Improving the Efficiency of Dairy Cattle Feeding

Tyler R. McGill

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science

In

Dairy Science

Mark Hanigan, Committee Chair

Michelle Rhoads

Mike Akers

April 26, 2017

Blacksburg, Virginia

Keywords: ruminant, nitrogen efficiency, model evaluation

ACADEMIC ABSTRACT

The aim of this work was to identify opportunities within the dairy industry whereby nitrogen (N) efficiency may be improved. As certain feedstuffs may be metabolized more efficiently, two trials were conducted where low protein rations were supplemented with rumen protected amino acids (RPAA). As inaccurate nutrient requirement predictions contribute to the inefficiency of feeding systems, a trial was conducted to evaluate model prediction equations. In the first feeding trial, NRC (2001) predicted a decrease milk production of 17% between low protein and high protein groups. However, milk yield was not different among treatment groups. The efficiency of N metabolism was higher in cows fed low protein diets. In the second trial, NRC (2001) predicted deficiencies in metabolizable protein (MP) and amino acids (AA). However, equivocal milk yields among treatment groups provides evidence of overestimated MP requirements. The efficiency of N metabolism was again improved in cows fed diets low in MP. These two trials demonstrate the need for improvement in nutrient requirement models. A third trial was performed to evaluate the PREP10 and Dairy NRC (2001) models using production data from literature to identify deficiencies in prediction equations. Overall, NRC (2001) over-predicted and PREP10 under-predicted milk production. For each model, slope bias exceeded mean bias. Backward elimination regression of residual errors identified correlations with nutrient supplies from both models. Correction of slope bias would considerably reduce prediction errors. Improvements in model predictions could act synergistically with RPAA supplementation to more closely match feed nutrient composition to dairy cow nutrient requirements, thus improving feed efficiency.

GENERAL AUDIENCE ABSTRACT

Biological functions that use amino acids (AA) are limited by AA supply. This concept was likened to staves in a barrel, where the shortest stave determines the barrel's ability to hold water (Mitchell and Block, 1946). Inaccuracies in models that predict nutrient supply and requirements of dairy cows result in inefficient feeding, as under-prediction of requirements results in deficiency, and over-prediction results in excess. To avoid limitations in production due to AA deficiencies, protein is fed in quantities that likely exceed requirements. Overfeeding of AA results in increased expenses for producers and increased N excretion to the environment, providing economic and environmental incentives to increase N-efficiency. Work presented in the following chapters evaluated the impact of AA supplementation on milk production in dairy cattle, and evaluated the PREP10 and NRC (2001) nutrient requirement model predictions. In two feeding trials (Chapter 2 and Chapter 3), low protein diets did not result in decreased milk production, indicating that protein requirements were overestimated. Although supplementation of AA did not increase milk production, low protein diets resulted in greater N-efficiency, especially when supplemented with Histidine. Evaluation of the PREP10 and NRC (2001) models (Chapter 4) used production data from the literature to identify deficiencies in prediction equations, and found that correction of model bias would considerably reduce prediction errors. Model inaccuracies affect the inefficiency of dairy cow feeding, and must be evaluated to improve feed efficiency. Such improvements could act synergistically with AA supplementation to more closely match nutrient supply to requirements.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Mark Hanigan, for his continued support and positivity. His steadfast commitment to never give up on his students has been the saving grace of my MS career. His patience and even temper are qualities that I hope to emulate in my own personal and professional life.

My committee members, past and present, have gone above and beyond to help develop my skills and knowledge as a scientist. Without their guidance, I would not have achieved the success I enjoy today.

To the entire Hanigan laboratory, I am forever grateful for the assistance I received, from feeding cows to writing SAS code, and even sifting feed samples out of manure for days on end.

Every aspect of my MS experience was made better by my fellow graduate students. The friendships I developed over the last several years made each day of school more enjoyable, even with a broken arm.

My family has stood behind every step and misstep of my life and career. I am so thankful to have such a loving family who has always empowered me to make my own dreams come true.

To Laura, I cannot properly express my gratitude for your patience and support. I am so glad that Virginia Tech brought us together. In spite of times that tested your limits, you never gave up on me. You helped me believe in myself.

I would be remiss to not thank dairy cows, themselves. Their loving nature, curiosity, patience and generosity are why I fell in love with them in the first place. Dozens of cows have helped me complete my graduate work, and many others have allowed me to succeed academically and professionally. I am so happy to have dedicated my career to such wonderful creatures.

To any current or prospective student reading this thesis: I spent 6 years completing my MS. Returning to this work after a four year absence was daunting and humbling. Time spent pursing other interests led to mounting anxiety and diminished confidence regarding my ability to finish. But this University is full of amazing people, and with their help I overcame my fears and completed my long-delayed defense. I hope that I may serve as an example that it is never too late for anyone to accomplish their goals.

Table of Contents

List of tables

List of Figures

List of abbreviations

AA	Amino acid	NDF	Neutral detergent fiber
ADF	Acid detergent fiber	NE_L	Net energy for lactation
AF	As fed	NFC	Non-fiber carbohydrate
Arg	Arginine	NPN	Non-protein nitrogen
BCA	Rumen protected citric acid	Phe	Phenylalanine
BIV	Binary indicator variable	ppb	Parts per billion
BW	Body weight	ppm	Parts per million
CP	Crude protein	RDP	Rumen degradable protein
DE	Digestible energy	RFI	Residual feed intake
DIM	Days in milk	RMP	Rumen protected methionine supplemented diet
DM	Dry matter	RMSPE	Root mean squared prediction error
DMI	Dry matter intake	RPAA	Rumen protected amino acid
EE	Ether extract (Fat)	RPH	Rumen protected histidine supplemented diet
His	Histidine	RPLys	Rumen protected lysine
Ile	Isoleucine	RPM/L	Rumen protected methionine and lysine supplemented diet
iNDF	Indigestible neutral detergent fiber	RPMet	Rumen protected methionine
K_d	Degradation rate	RPSBM	Heat treated soybean meal
K_p	Passage rate	SCC	Somatic cell count
Lys	Lysine	SNF	Solids non-fat
ME	Metabolizable energy	TDN	Total digestible nutrients
Met	Methionine	TEAA	Total essential amino acid
MFD	Milk fat depression	Thr	Threonine
MP	Metabolizable protein	TMR	Total mixed ration
MSE	Mean squared error	Val	Valine
MUN	Milk urea nitrogen	+Con	Positive control diet
N	Nitrogen	-Con	Negative control diet

Chapter 1

Introduction

Genomic Selection for Feed Efficiency

Historically, improvements in the feed efficiency of dairy cows have been achieved through increases in production which have proportionally diminished maintenance requirements (VandeHaar and St-Pierre, 2006). Since feed costs account for the largest expenditure on dairy farms (Tozer, 2000), great incentive exists to diminish inputs rather than relying on maximal outputs. As some Holstein cows currently consume feed at or above 4-times maintenance requirements to achieve maximal production, increased productivity is a less viable means of improving feed efficiency (VandeHaar and St-Pierre, 2006). More practical means include the development of tools to genetically select for more efficient animals, as well as improving the efficiency with which those animals are fed.

Koch et al. (1963) introduced residual feed intake (RFI), defined as the difference between actual and predicted energy intake, as a measure of feed efficiency for selection of more profitable beef cattle. This measurement nullifies otherwise confounding variables by accounting for all energy-consuming processes (Koch et al., 1963). Arthur et al. (2001) found that there is a genetic basis for RFI and that it is a moderately heritable (correlation coefficient=0.39) trait in Angus cattle (Arthur et al., 2001). Similarly, Pryce et al. (2012) calculated the heritability of RFI in dairy cattle to be 0.4 (Pryce et al., 2012). Certain pitfalls do exist in using RFI as a measure of efficiency; diets varying in nutrient density could allow animals to consume more or less of those nutrients as compared to their requirement. Inaccuracies in model predictions for intake could falsely alter calculations of RFI. These inaccuracies could account for a portion of RFI variation, which has

been reported to be as high as 30% in growing beef cattle (Nkrumah et al., 2007). However, given this variation in RFI between otherwise similar beef cattle, and given that this trait correlates to genotypic markers, a genetic component of feed efficiency likely exists in dairy cattle as well. Improvements in efficiency have been correlated with high production, suggesting that selecting for efficient animal would not come at the cost of high production (Prendiville et al., 2009). By identifying this genetic component explicitly, heritability of feed efficiency can be characterized and predicted. Such characterization may be capitalized upon by artificial insemination companies, who could allow breeders of dairy cows to select for improved genetics of feed efficiency. Through genetic selection for superior RFI, feed efficiency of dairy cattle as a whole could be improved through breeding of animals that achieve maximal production with minimal feed inputs.

Efficiency of Dairy Cow Feeding

In 2001, the National Research Council published the Seventh Revised Edition of Nutrient Requirements of Dairy Cows. It lists nutrient requirements for dairy cows, contains a database of feed nutrient values, and provides equations to calculate nutrient flows and animal production parameters. As a whole, these equations are used to evaluate and predict nutrient values supplied within rations and their suitability to meet the nutrient requirement of animals to achieve a specific level of production (NRC, 2001). The model predicts, among other values, dry matter intake (DMI), net energy for lactation (NE_L), and metabolizable protein (MP). The accuracy of performance predictions depends on accurate DMI estimation, as intake determines nutrient supply. In the NRC model, lactating cow DMI is calculated as a ratio of animal energy requirements and energy concentration within the ration. Thus, DMI serves as a perfect example of how critically the model relies on accurate animal and nutrient inputs (NRC, 2001).

Since energy is metabolized with similar efficiencies for maintenance and lactation, energy requirements can be expressed using a single unit of NE_L (Tyrrell and Moe, 1975). Different approaches to calculate NE_L, centered on the total digestible nutrients (TDN), digestible energy (DE), and metabolizable energy (ME) of diets. Values for digestible neutral detergent fiber (NDF), crude protein (CP), non-fiber carbohydrate (NFC), and fat are used to calculate DE and TDN. An energy-discount is applied to TDN and used with DE to calculate Discounted DE, which is converted to ME and then NE_L (NRC, 2001).

The remaining fraction of TDN is used along with RDP to calculate microbial protein. Previous editions of the Dairy NRC have quantified dietary protein as "absorbed protein". Protein itself is not absorbed; rather it is absorbed as amino acids (AA) and peptides after enzymatic and chemical digestion. Thus, the term "absorbed protein" was replaced by "metabolizable protein" in the last edition, which refers to rumen undegradable protein (RUP) and microbial protein available for digestion. Rumen undegradable protein refers to the CP of the diet that bypasses the rumen without conversion to microbial protein, comprised of true protein as well as non-protein nitrogen (NPN) sources. Microbial protein is calculated using Discounted TDN and RDP, assuming complete conversion of RDP to microbial protein. The rate at which protein that flows to the duodenum is metabolized and incorporated into milk protein is considered constant at 67%. These equations which predict supply of NE_L and MP are combined with equations that use animal characteristics to calculate requirement, ultimately allowing for the calculation of allowable milk production (NRC, 2001).

Another nutrient requirement model, PREP10, has been developed by Papillon Agricultural Company (Easton MD). PREP10 was developed for use by commercial nutrition consultants. Like NRC (2001), PREP10 provides an extensive feed library, and provides predictions of nutrient

flows and allowable production, but gives special emphasis to AA by predicting requirement, supply, and allowable production based on each of the 10 essential AA. The calculation of MP also differs from NRC (2001) in that it accounts for a certain fraction of RDP that escapes the rumen in the liquid fraction, and it considers protein use efficiency as a variable at different levels of intake and production (White et al., 2017c). It contains three binary indicator variables (BIV) which impact microbial growth predictions. These include effects of sugar, starch, and control of flow of the B1 protein fraction with liquid, which can be turned on and off in 8 unique arrangements.

Due to imprecise or biased estimation of nutrient requirements in models such as these, and in an effort to avoid nutrient limitations, nutrient requirements are often exceeded when formulating dairy cow rations (McNamara, 2004). While this practice may ensure an adequate supply of nutrients for maximal milk production, it does have disadvantages. Overfeeding of nitrogen (N) results in increased expense for producers and increased excretion of N to the environment (Li et al., 2009). By more accurately calculating animal requirements and dietary supply of nutrients, excess resource utilization and excretion to the environment may be greatly diminished. Thus, a more accurate and precise modeling program would provide a great environmental and economic advantage for producers.

Bibby and Toutenberg (1977) described a method for evaluating model accuracy from an analysis of residual errors (model prediction minus observed) which can be summarized as the root mean squared prediction error (RMSPE), and partitioned into mean bias, slope bias, and dispersion fractions. Mean bias represents a constant error across all predictions, whereas slope bias represents a variable error depending on the magnitude of the prediction (i.e. more or less error of prediction as predictions become larger or smaller) (Bibby and Toutenburg, 1977). A brief

evaluation of model predictions is reported in the 2001 NRC that includes 100 diets from 25 studies published from 1992 through 2001. This analysis showed RMSPE for DMI with no apparent slope bias. NE_L allowable milk was found to have a modest mean bias of 2.5%. However, NE_L allowable milk showed a more apparent slope bias, authors dismissed this as it would not likely apply to the range of NE_L concentrations found in rations normally fed within the United States. For most circumstances, MP allowable milk was found to contain substantial mean bias, as 67% of diets over-predicted MP allowable milk by more than 10%. Due to the limited number of studies used to evaluate the model, authors proposed that although many of the diets were not limiting in MP, AA content may have been improperly balanced. This resulted in an AA deficiency that limited production (NRC, 2001). Theses biases were revisited by St. Pierre (2003), who concluded that statistical methods for residuals analysis were valid and that error within these equations could be largely attributed to inherent imprecision in measurement of observed values. Although these model predictions were assessed appropriately, other equations predicting duodenal N-flows in NRC (2001) were originally evaluated by plotting residuals against observed values. Upon reevaluation by St-Pierre (2003), previous evaluation of duodenal N-flow predictions were deemed invalid (St-Pierre, 2003). From these model evaluations, authors conclude that more observations are needed from a more diverse range of nutritional scenarios to properly evaluate the model (NRC, 2001, St-Pierre, 2003).

Since the publication of NRC (2001), certain model equations have been evaluated more extensively. Seo et al. (2006) published a comprehensive evaluation of NRC (2001) equations which predicted passage rates (K_p) of wet forage, dry forage and concentrates out of the rumen. These K_p helped determine the supplies of RDP, RUP, and MP. A data set of 319 diet treatment means was used to evaluate these passage rate equations. Sensitivity analysis showed that these

model equations were most sensitive to DMI, rate of degradation (K_d) of protein-B fraction, and CP content of feed. Overall, it was concluded that the 2001 Dairy NRC ruminal Kp equations were adequate for lactating dairy cows (Seo et al., 2006).

Lanzas et al. (2007) performed a sensitivity analysis of NRC (2001) MP and AA supply and requirement predictions. Two diets were formulated; the first diet contained low protein and was formulated to meet 20kg/d milk production, whereas the second diet contained high protein and was formulated to meet 38kg/d production. A sensitivity analysis was performed based on Monte Carlo techniques (Helton and Davis, 2003) to assess the impact of CP and essential AA composition on model predictions of nitrogen flows out of the rumen. Flows of MP and essential AA were found to be sensitive to K_d of the protein B-fraction. Authors concluded that more data are needed on protein fractionation and K_d for forages and concentrates to more closely represent the effects of dietary changes on MP flow to the animal (Lanzas et al., 2007).

Krizsan et al. (2010) used 172 treatment means from published literature to perform a meta-analysis of the effect of forage type on K_p equations in NRC (2001). Regression of residuals on predicted values of K_p identified significant mean and slope bias of these equations. Slope bias was calculated to be -0.59, indicating that as the magnitude of K_p (expressed as %/h) predictions increase, true K_p becomes increasingly over-predicted. Mean bias was calculated to be -2.4%/h. From this analysis, authors concluded that K_p is affected by forage type, indicating the need for more precise equations to predict the passage rate of individual forage types (Krizsan et al., 2010).

Since milk production predictions of NRC (2001) and PREP10 have not been rigorously evaluated against experimental data, further work must include evaluation of predictions of production to determine: 1) the overall performance of each model, 2) correlations between nutrient flow and residual error, and 3) what deficiencies exist in prediction equations.

Efficiency of Feed Metabolism

Another manner in which feed efficiency may be improved is by utilizing feed ingredients that may be digested more efficiently. One popular approach has been to search for limiting amino acids based on the "most limiting nutrient" theory. Mitchell and Block (1986) applied this theory to AA, stating that the nutritive value of a protein is determined by its content of the first-most limiting AA (Mitchell and Block, 1946). Supplementation of limiting AA has been shown to decrease the amount of dietary CP needed while maintaining level of production, as seen in swine (Tuitoek et al., 1997) and in poultry (Edmonds et al., 1985). Unlike monogastric species such as poultry and swine, ruminant AA metabolism is much more complex; proteins and AA from the diet are first exposed to digestion in the rumen, where proteins are degraded and AA are metabolized to microbial protein. The AA profile of digesta reaching the duodenum is that of microbial protein plus RUP, and thus altered from the dietary AA profile of even an optimally formulated ration. Therefore, the AA profile of digesta reaching the duodenum does not often match AA requirements of high producing dairy cows (Kung and Rode, 1996).

In an effort to alter the AA profile of digesta reaching the duodenum, attempts have been made to protect AA from ruminal degradation by rendering them inert to rumen digestion. Results of this strategy have been mixed with respect to milk yield responses to rumen protected AA (RPAA) (Rogers et al., 1987, Polan et al., 1991, Robinson et al., 2010). Additional data are needed to better assess the mechanism of lactation response to RPAA.

Many studies have focused on lysine (Lys) and methionine (Met) as limiting AA. Rogers et al. (1987) provided evidence that Lys and Met may be first limiting amino acids, and thus provision of these may increase milk production when fed in rumen protected form. Diets deficient in CP were supplemented with RPMet and RPLys and fed to 8 Holstein cows in a Latin square design.

