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Estimating amino acid limitations in California dairy rations and the effect of feeding a ruminally protected lysine supplement on animal performance.

by

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CONTENTS

Page

Page

Page

Declaration

I hereby declare that this thesis, submitted for the MSc (Agric) Animal Nutrition degree at the University of Pretoria, is my own work and effort, conducted under the supervision of Prof. L.J. Erasmus and Dr. P.H. Robinson and that it has not previously been submitted by me for a degree at any other University.

N. Swanepoel
Pretoria
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Summary

Estimating amino acid limitations in California dairy rations and the effect of feeding a ruminally protected lysine supplement on animal performance

By

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The widespread increase in use of maize-based feedstuffs in California dairy cow rations has raised concerns of reduced efficiency of CP utilization due to the low lysine content of maize and maize by-products. The objectives of this research were to determine the impact of increased dietary maize CP levels on animal performance using three metabolic models of dairy cows in order to predict limiting AA's in California dairy rations to identify a ruminally protected AA package to supplement similar rations. Since lysine was the most consistently predicted limiting AA among dairies, and metabolic models, the dairy with the ration calculated to be the most limiting in lysine was chosen to determine effects of feeding an RPL product on milk production and composition, as well as on N balance. Nutrient profiles of 16 high multiparity cow rations were evaluated and limiting AA predicted by the metabolic models Amino Cow, CPM Dairy and Shield. Higher inclusion levels of maize products in rations increased the contribution of maize CP to the total CP content of the TMR, to between 20 – 40%, but had no impact on cow performance. Even

though the lysine to methionine ratio decreased as more maize CP was included in the TMR, it did not have a major impact on the final predicted AA profile of MP, or milk component levels, but, according to Shield, it had an effect on milk yield. Methionine, isoleucine and lysine were predicted to be most limiting according to Amino Cow, CPM Dairy and Shield respectively. The models suggested three dramatically different AA packages but the high degree of consistency within model in predicting the limiting AA sequence among dairies, suggest that there may be sufficient consistency in the nutrient profiles among rations to support production of a ruminally protected AA complex. The second experiment used a double (i.e., early and mid-lactation multiparity dairy cows) 2 x 2 factorial with 28 day experimental periods. Feeding the RPL, with estimated rumen escape of lysine between 18 and 23% suggesting an increased intestinal absorption of lysine between 8 and 22 g/d/cow, did not influence DMI or milk, true protein and lactose yields. Milk fat yield and concentration decreased, while MUN percentages increased when RPL was fed. Blood plasma levels of almost all AA's, except lysine, decreased when RPL was fed, suggesting that lysine was the limiting AA and that its supplementation led to increased absorption and utilization of other AA. The lack of response in milk protein synthesis and the decrease in plasma 3-MH concentrations when RPL was fed suggests that muscle protein synthesis was stimulated, and degradation reduced, with RPL feeding. It is possible that lysine had an effect, either directly or indirectly, on muscle protein turnover and energy metabolism that, impacted intakes, metabolism and absorption of AA and milk production in mid lactation cows, but it had no major impact on early lactation cows.

List of Abbreviations

AA	amino acid
AAS	atomic absorption spectrometry
ADF	acid detergent fibre
ADICP	acid detergent insoluble crude protein
ADIN	acid detergent insoluble nitrogen
AES	atomic emission spectrometry
ANR	UC Davis analytical laboratory
AP	absorbed protein
ARC	Agricultural Research Council (UK)
AUC	area under the curve
BCAA	branched-chain amino acid
BCS	body condition score
BHT	butylated hydroxytoluene
BUN	blood urea nitrogen
BW	body weight
CA	California
CLA	conjugated linoleic acid
CNCPS	Cornell Net Carbohydrate and Protein System
CP	crude protein
DC 305	Dairy Comp 305
DDDH2O	double distilled de-ionized water
DDG	dried distillers grains
DHIA	Dairy Herd Improvement Association
DIM	days in milk
DM	dry matter
DMI	dry matter intake
dNDF30	digested NDF after 30 h of in vitro rumen incubation
EAA	essential amino acid
ECP	endogenous crude protein
EE	ether extract
EN	endogenous nitrogen
ENS	endogenous nitrogen secretion
FA	fatty acid
GIT	gastro intestinal tract
GLM	general linear model of SAS
HMB	2-hydroxy-4-methylthiobutanoic acid
HMBi	isopropyl ester of HMB
HPLC	high-performance liquid chromatography
ICP-AES	inductively coupled plasma atomic emission spectrometry
INRA	Inland Northwest Research Alliance
LCFA	long chain fatty acid
MCP	microbial crude protein
MP	metabolizable protein
MPN	milk protein nitrogen
MRT	mean retention time
MUN	milk urea nitrogen
NAN	non-ammonia nitrogen
NDF	neutral detergent fibre
NDFD	neutral detergent fibre digestibility
NE	net energy
NEAA	non-essential amino acid
NEFA	non-esterified fatty acid
NEL	net energy for lactation
NIR	near-infrared spectroscopy
NLIN	nonlinear regression
NPN	non-protein nitrogen
NRC	National Research Council
OM	organic matter

