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## Reduced protein for late-lactation dairy cows

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# REDUCED PROTEIN FOR LATE-LACTATION DAIRY COWS

A thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

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by

Andre de Barros Duarte Pereira  
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## **ABSTRACT**

Excess protein in dairy cattle diets unnecessarily increases the cost of production and may contribute to environmental pollution. The objective of this research was to evaluate the effect of feeding dairy cows with two levels of dietary protein on animal performance and manure characteristics. Two experiments were carried out with 24 lactating dairy cows each. Experiment 1 was corn silage based and had a control TMR (HP1) estimated to contain 16.5% CP with SBM and treatment TMR (LP1; 13.5% CP) using DDGS and rumen protected Lys and Met. Experiment 2 was ryegrass haylage based and had a control TMR (HP2) with 15.5% CP with Met and a treatment TMR (LP2) with 13.5% CP with Lys and Met. Experiments were analyzed as a crossover design using the MIXED procedure of SAS with pen as the experimental unit. Experiment 1 had no significant difference between treatments in DMI (21.0 for HP1 and 20.4 kg/cow/d for LP1;  $P=0.46$ ) and milk yield (20.7 for HP1 and 20.5 kg/cow/d for LP1;  $P=0.91$ ). Percentage of milk components averaged 4.21, 3.72, 4.54, and 9.15, respectively for fat, protein, lactose, and solids non-fat ( $P>0.60$ ). Milk urea nitrogen (MUN) decreased ( $P<0.01$ ) from 17.2 with HP1 to 9.93 mg/dL with LP1. Manure pH was significantly higher for HP1 than LP1 (7.87 and 7.53 respectively,  $P<0.05$ ). Experiment 2 had no significant difference in cow performance (DMI: 21.4 for HP2 and 20.9 kg/cow/d for LP2;  $P=0.51$ ; milk yield: 26.4 for HP and 24.4 kg/cow/d for LP2;  $P=0.19$ ; percentage of milk components averaged 3.48, 3.29 and 4.71, respectively for fat, protein and lactose;  $P>0.30$ ; MUN decreased ( $P<0.01$ ) from 9.85 with HP2 to 6.40 mg/dL with LP2). Manure pH was



significantly higher for HP2 than LP2 (7.50 for HP and 7.13 for LP,  $P=0.05$ ). There was no difference in volatilized N between HP2 and LP2. This experiment suggests that performance of late-lactation dairy cows can be maintained with low-protein DDG based diets supplemented with Lys and Met.

## **CHAPTER 1: INTRODUCTION**

Intensification of animal production results in environmental damage. Greenhouse gas production by farmers and animals, nutrient excretion and lack of management practices are the key factors. The most important nutrients found in cattle manure that may cause potential harm to the environment are nitrogen (**N**), phosphorus (**P**) and potassium (**K**). To reduce the environmental impact caused by cattle production, it is necessary to understand what factors are involved in the amount of nutrients emitted to the environment. This will lead to the prediction and consequent control of these excreted nutrients.

Animal feeding operations have an important role in environmental impact. Environment is a growing topic of discussion and livestock's impact on it is of great concern (FAO, 2006). An adequate level of N in the diet of dairy cows is essential to maximize production and profitability of producers (Pfeffer and Hristov, 2005) and to avoid N pollutants (Cheng et al., 2011).

Commodities prices are increasing. The cost of producing one acre of soybean meal has increased from 178 dollars in 2001 to 450 dollars in 2010, an increase of 153% (USDA, 2011). This increase in prices of production has a direct impact in farmers, which are paying more for an important product. Soybean meal is the most used source of crude protein for livestock in United States. Corn dry distillers' grain is an alternative source of crude protein that can be used without decrease in cattle performance. This product from ethanol plants is broadly

available in the country and has a good proportion of crude protein, with a high content of rumen undegraded protein (**RUP**).

The world population is increasing and recently just passed the seven billion mark, and the number of undernourished is close to 13% of all the people in our planet. The available land for agriculture purposes has little or no space to grow anymore and a tendency to decrease as the soil quality and other climate conditions change it. Agriculture and livestock production must be focused now on efficiency rather than only thinking about increasing in production. This way people in the world can be fed and have a perspective of a sustainable future.

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## **CHAPTER 2: LITERATURE REVIEW**

### **Protein and Nitrogen**

Protein is constructed from the same set of 20 amino acids, from which some are essential (cannot be produced by mammals and need to be offered in diet) and others are not essential (can be synthesized by the body and does not need to be in diet) (Lehninger, 2004). The amino group consists of one atom of nitrogen and three atoms of hydrogen. The nitrogen atom is part of the peptic bond, done between the amino group ( $\text{NH}_3$ ) of one amino acid and the carboxyl group ( $\text{CH}_3$ ) of the other amino acid. Some polar uncharged amino acids (asparagine and glutamine) and some polar positively charged amino acids (Lysine, Arginine and Histidine) contain one atom of nitrogen in their R chain as well. Protein from diet that escapes ruminal degradation and that synthesized from rumen microbes is used for animal maintenance; to build tissues, produce milk and meat, enzymes and hormones. Nitrogen metabolism in ruminants is a more complex process than in non-ruminant animals because of extensive breakdown and modification of proteins in the reticulo-rumen compartment. Bacteria in this compartment can use all N not linked to protein and about 65 to 85% of nitrogen consumed by cattle to produce their protein (NRC, 2001). This process is dependable not only from the protein, but from energy as well. Energy comes from organic matter such as soluble carbohydrates, digested by the microbial flora, and from fermentation of celluloses, hemicelluloses and pectin. Carbohydrates are the most important energy sources used to increase protein utilization efficiency by ruminants (Wattiaux, 1998). Microbial protein will enter the abomasum and complete digestion in the intestine. Protein not utilized by

microbes in the rumen and a small quantity of endogenous protein (NRC, 2001) can be digested post ruminally. Another source of nitrogen is synthetic amino acids bound to specific chemical groups that reduce the ruminal degradation and allow most part of it to be available at the duodenum for enzymatic hydrolytic digestion. Proteins contain approximately 16% of nitrogen. The conversion factor commonly used is the amount of nitrogen times 6.25 equals the amount of crude protein in the sample. For milk, the conversion factor is 6.38, since it has a different amino acid profile and contain a different amount of nitrogen in the protein (Jones, 1931).

### **Environmental Concerns**

Biochemical reactions and mass transfer reactions release ammonia to atmosphere. Biochemical processes are done by a hydrolytic reaction catalyzed by urease, an enzyme found in feces and soil (Muck and Steenhuis, 1980). An enzyme substrate biochemical process is limited by the amount of enzyme or substrate available in the system. In this case, urea is the limiting factor for ammonia production (Monteny and Erisman, 1998). The reaction can only happen if the enzyme is present. Without it, the reaction would need a certain amount of energy not available in a common dairy barn (Kaminskaia and Kostic 1997). Feces have a low proportion of urea readily available for hydrolysis because bacteria in the large intestine use it as substrate to produce protein.

Mass transfer reaction is the release of N to ambient from manure surface. This reaction is a function of wind speed over the surface, manure and ambient temperature and the characteristic of the manure surface (Hristov et al., 2011). All

these factors may contribute to increase or decrease the amount of ammonia released to air. Wind over manure decreases the partial pressure of ammonia over it moving the equilibrium between manure and atmosphere to the release of ammonia to the air. Higher ambient temperature lowers the partial pressure over manure by greater movement of molecules. Larger contact surface between manure and air increase the area for ammonia release from manure.

Ammonia in atmosphere reacts with sulfur and nitrogen oxides and produces harmful particles to human health. It is pollutant for the environment because it can cause soil acidification and damage to plant populations. Ammonia may cause over-fertilization of water bodies resulting in biodiversity reduction (Wolfe and Patz, 2002; Kaiser, 2005, Carpenter et al., 1998; Tilman, 1999; Rabalais, 2002). Livestock operations account indirectly for 18% of greenhouse gases emissions (**GHG**), because 1 mole of nitrogen dioxide (**NO<sub>2</sub>**), a product of ammonia reaction in atmosphere and bacteria in soil (USEPA, 2010) have a GHG effect of 296 times the effect 1 mole of carbon dioxide does (FAO, 2006). Ammonia is predicted (Pye et al., 2009) to have an atmospheric lifetime of 3 to 4 days, thus reacting with other atmospheric substances for a long period of time near source of emission or away from it (Wu et al., 2008) making it difficult to punctuate sources of emission and control them (Hristov et al., 2011).

Urinary N is identified as the principal (79 to 90%) source of ammonia volatilized from cattle manure in the first 7 to 10 days of manure storage (Lee and Hristov, 2010; Thomsen, 2000). Ammonia emissions are linearly dependent on urinary urea excretion. This is dependent on the amount of protein in the diet of

dairy cattle and from factors that influence urine volume, such as ambient temperature and ion intake (James et al., 1999; Cassel et al., 2005a, 2005b; Bannink et al., 1999). Diet manipulation can increase efficiency of nitrogen utilization in ruminants. Degradability of carbohydrates and sources of protein in the diet can alter animal nitrogen utilization and consequent urea excretion (Broderick et al., 2008a). Prediction of N utilization in the rumen and correct utilization of degradable and undegradable protein sources in the diet of ruminant animals are important aspects of nutrition that may reduce ammonia release to environment (Hristov et al., 2011). Nitrogen recycling in animals is a new topic that must be considered in all new nutritional models so overfeeding and excess nitrogen excretion on environment can be avoided. (Lapierre and Lobley, 2001).

In a recent work, Capper et al. (2009), using a deterministic model based on NRC (2001) nutrient requirements evaluated the environmental impact of historical US milk production as exemplified by the US dairy system in 1944, compared with modern (2007) practices. According to the authors, pollution coming from agriculture increased from 1944 to 2007 in total numbers. But the authors show that the productive efficiency increased as well, using fewer cows to produce more milk by genetic and nutritional improvements. The authors concluded that even polluting more, the ratio between milk production and pollution produced to yield 1 kg of milk has increased considerably, increasing efficiency of production when considering pollution yield.

## **Ryegrass and Corn Silage**

Corn silage is the primary forage used in dairy rations in the southeastern United States. In the Coastal Plain and southeast states, annual ryegrass and bermudagrass are commonly grown in addition to corn silage or as a replacement for corn (Newton et al., 2003). Ryegrass can be used as green forage source in late fall and spring or can be used as haylage in summer. Forages have some important characteristics for use in ruminant diets. A good proportion of effective fiber is important to increase rumination time and consequent rumen buffering by increasing saliva and bicarbonate production. Organic matter digestibility gives animals most of the energy required. Total digestibility in rumen increases passage rate for intestines, resulting in greater intake and absorption of nutrients (Newton et al., 2003).

Silage made from grass has a different nitrogen and energetic content when compared to grain silages. Corn silage (and other grains silages) has a greater proportion of organic matter readily fermentable in the rumen. Corn silage has amylase and amylopectin in the grain. These two substances generate faster ruminal starch degradability when compared with fiber. It has a similar digestibility of cracked corn when well chopped and ensiled (NRC, 2001). This allows energy for microbes to capture  $\text{NH}_3$  in the rumen and process it to produce microbial protein. However, corn silage is low in CP and high in RDP; therefore, protein supplementation is often required to maintain dietary CP (O'Mara et al., 1998; Burke et al., 2007) or provide enough true protein to animals that require more than only microbial protein to meet their production requirements.



Grass silage, on the other hand, has a low proportion of fast degradable organic matter. It has mostly cellulose and hemicellulose as energy source for microbes and these have slow degradability in the rumen. Grass silage requires more energy from bacteria to be degraded in the rumen. It has a greater proportion of highly degraded crude protein, and almost all of it is transformed to  $\text{NH}_3$  by some microorganisms to be available for other bacteria. Consequently, high levels of N will be excreted if an energy source is not added to the diet. Dry distillers' grains (**DDG**) are a source of energy and protein and can be added to diets with grass forage or grain forage to compensate for deficiencies of these nutrients (Whelan et al., 2011).

According to NRC (2001), the average nutrient composition of ryegrass haylage (*Lolium perenne*) (Grass silage, cool season) is the following: 36.2% of DM, 1.29 MCal of  $\text{N}_{\text{EL}}$ , 16.8 % of CP, 51.0% of NDF, 32.9% of ADF, 9.9% of ash. Ryegrass also has 0.57% of calcium, 0.36% of phosphorus and 3.11% of potassium and 3.28% of Lys and 1.21% of Met in total CP. The average nutrient composition of corn silage is the following: 35.0% of DM, 1.45 MCal of  $\text{N}_{\text{EL}}$ , 8.8 % of CP, 45.0% of NDF, 28.1% of ADF, 4.3% of ash. Corn silage also has 0.28% of calcium, 0.26% of phosphorus and 1.20% of potassium and 2.51% of Lys and 1.53% of Met in total CP. These values are mean values collected from several analyzes throughout the country and are a good source of information to be used when formulating diets to compare these numbers with actual analysis of feed utilized.

### **Dry Distillers' Grains and Rumen Protected Amino Acids**

Availability of corn distillers' grains (**CDG**) for livestock has increased recently with the use of ethanol in the country's automobiles and with government stimulus to use it replacing part of fossil fuel dependency. Dry distillers' grains are CDG processed by the industries to increase dry matter (**DM**) of the by-product and avoid losses with mold and rancidity. Recommendations suggest that as much as 20% to 30% of the ration DM (about 4.5 to 5.9 kg/cow per day) can be fed to dairy cows with no ill effects on milk yield (**MY**) and composition compared with that from lower dietary concentrations, if diets contain at least 50% forage (Schingoethe et al., 2009). DDG contains a considerable amount of fat and it may decrease fat content in milk if effective fiber is lacking.

Lysine (**Lys**) was identified as the first limiting amino acid for lactating dairy cattle when corn and feeds of corn origin provided most of all dietary RUP. In contrast, Methionine (**Met**) was identified as first limiting amino acid for lactating cows when small amounts of corn were fed or when soybean meal (**SBM**) products provide most of the supplemental RUP (Armentano et al., 1997; NRC, 2001). Rumen microbes have a concentration of Lys in total essential amino acids of between 15.8 and 17.3%. The concentration of Met is between 4.9 and 5.2%. The ratio is approximate 3:1. This is considered the optimal ratio for duodenal availability of amino acids in dairy cows according to NRC (2001) model. Corn DDG have a concentration of 5.9% of Lys and 4.8% of Met. Dry distillers' grains lack Lys for optimal bacterial growth in the rumen. Soybean meal has a concentration of 13.9% Lys and 3.2% Met (NRC, 2001). Soybean meal lacks Met for optimal bacterial

growth. The ratio Lys : Met is not close to 3 : 1 in these feedstuffs. To improve amino acid utilization by ruminants, this ratio of Lys and Met must reach the duodenum. If the diet is not providing enough good quality protein for the rumen, protected amino acids that are mostly digested after the abomasum can be added to the diet to supply total protein needs.

### **Protein Levels and Nitrogen Efficiency**

Protein and energy should be fed to animals close to their needs to reduce environmental impact and unnecessary costs (Hristov et al., 2011). Low levels of protein may compromise animal production (Olmos Colmenero et al., 2006; Cabrita et al., 2011). High levels will have a significant portion of N not being utilized and processed in the body leading to excess excretion (Hristov et al., 2011). Farm animals are considered the greatest contributor of gaseous NH<sub>3</sub> emissions accounting for 50% of NH<sub>3</sub> emissions in the United States (NRC, 2003). Overfeeding of protein to dairy cows can also have an energetic toll (Milano et al., 2000) and potentially a negative effect on reproductive performance (Ferguson and Sklan, 2005).

