenate linoleic and oleic acid at the same rate than normal sheep; from this it was concluded that protozoa are not essential in the biohydrogenation (**BH**) process (Dawson and Kemp, 1969). Therefore, it appears that rumen bacteria are responsible for the major proportion of BH and this activity is primarily associated with bacteria attached to the particulate matter (Dehority, 2003). The major species involved in rumen biohydrogenation is

Butyrivibrio fibrisolvens and some other in the genera *Treponema*, *Micrococus*, *Ruminococus*, *Eubacterium* and *Fusicillus* (Harfoot and Hazlewood, 1988).

Linolenic, linoleic and oleic acid are the major unsaturated fatty acids in current ruminant diets which include grains and oils as supplemental sources of energy. Figure 1 depicts the biohydrogenation pathways of these fatty acids, which through full hydrogenation are turned into stearic acid. The biohydrogenation process is complex and involves interactions among several bacterial species. Pure cultures have shown partial hydrogenation whereas culture of mixed rumen contents yields stearic acid (Kemp and Lander, 1984).

The process of bacterial hydrogenation of UFA is a phenomenon that is relatively well understood in terms of metabolic pathways, bacterial communities involved, effects on animal performance and composition of ruminant food products. In spite of all the knowledge surrounding this process, a very basic question remains unanswered. The question is, "why have bacteria evolved to carry out such a biochemical process?" Lennarz (1966) proposed that BH is a mechanism to dispose of reducing power. Another explanation by Kemp and Lander (1984) is that BH is a detoxification mechanism. Jenkins et al. (2008) mention that the reducing power hypothesis seems difficult to sustain and indicate that the detoxification hypothesis seems to be more plausible. Disposing of hydrogen is essential for fermentation in an anaerobic ecosystem; however, the relative contribution of BH to the removal of hydrogen is a small proportion of total metabolism. One experiment that seems to support the toxic effects of fatty acids on bacterial cell was conducted by Maia et al. (2006) who demonstrated that linoleic acid

damaged the integrity of different bacterial cells at different extents and in fact, *Clostridium proteoclasticum* was inhibited by linoleic acid.

Milk fat synthesis

The mammary gland in an organ with a complex metabolism which is capable of synthesizing milk which is a secretion with physical and chemical properties of a solution, colloidal suspension and emulsion; all three in a homogeneous stable form within the mammary gland. The fat in milk accounts for the emulsion properties of milk. This component is the most variable in milk composition and is greatly affected by the diet that the animal consumes. Milk fat accounts for approximately fifty per cent of the calories in milk (Van Soest, 1994) and its composition is characterized by high content of short chain fatty acids, though it also contains long chain fatty acids.

The source of fatty acids for the mammary are: glucose which is converted to pyruvate, citrate and acetyl CoA; triglycerides contained in chylomicrons; and de novo synthesis within the mammary gland from non-glucose sources (Akers, 2002). These three sources make up the fatty acid profile of milk; however each one contributes differently. The main lipogenic activity of the mammary gland is the synthesis of short chain fatty acids (SCFA) (Van Soest, 1994: Akers, 2002). The carbon sources for synthesis of SCFA are β-hydroxybutyrate (BHBA) and acetate. The mammary gland can utilize BHBA to incorporate into SCFA via the enzyme fatty acid synthetase which utilizes butyryl CoA as a primer (Van Soest, 1994); therefore, BHBA appears in the first four carbons of the majority of SCFA (Akers, 2002). Acetate is a carbon donor for the synthesis of fatty acids in the mammary gland. Most of the fatty acids synthesized within

the mammary gland range from 4 carbons to 14 and some have 16 carbons(Akers, 2002). These chains are elongated two carbons at a time via the malonyl CoA pathway. Long chain fatty acids (LCFA) are mostly derived from dietary sources. In dairy rations LCFA are comprised primarily by stearic, olei and linoleic.

In summary, milk fat contains SCFA derived from no novo synthesis in the mammary gland and the majority of LCFA are provided by the diet and removed from the blood stream by the mammary gland.

Milk Fat Depression

Fat is the most variable component in milk and can be modified by changes in the feeding regimen (Palmquist et al., 1993). Nutritional changes impact the amount and composition of milk fat either increasing the concentration or in some instances decreasing it. Low milk fat syndrome or milk fat depression (MFD) is one of the major changes that may occur in milk fat concentration. It is characterized by abnormally low levels of milk fat (i.e. reduction up to 50%) without any changes in milk yield or yield of other components (protein, lactose). Milk fat depression has been a common problem in dairy farms for more than a century (Bauman et al., 2008). Shingfield and Griinari (2007) mention that when diets contain high proportions of rapidly fermentable carbohydrates (for example starch > 25% of DM) or supplements of vegetable oil or marine lipids (> 5% of DM) it is likely that these diets can induce MFD. Similarly, Palmquist et al. (1993) point out that rations high in grains (≥ 50% of feed DM) increase starch and results in MFD.

Throughout the years there have been several theories that explain the causative factors that trigger MFD; some have attributed the disorder to limited mammary *de novo* synthesis of fatty acids because of reduced production of β hydroxybutyrate and acetate in the rumen. Another mechanism that has been proposed is the partitioning of fatty acids toward adipose tissue due to an insulin response lowering the amount of fatty acids available in the mammary gland; one more is the inhibition of *de novo* synthesis in the mammary gland by methylmalonate arising from decreased vitamin B12 and increased propionate in the rumen; and more recently a direct inhibition in the mammary gland by *trans* fatty acids derived from incomplete biohydrogenation of dietary fat in the rumen (Shingfield and Griinari, 2007). The latter mechanism has provided better support to explain MFD since experiments dealing with geometrics isomers of conjugated linoleic acid (CLA) have resulted in MFD reducing the concentration on milk fat dramatically whereas the other theories, also supported by data, only accounted for less than 10% of the fatty acids mobilized for milk synthesis (Griinari and Bauman, 2005). One key observation that arouse thoughts about the biohydrogenation theory was made by Davis and Brown (1970) when they suggested that milk from cows under MFD has higher concentration of *trans* 18:1 fatty acids. With this background, recent research has focused on biohydrogenation of unsaturated fatty acids and production of CLA isomers that inhibit milk fat synthesis (Figure 2).

Chouinard et al. (1999) demonstrated that CLA alters the fatty acid profile of milk fat and inhibits milk fat secretion in dairy cows. In their study they infused a mixture of CLA isomers into the abomasum of dairy cows to bypass rumen biohydrogenation. Their results showed very clearly that the infusion of CLA isomers dramatically reduced (by

more than 50%) the concentration of milk fat at three levels of infusion 50, 100 and 150 g/d of the CLA. Observed mean milk fat concentrations were 1.43, 1.38 and 1.23% for 50, 100 and 150 g/d of the CLA mixture whereas the control treatment resulted in 2.81%. As expected and consistent with previous observations (Davis and Brown, 1970) milk from cows with MFD had higher concentration of *trans* isomers of CLA. Four isomers were reported to have increasing concentrations during the infusion period; formerly, 8, 10; 9, 11; 10, 12; and 11,13, all in a *cis*, *trans* configuration.

Since the observations of Chouinard et al. (1999) pointed out the relevance of CLA *trans* isomers during MFD, Baumgard et al. (2000) focused their research on two CLA isomers that are commonly found in ruminant fat (*cis* 9, *trans* 11 CLA) and also a *trans* 10, *cis* 12 CLA isomer which is the putative source of *trans* 18:1 fatty acids which the concentration is typically higher in cows experiencing MFD. The results of the infusion of these isomers revealed that *trans* 10, *cis* 12 is a potent inhibitor of milk fat synthesis as it took about 24 h to exert its effect and by days 3 and 4 milk fat concentration dropped drastically whereas *cis* 9, *trans* 11 did not affect milk fat secretion. The control treatment resulted in 3.04% and 1.068 Kg/d milk fat whereas *trans* 10, *cis* 12 was 1.92% and 0.696 Kg/d milk fat. Altough *trans* 10, *cis* 12 CLA resulted in a dramatic reduction in milk fat, Perfield et al. (2007) mentions that there is a curvilinear response in the reduction of fat suggesting that there may be additional CLA isomers responsible for MFD as *trans* 10, *cis* 12 is inadequate to account for the total reduction in milk fat secretion. Another isomer associated with this disorder is *trans* 9, *cis* 11 CLA; this isomer accounted for 15% of the reduction in milk fat when it was infused into the abomasum of dairy cows (Perfield et al., 2007).

In is important to note that these experiments have not shown the results of dietary treatments per se but rather the response to abomasal infusions of CLA, therefore one ought to assume that under certain dietary conditions, some of these CLA isomers arise in the rumen and cause MFD. One experiment that clarified and confirmed the results from the trials with abomasally infused dairy cows was conducted by Peterson et al. (2003). Their approach was to induce MFD through dietary treatments. Briefly, the treatment responsible for MFD was high in concentrate (i.e. cracked corn was fed at 64.4% of the dietary DM) and low in fiber (14.9 % in the MFD treatment, compared to 31.1% NDF in the control diet). Results from this work confirmed that during diet induced MFD, the fatty acids profile of milk fat is changed with a remarkable increased concentration of *trans* 10, *cis* 12 CLA in milk fat.

Another key finding by Peterson et al. (2003) pertains to a coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. This was the first investigation that linked gene expression with diet induced MFD. In short, MFD coincided with lower expression of genes encoding for proteins responsible for uptake of fatty acids from the bloodstream and proteins associated with *de novo* synthesis of fatty acids. These findings illustrate that MFD is the result of both, lower absorption and lower synthesis of fatty acids in the mammary gland. More recent research has elucidated more about the molecular mechanism by which CLA is responsible for MFD. Harvatine et al. (2009) utilized molecular techniques to measure gene expression of enzymes and key regulators of lipid synthesis in adipose tissue of cows under MFD. They reported that the infusion of *trans* 10, *cis* 12 CLA in to the abomasums of dairy cows resulted in up

regulation of lipoprotein lipase, fatty acid synthase, stearoyl CoA desaturase, fatty acid binding protein 4 in adipose tissue.

Taken together, observations of these last studies (Peterson et al., 2003; Harvatine et al., 200) strongly support the biohydrogentation theory and its relationship to MFD. During MFD there is a lipid repartitioning process that accounts for the reduction in mammary uptake and synthesis of fatty acids while concomitantly there is lipid accretion in adipose tissue which was not perceived in previous studies because of the short duration of the trials and was possible to detect only through molecular techniques. Figure 3 depicts a simplistic diagram of an overview of metabolic events during MFD derived from the literature reviewed in this chapter.

Feeding *bm3* corn silage to dairy cows

The *bm3* mutation in corn causes chemical and physical changes in the plant. This mutation is linked to the characteristic reddish brown pigmentation of the midrib of the leaf (Barriere and Argillier, 1993), hence the common name brown midrib corn (BMR). Perhaps the most important change caused by the *bm3* mutation is the content of lignin which is typically between 30 to 40% lower (Sheldrick, 1979) compared to the genetic counterpart without this mutation. This makes *bm3* corn silage valuable as forage for dairy cows as it has the potential to be more digestible possibly yielding more energy available for higher milk production (Gehman et al., 2008)

Lignin is formed when p-coumaryl, coniferyl and synapyl are polymerized in the cell wall of plants to form a three dimensional macromolecule (Chesson and Forsberg, 1988). This polyphenolic compound has various types of linkages, most of which are aryl-ether and carbon-carbon linkages which are very strong, thus lignin provides rigidity

to plants and confers resistance to chemical and microbial degradation (Van Soest, 1994; Chesson and Forsberg, 1988). These two functions of lignin are important to plants as they may have evolved as part of a system that plants use to protect themselves against herbivores (Van Soest, 1994).

Lactational performance of dairy cows fed *bm3* corn silage: a meta-analysis

Lignin is a major constraint for fiber digestion as it forms complex matrices with carbohydrates that result in indigestible material by rumen bacteria. Published dairy research testing the effects of *bm3* corn has been conducted as early as 1976 (Frenchick et al., 1976) and continues to be the subject of investigations (Castro et al., 2010). Throughout the years there has been a variety of dietary treatments associated with *bm3* corn silage (e.g. combination with other forages or additives such as monensin); therefore productive responses vary from one experiment to another. One approach to analyze the overall effects of *bm3* corn silage derived from the various experiments reported is to conduct a meta-analysis (St-Pierre, 2001). For such analysis data from 23 published nutrition experiments (Table 3), which included a total of 83 treatment observations (Appendix 1), were used. Data were analyzed using SAS and a random coefficient model to account for the random effects of different experiments. The model included a fixed effect of corn hybrid (*bm3* versus the control) and the level of corn silage included in the observation was also modeled as continuous independent variable; study was considered random. The covariance structure used in the analysis was the Variance Components option of SAS which was included in the model's random statement. To fully clarify, data were fit to the following model, $Yijkl = \mu + \rho i + \alpha j + \beta k + eijk$. Where Yijkl is the

observation for the j^{th} hybrid and k^{th} level of hybrid within the i^{th} study, μ is the overall mean, ρi is the random effect of the i^{th} study, αj is the fixed effect of the j^{th} hybrid, βk is the fixed effect for level of corn silage and eijk is the normally identical and independently distributed error term.