List of Abbreviations

PDI	protein truly digestible in the small intestine
PN	protein nitrogen
RDP	rumen-degradable protein
RPAA	rumen protected amino acid
RPL	rumen protected lysine
RPM	rumen protected methionine
RUP	rumen-undegradable protein
SAS	Statistical Analysis Software
SCC	somatic cell count
SE	standard error
SG	specific gravity
SmM	Smartamine M
SolCP	soluble crude protein
TMR	total mixed ration
UN	urea nitrogen
UUN	urinary urea nitrogen
VFA	volatile fatty acid
VMTRC	Veterinary Medicine Teaching and Research Centre

List of Products

Product	Manufacturer	Description
RPL product	Ajinomoto Inc., Tokyo, Japan	53% L-Lys monohydrochloride (L-LysHCL), 42% vegetable fat, 1% lechithin and 4% water.
Ener GII®	Nutritech Solutions, Ltd., Abbotsford, British Columbia, Canada	A concentrated source of rumen escape energy in the form of calcium salts of long-chain fatty acids delivering a net energy of lactation of 27 MJ/kg
XPTM Yeast culture	Diamond V Mills, Inc., Cedar Rapids, IA, USA	Contains *Saccharomyces cerevisiae* bakers yeast, cereal grain raw ingredients on which it was grown, B-vitamins and other fermentation products. It also is guaranteed to contain no less than 12 g/kg CP, 30 g/kg fat, and 65 g/kg crude fibre.
AliMet®	Novus International, Inc., St. Louis, MO, USA	An 880 g/kg aqueous solution of DL-HMB, a source of L-Met
Met-Plus™	Nisso America, Inc., New York, NY, USA	A lipid-protected product containing 650 g/kg DL-Met in a mixture of calcium salts of long-chain fatty acids, lauric acid, and a FA preservative (i.e., butylated hydroxytoluene)
Mepron® M85	Degussa AG, Henau, Germany	These pellets are surface coated products with a core of 850 g/kg DL-Met and starch, coated with layers of ethylcellulose and stearic acid. It contains 85 g/kg non-structural carbohydrates, 35 g/kg NDF, 15 g/kg ash, 10 g/kg moisture and 5 g/kg fat.
Smartamine™ M	Adisseo, Inc., Antony, France	This pellet is a lipid/pH-sensitive polymer-protected product. It is surface-coated with a 750 g/kg DL-Met core, coated with stearic acid containing 30 g/kg poly (2-vinylpyridine-styrene) droplets which solubilizes at a low pH, releasing Met into the abomasum.
Generator D™	Bio-Vet Inc., Blue Mounds, WI, USA	Direct fed microbial for ruminants. Live Bacterial Count: 2 Billion (2 x 109) CFU/gram (*Propionibacterium freudenreichii, L. acidophilus, L. casei, E. faecium, L. lactis, Pediococcus cerevisiae, B. subtilis, B. licheniformis*) Live Yeast Count: 10 Billion (1 x 1010) CFU/gram (*Saccharomyces cerevisiae*)

List of Programs

Program	Manufacturer	Description
EZfeedTM	DHI-Provo, Provo, Utah, USA	A feed management tool to control rations, monitor feed delivery, track feed inventories and reduce waste.
Feed Watch	Valley Ag Software, Tulare, CA, USA	A complete feed management software package used to control what and how much is fed to animals, thereby controlling and minimizing feed waste.
Dairy comp 305	Valley Ag Software, Tulare, CA, USA	On-farm dairy management software program used to keep track of all cow information (i.e., reproduction, production and health)
CPM-Dairy	Joint product from Cornell University, University of Pennsylvania and the Miner Agricultural Research Institute	A computer program to evaluate and formulate Dairy cattle rations for all types of cattle, control feed cost, promote animal health and production and minimize environmental pollutants. It makes use of mathematical techniques to formulate least cost rations and ensure that nutrient requirements of rumen microbes and cows are Met.
Shield	Dr. Peter H. Robinson UC Davis Cooperative Extention, Davis, CA, USA	A mathematical model used to evaluate energy, protein, amino acid, mineral and vitamin status and biological feasibility of rations proposed to be fed to lactating dairy cattle.
Amino cow®	Degussa AG, Henau, Germany	A computer program used to evaluate all aspects of dairy nutrition and provide a comprehensive analysis of amino acid supply in a ration.
CNCPS	A team of 12 scientists and 40 graduate students from Cornell University, over the past 25 years	A nutrition model developed to predict requirements, feed utilization, animal performance and nutrient excretion for dairy and beef cattle, using accumulated knowledge about feed composition, digestion, and metabolism in supplying nutrients to meet requirements
MP system of ARC	Published in the United Kingdom	The system includes a number of changes designed to represent the extent of protein degradation in the rumen and the synthesis of microbial protein as variable functions. It also provides a more rational description of the energy available for microbial growth (fermentable metabolizable energy) by discounting the energy content of dietary lipids and fermentation end products

List of Tables

List of Figures

Chapter 1: General Introduction

Nutrient requirements are relatively well defined for most domesticated monogastric species. For ruminants, however, there is still a large amount of uncertainty, especially regarding protein and amino acid (**AA**) requirements. The goal of ruminant protein nutrition is to achieve optimal rumen efficiency, and desired animal productivity, with a minimum level of dietary crude protein (**CP**) (NRC, 2001). Feed CP can be divided into rumen-degradable protein (**RDP**), which is largely incorporated into micro-organisms in the rumen when they synthesize microbial crude protein (**MCP**), and rumen undegradable protein (**RUP**) which escapes rumen degradation and passes from the rumen with micro-organisms to potentially be digested in the small intestine.