When the levels of ammonia exceed the ruminal micro flora protein synthesis capacity, it is absorbed by the ruminal epithelium and transported through blood to the liver, processed to urea and then spread to salivary glands and other tissues, including mammary gland or it may be recycled back to the gut (Lapierre and Lobley, 2001). On the other hand, some animals have high protein requirements. These animals require true protein not degraded in the rumen (RUP) that will be degraded in the duodenum by enzymatic hydrolysis. To reach animal genetic

potential, producers make use of protein sources such as soybean meal and dry distillers' grains, which have higher concentrations of RUP (NRC, 2001).

Since Clark (1975) and Chalupa (1975), ruminant nutritionists embraced the concept that feeding animals accordingly to their amino acid (**AA**) requirements and true protein available at the duodenum can improve performance. Balancing diets for limiting AA could improve efficiency of low CP diets without increasing N losses in urine (Leonardi et al, 2003) consequently affecting less negatively the environment. Ruminants are not as efficient N utilizers as non-ruminants because N passes through more processes from the mouth until being absorbed in the lower intestine, resulting in a capture of only 23 to 33% of all N consumed (Robinson, 2010). This rate is lower than poultry and swine.

Methionine deficiency can cause decrease in milk protein yields from high producing dairy cows (Broderick et al., 2008b; NRC, 2001). Lysine deficiency can decrease milk true protein yield and concentration, and, to a lesser degree, milk yield (NRC, 2001). Rumen protected amino acids (**RPAA**), most importantly Lys (**RPL**) and Met (**RPM**), have been incorporated in low crude protein diets in several studies to test if animal performance can be maintained using these diets as has been done for poultry and swine for 40 years (Broderick et al., 2008b; Robinson, 2010).

Nitrogen use efficiency (**NUE**) is the efficiency of converting feed N into milk N and can be assessed by doing a ratio between N in milk and N intake (Cheng et al, 2011). However, this technique tends to overestimate N retention because some of

the nitrogen will go to other tissues and can be released from the animal from different routes (MacRae et al., 1993; Spanghero and Kowalski, 1997). Sick et al. (1997) demonstrated relationships between amino acid utilization and isotopic fractionation in the liver of rats fed different proteins. Sponheimer et al. (2003) provided preliminary evidence of increased enrichment of  $^{15}\text{N}$  in hair protein when ruminants were fed high protein diets. Animals in heat stress secrete nitrogen through sweat and saliva as well, making these measurements more difficult to be established.

Dietary CP content is the most important factor determining milk N efficiency in dairy cows (Hristov et al., 2011). Increase in dietary N content can increase total milk N yield, but will decrease efficiency because each g of N increased in milk results in 12.75 g not used and excreted in urine as urea (Hristov and Huhtanen, 2008).

### **Nitrogen Metabolism**

After ingested, CP, which includes true protein and non-protein compounds, is degraded to peptides, amino acids and mostly to  $\text{NH}_3$  by some rumen microbial species (Hristov and Jouany, 2005). Other species utilize this  $\text{NH}_3$  as an N source to synthesize protein and genetic material while they are reproducing. All this process depends upon presence of carbon skeletons from carbohydrate sources. Break down of these CHO compounds release energy necessary for microbes' processes and make carbon, oxygen and hydrogen available to build amino acids and proteins. Ammonia can also be absorbed through rumen wall or other sections of

gastrointestinal tract into blood stream (Reynolds and Kristensen, 2008). Microbial protein profile is similar to animal needs when considering tissues and milk protein composition (NRC, 2001; Lapierre et al. 2006), which makes it an ideal source of AA for the animal when in a maintenance state. High producing cows, in the other hand, need a profile of AA more complete than microbial and should be fed high quality proteins that bypass ruminal degradation and have a good biological value. (Chalupa, 1975; Clark, 1975).

There is an energetic toll when the excess of amino acids in the blood stream is transformed to urea in hepatic cells (Milano, 2000).  $\text{NH}_3$  is a neurological toxin and must be processed to a non-toxic substance such as urea in mammals and uric acid in birds. In mammals, this transformation consumes 3 ATP. Two ATP are consumed to incorporate the nitrogen atom taken from the AA in the urea cycle as carbamoyl phosphate. The other ATP is used in the reaction of citruline and aspartate to produce argininosuccinate (Lehninger, 2004). Blood urea is excreted by the kidneys or is recycled back to rumen or guts, utilized by microbes to produce protein or broken down to form ammonia again.  $\text{NH}_3$  will be reabsorbed in the body and, in excess, will pass through the liver and spend more energy to be reconverted to urea (Lapierre and Lobley, 2001). The same N atom can pass through this cycle several times (Lobley, 2000).

Urea recycling to the digestive tract of the ruminant animal is an important N preservation mechanism. Ruminal  $\text{NH}_3$  concentrations and plasma urea-N concentrations appear to be important factors although the processes controlling

this mechanism are not well understood (Reynolds and Kristensen 2008; Lapierre and Lobley, 2001).

Agriculture and livestock production today must focus on improving production efficiency rather than emphasizing increases in production only. Low CP diets must be studied in order to increase efficiency of conversion of dietary protein into milk protein with minimal harm to environment. Animal feeding practices that lead to high productivity and efficiency support both world feed needs and environmental sustainability.

### **Objective**

The objective of this research was to test if performance of late-lactation dairy cows can be maintained by changing the main source of dietary protein from soybean meal to dry distillers' grains and correcting the amino acid profile with rumen protected amino acids using corn silage or ryegrass haylage as forage sources.

### **Hypothesis**

The hypothesis of this research is that late-lactating dairy cows will maintain their performance when fed low crude protein diets using dry distillers' grains as main source of dietary protein and ruminal protected amino acids to correct availability of limiting amino acids in the duodenum. This hypothesis was tested in successive experiments using corn silage or ryegrass haylage as forage sources.

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## **CHAPTER 3: EXPERIMENT 1**

### **Materials And Methods**

#### **Introduction**

One experiment was conducted in the winter of 2010/2011 to evaluate performance of cows supplemented with diets based on dry distillers grains and rumen protected amino acids compared with control diets using soybean meal as the main protein source. In the present experiment, dietary treatments were evaluated in corn silage-based total mixed rations (TMRs).

#### **Location**

The experiment was conducted at the Louisiana State University Agricultural Center Southeast Research Station (SERS), located in Franklinton, Louisiana, USA (Coordinates 30°50'55"N, 90°9'0"W). The city has an elevation of 46.9 meters from sea level and is in a humid subtropical climate (Peel et al., 2007) with long, hot and humid summers and short winters. The experiment was conducted during the winter of 2010/2011. Start date was 12/17/2010 and end date 01/27/2011.

#### **Weather and Environment**

The average weather records of experiment 1 is summarized in table 1.

#### **Cows, Study Design, and Treatments for Experiment 1**

Twenty-four lactating Holstein cows averaging  $334 \pm 43$  DIM and  $22.2 \pm 3.79$  kg milk per day were brought to a free-stall barn equipped with electronic gates (American Calan Inc., Northwood, NH) for individual TMR feeding. The barn has a length of approximately 50 meters by 25 meters.

TABLE 1. Weather records for experiment 1 from LSU Southeast Research Station, located at Franklinton, LA.<sup>1</sup>

<b>Experiment 1</b>	<b>Max Air Temp (°C)<sup>2</sup></b>	<b>Min Air Temp (°C)<sup>3</sup></b>	<b>Average Air temp (°C)<sup>4</sup></b>	<b>Rain daily average (mm)</b>	<b>Average Wind Speed (km/h)</b>	<b>Average Relative Humidity</b>
Sampling Period 1 <sup>5</sup>	17.9	5.48	11.7	6.57	10.3	95.24
Sampling Period 2 <sup>6</sup>	13.1	-0.08	6.51	2.83	9.15	100.0

<sup>1</sup>Data was taken from LSU Southeast research station weather station (LSU AgCenter, 2011).

<sup>2</sup> Max Air Temp: Maximum ambient temperature. <sup>3</sup> Min Air Temp: Minimum ambient temperature. <sup>4</sup> Average Air temp: Average ambient temperature. <sup>5</sup> Sampling period 1 was from 12/31/2010 until 01/06/2011. <sup>6</sup> Sampling period 2 was from 01/21/2011 until 01/27/2011.

Each side (assigned as north and south) of the barn has 32 electronic gates but only 12 gates were used from each side divided in two pens, 6 in each extreme of the barn. Pens were assigned as NE, NW, SE and SW. Each pen had approximately 16.5 x 8.5 meters. Cows were brought to the barn 2 weeks prior to experiment start to be trained to use the gates. Each cow had a specific transponder on the neck that, when in contact with the respective gate opens it and allowed the cow access to the TMR. One cow had access to one gate only. Each group of cows had free access to a water trough linked to a water meter (Recordall model 40, Badger Meter, Milwaukee, Wisconsin). Heat abatement procedures, such as fans and water soakers, were unnecessary during this experiment. Rubber mats strips were used as containment apparatus to prevent manure runoff. The mats were placed on the concrete alley downslope at the limit of each experimental pen. Strips were fixed with nails in the concrete and sealed with a mastic asphalt to prevent urine and feces losses from the pens.

Cows were randomly distributed according to DIM and initial MY to keep homogeneity of pens among 4 pens to allow manure collection and sampling and to measure group water intake (**WI**). Each dietary treatment was assigned to two pens, one in the north and one in the south sides, for an experimental period of 21 days. Treatments were shifted to the other two pens for the second experimental period in a cross-over design. The two treatments were:

- Control TMR (**HP1**) estimated (NRC, 2001) to contain 16.5% CP with SBM as the main protein source;

- Treatment TMR (**LP1**) estimated (NRC, 2001) to contain 13.5% CP with DDG as the main protein source, supplemented with rumen protected lysine (AminoShure-L<sup>®</sup>, Balchem) and methionine (Metasmart<sup>®</sup>, Adisseo) to offset amino acid deficiencies in the diet.

Diets contained nearly 55% forage as corn silage and bermudagrass hay. Diets had AA profiles (lysine and methionine) corrected according to estimations of AA availability and flow in the duodenum (NRC, 2001). Rumen protected Lys (AminoShure-L<sup>®</sup>, Balchem) was 50% RUP according to product's label. Rumen protected Met (Metasmart<sup>®</sup>, Adisseo) was 50% RUP according to product's label. Dietary ingredients in the diets are presented in Table 2.

### **Diets of Experiment 1**

Diets were fed as TMRs. Nutrient contents of feeds were kept the same over the course of the study for each dietary treatment. Batches of grain mix were prepared once a week at the station and stored in dedicated grain silos. Grain mix formulations are shown in Table 3.

Silos were emptied and cleaned prior to experiment initiation. Two silos were used, one for each dietary treatment's grain mix. Premixes of DDG only for control diet (HP1 premix) and DDG plus ruminal protected amino acids for the treatment diet (LP1 premix) were formulated in the beginning of the experiment and stored in the barn, inside plastic cans holding 68 kg each. Premix formulations are shown in Table 4.



TABLE 2. Ingredient contents of diets containing different crude protein levels.

<b>Ingredients (% dry matter)</b>	<b>Adaptation Period</b>	<b>HP1<sup>1</sup></b>	<b>LP1<sup>1</sup></b>
Bermuda grass hay	10.2	11.1	11.1
Corn silage	43.3	43.5	43.5
Cottonseed, whole with lint	7.55	7.30	7.30
Corn grain, ground, dry <sup>§</sup>	10.0	16.2	16.2
Dry distillers' grain <sup>§</sup>	14.5	0.00	13.5
Soybean meal (48% CP) <sup>§</sup>	10.9	17.0	3.50
AminoShure-L <sup>2,*</sup>	0.00	0.00	0.30
Metasmart Dry <sup>3,*</sup>	0.00	0.00	0.10
Dry distillers' grain <sup>*</sup>	0.00	2.10	1.70
Calcium Carbonate <sup>§</sup>	0.82	0.80	0.80
Sodium Bicarbonate <sup>§</sup>	1.01	0.00	0.00
SE Sta. Mineral <sup>4,§</sup>	1.46	1.50	1.50
Salt <sup>§</sup>	0.30	0.50	0.50

<sup>1</sup>"HP1" diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5% CP). "LP1" diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5% CP).

<sup>2</sup> AminoShure-L is a product containing rumen-protected lysine (Balchem Corp., New Hampton, NY).

<sup>3</sup> Metasmart dry is a product containing rumen-protected methionine (Adisseo S.A.S., Antony, France).

<sup>4</sup> SE Sta. Mineral = Southeast Experiment Mineral Concentrate contained Ca = 20%; P = 3%, Mg = 7%; K = 6%; S = 3%; Co = 15 ppm; Cu = 650 ppm; I = 50 ppm; Mn = 1200 ppm; Se = 18 ppm; Zn = 3,700 ppm; vitamin A = 300 KIU/kg; vitamin D = 30 KIU/kg; and vitamin E = 1.5 KIU/kg; manufactured for Kentwood Co-op (Kentwood, LA).

Feedstuffs indicated by the symbol (\*) were added to the TMR mixer as premixes.

Feedstuffs indicated by the symbol (§) were added to the TMR mixer as grain mixes.

Cottonseed, DDG and both dietary treatments' grain mixes were stored in the barn, inside troughs that could hold approximately one ton. Bermuda grass hay was ground once a week and stored in a clean, dry barn near the Calan gate barn. Bermuda grass hay was grown and harvested in the summer of 2010 at SERS. Corn silage was taken from only one silo trench for the entire experiment to minimize changes in nutrient compositions and dry matter. Corn for silage was planted on 3/16/2010 and harvested on 7/5/2010. Corn variety utilized was Dekalb 6786 Roundup Ready. Corn was harvested with a John Deere Forage Harvester 3975 pull-type equipped with corn head for silage cutting mounted with a corn processor. Weekly fresh corn silage samples were used for DM adjustment of the diets. Each group of cows was offered feeds from a single batch or harvest from the beginning to the end of their experimental period to limit changes in nutrient composition. Dietary treatments were mixed twice daily using a Data Ranger (American Calan Inc., Northwood, New Hampshire) at 7:00 am and 1:30 pm. A single TMR batch for each dietary treatment was prepared in the morning and another in the afternoon. All cows from one treatment received the dietary treatment from one batch only, to avoid differences in nutrient intake between them.

### **Sampling, Laboratory Analyses and Data Collection for the Experiment**

Dry matter intake was estimated as the difference of the amount of feed offered and the weight back. Leftover TMR was collected every morning at 6:30 a.m., before feeding the animals the morning TMR and measured as weight back. The collection was made using a scoop shovel (37.5 x 47.6 cm) until the manger was clean.

TABLE 3. Ingredient contents of grain mixes for each dietary treatment.

<b>Ingredients (% dry matter)</b>	<b>HP1<sup>1</sup></b>	<b>LP1<sup>1</sup></b>
Corn grain, ground, dry	45.0	72.0
Soybean meal (48% CP)	47.2	15.5
Calcium carbonate	2.23	3.55
SE Sta. custom mineral <sup>2</sup>	4.16	6.67
Salt	1.39	2.23

<sup>1</sup>“HP1” diet indicates grain mix of control TMR. “LP1” diet indicates grain mix of treatment TMR.