Though the increase in milk yield observed in this study encouraged further investigation of Met and Lys, the effect was not statistically significant (P=0.12) (Rogers et al., 1987). King et al. (1991) provided further evidence that Lys is the first limiting AA in dairy cows. Two experiments were performed using Latin square designs where 6 and 12 animals, respectively, were infused with graded levels of Lys. Supplementation had no effect on milk yield in the first trial, but produced a linear increase in milk yield as increasing levels were infused to the second group. Furthermore, milk protein yield increased linearly with increased level of Lys infusion in both trials (King et al., 1991). Guinard and Rulquin (1995) fed CP deficient diets to 4 animals and infused graded levels of Met duodenally. Production was unaffected by these treatments, though mammary blood flow significantly decreased in the highest level of Met infusion. In this case, unchanged production in the face of decreased blood flow may suggest improved efficiency of mammary AA uptake. Although this study revealed a statistically significant effect of Met on mammary blood flow, it provided more questions than answers about the mechanism of milk yield response to changes in dietary AA profile (Guinard and Rulquin, 1995). Polan et al. (1991) summarized data from 259 cows fed diets of varying protein quality and protein source at 6 universities. Diets using corn gluten meal as the major protein source and supplemented with RPLys and RPMet were observed to increase milk yield. Supplementation with RPLys and RPMet did not, however, increase milk yield in similar experimental groups that used soybean meal as the major protein source. As one may have suspected given varying AA profiles of differing protein sources, this study suggests that protein source must be considered when determining AA supplementation rates (Polan et al., 1991).

Recognizing the impact of varying feedstuffs, more recent trials have provided more in depth analysis of diet composition and have expanded to evaluate additional AA. Vanhatalo et al. (1999)

supplemented grass silage-based diets with a variety of AA, including histidine (His) alone, as well as combinations of His, Lys and Met. Infusions of Lys and Met had no effect on milk yield. Infusions of His, however, increased milk yield and milk protein, while decreasing milk fat and lactose production. This study suggests that in this case, histidine was limiting, thus adding to the complexity of the mechanism of limiting AA in dairy cow diets (Vanhatalo et al., 1999). This concept was described further by the 2010 study by Robinson et al., where CP deficient diets were supplemented with RPLys alone, as well as a combination of RP Lys, His, isoleucine (Ile) and valine (Val). No effect on milk production was produced by the RPLys alone, whereas the supplement of multiple RPAA produced a small increase (Robinson et al., 2010). A subsequent study by Robinson et al in 2011 used 1,400 lactating cows of variable lactation stages to further observe the effect of RPLys supplementation. Rumen protected lysine increased milk yield and milk fat, protein and lactose production in mid lactation animals, but failed to produce a response in early lactation animals. Results of this study suggest that AA were not supplemented at a sufficient level to meet the requirements of early lactation, but that RPLys produced increased milk yields when requirements were sufficient for other AA (Robinson et al., 2011).

Traditionally, RPAA are supplemented to diets deficient in RUP, but sufficient in RDP, as limiting protein supply to the rumen could impact microbial growth and nutrient digestibility. Lee et al. (2012) fed combinations of RPAA to animals consuming diets of moderate forage content (60%) that were limiting in both RUP and RDP. From the positive control (sufficient RUP and RDP) to the negative control (deficient RUP and RDP), a 3kg decrease in milk production was observed. Supplementation of Lys and Met to the negative control provided a modest increase in milk yield as compared to control groups. Supplementation with RP lysine, methionine and histidine completely corrected the depression of production (Lee et al., 2012).

Arriola et al. (2014) sheds some light on this history of conflicting responses. It may prove effective to break from the theory of "most limiting nutrient", as different nutrients are limiting in different settings, and different nutrients (AA, specifically) can play a compensatory role (Arriola Apelo et al., 2014). According to the conventional wisdom of limiting-AA theory, if a specific AA is deficient and production is depressed, then only supplementation of that AA would allow for production to increase. A review by Cant et al. (2003) reports many instances where this is not the case, and attributes recovery of production in the face of AA deficiency to compensatory mechanisms. These mechanisms may include changes in partitioning and absorption of AA at the level of the mammary gland, splanchnic, and peripheral tissues (Cant et al., 2003). If this is the case, it would be most practical to supplement diets with whatever is most readily available and economical, rather than what is "most limiting".

Studies such as these reflect the complex nature of amino acid metabolism in the dairy cow. Additional data are needed to elucidate the mechanism of lactation response to RPAA, to evaluate the efficacy of RPAA products for increasing milk yield, and to develop improved representations of AA supplies and requirements in our ration balancing models.

Summary

Great environmental and economic incentives exist for improving the efficiency of dairy cows and dairy cow feeding. Feed costs account for the largest expenditure on dairy farms. Lack of knowledge of the true AA requirements of animals results in overfeeding of dietary N to prevent inadvertent losses in production. These N losses result in increased expenditure for producers and high levels of N excreted to the environment. As many dairy cows already produce large quantities of milk, genetic identification of more efficient animals would allow producers to select not just for cows with high production to achieve greater efficiency, but to select for efficiency directly.

By improving the accuracy and precision of nutrient requirement models, nutrients delivered in rations may more closely match nutrients required. The improved models would also allow more precise identification of efficient animals than the current RFI approach. Improved accuracy and precision would decrease excess expenditures for producers, and decrease excess that is otherwise excreted to the environment. Providing feed ingredients that are metabolized more efficiently, such as RPAA, may allow for lower input of N-containing feeds. Such advancements would lead to improvements in air and water quality, while decreasing financial inputs and demand on land and other natural resources.

Chapter 2

Improvements in feed efficiency via rumen protected amino acid supplementation limited by ration formulation software

ABSTRACT

To avoid limitations in production due to amino acid (AA) deficiencies, metabolizable protein (MP) is fed in quantities that likely exceed requirements for all of the AA across a range of diets. Overfeeding of AA, and thus nitrogen (N), results in increased expenses for producers and increased N excretion to the environment. Thus, improvements in the N efficiency of milk production provide economic and environmental incentives. Previous improvements in N efficiency have come via the dilution of maintenance through increased production. To achieve further improvements, it is necessary to explore approaches such as supplementation of limiting AA in rumen protected form (RPAA) to diets low in MP. The goal of this experiment was to evaluate the impact of specific RPAA products on milk yield. The 2001 NRC ration formulation software was used to formulate diets that were fed to 36 multiparous mid-lactation Holstein dairy cows in a 4x4 Latin square design. At the time of selection, cows produced an average of 35 kg of milk/d. Diets were: (1) a positive control diet with 17.1% CP (+Con), (2) a negative control diet containing 15.4% CP (-Con), (3) -Con supplemented with 20 g/d/cow of RP methionine (RPM), and (4) -Con supplemented with 20 g/d/cow of RP methionine and 60 g/d/cow of RP lysine (RPM/L). Data were analyzed using the GLIMMIX procedure in SAS (v9.3). Milk yield was not different among any of the diets, suggesting that protein was not sufficiently limiting in –Con to affect production. This likely explains the lack of treatment responses on milk production. However, concentrations of MUN from cows fed –Con, RPM and RPM/L diets were lower than that of cows fed +Con diet ($P<0.0001$), suggesting improved efficiency of N metabolism in cows

fed diets low in MP. The NRC Predicted MP balance of the –Con diet was -262 g/d which equated to a predicted decrease in MP allowable milk of 17% as compared to +Con. This model inaccuracy demonstrates the need for improvement in dairy nutrition models, as clearly the MP requirements were over-specified for this group of cows. Model inaccuracies contribute significantly to the inefficiency of dairy cow feeding, and must be evaluated in an effort to improve feed efficiency. They also contribute to failures in predicting responses to RPAA supplementation under conditions of MP deficiency to achieve greater N efficiency.

INTRODUCTION

Past improvements in the nitrogen (N) efficiency of dairy cows have been achieved through increases in production which have proportionally diminished maintenance requirements (VandeHaar and St-Pierre, 2006). As some cows currently consume feed at or above 4-times maintenance requirements, additional improvements become increasingly difficult due to declining marginal dilutions of maintenance. Development of tools to genetically select for more efficient animals, as well as improving the efficiency with which those animals are fed is likely a more productive approach moving forward.

Feed efficiency may also be improved by utilizing feedstuffs that may be metabolized more efficiently. According to the limiting AA theory, production is determined by the most-limiting AA, likened to the shortest stave in a barrel determining its water carrying capacity (Mitchell and Block, 1946). As such, crude protein (CP) is often provided in excess of requirements to ensure that requirements are met for each AA. Supplementation of limiting amino acids (AA) decreases the amount of dietary CP needed while maintaining level of production, as demonstrated in swine (Tuitoek et al., 1997) and in poultry (Edmonds et al., 1985). Supplementing AA may provide a similar benefit in dairy cattle, although ruminant AA supplementation is more complicated than

for monogastric species. Proteins and AA from the diet are first exposed to digestion in the rumen, where proteins are degraded and a portion are remodeled into microbial protein. The AA profile of digesta reaching the duodenum is thus the sum of AA in microbial protein plus rumen undegradable protein (RUP) and secretions into the rumen. As such, it is altered from the dietary AA profile. Therefore, the AA profile of digesta reaching the duodenum does not often match AA requirements of high producing dairy cows (Kung and Rode, 1996).

Multiple studies have shown that graded increases in Lys supplementation increase milk and milk protein yield (King et al., 1991, Guinard and Rulquin, 1995), though low level Lys supplementation to CP deficient diets does not appear to improve production. The effect of RPLys supplementation has also been shown to vary based on stage of lactation, as similar levels of RPLys supplementation were found to increase milk and milk component yields in mid-lactation cows whereas no effect was observed in early lactation cows. As animals in early lactation are often in negative protein balance, increased mobilization of protein from tissues add to the supply of AA absorbed by the gut (Robinson et al., 2011). Thus, the success of RPAA supplementation is contingent on accurate estimation of AA requirements.

Other studies have focused on the combination of rumen protected lysine (RPLys) and methionine (RPMet) as limiting AA. Conflicting evidence exists regarding the effect of RPLys and RPMet supplementation on milk production when added to diets consisting of corn silage and soybean meal or corn gluten meal. This suggests that dietary protein source and quality impacts the effect of RPAA supplementation (Rogers et al., 1987, Polan et al., 1991, Robinson et al., 2011).

Additional data are needed to better understand lactational responses to RPAA particularly in protein limiting diets. Thus, the objective of this study was to evaluate the impact of supplementing protein deficient diets with RPMet and RPLys on milk production in mid-lactation dairy cows

consuming diets composed of corn silage and high quality protein sources. We hypothesized that feeding diets deficient in MP will result in a decrease in milk production, and that decreased production will be partially alleviated by feeding RPMet or a combination of RPLys and RPMet.

MATERIALS AND METHODS

Animals and Housing

Feeding Trial. All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. Thirty-six lactating primiparous (N=18) and multiparous (N=18) Holstein dairy cows from the Virginia Tech Dairy herd were allocated to the trial. Cows were chosen based on milk production, DIM, and stage of gestation. Animals ranged from 70-230 days in milk (DIM) with daily milk production ranging from 21 kg-60 kg. Cows were used in a Latin square experimental design consisting of 4 treatments and 4 periods. Animals were blocked by DIM and randomly assigned to treatment groups. Experimental periods lasted 21 days and consisted of 14 days of diet adaptation and 7 days of sample collection. Animals were housed in the free stall barn of the Virginia Tech Dairy Facility in 4 pens equipped with Calan Broadbent individual animal feeders (American Calan Inc., Northwood, NH). All animals had free access to water, and were milked 2 x/d. One cow was removed from the study for health problems unassociated with the study.

Mobile Bag Trial. Two ruminally cannulated, mid-lactation Holstein cows were used to assess ruminal and intestinal protein degradation kinetics. Animals were housed in tie stalls of the nutrition research barn at the Virginia Tech Dairy Center. Regular lactating dairy cow rations were provided ad-libitum and water was freely accessible. Cows were milked twice daily at 0300 and 1400 h using a portable milking unit.

Treatments and Diets

Feeding Trial. Diets were formulated using the NRC 2001 software. Diets were: (1) a high MP, positive control diet containing 17% CP (+Con) and meeting all requirements of a lactating cow producing 35 kg/d, (2) a low MP, negative control diet containing 15.5% CP (-Con) that was isoenergetic with +Con but limiting in MP (MP allowable milk = 30 kg/d), (3) -Con supplemented with RPMet (RPM) to match the duodenal Met flow of +Con , (4) -Con supplemented with RPMet and RPLys (RPM/L) to match the duodenal Lys and Met flows of +Con. Based on NRC (2001) predictions, -Con provided 6 grams less Met and 12 grams less lysine flow to the duodenum per day than +Con. Diet composition is listed in Table 2.1 and Table 2.2. Diets were mixed using a Data Ranger (American Calan Inc., Northwood, NH), and fed as total mixed rations (TMR) once daily to achieve 5-10% refusals. The grain mixes were fed in pelleted form and constructed according to Table 2.2. Diets were mixed a minimum of 15 minutes. Rumen protected AA were added to the mixer to deliver 0.9 g of RPMet/kg of DM (70% Met in DM; Balchem Corp., New Hampton, NY), and 2.6 g of RPLys/kg of DM (55% Lys in DM; AminoShure-L; Balchem Corp.).

Sample Collection and Analyses

Feeding Trial. Total mixed rations were sampled on days 3, 6, 10, 13, 17, and 20 of each period. Individual feed components were sampled on days 3, 10, and 17 of each period. Samples of TMR were collected using the quartering technique (Dairyland Laboratories, Inc., Arcadia WI), and stored in 1 gallon air tight plastic bags. Feed samples were stored at -20^0 C and subsequently thawed for drying. Samples of TMR were composited for the first two weeks of each period, while samples from the third week of each period were handled separately. Samples (~200g each) were dried in a forced air oven at 55^0 C until daily variation in weight was less than 1 g. Dry samples were ground in a Wiley mill through a 2-mm screen (Arthur H. Thomas Co., Philadelphia, PA),

and subsamples submitted to Dairyland Laboratories, Inc. (Arcadia WI) for nutrient analysis. Daily dry matter intake (DMI) was calculated as the difference between fed offered and refusal for each animal adjusted using the 55^0 C DM content of each ration.

Cows were milked at 0300 and 1300 h. Milk weights were recorded at each milking, and body weights (BW) were recorded as cows exited the milking parlor. Milk samples were taken on days 4, 11, 18, 19, and 20 of each period at the morning and afternoon milkings. Sample analysis was performed at the DHIA laboratory at Virginia Tech. Samples were analyzed for lactose, protein, fat, milk urea nitrogen (MUN), and somatic cell count (SCC). Milk weight, fat content, protein content, lactose content, and SCC were measured at each milking using the Afimilk system (Afimilk Ltd, Kibbutz Afikim, Israel). Body condition scores were assessed by two individuals at the beginning of the study and at the end of each period

Fecal samples were collected trans-rectally from each animal on days 19 and 20 of each period. Fecal samples were freeze-dried, composited by animal and period, ground in a Wiley mill through a 2-mm screen (Arthur H. Thomas Co., Philadelphia, PA), and analyzed for N, neutral detergent fiber (NDF), and indigestible NDF (iNDF) content. Content of iNDF in feed and feces was determined by in situ incubation in the rumen of two cannulated cows for 12 days. The cannulated cows were housed with other lactating animals in the main free stall barn at the Virginia Tech Dairy facility and were fed a standard lactating cow ration.

Spot urine samples were collected from each animal over the course of the third week of each period. Urine was acidified with H_2SO_4 to a pH < 2.5, and analyzed for total N and creatinine concentrations.

Mobile Bag Trial. Mobile bag sample preparation and analysis followed methods described in Forage Evaluation in Ruminant Nutrition (Hvelplund and Weisbjerg, 2000). Since heat treated

soybean meal is a reliably degraded N-containing feedstuff, it was used as a positive control (RPSBM) (Soyplus, Southern States, Christiansburg, VA). Rumen protected citric acid was chosen as a negative control (BCA) since it is a N-free, lipid encapsulate. Thirty-two samples of each RPAA and control product, 1.5g each, were weighed into 5 × 5-cm, 50-μm-poresize Dacron bags (Ankom Technology Corp., Macedon NY). After sample addition, the bag was heat sealed, labeled, and weighed.

In situ samples of RPSMB, BCA, RPMet and RPLys were ruminally incubated for 8h, and then manually placed in the abomasum of the same cow via the reticulo-omasal orifice. Sample placement occurred over a 16 hour period. At each hour, 3 samples were placed in the abomasum of each animal. The time of placement of each bag was recorded. Total fecal collection commenced 8h after abomasal placement of the first samples, and continued until 56h after the placement of the last samples. Fecal collections occurred at 4-hour intervals. Feces were filtered through a 5-mm pore size mesh to retrieve the samples. In addition to total fecal collection, fecal grab samples were collected at each sampling time to recover any bags present in the rectum. To determine rumen degradation of each product, 4 of the ruminally incubated samples of each ingredient were retained without introduction into the abomasum.

Upon recovery, samples were washed in three cold water baths (30 seconds in each bath), spun dry in a salad spinner and immediately frozen at -20^0 C. Samples were freeze dried in a lyophilizer at -80^0 C and weighed to determine dry matter content. Contents of each bagged sample were analyzed in duplicate for total N via combustion analysis. Visual observations of products were made before and after the freeze drying process. No evidence of product degradation was observed.

Statistical Analysis

Feeding Trial. Nitrogen content of TMR and observed DMI were used to determine N intake. Assuming 29 mg of creatinine excretion per kg body weight per day as reported by Valadares et al. (1999), daily urinary output was estimated as:

$Vol_u = (BW * 29) / Conc_u$,

where Vol_u = daily urine production (L/d), BW = body weight (kg), and $Conc_u$ = concentration of urinary creatinine (mg/L) (Valadares et al., 1999). Urinary N content was used with total urinary output to determine total urinary N output.

Fecal concentrations of indigestible neutral detergent fiber (iNDF) were used with TMR concentrations of iNDF to calculate total fecal output:

$F_{total} = (iNDF_{intake}) / (iNDF\%_{feces})$,

where F_{total} = fecal output (kg/d), $iNDF_{intake}$ = iNDF intake (kg/d), and $iNDF\%_{feces}$ = iNDF content of feces (% of DM) (Huhtanen et al., 1994). Total fecal output and the N content of feces were used to calculate fecal N output.

Milk protein and MUN content was used with observations of milk yield to calculate total milk N output. Total N output was calculated as the sum of urinary, fecal, and milk N output. Nitrogen balance was then calculated using total N intake and total N output.