Ruminants have the unique ability to transform low quality forage based diets, partly indigestible by monogastric species, into high quality consumer products such as milk, meat and fibre. A lot of the dietary CP that is ingested and absorbed is used for body protein synthesis which, in mature animals, is mostly for replacing tissue (turnover). A part of ingested CP gets deaminated to be used for glucose synthesis. Faecal and urinary N therefore consists of a mixture of undigested or unabsorbed dietary CP and detritus of metabolic processes. However, CP is utilized with a relative low efficiency and only 250-350 g/kg of ingested CP is secreted as milk protein. Even though some of the protein is used by the cow for maintenance, growth, health and reproductive processes, a large amount is excreted in urine and faeces (Lapierre *et al,* 2002). Poor efficiency of CP use by ruminants may be due to energy limitations, reduced growth of microorganisms in the rumen, catabolism and partitioning of AA's, imbalances in AA supply to the intestinal absorptive site or genetic limitations (Bequette, 2002).

To improve the efficiency of CP use by ruminants, diets need to be balanced according to the specific AA requirements of the animals, instead of simply balancing for RUP and RDP, as is common in practice. Balancing for post ruminal AA delivery could allow use of lower CP rations because they would be balanced to supply individual AA's to the intestinal absorptive site. Metabolic costs of deamination of excess AA's, and excretion of excess N, would be lower, and removal of CP from the ration leaves space to supply other nutrients, such as those that more efficiently supply energy (Lapierre *et al*, 2002).

Prior to 1970, diets fed to ruminants were largely formulated and expressed according to the CP content (the 'CP System'), without taking ruminal CP degradability into account, at least in any systemized manner. There was a general belief that all dietary CP, regardless of quality (which includes AA composition and availability of the undegraded CP), could be converted to high quality MCP, and that this would complement any deficiencies in CP intake. This belief changed during the 1980's, when researchers reported that the proportion of ruminant CP requirements met by ruminally synthesized MCP declined as milk production increased (Santos *et al*, 1998b) and more protein needed to escape rumen degradation to meet animal needs (Clark *et al*, 1987). More emphasis was therefore placed on determining CP quality in terms of its RDP and RUP proportions.

In 1985, the National Research Council (**NRC**) recommended a system that considered the N requirements of rumen microbes, since ruminal digestion is an essential component of feed utilization and microbes depend on energy intake of the animal, but also adequate supplies of rumen degradable CP, for optimal fermentation (Dugmore, 1995).

This led to the development of other published systems to better predict animal protein requirements; these include:

→ The metabolizable protein system of the Agricultural Research Council (**ARC**) of the United Kingdom which estimates the amount of CP degraded in the rumen, thereby calculating protein flow to the small intestine (ARC, 1992).

→ The absorbed protein (**AP**) model of NRC which uses a factorial method to determine AP and N requirements. By examining differences in the proportion of dietary CP that escapes ruminal fermentation, it introduced the concept of RUP and RDP (NRC, 1985; 1989).

→ The Cornell Net Carbohydrate and Protein System (**CNCPS**) that has a fermentation sub-model to compare rates of CP degradation and carbohydrate fermentation. It also predicts ruminally digestible organic matter (**OM**), MCP synthesis, ammonia production and protein flow to the small intestine, providing a tool

to evaluate diets for adequacy of RDP, RUP, and AA (Russel *et al*, 1992; Sniffen *et al,* 1992).

The nutritional value, and chemical composition, of feeds are greatly influenced by the vegetative stage of the plant, weather during growth, time of day during harvest, soil fertility, storage and even feed bunk management (Van Soest *et al*, 1994), and vary widely in their proportions of protein and non-protein N (**NPN**), rate and extent of CP degradation in the rumen, digestion in the intestine and the AA composition of undegraded feed CP (NRC, 2001).

Since 1989, protein requirements have often been expressed in terms of AP or total AA reaching the small intestine, which includes protein synthesized by rumen micro-organisms and feed CP that escapes rumen degradation (Dugmore, 1995). Even though the NRC (1989) recognized that intestinal digestion of proteins differed, they used a constant digestibility value of 800 g/kg for RUP in all feedstuffs due to the lack of data to differentiate among feeds. Other shortcomings of this model, as pointed out by several research groups (e.g., Satter 1986; Clark 1987), are the prediction of increased milk yield when a protein source high in RDP is substituted by a source high in RUP, when many research studies reported a lack of response. Possible reasons for this, as reviewed by Santos *et al* (1998a), may be decreased microbial synthesis due to removal of RDP from the diet, a poor AA profile, or low digestibility of the RUP source. Some studies suggested that the source of RUP should have an AA profile to complement that of MCP (Clark *et al,* 1992; Chen *et al*, 1993). The NRC model also failed to consider the AA profile of AP (NRC, 1989), mainly due to variability in feedstuffs fed to ruminants, while growth of rumen microorganisms changes the AA profile of the proteins fed, thereby making it difficult to predict exactly the quality and quantity of AA fed and absorbed from the small intestine (Rode and Vazquez-Anon, 2006).