<sup>2</sup> SE Sta. Mineral = Southeast Experiment Mineral Concentrate contained Ca = 20%; P = 3%, Mg = 7%; K = 6%; S = 3%; Co = 15 ppm; Cu = 650 ppm; I = 50 ppm; Mn = 1200 ppm; Se = 18 ppm; Zn = 3,700 ppm; vitamin A = 300 KIU/kg; vitamin D = 30 KIU/kg; and vitamin E = 1.5 KIU/kg; manufactured for Kentwood Co-op (Kentwood, LA).

TABLE 4. Ingredient contents of premixes for each dietary treatment.

<b>Ingredients (% dry matter)</b>	<b>HP1<sup>1</sup> premix</b>	<b>LP1<sup>1</sup> premix</b>
Dry distillers' grain	100.0	81.8
AminoShure-L <sup>2</sup>	0.00	13.9
Metasmart Dry <sup>3</sup>	0.00	4.30

<sup>1</sup>“HP1 premix”: premix for amino acid delivery for control TMR. “LP1 premix”: premix for amino acid delivery for treatment TMR.

<sup>2</sup> AminoShure-L is a product containing rumen-protected lysine (Balchem Corp., New Hampton, NY).

<sup>3</sup> Metasmart dry is a product containing rumen-protected methionine (Adisseo S.A.S., Antony, France).

$$\text{DM intake} = \{[(\text{morning TMR offered}) + (\text{afternoon TMR offered})] \times \text{TMR DM}\} - [(\text{weigh back}) \times \text{DM of TMR refusals}]$$

Excreted nitrogen in manure is estimated by the nitrogen to phosphorus ratio (N:P ratio) methodology as the difference between N and P intake and the amounts of N and P secreted in milk. The difference gives the amount of N and P excreted in the manure (Moreira and Satter, 2006).

Volatile N loss was calculated according to Moreira and Satter (2006). The authors calculations are based on the difference between estimated nitrogen : phosphorus ratio (N:P) of fresh excreta and measured N:P in scraped manure. Nitrogen : potassium (Hristov et al., 2009), nitrogen : calcium and nitrogen : magnesium ratios were used in this experiment and compared. Excretion of N, P, K, Ca and Mg were estimated as amount of ingested nutrients minus the amount of nutrients secreted in milk, according to Van Horn et al. (1994). Dietary DMI was calculated as total amount fed to animals in the morning and afternoon minus recorded weight backs as shown in the formula above. Feed CP and P content were adjusted with measured weekly intake of the animals according to actual analysis of each feed and DMI. Individual cow data for milk fat, protein and minerals and CP content in diet was used for calculations of intake and excretion in the trial and were based on DHIA data. Milk P was fixed at 0.09%, milk K at 0.15%, milk Ca at 0.122% and milk Mg at 0.15%, according to the NRC (2001). Milk nutrient yields were calculated and subtracted from daily intakes to give excreted nutrient loads. The estimated amount of N excreted ( $N_{\text{exc}}$ ) was divided by the estimated excreted P to determine the N : P as excreted by the animal ( $N:P_{\text{exc}}$ ). Scraped manure was

analyzed for N and P and ratios were calculated for each sample ( $N:P_{\text{man}}$ ). Nitrogen loss as percentage of  $N_{\text{exc}}$  was estimated as 1 minus the quotient of  $N:P_{\text{man}}$  and  $N:P_{\text{exc}}$ :  $[1 - (N:P_{\text{man}}/N:P_{\text{exc}})] \times 100$ . The fractional N loss was multiplied by  $N_{\text{exc}}$  to obtain an estimate of the amount of N lost (Moreira and Satter, 2006). The same methodology was used with K, Ca and Mg in this experiment to compare results of volatilization between these markers.

Milk production was measured twice a day in AfiFlo milk meters (SAE Afikim, Kibbutz Afikim, Israel) and recorded daily from each milking. Data was downloaded from Afimilk software (Afifarm version 3.01A, SAE Afikim) every 7 days. Milk samples were collected for 3 consecutive days in the morning and afternoon milking, from days 16 to 18 of each period. The Mid-South dairy records DHIA Laboratory (Springfield, MO) analyzed the milk samples for fat and true protein by near-infrared spectroscopy (Bentley 2000, Bentley Instruments, Chaska, MN), and for somatic cells in a flow cytometer (Bentley Somacount 300). A colorimetric enzymatic assay analyzed milk urea nitrogen (Bentley Chemspec 150). Milk components were weighted based on morning and afternoon milk production on sampling dates according to milk yield at each milking.

Solids yield were estimated according to milk total production and milk percentage of each component analyzed.

Energy corrected milk was calculated according to Tyrrell and Reid (1965):

$$\text{SCM (kg)} = 12.3 (\text{F}) + 6.56 (\text{SNF}) - 0.0752 (\text{M})$$

(F) is fat yield in kg, (SNF) is solids non fat yield in kg and (M) is milk yield in kg.

Mixed feeds offered and refusals were weighed daily to estimate dry matter intake. Feed intake and milk yield were recorded daily and used as daily measurements for repeated measures statistical analyses. Forage, TMR and refusal samples were collected daily with 500mL cups, stored frozen in buckets and composited weekly for analyses and for dry matter intake estimation. Refusals were sampled during leftover collection in each manger every morning. TMR samples were also taken after feeds were added in each manger. Forage samples were taken randomly from the conveyor as silage was loaded in the Data Ranger. The treatment with lower protein content was always the first to be mixed in the Data Ranger for the entire experiment. After feeding all cows in a treatment, the Data Ranger TMR chamber was carefully emptied before mixing the other TMR treatment. No water was added to any ration in the experiment 1. Water was added using a hose over the TMR while mixing in the Data Ranger to achieve 52% DM in the TMR in both rations of experiment 2.

Manure samples for both experiments were collected during 3 consecutive days at the end of each experimental period, in days 19, 20 and 21. The collection was done at 5:00 am and 16:00 pm while the cows were at the milking parlor. The use of two scrapings per day is in accordance with previous study (Moreira and Satter, 2006) that found no differences in scraping a barn twice a day or six times a day in ammonia volatilization results. Manure was scraped, piled and mixed thoroughly on the alley of the barn using hand scrapers and a scoop shovel (37.5 x 47.6 cm). Temperature was measured in 4 to 8 points of the manure depending on the size of the pile and averaged. Four to eight samples from each pile were taken

with the shovel and placed in a 34 L plastic tub. Manure in the tub was thoroughly mixed using a power drill and paint mixer attachments. Four random subsamples were taken from the tub using a 500 mL cup and placed in an 18.9L bucket. Manure in this bucket was then hand mixed and pH was measured. Two random subsamples were taken from the bucket using a 500 mL cup with approximately 2/3 of it full. Each cup with the subsamples was acidified with 2 mL of a solution with 66.7% of sulfuric acid to lower the pH of the solution to 2 or below. Samples were subsequently stored in a freezer to, together with the reduction of the pH, minimize N loss.

Cottonseed, DDG, dietary treatments' grain mixes and premixes were sampled weekly. Samples were dried in air forced 60°C drying oven for 72 h. Manure samples were sliced in pieces of 2 cm and then lyophilized in a VirTis Freeze Dryer (SP Industries Co., Gardiner, NY). Feed samples were ground through a 2-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA), and then passed through a 1-mm screen in a Cyclotec 1093 Sample Mill (Foss Tecator, Höganäs, Sweden). Manure samples were ground through a 1-mm screen in a SK100 cross beater mill (Retsch, Haan, Germany). Feed ground samples were composited by week. Feed and manure ground samples were analyzed for nitrogen according to the combustion method (AOAC, 1990) in a Leco Fp-2000 Nitrogen/Protein Analyzer (Leco Co., St. Joseph, MI). Feed and manure samples were analyzed for Kjeldahl P in an automated colorimetric assay adapted for flow-injection analyzer (QuickChem 8000 FIA, Lachat Instruments, Milwaukee, WI). Fibers were analyzed according to the sequential NDF/ADF analysis utilizing heat-stable amylase and sodium sulfite

(Van Soest et al., 1991) modified for the Ankom<sup>200</sup> Fiber Analyzer (Ankom Technology, Fairport, NY) (AOAC, 1990). Calcium, Mg, and K in feed and fecal samples were determined by flame atomic absorption spectrophotometry (Perkin-Elmer Analyst 300, Norwalk, CT) after dry-ashing at 500°C overnight in porcelain crucibles. Ashed samples were dissolved over heat (before the sample started boiling) in 5 mL of 20% HCl, transferred to 100-mL volumetric flasks, and diluted to 100 mL with deionized water. Diet composition results were calculated based on the composition of individual feeds. Body weights were measured for two consecutive days, immediately after morning milking, at the beginning and end of each experimental period. Body condition scores were given by 3 individuals at the beginning of the experiment and at the end of each experimental period.

### **Statistical Analysis**

All the response variables were analyzed using the MIXED procedure of SAS (Littell et al., 1996, SAS Institute, 2003). Milk yield, milk composition and DMI were averaged over time to obtain one observation per animal within each period. The previously mentioned response variables, body weight changes and BCS changes were analyzed including in the model the fixed effect of treatment (HP1 or LP1), period (1 or 2) and sequence (1 or 2). Pen within sequence and pen within sequence by treatment were included in the model as random effects. Pen water consumption was analyzed including in the model the fixed effect of treatment, period and sequence. Pen within sequence was included in the model as a random effect. Nitrogen volatilization was analyzed including in the model the fixed effect of treatment, period, sequence, day (1, 2 or 3) and treatment by day interaction. Pen



within sequence and pen within sequence by treatment were included in the model as random effects. Manure pH and temperature were analyzed including in the model the fixed effect of treatment, period, sequence day, scraping (AM and PM) and two and three way interactions among treatment, day and scraping. Pen within sequence and pen within sequence by treatment were included in the model as random effects. The Kenward-Roger denominator degrees of freedom adjustment was used in all the analysis. Values reported are least square means. Significance was declared at  $P \leq 0.05$ , and a trend was reported if  $0.05 < P \leq 0.10$ .

## **Results And Discussion**

### **Diets**

Experiment 1 had dietary CP contents of  $16.7\% \pm 0.61\%$  and  $13.7\% \pm 0.20\%$ , respectively for HP1 and LP1 (Table 5). Diets were formulated according to balance of metabolizable protein (**MP**) for cows producing  $22.2 \pm 3.79$  kg of milk per day with  $334 \pm 43$  DIM. HP1 had a positive balance (429 g/d) of MP (Table 5). Diet HP1 was designed to contain typical crude protein used in dairy farms. Diet LP1 had MP balance close to zero (37 g/d) (Table 5). This was considered challenging level of metabolizable protein in the diet. The estimated (NRC, 2001) RUP was 5.97% and 5.15% of total DM respectively for HP1 and LP1. Estimated RDP were 10.6% (2,114 g/d) and 8.37% (1,674 g/d) of total DM respectively for HP1 and LP1. Phosphorus contents in the diets were 0.53% for HP1 and 0.50% for LP1. Calcium contents were 0.62% for HP1 and 0.72% for LP1. These values were in accordance with NRC (2001) recommendations. Estimated content of available Lys at the duodenum was 173 g/d and 143 g/d for HP1 and LP1 respectively. Estimated content of available

Met at the duodenum was 49 g/d and 47 g/d respectively for HP1 and LP1. Water was not added to any treatments. Dry matter as a percent of as fed for both diets was 65%. Diets HP1 and LP1 had similar contents of neutral detergent insoluble fiber (**NDF**) (35.8% for HP and 36.7% for LP) and acid detergent insoluble fiber (**ADF**) (20.8% for HP and 21.0% for LP).

Rumen degradable protein and RUP supplied for the treatments were at the recommended levels (NRC, 2001) for control diet and at lower levels for treatment diet. Diet HP1 supplied 2,114 g/d of RDP. The requirement was 2,000 g/d resulting in a positive RDP balance of 114 g/d. Rumen degradable protein content of LP1 was 1,674 g/d and the requirement was the same. The RDP balance was a negative 326 g/d. Supplied RUP for the control diet was 1,193 g/d with a requirement of 691 g/d resulting in a positive balance of 503 g/d of RUP.

Net energy for lactation (**N<sub>El</sub>**) and MP contents of the diets were not limiting according to the NRC (2001). Dietary **N<sub>El</sub>** allowed for 31.7 kg/d and MP allowed for 32.9 kg/d of milk for HP1. For LP1, **N<sub>El</sub>** allowed for 31.2 kg/d and MP allowed for 24.2 kg/d of milk.

### **Dry Matter Intake and Water Intake**

There was no significant difference in DMI in experiment 1. Cows in HP1 ingested 21.0 kg/d of DM while cows in LP1 ingested 20.4 kg/d ( $P=0.46$ ) (Table 6). Animals in diets with low amount of protein are expected to increase intake to be able to reach the requirements of the nutrient (Conrad, 1966). If the DMI is the same and cow performance is different, the animals may be limited by high amounts of fiber or energy in the diet. If the performance is maintained, the animals may not

have a high requirement or might not need the amount of protein of the high protein diet.

In both experiments, DMI was not significantly different. Leonardi et al. (2003) feeding cows with different concentrations of crude protein (16.1% CP as low protein content and 18.9% CP as high protein content) in the diets in a factorial design with or without addition of methionine (8% addition in DM basis) found no differences in DMI among treatments. The authors used cows with approximately 42 kg of milk yield/d.

Hollmann et al. (2011b) found that dry matter intake response to CDG increased linearly with increasing dietary CP concentration of control diets ( $R^2 = 0.22$ ;  $P_{\text{model}} < 0.01$ ). A concentration of CP greater than 16.5%, dry basis, was related to an increase in DMI of cows fed CDG diets, whereas a lower concentration was associated with a negative DMI response.

Hollmann et al. (2011a) had a positive DMI response to CDG in the diet when forage concentration in the diet was less than 49% in dry matter basis. In addition, a linear relationship between corn silage concentration and DMI response to CDG existed ( $R^2 = 0.09$ ;  $P = 0.04$ ;  $n = 44$ ).

Broderick et al. (2008b) described two trials designed to study rumen-protected Met supplementation and reducing dietary protein as follows: 18.6% CP and 0 g of RPM/d; 17.3% CP and 5 g of RPM/d; 16.1% CP and 10 g of RPM/d; or 14.8% CP and 15 g of RPM/ d. The authors found no differences in DMI in one of the trials.

TABLE 5. Nutrient contents of each experimental diet of experiment 1

<b>Composition</b>	<b>Adaptation Period<sup>1</sup></b>	<b>HP1<sup>2</sup></b>	<b>LP1<sup>2</sup></b>
Dry matter, % as fed	66.2	65.1 ( $\pm 0.26$ )	64.9 ( $\pm 0.20$ )
Crude protein, % DM	16.5	16.7 ( $\pm 0.61$ )	13.7 ( $\pm 0.20$ )
MP balance <sup>3</sup> (g/day)	-	429.0	37.0
RUP <sup>4</sup> , % of DM	-	5.97	5.15
RDP <sup>4</sup> , % of DM	-	10.57	8.37
Neutral detergent fiber, % DM	35.1	35.8 ( $\pm 1.12$ )	36.7 ( $\pm 1.09$ )
Acid detergent fiber, % DM	20.7	20.8 ( $\pm 0.72$ )	21.0 ( $\pm 0.63$ )
Calcium, % DM	0.59	0.62 ( $\pm 0.12$ )	0.72 ( $\pm 0.10$ )
Phosphorus, % DM	0.56	0.53 ( $\pm 0.01$ )	0.50 ( $\pm 0.01$ )
Potassium, % DM	1.26	1.23 ( $\pm 0.13$ )	1.09 ( $\pm 0.10$ )
Magnesium, % DM	0.28	0.28 ( $\pm 0.01$ )	0.28 ( $\pm 0.02$ )
Lysine g /day <sup>5</sup>	-	173	143
Methionine g/day <sup>5</sup>	-	49.0	47.0

<sup>1</sup> Adaptation period diet contents were estimated by NRC model (NRC, 2001).