The summarized results of the meta-analysis are presented in Table 4 and were generated using the LSMEANS statement of SAS. Dry matter intake was greater ($P<0.01$) for cows consuming *bm3*. Specifically, compared to the control hybrid, cows consumed 1.2 more kg (DM). Feed efficiency was similar between the two corn hybrids and averaged 1.49 ± 0.05. Dry matter intake is associated with physical and chemical characteristics of the feeds. Oba and Allen (1999) suggest that the NDF fraction of *bm3* corn is readily hydrolyzed in the rumen which contributed to less fill effect and greater intake. This is consistent with our meta-analysis in which estimated digestibility of NDF *in vitro* was greater ($P < 0.01$) for *bm3* corn silage by 10% units (48.3 VS 58.6 ± 3.57%). The same response was observed for total tract NDF digestibility only at a lower extent for *bm3*. Total tract digestibility of NDF was lower ($P = 0.01$) when cows consumed control corn silage, 45.4 ± 4.23% whereas cows on the *bm3* hybrid digested 50.2 ± 4.23%. Overall, feeding *bm3* corn silage results in greater DMI and fiber digestibility compared to its genetic counterpart.

Milk production was also greater ($P < 0.01$) for cows receiving dietary treatments with *bm3*corn silage; actual milk yield was 1.2 kg/d more. This is likely to be the result of more energy being available for milk synthesis derived from greater DMI. Similarly fat corrected milk (FCM) was greater ($P = 0.01$) when cows consumed *bm3* corn silage

which resulted in 34.2 and 33.2 ± 1.49 kg/d for *bm3* and control, respectively. Milk composition was observed to be similar between the two hybrids, the average milk fat concentration was 3.49 ± 0.09% and milk protein was 3.07 ± 0.06%. Nonetheless, milk protein yield was observed to be greater ($P < 0.01$) primarily due to the significantly higher milk yield.

Feeding *bm3* corn silage to dairy cows represents a nutritional strategy to increase DMI. Because of greater DMI and enhanced NDF digestibility, cows can obtain more energy which can be used for milk synthesis and synthesis of milk components. Since *bm3* corn silage can result in greater energy intake, cows in early lactation may benefit from consuming *bm3* corn silage to a greater extent compared to cows in mid-late lactation.

Feeding distillers grains to dairy cattle

In the last decade, the U.S. ethanol industry has grown dramatically. Today there are more than three times the number of operating ethanol plants compared to ten years ago and with a potential capacity of production equal to 13,028.4 millions of gallons per year (RFA, 2010), the ethanol industry represents a vast source of feed byproducts suitable for ruminants. Generally speaking, the byproducts resulting from the distillation of corn are known as distillers grains (DG); such byproduct is a source of protein and energy for ruminants (Ham et al., 1994).

The American dairy industry consumes about 42 to 46% (NCGA, N/D; RFA, 2008) of the total DG produced in the country. Several studies have shown the effects of utilizing DG in dairy rations. The responses are a function of the treatments tested; nonetheless DG has generally been demonstrated to be an useful feed when incorporated

into dairy feeding systems as it supports similar or higher milk yield than compared to control diets (Schingoethe et al., 2009). In feedlot diets inclusion of 20% DDGS (DM) has resulted in greater economic returns (Buckner et al., 2008), it is likely that in dairy rations inclusion of DDGS results in a similar situation as it can replace proportions of corn and soybean meal.

Anderson et al. (2006) pointed out that one of the concerns about dried distillers grains with solubles (DDGS), is how much to feed. Even though DDGS have a valuable nutritional composition, dairy nutritionists tend to limit the inclusion of DDGS to 10% of the dietary DM (Janicek et al., 2008; Schingoethe et al., 2009). One reason for this is that the fat content is high, generally ranges between 10 and 12% (Kleinschmit et al., 2006; Schingoethe et al., 2009). This may result in milk fat depression due to the high content of unsaturated fatty acids present in DDGS which can alter lipid metabolism in the rumen, thus altering the metabolism in the mammary gland so that milk fat synthesis is decreased as well as fatty acid uptake. Anderson et al. (2006) reported that when dairy cows were fed DG at 20% of the ration DM, milk yield was observed to be about 2.5 kg/d higher for cows consuming DG. In addition, milk fat and milk protein yield were also greater for DG diets compared to a control diet. Likewise, Kleinschmit et al. (2006) reported that cows consuming a ration with 20% DDGS increased milk yield, 4% FCM and ECM compared to the control ration.

In practice there is a common perception that high inclusion of DDGS in the ration reduces the concentration of milk fat (Kleinschmit et al., 2006; Janicek et al., 2008; Schingoethe et al., 2009; Hippen et al., 2010). Leonardi et al. (2005) reported a linear decrease in milk fat percentage as the inclusion of DDGS increased in the diet. This

reduction was only significantly different between 10 and 15% DDGS when milk fat dropped from 3.33 to 3.24%; however, Kleinschmit et al. (2006) suggest that this effect may not be of biological significance. Similarly Hippen et al. (2010) report that DDGS fed at 20% of the diet resulted in milk fat depression, hence reduction in milk fat percentage and milk fat yield. These changes were slight and not very dramatic as diets with no DDGS averaged 3.21% and 1.41 kg of milk fat whereas diets with DDGS averaged 3.03% and 1.27 kg. In contrast to the response observed by Leonardi et al. (2005), several other experiments reported no effect on milk fat percentage when DDGS were included at 20% in the ration (DM) (Kleischmit et al., 2006). Furthermore, Janicek et al. (2008) fed 30% DDGS with no differences in milk fat percentage. In both works, there was a significant increase in milk fat yield due to increased milk production.

The results from these works demonstrate that DDGS can be included effectively in dairy rations at levels between 20 and 30% of the dietary dry matter without adverse effects on milk fat concentration. However, in some peculiar circumstances DDGS may be linked to reduced milk fat percentage which does not necessarily implies a reduction in milk fat yield. Such instance is observed in the work done by Kleinschmit et al. (2007) where they included 15% DDGS with added alfalfa hay on a corn silage based diet. There was a linear decrease in milk fat percentage with the inclusion of alfalfa. Milk fat was 3.67 ± 0.08% on the diet with corn silage whereas the alfalfa hay diet resulted in 3.49 ± 0.08% indicating a possible interaction with the type of forage. Despite this reduction in milk fat percentage, actual yield of milk fat was observed to be unaffected. Further analysis showed that not all the cows responded the same way and there was an interaction with parity, resulting in primiparous cows producing milk with low fat content

whereas milk from multiparous cows did not differ across treatments. There is no explanation provided as to why this interaction may have such an effect. The authors also suggest that the reduction in milk fat percentage could be a diluting effect as milk fat yield remained similar across treatments.

Nitrogen utilization by dairy cows

It is generally assumed that the crude protein of feeds contains 16% N (Satter and Roffler, 1975; NRC 2001). Dietary protein for ruminants can be classified as rumen degradable protein (RDP) and rumen undegradable protein (RUP) (NRC, 2001). Rumen degradable protein is represented by the N (contained in dietary protein as well as non protein N) that enters the rumen via dietary sources, saliva and to a smaller extent across the rumen wall and is acted upon by microbial enzymes which yield free amino acids, peptides and ammonia (Hogan, 1975). As the terminology implies RUP is comprised of feed protein that escapes rumen degradation and reaches the abomasum for enzymatic digestion by the host.

Within the rumen there is a massive intervention of microorganism that matabolize dietary protein (e.g RDP) and resynthesize amino acids which are also incorporated into microbial crude protein (MCP) (Satter and Roffler, 1975; Russell and Hespell, 1981). Rumen microbes also have the ability to utilize non-protein N to synthesize MCP which represents and ample supply of protein for the ruminant animal. When the dietary supply of protein overwhelms the microbial capacity of ammonia utilization there is an “ammonia overflow” (Satter and Roffler, 1975). The surplus of ammonia is transformed to urea in the liver. Urea can be recycling via saliva; however a

large proportion is excreted in urine (Satter and Roffler, 1975) which is then converted back to ammonia which is the most environmentally labile for of N (Varel, 1999).

Dairy rations should be formulated to supply enough nutrients to meet the animal's requirements for maintenance and production while maximizing efficiency of utilization. Thus, feeding just enough protein to support optimal productivity is important because it has practical benefits such as reduced feed cost per unit of lean tissue produced, greater and more efficient yields of milk protein; it also allows more room into the diet for other nutrients that may enhance production and ultimately alleviate concerns about excessive N being excreted into the environment (NRC, 2001).

Nitrogen Excretion: fecal, urinary and milk N

Fecal nitrogen is comprised of endogenous N, undigested MCP and undigested RUP (Tamminga, 1992). Endogenous sources of N are secretions and epithelial debris from the intestines (Hogan, 1975). Undigested MCP is in part originated from bacterial synthesis occurring in the cecum (Hogan, 1975; Van Soest, 1994) where the cow has no enzymatic system to release amino acids for absorption from the colon and rectum (Hogan, 1975). Undigested RUP that is present in feces is quantitatively insoluble and it could be comprised of keratin, Maillard reaction products or protein bound to lignin thus resistant to peptic digestion (Van Soest, 1994).

As mentioned earlier, dietary N in excess of requirements is converted to urea which diffuses into the animal's fluid pools such as blood, milk and urine (Kauffman and St-Pierre, 2001). Urinary nitrogen can account for approximately 30-50% of N excreted

relative to N ingested (Gehman and Kononoff, 2010). The urea contained in urine is acted upon by microbial urease originated from bacteria found in fecal matter, fouled pen surfaces and slurry pits (van Duinkerken, 2011), this hydrolysis releases ammonia into the atmosphere which raises concerns about air pollution. Wang et al. (2007) refer to studies were approximately 70% of N excreted can be lost in the environment through volatilization, leaching and run-off. A similar figure is reported by Gehman and Kononoff; in their study manure N (urinary N+fecal N) ranged from 74 to 94% relative to N intake. Interestingly, the lowest proportion of manure N was achieved with a diet that contained wet distillers grains plus solubles and had higher CP than the corn silage control diet (18.5 VS 17% CP) indicating improved utilization of N. On the other hand Wang et al. (2007) reported increasing N excretion with increasing levels of CP in the diet of dairy cows. They report that a diet with 12% CP resulted in 469 mg/dL urinary N whereas a diet with 15% CP resulted in 713 mg/dL urinary N. These studies (Gehman and Kononoff, 2010; Wang et al. 2007) clearly show that urinary N excretion depends, in part, on protein level in the diet as well as the quality of the protein, and also, that nutritional strategies can be developed to reduce N excretion possibly through complimentary relationships between feedstuffs (Gehman and Kononoff, 2010).

The concentration of urea in blood can be utilized as an indicative of utilization of dietary N. Wang et al. (2007) reported the ratio of milk protein yield:CP intake as well as the concentration of BUN as indicators of N conversion by dairy cows fed increasing levels of CP (from 12 to 15). The N conversion of the low CP diet was 0.33 whereas the high CP diet resulted in significantly lower conversion, 0.28. The concentration of BUN was significantly different, for the high CP diet BUN was 15.7 mg/dL whereas the low

CP diet resulted in 8.6 mg/dL. Thus high concentrations of BUN (> 15 mg/dL) may be indicative of low N utilization (Nousiainen et al. 2004; Wang et al., 2007).However, measuring blood urea is not practical because of the difficulty to obtain regular and reliable samples. Instead, milk urea nitrogen (MUN) has been used as an indicator of dietary N utilization as it is greatly affected by the CP content of the diet (Nousiainen et al. 2004) as well as a mean to predict N emission into the environment (Burgos et al., 2010; van Duinkerken, 2011).

Feeding practices can influence animal performance and dictate the outcomes in terms of nutrient balance. Current and future feeding strategies focus on optimizing animal performance; Wang et al. (2007) suggest that the optimal content of metabolizable protein should be based not only on milk production but also on N excretion and associated environmental consequences.

SUMMARY

Plant cell walls are comprised of complex carbohydrates undigested by mammals, namely cellulose and hemicellulose, and a polyphenolic compound known as lignin which provide physical protection and rigidity to the cell wall. Ruminant animals harbor vast populations of microorganism including bacteria, protozoa, fungi and yeast. These different organisms confer the characteristic ability of ruminants to digest fibrous feeds such as forages and obtain energy contained in these feeds. Hydrolysis of plant carbohydrates yields simple sugars that are further digested by ruminal microbes which in turn produce volatile fatty acids (VFA) that serve as energy source to the host animal.

Current feeding practices in the dairy industry involve the inclusion of grains and or fat (animal or vegetal) as a means to increase the energy density of the diet to meet the energy requirement of today's high producing dairy cow. Rumen microbes, particularly bacteria, participate in metabolism of starch (derived from grains) and fatty acids. Starch is hydrolyzed to glucose and it is readily utilized by bacteria as an energy source. Dietary fats undergo an extensive transformation which includes hydrolysis of triacylglycerides and saturation of double bonds by bacteria. This latter process is known as biohydrogenation. Bacteria species associated with rumen lipid metabolism include *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica*.

