

APPLICATION OF RUMEN-PROTECTED LYSINE TO LOWER CRUDE PROTEIN DIETS
FOR LACTATING DAIRY COWS

by

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B.S., Kansas State University, 2009

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

Approved by:

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Abstract

The study objective was to evaluate the application of supplemental rumen-protected lysine (RP Lys) to maintain milk production when reducing the crude protein levels in a lactating dairy cow diet. Twelve lactating multiparous Holstein cows, averaging 129 DIM, 50.2 kg milk yield, 3.6% fat and 2.9% true protein were randomly assigned to one of four 3x3 Latin squares. Each 14-d period had 11 d for adaptation followed by 3 d of data collection. Cows were offered one of three experimental treatment rations formulated with CPM Dairy (v3.0); Positive control (PC) — formulated to meet all nutrient requirements; Test diet (Test) — negative control diet formulated to meet nutrient requirements, except deficient in metabolizable protein (MP) (approximately 200 g/d) and first limiting in metabolizable Lys (approximately 10 g/d); and Test+RPL — same basal diet as negative control + RP-Lys to provide 14.5 g/d of MP-Lys. For Test+RPL, 45g of RP-Lys (AminoShure-L®; Balchem Corp., New Hampton, NY, containing 23.4g Lys) was top-dressed on the TMR once daily. The PC diet resulted in lower dry matter intake ($P = 0.03$) as compared to either the Test or Test+RPL diet. PC, Test, and Test+RPL cows averaged 42.6, 42.9, 43.6 kg/d of milk and 27.3, 28.4, 28.8 kg/d of DMI, respectively. Crude protein intake for the PC, Test, and Test+RPL diets was 4.83, 4.67, and 4.74 kg/d respectively. MUN decreased ($P < 0.01$) for cows on Test and Test+RPL diets as compared to PC diet (12.5, 12.5 and 14.9 mg/dL, respectively). Milk yield, milk components, milk component yields, FCM, ECM, SCM and production efficiencies (milk, ECM, SCM and FCM) did not differ ($P > 0.05$) among treatments. A post-study CPM Dairy evaluation using final chemical composition analyses of the feedstuffs and average production data from the animals predicted that diets supported more than 47 kg of milk and Lys was not limiting. Cows on the study produced slightly less milk, however DMI was 5-8% more than predicted by initial formulations. Formulation accuracy of the MP and Lys deficient diet may have been improved if data had been available from an initial adjustment period measuring DMI, body weight, milk yield and milk composition. It is also possible that the bioavailability of the RP Lys was not as great as thought during the diet formulation process. However, given the fact that the post-trial CPM analysis did not indicate a deficiency of Lys, it is not very likely that this impacted the results of this trial.

Key words: amino acids, crude protein, dairy cattle

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Acknowledgements

I have been blessed with great opportunity, guidance, and comradery in my time here at Kansas State University. I wish to foremost thank my major professor, Dr. Micheal Brouk, for his advice, support, and encouragement throughout my degree. His knowledge, work ethic, character, and morals make him an exceptional mentor and I feel very fortunate to have had the opportunity to work with him.

I would also like to thank the members of my committee: Dr. Barry Bradford and Dr. Larry Hollis for their support and advice throughout my degree. Their comments on my thesis and commitment to my program have made this a very enjoyable and rewarding experience.

I have also been blessed with an outstanding support group throughout my college career, particularly my parents, George and Marjorie, and my siblings Eric, Katie, and Mark. Their encouragement and interest in my academic goals have been a great inspiration for me to succeed. I am eternally grateful to my mother and father for my upbringing. Through my experiences on the dairy farm just outside on Osawatomie, KS, I learned many life lessons and the value of hard work. If I would not have grown up on the farm, I might not have found this area of interest which is so very important to me today. There have been countless others who have helped me become who I am today, specifically, my fiancé and soon to be wife Jenna. Her undying love and support has helped me to go above and beyond what is expected and I cannot thank her enough. To my fellow graduate students, your friendships and sincerity has been a priceless asset to my education thus far.

Additionally, I want to thank those who have provided assistance with my research. This includes Dr. Laman Mamedova, Cheryl Armendariz, Mike Scheffel, and the numerous undergraduate students who have helped with tie-stall barn operations. Your help has been a blessing and I could not have done it without you.

And most importantly, I must thank God for the many opportunities he has blessed me with throughout my life and for the extraordinary individuals he has surrounded me with to enjoy those opportunities.

Dedication

This work is dedicated to my grandfathers,
Who have joined our Lord in Heaven,
George Pretz, Sr. and Charles Schneider.

As a young professional in the dairy industry, I could not have come from genetics any greater than those I have been given. Both of my parents come from dairy farms here in Kansas, which were established by my grandfathers George (Pretz Holstein Farm) and Charles (Kanza Holsteins). Through their actions, these men taught me something that I will carry with me forever. Take pride in what you have, work harder than others feel is necessary, do more than what is expected, and never shy away from your faith or family. The experience and knowledge gained from these individuals is priceless and I believe they have done more for me than I probably deserve, most of which by simply choosing the paths they chose. Without their decision to be a part of the dairy industry, I have no question that I would not be here today, with the bright path I have before me. For them, I am eternally grateful and I will strive to uphold their family names for as long as the good Lord allows.

Chapter 1 - Literature Review

Introduction: Rumen-Protected Lysine

Today's dairy farmers have two distinct goals in mind when asked about their operation: to maximize milk production and to increase economic efficiency. These two goals have been common for years and have become increasingly tough to achieve in current times of record high feed costs and low milk prices. Through scientific research we continue to find new techniques and methods to allow the dairy cow to maintain or even increase production with less expensive feed inputs and the inclusion of nutritional supplements.

One specific area of research that is growing in terms of importance due to the high cost of feed is the inclusion of nutritional supplements in dairy cattle rations. There are many different products on the market but we continue to look for products with extremely high effective quality. This review of literature will primarily focus on the use of rumen-protected lysine (RP Lys) within dairy cattle rations.

Great advances have been made in feeding dairy cattle over the years which has allowed these cows to better utilize their genetic potential. The protein requirements of lactating dairy cows have been researched for many decades and continue to be refined. Previous NRC recommendations (NRC, 1971; 1978) simply express dietary requirements as crude protein (CP) and metabolic requirements as digestible protein. The NRC (1989) moved forward by expressing dietary requirements as CP or degraded intake protein (DIP) and undegradable intake CP (UIP) and metabolic requirements as absorbed protein (AP). The most recent NRC (2001) expresses dietary requirements as rumen degradable

CP (RDP) and rumen undegradable CP (RUP) and metabolic requirements are expressed as metabolizable protein (MP).