<sup>2</sup>“HP1” diet indicates dietary protein concentration above NRC (2001) predicted requirement. “LP1” diet indicates dietary protein concentration below NRC (2001) predicted requirement.

<sup>3</sup> “MP balance”: Total metabolizable protein required in diet minus total metabolizable protein supplied in diet

<sup>4</sup> “RUP”: Rumen undegraded protein “RDP”: Rumen degraded protein; RUP and RDP were estimated according to NRC (2001) model.

<sup>5</sup> Amino acids are shown in g/day of availability in the duodenum according to NRC (2001) model.

In the other trial there was a tendency for greater DMI using diets with 17.3% and 16.1% of CP with 10 g of RPM added to the lower protein diet, but this trial had DMI lower than expected and the cows were in a negative balance for the three lower protein levels.

There was a tendency ( $P=0.09$ ) of difference in WI between treatments HP1 and LP1 in experiment 1 (Table 6). Experiment 1 had a difference of 3 percentage-units in CP between treatments. Several factors may increase or decrease water intake. Ambient temperature, presence or absence of heat abatement procedures such as fans and water soakers, intake of ions ( $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$ ) and concentration of protein in the diet (NRC, 2001) are some of the causes. Obitisu et al. (2011) found no differences in water intake (96.9 and 89.8 L/d,  $P=0.36$ ) when cows were exposed to different ambient temperatures (18°C and 28°C). Dinn et al. (1998) feeding cows with different levels of CP (18.3, 16.7, and 15.3%) found differences in water intake between the treatment with 18.3% of CP and 15.3% of CP. According to the authors, the greater intake of CP increases urea production in the liver and consequently urinary urea excretion. This increased excretion is accompanied by water excretion. Hence, animals intake more water to compensate the greater water excretion. Treatment of 16.7% CP was equal to the others.

### **Milk Yield**

Cows fed HP1 in experiment 1 had mean yield of milk of 20.7 kg/d. Cows fed LP1 had mean yield of 20.5 kg/d with no significant difference between treatments ( $P=0.91$ ) (Table 7).

Silva et al. (2009) carried out an experiment with cows producing 12 kg of milk per day using two treatments: a high protein diet with 13% of CP in the TMR and a low protein diet with 11% of CP in the TMR. With 13% of CP the cows were ingesting 275 g/d of N and the animals in the 11% CP diet were ingesting 248 g/d of N. In this experiment, milk yield did not change as a result of nitrogen levels in the diet. However, Arriaga et al. (2010) found significant differences in milk yield (18.2 kg/day versus 21.4 kg/day,  $P<0.05$ ) with cows in a low CP diet and a higher CP diet with different intake of nitrogen (405 grams/day versus 498 grams/day of ingested N respectively,  $P<0.05$ ). In this experiment, the authors did not find any difference in milk nitrogen excretion (7.7 mg/dl vs 8.2mg/dl,  $P=0.82$ ) but found differences in urine and feces nitrogen excretion (128.5 g/day vs 153.5 g/day,  $P<0.05$  and 144.8 g/day vs 162.8 g/day,  $P<0.05$ ), for low protein intake and high protein intake respectively). With high-producing cows, lowering dietary CP may, in certain situations, result in decreased milk yield (Broderick, 2003), which would be unacceptable to most producers and nutritionists in the field (Agle, 2010).

Leonardi et al. (2003) found no difference ( $P=0.51$ ) in milk yield among treatments with addition of Met (41.0 kg/d and 42.8 kg/d,  $P=0.51$ ) and between treatments with Met and without Met ( $P=0.63$ ). One trial with the same design of Leonardi et al. (2003), similar amount of Met added, and similar dietary CP, using soybean meal, showed a significant increase in milk yield (32.9 kg for control diet without Met and 35.2kg for the diet with Met added,  $P<0.01$ ) (Illg et al., 1987). Increase in milk yield may be a response to a better amino acid profile reaching the duodenum and being absorbed, but results are conflicting.

TABLE 6. Water intake and dry matter intake from late lactation cows fed two different levels of dietary crude protein levels in experiment 1.

Treatment	HP1 <sup>1</sup>	LP1 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Water consumption, liters/cow/day	69.0	64.0	0.88	0.09	1.00	0.02
Dry matter intake, kg/cow/day	21.0	20.4	1.30	0.46	0.37	0.07

<sup>1</sup>“HP1” diet indicates dietary protein concentration close to NRC (2001) predicted requirement. “LP1” diet indicates dietary protein concentration below NRC (2001) predicted requirement.

<sup>2</sup> “SEM”: Standard error for the mean.

No increase in milk yield was observed when dietary CP was either 15.7 or 16.2% and 17 g/d of Met was supplemented (Overton et al., 1998), or when dietary CP was 19.5% and 10.5 g/d of methionine was supplemented (41.5 kg/d of milk) (Armentano et. al., 1997). Dinn et al. (1998) found higher milk yield (34.2 kg/d of milk) for cows fed a high protein diet (18.3% CP) compared with other diets (32.8 kg/d of milk)(16.7% CP and 15.3% CP). Diets with SBM are low in Met and need supplementation to improve this profile. There is a high variability between experiments. Differences in feed and animal management forage quality and bioavailability of each nutrient in the diet may affect utilization and differentiate results between treatments.

Hollmann et al. (2011a) published a meta-analysis of published studies on CDG utilization with different percentages of CDG in the diet to evaluate its effects on cow performance. The authors found that MY was quadratic ( $P_{\text{quadratic}} = 0.02$ ) in response to increasing CDG concentration ( $R = 0.15$ ;  $P = 0.04$ ) with a maximum of 1.2 kg of milk per cow per day at 21% CDG, dry basis. This response was pronounced in experiments using high-producing cows, but did not occur in experiments with low-producing cows. The two experiments presented in this thesis did not use large proportions of distillers' grains (15.2% of DDG in DM in LP1 and 6.1% of DDG in DM in LP2).

Broderick et al. (2008b) did two 4 x 4 Latin square trials (4-wk periods; 16 wk total) with decreasing concentrations of crude protein in the diet supplemented with RPM. The authors found significant differences in MY and 3.5% FCM. Yields of



milk and FCM were greater at 17.3% CP plus 5 g/d of RPM and 16.1% CP plus 10 g/d of RPM than on the other 2 diets (18.6% CP and 0 g of RPM and 14.8% CP and 15 g of RPM). According to the authors, the best Lys : Met profile available for absorption at the duodenum occurred at 17.3% and 16.1% of CP in diet and the increased milk yield might be a result of this closer profile. According to the NRC (2001), a 3:1 ratio of Lys to Met may improve performance. In our experiments, the ratio of low protein diets LP1 and LP2 were respectively 3.04 and 2.93. Diets HP1 and HP2 had ratios of 3.53 and 3.03 respectively. Diets used in both studies described in this thesis were near or above the ratio recommended in the NRC (2001)

Broderick et al. (2008b) found that digestible Lys became limiting when the estimated supply fell from 162 to 154 g/d resulting in a drop in milk yield from 41.6 to 39.7 kg/ d ( $P < 0.05$ ) between the diets containing 16.1 and 14.8% CP.

According to the NRC (2001), milk yield responses to Lys and Met are more common in cows during early lactation than in mid or late lactation dairy cows. Production responses to increased supplies of Lys and Met in MP typically are greater when CP in diet DM approximates normal levels (14 to 18 percent) than when it is lower or higher (NRC, 2001).

### **Milk Components**

Experiment 1 had no significant difference between diets in milk fat percentage and yield (4.18%, 0.86 kg/d for HP1 and 4.25%, 0.86 kg/d for LP1,  $P = 0.70$  and  $P = 0.99$  respectively), milk protein percentage and yield (3.73%, 0.78

kg/d for HP1 and 3.71, 0.76 kg/d for LP1,  $P=0.77$  and  $P=0.70$  respectively) and milk lactose percentage and yield (4.55%, 0.96 kg/d for HP1 and 4.53%, 0.94 kg/d for LP1,  $P=0.77$  and  $P=0.80$  respectively) (Table 7).

Dinn et al. (1998) found no differences in milk fat output, milk protein percentage and milk protein output among experimental periods when using 3 levels of CP in the diet (18.3%, 16.7% and 15.3% of CP). Milk fat concentration and yield in milk may decrease as a result of addition of DDG. Diets with high concentration of fat may decrease fiber digestibility and consequently decrease fat content in milk (NRC, 2001). DDG diets have higher concentrations of fat when compared to SBM diets. Fat content from DDG may range from 10.2% to 11.7% (Spiehs et al., 2002). Hollmann et al. (2011a) found in his meta-analysis that milk fat concentration in all included experiments was not related to dietary concentration of CDG when experimental treatments had less than 21% CDG and adequate levels of effective fiber (eNDF).

Hollmann et al. (2011b) found that milk true protein concentration response to dietary CDG was related negatively to dietary CDG concentration ( $R^2 = 0.15$ ;  $P = 0.02$ ). In our experiments, DDG was used as 15.2% in LP1 and 6.1% in LP2. These concentrations of CDG in the diet would not reduce milk true protein content according to the authors.

Studies put together by NRC (2001) indicate that content of protein in milk is more responsive than milk yield to supplemental Lys and Met, particularly in post-peak lactation cows. Increases in milk protein percentage are independent of milk

yield. Casein is the most influenced milk protein fraction. Increases in milk protein production to increased supplies of either Lys or Met in MP are the most predictable when the resulting predicted supply of the other AA in MP is near or at estimated requirements.

A number of studies have shown that supplementing lactating dairy cows with RPM has improved milk protein synthesis. Feeding RPM increased milk concentrations of total protein (Armentano et al., 1997; Berthiaume et al., 2006), true protein (Berthiaume et al., 2006), and casein N (Overton et al., 1998), and yields of milk (Schmidt et al., 1999), total protein (Armentano et al., 1997), and true protein (Rulquin and Delaby, 1997). These experiments did not use negative balance of MP.

In experiment 1, milk urea nitrogen had a significant difference between diets with 17.2 mg/dL in HP1 diet and 9.93 mg/dL in LP1 diet ( $P < 0.01$ ) (Table 15). High MUN levels in HP1 could have happened because of its positive MP balance.

Leonardi et al. (2003) feeding cows with different concentrations of crude protein (16.1% CP as low protein content and 18.9% CP as high protein content) in the diets in a factorial design with or without addition of methionine (8% addition in DM basis) found a significant difference between protein levels in MUN. In that study, the authors found a significantly lower content of MUN in the low CP diets (4.44 g/d for low CP diet without methionine and 4.29 g/d for low CP diet with methionine included) and a higher content in the high CP diets (5.96 g/d in the high

CP diet without methionine and 6.10 g/d for high CP diet with methionine included) ( $P < 0.01$ ), but MUN was not affected by Met inclusion ( $P = 0.98$ ).

Burgos et al. (2010) studied the relationship between ammonia emissions from dairy cattle manure and MUN and dietary crude protein content affecting these two variables. According to the authors, there is a relationship between MUN and manure emissions of ammonia ( $R^2 = 0.85$ ). A shift from 9 mg/dL to 17 mg/dL of MUN could increase ammonia emissions from 70 g/d of N per cow to 110 g/d of N per cow.

Broderick et al. (2008b) found reduced ( $P \leq 0.01$ ) MUN when protein content in his trial decreased from 18.6% CP to 17.3% CP, 16.1% CP and 14.8% of CP with RPM added and was paralleled with increased apparent N efficiency (milk N : N intake). Broderick et al. (2008b) found a significant ( $P \leq 0.01$ ) negative linear relationship between dietary CP content and MUN and N efficiency. Apparent N efficiency improved by nearly 8 percentage units from the highest to lowest CP, and was greatest ( $P \leq 0.01$ ) on the diet containing the least CP and most RPM. However, the highest N efficiency on 14.8% CP occurred along with lost yields of milk and milk components relative to the 2 intermediate diets. The greatest N efficiency, accompanied by production and feed efficiency similar or equal to the highest observed in the author's trial, occurred on the RPM-supplemented diet containing 16.1% CP.

Broderick et al. (2008b) found in one trial using different concentrations of CP in the diet that apparent N balance, computed from observed N intake and milk N secretion (milk N = milk protein/6.38), and estimated manure N excretion showed a

significant diet effect ( $P \leq 0.01$ ); only cows fed the RPM – supplemented diet with 14.8% CP were in apparent negative N balance. In this trial, the authors concluded that if N utilization was considered optimal on the diet with 16.1% CP plus RPM, then the 72 g/d reduction in total urinary N compared to that on the 18.6% CP would correspond to approximately 22 kg/cow of N over 300 days lactation. Fecal N excretion was unaffected by the diet.

### **Body Weight and Body Condition Score**

Changes in body weight (**BW**) and body condition score (**BCS**) were also not significantly affected by reducing protein in this study (Table 15), although those observations should be analyzed with caution because diets were only fed for 21 days. Changes in body weight and body condition score may require longer periods to be detectable.

Broderick et al. (2008b) found no difference in body weight gain in the 2 trials with reducing levels of CP and addition of RPAA in diet. Cabrita et al. (2011) found a significant difference in body weight between treatments with different CP levels ( $P < 0.05$ ). They were lower in the low CP diet (14%) compared to the high CP diet (16%). There was no difference comparing diets with same level of CP with or without addition of RPAA in that study.

### **Manure Analyses**

In experiment 1, manure pH was significantly higher for HP1 than for LP1 (7.87 and 7.53 respectively,  $P = 0.02$ ), perhaps as a result of higher excretion of urinary urea in the latter (Table 9).

TABLE 7. Milk yield and milk composition from late lactation cows fed two different levels of dietary crude protein levels<sup>1</sup> for experiment 1.

Treatment	HP1 <sup>1</sup>	LP1 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Milk yield, kg/cow/day	20.7	20.5	2.60	0.91	0.45	0.18
Fat, %	4.18	4.25	0.23	0.70	0.89	0.74
Fat, kg	0.86	0.86	0.04	0.99	0.51	0.16
Protein, %	3.73	3.71	0.10	0.77	0.90	0.79
Protein, kg	0.78	0.76	0.03	0.70	0.26	0.15
Lactose, %	4.55	4.53	0.08	0.77	0.23	0.58
Lactose, kg	0.96	0.94	0.07	0.80	0.40	0.28
MUN <sup>3</sup>	17.2	9.93	0.71	0.01	0.40	0.72

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%).

“LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%).

<sup>2</sup> “SEM”: Standard error for the mean. <sup>3</sup> MUN = milk urea nitrogen.

TABLE 8. Body weight and body condition score changes from late lactation cows fed two different levels of dietary crude protein levels for experiment 1.

Treatment	HP1 <sup>1</sup>	LP1 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Body weight gain, kg/cow/period	84.2	82.0	14.2	0.91	0.63	0.40
Body condition score, units/period	0.14	0.10	0.06	0.69	0.97	0.12

<sup>1</sup>“HP1” diet indicates dietary protein concentration above NRC (2001) predicted requirement. “LP1” diet indicates dietary protein concentration below NRC (2001) predicted requirement. <sup>2</sup> “SEM”: Standard error for the mean.