Data were analyzed using the GLIMMIX procedure in SAS (v9.3, SAS Inst. Inc., Cary, NC, USA). The effect of dietary treatment was assessed using the model:

$$Y_{ijkl} = \mu + T_i + P_j + S_{ij} + (T_i + P_j) + \beta_{ij}(S_{ij}) + \varepsilon_{ijk,}$$

where Y_{ijkl} = the dependent variable, μ = the population mean of Y, T_i = the effect of the ith treatment (N=4), P_j = the effect of jth period (N=4), S_{ij} = the random effect of square, $\beta_{ij}(S_{ij})$ = the random effect of cow nested within square, and ε_{ijk} = residual error. Data are reported as least

square means with standard errors of the mean (SEM). Statistical significance was declared at $P < 0.05$.

Mobile Bag Trial. Recovery rate of in-sacco samples was not sufficient for statistical comparison among samples. Time of placement and time of recovery were used to calculate total retention time. The starting N content was determined for samples of each ingredient. Nitrogen content of ruminally and intestinally digested samples were compared to starting nitrogen to calculate residual nitrogen content. Residual nitrogen content and intestinal retention time were plotted to generate degradation curves of each product. Data were analyzed using the MEANS procedure in SAS (v9.3, SAS Inst. Inc., Cary, NC, USA) to determine mean intestinal retention time as well as mean nitrogen and dry matter retention after in-sacco incubation.

RESULTS AND DISCUSSION

In-sacco rumen incubation of 8 hours for RPAA samples has been shown to accurately assess rumen protection (Rossi et al., 2003). Data from ruminal incubations are presented in Table 2.5, which shows that greater than 100% of the original N content was present in RP Lys and RP Met samples after 8 hours of exposure to ruminal degradation. As such, rumen protection was determined to be adequate for each AA product, although ruminal N-contamination was evident in both products. The original objective was to correct for such potential contamination using the BCA product. However it is clear from the data that it did not act in the same manner as the RPAA products. This may have been due to large differences among the particle sizes of each of the products with the BCA product having much smaller particles.

Of the 96 mobile bag samples placed in the abomasum, only 15 were recovered in feces. Mean transit time and N content of each ingredient are reported in Table 2.5. Intestinal residence time of samples ranged from 32 to 77 hours. The mobile bag technique utilized in this study is novel, as

manual placement of in-sacco samples in the abomasum has not been described in literature. Though ruminant gastrointestinal motility has been well documented (Church, 1969), the abomasal responses to placement of relatively large in-sacco samples seems to be unpredictable. This probably explains why there were no reports of this method in the literature. The size, shape, and weight of Dacron bags may have resulted in backflow to the rumen or stagnation in the abomasum.

Analysis of RPSBM and BCA control products suggests that the mobile bag technique adequately represents digestion of feedstuffs, although relatively long residence times make this method more indicative of over-digestion. Figure 2.1 shows that N from RPSBM was partially degraded within the rumen, and almost completely digested post-ruminally. However, due to intestinal residence times above that of normal digesta, disappearance of bag contents may be misleading. This positive control serves as an adequate comparison to products that are not readily digested; Dacron bags did not serve as a barrier to prevent intestinal digestion of RPBCA, and thus any product that does not disappear after intestinal residence is not likely digestible. Nitrogen content of undigested BCA was found to be 0.2% on a DM basis, indicating that the product was virtually N-free. Contents of digested BCA sample bags showed almost complete intestinal digestion of dry matter, and analysis revealed N content even lower than that of virtually N-free undigested samples. Thus, bag contamination was not a relevant source of N among in-sacco samples, making any increase in N content of in-sacco samples due to microbial adherence to the product and not the Dacron bag.

Degradation versus time in the digestive tract of each product is shown in Figure 2.1, Figure 2.2, Figure 2.3, and Figure 2.4. Results indicate that the RPMet product used in this study was prone to ruminal contamination, as N content after removal from the rumen (0 time) was greater than 100% of starting N. Given the lack of Dacron bag N contamination demonstrated by BCA,

adherence of rumen microbes to the RPMet capsule were likely the source of this N contamination. The degradation curve reveals that once RPMet reached the intestine it was readily digested. The RPLys product had less N contamination in the rumen. However, digestion was incomplete post-ruminally, suggesting that this product was over-protected. Thus, the RP-Lys product likely delivered very little of the encapsulated Lys to the animal which compromises that treatment in the main trial.

To properly evaluate protected amino acids, it is necessary to establish a method for evaluating their digestibility and bioavailability. Placement of mobile bag samples in the abomasum via the ruminal cannula is much less challenging than introducing a duodenal cannula, however excessive transit times with such placement limit its practical usage. Thus, this method would likely be of greater value if samples could be placed in the duodenum and recovered at the ileum (Van Straalen et al., 1993, Ali et al., 2012).

Least square means of milk production parameters are presented in Table 2.4. Milk protein yield was significantly different between groups, as animals fed +Con diets produced significantly more protein than animals fed the RPM/L diet. However, milk protein yield was not significantly different between animals fed +Con and –Con diets, making the biological significance of the difference between +Con versus RPM/L milk protein yield difficult to interpret. Milk yield was not significantly different among all treatment groups. Milk fat yield and milk fat percentage was not different between treatment groups, nor was SCC.

Although not different among treatment groups, milk fat percentage was found to be lower than expected; cows consuming corn silage-based diets produce milk with an average milk fat content of 3.5% (Oba and Allen, 1999), whereas milk fat content in this experiment ranged from 2.83% in the +Con treatment group to 3.02% in the RPM/L treatment group. Furthermore, DMI was lower

for cows consuming the RPM/L diet than that of cows consuming the +Con and -Con diet. As mycotoxin contamination of diets has been shown to negatively impact intake and milk fat production (Applebaum et al., 1982), corn silage mycotoxin contamination was considered as a possible cause of unexpected DMI and milk fat content. Mycotoxins of particular interest were aflatoxin, which can negatively impact production at levels of 100 parts per billion (ppb) in feed (Patterson and Anderson, 1982), and vomitoxin, which can negatively impact production at levels of 2.5 parts per million (ppm) (Whitlow et al., 1994). However, analysis of corn silage samples by Dairyland Laboratories, Inc. (Arcadia WI) revealed aflatoxin and vomitoxin levels of less than 12 ppb and 1 ppm, respectively, and these levels are not generally considered to cause problems. As reviewed by Bauman and Griinari (2003), high levels of dietary carbohydrates that are readily fermented in the rumen can result in milk fat depression (MFD). Nutrient composition of forages, concentrates, and TMR fed in this experiment are reported in Table 2.3. Composition of TMR from all treatment groups included 30% starch and greater than 40% non-fiber carbohydrate (NFC), which is near the limit of dietary inclusion to result in MFD (Bauman and Griinari, 2003), and thus this appears to be the cause of the milk fat depression in the current study.

Nitrogen intake and output are reported in Table 2.4. Consistent with the design of these rations, N intake was higher in the +Con ration than the –Con. As a result of the significant difference in DMI between –Con and RPM/L groups, N intake was significantly lower in cows fed the RPM/L diet than the –Con diet. Output of MUN was higher in the +Con treatment group than all other treatment groups, suggesting improved N efficiency in cows consuming low protein diets. This improved efficiency is also reflected by N-capture in milk protein; animals consuming lower amounts of N utilized a higher percentage of dietary N for milk protein production.

Nitrogen balance was calculated by subtracting grams of N output (sum of N in feces, urine and milk) from grams of N intake. The +Con treatment group excreted more N without producing more milk protein than animals consuming low protein rations, with the exception of the RPM/L treatment group. This suggests that, although the 2001 NRC ration formulation software predicted the low protein rations to limit milk production, no such limitation was observed. Considering the high N output of animals consuming a high protein diet, these data serve as evidence that protein requirements are overestimated.

Given that milk protein yield was not different between control groups, protein supply may not have been sufficiently limiting to affect production. Predictions of MP, Met and Lys supplies are listed in Table 2.1. The predicted supply of Lys was over-predicted due to the low intestinal availability of the RP Lys product. Predictions from NRC (2001) indicated that MP and AA flow to the duodenum would be greatly diminished by the –Con diet. The predicted MP balance of the –Con diet was -262 g/d, and predicted a 17% decrease in MP allowable milk compared to the +Con. Diets were predicted to provide sufficient energy (to match +Con MP allowable milk), however observed production showed that both MP and NE_L requirements were over-predicted. These inaccuracies demonstrate the need for improvement in dairy nutrition models.

Moe and Tyrell (1975) found that increasing DMI results in depressed nutrient digestibility. As modern dairy cows commonly consume 3-4 times maintenance requirements, this decrease in digestibility is of increasing relevance (Tyrrell and Moe, 1975). The NRC (2001) nutrient requirement model assumes a fixed MP conversion efficiency of 67%, whereas White et al. (2017) demonstrated that it is more accurately represented as varying with level of intake and production (White et al., 2017c). Thus, as intake and production increase, so does the degree of error for NRC (2001) predictions.

Model inaccuracies contribute substantially to inefficiency of dairy cow feeding, and must be evaluated in the effort to improve dairy cow feed efficiency. Such improvements could act synergistically with RPAA supplementation to more closely match nutrient composition in feed to nutrient requirements of dairy cows.

TABLES

Table 2.1 Composition of diets on a DM basis.

Item	+ Con	- Con	RPM	RPM/L
Corn silage[1]	34.8	34.8	34.8	34.8
Alfalfa haylage[1]	16.5	16.5	16.5	16.5
High CP grain mix[1]	48.7	0.00	0.00	0.00
Low CP grain Mix[1]	0.00	48.7	48.7	48.7
RP Lys[1,4]	0.00	0.00	0.00	0.26
RP Met[1,4]	0.00	0.00	0.09	0.09
NE_L allowable milk, kg/d[2]	43.2	43	43.3	43.5
MP allowable milk, kg/d[2]	43.6	35.8	36.2	36.7
MP supply, g/d[2]	2709	2377	2387	2409
Met flow, g/d[2]	50	44	53	52
Met, %[2,3]	1.83	1.86	2.2	2.18
Lys flow, g/d[2]	162	150	150	165
Lys, %[2,3]	6	6.3	6.29	6.86

[1]Percent of diet dry matter.
[2]Predicted values based on observed dry matter intake, body weight, and milk production.
[3]Values reported as a percentage of predicted total MP supply.
[2]Rumen protected lysine (38% Lys in DM, Balchem Corp, New Hampton, NY).
[3]Rumen protected methionine (70% Met in DM, Balchem Corp, New Hampton, NY).

Table 2.2 Composition of the grain mixes.

	High CP	Low CP
Ingredient	% of grain mix AF	
Corn grain, ground, dry	40.6	40.6
Soybean, meal, solvent extracted	12.5	13.4
Corn dry distiller grain + solubles	13.4	13.4
Corn gluten meal	4.91	0.00
Corn gluten feed	1.79	6.24
Blood meal, sprayed	2.23	0.00
Soybean, hulls	13.4	14.3
Urea	0.803	0.803
Energy Booster 100	3.57	4.46
Calcium carbonate	2.21	2.21
Calcium phosphate (Di-)	0.892	0.892
Sodium bicarbonate	1.02	1.02
Salt	1.02	1.02
0.06% Selenium Premix	0.060	0.060
Trace mineral premix[1]	0.031	0.031
Vitamin A-D3-E PMX[2]	0.018	0.018
Vitamin E (6000)	0.018	0.018
Magnesium oxide (Ore)	0.419	0.419
Magnesium sulfate (H2O)	0.419	0.419
Potassium carbonate	0.839	0.839
Total	100	100

[1]Composition on an as fed basis : 1,603.21 mg/kg Co, 40,080.16 mg/kg CU, 3,507.01 mg/kg I, 30,060.00 Fe, 150,300.60 Mn, 160,320.60 mg/kg Zn.

[2]Composition on an as fed basis: 26,484.58 IU/kg vitamin A, 8,836.16 vitamin D, 66,238.10 IU/kg vitamin E

Table 2.3 Observed nutrient composition of forages, grain mixes, and total mixed rations.

Item	Forage		Grain		TMR			
	Corn Silage	Alfalfa Haylage	High CP	Low CP	+Con	-Con	RPM	RPML
Moisture[1]	8.55	6.95	6.96	6.83	8.76	8.77	8.77	8.41
Dry Matter[1]	91.5	93.1	93.0	93.2	91.2	91.2	91.2	91.6
CP[2]	8.38	16.9	25.1	20.7	17.5	15.9	15.9	16.1
ADF[2]	18.7	45.2	10.5	11.3	19.0	20.5	20.3	19.6
NDF[2]	34.8	50.1	19.4	21.6	27.8	30.2	30.6	29.9
Lignin[3]	2.69	10.1	1.08	1.07	3.77	4.09	3.19	3.18
Starch[2]	37.2	2.20	29.5	31.0	30.6	29.9	29.6	29.9
Fat[2]	3.97	2.12	4.85	4.89	5.63	5.85	5.36	5.86
Ash[2]	3.40	14.7	8.23	8.79	7.88	7.98	8.09	8.20
Ca[2]	0.17	0.88	1.01	1.02	0.81	0.74	0.78	0.88
P[2]	0.24	0.27	0.58	0.59	0.43	0.41	0.43	0.45
Mg[2]	0.15	0.25	0.41	0.51	0.30	0.34	0.34	0.36
K[2]	0.90	2.64	1.29	1.30	1.29	1.31	1.38	1.37
TDN[1]	75.3	46.4	76.9	76.3	71.8	70.8	71.4	71.9
NFC[1]	49.9	17.1	42.4	43.9	42.1	40.9	40.8	40.7

[1]Values reported as % as sampled.
[2]Values reported as % DM.
[3]Values reported as % NDF.

Table 2.4 Predicted and observed production with N intake and output

Parameter	+Con	-Con	RPM	RPM/L	SEM	*P*<
DMI, kg/d	24.3[a]	24.5[a]	23.4[ab]	22.5[b]	0.56	0.003
Body weight, kg	598	596	593	591	13.9	0.94
Milk, kg/d	34.8	33.8	33.5	33.8	1.42	0.09
Feed efficiency, kg/kg	1.45[ab]	1.39[a]	1.43[ab]	1.53[b]	0.06	0.003
Milk fat, kg/d	0.96	0.99	0.98	1.00	0.05	0.69
Milk fat, %	2.83	2.98	2.98	3.02	0.11	0.09
Milk protein, kg/d	1.09[a]	1.05[ab]	1.02[ab]	1.04[b]	0.04	0.04
Milk protein, %	3.14	3.13	3.11	3.13	0.03	0.99
Lactose, kg/d	1.70	1.64	1.60	1.62	0.08	0.06
Lactose, %	4.85	4.80	4.82	4.81	0.05	0.99
Somatic cell count, ×1,000 cells/mL	146	144	155	170	27.8	0.99
MUN, mg/dL	12.9[a]	11.5[b]	11.1[b]	11.1[b]	0.29	0.0001
Body condition score[1]	3.18[ab]	3.22[a]	3.17[ab]	3.08[b]	0.08	0.03
N intake, g/d	644[a]	546[b]	518[bc]	506[c]	14.2	0.02
Milk protein N output, g/d	170[a]	165[ab]	160[b]	163[ab]	6.72	0.04
Milk protein N Capture, %[2]	26.8[a]	31.0[b]	31.3[b]	32.6[b]	1.12	0.0001
Milk MUN output, g/d	4.52[a]	3.84[b]	3.71[b]	3.74[b]	0.17	0.0001
Urinary N output, g/d	60.6	61.8	56.2	57.1	3.31	0.99
Fecal N output, g/d	285	337	300	307	18.6	0.99
Total N output, g/d	518	585	519	530	24.0	0.97
N Balance, g/d[3]	142[a]	-32.4[b]	-5.34[b]	-42.6[b]	20.7	0.0001

[a-c]Values within a row with different superscripts differ ($P < 0.05$).

[1]Values determine by 2 observers during week 3 of each period.

[2] Values reported as a percentage of N intake.

[3]Values calculate as (N intake – (Fecal N output + Urinary N output + Milk protein N output + Milk MUN output)

Table 2.5 Mean transit time and analysis of residual nitrogen in intestinally digested samples

	RPSBM[2]	SEM	Citric Acid[3]	SEM	RP Lys[4]	SEM	RP Met[5]	SEM
Expected starting N, %[1]	7.50	---	0.00	---	8.80	---	12.0	---
Actual starting N, %[1]	7.20	0.37	0.20	0.09	9.10	0.52	12.1	0.97
Mean rumen undigested N, %[6]	76.9	0.35	31.1	4.01	103.6	1.58	183.4	1.54
Mean intestinal transit time[7]	57.3	2.07	61.0	3.48	57.2	4.49	61.6	3.55
Mean intestine undigested N, %[8]	1.71	0.34	17.8	1.18	46.7	7.32	3.70	0.68

[1]Values reported as a % of dry matter.
[2]Soybean meal (Soyplus, Southern States, Christiansburg, VA).
[3]Rumen protected citric acid (Balchem Corp, New Hampton, NY).
[4]Rumen protected (RP) lysine (Lys) (Balchem Corp, New Hampton, NY).
[5]Rumen protected (RP) methionine (Met) (Balchem Corp, New Hampton, NY).
[6]Values calculated as (N content of ruminally digested sample) / (N content of undigested sample) * 100 after 8 hour rumen incubation.
[7]Values reported as number of hours between sample placement and sample collection.
[8]Values calculated as (N content of intestinally digested sample) / (N content of undigested sample) * 100 after intestinal digestion.