There are many benefits that have come with these changes in the way protein is added to the diet. Each one of these steps forward allows nutritionists and scientists to better supply the animal with a more precise measurement of needed protein. With the move from CP to RDP and RUP, it is much easier to understand how protein is utilized once it is inside the cow. The goal as a ration formulator is to provide sufficient RDP in the ration to support microbial growth and synthesis within the rumen, while at the same time, providing sufficient RUP to support production.

Moving from balancing rations by looking at RUP and RDP to balancing rations for specific amino acids (AA) helps us to more economically and efficiently feed cows. This in turn allows us to minimize losses of excess rumen ammonia from degradation of over-supply of RDP. More importantly, by using small amounts of rumen-protected (RP) AA we can substitute for a substantially greater amount of RUP. Another advantage is being able to better utilize by-product feeds that are low in methionine (Met) and lysine (Lys), knowing that RP AA could overcome AA limitations in these feed stuffs. We can do this by predicting the amount of microbial protein needed and then balancing for the additional needs with RUP and RP AA.

Amino acids can be added directly to the diets of monogastric animals to overcome nutritional deficiencies. However, in ruminants AA are readily degraded in the rumen and are of little or no practical benefit in alleviating AA deficiencies. This in turn makes it difficult to predict the quality and quantity of AA that are absorbed by the animal. Therefore, much research has been conducted in an attempt to increase the

postruminal passage of protein and amino acids (Donkin et al., 1989; Piepenbrink et al., 1996). The proportion of dietary protein that is not degraded in the rumen first enters the abomasum, then omasum, and then enters the small intestine, where nutrients are absorbed and additional RP AA can then be used to meet the nutrient requirements of the animal (NRC, 2001). Ruminally synthesized microbial protein can supply up to 50% or more of the absorbable AA in diets (Schwab, 1996). Research conducted in the 1960s showed that the rumen was capable of supplying all of the protein required by cows producing up to 4,500 kg of milk per lactation (Virtanen, 1966). Microbial protein is the cellular protein of the bacteria, fungi, and protozoa that multiply in the rumen then, along with unfermented feed, pass along the small intestine. Bacteria provide the majority of the total microbial protein leaving the rumen. Microbial protein is considered to be a high quality source of absorbable AA (Rode & Kung, 1996), although ruminally synthesized microbial protein still does not possess a perfect essential AA balance (Schwab, 1996).

Amino acids function as the building blocks for tissue and milk proteins. Amino acids are organic compounds which contain an amino group and a carboxyl group. There are two different classifications of amino acids: essential and nonessential. Essential amino acids must be supplied as part of the diet as body synthesis is inadequate to meet metabolic need. The ten essential amino acids in dairy cows include leucine, isoleucine, valine, tryptophan, phenylalanine, methionine, threonine, lysine, histidine and arginine. Amino acids that are synthesized by the body are termed nonessential amino acids and include alanine, aspartic acid, asparagine, glutamic acid, glutamine, glycine, proline and serine.

Lysine and Met have been reported to be co-limiting AA for milk synthesis and growth in dairy cattle (Clark, 1975; Schwab et al., 1976; Nichols et al., 1998; Socha et al., 2005). For this reason, they are the most commonly researched and supplemented AA in dairy nutrition and are often researched while being used together. It is commonly known that Lys and Met work together in dairy rations in a ratio of 3 to 1 respectively. If Lys and Met are not balanced this way within the ration, there may be a decrease in milk yield and components.

Due to rapid degradation of rumen degradable Lys and Met by microorganisms in the rumen, there is no positive effect on production (Piepenbrink et al., 1996) when they are supplemented. However, these AA can potentially be used for microbial protein when digested in the rumen. In order for these supplemented AA to be productive and worth the additional input expense, they must be ruminally protected from degradation allowing them to reach the small intestine (Rogers et al., 1987). Increased postruminal supply of AA in lactating dairy cows may improve milk production if the AA supplied are, in fact, the most limiting nutrient for the response being measured (Rogers et al., 1987). In addition, rumen-protected AA fed in the ration must supply those AA that are limiting if a response is to be expected.

Over the past three decades, considerable research has been conducted to develop strategies to protect AA (Chalupa, 1975; Kaufmann & Luppig, 1982). A potential problem is that AA can be over-protected (Rode & Kung, 1996). Complexes that are extremely inert in the rumen can be indigestible in the small intestine as well. Furthermore, a trade-off exists between good ruminal protection and bioavailability (Rode & Kung, 1996).

Ruminal-protected Met has been available for several years (Donkin et al., 1989) although producing ruminally protected Lys and other AA has been less successful (Robinson et al., 1998). Current protection strategies include: fats, binders, carbohydrates, minerals, heat treating, and formaldehyde treating. When compared to the swine and poultry industries, our ability to formulate ruminant rations while balancing for AA requires further refinement. However, the use of RP Lys and other AA gives us a direct mechanism to formulate lower protein diets that should support or improve milk yield and components while increasing nitrogen utilization efficiency.

Rumen-protected Lys products have the greatest potential to improve milk yield for high producing cows in early lactation. Most commonly, researchers have seen significant increases in milk protein (Wu et al., 1997; Socha et al., 2005; Donkin et al., 1989). Research conducted in close-up dry cows has suggested potential health improvements, but additional research is needed (Xu et al., 1998).

Supplemented Rumen-Protected Amino Acids during the Transition Period

Research on feeding strategies for high yielding dairy cows over the last three decades has focused primarily on postparturient cows. A common strategy is to increase energy density to overcome low feed intake during the first few weeks of lactation. Intake depression can be initiated by a number of different transition disorders which in turn affect the cow's production throughout lactation. Researchers have a theory that supplementation of necessary AA during the transition period (3 weeks prepartum to 3 weeks postpartum) can mitigate such disorders.

In the late 1990s, Wu et al. (1997) evaluated the lactational performance of cows fed low or high RUP prepartum and supplemental Met and Lys postpartum. Researchers used 24 multiparous Holstein cows assigned to six outcome groups based on mature equivalent milk yield and parity. Two cows in each group were randomly prescribed to a diet supplemented with soybean meal while the other two were fed a diet in which fish meal partially replaced soybean meal for the last 30 days of gestation (increasing RUP from 34 to 41% of CP). After parturition, each of the pairs were split and supplemented with or without RP Met (10.9 g/d) and RP Lys (15.2 g/d) which when supplemented increased Met to 5.1% and Lys to 15.3% of predicted total absorbable essential AA (Lys/Met ratio of 3 to 1).