Manure temperature was not affected by treatments. Burgos et al. (2010) found in his experiment to estimate amount of N in manure that initial urea N concentration increased linearly with dietary CP from 153.5 to 465.2 mg/dL in manure slurries from cows fed 15 to 21% CP diets. Increased amount of urea in manure results in an increase of the pH due to N atom being in the form of  $\text{NH}_3$  and  $\text{NH}_4^+$ .

Although cows ingested more water in the treatment HP1 ( $P=0.09$ ), there was no difference in dry matter content of manure between dietary treatments (Table 10). Maltz and Silanikove (1996) demonstrated that cows strive to maintain urinary osmotic load. Cows spend energy to transform excess ammonia to urea in the liver, which is excreted in urine. When excess protein is fed, more urea is produced. That may require greater urine volume (thus, greater water ingestion) to maintain urine osmolality, assuming the consumption of ions ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ ) remains similar.

Leonardi et al. (2003) found no effects of methionine supplementation on urine volume or urinary N excretion. When CP level increased from 16.1 to 18.9%, urine output increased from 21.8 to 24.6 L/d. Dinn et al. (1998) measured urine output through total collection and reported that urine output increased approximately 2 L/d for every 1 percentage unit increment in dietary protein. Dinn et al. (1998) and Cressmann et al. (1980) reported an increment of approximately 70 g/d of urinary N when increasing dietary protein by 1.6 or 2.6 percentage units, respectively.

In experiment 1, the reduction in CP content from 16.7% to 13.7% resulted in



a significant reduction in manure pH (Table 10) and a reduction of the concentration of N in the manure from 2.11 % to 1.64% (Table 11).

Broderick et al. (2008b) found a significant ( $P < 0.01$ ) linear relationship between dietary CP content (18.6%, 17.3%, 16.1% and 14.8% of CP) and urinary urea N and total N. Reducing dietary CP concentration resulted in highly significant ( $P < 0.01$ ) reductions in estimated urine volume and proportion of urea N in total urinary N, which fell from 78 to 53%. As dietary CP decreased from 18.6 to 14.8%, urea N and total N excreted in the urine, as estimated by spot urine sampling, declined by, respectively, 125 and 110 g/d. The authors suggested that this lower N output was a result of lower urea N. Studies from Broderick (2003) and Olmos Colmenero and Broderick (2006) have observed a similar correspondence between the reductions in urea N and total N in the urine when using the same methodology.

Manure volatilization data is summarized in table 12 for experiment 1. No difference was found between treatments in N volatilization for all mineral markers analyzed in this experiment ( $P > 0.37$ ).

Data had some negative values in N : K ratios calculated before analyzing the data with SAS. Manure was not perfectly retained by the mats and some runoff was visually detected. Despite repeated attempts to caulk the gaps between mats and concrete alleys, spills continued to occur. Nitrogen and minerals were probably lost in result of retention failure.

Negative values of volatilized nitrogen as estimated by ratios of mineral markers can happen for two reasons: incorporation of nitrogen to the manure or loss of mineral markers (P, K, Ca, Mg) in scraped manure. Potassium might have

been lost because this mineral is highly soluble in water. According to Nennich et al. (2005) and Nennich et al. (2006), 75% of total excreted K is solubilized in urine. Urine in contact with feces may solubilize more potassium and be leached away with urine and N. Volatilization based on Mg data shows values of volatilized nitrogen closed to what was expected (around 30% of nitrogen volatilization).

Conversion of dietary protein into milk protein in experiment 1 was 27.9% for HP1 and 30.6% for LP1. Leonardi et al. (2003) found that feeding the higher protein diets resulted in a conversion of dietary protein into milk protein of 30.3% vs. 37.1% for the lower protein diets. The authors found that for the higher CP diets, 44% of the N excreted was excreted as urine and milk urea compared with 38% ( $P < 0.01$ ) with the lower protein diets.

Hristov (2009) found that 26% of the N consumed was secreted in milk. Meta-analyses of large North American and North European datasets (Hristov et al., 2005; Huhtanen and Hristov, 2009) found similar results. Experiment 1 had a low ratio, probably a result of the higher amount of phosphorus in the diets of experiment 1.

Percentages of total milk secreted minerals over total mineral intake are summarized in table 13. Phosphorus secretion had a low efficiency, probably due to the excessive amount of phosphorus in HP1 and LP1 diets. K values of milk secretion percentages over K intake were lower than the ones in Hristov et al. (2009). Values of minerals in milk were not analyzed but estimated according to NRC (2001).

TABLE 9. Manure temperature and manure pH measured onsite from pens with late lactation cows fed two different levels of dietary crude protein levels in experiment 1.

Treatment	HP1 <sup>1</sup>	LP1 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Manure Temperature	12.3	12.4	0.33	0.92	0.56	<0.01
Manure pH	7.87	7.53	0.06	0.02	0.71	0.47

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%).

“LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%).

<sup>2</sup>“SEM”: Standard error for the mean.

TABLE 10. Actual milk yield and estimated nutrient intake and excretion by pens during each trial (winter 2010/2011 and summer 2011; means  $\pm$ SD) evaluating the effect of manure scraping on N volatilization from a freestall barn.

<b>Composition</b>	<b>Experiment 1</b>	
	<b>HP1<sup>1</sup></b>	<b>LP1<sup>1</sup></b>
DMI <sup>2</sup> , kg/d	21.0 ( $\pm$ 3.25)	20.4 ( $\pm$ 3.13)
N intake <sup>2</sup> , kg/d	0.55 ( $\pm$ 0.06)	0.45 ( $\pm$ 0.03)
P intake <sup>2</sup> , kg/d	0.11 ( $\pm$ 0.01)	0.11 ( $\pm$ 0.01)
K intake <sup>2</sup> , kg/d	0.24 ( $\pm$ 0.04)	0.24 ( $\pm$ 0.04)
Ca intake <sup>2</sup> , kg/d	0.13 ( $\pm$ 0.01)	0.13 ( $\pm$ 0.01)
Mg intake <sup>2</sup> , kg/d	0.06 ( $\pm$ 0.003)	0.06 (0.003)
Milk yield, kg/d	20.7 ( $\pm$ 5.30)	20.5 ( $\pm$ 4.83)
Milk N, kg/d	0.15 ( $\pm$ 0.02)	0.14( $\pm$ 0.004)
Milk P, kg/d	0.02 ( $\pm$ 0.002)	0.02 ( $\pm$ 0.001)
Milk K, kg/d	0.03 ( $\pm$ 0.003)	0.03 ( $\pm$ 0.001)
Milk Ca, kg/d	0.03 ( $\pm$ 0.003)	0.03 ( $\pm$ 0.001)
Milk Mg, kg/d	0.004 ( $\pm$ 0.003)	0.004 ( $\pm$ 0.001)
Excreted N <sup>3</sup> , kg/d	0.40 ( $\pm$ 0.04)	0.31 ( $\pm$ 0.03)
Excreted P <sup>3</sup> , kg/d	0.10 ( $\pm$ 0.11)	0.10 ( $\pm$ 0.07)
Excreted K <sup>3</sup> , kg/d	0.24 ( $\pm$ 0.08)	0.20 ( $\pm$ 0.06)
Excreted Ca <sup>3</sup> , kg/d	0.09 ( $\pm$ 0.003)	0.10 ( $\pm$ 0.008)
Excreted Mg <sup>3</sup> , kg/d	0.03 ( $\pm$ 0.002)	0.03 ( $\pm$ 0.001)
Excreted N:P <sup>4</sup>	3.84 ( $\pm$ 0.02)	3.16 ( $\pm$ 0.04)
Excreted N:K <sup>5</sup>	1.81 ( $\pm$ 0.46)	1.61 ( $\pm$ 0.34)
Excreted N:Ca <sup>6</sup>	4.30 ( $\pm$ 0.35)	2.96 ( $\pm$ 0.44)
Excreted N:Mg <sup>7</sup>	7.56 ( $\pm$ 0.36)	5.98 ( $\pm$ 0.23)

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%). “LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%). <sup>2</sup> Nutrient intake estimates are based in actual animal intakes and nutrient content of each component in diet <sup>3</sup> Intake of nutrient minus nutrient excreted in milk. <sup>4</sup> Ratio between excreted N and excreted P. <sup>5</sup> Ratio between excreted N and excreted K. <sup>6</sup> Ratio between excreted N and excreted Ca. <sup>7</sup> Ratio between excreted N and excreted Mg.

TABLE 11. Estimated volatilized N percentages using method described in Moreira and Satter (2006) in experiment 1 evaluating the effect of different dietary CP concentrations on N volatilization from a free-stall barn.

Treatment	HP1 <sup>1</sup>	LP1 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Volatilized N (P) <sup>3</sup>	10.4	9.92	7.24	0.96	0.90	0.25
Volatilized N (K) <sup>4</sup>	-18.0	-22.4	10.3	0.78	0.50	0.35
Volatilized N (Ca) <sup>5</sup>	39.4	40.7	9.00	0.92	0.99	0.16
Volatilized N (Mg) <sup>6</sup>	33.6	29.9	7.46	0.74	0.92	0.62

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%). “LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%). <sup>2</sup> “SEM” Standard error of the mean. <sup>3</sup> Values calculated using N : P ratio and presented as a % of excreted N. <sup>4</sup> Values calculated using N : K ratio and presented as a % of excreted N. <sup>5</sup> Values calculated using N : Ca ratio and presented as a % of excreted N. <sup>6</sup> Values calculated using N : Mg ratio and presented as a % of excreted N.

TABLE 12. Manure analysis for each trial (winter 2010/2011 and summer 2011; means  $\pm$ SD) evaluating the effect of two levels of protein in the diet of lactating dairy cows on manure composition and physical-chemical properties

<b>Composition</b>	<b>Experiment 1</b>	
	<b>HP1<sup>1</sup></b>	<b>LP1<sup>1</sup></b>
Manure dry matter, % of as collected	95.5 ( $\pm$ 0.94)	95.5 ( $\pm$ 1.29)
Ash (%)	54.4 ( $\pm$ 6.57)	53.8 ( $\pm$ 8.89)
Nitrogen (%)	2.11 ( $\pm$ 0.24)	1.64 ( $\pm$ 0.24)
Calcium (%)	0.89 ( $\pm$ 0.16)	0.93 ( $\pm$ 0.21)
Phosphorus (%)	0.50 ( $\pm$ 0.04)	0.49 ( $\pm$ 0.04)
Potassium (%)	0.97 ( $\pm$ 0.16)	0.77 ( $\pm$ 0.16)
Magnesium (%)	0.38 ( $\pm$ 0.06)	0.39 ( $\pm$ 0.09)

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%). “LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%).

TABLE 13. Estimated nutrient secretion percentages in milk by pens during each trial (winter 2010/2011 and summer 2011; mean percentage) evaluating the effect different concentrations of CP in the diet in nutrient efficiency.

<b>Composition</b>	<b>Experiment 1</b>	
	<b>HP1<sup>1</sup></b>	<b>LP1<sup>1</sup></b>
Milk N / N intake <sup>2</sup> (%)	27.9	30.6
Milk P / P intake <sup>2</sup> (%)	17.1	17.0
Milk K / K intake <sup>2</sup> (%)	12.7	12.8
Milk Ca / Ca intake <sup>2</sup> (%)	17.8	18.6
Milk Mg / Mg intake <sup>2</sup> (%)	6.67	6.67

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%). “LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%).

<sup>2</sup> Nutrient intake estimates are based in actual animal intakes and nutrient content of each component in diet.

## **Conclusion**

Substituting soybean meal based diets (16.7% and 15.4% CP) for dry distillers' grains based diets adjusted for rumen-protected lysine and methionine (diets containing 13.7% and 13.8% CP) in corn silage-based and ryegrass haylage-based diets maintained performance and decreased environmental impact of late-lactation dairy cows yielding 20.6 and 25.4 kg of milk per day respectively.

Animals in a zero metabolizable protein balance had the same performance of animals in positive metabolizable protein balance, a diet commonly used in dairy farms that overfeed protein to animals.

Animals in a negative metabolizable protein balance maintained performance and had a tendency to emit less nitrogen to the environment through manure volatilization when compared to animals in a zero balance of metabolizable protein.

Both, MUN and manure pH, are indicative of less nitrogen loss to the environment when cows were fed a low CP ration with inclusion of DDG when compared to high protein diets using soybean meal as main protein source.

The experiments suggest that efficiency of protein utilization of late-lactation dairy cows can be maintained with low-protein diets based on dry distillers' grains and supplemented with Lys and Met when compared to a soybean meal diet containing higher levels of CP.

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## **CHAPTER 4: EXPERIMENT 2**

### **Materials And Methods**

#### **Introduction**

One experiment was conducted in the summer of 2011 to evaluate performance of cows supplemented with diets based on dry distillers grains and rumen protected amino acids compared with control diets using soybean meal as the main protein source. In the present experiment, treatments were assessed in ryegrass haylage-based TMRs.

#### **Location**

The experiment was conducted at the Louisiana State University Agricultural Center Southeast Research Station (SERS), located in Franklinton, Louisiana, USA (Coordinates 30°50'55"N 90°9'0"W). The city has an elevation of 46.9 meters from sea level and is in a humid subtropical climate (Peel et al., 2007) with long, hot and humid summers and short winters. The experiment was conducted during the summer of 2011. Start date was 06/17/2011 and end date 07/29/2011.

#### **Weather and Environment**

The average weather records of experiment 2 is summarized in table 14.

#### **Cows, Study Design, and Treatments for Experiment 2**

Twenty-four lactating Holstein cows averaging  $244 \pm 55$  DIM and  $30.7 \pm 4.81$  kg milk per day were brought to the same free-stall barn described for experiment 1.

TABLE 14. Weather records for experiment 1 from LSU Southeast Research Station, located at Franklinton, LA. <sup>1</sup>

<b>Experiment 2</b>	<b>Max Air Temp (°C)<sup>2</sup></b>	<b>Min Air Temp (°C)<sup>3</sup></b>	<b>Average Air temp (°C)<sup>4</sup></b>	<b>Rain daily average (mm)</b>	<b>Average Wind Speed (km/h)</b>	<b>Average Relative Humidity</b>
Sampling Period 1 <sup>5</sup>	35.2	21.7	28.5	5.95	5.94	94.5
Sampling Period 2 <sup>6</sup>	30.8	22.8	26.8	9.36	5.70	100.0

<sup>1</sup>Data was taken from LSU Southeast research station weather station (LSU AgCenter, 2011).<sup>2</sup> Max Air Temp: Maximum ambient temperature. <sup>3</sup> Min Air Temp: Minimum ambient temperature. <sup>4</sup> Average Air temp: Average ambient temperature. Sampling period 1 was from 07/01/2011 until 07/07/2011. <sup>6</sup> Sampling period 2 was from 07/22/2011 until 07/28/2011.

Cows were brought to the barn two weeks prior to experiment start to be trained to use the gates. Each cow had a specific transponder on the neck that, when in contact with the respective gate, open it and allow the cow access to the TMR. One cow had access to one gate only. Each group of cows had free access to a water trough linked to a water meter (Recordall model 40, Badger Meter, Milwaukee, Wisconsin). Cows were randomly distributed according to DIM and MY values to keep homogeneity of pens among 4 pens to allow manure collection and sampling and to measure group water intake. Rubber mats strips were used as containment apparatus to prevent manure runoff. The mats were placed on the concrete alley downslope at the limit of each experimental pen. Strips were fixed with nails in the concrete and sealed with insulation foam (Big Gap Filler Insulating Foam Sealant, Dow Chemical, Hayward, CA) to prevent urine and feces losses from the pens.