FIGURES

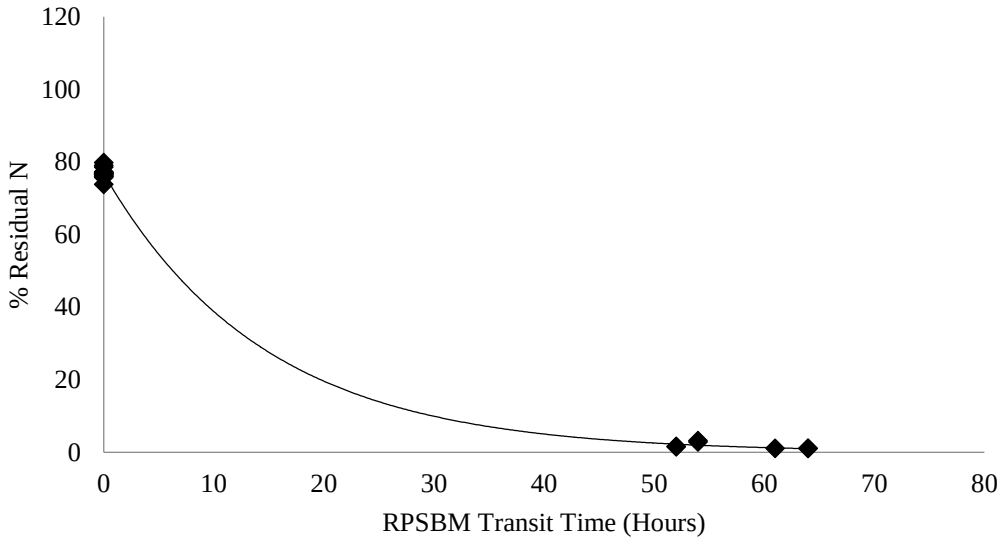

Figure 2.1 Nitrogen degradation versus intestinal residence time for heat treated soybean meal (RPSBM). Hour zero represents samples that were exposed to 8 hour rumen digestion only.

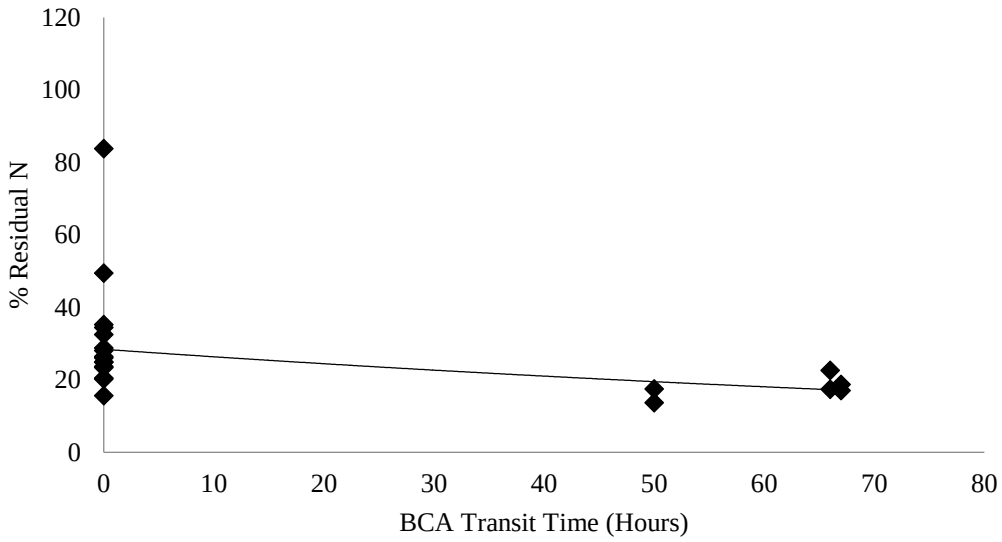

Figure 2.2 Nitrogen degradation versus intestinal residence time for rumen protected citric acid (BCA). Hour zero represents samples that were exposed to 8 hour rumen digestion only.

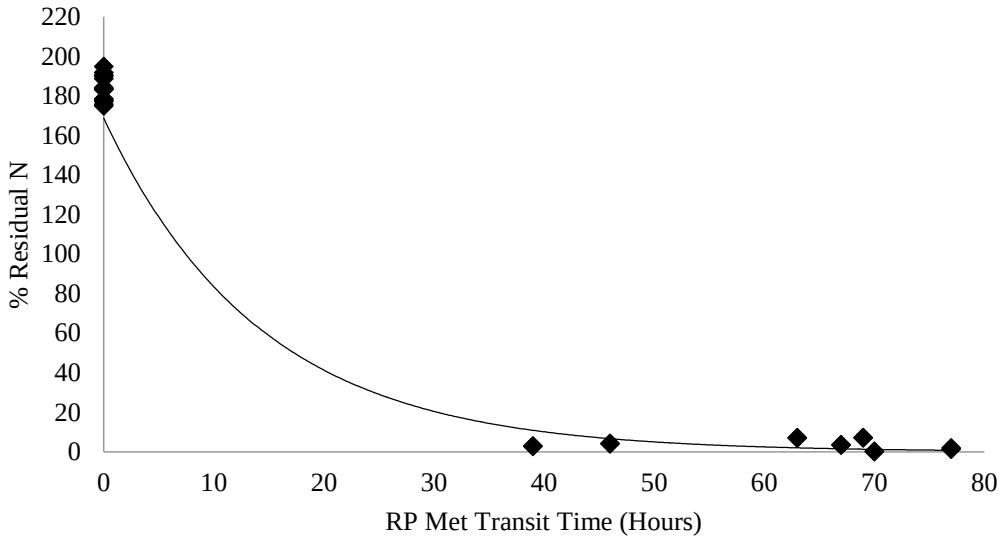

Figure 2.3 Nitrogen degradation versus intestinal residence time for rumen protected methionine (RP Met). Hour zero represents samples that were exposed to 8 hour rumen digestion only.

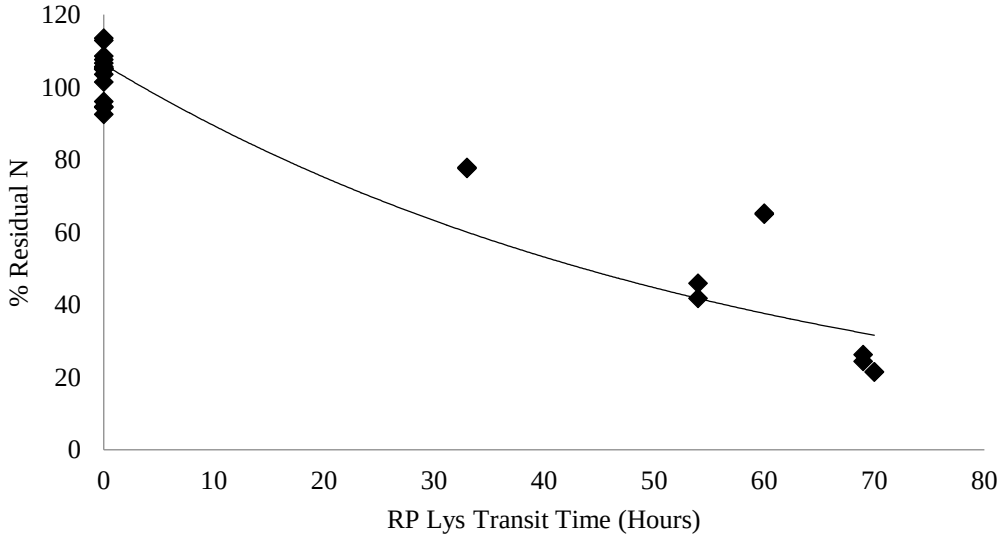

Figure 2.4 Nitrogen degradation versus intestinal residence time for rumen protected lysine (RP Lys). Hour zero represents samples that were exposed to 8 hour rumen digestion only.

Chapter 3

Improvements in efficiency of dairy cow feeding via rumen protected histidine and methionine in diets of moderate forage inclusion.

ABSTRACT

Modern dairy cows are often fed to meet metabolizable protein (MP) requirements predicted by the 2001 NRC ration formulation software. Due to overestimation of such requirements, excess dietary protein results in increased feed costs and excretion of nitrogen (N) to the environment. Low MP diets supplemented with limiting amino acids (AA) in rumen protected (RP) form have been shown to support high levels of production through improved N utilization efficiency. However, the success of RPAA supplementation varies with diet composition as well as RPAA source. The goal of this experiment was to evaluate the impact of specific RP Met and RP His products on milk yield of mid-lactation cows consuming diets of moderate forage inclusion. The 2001 NRC formulation software was used to formulate an MP deficient diet that was fed to 36 mid-lactation Holstein dairy cows producing an average of 35 kg of milk/d in a replicated, 3x3 Latin square design. An additional MP sufficient diet was formulated and fed as a positive control ration to 12 cows in all periods. Diets were: (1) a high MP, positive control diet containing 19.9% crude protein (CP) (+Con) and meeting all requirements of a lactating cow producing 35 kg/d, (2) a low MP, negative control diet containing 15.3% CP (-Con) that was isoenergetic with +Con but limiting in MP (MP allowable milk = 30 kg/d), (3) -Con supplemented with RP Met (RPM) to match the duodenal Met flow of +Con , (4) -Con supplemented with RP His (RPH) to match the duodenal His flow of +Con. Milk yield was not different among any of the diets, suggesting that protein was not sufficiently limiting for –Con to affect production, and thus no responses could be

expected from AA supplementation. Concentrations of MUN from cows fed –Con, RPM and RPH diets were lower than that of cows fed +Con (P<0.0001), suggesting improved efficiency of N metabolism in cows fed diets low in MP. Although dry matter intake was lower in cows consuming the RPH diet than –Con, increased dietary N capture in milk protein indicates improved AA efficiency in the RPH diet. Although the NRC (2001) predicted deficiencies in MP and AA, equivocal milk yields among treatment groups provides evidence that MP requirements are overestimated. This inaccuracy demonstrates the need for improvement in dairy nutrition models. Such improvements could act synergistically with RPAA supplementation to more closely match nutrient composition in feed to nutrient requirements of dairy cows. Although previous work demonstrated a response to histidine when lysine and methionine were adequate, the present study did not indicate that histidine was limiting for the diet used in this trial.

INTRODUCTION

Supplementation of rumen protected (RP) amino acid (AA) products has shown varying degrees of effectiveness. Studies by Rogers et al. (1987), Polan et al. (1991), and King et al. (1991) provided strong evidence that methionine (Met) and lysine (Lys) were effective candidates to stimulate increased milk production, or to maintain production with decreasing protein content of diets (Rogers et al., 1987, King et al., 1991, Polan et al., 1991). Although methionine and lysine supplementation provided a benefit to milk production in some circumstances, it was ineffective in others. Vanhatalo et al. (1999) supplemented grass forage based diets with infusions of Met, Lys and histidine (His) and found no effect of Met or Lys supplementation on milk production. However, His did increase milk yield. This study demonstrates that AA other than Lys and Met must be considered. Diets high in grass forages, such as grazing operations, can benefit from supplementation of RP His, which was found to be limiting on such diets (Vanhatalo et al., 1999).

Metabolizable protein reaching the duodenum is the sum of microbial protein plus rumen undegradable protein (RUP) and secretions into the rumen. Since limiting protein supply to the rumen could impact microbial growth and nutrient digestibility, many studies have supplemented RPAA to diets deficient in RUP, but sufficient in rumen degradable protein (RDP). However Lee et al. (2012) fed combinations of RPAA to animals consuming diets of moderate forage content (forage = 60% of dry matter) that were limiting in both RUP and RDP. From the positive control (sufficient RUP and RDP) to the negative control (deficient RUP and RDP), a 3 kg decrease in milk production was observed. Supplementation of Lys and Met to the negative control provided a modest increase in milk yield as compared to control groups, however supplementation with RP lysine, RP Met and RP His completely recovered the depression of production (Lee et al., 2012).

Studies such as these reflect the complex nature of AA metabolism in the dairy cow. Additional data are needed to elucidate the mechanism of lactation response to RPAA's, and to evaluate the efficacy of rumen protected amino acid (RPAA) products for increasing milk yield.

The objective of this study was to determine the effect of RP Met and RP His supplementation to MP deficient diets on milk and component production on mid-lactation dairy cows. We hypothesized that limiting dietary RUP supply would limit production and that supplementing RUP deficient diets with limiting AA (Met or His) to meet requirements would partially rescue losses in milk production.

MATERIALS AND METHODS

Animals and Housing

All experimental procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. Forty-eight lactating primiparous (N=32) and multiparous (N=16) Holstein Dairy cows from the Virginia Tech Dairy herd were allocated to the trial. Cows were chosen based

on current milk weights, days in milk (DIM), and stage of gestation. Animals ranged from 87-243 DIM with daily milk production ranging from 16-59 kg. Cows were used in a Latin square experimental design consisting of 3 experimental treatments and 3 periods, with a covariate group consuming the same positive control ration during all 3 periods. Animals were blocked by milk production and randomly assigned to treatment groups. Experimental periods lasted 21 days and consisted of 14 days of diet adaptation and 7 days of sample collection. Animals were housed in the free stall barn of the Virginia Tech Dairy Facility in 4 pens equipped with Calan Broadbent individual animal feeders (American Calan Inc., Northwood, NH). All animals had free access to water, and were milked 2 x/d. One cow was removed from the study for health problems unassociated with the study.

Treatments and Diets

Diets were formulated using the NRC 2001 software. Diets were: (1) a high metabolizable protein (MP), positive control diet containing 17% crude protein (CP) (+Con) and meeting all requirements of a lactating cow producing 35 kg/d, (2) a low MP, negative control diet containing 14.5% CP (-Con) that was isoenergetic with +Con but limiting in MP (MP allowable milk = 30 kg/d), (3) -Con supplemented with RP Met (RPM) to match the duodenal Met flow of +Con , (4) -Con supplemented with RP His (RPH) to match the duodenal His flow of +Con. Based on NRC (2001) predictions, -Con provided 400 grams less MP, 8 grams less duodenal Met, and 7 grams less duodenal His flow per day than +Con. As the positive control diet was predicted to provide limiting amounts of Lys, RP Lys was added to all rations to deliver 2.5 g of RP Lys/kg of DM (38% Lys in DM; AminoShure-L; Balchem Corp., New Hampton, NY). Diet composition is listed in Table 3.1 and Table 3.2. Diets were mixed using a Data Ranger (American Calan Inc., Northwood, NH), and fed as total mixed rations (TMR) once daily to achieve 5-10% refusals. The

grain mixes were fed in pelleted form and constructed according to Table 3.2. Diets were mixed a minimum of 15 minutes. Rumen protected AA were added to the mixer to deliver 0.9 g of RP Met/kg of DM (70% Met in DM; Balchem Corp., New Hampton, NY), and 1.9 g of RP His/kg of DM (40% His in DM; Balchem Corp., New Hampton, NY).

Sample Collection and Analyses

Total mixed rations were sampled on days 3, 6, 10, 13, 17, and 20 of each period. Individual feed components were sampled on days 3, 10, and 17 of each period. Samples of TMR were collected using the quartering technique (Dairyland Laboratories, Inc., Arcadia WI), and stored in 1 gallon air tight plastic bags. Feed samples were stored at -20^0 C and subsequently thawed for drying. Samples of TMR were composited for the first two weeks of each period, while samples from the third week of each period were handled separately. Samples were dried in a forced air oven at 55^0 C until daily variation in weight was less than 1 g. Dry samples were ground in a Wiley mill through a 2-mm screen (Arthur H. Thomas Co., Philadelphia, PA), and subsamples submitted to Dairyland Laboratories, Inc. (Arcadia WI) for nutrient analysis. Observed nutrient composition of feed samples are reported in Table 3.3. Daily dry matter intake (DMI) was calculated from as fed offered and refusals by animal using the 55^0 C DM content of each ration.

Cows were milked at 0300 and 1300 h. Milk weights were recorded at each milking, and body weights (BW) were recorded as cows exited the milking parlor. Milk samples were taken on days 4, 11, 18, 19, and 20 of each period at the morning and afternoon milkings. Sample analysis was performed at the DHIA laboratory at Virginia Tech. Samples were analyzed for lactose, protein, fat, solids non-fat (SNF), milk urea nitrogen (MUN), and somatic cell count (SCC). Milk weight, fat content, protein content, lactose content, and SCC were measured at each milking using the

Afimilk system (Afimilk Ltd, Kibbutz Afikim, Israel). Body condition scores were assessed by 2 individuals at the beginning of the study and at the end of each period.

Mobile Bag Technique. In-sacco analyses of the RP Met product used in this experiment are described in detail in Chapter 3, and results are reported here in Table 3.5. For evaluation of digestibility of RP His, twenty-four samples (1.5 g) of RP His were placed in 5 × 5-cm, 50-µm-poresize bags (Ankom Technology Corp., Macedon NY) and bags were heat sealed. Since in-sacco rumen incubation of 8 hours for RPAA samples has been shown to accurately assess rumen protection (Rossi et al., 2003), bags were incubated for 8 h in the rumen of 2 ruminally cannulated dairy heifers. Four samples were subjected to only rumen degradation to assess N loss in the rumen. After rumen incubation, the other 20 samples were soaked for 1 h in HCl (pH 2.4) and then 2 h in HCl (pH 2.4) solution supplemented with 100 mg of pepsin/L (Ali et al., 2012). Four of the pepsin-digested samples were washed and frozen for analysis. Sixteen samples were placed in the duodenal cannula of 2 fistulated steers at a rate of 3 every half hour, and recovered in feces. Recovered bags were washed in a series of 3 cold-water baths then placed into a salad spinner to remove excess water. Samples were freeze-dried and weighed to determine DM recovery and analyzed for total N via combustion analysis.

Statistical Analysis

Feeding Trial. Data were analyzed using the GLIMMIX procedure in SAS (v9.3, SAS Inst. Inc., Cary, NC, USA). The effect of dietary treatment was assessed using the model:

$$Y_{ijkl} = \mu + T_i + P_j + S_{ij} + (T_i + P_j) + \beta_{ij}(S_{ij}) + \varepsilon_{ijk,}$$

where Y_{ijkl} = the dependent variable, μ = the population mean of Y, T_i = the effect of the ith treatment (N=4), P_j = the effect of jth period (N=3), S_{ij} = the random effect of square, $\beta_{ij}(S_{ij})$ = the random effect of cow nested within square, and ε_{ijk} = residual error. Data are reported in Table 3.4

as least square means with standard errors of the mean (SEM). Statistical significance was declared at $P < 0.05$.

Mobile Bag Trial. Recovery rate of in-sacco samples was not sufficient for statistical comparison among samples. Time of placement and time of recovery were used to calculate total retention time. Nitrogen content of ruminally, HCl/pepsin, and intestinally digested samples were compared to starting nitrogen to calculate residual nitrogen content. Data were analyzed using the MEANS procedure in SAS (v9.3, SAS Inst. Inc., Cary, NC, USA) to determine mean intestinal retention time as well as mean nitrogen and dry matter retention after in-sacco incubation.