Cows fed low RUP diets with supplemented Lys and Met had increased milk yields, but milk yields were similar for high RUP fed cows both with and without AA supplementation. Milk protein percentage increased numerically from 2.83 to 2.96 for cows previously fed the high RUP diet. Milk protein yield increased from 1.13 to 1.21 kg/d when RP Met and Lys were fed. Post study analysis suggest that supplementation of Met and Lys corrected a Met limitation.

Xu et al. (1998) studied the effect of rumen bypass Lys and Met on milk yield and composition of lactating cows. Researchers utilized 56 multiparous Holstein cows split into 4 treatment rations beginning at 3 weeks prior to predicted calving. Two dry cow rations were utilized prepartum, resulting in four dietary treatment groups, two of which utilized a Lys/Met ratio of 3 to 1. Prepartum diets were based on grass silage with: 1) corn distillers grains to provide 86 and 90% of estimated required metabolizable Lys and Met, respectively (NCR); 2) a blend of blood meal, fish meal, and meat and bone meal as

AA sources to provide 112 and 103% of required metabolizable Lys and Met, respectively (PCR); 3) negative control plus RP Lys (27 g/d) and Met (8 g/d) (NCR plus RP Lys + Met); and 4) negative control plus high amount of RP Lys (40 g/d) and Met (13g/d) (NCR plus HRP Lys + Met). Cows on ration 3 and 4 were offered 13.5 g/d of duodenally available Lys and 4 g/d of Met for 3 weeks prepartum. The total length of the study was 43 weeks.

Researchers found that cows fed ration 4 (NCR plus HRP Lys + Met) consumed 3 to 4 kg/d more dry matter than cows on any of the other 3 rations, and milk yield and the percentage of milk protein and fat significantly increased during the first 8 weeks of lactation. In early lactation, cows fed ration 3 (NCR plus RP Lys + Met) had a higher milk fat percentage but similar dry matter intake, protein percentage and yield of FCM when compared to the cows fed ration 2 (PCR). Researchers concluded that high concentrations of AA (NCR plus HRP Lys + Met) in the rations during early lactation may reduce the risk of metabolic disorders. Post study analysis of diets based on actual intake and nutrient compositions showed that Met was limiting and Lys was co-limiting for milk yield when cows were fed grass silage based rations.

More recently, Socha et al. (2005) looked at improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected Met and Lys. Researchers assigned 84 Holstein cows to a randomized complete block experiment (14 blocks) to determine effects of supplementing diets containing high Lys protein supplements with RP Met and Lys. Prior to calving (2 weeks prior), cows received 1 of 3 corn based, basal diets: 1) no RP AA; 2) 10.5 g/d of RP Met; 3) 10.2 g/d of RP Met and 16 g/d of RP Lys (Lys/Met ration of 3 to 2). After calving, cows continued to receive

their respective RP AA treatment but were switched to either a 16 or 18.5% CP postcalving diet. This in turn formed a 2 x 3 factorial arrangement of treatments during lactation. Cows remained on their specific diet through 15 weeks of lactation.

Met + Lys diet supplementation increased yield of ECM, fat, and protein, and tended to increase production of FCM when compared to the basal or basal + Met diet. Supplementation of the 16% CP diet with Met or Met + Lys had no significant effect on milk true protein or fat content. The 18.5% CP did however, significantly increase ($P < 0.05$) milk protein content by 0.21 and 0.14 percentage units for Met and Met + Lys supplementation respectively. Methionine supplementation also increased fat content of the milk by 0.26 percentage units.

Research conducted on supplementation of Lys over the transition period is limited. From the research previously represented, supplemental RP AA can be advantageous to cows in the transition period by increasing milk yield initially and protein yield during lactation when RP Lys is supplemented throughout and beyond the transition period. Xu et al. (1998) also stated that high concentrations of AA (40 g of Lys and 13 g of RP Lys and Met) in the rations during early lactation (wk 1 to 8) may reduce the risk of metabolic disorders.

Lactation Performance

If an essential AA is the key limiting substrate for milk protein synthesis and the amino acid transport system is operating well below saturation in the mammary gland, then increased delivery of a limiting AA should increase milk protein synthesis (Donkin

et al., 1989). Therefore, our goal in supplementing rumen-protected Lys is to increase milk protein yield as milk protein is the most valuable component of the milk, by pound, to the dairy farmer.

Supplemented Rumen-Protected Amino Acids during Lactation

Over the last 3 decades, there has been a lot of research conducted in the area of amino acid supplementation to dairy cows in early to mid lactation. Much of this research includes the use of a combination of Lys and Met; however, some studies have evaluated Lys specifically.

In the late 1980s, Donkin et al. (1989) published a manuscript reporting the effects of supplementing RP Met and Lys on milk protein yield. They used eight mid lactation Holstein cows in a three-period (28 d) switchback design to evaluate addition of a combination of ruminally protected Met (15 g/d) and Lys (40 g/d) on milk protein yield. Cows were paired on pre-experimental milk yield and days postpartum, and assigned to one of two treatments. Animals were fed for *ad libitum* intake a total diet consisting of 50% concentrate and 50% corn silage (DM basis) with inclusion of amino acids in the test diet.

There were no significant differences between treatments in DM intake, milk yield, fat yield, milk fat percentage, or 4% FCM yield. Addition of AA increased milk protein yield from 3.15 to 3.25% and increased yield of α - and β -casein proteins in the milk. These data show that RP Met and RP Lys added to corn based diets fed to mid lactation dairy cattle resulted in a significant increase of 7.5% in total milk protein yield

from 0.80 kg/d to 0.86 kg/d. These data suggest that Lys and probably Met were limiting for casein protein synthesis in the corn-based diets used. The use of RP Lys and RP Met in these diets is an effective method of improving the supply of post ruminal amino acids in favor of increasing milk protein production.

Rogers et al. (1989) conducted an experiment using 130 cows on three different university farms to evaluate production responses of dairy cows fed various amounts of RP Met and RP Lys. Researchers assigned cows to a 3 x 3 factorial response surface design conducted during the 305 day lactation. Cows were blocked according to expected calving date and randomly assigned to a treatment. The treatments included an unsupplemented control diet (CN) made up of corn silage and corn grain containing either soybean meal or corn gluten meal and urea, and the CN supplemented with nine different combinations of RP Met and RP Lys, with three different concentrations each of RP Met and RP Lys. Supplements were fed immediately following the transition period from 22 to 305 days of lactation. The nine treatment combinations included RP Met at 3.4, 7.8, or 12.2 g/d and RP Lys at 5.9, 13.5 or 21.2 g/d.