Proteins digested to AA that are actually available for absorption in the small intestine are largely a combination of RUP, MCP and some endogenous secretions (**ECP** - proteins secreted into the digestive tract) – collectively known as metabolizable protein (**MP**) (CNCPS, 2000; NRC 2001). As milk production increased, the proportion of the total CP requirements met by MCP was predicted to decrease and substantial amounts of dietary CP must escape rumen degradation to

meet predicted protein needs (Santos *et al,* 1998b). Reviews by Satter *et al* (1986); Clark *et al* (1987) and studies by Higginbotham *et al* (1989) and Taylor *et al* (1991) showed that increasing the amount of RUP in the ration could improve milk production, but only up to 30-40% of total CP (Santos *et al*, 1998b), after which RDP becomes limiting, decreasing MCP production and non-ammonia N (**NAN**) supply to the intestine (Clark *et al,* 1992; Ferguson *et al,* 2000). Further increases in RUP could also cause a reduction in diet fermentability, dry matter (**DM**) intake (**DMI**) (Olmos Colmenero and Broderick, 2006) and milk production. Santos *et al* (1998a) published a comprehensive review of effects of replacing soybean meal (high in RDP) with various RUP sources on animal protein metabolism. In 76% of the studies, such a substitution decreased MCP flow to the small intestine, but there were no net changes in total essential AA (**EAA**) flow to the duodenum and milk production only increased in 17% of the studies. This suggests that the nutrients required for milk synthesis are not protein *per se*, but the AA in the protein, and that the range of AA in rumen escape protein is far more important in determining the quality of intestinally delivered protein than the amount of RUP in the diet. Trying to provide additional AA by adding more rumen escape CP, without considering its AA profile, can lead to oversupply of metabolically unnecessary AA. Amino acid deamination is an energetically wasteful process (Russel *et al,* 1988; Wallace, 1996) and often yields ammonia in excess of rumen bacterial needs for growth (Annison, 1956). Surplus N is converted to urea and excreted in the urine, thereby reducing efficiency of protein utilization. It also has the potential of increased DMI, without improvements in milk production, thereby reducing efficiency further (Olmos Colmenero and Broderick, 2006).

Research in poultry (NRC, 1994) and swine (NRC, 1998) revealed that each physiological state in an animal (i.e., maintenance, growth, lactation) requires a unique profile of absorbed AA. However, these profiles still need to be established for ruminants.

As new information became available, the NRC (2001) protein model was broadened to include a number of regression equations to consider:

→ differences in efficiency of MCP production, including factors modifying microbial responses and conditions for optimum rumen fermentation, as reviewed by Wallace (1986), Hoover and Stokes (1991) and Clark *et al* (1992). Since MCP is a

main source of absorbed protein for ruminants, digestibility of each AA from MCP needed to be estimated to determine AA requirements (Storm *et al*, 1983),

→ factors affecting rate of ruminal passage (i.e., DMI, concentrate and fibre ratios and fibre length (Yang *et al,* 2002)) and RUP content of feed were reviewed by Satter (1986), in order to assign RUP digestibility values to individual feedstuffs and account for differences in their nutritive value,

→ the contribution of endogenous protein and NPN to the intestinal N supply (Hannah *et al*, 1991; Lintzenich *et al*, 1995), and

→ the amount of each EAA in total AA, thereby predicting, as accurately as possible, the duodenal AA composition using the **PDI** system (Protein truly digestible in the small intestine; INRA, 1989) as a basis for this model (Rulquin *et al,* 1997).

Some of these prediction equations are based on a limited number of experiments and models may lack accuracy if used under diverse conditions. Metabolic pathways need to be investigated more closely, and under different conditions, in order to improve model accuracy.

Since Lysine (**Lys**) and Methionine (**Met**) are generally considered to be the most limiting AA for milk production in ruminants (King *et al*, 1990; Schwab *et al*, 1992a and 1992b), it is common to feed for higher milk protein yield by balancing diets to maximize absorbable Lys and Met delivery. This attempts to achieve an 'ideal' 3:1 Lys to Met ratio (Rulquin *et* al, 1993) through complementary RUP sources. However, many RUP sources are low in Lys and/or Met with AA profiles that are generally inferior to MCP, making it difficult to formulate a ration to achieve the optimum concentration of both Lys and Met in MP, in order to satisfy the animal's requirement for limiting AA, without oversupplying N (Santos *et al,* 1998a; Rode and Vazquez-Anon, 2006). This reality directed attention toward supplementing only the limiting AA in the diet, leading to numerous studies to determine effects of adding ruminally protected, and free AA to dairy rations, as well as infusing specific AA (or AA mixtures) to the duodenum.