Fans and water soakers (sprinklers) were used to attenuate heat stress effects in cows. The Calan gate barn is equipped with three fans on each side approximately 11 meters apart. One fan on southeast side was not working so it stayed off for the whole experiment. Each side is equipped with water sprinklers on the entire extension of the barn. Sprinklers were programmed to turn on every 5 minutes when the temperature was over 25°C and every 10 minutes when the temperature was between 20°C and 25°C. The sprinklers did not turn on if the temperature was under 20°C. Sprinklers were distributed in a one sprinkler per gate pattern. They were settled over the gate to soak cow's back and neck. Sprinklers were turned off on the evening of day 18 and remained off on days 19, 20 and 21 for manure sampling. There were two reasons for turning sprinklers off. One

was to reduce risk of manure overflow over the containment apparatus. If the sprinklers were on during the period of manure collection, excess water would overflow the containment apparatus. The other reason was to prevent errant manure pH and dry matter measurements (If water were added to the floor, pH and dry matter measurements would not be correct and could not be used.) Fans remained on during the manure sampling days to help with cow comfort in high temperatures (Table 14).

Each dietary treatment was assigned to two pens (one on the north and one on the south sides) for an experimental period of 21 days. Treatments were shifted to the other two pens for the second experimental period in a crossover design. The two treatments were:

- Control TMR (**HP2**) estimated (NRC, 2001) to contain 15.5% CP with soybean meal as the main protein source and rumen protected methionine (Metasmart®, Adisseo) to balance the amino acid profile;
- Treatment TMR (**LP2**) estimated (NRC, 2001) to contain 13.5% CP, prepared with DDG as the main protein source, supplemented with rumen protected lysine (AminoShure-L®, Balchem) and methionine (Metasmart®, Adisseo) to offset amino acid deficiencies in the diet and balance the amino acid profile according to estimated duodenal amino acid availability.

Diets contained approximately 41% forage as ryegrass haylage to limit NDF content in the TMR. This low amount of ryegrass haylage was necessary as well to reach 13.5% of crude protein in the LP2 diet. Dietary amino acid profiles (Lys and Met)



were corrected according to estimations of amino acid availability and flow in the duodenum (NRC, 2001). Rumen protected Lys (AminoShure-L<sup>®</sup>, Balchem) contained 80% RUP according to product's label. Rumen protected Met contained 50% RUP according to product's label. Dietary ingredients in the diets are presented in Table 15.

## **Diets of Experiment 2**

Diets were fed as TMR. Nutrient contents of feeds were kept the same in the course of the study for each dietary treatment. Batches of grain mix for each diet were mixed once a week at SERS and stored in dedicated grain silos. Grain mix formulations are shown in Table 16. Grain bins were emptied and cleaned prior to experiment beginning. Two bins were used, one for each dietary treatment's grain mix. Premixes of DDG and ruminal protected methionine (Metasmart<sup>®</sup>, Adisseo) (**HP2 Premix**) for control diet and DDG plus ruminal protected lysine (AminoShure-L<sup>®</sup>, Balchem) and methionine (Metasmart<sup>®</sup>, Adisseo) (**LP2 Premix**) for the treatment diet were formulated in the beginning of the experiment and stored in the barn, inside plastic cans holding 68 kg each. Premix formulations are shown in Table 17. Cottonseed, DDG and both dietary treatments' grain mixes were stored in the barn, inside troughs that could keep approximately one ton. When a trough was emptied, it was refilled from grain mix bins or from cottonseed or DDG bays in the station's commodity barn. Ryegrass haylage was planted on 10/14/2010, harvested on 4/5/2011 and wilted for two days before pickup.

TABLE 15. Ingredient contents of diets containing different crude protein levels.<sup>1</sup>

<b>Ingredients (% dry matter)</b>	<b>Adaptation period</b>	<b>HP2<sup>1</sup></b>	<b>LP2<sup>1</sup></b>
Ryegrass haylage	40.4	41.0	41.0
Cottonseed, whole with lint	7.34	6.70	6.70
Corn grain, ground, dry <sup>§</sup>	23.3	32.5	32.5
Dry distillers' grain <sup>§</sup>	10.6	0.00	4.00
Soybean meal (48% CP) <sup>§</sup>	7.96	7.03	0.00
Soybean Hulls <sup>§</sup>	8.11	7.50	10.2
AminoShure-L <sup>2,*</sup>	0.00	0.00	0.25
Metasmart Dry <sup>3,*</sup>	0.00	0.10	0.18
Dry distillers' grain <sup>*</sup>	0.00	2.10	2.10
Calcium Carbonate <sup>§</sup>	0.45	0.34	0.34
Sodium Bicarbonate <sup>§</sup>	0.90	0.90	0.90
SE Sta. custom mineral <sup>4,§</sup>	0.65	1.25	1.25
Salt <sup>§</sup>	0.29	0.58	0.58

<sup>1</sup>"HP2" diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5% CP). "LP2" diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5% CP).

<sup>2</sup> AminoShure-L is a product containing rumen-protected lysine (Balchem Corp., New Hampton, NY).

<sup>3</sup> Metasmart dry is a product containing rumen-protected methionine (Adisseo S.A.S., Antony, France).

<sup>4</sup> SE Sta. Mineral = Southeast Experiment Mineral Concentrate contained Ca = 20%; P = 3%, Mg = 7%; K = 6%; S = 3%; Co = 15 ppm; Cu = 650 ppm; I = 50 ppm; Mn = 1200 ppm; Se = 18 ppm; Zn = 3,700 ppm; vitamin A = 300 KIU/kg; vitamin D = 30 KIU/kg; and vitamin E = 1.5 KIU/kg; manufactured for Kentwood Co-op (Kentwood, LA).

Feedstuffs indicated by the symbol (\*) were added to the TMR mixer as premixes.

Feedstuffs indicated by the symbol (§) were added to the TMR mixer as grain mixes.

Ryegrass was conditioned at cutting with a flail-type mower centerline. Wilted forage was picked up and chopped with a John Deere Forage Harvester 3975 pull-type equipped with haylage head for ryegrass pickup. Ryegrass haylage was taken from only one silo bag for the entire experiment to limit changes in nutrient compositions and dry matter. Weekly ryegrass haylage samples were used for DM adjustment of the diets. These samples were collected daily, stored in a freezer, subsampled and dried on a weekly basis. Each group of cows was offered feeds from a single batch or harvest from the beginning to the end of the experimental period to minimize variability in nutrient composition. Dietary treatments were mixed twice daily using a Data Ranger (American Calan Inc.) at 7:00 am and 1:30 pm. A single TMR batch for each dietary treatment was prepared in the morning and another in the afternoon. All cows from one treatment received the dietary treatment from one batch only, to avoid differences in nutrient intake between them.

### **Sampling, Laboratory Analyses and Data Collection for the Experiment**

Sampling, laboratory analyses and data collection for the experiments were done accordingly with the methodology described in chapter 3 for experiment 1.

### **Statistical Analysis**

Statistical analysis was done accordingly to the procedure explained in chapter 3 of experiment 1.

TABLE 16. Ingredient contents of grain mixes for each dietary treatment.<sup>1</sup>

<b>Ingredients (% dry matter)</b>	<b>HP2<sup>1</sup></b>	<b>LP2<sup>1</sup></b>
Corn grain, ground, dry	65.3	72.1
Soybean meal (48% CP)	14.3	0.00
Soybean hulls	14.8	21.7
Calcium carbonate	0.60	0.68
Sodium bicarbonate	1.60	1.78
SE Sta. custom mineral <sup>2</sup>	2.38	2.63
Salt	1.08	1.18

<sup>1</sup>“HP2” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (15.5%). “LP2” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement.

<sup>2</sup> SE Sta. Mineral = Southeast Experiment Mineral Concentrate contained Ca = 20%; P = 3%, Mg = 7%; K = 6%; S = 3%; Co = 15 ppm; Cu = 650 ppm; I = 50 ppm; Mn = 1200 ppm; Se = 18 ppm; Zn = 3,700 ppm; vitamin A = 300 KIU/kg; vitamin D = 30 KIU/kg; and vitamin E = 1.5 KIU/kg; manufactured for Kentwood Co-op (Kentwood, LA).

TABLE 17. Ingredient contents of premixes for each dietary treatment.<sup>1</sup>

<b>Ingredients (% dry matter)</b>	<b>HP2 premix <sup>1</sup></b>	<b>LP2 premix <sup>1</sup></b>
Dry distillers' grain	95.65	81.74
AminoShure-L <sup>2</sup>	0.00	10.43
Metasmart Dry <sup>3</sup>	4.35	7.83

<sup>1</sup>“HP1 premix”: premix for amino acid delivery for control TMR. “LP1 premix”: premix for amino acid delivery for treatment TMR.

<sup>2</sup> AminoShure-L is a product containing rumen-protected lysine (Balchem Corp., New Hampton, NY).

<sup>3</sup> Metasmart dry is a product containing rumen-protected methionine (Adisseo S.A.S., Antony, France).

## Results And Discussion

### Diets

Experiment 2 had dietary CP contents of  $15.4\% \pm 0.2\%$  and  $13.8\% \pm 0.4\%$ , respectively for HP2 and LP2 (Table 18). Diets were formulated according to balance of MP for cows producing  $30.7 \pm 4.81$  kg of milk per day with  $244 \pm 55$  DIM. Diet HP2 had a balance of MP close to zero (3.90 g/d) of MP (Table 18). Diet LP2 had a negative MP balance (-656 g/d) (Table 18). A negative value indicates a diet containing less MP than NRC (2001) recommended level and could limit animal production. The intention with the two diets was to observe if using a low MP diet with addition of rumen protected amino acids and DDG could maintain animal performance. Dry distillers' grains have a protein profile close to 50% RDP and 50% RUP. Rumen protected Lys (AminoShure-L®, Balchem) has 80% total CP as RUP. The estimated (NRC, 2001) RUP was 5.35% and 4.71% of total DM respectively for HP2 and LP2. Estimated RDP was 10.2% and 8.91% of total DM respectively for HP2 and LP2. Phosphorus contents were at the recommended levels (NRC, 2001) with 0.40% and 0.41% of ration DM. Calcium concentration was 0.74 and 0.73% of DM for HP2 and LP2. The ratio Ca : P for the treatments was 1.8. Estimated content of available Lys at the duodenum was 193 g/d and 184 g/d for HP2 and LP2 respectively. Estimated content of available Met at the duodenum was 63 g/d for HP2 and LP2.

Net energy for lactation and MP contents of the diets were not limiting. The  $N_{El}$  content allowed for 40.7 kg/d of milk yield and MP allowed 36.0 kg/d of milk yield for HP2 (NRC, 2001). Diet LP2  $N_{El}$  supplied enough energy for 40.3 kg/d and MP for 29.0 kg/d of milk yield (NRC, 2001).

Broderick et al. (2008b) had levels of duodenal Lys ranging from 154 g/d to 172 g/d in trial 1 and 144 g/d to 155 g/d in trial 2. Low Lys treatment in trial 1 resulted in lower milk yield. According to the authors, values of NRC (2001) estimated digestible Lys less than 160 g/d can decrease milk yield for cows producing around 40kg of milk/d. Addition of rumen-protected Lys cannot compensate for reduction in dietary CP in some cases and results in lower animal performance. Leonardi et al. (2003) had Lys available in the duodenum ranging from 139 g/d to 211 g/d from the lower protein diet with addition of methionine to the higher CP diet plus methionine. The authors found no difference in MY between treatments. Results seem to be variable in the literature and depend on several variables including type of forage and feed used, variability in RDP and RUP between feeds and animal variability (Broderick et al., 2008b, Leonardi et al., 2003; Cabrita et al., 2011; Olmos Colmenero and Broderick, 2006).

### **Dry Matter Intake and Water Intake**

There was no significant difference in DMI in experiment 2. In experiment 2, cows in HP2 ingested 21.4 kg/d of DM. Cows in LP2 had a mean intake of 20.9 kg/d of DM ( $P=0.51$ ) (Table 19). Animals in diets with low amount of protein are expected to increase intake to be able to reach the requirements of the nutrient (Conrad, 1966).

If the DMI is the same and cow performance is different, the animals may be limited by high amounts of fiber or energy in the diet. If the performance is maintained, the animals may not have a high requirement or might not need the

amount of protein of the high protein diet. In this experiment, DMI was not significantly different. Leonardi et al. (2003) feeding cows with different concentrations of crude protein (16.1% CP as low protein content and 18.9% CP as high protein content) in the diets in a factorial design with or without addition of methionine (8% addition in DM basis) found no differences in DMI among treatments. The authors used cows with approximately 42 kg of milk yield/d. Hollmann et al. (2011b) found that dry matter intake response to CDG increased linearly with increasing dietary CP concentration of control diets ( $R^2 = 0.22$ ;  $P$  model  $< 0.01$ ).

A concentration of CP greater than 16.5%, dry basis, was related to an increase in DMI of cows fed CDG diets, whereas a lower concentration was associated with a negative DMI response.

Hollmann et al. (2011a) had a positive DMI response to CDG in the diet when forage concentration in the diet was less than 49% in dry matter basis. In addition, a linear relationship between corn silage concentration and DMI response to CDG existed ( $R^2 = 0.09$ ;  $P = 0.04$ ;  $n = 44$ ).

Broderick et al. (2008b) described two trials designed to study rumen-protected Met supplementation and reducing dietary protein as follows: 18.6% CP and 0 g of RPM/d; 17.3% CP and 5 g of RPM/d; 16.1% CP and 10 g of RPM/d; or 14.8% CP and 15 g of RPM/ d. The authors found no differences in DMI in one of the trials. In the other trial there was a tendency for greater DMI using diets with 17.3%

TABLE 18. Nutrient contents of each experimental diet of experiment 2

<b>Composition</b>	<b>Adaptation Period<sup>1</sup></b>	<b>HP2<sup>2</sup></b>	<b>LP2<sup>2</sup></b>
Dry matter, % as fed	62.4	50.8 (±0.01)	50.6 (±0.01)
Crude protein, % DM	17.0	15.4 (±0.002)	13.8 (±0.004)
MP balance <sup>3</sup> (g/day)	-	3.9	-656
RUP <sup>4</sup> , % DM	-	5.35	4.71
RDP <sup>4</sup> , % DM	-	10.2	8.91
Neutral detergent fiber, % DM	44.1	33.0 (±0.59)	33.9 (±0.47)
Acid detergent fiber % DM	27.8	22.5 (±0.41)	23.0 (±0.33)
Calcium % DM	0.64	0.74 (±0.003)	0.73 (±0.002)
Phosphorus % DM	0.43	0.40 (±0.02)	0.41 (±0.02)
Potassium % DM	1.51	1.59 (±0.02)	1.47 (±0.02)
Magnesium % DM	0.25	0.26 (±0.002)	0.24 (±0.001)
Lysine g /day <sup>5</sup>	-	193	184
Methionine g/day <sup>5</sup>	-	63.0	63.0

<sup>1</sup> Adaptation period diet contents were estimated by NRC model (NRC, 2001).

<sup>2</sup>“HP2” diet indicates dietary protein concentration above NRC (2001) predicted requirement. “LP2” diet indicates dietary protein concentration below NRC (2001) predicted requirement.

<sup>3</sup> “MP balance”: Total metabolizable protein required in diet minus total metabolizable protein supplied in diet

<sup>4</sup> “RUP”: Rumen undegraded protein “RDP”: Rumen degraded protein; RUP and RDP were estimated according to NRC (2001) model.