Trial results demonstrated that RP Met and RP Lys did not affect DMI for cows fed either of the basal diets. Cows on the soybean meal diet had increased milk protein percentage when RP Met and RP Lys were supplemented; however milk and milk protein yields were not improved. In comparison, milk and milk protein yields were improved with the corn gluten meal and urea diet with the supplementation of RP Met and RP Lys.

Piepenbrink et al. (1996) researched the response of 10 cows fed a low crude protein diet to RP Met and RP Lys. Cows were utilized in a replicated 5 x 5 Latin square design with periods of 14 days. Cows were fed diets formulated to be adequate (18% CP)

or inadequate (14% CP) in Met and Lys. RP Met and RP Lys was added to the 14% CP diet to provide 0, 50 100, and 150% of the predicted deficiency of Met and Lys using the Cornell Net Carbohydrate and Protein System (CNCPS). Cows averaged 128 DIM (110 to 149 DIM) and 589 kg of BW (532 to 683 kg) at the beginning of the experiment.

Supplementing RP Met and RP Lys to the 14% CP diet did not affect DMI or yields of milk, 3.5% FCM, milk CP, and milk SNF. It is suggested that the 14% CP diet allowed other nutrients to be more limiting than Met and Lys for synthesis of milk and milk protein. Increasing the CP to 18% in the diet increased milk yield and milk protein numerically.

In the late 1990s, Armentano et al. (1997) tested the response of lactating cows to RP Met or a combination of RP Met and RP Lys when added to high protein diets. Researchers utilized 16 cows (early lactation) in a 4 x 4 Latin square design with 21-d periods where days 17 to 21 were used to collect data on milk production, milk composition, and dry matter intake.

Supplemented amino acids had no effect on milk production, dry matter intake, or milk fat concentration. The addition of RP Met increased milk protein concentration and yield linearly although the addition of RP Lys did not elicit a response. Total mixed rations based on alfalfa haylage, heated soybeans, and small amounts of animal proteins utilized in this study were limited in RP Met content but adequate in their RP Lys content even after substantial amounts of RP Met were supplemented.

Robinson et al. (1998) carried out a trial to separate the effects of RP Lys from effects of RP Met fed a ration first limiting in Lys and second limiting in Met. Researchers used 30 multiparous Holstein cows in a 20 week study that started 5 weeks

postpartum. Rations consisted of timothy silage, corn silage, barley, corn, corn gluten meal, and soybean meal. Four treatments were used including: 1) no supplemental AA, 2) 21 g/d available RP Lys, 3) 22 g/d available RP Lys and 6 g/d available RP Met. Post study calculations suggested that the diet was actually first limiting in histidine (His) (0.96 of requirement), followed by Lys (1.00), digestible RUP (1.01), Ile (1.03), Arg (1.04), Val (1.10), and finally Met (1.14).

Researchers determined that dairy cows did not respond to supplemented RP Lys when Lys was not calculated to be the first-limiting nutrient. Upon further analysis, in cows supplemented with both RP Lys and RP Met, the production of both milk protein (40 g/d) and fat (40 g/d) was numerically increased to a similar level as compared to other studies. Final results of this study suggested Met, unlike Lys, may enhance the production of milk components because of its vital role as a limiting amino acid.

Nichols et al. (1998) evaluated RP Lys and RP Met when supplemented to soybean meal or corn distiller grain diets. Researchers utilized 12 Holstein cows averaging 57 DIM in a replicated 4 x 4 Latin square with four different dietary protein supplements including: 1) soybean meal, 2) soybean meal plus RP Lys (20 g/d) and RP Met (6 g/d), 3) corn distillers grains, and 4) corn distillers grains plus RP Lys (20 g/d) and RP Met (6 g/d).

Milk fat yield and percentage were unaffected by diet. Lys, Met, and phenylalanine (Phe) were determined by researchers to be the most limiting amino acids in all diets by using amino acid extraction efficiency and transfer efficiency. After final analysis, researchers determined higher milk yield increases in corn distiller grain diets with larger increases when supplemented with RP Lys and RP Met when compared to

soy bean meal. Milk protein yield and percentage were also increased with AA supplementation because the diet containing corn distillers grains was probably deficient in Lys although blood concentrations of Lys were not evaluated.

More recently, Lee et al. (2012) supplemented a combination of RP Lys, RP Met and RP His to dairy cows fed MP deficient diet. The study was 12 weeks in length and included 48 Holstein cows blocked by DIM and milk yield and randomly assigned to one of four diets. The different diets included a MP adequate diet (ADMP, control), MP deficient diet (-317 g/d MP) (DMP), and DMP diet supplemented with RP Lys (DMPLM) (AminoShure-L®, Balchem Corp., New Hampton, NY), RP Met (Mepron; Evonik Industries AG, Hanau, Germany); and RP His (DMPLMH). All diets were based on corn silage and alfalfa haylage.

Milk yield was decreased by the DMP diet (35.2 kg/d) but remained similar to ADMP (38.8 kg/d) for DMPLM and DMPLMH (36.9 and 38.5 kg/d respectively), which paralleled the same trend in DMI. Researchers found that the inclusion of RP Lys and RP Met diminished any loss of DMI and milk yield when compared to the DMP diet. Researchers determined that increased DMI lead to increased milk and milk protein yields due to the inclusion of AA in the diet. It was also established that AA play a role in DMI regulation in dairy cows. This study further clarified that dairy cattle can in fact be limited in more than one or two specific AA.

Impact of Amino Acids on Calf Growth

Recent studies have begun to show the importance of AA to the dairy animal at a younger age, although the dairy NRC (2001) currently does not consider individual AA for calves. The most recent summaries of the AA requirements of calves are Williams and Hewitt (1979), van Weerden and Huisman (1985), Toullec (1989), and Gerrits et al. (1997). A more recent study by Hill et al. (2008) evaluated varying concentrations of Lys, Met, and threonine (Thr) in milk replacer to estimate optimal concentrations of these AA for calves less than 5 wk of age. Their hypothesis was that Lys, Met, and Thr would be limiting. Feeding calves 0.68 kg/d of a whey-based milk replacer with synthetic Lys and Met that was 26% CP, 17% fat, 2.34% Lys, 0.72% Met, 1.27% Met+Cys, and 1.8% Thr maximized average daily gain and efficiency significantly ($P = 0.018$). This response to added Lys and Met was large which shows there is a need to formulate milk replacer for Lys and Met and not just CP.