Amino acid requirements are calculated based on milk protein yield responses to different levels of post-ruminally infused AA, using a fixed coefficient for transfer of absorbed AA into milk. Differences between duodenal and faecal AA flows are sometimes considered to represent

AA available for utilization by the animal, but this is a misestimation since various amounts of AA are absorbed from, synthesized in, and secreted into this portion of the gastro intestinal tract (**GIT**) (Lapierre *et al*, 2006).

There have been few attempts to quantify AA requirements in ruminants (Williams and Smith, 1974; Fendersen and Bergen, 1975; Titgemeyer *et al*, 1988), and results obtained from infusion studies have been inconsistent, thereby limiting the confidence with which AA can be supplemented to rations.

There are currently a number of ruminally protected Met (**RPM**) products commercially available and extensive research has been completed in this area. Ruminal protection of Lys, however, has largely not been successful so far. A ruminally protected Lys (**RPL**) product was available in the past but has since been removed due to instability of the product. Currently there are no RPL products commercially available, and even though other RPL products are starting to enter the market now, information regarding the effect of such supplements on milk production and composition is limited.

Genetic improvements in modern dairy cows have lead to increased milk production, which requires higher intake of dietary CP to meet the needs of milk protein synthesis. Due to the expanding ethanol industry in the Midwestern USA, large amounts of maize distiller's by-products are being used in California dairy rations, in addition to conventional maize products such as silage, grain, gluten feed and gluten meal. Lys and Met have been suggested to be the most limiting AA for milk protein synthesis and increasing the proportion of total dietary CP coming from low Lys dietary protein sources (i.e., maize and maize by-products) has raised concerns that milk and milk protein yields may be limited by supplies of intestinally absorbable lysine.

To determine the potential impact of increased dietary maize protein levels on animal performance, TMR's from various dairies throughout California were sampled, analyzed and evaluated in order to link ration nutrient profiles to milk production data and predicted intestinally delivered AA profiles, which was determined using three computer models of dairy cattle metabolism.

Since Lys was the most consistently predicted limiting AA among dairies (i.e., estimated by Shield to be limiting in 14/16 of the evaluated TMR's), the dairy that was calculated to be most limiting in Lys was chosen to determine effects of feeding an RPL product on milk production and composition, as well as on nitrogen balance.

Chapter 2: Literature Review: Protein and amino acid nutrition of dairy cows

2.1 Feed protein

Dietary protein, generally referred to as CP in ruminant nutrition, are the major feed compounds containing N. Feed protein is degraded by microbes and their proteases in the rumen, or hydrolyzed by enzyme proteases in the small intestine, into shorter polypeptide chains and AA. These AA undergo deamination and the amino groups are removed and converted to ammonia (NH_3) which can then be used by some rumen microbes to synthesize MCP, other AA or be converted to urea to be recycled in saliva or excreted in urine. The average N content of feedstuffs is fairly constant at 16 g per 100 g of protein (160 g/kg) and the CP content of feed is calculated from the analyzed N content x 6.25 (Dugmore, 1995). However, CP includes both true protein and NPN in proportions that vary considerably among feedstuffs, the latter being a source of N that is largely and rapidly degraded in the rumen. These include AA, amines (e.g., histamine), amides (e.g., asparagine, glutamine, urea, and uric acid), ammonia, nitrates, alkaloids, nucleic acids and others. The NRC (2001) developed a system, based on the CNCPS, to better evaluate the N value of forages by categorizing them into soluble (a), potentially rumen degradable (b) and indigestible (c) fractions.

2.2 What are amino acids?

Amino acids are the key components, or building blocks, of all proteins. They are linked by dipeptide bonds to form protein chains. Each protein chain has a specific sequence of AA that determines its integrity and functionality with functions that include production of enzymes, immunoglobins, hormones and milk proteins, making them vital to the maintenance, growth, reproduction and lactation of dairy cattle (Schwab *et al,* 1995; Rode and Vazquez-Anon, 2006; Appendix A2; Table A2.1).

The AA that are absorbed, but not used for protein synthesis, are catabolized and serve as a source of metabolic energy when oxidized to CO_2, while the amino groups are used to synthesize other deficient AA. They can also be converted to fatty acids or serve as precursors of other

metabolites in pathways within the body, such as gluconeogenesis (Vanhatalo *et al,* 2003; Rulquin *et al,* 2004). An estimated 110-180 g/kg of glucose flux was synthesized from the glucogenic AA's glutamic acid (**Glu**), aspartic acid (**Asp**), serine (**Ser)** and glycine (**Gly**). Alanine (**Ala**) was quantitatively the most important (Wolff *et al*, 1972), but it is much less efficiently used than propionate. Catabolism (oxidation) of AA becomes more important when animals are underfed and need additional energy. It is possible that amino and organic acids produce more than half of glucose turnover when sheep are starved or fed energy maintenance rations, but this may be overcome by providing gluconeogenic precursors (e.g., propionate), as intimated by studies in which glucose or propionate was abomasally or intravenously infused (Fisher and Elliot, 1966; Vik-Mo *et al*, 1974; Rulquin *et al,* 2004).