RESULTS AND DISCUSSION

Observed nutrient composition of TMR as well as forage and concentrates are reported in Table 3.3. Although +Con rations were formulated to contain 17% CP and –Con rations were formulated to contain 14.5% CP, actual CP content of TMR was higher; +Con contained 19.9% CP and –Con contained 15.3% CP. Dry matter content of forages was measured twice weekly and as fed (AF) ration recipes were adjusted accordingly in an effort to avoid fluctuations in nutrient concentrations resultant from moisture variability. In spite of this, excess dietary protein was delivered to all treatment groups. Although 15.3% CP in –Con rations exceeded intended MP supply, NRC (2001) predictions still indicated that MP supply would be limiting.

In-sacco analysis of RPAA products is reported in Table 3.5, which indicate that RP Met and RP His products were adequately protected in the rumen and readily digested in the intestine. Thus, these products appear to have been suitable for contributing to the predicted AA flow presented in Table 3.1.

Although milk and milk protein yields were not different among +Con versus RPH or RPM diets, these yields were also not different among animals fed +Con and -Con diets. Thus, any effect

of experimental treatment is difficult to assess since there was no decrease in production to recover. Dry matter intake was significantly higher in cows fed the –Con diet than in cows fed the RPH diet. These results conflict with previous evidence that His supplementation increases voluntary DMI and milk protein yield (Huhtanen et al., 2002, Lee et al., 2012) A recent study by Giallongo et al. (2015) reported significantly increased milk protein yield and significantly increased DMI in cows consuming MP deficient diets supplemented with RP His. Authors attribute increased milk protein content to increased voluntary DMI, which may partially explain why no increase in milk protein was observed in cows supplemented with RP His in the present study (Giallongo et al., 2015). Dry matter intake is largely determined by body weight, stage of lactation, and milk production (NRC, 2001). Since no differences existed among RPH and other treatment groups, no readily apparent mechanism for decreased DMI in the RPH group is evident.

Predictions from NRC (2001) indicated MP allowable milk production would be 34 kg/d, and that NE_L allowable milk would be 29.6 kg/d. As evidenced by the observed milk production of 27.7 kg/d, animals in the +Con treatment group appear to lack the capacity to respond to added dietary protein. Reported in Table 3.4, MUN concentrations were significantly higher in animals consuming the +Con ration than all other treatment groups. In addition, a greater percentage of N intake was captured in milk protein among the low protein treatment groups as compared to +Con. This demonstrates increased N efficiency in animals consuming low protein diets, as milk protein was not different among treatment groups while excess nitrogen (in MUN form) was greater in animals consuming a high protein diet. Although N efficiency appears greater in low protein treatment groups, feed efficiency (calculated as milk yield kg / DMI kg) was not different among treatment groups. Given the greater N efficiency of low protein diets in addition to decreased DMI of the RPH treatment group, N capture in milk among RPH was significantly greater than that of

-Con. In high forage diets, His has been shown to be a limiting AA for milk protein production (Lee et al., 2012) and removed from plasma by mammary tissues with an efficiency of 43% (Kim et al., 2001). Supplementation of His may have allowed for sustained milk protein production at lower levels of DMI by increasing plasma concentrations of His. However, N balance and tissue AA were not measured in this study.

Although milk fat yield was not different among treatment groups, milk fat content was higher than that of average mid-lactation cows consuming corn silage-based diets. As reported in**Error! eference source not found.**, non-fiber carbohydrate (NFC) and starch content of all diets were relatively low compared to neutral detergent fiber (NDF) content. As reviewed by Bauman and Griinari (2003), such diet composition has been shown to promote high milk fat content (Bauman and Griinari, 2003)

Diets were formulated to be non-limiting in energy and limiting in RUP, as shown by NRC predictions of production in Table 3.1. The +Con diet was predicted to allow for over 9 kg more milk production than the –Con. Predictions from NRC (2001) indicate that Met and His flow decrease by 8 and 7 grams/day, respectively, from the +Con diet to the –Con diet, and that the +Con diet delivered over 400 grams/day of MP more than the –Con diet. Furthermore, the –Con diet was predicted to provide 47 g/d less RUP than required. Observed milk production exceeded MP allowable milk predictions in all diets, providing further evidence of inaccuracies contained within the NRC (2001) model. White et al. (2017) reported that the fixed protein utilization efficiency of 67% assumed by NRC (2001) would be better represented as variable, given that nutrient digestibility decreases with increased levels of DMI (Tyrrell and Moe, 1975, White et al., 2017c). Since DMI was relatively low among treatment groups, increased digestibility may partially account for over-prediction of nutrient requirements.

Imprecise and biased estimations of nutrient requirement models hinder production in industry and academia alike. Improvements in the NRC (2001) predictions would allow for more unbiased and effective evaluations of dietary treatments such as rumen protected amino acids. Furthermore, additional work must be done to identify conditions under which RPAA supplementation is effective. Although the present study did not provide conclusive evidence of RP His supplementation improving lactational performance, previous work reflects that amino acids beyond lysine and methionine must be explored (Vanhatalo et al., 1999, Lee et al., 2012, Giallongo et al., 2015), and that histidine may be an effective supplement to more than just grass forage based diets.

TABLES

Table 3.1 Diet composition (% of DM) and NRC (2001) predictions.

Ingredient	+Con	-Con	RPM	RPH
Corn Silage	41.9	41.9	41.9	41.8
Grass Hay	6.00	6.00	6.00	6.00
Alf Hay	12.0	12.0	12.0	11.9
Low CP Grain Mix	0.00	39.9	39.9	39.8
High CP Grain Mix	39.9	0.00	0.00	0.00
RP Lys[1]	0.25	0.25	0.25	0.25
RP His[2]	0.00	0.00	0.00	0.19
RP Met[3]	0.00	0.00	0.09	0.00
NE_L intake, Mcal/d[4]	31.4	33.7	32.3	30.8
MP Supply, g/d[5]	2395	1986	1949	1840
MP supply/NE_L intake, g/Mcal	76.3	58.9	60.3	59.7
NE_L Allowable Milk, kg/d[5]	29.6	34.0	28.4	31.8
MP Allowable Milk, kg/d[5]	34.7	25.9	22.1	24.2
RUP requirement, g/d[5]	1033	986	1077	994
RUP supply, g/d[5]	1475	939	941	900
Met Flow, g/d[5]	47.0	39.0	49.0	36.0
Met%[6]	1.95	1.95	2.50	1.94
His Flow, g/d[5]	49.0	42.0	41.0	49.0
His%[6]	2.07	2.12	2.10	2.66
Lys Flow, g/d[7]	137	132	129	123
Lys%[6]	5.70	6.65	6.62	6.68

[1]Rumen protected lysine (38% Lys in DM, Balchem Corp, New Hampton, NY).
[2]Rumen protected histidine (40% His in DM, Balchem Corp, New Hampton, NY).
[3]Rumen protected methionine (70% Met in DM, Balchem Corp, New Hampton, NY).
[4]Observed NE_L intake, calculated as (Observed TMR NE_L Mcal/kg) * (Observed DMI kg/d).
[5]Predicted values based on observed nutrient values of forages as well as observed dry matter intake, body weight, and milk production. Tabular values were used for concentrates.
[6]Values reported as a percentage of predicted total MP supply.

Table 3.2 Grain mix composition (% of grain mix as fed).

Ingredient	High CP	Low CP
	% of grain mix as fed	
Corn grain, ground, dry	52.0	60.5
Corn gluten feed	0.00	11.1
Corn gluten meal	18.0	3.36
Corn dry distiller grain + solubles	9.80	0.00
Cotton seed meal	0.00	4.35
Soybean, meal, solvent extracted	3.43	0.00
Soybean, hulls	11.8	11.9
Urea	0.98	1.58
Tallow	0.98	4.25
Calcium Carbonate	0.49	0.49
Calcium Phosphate (Di-)	0.39	0.40
Sodium Bicarbonate	0.49	0.49
Salt	0.88	0.89
0.06% Selenium Premix	0.10	0.10
Trace mineral premix[1]	0.05	0.05
Vitamin A-D3-E PMX [2]	0.05	0.05
Vitamin E (60000)	0.05	0.05
Calcium Sulfate (2H2O, 74% CaSO4)	0.49	0.49

[1]Composition on an as fed basis : 1,603.21 mg/kg Co, 40,080.16 mg/kg CU, 3,507.01 mg/kg I, 30,060.00 Fe, 150,300.60 Mn, 160,320.60 mg/kg Zn.
[2]Composition on an as fed basis: 26,484.58 IU/kg vitamin A, 8,836.16 vitamin D, 66,238.10 IU/kg vitamin E.

Table 3.3 Observed nutrient composition of forages, grain mixes, and total mixed rations.

Item	Forage			Grain		TMR			
	Corn Silage	Alfalfa Hay	Grass Hay	High CP	Low CP	+Con	-Con	RPM	RPH
Moisture[1]	8.96	7.55	7.66	7.32	7.09	9.30	9.41	9.71	9.64
Dry Matter[1]	91.0	92.5	92.3	92.7	92.9	90.7	90.6	90.3	90.4
CP[2]	9.40	17.2	11.1	27.1	19.0	19.9	15.3	15.2	15.2
ADF[2]	19.2	41.8	46.4	11.4	11.9	22.9	22.6	23.1	24.2
NDF[2]	35.3	50.3	70.7	17.8	20.6	35.0	34.7	35.9	37.8
Lignin[3]	1.95	9.17	7.24	1.62	2.25	3.80	3.65	3.48	4.40
Starch[2]	41.3	2.60	2.30	38.5	43.0	28.3	30.7	29.5	28.7
Fat[2]	3.71	1.70	1.80	5.03	8.14	4.33	5.79	5.77	5.73
Ash[2]	3.44	8.51	8.63	4.98	4.99	6.23	6.28	6.29	6.35
Ca[2]	0.19	0.89	0.58	0.65	0.55	0.57	0.52	0.51	0.53
P[2]	0.26	0.26	0.23	0.46	0.49	0.35	0.36	0.35	0.36
Mg[2]	0.17	0.20	0.20	0.15	0.18	0.18	0.20	0.20	0.20
K[2]	0.82	1.72	1.84	0.54	0.63	0.91	0.95	1.00	0.98
TDN[1]	76.0	52.9	49.6	79.7	82.4	69.9	71.6	71.5	69.5
NFC[1]	48.7	23.1	8.3	45.1	47.3	35.6	38.6	37.6	35.6

[1]Values reported as % as sampled.
[2]Values reported as % DM.
[3]Values reported as % NDF.

Table 3.4 Mean observed production by treatment group.

Parameter	+Con	-Con	RPM	RPH	SEM	*P*<
DMI, kg/d	19.8[ab]	20.7[a]	19.8[ab]	19.5[b]	0.85	0.05
Body weight, kg	586	579	577	577	11.5	0.99
Milk, kg/d	27.7	27.2	26.1	27.4	2.06	0.99
Feed efficiency, kg/kg	1.47	1.30	1.33	1.41	0.08	0.98
Milk fat, kg/d	1.14	0.95	0.96	0.96	0.08	0.93
Milk fat, %	4.12	3.54	4.09	3.52	0.25	0.99
Milk protein, kg/d	0.87	0.82	0.81	0.83	0.06	0.98
Milk protein, %	3.18	3.04	3.42	3.06	0.19	0.99
N Intake, g/d[1]	629[a]	508[b]	481[bc]	474[c]	23.3	0.04
N capture in milk protein, %[2]	22.6[a]	25.0[b]	26.6[bc]	27.7[c]	1.51	0.003
Lactose, kg/d	1.35	1.30	1.28	1.33	0.09	0.99
Lactose, %	4.87	4.77	5.38	4.84	0.31	0.99
Somatic cell count, ×1,000 cells/mL	95	157	178	164	28.5	0.94
MUN, mg/dL	17.9[a]	10.5[b]	10.0[b]	10.5[b]	0.29	0.0001
SNF, kg/d	2.47	2.37	2.34	2.42	0.18	0.98
SNF, %	8.95	8.71	9.82	8.83	0.56	0.99

[a-c]Values within a row with different superscripts differ ($P < 0.05$).

[1]Nitrogen intake calculated assuming CP content of 16% N

[2]Nitrogen capture calculated as a percent of N intake and assuming milk protein containing 15.67% N.

Table 3.5 Mean transit time and analysis of residual nitrogen in intestinally digested RP Met and RP His.

	RP Met[2]	SEM	RP His[3]	SEM
Expected starting N, %[1]	12.0	---	6.40	---
Actual starting N, %[1]	12.1	0.97	---	---
Mean rumen undigested N, %[4]	183.4	1.54	74.6	1.64
Mean HCl/Pepsin undigested N, %[5]	---	---	39.3	8.80
Mean intestinal transit time	61.6[6]	3.55	48.63[7]	1.47
Mean intestine undigested N, %[8]	3.70	0.68	3.19	1.06

[1]Values reported as a % of dry matter.
[2]Rumen protected (RP) methionine (Met) (Balchem Corp, New Hampton, NY).
[3]Rumen protected (RP) histidine (His) (Balchem Corp, New Hampton, NY).
[4]Values calculated as (N content of ruminally digested sample) / (N content of undigested sample) * 100 after 8 hour rumen incubation.
[5]Values calculated as (N content of samples after rumen and HCl/pepsin incubation) / (N content of undigested sample) * 100 after 8 hour rumen incubation, 1 hour HCl incubation, and 2 hour pepsin incubation.
[6]Values reported as number of hours between sample placement in the abomasum and sample collection in feces.
[7]Values reported as number of hours between sample placement in the duodenum and sample collection in feces.
[8]Values calculated as (N content of intestinally digested sample) / (N content of undigested sample) * 100 after intestinal digestion.

Chapter 4

An evaluation of the Dairy NRC 2001 and Papillon PREP10 nutrient requirement models

ABSTRACT

Imprecise or biased estimation of nutrient requirements of dairy cows can result in reduced income for producers and increased nutrient excretion to the environment. Improvements in the accuracy and precision of nutrient requirement models can improve feed efficiency. The aim of this study was to evaluate PREP10 (Papillon Agricultural Company, USA) and Dairy NRC (2001) models using production data from the literature to identify deficiencies in prediction equations. Data from 99 published studies with 305 treatment means were used for this work, with publication dates ranging from 1983 through 2011. The models were each coded in Microsoft Excel. Nutrient inputs included dry matter intake (DMI) of each ingredient and the ingredient composition. If nutrient composition was not available, the source model feed library was used to estimate nutrient availability. Model outputs of allowable milk production were collected based on the following nutrient supplies: metabolizable protein (MP), net energy of lactation (NE_L; NRC (2001) only), and amino acids (PREP10 only). The PREP10 model was further evaluated for the effect of binary indicator variable (BIV) settings controlling the effects of sugar and starch supply on microbial growth, as well as a BIV that alters the liquid passage rate (K_p) of the B1 protein fraction (buffer-soluble true protein). Residual errors were only considered for the most limiting nutrient. The error of prediction for PREP10 amino acid (AA) and MP allowable milk exceeded that of NRC (2001). However, the lowest root mean squared prediction error (RMSPE) observed for PREP10 MP and AA allowable milk (23.4% and 24.2%, respectively) were lower than that of NRC (2001) NE_L allowable milk (24.67%). Overall, NRC (2001) over-predicted milk production, with average predicted values ranging from 0.54-2.93 kg greater than observed production. The PREP10 model

under-predicted milk production, with average predicted values ranging from 0.2-3.4 kg less than observed production. For each model, slope bias was greater than mean bias. Backward elimination regression of residual errors for dietary nutrients with publication included as a random variable identified correlations with various nutrient supplies from both models. On the basis of RMSPE%, MP allowable milk was the best predictor of production in both NRC (2001) and PREP10 models (21.51% and 23.4%, respectively). Correction of slope bias would considerably reduce prediction errors.

INTRODUCTION

Due to imprecise or biased estimations, and in an effort to avoid nutrient limitations, nutrient requirements are often overestimated when formulating dairy cow rations. Overfeeding of N results in increased expense for producers and increased excretion of N to the environment (McNamara, 2004). Thus, improved accuracy and precision of predicting nutrient requirements of dairy cows and nutrient content of feed would contribute substantially to minimizing excess resource utilization and maximizing efficiency of dairy cow feeding. Such improvements would provide an economically and environmentally advantageous modeling approach.

In 2001, the National Research Council published the Seventh Revised Edition of Nutrient Requirements of Dairy Cows. This publication describes nutrient requirements for dairy cows, and contains a database of feed nutrient values. These equations predict nutrient supply, intake, as well as animal requirements and production (NRC, 2001). By inputting individual components of feeds and their nutrient profiles, the model evaluates the suitability of the ration for meeting animal requirements for a specified level of production. The model predicts, among other values, dry matter intake (DMI), net energy for lactation (NE_L) required and provided, and metabolizable protein (MP) required and provided (NRC, 2001). Since the accuracy of predictions for production

is contingent on a specific level of DMI, special care must be taken to ensure that actual DMI matches model predictions. Animal inputs such as body weight, production, and stage of lactation are the major factors that affect DMI predictions, making accurate animal inputs of vital importance (Conrad et al., 1964). Equations which predict supply and requirement of NE_L and MP, in addition to equations representing other nutrients and feed fractions, are combined to produce the overall NRC (2001) model. Animal inputs are used in conjunction with MP and NE_L supply to calculate allowable production (MP allowable Milk, NE_L allowable Milk). Of particular interest in the present study is MP; this is calculated as the sum of microbial protein and RUP, and MP use efficiency for maintenance and lactation is assumed to be constant at 67%. Fractions of dietary crude protein are used to calculate microbial protein and RUP; microbial protein is synthesized from the A-fraction and microbially degraded portion of the B-fraction, and is contingent upon energy supply (TDN). The remaining portion of fraction-B as well as all of fraction-C represent RUP (NRC, 2001).

Another nutrient requirement model is PREP10, developed by Papillon Agricultural Company. The PREP10 model was developed for use by commercial nutrition consultants. Like NRC (2001), it provides an extensive feed library and comprehensive nutritional predictions. The PREP10 model also predicts nutrient flows and allowable production, but gives special emphasis to amino acids (AA) by predicting requirement, supply, and allowable production based on each of the 10 essential AA. As compared to the NRC (2001), PREP10 accounts for the fraction of RDP that escapes the rumen in the fluid fraction in addition to RUP and microbial protein to calculate MP. Supplies of MP and AA are used to calculate allowable milk production (MP allowable Milk, AA allowable Milk). Furthermore, PREP10 accounts for the increase in protein use efficiency with increased production (Hanigan et al., 2013). It contains three binary indicator variables (BIV)

which impact microbial growth predictions and can be turned on and off, yielding 8 unique arrangements.