Conclusions

After evaluating published research, balancing diets on specific AA should increase protein yield. The specific type of response was dependent on stage of lactation, parity, and DMI. When RP Lys is supplemented at or prior to parturition, there is typically an increase in milk yield. If the RP AA is supplemented beginning around peak lactation, there will typically be an increase in milk protein concentration. A dairy cow's lactation performance can be enhanced by optimizing Met and Lys nutrition. Lack of

response to RP Met and RP Lys helps researchers to understand the importance of characterizing protein fractions of protein sources utilized in diet formulation.

Milk protein levels are significantly reduced when diets provide less than 2.1% Met or 6.7% Lys in metabolizable protein, thus these are considered minimum levels. Rulquin et al. (1993) suggest that response of milk protein to Met may be negative if Met is limiting ($Lys/MP > 6.57$). Methionine at 150% of requirements depressed DMI and milk yield even when Lys was decreased (Piepenbrink et al., 1996). To avoid potential negative impacts of excess Met, the Lys:Met ratio should always be 3:1.

It is important to optimize Lys and Met when balancing diets to maximize milk and milk protein. Further research shows it is also important to keep calves in mind when looking at amino acid balance in the diet. Establishing relationships between predicted supplies and most limiting AA in the diet and milk or milk protein yield will allow for more accurate prediction of changes in milk protein production when changes in protein nutrition are made (NRC, 2001). With a lack of reliable RP Lys products and the inability to achieve desired concentrations of Lys in corn based diets, research in the area of RP Lys has significantly increased in the last couple of years. It is important that researchers further pursue a commercially viable RP Lys product in order to reach higher goals of lactation performance.

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Chapter 2 - Application of Rumen-Protected Lysine to Lower Crude Protein Diets for Lactating Dairy Cows

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Abstract

The study objective was to evaluate the application of supplemental rumen-protected lysine (RP Lys) to maintain milk production when reducing the crude protein levels in a lactating dairy cow diet. Twelve lactating multiparous Holstein cows, averaging 129 DIM, 50.2 kg milk yield, 3.6% fat and 2.9% true protein were randomly assigned to one of four 3x3 Latin squares. Each 14-d period had 11 d for adaptation followed by 3 d of data collection. Cows were offered one of three experimental treatment rations formulated with CPM Dairy (v3.0); Positive control (PC) — formulated to meet all nutrient requirements; Test diet (Test) — negative control diet formulated to meet nutrient requirements, except deficient in metabolizable protein (MP) (approximately 200 g/d) and first limiting in metabolizable Lys (approximately 10 g/d); and Test+RPL — same basal diet as negative control + RP-Lys to provide 14.5 g/d of MP-Lys. For Test+RPL, 45g of RP-Lys (AminoShure-L; Balchem Corp., New Hampton, NY, containing 23.4g Lys) was top-dressed on the TMR once daily. The PC diet resulted in lower dry matter intake ($P = 0.03$) as compared to either the Test or Test+RPL diet. PC, Test, and Test+RPL cows averaged 42.6, 42.9, 43.6 kg/d of milk and 27.3, 28.4, 28.8 kg/d of DMI, respectively. Crude protein intake for the PC, Test, and Test+RPL diets was 4.83, 4.67, and 4.74 kg/d respectively. MUN decreased ($P < 0.01$) for cows on Test and Test+RPL diets as compared to PC diet (12.5, 12.5 and 14.9 mg/dL, respectively). Milk yield, milk components, milk component yields, FCM, ECM, SCM and production efficiencies (milk, ECM, SCM and FCM) did not differ ($P > 0.05$) among treatments. A post-study CPM Dairy evaluation using final chemical composition analyses of the

feedstuffs and average production data from the animals predicted that diets supported more than 47 kg of milk and Lys was not limiting. Cows on the study produced slightly less milk, however DMI was 5-8% more than predicted by initial formulations.

Formulation accuracy of the MP and Lys deficient diet may have been improved if data had been available from an initial adjustment period measuring DMI, body weight, milk yield and milk composition. It is also possible that the bioavailability of the RP Lys was not as great as thought during the diet formulation process. However, given the fact that the post-trial CPM analysis did not indicate a deficiency of Lys, it is not very likely that this impacted the results of this trial.

Key words: amino acids, crude protein, dairy cattle

Introduction

Increased feed costs and low milk prices have taken their toll on U.S. dairy farms. As many rations are formulated on a least-cost basis, researchers and producers continue to search for novel ways to feed cows more economically. One specific area of research that is continuing to grow is the use of additives within rations. More specifically, nutritional companies and researchers continue to evaluate various types of rumen-protected amino acids, in this case rumen-protected lysine (RP Lys), in order to better balance dairy cow rations. Gone are the days of feeding only a set amount of crude protein; we now know that we must balance for specific amino acids. By doing so we are better equipped to use resources more efficiently and reduce environmental emissions of nitrogen (Wang et al., 2010).

Lysine (Lys) and methionine (Met) have been reported, in several instances, to be co-limiting amino acids (AA) for milk synthesis and overall growth in dairy cattle (Clark, 1975; Schwab et al., 1976; Nichols et al., 1998; Socha et al., 2005). It is commonly known that Lys and Met work together in dairy rations in a ratio of 3 to 1 respectively with digestible Lys recommended at 7.2 % of MP and digestible Met at 2.4 % of MP (NRC, 2001; Vyas and Erdman, 2009).

Amino acids can be added directly to the diets of monogastric animals to overcome nutritional deficiencies. However, in ruminants, ruminally available AA are readily degraded in the rumen and are of little or no practical benefit in alleviating AA deficiencies. This in turn makes it difficult to predict the quality and quantity of AA that are absorbed by the animal. Therefore, much research has been conducted in an attempt to increase the postruminal passage of protein and amino acids (Donkin et al., 1989;

Piepenbrink et al., 1996). The proportion of dietary protein that is not degraded in the rumen enters the small intestine where it is digested and provides additional AA that could be used to meet the nutrient requirements of the animal (NRC, 2001). Many dietary proteins and AA are readily degraded by microorganisms in the rumen, therefore methods are needed to protect amino acids from bacterial degradation (Chalupa, 1975). A potential problem is that AA can be over-protected (Rode & Kung, 1996). Complexes that are extremely inert in the rumen can be indigestible in the small intestine as well. Furthermore, a trade-off exists between good ruminal protection and bioavailability (Rode & Kung, 1996). RP Met products have been available for several years although production responses to supplementation of RP Lys have not always been successful (Piepenbrink et al., 1996; Armentano et al., 1997; Robinson et al., 1998; Lobos et al., 2012; Paz et al., 2012). Several investigators reported an increase in milk protein when incorporating RP Lys into dairy cattle diets (Xu et al., 1998; Socha et al., 2005; Polan et al., 1991; Donkin et al., 1989; Rogers et al., 1989; Nichols et al., 1998; Lee et al., 2012). Milk components and milk yield varied both numerically and significantly for different experiments.