Even though most biochemical pathways are well-established, rates of the individual reactions in ruminants are mostly unknown. Researchers rely mainly on *in vitro* studies because the regulatory aspects of AA metabolism *in vivo* have been poorly studied (Baumrucker, 1985; Lobley, 1992).

Methionine and Lys are considered to be first limiting, but surprisingly little is known of their metabolic fates. Methionine is a precursor for cysteine (i.e., a component in intestinal secretions and the immune system of the animal) by donating a sulfur group. As an intermediate in transmethylation reactions, it donates a methyl group to synthesize choline, vitamin B_{12}, phospholipids in cell membranes, creatine production for energy storage and transfer, and the carnitine required in lipid metabolism and fatty acid mobilization (Campbell and Farrell, 2003).

Lysine is an anomaly since it is almost always taken up by the udder in excess of requirements. Excess Lys is oxidized to produce Glu, an energy source for intestinal mucosa cells (Windmueller and Spaeth, 1980) and a precursor for *de novo* Arg and Pro synthesis (Bequette, 2002). Understanding these roles could aid in improving milk yields, milk component yields and overall efficiency of nutrient use by dairy cows.

Amino acids synthesized by rumen bacteria and cells in the animal body, using metabolites from surplus AA catabolism, are known as non-essential amino acids (**NEAA**) or dispensable AA and do not necessarily have to be provided in the diet. Ten of the 20 primary AA in proteins

however, are classified as essential, or indispensable, amino acids (EAA) and need to be supplemented in the diet (in the form of rumen escape protein) since they cannot be synthesized by animal tissues in sufficient quantities to fulfil metabolic requirements for growth and high levels of production. These include Lys, Met, arginine (**Arg**), histidine (**His**), isoleucine (**Ile**), leucine (**Leu**), phenylalanine (**Phe**), threonine (**Thr**), tryptophan (**Trp**) and valine (**Val**) (NRC, 2001).

Classification of AA as essential or non-essential was based on research completed with non-ruminant animals, but was shown to be similar to that of ruminants (Black *et al*, 1957). Essential AA are the focus of most nutritional studies, since there is little evidence that NEAA profiles are important for efficiency, or that NEAA would ever become more limiting than EAA (Schwab *et al*, 1976; NRC, 2001). A number of studies, where mixtures of AA were administered post-ruminally, indicated that requirements for NEAA were met before requirements for EAA, and that individual NEAA absorbed below requirements can be synthesized from excess AA in adequate amounts to maintain animal performance (Oldham *et al*, 1979; Fraser *et al,* 1991)

Studies have shown that the total uptake of certain EAA (i.e., Arg, Lys, and some branched-chain AA (**BCAA**)) by the mammary gland is higher than their output in milk (Clark, 1975). Uptake of NEAA such as Glu, Asp and Pro, in contrast, is less than in milk. Since uptake of total AA is similar to output, it is clear that excess EAA are used to synthesize deficient NEAA. For example, the BCAA Leu, Val, and Ile are catabolized to provide a carbon skeleton and amino group for synthesis of Ala, Glu and Gln (Bequette, 2002), and the AA profile needed for milk protein synthesis therefore differs from the AA composition of protein in milk. The mammary gland appears to have the ability to regulate its supply of nutrients (Cant and McBride, 1996), based on the relationship between AA supply and demand, by altering blood flow rates, using nitric oxide as a vasorelaxant (Lacasse *et al*, 1996) and regulating the amount of AA extracted by splanchnic tissues to meet requirements (Maas *et al*, 1998; Hanigan 2005).

There is a need for further research to determine the importance of selected NEAA in dairy production. Two NEAA, proline and glutamine, have received some attention due to their importance in milk production (Bruckental *et al*, 1991) and glutamine has been hypothesized to be a limiting AA for milk production during early lactation (Meijer *et al*, 1995). However, because

NEAA are synthesized from EAA by the mammary gland and somatic tissue, the availability, or deficiencies, of EAA are highly dependent on adequate NEAA supplies.

2.3 Sources of amino acids

Amino acids utilized by the mammary gland are provided by MP, primarily composed of:

→ Microbial CP containing an estimated 800 g/kg of true protein (the remainder being nucleic acids), and with 800 g/kg digestibility about 640 g/kg of MCP is therefore converted to MP (NRC, 1989; Clark *et al,* 1992; Verbic, 2002).

→ Rumen-undegradable protein, assumed to be 1000 g/kg true protein, but the contribution to MP is variable depending on feed type since intestinal digestibilities were assigned to each individual feedstuff range from 500 to 1000 g/kg (NRC, 1989).

→ Endogenous CP. Data on the proportion and digestibility of true protein in ECP is extremely limited, but its true protein content is estimated to be 500 g/kg and digestibility is assumed to be 800 g/kg, resulting in a 400 g/kg conversion to MP.

→ Peptides and free AA from soluble CP in the feeds, if it escapes rumen degradation.