Since milk production predictions of the 2001 Dairy NRC and PREP10 have not been rigorously evaluated against experimental data, the aim of this study was three fold: 1) to determine the overall performance of each model, 2) to identify correlations between nutrient flow and residual errors, and 3) to determine what deficiencies exist in prediction equations. We hypothesize that biases exist in each model which could be corrected after identifying nutrient flows that correlate with residual error.

MATERIALS AND METHODS

A database of observed values from 99 published studies was developed. Seventy of these studies were included in the database assembled by the NRC committee to develop and test predictions of ruminal outflow of N, and 29 studies were added to the database throughout the present study. Publication dates ranged from 1983 through 2011. The 99 studies reported 374 treatments and included diet composition, animal characteristics, nutrient intake and nutrient digestibility data. Milk production data was reported in 305 treatments.

Equations listed in the NRC (2001) publication were used to reconstruct the model in Microsoft Excel. Simulations were conducted using a macro loop in SAS (v9.3, SAS Inst. Inc.), whereby each diet was written into an Excel spreadsheet version of each model. Nutrient inputs included DMI of each ingredient and the ingredient composition, if available. If nutrient composition was not available, the source model feed library was used to estimate nutrient availability. Animal inputs for NRC (2001) included observed milk production, DMI, days in milk (DIM), BW, milk fat content, milk protein content, milk lactose content, and days pregnant. Animal inputs for

PREP10 included milk production, BW, DIM and milk protein content. If animal inputs were unavailable, the mean of database values was used.

Predictions of allowable milk production and nutrient supply for each treatment were collected and stored in a separate Excel spreadsheet. Protein and energy balances were calculated by subtracting predicted requirements from predicted supply and used to create treatment mean subsets. Model predictions were evaluated using these subsets; NRC (2001) predictions for NE_L allowable milk were evaluated using the subset of 155 treatment means where NE_L supply was predicted to limit production, and NRC (2001) MP allowable Milk predictions were evaluated using 150 treatment means where MP supply was predicted to limit production. Evaluation of the overall NRC (2001) model was performed by including all 305 treatment means and comparing observed production to whichever milk production prediction was smallest, thus creating a "Most-Limiting Nutrient allowable Milk" prediction. The PREP10 model includes three BIV which yields 8 unique model settings, and thus it was evaluated for each of those states. These BIV are: 1) Starch 2) Sugar, and 3) B1 K_p. The "Starch" BIV adds 96.4g of MP for each kg of dietary starch when dietary starch exceeds 25% of a ration. The "Sugar" BIV adds 160.6g of MP for every kg of dietary sugar when dietary sugar exceeds 3% of a ration. The "B1 K_p" BIV calculates the amount of the protein-B1 fraction that escapes the rumen by the flushing action of liquid turnover and adds this protein to the RUP content of the diet. The PREP10 model provides milk production predictions based on MP allowance milk as well as a function of the most limiting AA, thus subsets of treatment means were compared based on those that were MP versus AA limiting.

Having calculated model predictions for each diet, residual errors were calculated (observed-predicted) and used to determine root mean square prediction error (RMSPE) and the contributions of errors in mean and slope bias to mean squared error (MSE) (Bibby and Toutenburg, 1977).

From the results of analysis of residual errors of PREP10, the predictions of AA allowable milk and MP allowable milk with the least value for root mean square prediction error expressed as a percent of observed production (RMSPE %) were chosen for further analysis.

Stepwise, backward elimination regression of residual errors for DMI and dietary concentrations of 15 nutrients (12 basic nutrients plus methionine (Met), lysine (Lys), and total essential AA (TEAA)) in NRC (2001) and 22 (12 basic nutrients as with NRC (2001), plus 10 individual essential AA (EAA)) nutrients in PREP10 was performed with publication as a random independent variable using the GLIMMIX procedure of SAS (V. 9.3). Dependent variables were sequentially removed based on greatest *P*-value until all *P*-values were less than or equal to 0.15.

RESULTS

Analyses of residuals for MP, NE_L, and Most-Limiting Nutrient allowable milk of NRC (2001) are presented in Table 4.1. As a percent of observed production, RMSPE ranged from 21.5% for MP allowable milk to 24.7% for NE_L allowable milk. Mean bias associated with all three predictions was negative (over-prediction), as shown in Figure 4.1, Figure 4.2 and Figure 4.3. These plots provide visual representations of mean and slope bias of residual error; observed production (y-axis) is plotted against predicted production (x-axis) over the line of unity (slope = 1). For every unit of observed production, a truly accurate model would predict the same level of production, resulting in data points laying on the line of unity. A model that contains mean bias would result in the majority of data points laying above or below the line of unity. Residuals (y-axis), calculated as (observed milk (kg) – predicted milk (kg)), are plotted against predicted production (x-axis). These data points allow for easy visual recognition of slope bias, as a model that predicts accurately at all levels of production would result in a zero value for residuals, and data points would lay along the x-axis. If model prediction error varies with level of predicted

production, then the distribution of data points (above or below the x-axis) would change with increasing magnitude of production predictions, and a line plotted along these data points would represent the slope bias of model predictions. In the NRC (2001), the predominant component of residual error, was slope bias, ranging from 10.3% of MSE for MP allowable predictions to 13.9% for Most-Limiting Nutrient allowable predictions.

Results from the backwards stepwise regression of residual errors for dietary nutrients for NRC (2001) are presented in Table 4.3. Regression of residual errors identified correlations among nutrient supply (% of DMI) and residual errors for both MP and NE_L allowable milk predictions. Significant nutrient correlations with residual errors for NE_L allowable milk predictions included neutral detergent fiber (NDF) (-.5kg), RDP (0.6kg), and Met (-0.1kg). Significant nutrient correlations with residual errors for MP allowable milk predictions included MP (-4.8kg), lignin (-1.2kg), non-fiber carbohydrates (NFC) (0.8kg), RUP (1.4kg), RDP (1.8kg), and fat (EE) (1.2kg).

Predictions from PREP10 for all BIV settings were ranked based on RMSPE%, and the six smallest values are reported in Table 4.2. Values of RMSPE% ranged from 23.4 for MP allowable Milk with the "Starch Off" setting, to 26.3 for MP allowable Milk with the "Starch & B1-K_p Off" setting. A positive mean bias (under-prediction) for AA and MP allowable milk predictions for every combination of BIV settings can be observed in Table 4.2, ranging from 0.1% of MSE for "All On" setting to 16.6% for "Starch & B1 K_p Off" setting. For every BIV setting, slope bias for AA and MP allowable milk predictions was identified. Slope bias ranged from 37.0% of MSE for AA allowable Milk with the "B1-K_p Off" setting to 17.7% for MP allowable Milk with the "Starch & Sugar Off" setting. Bias associated with these predictions can be observed in Figure 4.4 and Figure 4.5.

Results from the backwards stepwise regression of residual errors for dietary nutrients for PREP10 are presented in Table 4.4. Regression of residual errors identified correlations among nutrient supply (% of DMI) and residual errors for both MP and AA allowable milk predictions. Significant nutrient correlations with residual errors for MP allowable milk predictions included DMI (-1.0 kg), MP (-9.8 kg), acid detergent fiber (ADF) (-1.0 kg), NFC (-1.4 kg), arginine (Arg) (-108.4 kg), and threonine (Thr) (-272.8 kg). Significant nutrient correlations with residual errors for AA allowable milk predictions included DMI (-1.1 kg), starch (-0.3 kg), RDP (-2.1 kg), Arg (-103.7 kg), valine (Val) (-334.3 kg), histidine (His) (190.0 kg), and phenylalanine (Phe) (194.3 kg).

DISCUSSION

An evaluation of predictions from the NRC 2001 model was reported by NRC (2001) that included 100 diets treatment means from 25 studies published from 1992 through 2001. This analysis showed root mean square prediction error of 2.0 kg/d over-prediction for DMI with no apparent slope bias. A plot of NE_L intake vs NE_L utilization was used to evaluate NRC (2001) energy predictions. This revealed a modest mean bias of 2.5%, while showing a more substantial slope bias (NE_L Intake = 7.8 + 0.8 x NE_L Use). However, this bias was dismissed as it would not likely apply to the range of NE_L concentrations found in rations normally fed within the United States. Predictions of MP allowable milk had substantial mean bias in most circumstances, as 67% of diets over-predicted MP allowable milk by more than 10%. Because of the limited number of studies used to evaluate the model, it was proposed that although many of the diets were not limiting in MP, AA content may have been improperly balanced and thus resulted in AA deficiency that limited production. Conclusions based on evaluation of NRC (2001) were that more observations were needed from a more diverse range of nutritional scenarios in order to properly evaluate the model (NRC, 2001). Although these model predictions were assessed appropriately,

other equations predicting duodenal N-flows in NRC (2001) were evaluated by plotting residuals against observed values. This approach was subsequently reevaluated and deemed inappropriate (St-Pierre, 2003).

In 2006, Seo et al. published a thorough evaluation of 2001 Dairy NRC equations which predicted passage rates (K_p) of wet forage, dry forage and concentrates out of the rumen. These K_p values are used to calculate supplies of rumen degraded protein (RDP), rumen undegraded protein (RUP), and MP. Passage rate equations were evaluated using 319 diet treatment means from published literature. Sensitivity analysis showed that the model was most sensitive to DMI, rate of degradation of CP B-fraction, and CP content of feed, and thus the accuracy of MP predictions are contingent on the accuracy of these values. This finding is in agreement with another study that concluded that improvements in model calculation of RUP would improve predictions of rumen degradation of CP, and may be achieved by better estimates of protein-B fractions (Edmunds et al., 2012). Overall, it was concluded that the 2001 Dairy NRC ruminal K_p equations were adequate to predict flow rates for lactating dairy cows (Seo et al., 2006).

The present study analyzed NRC (2001) further, as well as providing a comparison to PREP10. On the basis of RMSPE%, MP allowable milk was the best predictor of production in the NRC (2001) model (21.5%). Overall, NRC (2001) over-predicted allowable milk yield (0.5-2.9kg/d). Predictions of NE_L allowable milk had the greatest mean bias (15.1%), followed by Most-Limiting Nutrient allowable milk (6.5%), while MP allowable milk had negligible mean bias (0.8%). MP allowable milk had the least slope bias (10.3%), while Most-Limiting Nutrient allowable milk predictions had the most slope bias (13.9%).

Residual error regression found nutrients correlated with residual error for NE_L allowable milk and MP allowable milk. In both cases, increased RDP supply was found to contribute to under-

prediction of milk production, suggesting that improved predictions of RDP supply and availability could improve model performance on the basis of NE_L and MP alike. Although the calculation of NE_L is independent of RDP supply in NRC (2001), energy and protein are not independent of one another in a biological setting; microbial protein synthesis is limited by energy supply (Hackmann and Firkins, 2015), and milk protein synthesis is dependent upon AA and energy supply (Vyas and Erdman, 2009). Residual errors for NE_L allowable milk predictions also significantly correlated with NDF and Met. With each percent dry matter (%DM) increment increase of dietary NDF, milk production was over-predicted by 0.5kg. Since dietary NDF content normally ranges from 25-33% (NRC, 2001), variation in NDF supply could substantially impact model predictions. Conversely, variation in dietary Met content contribute modestly to residual error for NE_L allowable milk prediction; for each %DM increase in dietary Met, production is under-predicted by 0.1kg. Furthermore, since Met supply varies by mere grams per day, measurable variations of %DM are unlikely. Residual errors of MP allowable milk significantly correlated with MP supply, lignin, NFC, RUP, and Fat. Although lignin supply contributes to over-prediction while RUP, NFC and fat contribute to under-prediction, MP supply appears to impact model predictions most. The NRC (2001) model estimates MP requirements to be approximately 1.16g MP/kg body weight (BW). For a 680kg Holstein at peak lactation, the NRC (2001) predicts this requirement to be 3.14kg/d of MP. Assuming DMI to be within the higher end of average DMI reported by NRC (2001) (15-27kg/d), MP requirements would compose approximately 10-15% of DMI. With each %DM increase in MP supply, MP allowable milk predictions are over-predicted by 4.8kg. This provides further evidence that the fixed protein use efficiency of 67% assumed by NRC (2001) overestimates protein digestibility at higher rates of DMI.

Overall, PREP10 under-predicted milk production. Predicting milk production on the basis of MP versus AA supply provides equivocally accurate and precise predictions (23.4% versus 24.2% of RMSPE, respectively). Since residual error regression for AA allowable milk identified multiple AA as contributing to residual error, correction of AA estimates would likely result in a more precise prediction of production than predicting from MP supply alone. The PREP10 model accounts for the fact that the efficiency of AA conversion to milk protein is variable at different levels of AA supplementation (Vyas and Erdman, 2009), and that efficiency of protein utilization varies with intake and production (Erasmus et al., 1994, Hanigan et al., 1998). By accounting for varying levels of CP and AA intake, production may be more accurately predicted. However, large slope bias of this prediction indicates that improvement is needed. Some of this variation could be explained by principles discussed by Arriola Apelo et al. (2014), which suggested that the "most-limiting nutrient" theory may not completely translate to amino acids. If an excess of certain AA in a diet can compensate for deficiencies of other AA, the concept of production limited by AA supply could be undermined (Arriola Apelo et al., 2014).

In both MP and AA allowable milk predictions, residual error analysis showed correlations with DMI and multiple AA. With each kg increase of DMI, MP and AA allowable milk resulted in over-prediction of milk production (0.9kg and 1.1kg, respectively). Given large variations in DMI between animals, improved accuracy of ration TDN predictions could contribute substantially to improved model precision. With regards to AA supply, large estimates of contribution of residual error suggest that AA supply may have a measurable impact on model predictions in spite of proportionally low inclusion in rations. For example, incremental increases in %DM of Val result in 334.3kg over-prediction of AA allowable milk. Although Val daily supply ranges on the order of 100-200g, even a fraction of 334.3kg could measurably impact model accuracy. Further

research into the metabolism and requirements of AA such as His, Arg, Val, Thr, and Phe, combined with greater understanding of limiting nutrient concepts, could greatly improve model performance. In contrast to previous work demonstrating duodenal flows of nitrogen to be similar in forage versus concentrate based diets (Cunningham et al., 1993), recent evidence suggests that feedstuff plays a role in determining nitrogen flows, rather than nutrient composition alone (White et al., 2017b). As such, correlations of His and Arg with residual errors may further indicate that individual feedstuffs (such as forages rich is His) may behave differently in the model. Improved accuracy of predictions of AA allowable milk versus MP allowable milk may also be evident in the fact that MP allowable milk residual errors correlated significantly with MP supply. Increased MP supply contributed to over-prediction (estimate = 9.8kg), suggesting that calculating predictions based on MP supply alone do not account for limiting AA, thus resulting in over-prediction.

The error of prediction for PREP10 AA and MP allowable milk exceeds that of NRC (2001) MP allowable Milk, though the least RMSPE% for PREP10 MP and AA allowable milk are less than that of NRC (2001) NE_L allowable Milk. Analysis of PREP10 revealed slope bias for AA and MP allowable milk predictions for every BIV setting. On the basis of RMSPE%, MP allowable milk was the best predictor of production in both the NRC (2001) and PREP10 models. Despite the substantial slope bias of PREP10 AA allowable milk, regression analysis identified certain nutrients correlated with residual errors that may provide a greater opportunity to take a more targeted approach to correcting residual errors than any other prediction. Correcting inaccuracies resultant from the nutrients correlated with AA allowable milk residual errors could decrease RMSPE% considerably; as slope bias accounts for 1/3 of MSE in the "Starch & B1 K_p Off" setting, correction of this bias would reduce AA allowable milk RMSPE to ~16% of observed values.

Thus, AA allowable milk has great promise for predicting milk production, with the ability to correct for varying AA composition rather than simply MP supply. Greater understanding of rumen flora may allow improvements to be made upon this system; yeast supplementation, for instance, has been shown to impact rumen microbes enough to alter the AA profile and quantity of microbial protein flow to the duodenum (Erasmus et al., 1992). Rumen pH and rumen microbes are impacted by diet composition, and proteolytic activity decreases as rumen pH decreases in dairy cows (Bach et al., 2005). Although it is impractical to incorporate microbial populations into these models directly, accounting for the aforementioned impact of feedstuffs (rather than nutrient composition alone) on microbial flow and AA composition may allow for improved estimates of protein utilization.

Accurate estimation of AA and protein flows to the duodenum also depends on accurate calculation of RUP, RDP, and microbial protein. The NRC (2001) model calculates RDP from protein fractions A and B, and uses energy supply to estimate conversion to microbial protein. This calculation does not account for N-recycling within the rumen (White et al., 2017a). Calculation of RUP uses undegraded B fraction and C fraction protein (Ørskov and McDonald, 1979). The PREP10 model accounts for a portion of the A protein fraction escaping the rumen with liquid outflow, rather than assuming complete degradation and potential use for microbial protein as in NRC (2001). Tabular values for RUP content of individual feedstuffs are used to predict RUP supply in NRC (2001), but Bateman et al. (2005) reported that RUP content of individual feedstuffs varied with level of intake. Accounting for correlations between RUP and DMI were shown to improve predictions of nitrogen flow to the duodenum. This suggests that calculation of RUP content of feedstuffs as varying with intake rather than calculated from fixed tabular values would better predict protein flow and metabolism (Bateman et al., 2005). Since animal-sourced protein

is often utilized as a source of RUP and to replace a portion of CP in dairy rations (Santos et al., 1998), the suitability of these feedstuffs to be metabolized predictably warrants further consideration (Ipharraguerre and Clark, 2014, White et al., 2017b).