When dairy cows consume a ration with high content of starch (i.e. >25% of dietary DM) and high lipid content (>5 % of dietary DM) rumen environment is altered which leads to shifts in bacterial populations and also results in alterations of lipid metabolism by these microbes. During these alterations, microbial pathway of lipid

biohydrogenation results in increased concentration of C: 18 fatty acids. One intermediate form of these fatty acids is *cis*- 10, *cis*-12 conjugated linoleic acid (*cis*-10,*cis*-12 CLA). This CLA isomer has been identified as a potent inhibitor of milk fat synthesis, thus a major cause of milk fat depression. This disorder is characterized by abnormal low concentration of milk fat and normal milk yield and protein and lactose concentrations. The biohydrogenation theory links *cis*-10, *cis*-12 CLA and milk fat depression; this involves a direct down regulation of lipogenic enzymes in the mammary gland which is then reflected in the low concentration of milk fat typical of milk fat depression.

Current dairy rations contain typically between 50-55% forage (DM basis) and the rest is concentrate feeds. Since forages comprise such a large proportion of the ration it is necessary that these are of high quality to support higher milk yield. High quality attributes can be assessed by nutrient content, digestibility of nutrients and ultimately by the capacity of producing productive responses such as increased milk yield or components (i. e. milk fat and protein). Corn silage is commonly utilized in the dairy industry and it provides forage and grain. The grain part is digested to a greater extent compared to the fodder part. In order to improve quality of corn silage there have been hybrids developed based on density of nutrients and other compounds. One of these hybrids is known as brown midrib corn. This type of hybrid has a mutation identified as *bm3*; this mutation causes a plant that synthesizes less lignin. While lignin plays an important role in the plant's anatomy and physiology, it represents a constraint in terms of fiber digestion by ruminants. Since the *bm3* corn silage has lower content of lignin compared to the same type of corn without the mutation it is valuable as a forage source

for dairy cows as it has been shown to increase dry matter intake and digestibility, possibly driving higher milk yield.

The USA corn derived ethanol industry has been growing, thus there is a concomitant increasing supply of feed co-products derived from this industry. Dried distillers grains plus solubles (DDGS) is one of the major feed co-products currently available. This feedstuff is commonly fed to dairy cows as a source of energy and protein. Inclusion of DDGS in dairy rations typically ranges between 10 to 15% (DM); however, including up to 30% of dietary DM as DDGS has been reported to increase milk yield, thus the dairy industry has the opportunity to take advantage of the increasing supply of this feed and include it at higher levels in dairy rations and improve productive performance.

Dairy Cows require protein for maintenance of metabolic functions as well as deposition of protein into milk. Dairy rations should be formulated to meet these requirements to support adequate lactation. Current protein feeding practices are focused on meeting or exceeding the protein requirement established by the NRC. When dietary supply of protein surpasses the cow's needs, any excess protein is metabolized and nitrogen is excreted mainly in urine which contributes to environmental pollution. Nowadays dairy rations are formulated to maximize milk production, nonetheless over feeding protein can lead to increased N excretion, therefore it is necessary to develop nutritional strategies that maximize lactational performance while trying to minimize N excretion.

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Table 1. Prominent rumen bacterial species grouped according to their affinitive substrate guild (Williams et al. 2010)

	Substrate[1]									
Species	C	H	St	Pec	Su	Pro	Li	FC	NFC	La
Anaerovibrio lipolyticus							Li			
Bacteroides sp.									NFC	
Butyrivibrio sp.	C	H	St	Pec		Pro		FC	NFC	
Clostridium aminophilum						Pro				
Eubacterium ruminantium			St		Su				NFC	
Fibrobacter sp.	C							FC		
Lachnospira sp.				Pec						
Lactococcus lactis					Su				NFC	
Lactococcus sp.					Su				NFC	
Megasphaera elsdenii						Pro				La
Prevotella bryantii		H		Pec				FC	NFC	
Prevotella sp.		H	St	Pec		Pro		FC	NFC	
Ruminococcus sp.	C	H						FC		
Selenomonas sp.					Su				NFC	La
Streptococcus sp.			St	Pec	Su				NFC	
Succinimonas sp.			St						NFC	
Succinivibrio dextrinosolvens				Pec					NFC	
Treponema bryantii				Pec	Su		Li		NFC	

[1] C= cellulose, H= hemicellulose, St= starch, Pec= pectin, Su= sugar, Pro= protein, Li= lipid, FC= fiber carbohydrate, NFC= non fiber carbohydrate

Table 2. Summary of the aerobic fungal cellulase system (Wood, 1985)

Enzyme	Action	End product
Endo-1,4-β gluconase (endo-1,4, β-D Glucan 4-gluconohydrolase, endoglucanase, endocellulase)	Attacks carboxymethyl cellulose or phosphoric acid-swollen cellulose at random	Cello-oligosaccharides
Cellobiohydrolase (1,4-β-D-glucan cellobiohydrolase, exoglucanase, exocellulase)	Attacks the non reducing end of cellulose	Cellobiose
B-Glucosidase (Cellobiose)	Hydrolyzes cellobiose and cello-oligosaccharides	Glucose

Table 3. References consulted to compile data used in the meta-analysis of the effects of *bm3* corn silage on DMI and milk production

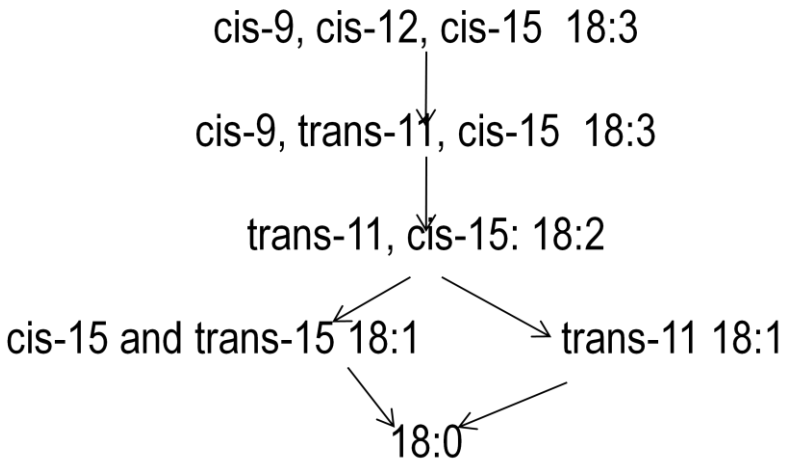
Frenchik et al. (1976)
Rook et al. (1977)
Keith et al. (1979)
Sommerfeltd et al. (1979)
Block et al. (1981)
Stallings et al. (1982)
Weller and Phipps (1986)
Oba and Allen (1999)
Oba and Allen (2000)
Oba and Allen (2000)
Bal et al. (2000)
Tine et al. (2001)
Greenfield et al. (2001)
Ballard et al. (2001)
Schwab et al. (2002)
Qiu et al. (2003)
Dominguez and Satter (2003)
Ebling and Kung (2004)
Barriere et al. (2004)
Taylor and Allen (2005)
Weiss and Wyatt (2006)
Kung et al. (2008)
Gehman et al. (2008)
Castro et al. (2010)

Table 4. Effects of *bm3* corn silage on productive performance of lactating dairy cows[1]

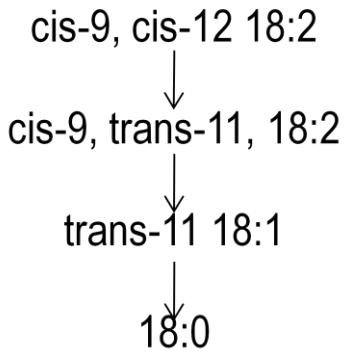
Item	Corn hybrid		SEM	*P-value*
	Control	*bm3*		
Production				
DMI, kg	22.3	23.5	0.73	<0.01
Milk yield, kg	34.3	35.5	2.32	<0.01
FCM, kg	34.5	35.6	2.39	0.05
Fat, %	3.64	3.34	0.09	0.16
Fat yield, kg	1.22	1.24	0.07	0.43
Protein, %	3.07	3.08	0.06	0.60
Protein yield, kg	1.10	1.14	0.06	<0.01
Digestibility				
In vitro	48.3	58.6	3.57	<0.01
Total Tract	45.4	50.2	4.23	0.01

[1] Least-square means from meta-analysis (St. Pierre, 2001) performed on data from the experiments referenced in Table 3.

A)



B)



C)

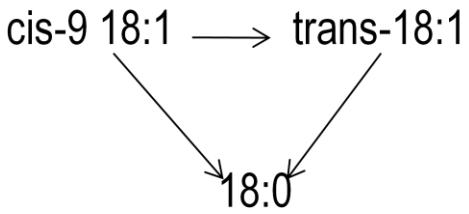


Figure 1. Biohydrogenation pathway of A) α linolenic acid, B) Linoleic acid and C) Oleci acid to fully saturated stearic acid. (Adapted from Hartfoot and Hazlewood, 1988).

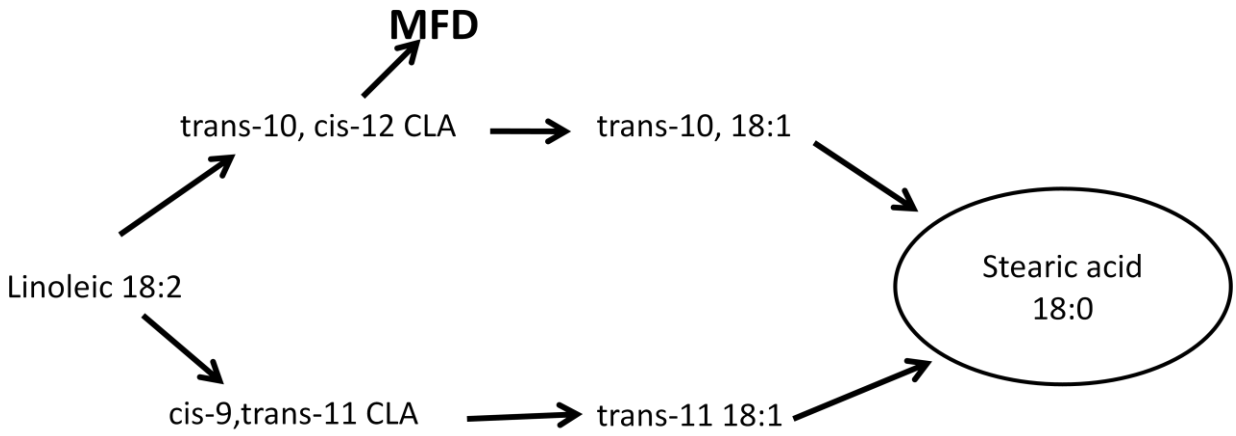


Figure 2. Biohydrogenation pathway of linoleic acid. The upper pathway represents the shift during MFD having *trans* 10, *cis*-12 CLA as the putative source of *trans* 10, 18:1. Adapted from Shingfield and Griinari, 2007.

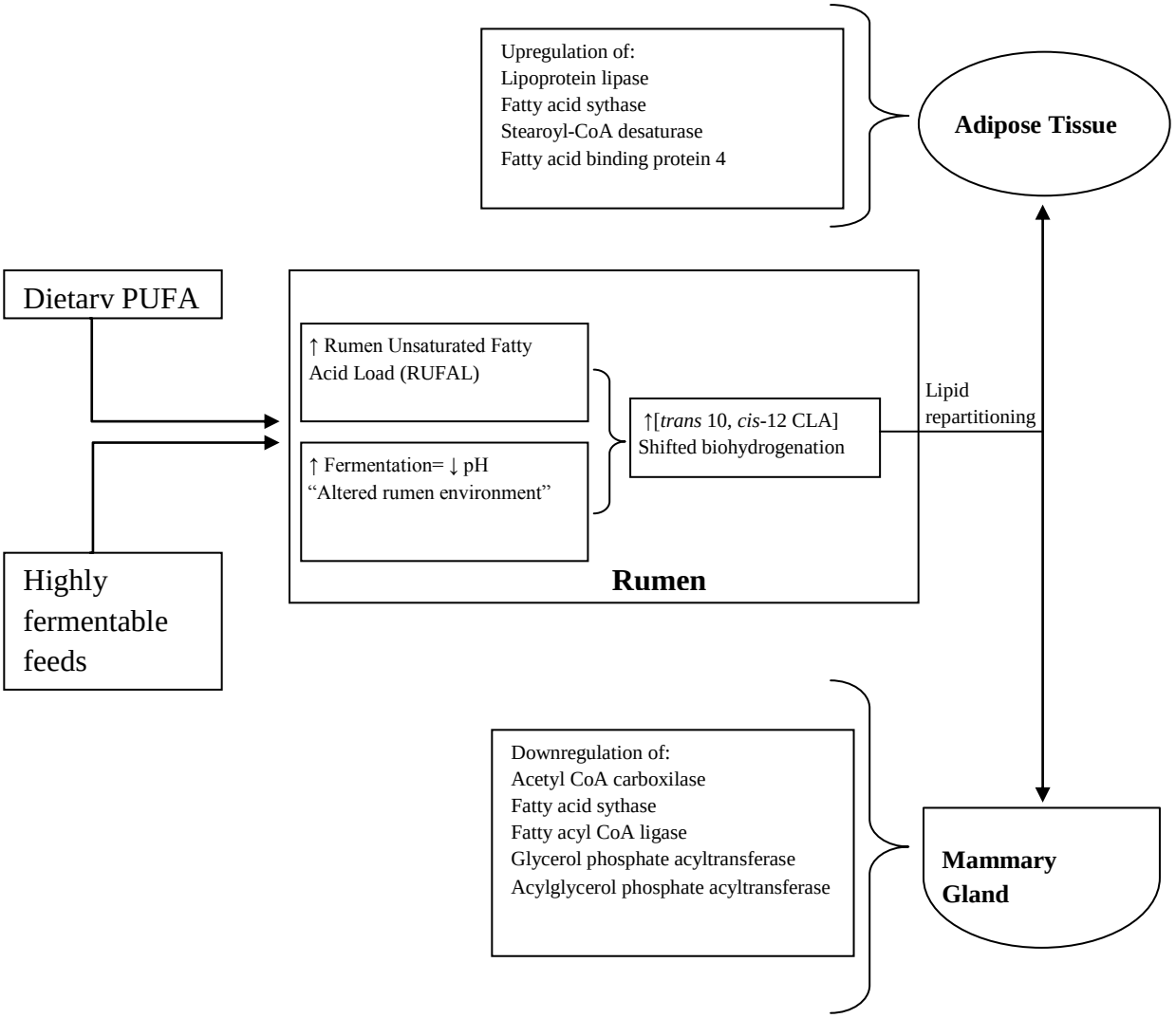


Figure 3. Schematic representation of the factors and events that cause MFD in dairy cows according to the biohydrogenation theory.