Milk protein synthesis may be limited by the supply of precursors reaching the mammary gland, in particular the essential amino acids (Clark, 1975). When an essential amino acid is the key limiting substrate for milk protein synthesis and the amino acid transport systems are operating well below saturation in the mammary gland, increased delivery of a limiting amino acid should increase milk protein synthesis (Donkin et al., 1989). The objective of this study was to investigate the effects on feed intake, milk

yield, and milk composition when RP Lys was added to a MP deficient ration fed to lactating Holstein cows.

Materials and Methods

Twelve lactating multiparous Holstein cows averaging (mean \pm SD) 50.2 ± 10 kg of milk/d, 129 ± 38 DIM, 670 ± 73 kg of BW, and a BCS of 2.63 ± 0.39 were randomly assigned to one of four 3 x 3 Latin squares balanced for carry over effects, although two of the replications had the same pattern. Treatment periods were 14 d and included 11 d for adaptation to treatments with samples collected in the final 3 d of each period.

Cows were housed in individual tie stalls at the Kansas State University Dairy Teaching and Research unit with free access to water, milked three times daily (0200, 1000, and 1800 h), and fed twice daily (0700 and 1800 h) for *ad libitum* intake through individual mangers located in front of each stall. Total daily feed offerings were adjusted based on previous 24-h intake so refusals were approximately 5%. Amounts fed and refused were recorded daily. The experimental cows were cared for according to the guidelines stipulated by Kansas State University Animal Care and Use Committee (Manhattan). The health status of each animal was evaluated and recorded daily.

Treatments consisted of three separate diets (Table 1) fed as TMR, composed from a common basal mix that consisted primarily of corn silage, alfalfa hay, wet corn gluten feed, and dry rolled corn. Treatments were as follows; Positive control (PC) – Diet formulated to meet all nutrient requirements, including ME, MP, and individual

amino acids; Test Diet (Test) – Diet formulated to meet all NRC recommendations, except deficient in MP (~200 g/d) and first limiting in metabolizable Lys (~10 g/d); and Test plus RP Lys (Test + RPL) – the same basal diet as test diet plus supplemental RP Lys (AminoShure-L, Balchem Corp., New Hampton, NY) to provide 10 g/d of MP Lys. Supplemental RPL (provided ~14.5g metabolizable Lys) was top-dressed on the TMR at the morning feeding and mixed with top layer of Test + RPL TMR in the bunk. All diets were formulated using CPM dairy model (Cornell-Penn-Minor, Cornell University, Ithaca, NY, USA), an applied mathematical nutritional model to predict lactating dairy cow performance.

Experimental Measures

Prior to the start of the experiment, samples of forages were analyzed and initial diets were formulated based on the feed analysis. Grain mixes for control and test diets were then formulated and tested for nutrient content prior to the start of the feeding study. Samples of the basal mix and TMR were collected and frozen (-20°C) weekly then composited by experimental period prior to analysis. Daily intake was calculated from feed offered and refused and recorded daily. Water intake and total milk production was measured and recorded daily throughout the experiment. Milk samples were collected (25 mL) at each milking during the final 3 d of each period, preserved using 2-bromo-2-nitropropane-1,3 diol, stored at 4°C after collection and analyzed for fat, true protein, lactose, MUN, SNF and somatic cells within 24 h. BW and BCS (1 – 5 scale) were

measured and recorded once each morning (0600 h) of the final two days of each experimental period.

Sample Analysis

Composited samples of individual feeds and TMRs were shipped frozen in insulated shippers to Dairy One Forage Laboratory (Ithaca, NY) for analysis. The Ration Balancer Plus Package which included DM, CP, SP, unavailable protein (ADICP on haylages), ADF, NDF, lignin, fat, ash, NFC and minerals was utilized for standard feed analysis. DM content was determined by drying samples at 105°C for 24 h in a forced-air oven. The wet chemistry techniques of Van Soest et al. (1991) were used to quantify NDF (with α -amylase and sodium sulfite) and ADF (nonsequential). CP analysis was performed with a Leco FP-528 Nitrogen/Protein Analyzer (Leco Corporation, St. Joseph, MI; AOAC 990.03). Soluble protein, ADICP, lignin, fat, ash, NFC, and minerals were determined using a Leco TruMac N Macro Determinator, NIRS-Fose NIRSystems Model 6500 with Win ISI II v1.5 software - (AOAC 989.03). Individual composited feed samples were also analyzed at Kansas State University for amino acid content by first being thawed at room temperature (22°C) and subsequently dried in a 55°C forced-air oven for 72 h, when partial DM was determined. Samples were then ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). True DM content was determined by drying samples at 105°C in a forced-air oven for 24 h. Samples were then analyzed by wet chemistry analysis in the Kansas State University

ruminant nutrition laboratory for amino acid content via acid hydrolysis (6 N HCL, 105°C, 24 h) and quantified by HPLC.

Milk samples were analyzed for concentrations of fat, true protein, SNF, and lactose via infrared absorbencies (B-2000 Infrared Analyzer; Bentley Instruments, Chaska, MN). Milk urea nitrogen was quantified colorimetrically (MUN spectrophotometer, Bentley Instruments) and somatic cells were counted using dual laser flow cytometry (SCC 500, Bentley Instruments; Heart of America DHIA, Manhattan, KS). Energy-corrected milk yield was calculated as follows: $0.327 \times \text{milk yield} + 12.95 \times \text{fat yield} + 7.2 \times \text{protein yield}$. Solids-corrected milk production was calculated as: $12.3 \times \text{fat yield} + 6.56 \times \text{SNF yield} + 0.0752 \times \text{milk yield}$. Fat corrected milk was calculated as: $0.4 \times \text{milk yield} + 15 \times \text{milk fat yield}$. Prior to statistical analysis, milk component data was averaged by cow within period.

Statistical Analysis

The PROC MIXED procedure of SAS (Version 9.1, SAS Institute, Inc., Cary, NC) was used for all statistical analysis. Feed intake, milk production, milk composition, milk component yield, BW and BCS data were analyzed as a replicated 3 x 3 Latin square with rep, treatment, day and all interactions as fixed effects. Random effects included period and the interaction of period with the effect of cow within rep and the effect of treatment within cow within rep. Significance was declared at $P < 0.05$.