The advancement of scientific knowledge relies entirely on accurate and precise research data. Publications used for the work herein included data that compared positive and negative control groups to experimental treatment groups. The aim of these studies was to determine if losses in production in negative control groups as compared to positive controls could be rescued by changing the composition of diets fed to treatment groups. The success of these studies was often contingent on accurate and precise predictions of nutrient requirements to ensure the formulated test diets were indeed deficient in the nutrient of interest. If, for example, a negative control diet was formulated using the NRC (2001) model to be deficient in protein and while sufficient in energy, the researcher would aim to attribute decreases in production to the insufficient protein supply. However, the model tends to over-predict NE_L allowable milk, and thus the formulated diets were often energy-deficient. As such, assignment of treatment effects is potentially confounded. These effects would be further complicated at varying levels of DMI, as shown by the substantial slope bias in each model. Slope bias in these models causes predictions to become more variable as intake and production increase. Moe and Tyrell (1975) found that increasing DMI results in depressed nutrient digestibility. As modern dairy cows commonly consume 3-4 times maintenance requirements, this decrease in digestibility is of increasing relevance (Tyrrell and Moe, 1975). As production increases, so does the margin by which energy allowable milk is over-predicted. Thus, a large portion of slope bias of model predictions could be attributed to an insufficient discount of digestibility for increased DMI.

Furthermore, adjustments should be considered for estimates of microbial growth per unit of fermentable energy, protein degradability and digestion in the rumen and SI, and the use of protein for non-milk functions (Hanigan, 1998). Specifically, the efficiency of conversion of MP for maintenance and milk production has been shown to vary with level of intake and production, rather than the current assumed constant of 67% (White et al., 2017c). With increasing intake, error resultant from this estimation and the overestimation of nutrient digestibility becomes more apparent.

Thus, work to be done in the future includes identifying and correcting the contributors of the error of MP, AA and NE_L systems. As evidenced by this study, RDP supply and digestibility appear to be underestimated by NRC (2001) and overestimated by PREP10. Predictions of NRC (2001) NE_L allowable milk overestimate the impact of NDF on production. Predictions of NRC (2001) over-estimate MP digestibility, as evidenced by superior PREP10 predictions. The PREP10 model over-predicts production with increasing DMI, suggesting a need to discount total tract digestibility of rations with increasing levels of intake. Predictions of AA allowable milk by PREP10 could be greatly improved by more precise estimations of AA supply and requirement. In both models, errors in predicting AA or RUP supply may relate to specific feedstuffs, suggesting a need for better characterization of individual feeds. It is ever important to address such issues in order to achieve greater efficiency, productivity and profitability in dairy herds.

Tables

Table 4.1 Analysis of Residual Errors for NRC (2001) Model Predictions of Milk Production.

Prediction	N	Mean Observed[1]	Mean Predicted[1]	RMSPE%[2]	Mean Bias Error%	Slope Bias Error%
NE_L allowable[3]	155	30.5	33.4	24.7	15.1	12.6
MP allowable[4]	150	28.6	29.2	21.5	0.8	10.3
Most-Limiting	305	29.6	31.4	23.3	6.5	13.9

[1]Values reported in kg.

[2]Root mean square prediction error as a percent of observed milk production

[3]Net energy for lactation allowable milk production

[4]Metabolizable protein allowable milk production

Table 4.2 Analysis of Residual Errors for the PREP10 Model Predictions of Milk Production.

BIV Setting	Prediction	N	Mean Observed[1]	Mean Predicted[1]	RMSPE %	Mean Bias Error%	Slope Bias Error%
Starch Off	MP Allow.	178	30.0	29.3	23.4	1.2	20.8
Starch & B1 K_p Off	AA Allow.	179	28.1	26.7	24.2	4.2	33.7
All On	MP Allow.	176	29.9	29.7	24.6	0.1	22.2
B1 K_p Off	AA Allow.	177	28.3	27.4	24.8	1.6	37.0
Starch & Sugar Off	MP Allow.	181	30.6	28.1	25.6	10.9	17.7
Starch& B1 K_p Off	MP Allow.	83	31.7	28.3	26.3	16.6	19.8

[1]Values reported in kg.
[2]Root mean square prediction error as a percent of observed milk production.

Table 4.3 Results from Regression of Residual Error of Prediction from NRC (2001) on Predictions of NE_L allowable Milk, MP allowable Milk, and allowable milk based on the most-limiting nutrient.

	NE_L allowable			MP allowable			Most-Limiting		
Effect[1]	Estimate[4]	DF	*P*-Value	Estimate[4]	DF	*P*-Value	Estimate[4]	DF	*P*-Value
Intercept	16.7	48	0.002	-24.8	48	0.372	26.4	78	<.0001
DMI[1]	-----	-----	-----	-----	-----	-----	-0.40	219	<.0001
MP[2]	-----	-----	-----	-4.80	91	<.0001	-3.10	219	<.0001
NDF[2]	-0.50	97	<.0001	0.50	91	0.0721	-----	-----	-----
ADF[2]	-----	-----	-----	0.30	91	0.0992	0.20	219	0.0428
Lignin[2]	-----	-----	-----	-1.20	91	0.0154	-----	-----	-----
NFC[2]	-----	-----	-----	0.80	91	0.0146	-----	-----	-----
RUP[2]	-----	-----	-----	1.40	91	0.0199	1.00	219	0.0165
RDP[2]	0.60	97	0.0492	1.80	91	0.0005	0.50	219	0.0096
Methionine[2]	-0.10	97	0.0244	-0.10	91	0.0636	-----	-----	-----
TEAA[2,3]	0.00	97	0.1259	0.00	91	0.1057	0.00	219	0.0304
EE[2]	-----	-----	-----	1.20	91	0.0003	-0.30	219	0.113

[1]Analysis conducted based on reported kg of DMI.

[2]Analysis conducted based on reported percent of DM intake.

[3]TEAA=Total essential amino acids.

[4]Values represent kg predicted milk production per unit increase of Effect variable.

Table 4.4 Results from Regression of Residual Errors from the PREP10 Model on Predictions of MP and AA allowable milk.

	MP allowable Milk			AA allowable Milk		
Effect	Estimate[4]	DF	*P*-Value	Estimate[4]	DF	*P*-Value
Intercept	142	61	0.0153	140	58	<.0001
DMI[1]	-0.90	102	<.0001	-1.10	110	<.0001
MP[2]	-9.80	102	0.0171	-----	-----	-----
ME supply[3]	103	102	0.1126	-----	-----	-----
Fat[2]	-2.90	102	0.145	-----	-----	-----
ADF[2]	-1.00	102	0.0336	-----	-----	-----
NFC[2]	-1.40	102	0.018	-----	-----	-----
Starch[2]	-----	-----	-----	-0.30	110	0.0013
Sugar[2]	-----	-----	-----	-0.80	110	0.1482
RDP[2]	2.70	102	0.1179	-2.10	110	0.0489
Lysine[2]	72.9	102	0.0862	-----	-----	-----
Arginine[2]	-108.4	102	0.0027	-103.7	110	<.0001
Threonine[2]	-272.7	102	0.0344	118.4	110	0.0928
Leucine[2]	-----	-----	-----	-16.9	110	0.1361
Isoleucine[2]	137.9	102	0.0756	-----	-----	-----
Valine[2]	-148.9	102	0.1023	-334.3	110	0.0002
Histidine[2]	207.0	102	0.0705	190.0	110	0.0009
Phenylalanine[2]	-----	-----	-----	194.3	110	0.0004

[1]Analysis conducted based on reported kg of DMI.

[2]Analysis conducted based on reported percent of DM intake.

[3]Analysis conducted based on reported Mcal/kg of DM intake.

[4]Values represent kg predicted milk production per unit increase of Effect variable.

FIGURES

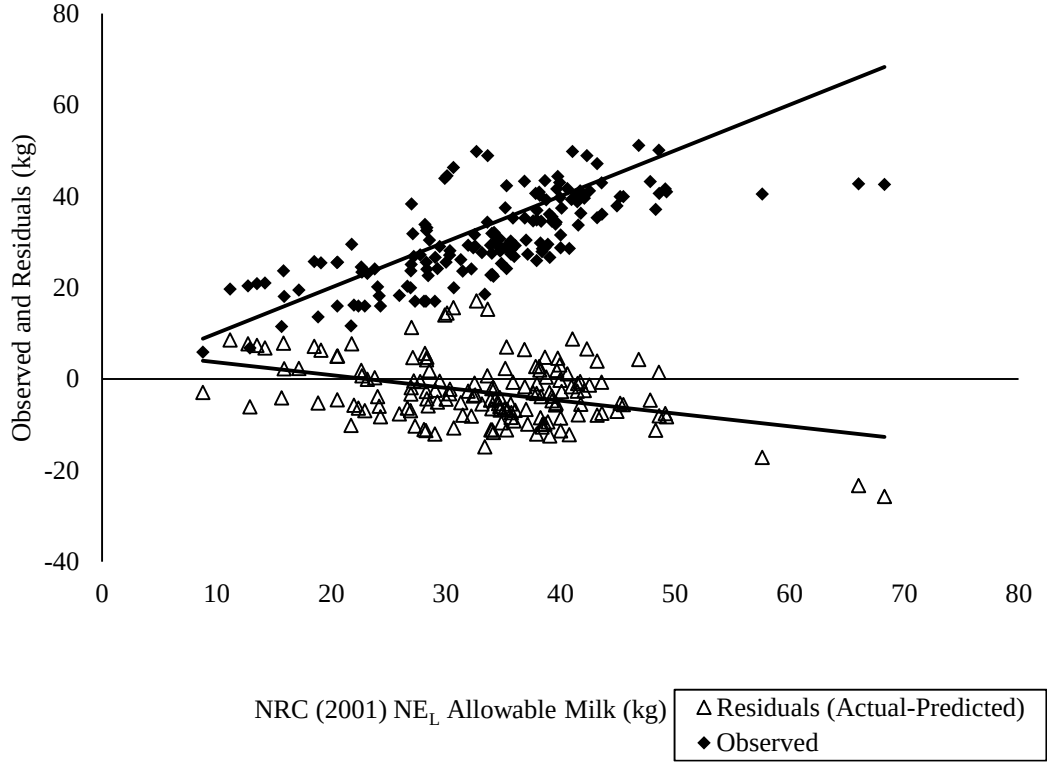

Figure 4.1

Residual errors of prediction of NE_L allowable milk production by the NRC (2001) model and observed production versus NE_L allowable milk. Data shown represent individual treatment means where NE_L allowable milk was limiting. Residual=6.44-(0.28*NEL allowable Milk).

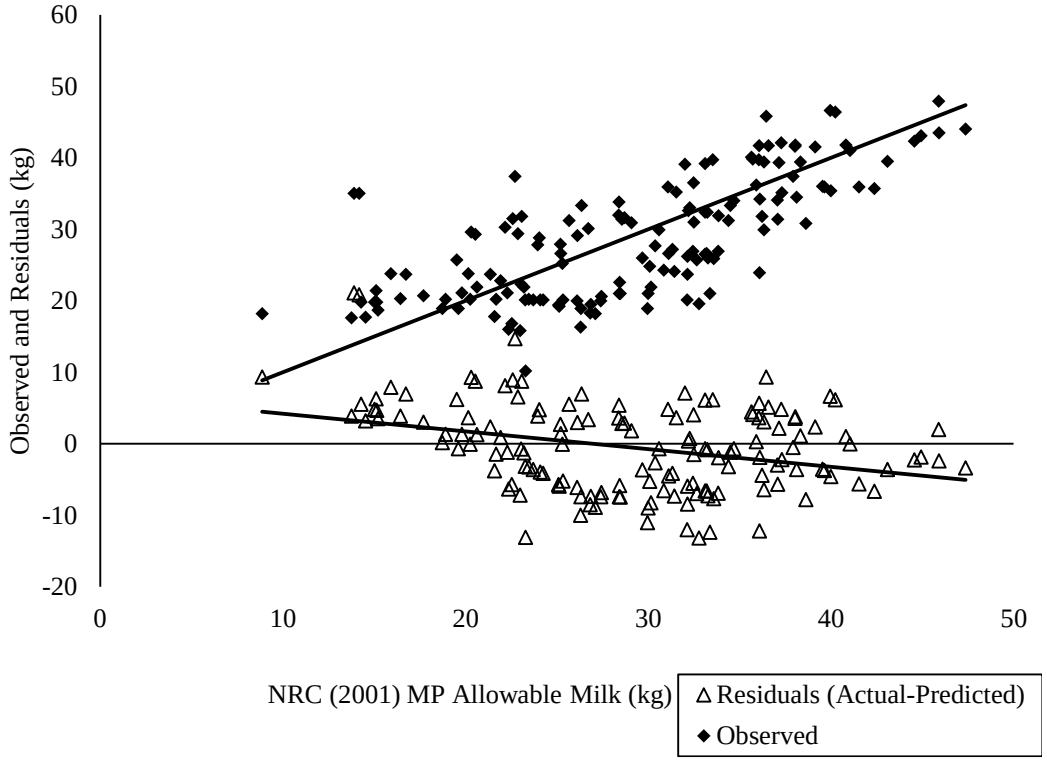

Figure 4.2 Residual errors of prediction of MP allowable milk production by the NRC (2001) model and observed production versus MP allowable milk. Data shown represent individual treatment means where MP allowable milk was limiting. Residual=6.69-(0.25*MP allowable Milk).

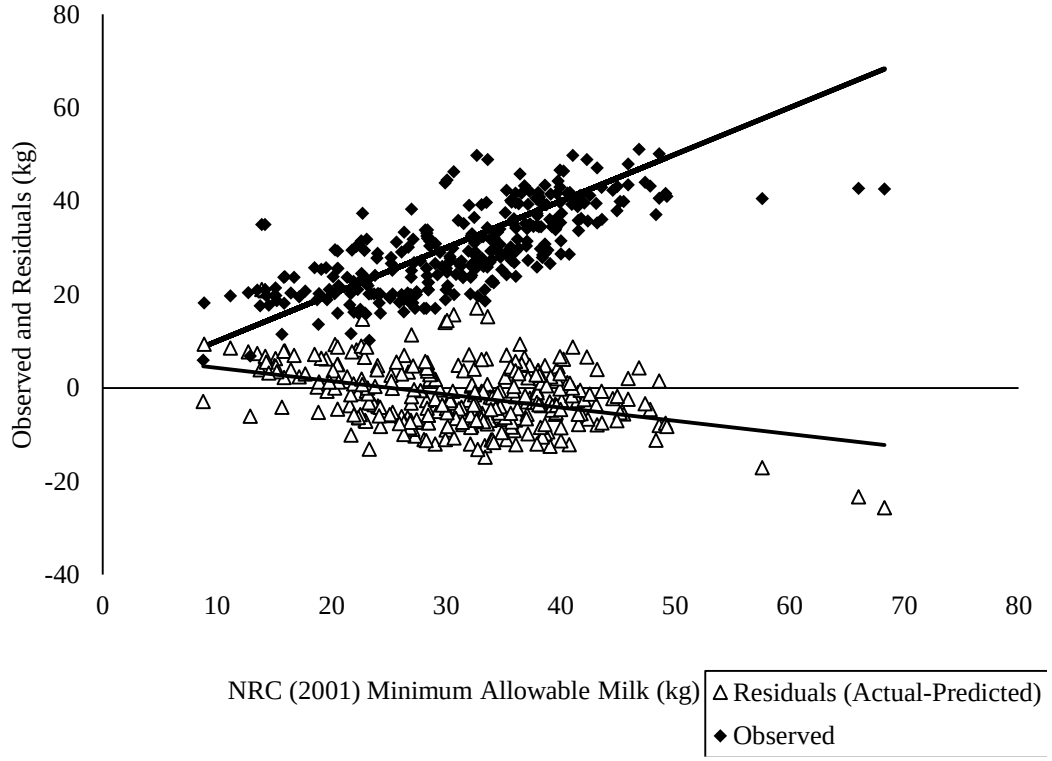

Figure 4.3 Residual errors of prediction of Most-Limiting Nutrient allowable milk production by the NRC (2001) model and observed production versus Most-Limiting Nutrient allowable milk. Data shown represent individual treatment means. Residual =7.12-(0.28*Most-Limiting Milk).

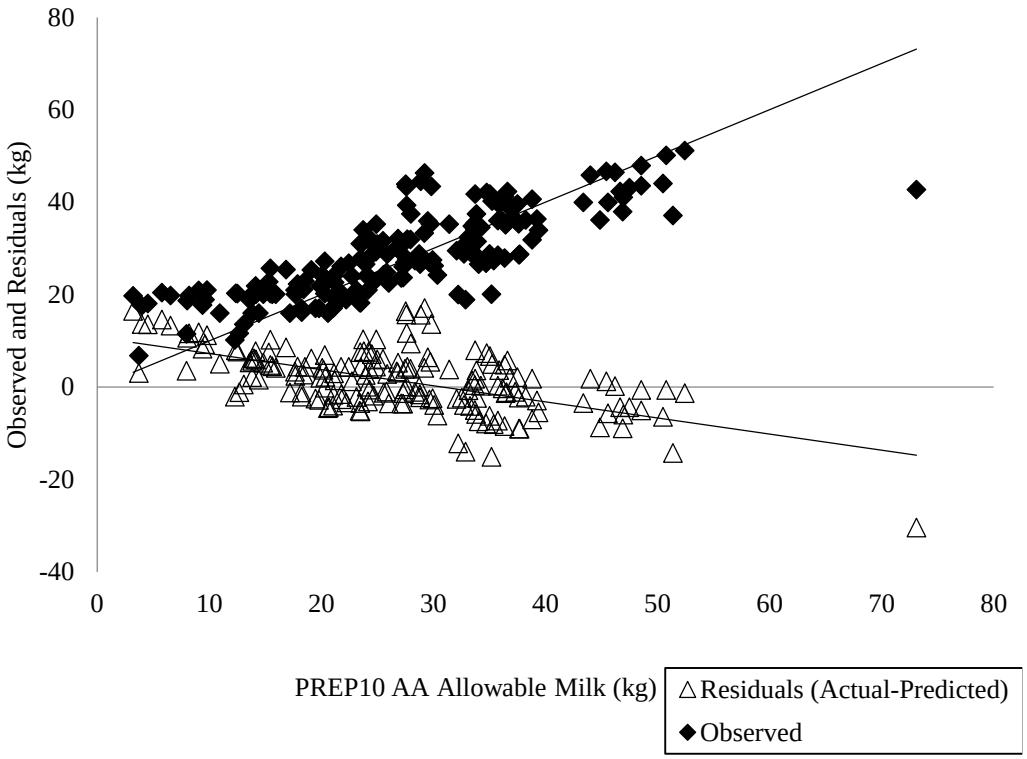

Figure 4.4 Residual errors of prediction of AA allowable milk production by PREP10 and observed production versus AA allowable milk. Data shown represent individual treatment means where AA allowable milk was limiting. Residual = 10.64-(0.35*AA allowable Milk).