Appendix 1. Data set used in the meta-analysis performed to test the effect of *bm3* corn silage on the productive response of dairy cows

Author	Study	Year	Hybrid	Level	DMI	Milk_Y	Fat_p	Prot_p	NDFDig	IVNDFDig	NDF_CS	Lig_CS	NDF_diet	CP_diet
Oba and Allen	1	1999	Cont	44.6	23.5	38.9	3.46	2.95	30.9	39.4	40.1	2.5	31.6	19.6
Oba and Allen	1	1999	bm3	44.6	25.6	41.7	3.44	2.99	33.1	49.1	38.3	1.7	30.8	19.6
Oba and Allen	2	2000	Cont	32.1	23.9	.	.	.	30.3	46.5	42.9	2	29.1	17.7
Oba and Allen	2	2000	bm3	35.8	24.7	.	.	.	30.2	55.9	41.4	1.3	28.7	17.7
Oba and Allen	2	2000	Cont	50.5	21.5	.	.	.	42.1	46.5	42.9	2	38.4	18
Oba and Allen	2	2000	bm3	55.9	22.9	.	.	.	38.1	55.9	41.4	1.3	37.5	18.1
Tine et al	3	2000	Cont	60	22.8	32.3	3.93	3.2	48.8	.	46.2	3.6	32	19.7
Tine et al	3	2000	bm3	60	25.2	35.4	3.76	3.32	55.8	.	43.8	2.47	31.3	19.7
Weiss and Wyatt	4	2006	bm3	55	23.9	.	.	.	50.6	65.2	39.8	1	32.3	14.4
Weiss and Wyatt	4	2006	bm3	55	24.6	.	.	.	53.8	65.2	39.8	1	32.3	17.2
Weiss and Wyatt	4	2006	Cont	55	23.8	.	.	.	50.8	58.3	40.3	1.96	32.6	14.2
Weiss and Wyatt	4	2006	Cont	55	24.9	.	.	.	51.7	58.3	40.3	1.96	32.6	17.1
Weiss and Wyatt	4	2006	bm3	55	24.5	36.4	3.64	3.08	.	65.2	39.8	1	32.3	14.4
Weiss and Wyatt	4	2006	bm3	55	25.2	37.4	3.71	3.14	.	65.2	39.8	1	32.3	17.2
Weiss and Wyatt	4	2006	Cont	55	24.8	34.9	4.04	3.13	.	58.3	40.3	1.96	32.6	14.2
Weiss and Wyatt	4	2006	Cont	55	25	35.7	4.17	3.13	.	58.3	40.3	1.96	32.6	17.1
Frenchik et al	5	1976	bm3	49	20.91	22.54	3.6	.	.	.	.	3.1	.	13.3
Frenchik et al	5	1976	Cont	49	20	21.72	3.68	.	.	.	.	4.4	.	13.1
Rook et al	6	1977	bm3	60	20.24	31.3	4.52	3.22	61.8	.	57.6	4.9	.	.
Rook et al	6	1977	Cont	60	18.63	32.9	4.23	3.12	58.9	.	57.4	6.4	.	.
Rook et al	6	1977	bm3	85	20.69	24.3	3.82	2.87	45.5	.	55.6	4.6	.	.
Rook et al	6	1977	Cont	85	17.63	22.6	3.84	2.89	53	.	60	6	.	.
Keith et al	7	1979	bm3	75	22	28.6	3.58	3.41	.	65.29	47.54	2.5	.	.
Keith et al	7	1979	Cont	75	21.4	27.3	3.66	3.41	.	54.75	49.3	3.29	.	.
Keith et al	7	1979	bm3	40	22	28.4	3.55	3.45	.	65.29	47.54	2.5	.	.
Keith et al	7	1979	Cont	40	21.6	26.8	3.69	3.45	.	54.75	49.3	3.29	.	.
Stallings et al	8	1982	bm3	49.4	17.6	21	3.24	.	.	.	.	2.4	.	.
Stallings et al	8	1982	Cont	47.3	17.1	21.4	3.37	.	.	.	.	4	.	.
Ebling and Kung	9	2004	Cont	40	23.4	41.4	3.16	2.79	22.7	39.9	41.6	2.25	33.9	16.3
Ebling and Kung	9	2004	bm3	40	25.9	44.3	2.94	2.76	31.9	54	41.8	1.02	33.5	17.1
Ebling and Kung	9	2004	bm3	40	24.5	42.5	3.17	2.75	35.5	51	41	0.87	33.8	17.3
Bal et al	10	2000	Cont	32	28.4	44.5	3.18	3.27	.	.	41.6	2.5	27.5	17.2
Bal et al	10	2000	bm3	40.2	28.4	43.1	3.46	3.2	.	.	38.1	1.6	29.4	17.1
Block et al	11	1981	bm3	65	22.9	34.38	2.58	3.04	.	.	39.5	3.3	33.2	16.1
Block et al	11	1981	bm3	65	20.9	36.44	3.16	2.88	.	.	39.5	3.3	35.4	15.8
Block et al	11	1981	Cont	65	19.4	33.5	2.46	2.96	.	.	45.5	4.9	36.3	16.2
Block et al	11	1981	Cont	65	19.4	32.77	3.84	2.81	.	.	45.5	4.9	38.5	15.7
Kung et al	12	2008	Cont	45	26.9	46.8	3.6	2.88	.	.	42.91	3.17	34.3	17.5
Kung et al	12	2008	Cont	45	27.3	47.7	3.48	2.87	.	.	39.57	2.76	34.1	17.5
Kung et al	12	2008	bm3	45	26.8	48.5	3.5	2.87	.	.	44.72	2.2	33.9	17.5

Oba and Allen	13	2000	bm3	35.8	23.6	.	.	.	.		55.9	41	1.3	28.7	17.7
Oba and Allen	13	2000	Cont	32.1	22.8	.	.	.	.		46.5	42.9	2	29.1	17.7
Oba and Allen	13	2000	bm3	55.9	22	.	.	.	.		55.9	41.4	1.3	37.5	18.1
Oba and Allen	13	2000	Cont	50.5	20.5	.	.	.	.		46.5	42.9	2	38.4	18
Schwab et al	14	2002	bm3	40	26.6	42.7	3.32	3.17	50.7	.		36.6	.	25.5	15.2
Schwab et al	14	2002	bm3	40	25.5	43	3.36	3.18	51	.		39.5	.	26.6	15.3
Schwab et al	14	2002	bm3	40	25.9	43.5	3.11	3.2	41.8	.		34.5	.	24.6	15.2
Schwab et al	14	2002	bm3	40	25.1	43.7	3.18	3.2	45.4	.		36.7	.	25.4	15.1
Greenfield et al	15	2001	Cont	60	19.8	24.3	4.3	3.4	52.4	.		46.32	2.69	30.03	18.21
Greenfield et al	15	2001	bm3	60	21.2	24.7	4.3	3.4	56.8	.		45.02	1.5	29.15	18.6
Qiu et al	16	2003	bm3	31.5	26.2	35.5	3.76	3.31	68	.		41.6	1.13	40	17.2
Qiu et al	16	2003	bm3	39.12	26.8	35.5	3.89	3.29	63.3	.		41.6	1.13	37.2	17.4
Qiu et al	16	2003	Cont	31.5	25	34	3.93	3.28	65	.		41.1	2.34	38.1	17.3
Qiu et al	16	2003	Cont	39.12	23.5	34.1	3.79	3.35	56.3	.		41.1	2.34	38.5	17.4
Dominguez and Satter	17	2003	bm3	61.5	20.55	35.25	.	.	.	.		40.6	.	.		.
Dominguez and Satter	17	2003	Cont	58.5	20.35	36.65	.	.	.	.		38.2	.	.		.
Dominguez and Satter	17	2003	Cont	61.5	19.2	35.1	.	.	.	.		35.8	.	.		.
Taylor and Allen	18	2005	Cont	37.5	23.6	39.8	3.51	2.95	46.3	.		41.2	2.25	26	17.3
Taylor and Allen	18	2005	Cont	37.3	25.5	40.6	3.62	2.95	45.3	.		41.2	2.25	25.8	17.2
Taylor and Allen	18	2005	bm3	39.6	25.2	42.5	3.49	3	52.4	.		38.9	1.22	25.7	17.5
Taylor and Allen	18	2005	bm3	39.2	24.9	40.6	3.44	2.93	49.7	.		38.9	1.22	25.5	17.4
Sommerfeltd et al	19	1979	bm3	57	18.36	25	3.79	2.99	.	.		53.5	5.3	.		.
Sommerfeltd et al	19	1979	Cont	55	17.71	25.5	3.88	3.03	.	.		50.9	5.5	.		.
Sommerfeltd et al	19	1979	bm3	52.96	18.39	24.8	3.94	2.95	60.1	.		53.5	5.3	.		.
Sommerfeltd et al	19	1979	Cont	47.65	17.25	24	4.47	3.11	50.5	.		50.9	5.5	.		.
Ballard et al	20	2001	Cont	31.14	.	31.1	4.27	3.26	.		28.2	42.2	2.6	35.3	17
Ballard et al	20	2001	bm3	31.14	.	33.4	4.13	3.2	.		45.7	41.7	2	34.7	17.3
Ballard et al	20	2001	Cont	31.14	.	31.2	4.2	3.21	.		32.1	41.5	3.1	35.1	17.7
Barriere et al	21	2004	bm3	.	18.2	.	.	.	.	.	.		1.66	.		.
Barriere et al	21	2004	Cont	.	16.9	.	.	.	.	.	.		2.6	.		.
Barriere et al	21	2004	Cont	.	16.8	.	.	.	.	.	.		2.57	.		.
Barriere et al	21	2004	Cont	.	15.6	.	.	.	.	.	.		2.9	.		.

Barriere et al	21	2004	Cont	.	13.9	.	.	.	.	.	.	.	2.37	.		.
Barriere et al	21	2004	Cont	.	15.2	.	.	.	.	.	.	.	2.51	.		.
Gehman et al	22	2008	bm3	54.3	21.1	39.5	3.65	3.03	40	61	40.2	2.4		33.7	16.7	
Gehman et al	22	2008	Cont	49.2	20.1	36.4	3.58	3.05	39.8	49.1	42.3	3.5		33.4	16.3	
Gehman et al	22	2008	bm3	54.3	21.5	37.1	3.55	3.07	41.3	61	40.2	2.4		33.7	16.7	
Gehman et al	22	2008	Cont	49.2	20.2	37.8	3.63	3.02	40.7	49.1	42.3	3.5		33.4	16.3	
Castro et al	23	2010	Cont	40	24.7	40.6	3.12	2.94	.	.	43.8	1.6		36.1	18.8	
Castro et al	23	2010	bm3	40	26.4	41	3.31	2.86	.	.	42.3	0.9		35.2	18.7	
Weller and Phipps	24	1986	Cont	70	11.03	13.4	4.96	3.28	39.1	.	44.3	3.9	.		.	
Weller and Phipps	24	1986	bm3	70	11.74	15.9	5.15	3.22	51.9	.	49.1	3.4	.		.	