2.3.1 *Endogenous crude protein*

Endogenous CP originates from various sources (Tamminga *et al*, 1995):

→ Mucoproteins in saliva

→ Epithelial cells from the respiratory tract

→ Cellular debris abraded from the mouth

→ Cellular debris from the omasum and abomasum

→ Enzyme secretions into the abomasums

→ Enzyme secretions into the ileum

The first three don't contribute to protein passage to the intestine since most is probably degraded by rumen microorganisms (NRC, 2001). A number of studies to identify the sources of endogenous N secretions (**ENS**) have been reported for sheep but, due to the complexity of N

exchanges, these studies are rare for dairy cows (Ouellet *et al*, 2007). It is technically tedious to distinguish between endogenous, microbial and feed N in the duodenal digesta, hampering attempts to determine passage of ECP to the small intestine. Most studies ignore the contribution from these recycled materials, probably overestimating the 'true' AA supply from the diet and MCP.

However, some approaches measured the flow of endogenous N (**EN**) through the rumen and abomasum by using cows fed diets low in CP that were considered to be free of RDP (Hannah *et al,* 1991; Lintzenich *et al* 1995), or ruminants solely nourished by volatile fatty acids (**VFA**) infused into the rumen (Ørskov *et al*, 1986). The NRC (2001) adopted an average value of 1.9 g of N/kg of DMI based on data from these studies. The French system developed a regression equation estimating contributions from all N fractions (Verite and Peyraud, 1989) and, assuming EN flow is closely correlated to intake of indigestible OM, it predicted passage of EN as 1.7 g of N/kg DMI. Mathematical approaches taking AA composition of each fraction into account estimated EN values varying from <10 g/kg to 320 g/kg of total N reaching the abomasum (Shabi *et al*, 2000).

Other estimations of ECP in dairy cows involve techniques using ^{15}N-isotope tracers (Leng and Nolan, 1984; Firkins *et al,* 1992). Values of 4.4 g and 5.9 g of N/kg DMI (150 g/kg and 200 g/kg of total N flow) were reported depending on rations fed (Van Bruchem *et al*, 1997). A number studies done on sheep by Sandek *et al* (2001) reported similar values.

The endogenous fraction potentially contributes 150-250 g/kg of the total flow of CP out of the rumen (Bequette, 2002) and, due to differences in AA composition between MCP, RUP and ECP (Table 2.1); it cannot be ignored since it will alter the proportion of AA in intestinally delivered EAA (Ørskov *et al*, 1986). Endogenous secretions to the abomasum and ileum are not completely re-absorbed, half of it appearing in faeces and, since the secretions are rich in Thr, Pro, Cys and Val, it reduces the amount of these AA available to the animal (Bequette, 2002). Only by understanding this additional loss of N can it be manipulated and minimized, thereby increasing the accuracy of estimating N and AA requirements.

2.3.2 *Microbial crude protein*

Microbial CP synthesis involves degradation of RDP, by proteases synthesized by various strains and species of bacteria, protozoa and anaerobic fungi in the rumen, and incorporation of the resulting peptides, AA and ammonia into microbial protein. It also allows ruminants to convert external NPN sources, such as urea, into ammonia and subsequently into MCP.

Bacteria are the most abundant micro-organism in the rumen and protein degradation occurs through extra-cellular proteolysis, in which soluble or insoluble proteins adsorb to bacteria (Nugent and Mangan, 1981; Wallace, 1985) which hydrolyzes it to small peptides and free AA which are finally absorbed for further degradation and utilization.

Protozoa are fewer in number, but larger in size, contributing significantly to microbial biomass. Due to a higher digestibility, and higher EAA content, of protozoa compared to bacteria, it has been speculated that increased synthesis and passage of protozoal protein from the rumen may have nutritional benefits for animals. However, Weller and Pilgrim (1974) found that sequestration of protozoa in the rumen results in a lower protozoa concentration in effluent than in corresponding rumen fluid, contributing too little to outflow protein to significantly affect the composition of the total protein mixture. Unlike bacteria, protozoa make use of intracellular hydrolysis of protein, obtained from ingesting small feed particles, fungi or, primarily, bacteria. Amino acids are incorporated into protozoal protein but they are not able to synthesize AA from ammonia as do bacteria. To determine the effect of protozoa on protein and fibre digestion, a defaunation method was used to eliminate them, resulting in reduced rumen ammonia concentrations. The increase in NAN flow to the duodenum can be attributed to increased MCP and dietary protein flow, thereby increasing efficiency of MCP synthesis (Ushida *et al,* 1990).

The contribution of anaerobic fungi to protein degradation is considered negligible, due to relatively low concentrations in rumen digesta (NRC, 2001). They do produce cellulases, hemicellulases and xylanases to degrade plant cell walls, and are more effective in degrading the lignin-containing tissues than bacteria since they are able to penetrate the plant cuticle (Akin and Borneman, 1990).