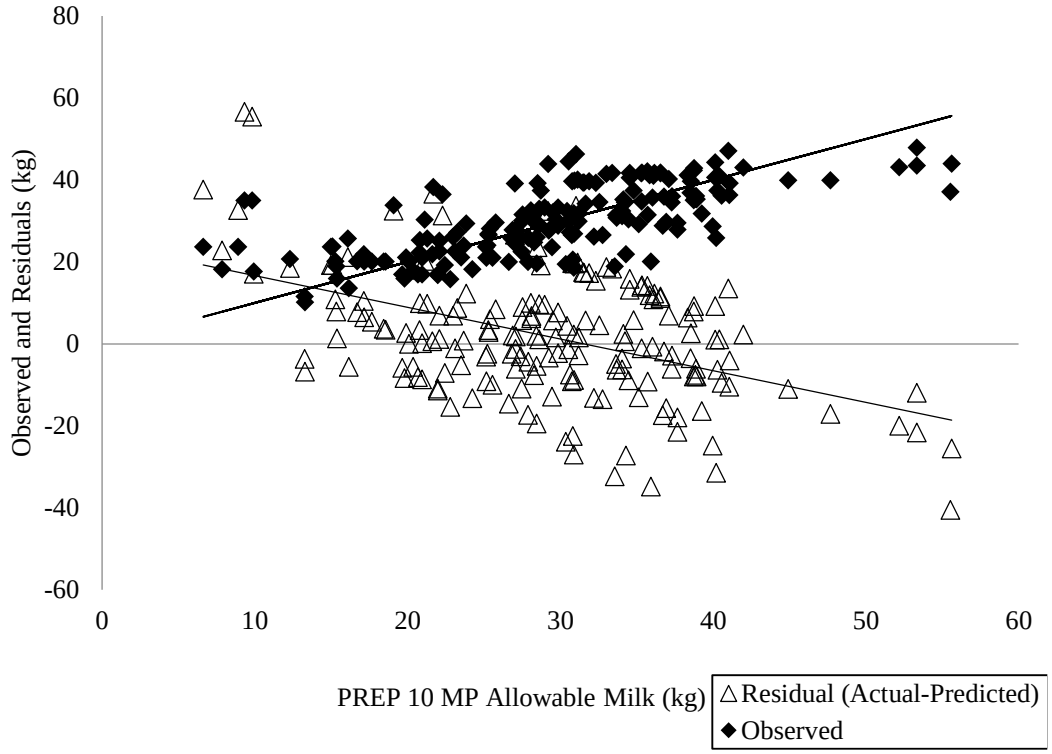

Figure 4.5 Residual errors of prediction of MP allowable milk production by PREP10 and observed production versus MP allowable milk. Data shown represent individual treatment means where MP allowable milk was limiting. Residual= 24.35-(0.773*MP allowable Milk).

Chapter 5

Conclusions and future work.

In the preceding chapters, several action points have been identified through which feed efficiency may be improved. Revision of MP requirements for lactating dairy cattle would greatly improve nitrogen efficiency, as feeding diets low in CP resulted in improved N efficiency without limiting milk production. This efficiency may be further improved by supplementing RPAA, as animals consuming diets supplemented with RP His showed superior N efficiency than animals consuming diets low in CP alone. This research was limited by inaccurate predictions of nutrient requirements and allowable milk production, making it difficult to interpret the biological impact of treatments that were applied. Improvements in these models would improve efficiency directly, and would also act synergistically with feedstuffs such as RPAA by allowing for more accurate assessment of their biological impact.

Predictions of milk production based on limiting AA was not superior in preceding chapters. However, the present work did identify improvements to be made; increased accuracy of AA supply and requirement would make this prediction more accurate than all others evaluated presently.

These improvements are subject to limitations; RPAA digestibility cannot currently be measure in-vivo. This study utilized two techniques to assess nutrient digestibility via in-sacco analysis, which proved less practical than expected. Refinement of this technique would allow for more timely and economic determination of feedstuff digestibility, and prevent improperly protected products from entering trials such as those in this work.

Feeding trials reported here contributed genetic and phenotypic information to be used to identify genomic markers of feed efficiency. Analysis is ongoing. This work aims to identify linear

traits of feed efficiency that can be selected for by industry professionals. This genetic variation may be accounted for by the presence or absence of genes that affect efficiency, but also may be the result of differences in epigenetic modifications or microbiomes. Previous work has shown phenotypic variations in cloned bovine fetuses as a result of epigenetic modifications, making gene activation of interest to this work (Cezar et al., 2003). Other phenotypic characteristics that effect feed efficiency, such as methane emissions, have been shown to be impacted by differences in the microbiome of individual dairy cattle (Tapio et al., 2017). Thus, increased understanding of dairy cow genomes, epigenomes, and microbiomes may allow for more rapid identification and selection for more efficient animals.

Acknowledgements

Funding for this project was provided by Agriculture and Food Research Initiative Competitive Grant no. 2011-68004-30340 from the USDA National Institute of Food and Agriculture, the Papillon Agricultural Company, and Balchem Corporation.

REFERENCES

Ali, M., M. R. Weisbjerg, J. Cone, G. Van Duinkerken, M. Blok, M. Bruinenberg, and W. Hendriks. 2012. Postruminal degradation of crude protein, neutral detergent fibre and starch of maize and grass silages in dairy cows. Animal feed science and technology 177(3):172-179.

Applebaum, R. S., R. E. Brackett, D. W. Wiseman, and E. H. Marth. 1982. Responses of dairy cows to dietary aflatoxin: Feed intake and yield, toxin content, and quality of milk of cows treated with pure and impure aflatoxin. Journal of Dairy Science 65(8):1503-1508.

Arriola Apelo, S. I., J. R. Knapp, and M. D. Hanigan. 2014. Invited review: Current representation and future trends of predicting amino acid utilization in the lactating dairy cow. J. Dairy Sci 97.7:4000–4017.

Arthur, P., J. Archer, D. Johnston, R. Herd, E. Richardson, and P. Parnell. 2001. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in angus cattle. Journal of animal science 79(11):2805-2811.

Bach, A., S. Calsamiglia, and M. Stern. 2005. Nitrogen metabolism in the rumen. Journal of dairy science 88:E9-E21.

Bateman, H., J. Clark, and M. Murphy. 2005. Development of a system to predict feed protein flow to the small intestine of cattle. Journal of dairy science 88(1):282-295.

Bauman, D. E. and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annual review of nutrition 23(1):203-227.

Bibby, J. and H. Toutenburg. 1977. Prediction and improved estimation in linear models. Wiley, Chichester ; New York.

Cant, J., R. Berthiaume, H. Lapierre, P. Luimes, B. McBride, and D. Pacheco. 2003. Responses of the bovine mammary glands to absorptive supply of single amino acids. Canadian journal of animal science 83(3):341-355.

Cezar, G. G., M. S. Bartolomei, E. J. Forsberg, N. L. First, M. D. Bishop, and K. J. Eilertsen. 2003. Genome-wide epigenetic alterations in cloned bovine fetuses 1. Biology of reproduction 68(3):1009-1014.

Church, D. C. 1969. Digestive physiology and nutrition of ruminants. Volume 1. Digestive physiology and nutrition of ruminants. Volume 1.

Conrad, H., A. Pratt, and J. W. Hibbs. 1964. Regulation of feed intake in dairy cows. I. Change in importance of physical and physiological factors with increasing digestibility. Journal of Dairy Science 47(1):54-62.

Cunningham, K., M. Cecava, and T. Johnson. 1993. Nutrient digestion, nitrogen, and amino acid flows in lactating cows fed soybean hulls in place of forage or concentrate1. Journal of Dairy Science 76(11):3523-3535.

Edmonds, M., C. Parsons, and D. Baker. 1985. Limiting amino acids in low-protein corn-soybean meal diets fed to growing chicks. Poultry Science 64(8):1519-1526.

Edmunds, B., K.-H. Südekum, H. Spiekers, and F. Schwarz. 2012. Estimating ruminal crude protein degradation of forages using in situ and in vitro techniques. Animal feed science and technology 175(3):95-105.

Erasmus, L., P. Botha, C. Cruywagen, and H. Meissner. 1994. Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feedstuffs1. Journal of dairy science 77(2):541-551.

Erasmus, L., P. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows1. Journal of Dairy Science 75(11):3056-3065.

Giallongo, F., A. Hristov, J. Oh, T. Frederick, H. Weeks, J. Werner, H. Lapierre, R. Patton, A. Gehman, and C. Parys. 2015. Effects of slow-release urea and rumen-protected methionine and histidine on performance of dairy cows. Journal of dairy science 98(5):3292-3308.

Guinard, J. and H. Rulquin. 1995. Effects of graded amounts of duodenal infusions of methionine on the mammary uptake of major milk precursors in dairy cows. Journal of dairy science 78(10):2196-2207.

Hackmann, T. J. and J. L. Firkins. 2015. Maximizing efficiency of rumen microbial protein production. Frontiers in microbiology 6:465.

Hanigan, M., J. Cant, D. Weakley, and J. Beckett. 1998. An evaluation of postabsorptive protein and amino acid metabolism in the lactating dairy cow. Journal of dairy science 81(12):3385-3401.

Hanigan, M. D. 1998. An evaluation of postabsorptive protein and amino acid metabolism in the lactating dairy cow. Journal of Dairy Science:81:3385-3401.

Helton, J. C. and F. J. Davis. 2003. Latin hypercube sampling and the propagation of uncertainty in analyses of complex systems. Reliability Engineering & System Safety 81(1):23-69.

Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Animal Feed Science and Technology 48(3-4):211-227.

Huhtanen, P., A. Vanhatalo, and T. Varvikko. 2002. Effects of abomasal infusions of histidine, glucose, and leucine on milk production and plasma metabolites of dairy cows fed grass silage diets. Journal of dairy science 85(1):204-216.

Hvelplund, T. and M. Weisbjerg. 2000. In situ techniques for the estimation of protein degradability and postrumen. Forage evaluation in ruminant nutrition 233.

Ipharraguerre, I. R. and J. H. Clark. 2014. A meta-analysis of ruminal outflow of nitrogen fractions in dairy cows. Advances in Dairy Research:1-13.

Kim, C. H., J. J. Choung, and D. G. Chamberlain. 2001. Estimates of the efficiency of transfer of l-histidine from blood to milk when it is the first-limiting amino acid for secretion of milk protein in the dairy cow. Journal of the Science of Food and Agriculture 81(12):1150-1155.

King, K., W. Bergen, C. Sniffen, A. Grant, D. Grieve, V. King, and N. Ames. 1991. An assessment of absorbable lysine requirements in lactating cows. Journal of Dairy Science 74(8):2530-2539.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. Journal of animal science 22(2):486-494.

Krizsan, S., S. Ahvenjärvi, and P. Huhtanen. 2010. A meta-analysis of passage rate estimated by rumen evacuation with cattle and evaluation of passage rate prediction models. Journal of dairy science 93(12):5890-5901.

Kung, L. and L. M. Rode. 1996. Amino acid metabolism in ruminants. Animal Feed Science and Technology 59(1-3):167-172.

Lanzas, C., L. Tedeschi, S. Seo, and D. Fox. 2007. Evaluation of protein fractionation systems used in formulating rations for dairy cattle. Journal of dairy science 90(1):507-521.

Lee, C., A. Hristov, T. Cassidy, K. Heyler, H. Lapierre, G. Varga, M. De Veth, R. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. Journal of dairy science 95(10):6042-6056.

Li, L., J. Cyriac, K. F. Knowlton, L. C. Marr, S. W. Gay, M. D. Hanigan, and J. A. Ogejo. 2009. Effects of reducing dietary nitrogen on ammonia emissions from manure on the floor of a naturally ventilated free stall dairy barn at low (0–20 c) temperatures. Journal of environmental quality 38(6):2172-2181.

McNamara, J. P. 2004. Research, improvement and application of mechanistic, biochemical, dynamic models of metabolism in lactating dairy cattle. Animal Feed Science and Technology 112(1):155-176.

Mitchell, H. and R. J. Block. 1946. Some relationships between the amino acid con-tents of proteins and their nutritive values for the rat. J. biol. Chem 163:599.

Nkrumah, J., J. Basarab, Z. Wang, C. Li, M. Price, E. Okine, D. Crews, and S. Moore. 2007. Genetic and phenotypic relationships of feed intake and measures of efficiency with growth and carcass merit of beef cattle. Journal of animal science 85(10):2711-2720.

NRC. 2001. Nutrient requirements of dairy cattle. 7Th Revised ed. National Academy Press, Washington, DC.

Oba, M. and M. Allen. 1999. Effects of brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. Journal of Dairy Science 82(1):135-142.

Ørskov, E. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science 92(02):499-503.

Patterson, D. and P. Anderson. 1982. Recent aflatoxin feeding experiments in cattle. Veterinary Record 110(3):60-60.

Polan, C. E., K. Cummins, C. Sniffen, T. Muscato, J. Vicini, B. Crooker, J. Clark, D. Johnson, D. Otterby, and B. Guillaume. 1991. Responses of dairy cows to supplemental rumen-protected forms of methionine and lysine. Journal of dairy science 74(9):2997-3013.

Prendiville, R., K. Pierce, and F. Buckley. 2009. An evaluation of production efficiencies among lactating holstein-friesian, jersey, and jersey× holstein-friesian cows at pasture. Journal of Dairy Science 92(12):6176-6185.

Pryce, J., J. Arias, P. Bowman, S. Davis, K. Macdonald, G. Waghorn, W. Wales, Y. Williams, R. Spelman, and B. Hayes. 2012. Accuracy of genomic predictions of residual feed intake and 250-day body weight in growing heifers using 625,000 single nucleotide polymorphism markers. Journal of dairy science 95(4):2108-2119.

Robinson, P., N. Swanepoel, and E. Evans. 2010. Effects of feeding a ruminally protected lysine product, with or without isoleucine, valine and histidine, to lactating dairy cows on their productive performance and plasma amino acid profiles. Animal feed science and technology 161(3):75-84.

Robinson, P., N. Swanepoel, I. Shinzato, and S. Juchem. 2011. Productive responses of lactating dairy cattle to supplementing high levels of ruminally protected lysine using a rumen protection technology. Animal feed science and technology 168(1):30-41.

Rogers, J., U. Krishnamoorthy, and C. Sniffen. 1987. Plasma amino acids and milk protein production by cows fed rumen-protected methionine and lysine. Journal of Dairy Science 70(4):789-798.

Rossi, F., M. Maurizio, M. Francesco, C. Giovanna, and P. Gianfranco. 2003. Rumen degradation and intestinal digestibility of rumen protected amino acids: Comparison between in situ and in vitro data. Animal feed science and technology 108(1):223-229.

Santos, F., J. Santos, C. Theurer, and J. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review1. Journal of dairy science 81(12):3182-3213.

Seo, S., L. Tedeschi, C. Schwab, B. Garthwaite, and D. Fox. 2006. Evaluation of the passage rate equations in the 2001 dairy nrc model. Journal of dairy science 89(6):2327-2342.

St-Pierre, N. 2003. Reassessment of biases in predicted nitrogen flows to the duodenum by nrc 2001. Journal of Dairy Science 86(1):344-350.

Tapio, I., T. J. Snelling, F. Strozzi, and R. J. Wallace. 2017. The ruminal microbiome associated with methane emissions from ruminant livestock. Journal of animal science and biotechnology 8(1):7.

Tozer, P. 2000. Least-cost ration formulations for holstein dairy heifers by using linear and stochastic programming. Journal of dairy science 83(3):443-451.

Tuitoek, K., L. Young, C. De Lange, and B. Kerr. 1997. The effect of reducing excess dietary amino acids on growing-finishing pig performance: An elevation of the ideal protein concept. Journal of Animal Science 75(6):1575-1583.

Tyrrell, H. and P. Moe. 1975. Effect of intake on digestive efficiency. Journal of Dairy Science 58(8):1151-1163.

Valadares, R., G. Broderick, S. Valadares Filho, and M. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives1. Journal of dairy science 82(12):2686-2696.

Van Straalen, W., F. Dooper, A. Antoniewicz, I. Kosmala, and A. Van Vuuren. 1993. Intestinal digestibility in dairy cows of protein from grass and clover measured with mobile nylon bag and other methods. Journal of dairy science 76(10):2970-2981.

VandeHaar, M. J. and N. St-Pierre. 2006. Major advances in nutrition: Relevance to the sustainability of the dairy industry. Journal of Dairy Science 89(4):1280-1291.

Vanhatalo, A., P. Huhtanen, V. Toivonen, and T. Varvikko. 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. Journal of Dairy Science 82(12):2674-2685.

Vyas, D. and R. Erdman. 2009. Meta-analysis of milk protein yield responses to lysine and methionine supplementation. Journal of dairy science 92(10):5011-5018.

White, R., Y. Roman-Garcia, J. Firkins, P. Kononoff, M. VandeHaar, H. Tran, T. McGill, R. Garnett, and M. Hanigan. 2017a. Evaluation of the national research council (2001) dairy model and derivation of new prediction equations. 2. Rumen degradable and undegradable protein. Journal of Dairy Science.

White, R., Y. Roman-Garcia, J. Firkins, M. VandeHaar, L. Armentano, W. Weiss, T. McGill, R. Garnett, and M. Hanigan. 2017b. Evaluation of the national research council (2001) dairy model and derivation of new prediction equations. 1. Digestibility of fiber, fat, protein, and nonfiber carbohydrate. Journal of Dairy Science.

White, R. R., T. McGill, R. Garnett, R. J. Patterson, and M. D. Hanigan. 2017c. Short communication: Evaluation of the prep10 energy-, protein-, and amino acid-allowable milk equations in comparison with the national research council model. Journal of Dairy Science 100(4):2801-2806.

Whitlow, L. W., R. L. Nebel, and W. M. Hagler Jr. 1994. The association of deoxynivalenol in grain with milk production loss in dairy cows. Pages 131-139 in Mycotoxins, wood decay, plant stress, biocorrosion, and general biodeterioration. Springer.