Effect of Brown Midrib Corn Silage and Dried Distillers Grains Plus Solubles on Lactational Performance and Nitrogen Utilization by Dairy Cows

INTRODUCTION

The U.S. ethanol industry provides many co-products that may be utilized as animal feedstuffs. One of the most widely used co-products is dried distillers grains with solubles (DDGS). This feed ingredient is characterized by having a high content of crude protein (32% CP, DM basis), fat (11.2%, DM basis) and fiber (59% NDF, 23.7 ADF, DM basis) (Belyea et al., 2010). For these reasons, it is commonly fed to lactating dairy cows. When DDGS is included in dairy rations it typically replaces soybean meal as a source of protein; moreover, DDGS can serve as a source of energy as it replaces corn (Ranathunga et al., 2010) and also a portion of forages (Kelzer et al., 2009). Janicek et al. (2008) mention that dairy nutritionists tend to limit the inclusion of DDGS to 10% of the diet (DM basis) and this is due to the high content of fat which is thought to be detrimental for ruminal fiber digestion (Van Soest, 1994) and may also trigger factors affecting milk fat. Nonetheless several studies have demonstrated that DDGS can be effectively included in dairy rations maintaining high milk yield (Kleinschmit et al., 2006, Anderson et al., 2006); furthermore, Janicek et al. (2008) were able to feed up to 30% of the ration (DM) as DDGS resulting in a linear increase in milk yield without negatively affecting milk fat. In addition, Gehman and Kononoff (2010b) reported lower N excretion when cows were fed distillers grains in corn silage based diets; this finding indicates the potential to mitigate environmental pollution through nutritional strategies.

Another industry that is rapidly evolving and adapting to the new challenges of the animal and fuel industries is the seed manufacturing industry. Many corn hybrids have dual purpose, that is, they are grown for grain or forage; however, forage-specific hybrids also exist. Among the forage-specific hybrids the *bm3* mutation it produces plants with a low lignin content. This is beneficial to ruminant animals as lignin is the most significant factor limiting the availability of plant cell walls materials (Van Soest, 1994) thus, fiber in *bm3* corn hybrids has the potential to be more digestible (Oba and Allen, 2000, Ebling and Kung, 2004). The hybrids that carry the *bm3* mutation are also known as brown midrib corn (BMR) because of the characteristic pigmentation of this part of the leaf. This type of hybrid as forage for dairy cows has been investigated since 1976 (Frenchik et al., 1976) and since then many other experiments have been conducted Typically, when ensiled and fed to dairy cows, it has been proven that *bm3* corn hybrids increase DMI (Oba and Allen, 1999; Qiu et al., 2003; Castro et al. 2010) as wells as digestibility of NDF (Oba and Allen, 1999; Gehman et al., 2008), which represents a potential to increase milk yield. Furthermore, when cows are fed *bm3* corn silage nitrogen digestibility has been observed to be greater (Gehman et al., 2008) and nitrogen excretion has been reported to be lower (Weiss and Wyatt, 2006).

Because the ethanol industry continues to grow, the supply of DDGS will also grow and this may represent an advantageous opportunity to the dairy industry as it offers nutritious feedstuff at lower costs than conventional commodities. Also, the development of improved corn hybrids specific for silage offers an alternative to improve the nutrition of the high-producing dairy cow. It is important to understand how dairy cows respond to

high inclusions of these feed products to develop strategies that facilitate utilization of both, DDGS and *bm3* corn silage, while maintaining adequate milk yield as well as minimizing environmental impacts, namely N excretion by dairy cows. We are not aware of any published experiment combining DDGS and *bm3* corn silage. Thus, the objectives of the present study were: 1) to evaluate the effect of DDGS and *bm3* on lactational performance of dairy cows, and 2) to measure utilization of N by lactating dairy cows fed the combination of DDGS+*bm3* corn silage. We hypothesized that feeding bm3 corn silage will result in improved fiber digestibility and that feeding DDGS and *bm3* corn silage will result in improved milk yield and N utilization.

MATERIALS AND METHODS

Silage, Experimental Treatments, and Design

Two corn hybrids, a dual-purpose (**DP**; Mycogen 7511 FQ, Dow AgroSciences LLC, Indianapolis, IN) and a *bm3* (Mycogen F2F797, Dow AgroSciences LLC) were planted during spring 2008 at the University of Nebraska Agricultural Research and Development Center located near Mead, Nebraska. Corn silage was harvested using a self-propelled forage harvester (model 6750, John Deere, Moline, IL). Approximately 50 ton of each hybrid were placed in bag silos (Ag/Bag International Ltd., Warrenton, OR) and ensiled for 210 d. Table 1 outlines the nutrient composition and particle size measurements of the corn silage used in the experimental diets. Thirty-six lactating Holstein cows (24 multiparous and 12 primaparous) averaging 111 ± 35 d (mean ± SD) DIM and weighing 664 ± 76.5 kg were randomly assigned to one of nine 4 × 4 Latin squares (using a 2 × 2 factorial arrangement of treatments). Prior to initiation of the study

four cows were ruminally cannulated as described by Laflin and Gnad (2008). During the experiment these cows were assigned to 1 Latin square and randomly assigned to experimental treatments. Remaining cows were blocked by DIM and randomly assigned to treatment sequences in 9 replicated 4 × 4 Latin squares. The square with cannulated cows was used for all rumen measurements, whereas all squares were used for milk production and intake data. The first 21-d of each period were considered as adaptation period and the remaining days were used for data collection. During each period, cows were offered 1 of 4 TMR that differed by corn silage hybrid (DP or *bm3*) and DDGS inclusion rate (0 or 30% DM). Treatments were 1) 0% DDGS and DP corn silage, 2) 0% DDGS and *bm3* corn silage, 3) 30% DDGS and DP corn silage, and 4) 30% DDGS and *bm3* corn silage. Cows were housed in individual stalls and milked at 0730 and 1930 h. Cows were fed at 0900 h for ad libitum consumption to allow for approximately 5% refusals. The experimental cows were cared for according to the guidelines stipulated by the University of Nebraska Animal Care Committee. In formulating experimental diets, the strategy was to develop diets similar in concentrations of NDF and NFC with diets high in forage content (≈ 50% DM). Preliminary forage analysis of green chop material showed that *bm3* corn silage had a similar concentration of NDF (46.8 vs. 48.85% DM) as well as similar concentration of starch (23.8 vs. 22.9% DM) than the DP hybrid. Table 1 lists the chemical composition of forages from samples collected throughout the experiment (n = 4). When diets were formulated to contain either hybrid in combination with DDGS, soybean meal, ground corn, alfalfa haylage and rumen protected protein

were partially substituted and included at lower levels than diets not containing DDGS (Table 2).

Experimental Measures

Particle Size, BW, BCS, and Milk Composition

The Penn State Particle Separator (**PSPS**) was used to measure particle size for both forages and TMR as described by Heinrichs and Kononoff (2002). Body weight and BCS (1 to 5 scale) were measured on d 27 and 28 of each period. Body condition score was measured by a single trained individual, and the scoring method used was similar to that of Wildman et al. (1982), but reported to the quarter point. Milk production was measured daily and milk samples were collected during the a.m. and p.m. milkings of d 26, 27, and 28 and preserved using 2-bromo-2-nitropropane-1,3 diol. During the last week of each period, daily DMI and milk yield were averaged. Milk samples were analyzed for fat and true protein (AOAC, 2000) using a B2000 Infrared Analyzer (Bentley Instruments, Chaska, MN) by Heart of America DHIA (Manhattan, KS).

Ruminal Sampling

Commencing at 0800 h on d 27 of each period ruminal contents were collected from the dorsal, ventral, and caudal area in the rumen at 0930, 1030, 1130, 1330, 1530, 1730, 2030, 2330, 0330, and 0830 h. Collected digesta were mixed and filtered through 4 layers of cheesecloth. Rumen liquid pH was immediately determined by using a hand-held pH electrode (model M90, Corning Inc., Corning, NY). The samples were prepared and analyzed as described by Lykos et al. (1997). Approximately 15 mL of filtered liquid

were placed into bottles containing 3 mL of 25% metaphosphoric acid and 3 mL of 0.6% 2-ethyl butyric acid (internal standard) and stored at −20°C. Samples were later centrifuged at 12,000 × *g* for 20 min at 4°C to obtain a clear supernatant that was analyzed for ammonia using a phenol-hypochlorite assay and VFA concentration using gas chromatography (Yang and Varga, 1989).

Apparent Digestibility and Nitrogen Excretion

The TMR and fecal samples were weighed (1.25 g) into 5 × 10 cm Dacron bags with 50 µm pores (No. R510; Ankom Technology, Fairport, NY) and incubated in the rumen of a lactating cannulated cow fed a diet containing 60% forage and 40% concentrate for 12 d for indigestible ADF determination. Apparent digestibility of nutrients was estimated based on the concentration of indigestible ADF in the TMR and feces, and N excretion in feces was calculated from the obtained N digestibility and N intake. Fecal and urine samples were collected on all cows at 0600 and 1800 h during the last 4 d of each period. Feces were sampled from the rectum and urine during urination with stimulation. Fecal samples were pooled to obtain a composite for individual cows in each period. Fecal samples were dried at 55°C in a forced air oven, ground (1-mm screen; Wiley mill, Arthur H. Thomas Co., Philadelphia, PA) and composited according to cow and period. Ground samples were analyzed for DM (100°C oven for 24 h).

Feed and fecal samples were analyzed for nitrogen (Leco FP-528, Leco Corp., St. Joseph, MI), ether extract (AOAC, 2000), and percentage ash (AOAC, 2000). Both NDF and ADF were analyzed using an Ankom Fiber Analyzer (Ankom Technology). Heat stable α-amylase (number A3306; Sigma Chemical Co., St. Louis, MO) was included in

the NDF procedure (100 mL per 0.50 g of sample). Whole-diet total digestible nutrient concentration was then determined (Weiss et al., 1992) and, based on these values, production levels of digestible energy, ME, and NEL were calculated as outlined by NRC (2001). Samples of urine were acidified to pH <4 using 4 *M* HCl and frozen (–20°C). Urine samples were later thawed and composited for each cow during each period. Urinary creatinine has been validated as a marker to estimate urine volume (Valadares et al., 1999; Leonardi et al., 2003). The ratio of urinary purine derivatives (**PD**) namely, allantoin, uric acid, xanthine, and hypoxanthine in urine are widely used to estimate the microbial protein flow (**MCP**) to the duodenum (Gonda, 1995; Shingfield and Offer, 1998). Purine derivatives and creatinine were analyzed by HPLC (Waters Corp., Milford, MA) according to the procedures of Shingfield and Offer (1999). Urine samples were analyzed for N. Urinary creatinine was used as a marker to estimate urine volume. In calculating urine volume we assumed that creatinine output averages 28 mg/kg of BW as estimated by Whittet (2004). Previous investigators have reported similar daily creatinine output ranging from 25 to 30 mg/kg of BW (McCarthy et al., 1983; Jones et al., 1990). The ratio of urinary PD allantoin and uric acid to creatinine (PD:C) was used to estimate relative differences in microbial protein production (Shingfield and Offer, 1998). Based on estimates of urinary excretion of PD, microbial protein supply was estimated by Chen and Gomes (1992).

Statistical Analyses.

Performance data were analyzed as a replicated 4 × 4 Latin square with model effects for square, period within square, and treatment as fixed effects, as well as cow

within square as a random effect. Sum of squares for all treatments were partitioned into single degree of freedom contrasts for corn silage hybrid and inclusion of DDGS and interaction as planned a priori. The first-order autoregressive covariance structure AR(1) and the MIXED procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC) were used to analyze all data. Repeated measurements of rumen ammonia, pH, and VFA concentration were analyzed by including a REPEATED model statement, as well as a term for time and interaction for treatment by time. Square by treatment interaction was tested but was not significant, and therefore it was removed from the model. Significance for all models was declared at $P \leq 0.05$, and trends are discussed at $P \leq 0.10$. All means presented are least-squares means.

RESULTS

Forage and ration chemical composition

Table 1 lists the composition of the forages fed during the trial. Dual purpose (DP) corn silage had slightly lower CP (6.8 ± 0.5), ADF (25.95 ± 2.14) and NDF (40.9 ± 1.28) compared to *bm3* (CP= 7.4 ± 0.39; ADF= 27.35 ± 1.09; NDF= 46.82 ± 3.13). The content of lignin, as expected, was lower for *bm3* (2.3 ± 0.63) compared to control (2.8 ± 0.53). When lignin was measured as proportion of NDF the results showed a difference of 2% less for *bm3* compared to DP (4.9 ± 1.33 *vs* 6.9 ± 1.11 % NDF).

The PSPS was used to determine estimates of particle size distribution of corn silages and the results are presented in Table 1. The 2 hybrids had similar particle size distribution and they are in accordance to the recommendations of Heinrichs and Kononoff (2002). In average, particles size distribution for both corn silages was 4.8% >

19 mm; 64.2% 19.0 – 8.0 mm; 28.5% 8.0 -1.18 mm and 2.25% < 1.18 mm. Ingredient and chemical composition of experimental diets are listed in Table 2. The CP content of the DP TMR was lower compared to *bm3* TMR (15.4 vs 16.1). Although when the diets contained DDGS the CP content increased, the difference between the 2 diets was consistent (17.5 vs 18.4 for DP and *bm3*). The starch content of DP and *bm3* TMR was similar (25.7 and 24.7 respectively). Similarly NDF, EE and NFC were similar between the 2 diets. When diets contained DDGS, NDF and EE were notably greater compared to diets with no DDGS; nevertheless the levels between the two diets with DDGS were similar (38.5 and 38.6% NDF; and 5.6 and 5.5% EE of DP+DDGS and *bm3*+DDGS, in that order).