Results and Discussion

Composition of diets offered and calculated chemical composition of the diets are found in Table 1. Control and Test diets contained similar amounts of forages but differed in sources and amounts of protein supplement. The Test diets were formulated to be slightly deficient in metabolizable protein as predicted by CPM dairy model as compared to Control. This formulation resulted in a lower percentage of dietary CP in the Test diets as compared to the Control diet.

Actual CP contents of the analyzed diets were slightly greater than the formulated diets. Test diets contained less Lys than the Control as predicted by the formulations.

When animals received the Control diet, dry matter intake was lower ($P = 0.01$) as compared to either the Test or Test+RPL diet (Table 2). However, the intakes of CP and fat were not different ($P > 0.05$) due to higher concentrations of CP and fat found in the Control diet compensating for lower intake. Fiber (ADF and NDF) intakes were greater ($P < 0.05$) for the Test and Test+RPL diets due to increased intake with similar diet fiber concentration as compared to the Control diet. Methionine intake was lower ($P < 0.05$) for the Control diet as compared to both of the other diets. Lysine intake was lower ($P < 0.001$) for the Test diet as compared to Control and Test+RPL diets. The Control diet contained a higher level of CP due to an increased inclusion of treated soybean meal as designed in the experiment. This increased level of soybean meal offset the decline in intake for Lys but it did not offset the Met intake as soybean meal naturally contains less Met than Lys.

Milk production, milk components, milk component production, FCM, ECM, and SCM did not differ ($P > 0.05$) between treatments (Table 2), which is in agreement with

Chung et al. (2006). Milk urea nitrogen increased ($P < 0.01$) when cows were fed the control diet as compared to the Test and Test+RPL diets (Table 3). However, all levels appeared to be adequate for optimal milk production. Numerically higher levels of milk production of the Test and Test+RPL diets with similar concentrations of milk components resulted in similar ($P > 0.05$) efficiencies of production (milk, ECM, SCM, and ECM) despite higher intakes. Numerically, SCC was greater for the Control diet due to a single cow that developed mastitis in the final period of the study. These nonsignificantly different SCC were in agreement with several other researchers (Wu, et al. 1997; Nichols et al., 1998; Rogers et al., 1987; Wang et al., 2010; Chung et al., 2006).

There was a significant ($P < 0.05$) rep x treatment interaction for milk, FCM, ECM, and SCM (Table 2). This was associated with a single replicate that experienced a 35% decline in milk production over the course of the experiment as compared to a 21% decline for the other three replications. Cows in the replication experiencing a greater decline in milk production were more advanced in lactation as compared to the other replications. Although, removing this replication from analysis did not change the overall significance of the treatment effect for the variables analyzed. There was no significant difference in BCS or water intake for diet or rep x diet.

Over the last 3 decades, there has been substantial research conducted in the area of amino acid supplementation to dairy cows in early to mid lactation. Much of this research includes the use of a combination of Lys and Met, however some studies have evaluated Lys specifically. These studies focused on elucidating the role of supplemental Lys and Met in the production of milk and milk constituents in lactating cows. Supplementing cows with greater amounts of RP Lys and RP Met can increase milk

production (Wu et al., 1997; Xu et al., 1998) which was not observed in our study. Some authors have reported increased concentrations of milk fat (Xu et al., 1998) and protein (Wu et al., 1997; Xu et al., 1998; Socha et al., 2005; Roger et al., 1989; Armentano et al., 1997; Nichols et al., 1998) in response to supplementation of Met and Lys which we did not observe. Others have indicated that yields of milk fat (Socha et al., 1995) and protein (Xu et al., 1998; Socha et al., 1995; Donkin et al., 1989; Armentano et al., 1997; Nichols et al., 1998; Lee et al., 2012) were increased due to AA supplementation; however other data demonstrated no improvements in milk yield (Donkin et al., 1989; Roger et al., 1989; Piepenbrink et al., 1996; Armentano et al., 1997; Lee et al., 2012) or percentage of milk protein (Lee et al., 2012) in response to supplementation of RP AA which is in agreement with our findings. Metabolizable Met and Lys generally increased milk protein yield, but this increase was typically associated with improvements in milk yields, which occurs less often than increases in concentration of milk protein (Patton, 2010). Our finding of no significant increases in milk, milk protein, protein percent as the result of feeding supplemental RP Lys could be partially explained if AA other than Lys and Met were in fact limiting although our CPM analysis shows adequate AA levels. There are many aspects of this trial which have led to unchanging production results. First, it is very difficult to balance a ration for only 14 g of metabolizable Lys and reasonably predict microbial production to need an estimated 14 g of Lys. Second, animal DMI would have to be consistent throughout the treatment period, which did not occur in our trial.

Post study review of feed costs for the control, test and test+RPL treatment rations were 7.79, 7.69, and 7.80 (less RP Lys cost; DM basis; United States dollar, USD)

per day, respectively. Trial results (Table 2) of significantly increased DMI ($P < 0.05$) and nonsignificantly increased milk, protein, and fat yield ($P = 0.43$) (Test+RPL treatment in comparison to the control treatment) lead us to believe that supplementation of the research product warrants further investigation, and in our trial specifically, showed no economic benefit.

Conclusions

Reducing dietary CP in the Test and Test+RPL diets resulted in similar milk production to the Control with lower MUN concentrations in the milk indicating that the crude protein levels of the Control diet were more than adequate to support the milk production level of cows in this study. The lack of response of cows to supplemental RP Lys was likely the result of adequate levels of metabolizable Lys in the Test diet. All diets were formulated to support 47 kg of daily milk production; however, average production was less than the amount expected in the diet formulation. This would be expected to prevent a milk response due to increased Lys supply in a protein deficient diet. Post study analysis using CPM Dairy, actual diet analysis, and DMI show that diet formulations were on target although an increase in feed intake and a slight variation of chemical composition between pre-trial and in-trial feed samples resulted in MP balance and Lys balance being higher than expected. Formulation accuracy of the MP and Lys deficient diet may have been improved if data had been available from an initial adjustment period measuring DMI, body weight, milk yield, and milk composition.

In an open letter written by Dana Putnam, Balchem Corp., after completion of our trial and on May 29th, 2012, stated that results from a separate study conducted at the same time as our trial evaluating the AminoShure-L® bioavailability were discussed. It is stated that the product bioavailability results were not in line with their expectations, being lower than they had originally expected. Several authors have recently reported results of unchanged production measures from the supplementation of RP Lys (Lobos et al, 2012; Gressley et al., 2013; Paz et al., 2012).