<sup>5</sup> Amino acids are shown in g/day of availability in the duodenum according to NRC (2001) model.



and 16.1% of CP with 10 g of RPM added to the lower protein diet, but this trial had DMI lower than expected and the cows were in a negative balance for the three lower protein levels.

No significant difference ( $P=0.47$ ) was found in experiment 2 for WI with diets HP2 and LP2 (Table 19). Experiment 2 had a difference of only 1.5% in CP between treatments. Several factors may increase or decrease water intake. Ambient temperature, presence or absence of heat abatement procedures such as fans and water soakers, intake of ions ( $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$ ) and concentration of protein in the diet (NRC, 2001) are some of the causes. Obitisu et al. (2011) found no differences in water intake (96.9 and 89.8 L/d,  $P=0.36$ ) when cows were exposed to different ambient temperatures (18°C and 28°C). Dinn et al. (1998) feeding cows with different levels of CP (18.3, 16.7, and 15.3%) found differences in water intake between the treatment with 18.3% of CP and 15.3% of CP. According to the authors, the greater intake of CP increases urea production in the liver and consequently urinary urea excretion. This increased excretion is accompanied by water excretion. Hence, animals intake more water to compensate the greater water excretion. Treatment of 16.7% CP was equal to the others.

### **Milk Yield**

Experiment 2 had mean milk yield for dietary treatment HP2 of 26.4 kg/d and yield for LP2 of 24.4 kg/d. with no significant difference between treatments ( $P=0.19$ ) (Table 20).

Silva et al. (2009) carried out an experiment with cows producing 12 kg of milk per day using two treatments: a high protein diet with 13% of CP in the TMR and a low protein diet with 11% of CP in the TMR. With 13% of CP the cows were ingesting 275 g/d of N and the animals in the 11% CP diet were ingesting 248 g/d of N. In this experiment, milk yield did not change as a result of nitrogen levels in the diet. However, Arriaga et al. (2010) found significant differences in milk yield (18.2 kg/day versus 21.4 kg/day,  $P<0.05$ ) with cows in a low CP diet and a higher CP diet with different intake of nitrogen (405 grams/day versus 498 grams/day of ingested N respectively,  $P<0.05$ ). In this experiment, the authors did not find any difference in milk nitrogen excretion (7.7 mg/dl vs 8.2mg/dl,  $P=0.82$ ) but found differences in urine and feces nitrogen excretion, (128.5 g/day vs 153.5 g/day,  $P<0.05$  and 144.8 g/day vs 162.8 g/day,  $P<0.05$ ), for low protein intake and high protein intake respectively). With high-producing cows, lowering dietary CP may, in certain situations, result in decreased milk yield (Broderick, 2003), which would be unacceptable to most producers and nutritionists in the field (Agle, 2010). Leonardi et al (2003) found no difference ( $P=0.51$ ) in milk yield among treatments with addition of Met (41.0 kg/d and 42.8 kg/d,  $P=0.51$ ) and between treatments with Met and without Met ( $P=0.63$ ). One trial with the same design of Leonardi et al (2003), similar amount of Met added, and similar dietary CP, using SBM, showed a significant increase in milk yield (32.9 kg for control diet without Met and 35.2kg for the diet with Met added,  $P<0.01$ ) (Illg et al., 1987).

TABLE 19: Water intake and dry matter intake from late lactation cows fed two different levels of dietary crude protein levels in experiment 2.

Treatment	HP2 <sup>1</sup>	LP2 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Water consumption, liters/cow/day	118	112	6.46	0.47	0.41	0.22
Dry matter intake, kg/cow/day	21.4	20.9	0.78	0.51	0.75	0.64

<sup>1</sup>“HP1” diet indicates dietary protein concentration close to NRC (2001) predicted requirement. “LP1” diet indicates dietary protein concentration below NRC (2001) predicted requirement.

<sup>2</sup> “SEM”: Standard error for the mean.

Increase in MY may be a response to a better AA profile reaching the duodenum and being absorbed, but results are conflicting. No increase in milk yield was observed when dietary CP was either 15.7 or 16.2% and 17 g/d of methionine was supplemented (Overton et al., 1998), or when dietary CP was 19.5% and 10.5 g/d of methionine was supplemented (41.5 kg/d of milk) (Armentano et. al., 1997). Dinn et al. (1998) found higher milk yield (34.2 kg/d of milk) for cows fed a high protein diet (18.3% CP) compared with other diets (32.8 kg/d of milk)(16.7% CP and 15.3% CP). Diets with SBM are low in Met and need supplementation to improve this profile. There is a high variability between experiments. Differences in feed and animal management forage quality and bioavailability of each nutrient in the diet may affect utilization and differentiate results between treatments.

Hollmann et al. (2011a) published a meta-analysis of published studies on CDG utilization with different percentages of CDG in the diet to evaluate its effects on cow performance. The authors found that MY was quadratic ( $P_{\text{quadratic}} = 0.02$ ) in response to increasing CDG concentration ( $R = 0.15$ ;  $P = 0.04$ ) with a maximum of 1.2 kg of milk per cow per day at 21% CDG, dry basis. This response was pronounced in experiments using high-producing cows, but did not occur in experiments with low-producing cows. The two experiments presented in this thesis did not use large proportions of distillers' grains (15.2% of DDG in DM in LP1 and 6.1% of DDG in DM in LP2).

Broderick et al. (2008b) did two 4 x 4 Latin square trials (4-wk periods; 16 wk total) with decreasing concentrations of crude protein in the diet supplemented with RPM. The authors found significant differences in MY and 3.5% FCM. Yields of

milk and FCM were greater at 17.3% CP plus 5 g/d of RPM and 16.1% CP plus 10 g/d of RPM than on the other 2 diets (18.6% CP and 0 g of RPM and 14.8% CP and 15 g of RPM). According to the authors, the best Lys : Met profile available for absorption at the duodenum occurred at 17.3% and 16.1% of CP in diet and the increased milk yield might be a result of this closer profile. According to the NRC (2001), a 3:1 ratio of Lys to Met may improve performance. In our experiments, the ratio of low protein diets LP1 and LP2 were respectively 3.04 and 2.93. Diets HP1 and HP2 had ratios of 3.53 and 3.03 respectively. Diets used in both studies described in this thesis were near or above the ratio recommended in the NRC (2001)

Broderick et al. (2008b) found that digestible Lys became limiting when the estimated supply fell from 162 to 154 g/d resulting in a drop in milk yield from 41.6 to 39.7 kg/ d ( $P < 0.05$ ) between the diets containing 16.1 and 14.8% CP.

According to the NRC (2001), milk yield responses to Lys and Met are more common in cows during early lactation than in mid or late lactation dairy cows. Production responses to increased supplies of Lys and Met in MP typically are greater when CP in diet DM approximates normal levels (14 to 18 percent) than when it is lower or higher (NRC, 2001).

### **Milk Components**

Experiment 2 had no significant difference between diets in milk fat (3.49%, 0.97 kg/d for HP2 and 3.47%, 0.90 kg/d for LP2,  $P = 0.83$  and  $P = 0.20$  respectively), milk protein (3.34%, 0.92 kg/d for HP2 and 3.25, 0.84 kg/d for LP2,  $P = 0.30$  and

P=0.12 respectively) and milk lactose (4.72%, 1.34 kg/d for HP2 and 4.69%, 1.25 kg/d for LP2, P=0.72 and P=0.31 respectively) (Table 20). There was a significant difference in fat percentage between experimental periods for this experiment (3.25% and 3.70%, P<0.01). Fat yield had no difference between experimental periods (0.91 kg/d and 0.95 kg/d, P=0.47). This might have happened as a result of increase in DIM, although there was no difference in milk yield between periods. It is common for animals in greater DIM have an increase in concentration of milk components. In this case it happened unexpectedly only with milk fat.

Dinn et al. (1998) found no differences in milk fat output, milk protein percentage and milk protein output among experimental periods when using 3 levels of CP in the diet (18.3%, 16.7% and 15.3% of CP). Milk fat concentration and yield in milk may decrease as a result of addition of DDG. Diets with high concentration of fat may decrease fiber digestibility and consequently decrease fat content in milk (NRC, 2001). DDG diets have higher concentrations of fat when compared to SBM diets. Fat content from DDG may range from 10.2% to 11.7% (Spiehs et al., 2002). Hollmann et al. (2011a) found in his meta-analysis that milk fat concentration in all included experiments was not related to dietary concentration of CDG when experimental treatments had less than 21% CDG and adequate levels of effective fiber (eNDF).

Hollmann et al. (2011b) found that milk true protein concentration response to dietary CDG was related negatively to dietary CDG concentration ( $R^2 = 0.15$ ;  $P = 0.02$ ). In our experiments, DDG was used as 15.2% in LP1 and 6.1% in LP2. These

concentrations of corn distillers' grains in the diet would not reduce milk true protein content according to the authors.

Studies put together by NRC (2001) indicate that content of protein in milk is more responsive than milk yield to supplemental Lys and Met, particularly in post-peak lactation cows. Increases in milk protein percentage are independent of milk yield. Casein is the most influenced milk protein fraction. Increases in milk protein production to increased supplies of either Lys or Met in MP are the most predictable when the resulting predicted supply of the other AA in MP is near or at estimated requirements.

A number of studies have shown that supplementing lactating dairy cows with RPM has improved milk protein synthesis. Feeding RPM increased milk concentrations of total protein (Armentano et al., 1997; Berthiaume et al., 2006), true protein (Berthiaume et al., 2006), and casein N (Overton et al., 1998), and yields of milk (Schmidt et al., 1999), total protein (Armentano et al., 1997), and true protein (Rulquin and Delaby, 1997). These experiments did not use negative balance of MP.

In experiment 2, milk urea nitrogen had a significant difference between diets with 9.85 mg/dL in HP2 and 6.40 mg/dL in LP2 ( $P < 0.01$ ) (Table 20). HP2 had a zero MP balance according to cows' requirements. Both values are lower than the values considered normal for the authors of this experiment.

Leonardi et al. (2003) feeding cows with different concentrations of crude protein (16.1% CP as low protein content and 18.9% CP as high protein content) in

the diets in a factorial design with or without addition of methionine (8% addition in DM basis) found a significant difference between protein levels in MUN. In that study, the authors found a significantly lower content of MUN in the low CP diets (4.44 g/d for low CP diet without methionine and 4.29 g/d for low CP diet with methionine included) and a higher content in the high CP diets (5.96 g/d in the high CP diet without methionine and 6.10 g/d for high CP diet with methionine included) ( $P < 0.01$ ), but MUN was not affected by Met inclusion ( $P = 0.98$ ).

Burgos et al. (2010) studied the relationship between ammonia emissions from dairy cattle manure and MUN and dietary crude protein content affecting these two variables. According to the authors, there is a relationship between MUN and manure emissions of ammonia ( $R^2 = 0.85$ ). A shift from 9 mg/dL to 17 mg/dL of MUN could increase ammonia emissions from 70 g/d of N per cow to 110 g/d of N per cow.

Broderick et al. (2008b) found reduced ( $P \leq 0.01$ ) MUN when protein content in his trial decreased from 18.6% CP to 17.3% CP, 16.1% CP and 14.8% of CP with RPM added and was paralleled with increased apparent N efficiency (milk N : N intake). Broderick et al. (2008b) found a significant ( $P \leq 0.01$ ) negative linear relationship between dietary CP content and MUN and N efficiency. Apparent N efficiency improved by nearly 8 percentage units from the highest to lowest CP, and was greatest ( $P \leq 0.01$ ) on the diet containing the least CP and most RPM. However, the highest N efficiency on 14.8% CP occurred along with lost yields of milk and milk components relative to the 2 intermediate diets. The greatest N efficiency, accompanied by production and feed efficiency similar or equal to the highest



observed in this trial, occurred on the RPM- supplemented diet containing 16.1% CP.

Broderick et al. (2008b) found in one trial using different concentrations of CP in the diet that apparent N balance, computed from observed N intake and milk N secretion (milk N = milk protein/6.38), and estimated manure N excretion showed a significant diet effect ( $P \leq 0.01$ ); only cows fed the RPM – supplemented diet with 14.8% CP were in apparent negative N balance. In this trial, the authors concluded that if N utilization was considered optimal on the diet with 16.1% CP plus RPM, then the 72 g/d reduction in total urinary N compared to that on the 18.6% CP would correspond to approximately 22 kg/cow of N over 300 days lactation. Fecal N excretion was unaffected by the diet.

### **Body Weight and Body Condition Score**

Changes in body weight (**BW**) and body condition score (**BCS**) were also not significantly affected by reducing protein in this study (Table 21), although those observations should be analyzed with caution because diets were only fed for 21 days.

Changes in body weight and body condition score may require longer periods to be detectable. In this experiment, there was a significant difference between BCS from period 1 to period 2 (0.20 and -0.14 for period 1 and 2 respectively,  $P < 0.01$ ) and a trend to decrease body weight from period 1 to period 2 (-4.58 kg/period to -27.5 kg/period). Body condition score is a difficult measurement and is not quantitative. It depends on who is measuring and if it is done with the same criteria over time. When animals use their reserves of fat and

protein to produce milk, as a result of a deficient diet, they can lose BCS.

Broderick et al. (2008b) found no difference in body weight gain in the 2 trials with reducing levels of CP and addition of RPAA in diet. Cabrita et al. (2011) found a significant difference in body weight between treatments with different CP levels ( $P < 0.05$ ). They were lower in the low CP diet (14%) compared to the high CP diet (16%). There was no difference comparing diets with same level of CP with or without addition of RPAA in that study.

### **Manure Analyses**

In experiment 2, manure pH was significantly higher for HP2 than LP2 (7.50 and 7.13 respectively for HP2 and LP2,  $P = 0.05$ ) (Table 22). Manure temperature was not affected by treatments.

Burgos et al. (2010) found in his experiment to estimate amount of N in manure that initial urea N concentration increased linearly with dietary CP from 153.5 to 465.2 mg/dL in manure slurries from cows fed 15 to 21% CP diets. Increased amount of urea in manure results in an increase of the pH due to N atom being in the form of  $\text{NH}_3$  and  $\text{NH}_4^+$ .

There was no difference in dry matter content of manure between dietary treatments in this experiment as expected because animals had no difference in water intake between treatments (Table 23). Maltz and Silanikove (1996) demonstrated that cows strive to maintain urinary osmotic load. Cows spend energy to transform excess ammonia to urea in the liver, which is excreted in urine. When excess protein is fed, more urea is produced.

TABLE 20. Milk yield and milk composition from late lactation cows fed two different levels of dietary crude protein levels<sup>1</sup> for experiment 2.

Treatment	HP2 <sup>1</sup>	LP2 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Milk yield, kg/cow/day	26.4	24.4	1.30	0.19	0.44	0.74
Fat, %	3.49	3.47	0.06	0.83	0.47	<0.01
Fat, kg	0.97	0.90	0.05	0.20	0.51	0.47
Protein, %	3.34	3.25	0.06	0.30	0.53	0.32
Protein, kg	0.92	0.84	0.04	0.12	0.45	0.34
Lactose, %	4.72	4.69	0.07	0.72	0.57	0.59
Lactose, kg	1.34	1.25	0.06	0.31	0.34	0.17
MUN <sup>3</sup>	9.85	6.40	0.22	<0.01	0.59	0.20

<sup>1</sup>“Control” diet indicates dietary protein concentration above NRC (2001) predicted requirement. “Treatment” diet indicates dietary protein concentration below NRC (2001) predicted requirement.

<sup>2</sup> “SEM”: Standard error for the mean. <sup>3</sup> MUN = milk urea nitrogen.