Apparent nutrient digestibility

Estimates of total tract nutrient digestibility are presented in Table 3. DDGS had no effect on DM and OM digestibility. In contrast the type of hybrid had an effect on both of these parameters. The diets that included *bm3* were significantly more digestible, average DM and OM were 62.1 ± 1.25% and 59.5 ± 1.3% for *bm3* and 59.3 ± 1.25% and 56.5 ± 1.3% for DP. Similarly, N digestibility was greater for diets with *bm3*. The most pronounced difference was observed when comparing DP to *bm3* diets; N digestibility of *bm3* was 61.3 ±1.14% and 57.7 ± 1.14%. Neutral detergent fiber (NDF) digestibility was greater for diets containing *bm3* alone or in combination with DDGS.

Rumen volatile fatty acids, rumen pH and ammonia

Effects of corn silage hybrid and DDGS on total and molar proportions of VFA, ruminal pH and ammonia concentrations are presented in Table 4. Ruminal pH was the

lowest when cows consumed *bm3*+DDGS (5.84 ± 0.07) and the highest when the diet only contained DP corn silage (6.26 ± 0.07). Rumen pH of cows on *bm3* and DP+DDGS was intermediate, 6.18 and 6.10 ± 0.07, respectively. The highest concentration of VFA (130 ± 4.99 mmol/dL) coincides with the lowest pH (5.84 ± 0.07) for *bm3*+DDGS; however this relationship is not held for DP+DDGS which showed an intermediate pH (6.1 ±0.07) and had the lowest concentration of total VFA (94.8 ± 4.99 mmol/dL).

Molar concentration of acetate was affected by treatment and the diets containing *bm3* had lower concentration of this fatty acid (61.8 and 55.0 mol/100 mol for *bm3* and *bm3*+DDGS) compared to the diets that contained the isogenetic hybrid (63.1 and 56.9 ± 0.77 mol/100 mol). In contrast, the molar concentration of propionate tended to be greater for *bm3*. The ratio of acetate to propionate was not affected by treatment and averaged 3.17 among treatments, likewise concentration of isovalerate was not affected by treatment averaging 1.75 mol/100 mol.

Purine derivatives, urinary creatinine and microbial crude protein

Table 5 shows the concentration and production of purine derivatives, namely allantoin and uric acid, and also concentration and production of creatinine. Diets containing *bm3* corn silage resulted in significantly higher ($P < 0.01$) urinary concentration of PD (9.61 vs 10.61 ± 0.302 mmol) and production (246.7 vs 275.7 ± 7.39 mmol d^{-1} for DP and *bm3*, respectively). When diets contained DDGS there also was a significant increase in PD concentration; however PD production was reduced when DDGS was included in the rations. When MCP was calculated, cows that consumed diets with *bm3* corn silage produced more MCP compared to cows consuming DP corn silage.

Even though no statistical test was performed on MCP due to statistical constraints, the data show that *bm3* corn silage increased production of PD and suggest that this increment results in greater production of MCP in dairy cows.

Nitrogen metabolism

Nitrogen metabolism and mass balance in presented in Table 6. The results show that N intake was greater when cows consumed diets containing *bm3* compared to DP; likewise, for diets containing DDGS. This is a direct result of greater DMI and increased concentration of CP in those rations.

Fecal N was greater when cows consumed *bm3* and accounted for 38.5 ± 1.15% of total N intake; whereas, cows consuming diets with DP corn silage excreted 36.9% of the N intake as feces. Urinary N was not affected by treatment and averaged 20.5 ± 1.4 among dietary treatments. There was a trend for a Hybrid × DDGS interaction for urinary N to be lower when diets included *bm3* and DDGS. Manure N was lower for diets with *bm3* corn silage (54.1 *VS* 58 ±1.75%); DDGS also accounted for a significant reduction (9 percentage units) compared to rations with no DDGS. Productive N (milk N + retained N) was significantly increased by approximately 4 per cent units when diets contained *bm3* corn silage compared with DP (45.8 vs 41.9 ± 1.75%). Productive N was also greater when diets included DDGS and averaged 48.4 ± 1.75%. There was a trend (P = 0.06) to increase productive N when diets included *bm3* and DDGS.

Dry matter intake

Dry matter intake of cows consuming *bm3* corn silage was greater ($P < 0.01$) than that of cows on the control diet (25.0 *vs* 23.6 ± 0.474 kg). When diets contained DDGS

(30% of DM) there was a significant increment in DMI of cows consuming *bm3* ($P < 0.01$) when compared to DP corn silage (26.6 *vs* 25.2 ± 0.474 for *bm3*+DDGS and DP+DDGS, respectively).

Milk production and composition

Milk yield, lactose percentage and lactose yield were not affected by either corn hybrid or by DDGS and averaged 30.6 ± 1.903 kg/d, 4.61 ± 0.056% and 1.40 ± .053 kg/d across treatments. However, when comparing milk yield on an iso-energetic basis (3.5 % FCM) there was a reduction ($P < 0.01$) due to DDGS. On average cows consuming diets with no DDGS produced 3.6 more kg of FCM than cows consuming diets containing DDGS. This response is due to a hybrid × DDGS interaction ($P = 0.02$) and its effect on milk fat percentage. The lowest milk fat percentage was observed when cows were fed *bm3*+DDGS (2.51 ± 0.1) followed by DP+DDGS (2.84 ± 0.1). When the diets contained no DDGS, milk fat percentages were considerably higher (3.46 and 3.59 for DP and *bm3*, respectively). As a result of this milk fat depression, fat yield (kg/d) was also affected by the same interaction and followed a similar pattern across treatments (0.78, 0.84, 1.03 and 1.08 ± 0.045 kg/d for *bm3*+DDGS, DP+DDGS, DP and *bm3* in that respectively).

Milk protein percentage was not affected by hybrid ($P = 0.47$); however, DDGS had a significant effect on this parameter ($P < 0.01$). The treatments with no DDGS averaged 3.12 ± 0.048% while the diets containing the co-product averaged 3.24 ± 0.048%. Both hybrid and DDGS had a significant effect on protein yield ($P < 0.01$). When the diets contained *bm3* alone or in combination with DDGS, protein yield was higher compared to DP corn silage alone or combined with DDGS (0.93 and 1.01 for

bm3 and *bm3*+DDGS *vs* 0.92 and 0.95 ± kg/d for DP and DP+DDGS). Milk urea nitrogen (MUN) was affected by corn hybrid and DDGS ($P < 0.01$). Milk from cows fed *bm3* and *bm3*+DDGS had lower MUN compared to that of cows fed DP and DP+DDGS (12.39 and 14.29 *vs* 12.95 and 14.72 ± 0.274 mg/dL).

Body weight and body condition score

Cows that consumed *bm3* corn silage were heavier ($P = 0.02$) than cows that consumed DP corn silage. On average, cows on diets with *bm3* weighed 7 kg more than cows on diets with DP. There were no differences in BCS and averaged 3.4 ± .041 on a scale from 1 to 5 across treatments.

DISCUSSION

Feeding *bm3* corn silage to lactating dairy cows improves total nutrient digestibility, specifically, fiber digestibility has been reported to increase with *bm3* corn silage (Oba and Allen, 2000; Ebling and Kung, 2004), interestingly digestibility of N may also be enhanced by *bm3* corn silage (Gehman et al., 2008) and reduced N excretion has also been reported when dairy cows consume this type of corn silage (Weiss and Wyatt, 2006). These effects are believed to be a result of the lower content of lignin which allows for greater fiber digestibility, thus greater energy supply which results in improved milk yield. Similarly, when cows are fed corn milling co-products Gehman and Kononoff (2010a; 2010b) reported lower N excretion, hence we hypothesized that feeding *bm3* corn silage would result in improved fiber digestibility and that feeding a combination of DDGS and *bm3* corn silage would result in greater milk yield and improved N utilization.

In the present study cows consuming diets with *bm3* corn silage had greater DMI than cows consuming DP corn silage; this response was expected and is in accordance to early and current experiments with *bm3* corn silage (Rook et al., 1977; Castro et al., 2010). The effect of *bm3* corn silage on DMI has not always been observed to be greater (Frenchik et al., 1976, Keith et al., 1979), in these studies, cows were described as being in late lactation and as a result energy requirements were lower and it is likely that DMI was not limited as much as it would be in high producing cows in early-mid lactation (Dado and Allen, 1995; Allen, 1996). The effects of *bm3* corn silage on increased DMI in this experiment are associated with lower content of lignin and higher digestibility of NDF. Chemical composition analyses confirmed that *bm3* corn silage utilized in this experiment is lower in lignin compared to DP corn hybrid. In addition, the proportion of lignin in NDF is greater in DP corn silage (6.9 ± 1.11) compared to *bm3* (4.9 ± 1.33 lignin as %NDF), suggesting that the latter provides more cellulose and hemicellulose and less lignin, thus making the cell wall more fragile resulting in enhanced particle size reduction, faster passage rate and ultimately allowing more fill capacity in the rumen (Oba and Allen, 1999; Oba and Allen, 2000; Tine et al., 2001). Cows consuming diets with DDGS also had greater DMI; this observation may be due to the fine particle size (Kononoff and Heinrichs, 2003) and is in agreement to the observations previously reported (Janicek et al., 2008; Zhang et al., 2010a; Zhang et al., 2010b). We measured the particle size distribution using the Penn State Particle Size Separator (Heinrichs and Kononoff, 2002). Diets containing 30% DDGS had a higher proportion of feed particles

<1.18 mm (33 *vs* 18%) compared to diets with no DDGS. This distribution of particle size may allow for greater rumen capacity and rate of passage (Janicek et al., 2008).

In the current experiment we did not observe any significant interactions between DDGS and corn hybrid on any measure of nutrient digestibility. Total tract digestibility of NDF was positively affected by *bm3* corn silage and it was 5% units greater that DP corn silage likely due to the lower lignin content of the *bm3* hybrid. Previous work done with *bm3* corn silage by Oba and Allen (2000) reported no difference in total tract digestibly of NDF, the same type of observation was reported Gehman et al. (2008).The proposed mechanism to explain no improvement in NDFD is that forages that result in higher DMI accelerate rate of passage which results in lower digestibility (Allen and Mertens, 1988). Contrary to this, Castro et al. (2010) reported higher DMI with cows being fed *bm3* corn silage with no effect on DM degradation rate, solid and liquid passage rate and rumen retention time. Although we did not measure rumen kinetics, based on the findings of Castro et al. (2010) it is possible that in our study passage rate was not affected and NDF digestibility was enhanced because of the lower lignin content of the *bm3* hybrid. In addition, we measured production of purine derivatives (PD) to indirectly estimate the production of microbial crude protein (Chen and Gomes, 1992) and our data indicate that rumen microbial population was stimulated by feeding *bm3* corn silage. Total production of PD was 246 mmol/d in cows fed DP corn silage whereas, cows that consumed *bm3* corn silage produced 275 mmol/d PD; this difference translated into approximately 130 g MCP greater supply of MCP. These observations suggest that greater supply of cellulose and hemicellulose by *bm3* corn silage provided more energy

for fiber digesting microbes which provided extra MCP to the cows (Oba and Allen, 2000).

Our observations on fiber digestibility and production of MCP seem to support the idea that enhanced fiber digestibility of *bm3* corn silage stimulates conversion of RDP into MCP thus reducing absorption of ammonia through the rumen and ultimately reducing N excretion. Reduction in manure N can be explained by reduced urinary N, reduced fecal N or both. In the present study the data show that N was more digestible in diets containing DDGS possibly due to the high content of RUP, which typically ranges between 50 and 70 RUP as % of CP (Kleinschmit et al., 2007; Janicek et al., 2008), that was metabolized in the small intestine thus yielding less N available for microbial synthesis in the hindgut which resulted in the lower fecal N. Some authors have reported a reduction in urinary N (Tine et al., 2001; Greenfield et al., 2001), opposite of this, in the present study the reduction was in fecal N. The proportion of N intake that was present in fecal matter was significantly reduced by *bm3*corn silage (36.94 vs 34.63% ± 0.86%, DP and *bm3* corn silage) and DDGS (40.44 vs 31.14 for 0 and 30% DDGS). Our observations are in agreement with Gehman and Kononoff (2010a) who reported the lowest fecal N when cows were fed 30% corn milling co-products. In our study urinary N remained unaltered by *bm3* and DDGS and averaged 20.5% across treatments. Consequently manure nitrogen (fecal + urinary) was reduced by *bm3* and DDGS by approximately 4 per cent units because of reduced fecal N excretion. Similarly Weiss and Wyatt (2006) reported a 4% reduction in N excretion at an equal N intake when dairy cows were fed *bm3*. We observed that productive N was in average 6% units greater

when DDGS and *bm3* were included in the rations. It is not very clear why productive N was higher; it may be driven by higher N retention which could be associated with tissue deposition as reflected in changes in body weight, although detecting changes in BW or BCS is limited in our experiment as animals were fed different diets for short periods of time and it was not part of our main objective, thus we can only make limited inferences about this response. As mentioned before, digestibility of N was observed to be significantly greater in diets with DDGS possibly because of the higher content of RUP provided by DDGS. It is also possibly that N was utilized for more active synthesis of microbial crude protein, stimulated by increased energy supply derived from more digestible NDF when the diets contained *bm3* corn silage (Oba and Allen, 2000; Weiss and Wyatt, 2006). Kleinschmit et al. (2007) report that the RUP content of DDGS is greater than that of soybean meal, in our experiment soybean meal was partly replaced by 30% DDG, thus the diets with DDGS provided more RUP than the other two diets. Oba and Allen (2000) reported increased microbial N flow to the duodenum possibly due to a faster passage rate, thus reducing microbial turnover in the rumen when cows were fed *bm3* corn silage. Our data show that *bm3* corn silage increases production of MCP while feeding DDGS resulted in lower amounts of MCP (1055 *vs* 948 20 ±34.3 g/d MCP for bm3 corn silage and 30% DDGS respectively.). This is likely the result of a shift in the energy available for microbial growth as the diets with DDGS were high in fat and low in carbohydrates, particularly starch, thus limiting bacterial growth in the rumen. In comparison, when DDGS were combined with *bm3* MCP was higher. It is important to point out that the current results demonstrate that *bm3* can support ruminal microbial

growth even if the diets are low in starch (14% of the dietary DM) compared to industry standards 23 -30% of the dietary DM (Grant, 2005) in part because of greater DMI and higher digestibility. One implication of this is that low starch in diets is mainly associated with lower inclusion corn which can have a positive economic impact in lowering the cost of the TMR. Altogether our results support the idea that *bm3* has the potential to reduce N excretion and increase productive N.