In conclusion of this trial, we have evaluated several aspects as to why we saw unchanged production measures. Upon analysis, available AA levels in the negative control diet were adequate according to post CPM dairy results. Additional RP Lys was not necessary and production data indicate that Lys was not the first limiting factor controlling milk production. It is also possible that the bioavailability of the RP Lys was not as great as thought during the diet formulation process. However, given the fact that the post-trial CPM analysis did not indicate a deficiency of Lys, it is not very likely that this impacted the results of this trial.

Acknowledgements

The authors express their appreciation to Mike Scheffel, Cheryl Armendariz, and all others who helped with this study for their assistance; and Balchem Corporation for partial financial support.

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Table 1 Ingredient and nutrient composition of diets

	Control	Test	Test + RPL
Ingredient, % of DM			
Alfalfa hay	17.4	17.3	17.3
Corn silage	29.9	31.3	31.1
Wet corn gluten feed ¹	26.9	26.9	26.9
Whole cotton seed	5.8	5.8	5.8
Dry-rolled corn	13.5	14.8	14.8
SoyBest® ²	3.0	1.0	1.0
Blood meal	0.43	0.0	0.0
MegaLac-R® ³	0.42	0.42	0.42
Limestone	1.22	1.22	1.22
Trace mineral salt ⁴	0.17	0.17	0.17
Sodium bicarbonate	0.52	0.52	0.52
Magnesium oxide	0.03	0.03	0.03
Rumensin 90® ⁵	0.01	0.01	0.01
Smartamine M® ⁶	0.02	0.02	0.02
Potassium carbonate	0.31	0.31	0.31
Zinpro 4-Plex® ⁷	0.05	0.05	0.05
Sodium Selenite	0.03	0.03	0.03
Diamond V XP® ⁸	0.18	0.18	0.18
Zinpro 100® ⁹	0.01	0.01	0.01
Vitamin premix ¹⁰	0.15	0.15	0.15
Nutrient, % of DM			
DM, % as-fed	64.64	63.94	63.94
CP	17.66	16.47	16.47
ADF	20.32	20.20	20.20
NDF	35.34	35.22	35.22
Crude fat	4.43	4.26	4.26
Lysine, % of MP	7.2	6.3	7.3
Methionine, % of MP	2.5	2.6	2.6
NE _L , Mcal/kg ¹¹	1.67	1.67	1.67

¹Sweet Bran®, Cargill, Inc. Minnetonka, MN.

²Grain States Soya, West Point, NE.

³Church and Dwight Co., Princeton, NJ.

⁴Contained 96% NaCl, 0.35% Zn, 0.2% Fe, 0.2% Mn, 0.03% Cu, 0.007% I, and 0.005% Co.

⁵Elanco, Greenfield, IN.

⁶Adisseo, Alpharetta, GA.

⁷Zinpro Corp., Eden Prairie, MN.

⁸Diamon V Mills, Inc, Cedar Rapids, IA.

⁹Zinpro Corp., Eden Prairie, MN.

¹⁰Provided to diets (DM basis) 3,400 IU of vitamin A/kg, 1,000 IU of Vitamin D/kg, and 57 IU of vitamin E/kg.

¹¹Estimated according to NRC (2001).

Does not include Lys from top dress treatment of 45g daily of RP Lys (56% Lys and assumed 64% bioavailability).

Table 2 Effects of treatments on performance of lactating cows

	Control	Test	Test + RPL	SEM	P Diet	P Rep*Diet
DMI, kg/d	27.31 ^a	28.36 ^b	28.77 ^b	2.04	0.03	0.58
Lysine intake, g/d	197 ^b	177 ^a	203 ^b	11	<0.01	0.68
Methionine intake, g/d	68 ^a	73 ^b	75 ^b	8	0.04	0.91
Milk, kg/d	42.59	42.69	43.57	3.63	0.43	0.05
FCM ¹	38.62	38.64	39.65	3.07	0.31	0.03
ECM ²	41.13	41.28	42.28	3.27	0.31	0.04
SCM ³	44.68	44.87	45.96	3.55	0.31	0.05

^{a-b}Means in rows with unlike superscripts are significantly different.

¹Fat Corrected Milk = (0.4 x kg of milk) + (15 x kg of milk fat).

²Energy Corrected Milk = (0.327 x kg of milk) + (12.95 x kg of milk fat) + (7.2 x kg of milk protein).

³Solid Corrected Milk = (0.0752 x kg of milk) + (12.3 x kg of milk fat) + (6.56 x kg of SNF).

Table 3 Effects of treatments on milk components

	Control	Test	Test + RPL	SEM	<i>P</i> Diet	<i>P</i> Rep*Diet
kg/d						
Fat	1.44	1.43	1.48	0.12	0.33	0.05
Protein	1.19	1.21	1.23	0.10	0.34	0.10
Lactose	2.04	2.06	2.09	0.17	0.39	0.09
SNF ¹	3.62	3.66	3.73	0.30	0.37	0.09
%						
Fat	3.38	3.42	3.44	0.18	0.66	0.46
Protein	2.81	2.84	2.85	0.09	0.29	0.53
Lactose	4.79	4.79	4.82	0.04	0.40	0.63
SNF ¹	8.53	8.56	8.60	0.12	0.16	0.91
Other measures						
SCC x 1,000, cells, ml	259	93	187	112	0.31	0.10
MUN, mg/dL	14.87 ^b	12.49 ^a	12.48 ^a	1.02	0.01	0.28
Body weight, kg	689 ^{ab}	685 ^a	690 ^b	22.8	0.02	0.01
Body condition score	2.75	2.75	2.73	0.13	0.39	0.46
Water intake, L/d	123.9	138.8	125.0	13.45	0.57	0.36

^{a-b}Means in rows with unlike superscripts are significantly different.

¹Solids Not Fat.

Table 4 Pre vs. post CPM Dairy results

	Control Diet		Test Diet	
	Formulated	Actual	Formulated	Actual
DMI, kg	25.9	27.2	25.9	28.3
MP balance, g/d	48.4	230.2	-141.9	134.2
Lysine balance, g/d	15.8	24.3	1.4	15.9
Methionine balance, g/d	13.1	16.2	10.7	16.1
Supported MP milk production, kg	46.4	47.9	42.3	46.0

Values calculated with CPM Dairy utilizing BW, milk production, feed intake, and milk composition.