That may require greater urine volume (thus, greater water ingestion) to maintain urine osmolality, assuming the consumption of ions ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ ) remains similar.

Leonardi et al. (2003) found no effects of methionine supplementation on urine volume or urinary N excretion. When CP level increased from 16.1 to 18.9%, urine output increased from 21.8 to 24.6 L/d. Dinn et al. (1998) measured urine output through total collection and reported that urine output increased approximately 2 L/d for every 1 percentage unit increment in dietary protein. Dinn et al. (1998) and Cressmann et al. (1980) reported an increment of approximately 70 g/d of urinary N when increasing dietary protein by 1.6 or 2.6 percentage units, respectively.

Experiment 2 had similar concentrations of N in manure (Table 23) but the pH was significantly different (7.50 for HP2 and 7.13 for LP2,  $P=0.05$ ) (Table 22).

Broderick et al. (2008b) found a significant ( $P < 0.01$ ) linear relationship between dietary CP content (18.6%, 17.3%, 16.1% and 14.8% of CP) and urinary urea N and total N. Reducing dietary CP concentration resulted in highly significant ( $P < 0.01$ ) reductions in estimated urine volume and proportion of urea N in total urinary N, which fell from 78 to 53%. As dietary CP decreased from 18.6 to 14.8%, urea N and total N excreted in the urine, as estimated by spot urine sampling, declined by, respectively, 125 and 110 g/d. The authors suggested that this lower N output was a result of lower urea N. Studies from Broderick (2003) and Olmos Colmenero and Broderick (2006) have observed a similar correspondence between

TABLE 21. Body weight and body condition score changes<sup>3</sup> from late lactation cows fed two different levels of dietary crude protein levels<sup>1</sup> for experiment 2.

Treatment	HP2 <sup>1</sup>	LP2 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Body weight gain, kg/cow/period	-18.1	-14.0	7.57	0.71	0.28	0.10
Body condition score, units/period	0.03	0.04	0.05	0.77	0.47	<0.01

<sup>1</sup>“HP2” diet indicates dietary protein concentration above NRC (2001) predicted requirement. “LP2” diet indicates dietary protein concentration below NRC (2001) predicted requirement. <sup>2</sup> “SEM”: Standard error for the mean. <sup>3</sup> Changes in body weight and body condition score are calculated as changes from beginning of experiment and end of each experimental period.

the reductions in urea N and total N in the urine when using the same methodology.

Manure volatilization data is summarized in Table 25 for experiment 2. No difference was found between treatments in N volatilization for all mineral markers analyzed in this experiments ( $P>0.37$ ).

Data had some negative values in N : K ratios calculated before analyzing the data with SAS. Manure was not perfectly retained by the mats and some runoff was visually detected. Despite repeated attempts to caulk the gaps between mats and concrete alleys, spills continued to occur. Nitrogen and minerals were probably lost in result of retention failure.

Negative values of volatilized nitrogen as estimated by ratios of mineral markers can happen for two reasons: incorporation of nitrogen to the manure or loss of mineral markers (P, K, Ca, Mg) in scraped manure. Potassium might have been lost because this mineral is highly soluble in water. According to Nennich et al. (2005) and Nennich et al. (2006), 75% of total excreted K is solubilized in urine. Urine in contact with feces may solubilize more potassium and be leached away with urine and N. Volatilization based on Mg data shows values of volatilized nitrogen closed to what was expected (around 25% of nitrogen volatilization).

In experiment 2 the conversion rate of dietary protein to milk protein was 31.5% for HP2 and 31.7% for LP2 (Table 26). Leonardi et al. (2003) found that feeding the higher protein diets resulted in a conversion of dietary protein into milk protein of 30.3% vs. 37.1% for the lower protein diets. The authors found that for the higher CP diets, 44% of the N excreted was excreted as urine and milk urea compared with 38% ( $P < 0.01$ ) with the lower protein diets. The conversion rate in

experiment 2 was similar between two levels of protein in the diet. The control diet (HP2) had MP balance of zero and the low crude protein diet (LP2) had a negative balance of MP. These values can explain the similar conversion rates observed in our experiment 2.

Hristov (2009) found that 26% of the N consumed was secreted in milk. Meta-analyses of large North American and North European datasets (Hristov et al., 2005a; Huhtanen and Hristov, 2009) found similar results. N : P ratios were close to ranges reported by Moreira and Satter (2006) (5.61–9.24) and Hristov et al. (2009) (6.57) for experiment 2.

Percentages of total milk secreted minerals over total mineral intake are summarized in table 23. Diets of experiment 2 were at recommended levels in phosphorus contents (NRC, 2001) and had secretion rates higher than reports from Hristov et al. (2009).

K values of milk secretion percentages over K intake were lower than the ones in Hristov et al. (2009). Values of minerals in milk were not analyzed but estimated according to NRC (2001).

### **Conclusion**

Substituting soybean meal based diets (16.7% and 15.4% CP) for dry distillers' grains based diets adjusted for rumen-protected lysine and methionine (diets containing 13.7% and 13.8% CP) in corn silage-based and ryegrass haylage-based diets maintained performance and decreased environmental impact of late-lactation dairy cows yielding 20.6 and 25.4 kg of milk per day respectively.

TABLE 22: Manure temperature and manure pH measured onsite from pens with late lactation cows fed two different levels of dietary crude protein levels in experiment 2.

Treatment	HP2 <sup>1</sup>	LP2 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Manure Temperature	25.0	24.9	0.23	0.68	0.50	<0.01
Manure pH	7.50	7.13	0.07	0.05	0.55	0.73

<sup>1</sup>“HP2” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (15.5%).

“LP2” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%).

<sup>2</sup>“SEM”: Standard error for the mean.



TABLE 23. Manure analysis for each trial (winter 2010/2011 and summer 2011; means  $\pm$ SD) evaluating the effect of two levels of protein in the diet of lactating dairy cows on manure composition and physical-chemical properties

<b>Composition</b>	<b>Experiment 2</b>	
	<b>HP2<sup>1</sup></b>	<b>LP2<sup>1</sup></b>
Manure dry matter, % of as collected	97.2 ( $\pm$ 0.13)	97.2 ( $\pm$ 0.14)
Ash (%)	75.9 ( $\pm$ 9.80)	74.0 ( $\pm$ 11.8)
Nitrogen (%)	0.93 ( $\pm$ 0.13)	0.93 ( $\pm$ 0.15)
Calcium (%)	0.33 ( $\pm$ 0.05)	0.34 ( $\pm$ 0.07)
Phosphorus (%)	0.18 ( $\pm$ 0.08)	0.20 ( $\pm$ 0.05)
Potassium (%)	0.67 ( $\pm$ 0.18)	0.70 ( $\pm$ 0.19)
Magnesium (%)	0.17 ( $\pm$ 0.02)	0.18 ( $\pm$ 0.04)

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%). “LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%).

“HP2” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (15.5%). “LP2” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%).

TABLE 24. Actual milk yield and estimated nutrient intake and excretion by pens during each trial (winter 2010/2011 and summer 2011; means  $\pm$ SD) evaluating the effect of manure scraping on N volatilization from a freestall barn.

<b>Composition</b>	<b>Experiment 2</b>	
	<b>HP2<sup>1</sup></b>	<b>LP2<sup>1</sup></b>
DMI <sup>2</sup> , kg/d	21.4 ( $\pm$ 2.8)	20.9 ( $\pm$ 3.1)
N intake <sup>2</sup> , kg/d	0.53 ( $\pm$ 0.085)	0.46 ( $\pm$ 0.09)
P intake <sup>2</sup> , kg/d	0.09 ( $\pm$ 0.001)	0.08 ( $\pm$ 0.003)
K intake <sup>2</sup> , kg/d	0.34 ( $\pm$ 0.002)	0.31 ( $\pm$ 0.02)
Ca intake <sup>2</sup> , kg/d	0.16 ( $\pm$ 0.002)	0.15 ( $\pm$ 0.007)
Mg intake <sup>2</sup> , kg/d	0.06 ( $\pm$ 0.001)	0.05 ( $\pm$ 0.002)
Milk yield, kg/d	26.4 ( $\pm$ 4.96)	24.3 ( $\pm$ 4.10)
Milk N, kg/d	0.17 ( $\pm$ 0.01)	0.15 ( $\pm$ 0.07)
Milk P, kg/d	0.02 ( $\pm$ 0.005)	0.02 ( $\pm$ 0.003)
Milk K, kg/d	0.04 ( $\pm$ 0.001)	0.04 ( $\pm$ 0.004)
Milk Ca, kg/d	0.03 ( $\pm$ 0.001)	0.03 ( $\pm$ 0.003)
Milk Mg, kg/d	0.004 ( $\pm$ 0.001)	0.004 ( $\pm$ 0.004)
Excreted N <sup>3</sup> , kg/d	0.36( $\pm$ 0.01)	0.31 ( $\pm$ 0.01)
Excreted P <sup>3</sup> , kg/d	0.06 ( $\pm$ 0.002)	0.06 ( $\pm$ 0.002)
Excreted K <sup>3</sup> , kg/d	0.30 ( $\pm$ 0.003)	0.27 ( $\pm$ 0.01)
Excreted Ca <sup>3</sup> , kg/d	0.13 ( $\pm$ 0.002)	0.12 ( $\pm$ 0.004)
Excreted Mg <sup>3</sup> , kg/d	0.01 ( $\pm$ 0.001)	0.01 ( $\pm$ 0.002)
Excreted N:P <sup>4</sup>	5.84 ( $\pm$ 0.10)	5.14 ( $\pm$ 0.02)
Excreted N:K <sup>5</sup>	1.20 ( $\pm$ 0.01)	1.17 ( $\pm$ 0.04)
Excreted N:Ca <sup>6</sup>	2.89 ( $\pm$ 0.05)	2.64 ( $\pm$ 0.04)
Excreted N:Mg <sup>7</sup>	7.14 ( $\pm$ 0.15)	6.79 ( $\pm$ 0.19)

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%). “LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%). “HP2” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (15.5%). “LP2” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%). <sup>2</sup> Nutrient intake estimates are based in actual animal intakes and nutrient content of each component in diet <sup>3</sup> Intake of nutrient minus nutrient excreted in milk. <sup>4</sup> Ratio between excreted N and excreted P. <sup>5</sup> Ratio between excreted N and excreted K. <sup>6</sup> Ratio between excreted N and excreted Ca. <sup>7</sup> Ratio between excreted N and excreted Mg.

TABLE 25: Estimated volatilized N percentages using method described in Moreira and Satter (2006) in experiment 2 evaluating the effect different dietary CP concentrations in the diet on N volatilization from a free-stall barn.

Treatment	HP2 <sup>1</sup>	LP2 <sup>1</sup>	SEM <sup>2</sup>	Effect (P > F)		
				Treatment	Sequence	Period
Volatilized N (P) <sup>3</sup>	13.2	14.4	6.18	0.90	0.29	0.37
Volatilized N (K) <sup>4</sup>	-37.6	-27.6	33.9	0.83	0.81	0.47
Volatilized N (Ca) <sup>5</sup>	6.59	0.45	6.70	0.55	0.84	0.16
Volatilized N (Mg) <sup>6</sup>	24.6	25.5	5.14	0.91	0.58	0.21

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%). “LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%). <sup>2</sup> “SEM” Standard error of the mean predicted requirement (13.5%).<sup>3</sup> Values calculated using N : P ratio and presented as a % of excreted N. <sup>4</sup> Values calculated using N : K ratio and presented as a % of excreted N. <sup>5</sup> Values calculated using N : Ca ratio and presented as a % of excreted N. <sup>6</sup> Values calculated using N : Mg ratio and presented as a % of excreted N.

TABLE 26. Estimated nutrient secretion percentages in milk by pens during each trial (winter 2010/2011 and summer 2011; mean percentage) evaluating the effect different concentrations of CP in the diet in nutrient efficiency.

<b>Composition</b>	<b>Experiment 2</b>	
	<b>HP2<sup>1</sup></b>	<b>LP2<sup>1</sup></b>
Milk N / N intake <sup>2</sup> (%)	31.5	31.7
Milk P / P intake <sup>2</sup> (%)	27.9	27.4
Milk K / K intake <sup>2</sup> (%)	11.7	12.5
Milk Ca / Ca intake <sup>2</sup> (%)	20.9	20.7
Milk Mg / Mg intake <sup>2</sup> (%)	6.67	6.67

<sup>1</sup>“HP1” diet indicates control TMR with dietary protein concentration above NRC (2001) predicted requirement (16.5%). “LP1” diet indicates treatment TMR with dietary protein concentration below NRC (2001) predicted requirement (13.5%).

<sup>2</sup> Nutrient intake estimates are based in actual animal intakes and nutrient content of each component in diet.

Animals in a zero metabolizable protein balance had the same performance of animals in positive metabolizable protein balance, a diet commonly used in dairy farms that overfeed protein to animals.

Animals in a negative metabolizable protein balance maintained performance and had a tendency to emit less nitrogen to the environment through manure volatilization when compared to animals in a zero balance of metabolizable protein.

Both, MUN and manure pH, are indicative of less nitrogen loss to the environment when cows were fed a low CP ration with inclusion of DDG when compared to high protein diets using soybean meal as main protein source.

The experiments suggest that efficiency of protein utilization of late-lactation dairy cows can be maintained with low-protein diets based on dry distillers' grains and supplemented with Lys and Met when compared to a soybean meal diet containing higher levels of CP.

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## **APPENDIX: RECOMMENDATIONS FOR FURTHER RESEARCH IN AREA**

Manure was not well retained in the barn in both experiments. Nitrogen volatilization had negative results because of mineral and nitrogen leakage. Insulation of mats and attempting to avoid leak of liquid material was not successful. Further research should be done using the same installations. One recommendation is use of concrete waves in the floor. Assembling a wave in a smooth way would be recommended so no leakage would occur from one pen to another or to outside and cows would be able to pass through it with no problems. This would be a permanent construction. Other problem would be with slippage. If the wave has a strong slope, cows may slip and fall.

Other recommendation is use of troughs under the concrete level after each pen. A metal grill could be used over them to prevent injuries in animals. Liquid phase of manure would leak to that trough. When manure was scraped and mixed, the trough could be taken out of the whole in the floor and the liquid phase would be mixed with the solid one. The negative aspect of this would be a smaller nitrogen volatilization. The liquid phase would not stay in contact with feces and would have less urease to react with. The trough would have a smaller area of contact with air and the air over it would stay saturated with ammonia, decreasing even more its' volatilization.

The use of fans in the summer increases evaporation of water and may increase the amount of nitrogen volatilized. Minerals that were solubilized in water would stay in the floor and, if no water passes through it while scraping the floors,

the mineral will stay there and not be mixed in the manure sample. Further research could be done in winter or seasons that don't have much heat stress so fans would not be needed. The use of a trough in the floor would decrease this problem as well.

## **VITA**

Andre de Barros Duarte Pereira was borne in Alfenas, state of Minas Gerais, Brazil, in 1984. He is son of Marcio Soares Pereira and Silvana Barros Duarte Pereira. Andre is the younger of two sons. He moved to Belo Horizonte, capital of Minas Gerais state, when he was 12 years old. Andre did his high school in Colegio Loyola, a Catholic private school, and graduated in December 2002. He was approved to school of veterinary medicine of federal university of Minas Gerais in January of 2005. Andre earned his bachelor's in veterinary medicine in January 2010 and received his DVM license in March 2010. He begun a Master of Science program in animal nutrition in the same university and did 6 months of it before being approved to Louisiana State University in the school of animal sciences master of science program. Andre is a Master of Science candidate.