Opposite to our hypothesis, the effects of *bm3* and DDGS on greater DMI and NDFD did not translate into higher milk yield which averaged 30.5 kg/d across treatments. This response suggests that the cows in our study were not deficient in energy and did not respond to increased DMI. It is possible that a portion of the energy consumed was partitioned towards milk protein synthesis and unexpected increased body weight as we observed that cows with MFD produced 6% more milk protein and were 7 kg heavier than cows with no MFD. Schingoethe et al. (2009) suggest that feeding DDGS to lactating dairy cows frequently results in similar or higher milk yield than cows fed control diets whereas the effect of *bm3* on milk production has been inconsistent. Some authors have reported increased milk yield (Frenchick et al., 1976; Keith et al., 1979; Oba and Allen, 1999, Weiss and Wyatt, 2006) while others have not observed effect of *bm3* on milk production (Rook et al., 1977; Bal et al., 2000; Gehman et al. 2008; Castro et al., 2010). Similar to our results Kelzer et al. (2009) reported no difference in milk yield when they fed three types of corn milling co-products. It is important to note that cows in the present study were in early to mid lactation as well as those in the experiments conducted by Kelzer et al. (2009), whereas in other studies where feeding corn milling

co-products resulted in greater milk yield (Janicek et al., 2008; Sasikala-Appukuttan et al., 2008; Gehman and Kononoff, 2010a; Gehman and Kononoff, 2010b) cows were in early lactation. Furthermore, Janicek at al. (2008) in a second experiment feeding 30% DDGS (dietary DM), utilized cows in mid lactation and there was no effect on milk production. These observations seem to indicate that cows that consume DDGS in early lactation are more responsive to increased energy intake that translates into higher milk yield compared to cows in mid to late lactation.

Milk protein synthesis is enhanced when energy and peptide substrates are available to the mammary gland (Rius et al., 2010). We observed increased milk protein synthesis which can be associated with increased protein level of the DDGS rations which increased the supply of amino acids (Kleinschmit et al., 2006; Mjoun et al., 2010) as well as increased energy supply by DDGS (Kelzer et al., 2009) and *bm3* corn silage which elicited higher milk protein synthesis. Hybrid did not have a significant effect on milk fat percentage, fat yield or 3.5% FCM. However, inclusion of DDGS resulted in significantly lower concentration of milk fat compared to diets with no DDGS (3.5 *vs* 2.7 ± 0.09% milk fat). Consequently milk fat yield was lower (1.05 *vs* 0.81 ± 0.04 kg/d) and 3.5% FCM was lower by 3.6 kg/d. Depressed synthesis of milk fat in our study may be related to an elevated intake of unsaturated fatty acids derived from the corn oil in DDGS and shifts in VFA concentration in the rumen as well as changes in ruminal pH as milk fat depression (MFD) is triggered when cows are fed highly fermentable diets or supplements of plant oils (Shingfield and Griinari, 2007; Bauman et al., 2008). We did not expect this type of response since previous research showed that inclusion of DDGS

up to 30% of the ration (DM) did not affect milk fat content (Kleinschmit et al., 2006, Anderson et al., 2006; Janicek et al. 2008; Schingoethe et al., 2009). Nonetheless, there have been other studies where DDGS are linked to MFD (Leonardi et al., 2005; Abdelqader et al., 2009; Hippen et al., 2010). Similar to our experiment, Abdelqader et al. (2009) fed a diet containing 30% DDGS which supplied 960 g/d unsaturated fatty acids whereas the control diet supplied 645 g/d. In such experiment they reported a trend towards decreasing milk fat concentration with DDGS and a significant reduction when corn oil was used. Ruminal fluid from cows consuming DDGS resulted in increased concentration of propionate and lower concentration of acetate which is the main source of carbon for fatty acid synthesis in ruminants (Van Soest, 1994); this changed in ruminal VFA may account for some of the observed reduction in milk fat, in addition, the treatment that resulted in the greatest total concentration of ruminal VFA was accompanied by lowest rumen pH (Figure 1)and interestingly this treatment also resulted in the lowest milk fat production. The EE in DDGS is mainly corn oil, which typically ranges between 54 and 60% linoleic acid (18:2) (CRA, 2006) It is possible that the MFD inducing diets overwhelmed the biohydrogenation capacity of the rumen microbes and there was a shift in the saturation pathway that led to increased synthesis of antilipogenic CLA isomers such as *cis*-10, *trans*-12 which has been identified as an inhibitor of milk fat synthesis and fatty acid uptake in the mammary gland (Baumgard et al., 2000; Peterson et al., 2003; Shingfield and Griinari, 2007, Hippen et al., 2010).

CONCLUSIONS

Feeding *bm3* corn silage and DDGS resulted in greater DMI and greater NDF digestibility but this did not translate into changes in milk yield but increased milk protein. When cows consumed *bm3* corn silage manure N excretion was lower and synthesis of microbial CP was grater. In the present study there was no improvement in milk production even though cows that consumed *bm3* and DDGS had greater energy intake; it is possible that cows in early lactation may benefit more of the greater DMI and NDF digestibility as energy is limiting in that phase of lactation. Our data indicates that *bm3* corn silage has the potential to increase microbial protein synthesis in the rumen and also improve N utilization when included in diets low in starch. Lastly, *bm3* with DDGS resulted in lower milk fat production presumably due to high ruminal fermentability of the diet and higher supply of unsaturated fatty acids.

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Table 1. Chemical composition of the control dual purpose (DP), *bm3* brown midrib corn silages, grass hay and alfalfa haylage (DM basis)[1] n=4

	DP[2]		*bm3*[2]		Grass Hay		Alfalfa Haylage	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chemical, % DM								
DM	34.3	1.50	30.7	1.58	92.1	0.28	35.9	0.70
CP	6.8	0.50	7.4	0.39	9.0	0.28	22.7	1.07
ADICP	--	--	--	--	--	--	0.95	0.44
Soluble Protein	53.5	4.20	51.5	4.51	34.5	2.65	70.3	1.26
ADF	25.95	2.14	27.35	1.09	44.08	2.10	30.6	1.76
NDF	40.9	1.28	46.82	3.13	68	1.64	38.9	0.88
Lignin	2.8	0.53	2.3	0.63	7.5	0.65	6.9	1.28
Lignin, % NDF	6.9	1.11	4.9	1.33	--	--	--	--
NFC	42.7	1.20	35.0	2.29	16.1	1.45	28.1	0.46
Starch	35.0	3.60	24.4	4.36	--	--	--	--
Ether extract	3.4	0.13	3.0	0.14	1.8	0.46	3.4	0.37
Ash	6.28	0.33	8.26	2.07	9.09	0.43	11.28	0.47
Ca, %	0.28	0.05	0.28	0.04	0.27	0.03	1.46	0.10
P, %	0.26	0.03	0.23	0.03	0.26	0.01	0.33	0.02
Mg, %	0.14	0.01	0.16	0.01	0.11	0.02	0.26	0.02
K, %	1.03	0.05	1.06	0.05	2.16	0.17	2.85	0.24
Na, %	0.01	0.00	0.01	0.01	0.02	0.01	0.03	0.01
Fe, ppm	279.5	101.1	408.9	495.83	133.75	32.17	435.75	36.38
Zn, ppm	30.25	2.63	30.25	3.30	21.25	2.99	35.50	5.20
Cu, ppm	5.50	0.58	6.75	0.50	6.50	1.00	10.00	1.15
Mn, ppm	62.25	2.22	72.50	13.40	33.25	3.86	52.25	3.10
Mo, ppm	1.10	0.14	0.88	0.22	0.65	0.06	1.15	0.19
S	0.09	0.02	0.11	0.01	0.13	0.03	0.29	0.01
30 h *In vitro* NDFD	52.7	1.4	60.3	0.73	.	.	.	.
Particle Size[3]								
> 19.0 mm, %	5.40	2.1	3.80	1.5	.	.	.	.
19.0 – 8.0 mm, %	65.60	1.8	62.80	1.7	.	.	.	.
8.0 – 1.18 mm, %	25.60	0.5	30.50	1.9	.	.	.	.
< 1.18 mm, %	3.20	0.4	1.30	0.5	.	.	.	.

[1] Values determined by Dairy One Forage Testing Laboratory, Ithaca, NY
[2] DP = dual purpose control hybrid; *bm3* = brown midrid hybrid
[3] Determined using the Penn State Particle Separator (Heinrichs and Kononoff, 2002)

Table 2. Ingredient and chemical composition of total mixed diets fed during lactation

	Diets[1]			
	0% DDGS		30% DDGS	
	DP	*bm3*	*DP*	*bm3*
Diet Ingredients				
DP Corn Silage	40.2	--	40.7	--
bm3 Corn Silage	--	40.2	--	40.7
DDGS	--	--	29.2	29.2
Ground Corn	20.1	20.1	6.3	6.3
Soybean Meal, 48%	12.5	12.5	4.4	4.4
Alfalfa haylage	10.4	10.4	3.6	3.6
Grass Hay	4.4	4.4	4.4	4.4
Soybean Hulls	4.2	4.2	4.2	4.2
Soypass[2]	4.2	4.2	3.5	3.5
Limestone	1.25	1.25	2.09	2.09
Sodium bicarbonate	0.97	0.97	0.94	0.90
CalciumPhosDi	0.84	0.84	--	--
Salt	0.42	0.42	0.42	0.42
Magnesium Oxide	0.33	0.33	0.15	0.15
Vitamin Premix[3]	0.13	0.13	0.13	0.13
Mineral Premix[4]	0.10	0.10	0.10	0.10
Chemical[5],				
CP,	15.4 (0.74)	16.1 (1.53)	17.5 (0.32)	18.4 (0.84)
Starch	25.7 (1.7)	24.7 (1.7)	16.4 (1.6)	14.6 (0.8)
NDF	33.0 (2.7)	33.2 (2.77)	38.5 (0.77)	38.6 (0.94)
Etherextract	2.2 (0.18)	2.1 (0.09)	5.6 (0.19)	5.5 (0.29)
NFC[6]	41.4 (3.37)	40.4 (3.16)	30.2 (1.56)	31.7 (0.39)
Ash	7.98 (0.61)	8.22 (0.82)	8.25 (0.66)	8.12 (0.31)

[1] Dual purpose corn silage (DP), brown midrib hybrid (*bm3*), dried distillers grains with solubles

[2] LignoTech, Overland Park, KS

[3] Formulated to supply approximately 120, 000 IU/d vitamin A, 24, 000 IU/d of vitamin D, and 800 IU/d Vitamin E in total ration.

[4] Formulated to contained 1.0% Ca, 0.50 % P, 0.36% Mg, 1.3% K

[5] Determined from composite samples collected through out the experiment, mean (SD)

[6] NFC = Nonfiber carbohydrate calculated by difference 100 – (%NDF + % CP + % Fat + % Ash)

Table 3. Effects of feeding dual purpose corn silage, *bm3* corn silage and DDGS on nutrient digestibility

	Treatment[1]				SEM[2]	Contrast[3]		
	DP	*bm3*	DP+DDGS	*bm3*+DDGS		Hybrid	DDGS	I
Digestibility								
DM, %	59.0	62.0	59.6	62.3	1.25	0.01	0.68	0.90
N, %	57.7	61.3	68.1	68.7	1.14	0.04	<0.01	0.14
NDF, %	32.5	38.1	37.8	42.2	1.79	<0.01	<0.01	0.67
EE, %	73.4	73.5	88.6	89.1	0.83	0.72	<0.01	0.81
OM, %	56.2	59.3	56.8	59.8	1.32	0.01	0.65	0.96
NFC, %[3]	88.3	89.6	85.4	87.4	0.87	0.03	<0.01	0.67
TDN, %[4]	52.7	54.9	56.3	58.7	1.1	0.02	<0.01	0.94
NE_L, Mcal/kg[5]	1.15	1.23	1.29	1.36	0.03	0.01	<0.01	0.96

[1]DP = dual purspose hybrid, *bm3* = brown midrib hybrid; DP+DDGS = dual purpose hybrid and DDGS 30% (DM); *bm3+DDGS*= brown midrib hybrid and DDGS 30% (DM).

[2] Highest standard error of treatment means is shown.

[3]Contrast *P*-values for effects of feeding corn silage hybrid (hybrid), DDGS and Hybrid by DDGS interaction (I).

[3] NFC = Nonfiber carbohydrate calculated by difference

[4] TDN (%) = tdNFC + tdCP + (tdEE X 2.25) + tdNDF

[5] NE_{Lp} (Mcal/kg) = Net energy of lactation at production levels, as described by NRC, (2001)

Table 4.Effects of feeding DP and *bm3* corn silages in combination with DDGS on ruminal concentration of volatile fatty acids and ammonia (NH_3N)

	0% DDGS		30% DDGS		SEM[1]	*P-Value*		
	DP	*bm3*	DP	*bm3*		Hybrid	DDGS	I
Rumen pH	6.26	6.18	6.10	5.84	0.07	< 0.01	< 0.01	0.03
Total VFA, (mM)	117.4	122.8	94.8	130.0	4.99	< 0.01	0.05	< 0.01
VFA mol/100 mol								
Acetate	63.1	61.8	56.9	55.0	0.77	< 0.01	< 0.01	0.29
Propionate	20.0	20.7	24.3	25.3	0.71	0.06	< 0.01	0.74
Isobutyrate	1.14	1.33	1.37	0.93	0.26	0.64	0.75	0.23
Butyrate	12.28	12.56	13.74	15.49	0.43	< 0.01	< 0.01	0.01
Isovalerate	1.86	1.87	1.91	1.38	0.21	0.21	0.28	0.19
Valerate	1.62	1.68	1.78	1.93	0.06	< 0.01	< 0.01	0.09
A:P[2]	3.17	3.77	3.55	2.20	0.77	0.63	0.44	0.20
NH_3N (mg/dl)	16.17	14.37	16.81	16.52	0.78	0.05	< 0.01	0.16

[1] Highest standard error of treatment means is shown.

[2] Ratio of acetate to propionate

Table 5. Effects of feeding different corn hybrids and dried distillers grains plus solubles and interactions between these factors (I) on daily excretion of urinary creatinine, allantoin, uric acid, rumen microbial crude protein production.

Item	0% DDGS		30% DDGS		SEM[2]	Contrast[3]		
	DP[1]	*bm3*	DP	*bm3*		Hybrid	DDGS	I
Concentration, mmol								
Creatinine	6.15	6.09	7.91	7.92	0.344	0.92	<0.01	0.89
Allantoin	8.10	8.91	9.95	10.87	0.384	0.02	<0.01	0.87
Uric Acid	0.60	0.74	0.40	0.49	0.048	0.02	<0.01	0.63
Hypoxanthine	ND[4]	ND	ND	ND	--	--	--	--
Xanthine	0.06	0.09	0.12	0.14	0.336	0.15	<0.01	0.96
PD[5]	8.76	9.73	10.46	11.50	0.408	<0.01	<0.01	0.92
Production, mmol/d								
Purine Derivative: Creatinine	1.48	1.63	1.39	1.54	0.051	<0.01	0.02	0.72
Allantoin : Creatinine	1.37	1.52	1.32	1.46	0.048	<0.01	0.18	0.89
Creatinine Production [6]	170.07	171.74	171.52	173.33	2.56	0.02	0.05	0.92
Allantoin Production [7]	235.04	260.49	228.06	252.58	8.68	<0.01	0.30	0.95
Uric Acid [8]	17.02	21.78	9.43	11.76	1.372	0.01	<0.01	0.37
Hypoxanthine [9]	ND	ND	ND	ND	--	--	--	--
Xanthine [10]	1.40	2.26	2.46	2.69	0371	0.14	0.04	0.39
Purine Derivative Production [11]	253.42	284.45	239.95	267.02	9.18	<0.01	0.04	0.79
Microbial CP, g/d[12]	955.72	1098.26	885.48	1011.56	42.96	--	--	--

[1] DP = dual purpose corn hybrid; *bm3*=brown midrib corn hybrid; DDGS= dried distillers grains plus solubles.
[2] Highest standard error of treatment means is shown.
[3] Contrast *P*-values for effects of feeding different corn hybrids (DP and *bm3*) and dried distillers grains plus solubles (DDGS) the interaction (I) between these factors.
[4] ND= Not detected
[5] PD = total purine derivatives (allantoin + uric acid+xanthine)
[6] Creatinine Production = (28* BW)/113.1
[7] Allantoin Production = (Creatinine Production * (Allantoin : Creatinine))
[8] Uric Acid Production = (Creatinine Production * (Uric Acid : Creatinine))
[9] Hypoxanthine Production = (Creatinine Production * (Hypoxanthine : Creatinine))
[10] Xanthine Production = (Creatinine Production * (Xanthine : Creatinine))
[11] Purine Derivative Production = (Creatinine Production * (Purine Derivative: Creatinine))

[12] Microbial Crude Protein as estimated by Chen and Gomes (1992). Statistical test of effect of treatment diet on ruminal microbial CP synthesis was not conducted because microbial CP was calculated based on excretion of PD (Firkins et al., 2006)

Table 6. Effects of feeding *bm3* corn silage and dried distillers grains with solubles and interactions between these factors (I) on N metabolism.

	0% DDGS		30% DDGS		SEM[1]	Contrast[2]		
	DP	*bm3*	DP	*bm3*		Hybrid	DDGS	I
Mass, g/d								
N Intake	599.0	667.0	730.3	783.5	14.40	<0.01	<0.01	0.41
Fecal N	253.6	257.9	228.3	244.4	9.86	0.20	0.02	0.46
Digested N	345.8	409.0	501.3	538.1	11.90	<0.01	<0.01	0.17
Urinary N	131.0	127.0	150.1	159.1	10.00	0.80	0.01	0.57
Manure N	379.7	384.7	378.7	400.8	14.09	0.26	0.53	0.48
Milk N[3]	150.3	148.2	153.4	162.9	5.55	0.32	0.02	0.12
N retained[4]	69.1	134.0	197.4	219.1	12.44	<0.01	<0.01	0.06
Productive N[5]	219.6	282.1	350.7	381.5	13.60	<0.01	<0.01	0.18
% N intake								
Fecal N	42.5	38.4	31.4	38.9	1.15	0.03	<0.01	0.09
Urinary N	22.2	18.8	20.5	20.5	1.40	0.21	0.98	0.12
Manure N	64.1	57.1	52.0	51.2	1.75	0.02	<0.01	0.06
Milk N	25.1	22.2	20.9	20.7	0.75	<0.01	<0.01	0.01
N retained	10.8	20.7	27.2	28.2	1.77	<0.01	<0.01	0.01
Productive N	35.8	42.8	48.0	48.9	1.75	0.02	<0.01	0.06

[1] Highest standard error of treatment means is shown.
[2] Contrast *P*-values for effects of feeding two corn silage hybrids (hybrid) and dried distillers grains (DDGS) and hybrid by DDGS interaction (I).
[3] Milk N yield (kg) per kg N intake*100.
[4] Retained N= intake N – (manure N + milk N)
[5] Productive N = milk N + retained N

Table 7. Effects of feeding dual purpose corn silage, Brown midrib corn silage and dried distillers grains plus solubles on milk production and composition

	0% DDGS		30% DDGS		SEM[2]	*P-Value*		
	DP[1]	*bm3*[1]	DP	*bm3*		Hybrid	DDGS	I
DMI, kg/d	23.6	25.0	25.2	26.6	0.474	<0.01	<0.01	0.98
Milk Yield, kg/d	30.4	30.1	30.4	31.6	1.093	0.55	0.31	0.28
3.5% FCM[3]	29.4	30.5	26.6	26.1	1.082	0.49	<0.01	0.13
Fat, %	3.46	3.59	2.84	2.51	0.100	0.09	<0.01	<0.01
Fat Yield, kg/d	1.03	1.08	0.84	0.78	0.045	1.00	<0.01	0.02
Protein, %	3.12	3.13	3.23	3.26	0.048	0.47	<0.01	0.71
Protein Yield, kg/d	0.92	0.93	0.95	1.01	0.031	<0.01	<0.01	0.14
MUN, mg/dl	12.95	12.39	14.72	14.29	0.274	0.01	<0.01	0.74
Body Weight	687.0	693.7	692.8	700.1	10.3	0.02	0.05	0.92
BCS[4]	3.41	3.44	3.38	3.41	0.041	0.88	0.28	0.32

[1] DP= dual purpose hybrid, *bm3*= brown midrib hybrid

[2] Highest standard error of treatment means is shown.

[3] 3.5 % Fat Corrected Milk

[4] MUN = Milk Urea Nitrogen

[5] BCS = Body Condition Score 1-5 scale.

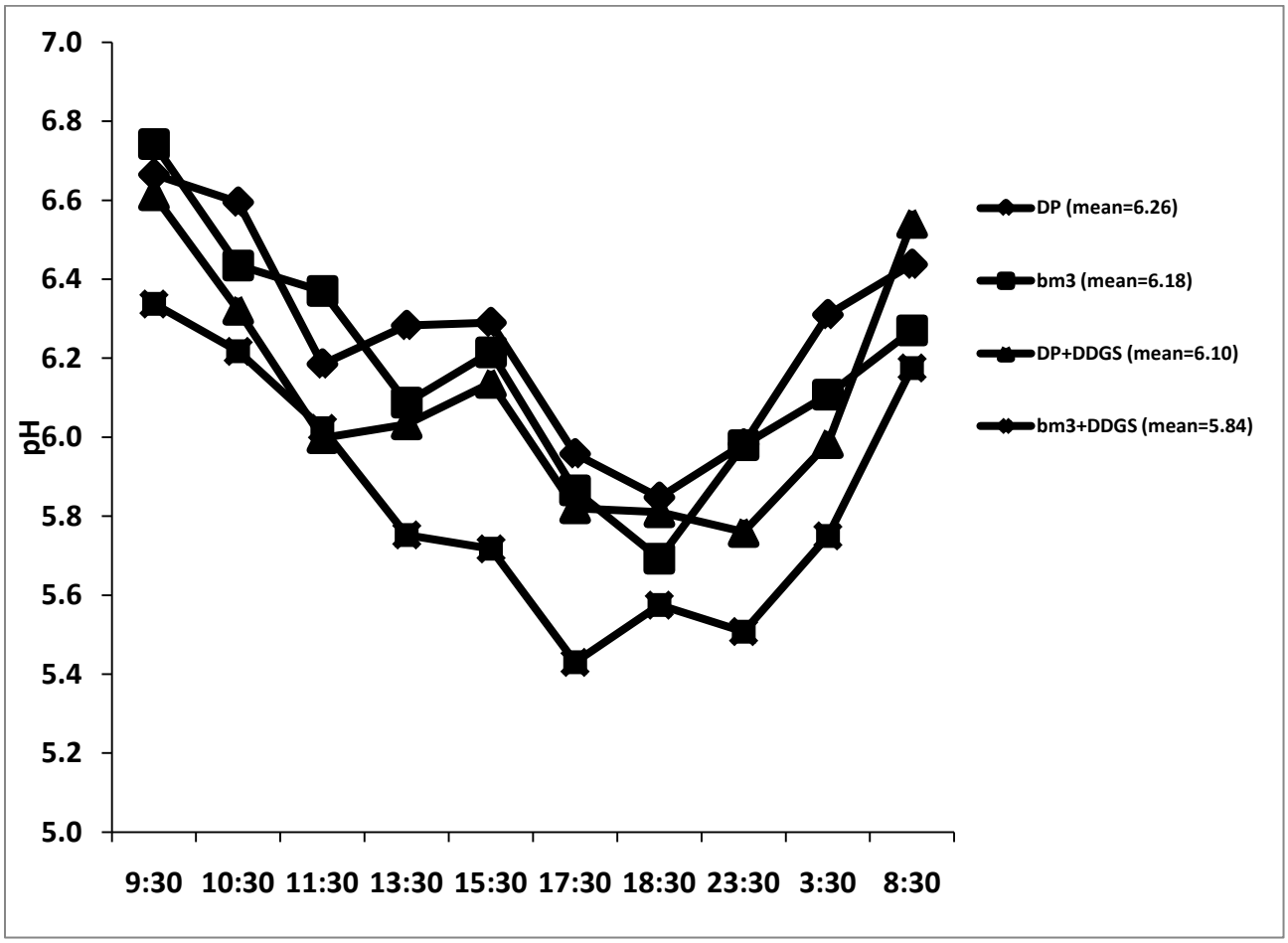

Figure 1. Ruminal pH measured in 10 time points over a 24-h period.