Microbial CP is considered to be the most important, and least expensive, MP source and it is the largest contributor of protein reaching the duodenum, providing about 100-150g MCP/kg of DMI (Verbic, 2002). It has a high quality AA profile (Clark *et al,* 1992) and apparent intestinal digestibility of about 847 g/kg (Storm *et al*, 1983). It has long been recognized that the EAA profile of MCP is fairly constant, because EAA profiles between different micro-organisms, and among predominant strains, vary little (Purser *et al*, 1966), and their contribution to postruminal protein supply is not proportional to the respective rumen biomass fractions (i.e., protozoa, bacteria and endogenous) (Weller and Pilgrim, 1974; Harrison *et al*, 1979). The AA composition of rumen bacteria was relatively constant regardless of sampling time post feeding (Martin *et al,* 1996) and diet composition (Prestlokken and Harstad, 2001), but a few studies reported large variations in AA composition of bacteria (Clark *et al,* 1992) at different levels of DMI (Rodriquez *et al*, 2000). Regardless, MCP has a relatively high proportion of NPN (150 g/kg nucleic acid-N) (Storm *et al*, 1983) and the AA composition of microbial true protein is very similar to that of milk and lean body tissue, ensuring high efficiency of AA utilization (Verbic, 2002) (Table 2.1). Microbial CP is mainly used for protein synthesis in the mammary gland, but also acts as a precursor in gluconeogenesis for lactose synthesis (Rode and Vazquez-Anon, 2006).

Table 2. 1: Comparison of EAA compositions of lean body tissue, milk protein, rumen micro-organisms and common lysine and methionine feed sources

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	EAA*	
Lean body tissue	16.8	6.3	7.1	17.0	16.3	5.1	8.9	9.9	2.5	10.1	-	1
	6.6	2.5	2.8	6.7	6.4	2.0	3.5	3.9	0.6	4.0	-	2
Milk protein	6.8	5.3	12.1	19.1	15.7	4.8	9.8	9.1	2.6	12.9	-	3
	7.2	5.5	11.4	19.5	16.0	5.5	10.0	8.9	3.0	13.0	-	1
	3.4	2.5	5.8	9.2	7.6	2.7	4.8	3.7	1.5	5.9	-	2
Rumen microbes												
Bacteria	10.2	4.0	11.8	14.9	16.5	4.9	9.4	11.4	2.9	11.8	-	3
	-	4.4	12.8	18.1	17.6	5.8	11.3	13.0	3.0	13.9	-	4
	10.4	4.1	11.5	15.9	16.5	5.1	10.1	11.3	2.7	12.4	-	1
Protozoa	8.7	3.6	12.8	15.4	19.8	3.8	11.2	9.9	2.8	9.4	-	3
	4.6	1.8	6.0	8.1	10.2	1.7	5.5	5.6	-	5.3	-	5
	9.3	3.6	12.7	15.8	20.6	4.2	0.0	10.5	2.8	9.7	-	1
Forages												
Lucerne hay	10.9	5.2	10.9	18.4	11.1	3.8	12.2	10.6	3.4	13.5	-	6
	12.5	4.7	10.3	17.9	12.4	3.8	11.6	10.6	3.6	12.7	41.2	1
Maize silage	6.4	5.5	10.3	27.8	7.5	4.8	12.0	10.1	1.4	14.1	-	6
	6.2	5.7	10.6	27.2	7.9	4.8	12.1	10.1	1.4	14.1	31.6	1
Grass silage	8.9	5.3	11.0	18.9	10.3	3.8	13.5	10.3	3.3	14.7	-	6
	9.4	5.1	10.9	18.8	10.1	3.7	13.4	10.2	3.3	15.0	32.6	1
Grain												
Maize	10.8	7.0	8.2	29.1	7.0	5.0	11.3	8.4	1.7	11.5	42.3	6
	11.5	7.8	8.2	27.9	7.1	5.3	11.5	8.8	1.8	10.0	40.1	1

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	EAA*	
Oats	15.6	5.4	9.5	18.1	10.0	4.3	11.5	9.2	3.2	13.3	42.8	6
	16.6	5.9	9.1	17.7	10.1	4.2	12.5	8.4	2.9	12.6	41.2	1
Plant protein												
Brewers grain,	8.9	6.4	10.6	17.6	11.4	4.8	10.3	11.4	3.0	15.6	46.3	6
dry	14.7	5.1	9.8	20.0	10.4	4.3	11.7	9.1	2.5	12.1	39.2	1
Maize gluten	6.9	4.7	9.3	36.4	3.8	5.5	13.8	7.5	1.5	10.7	44.2	6
meal	7.1	4.7	9.1	37.2	3.7	5.2	14.1	7.5	1.2	10.3	45.2	1
Maize DDG	7.7	7.2	9.8	26.3	6.2	5.2	11.1	10.3	2.7	13.4	37.7	6
w/sol	10.7	6.6	9.8	25.4	5.9	4.8	12.9	9.1	2.3	12.4	37.8	1
Soybean meal	16.3	5.7	10.8	17.0	13.7	3.1	11.0	8.6	3.0	10.6	47.6	6
	16.2	6.1	10.1	17.2	13.9	3.2	11.6	8.7	2.8	10.2	45.3	1
Animal protein												
Blood meal	7.6	11.2	2.1	22.8	15.7	2.1	12.3	8.1	2.7	15.4	49.4	6
	7.8	11.3	2.2	22.7	15.9	2.1	12.1	7.7	2.8	15.4	56.4	1
Fish meal,	13.1	5.7	9.3	16.5	17.0	6.3	8.8	9.5	2.4	11.3	44.8	6
(menhaden)	13.1	6.4	9.2	16.2	17.2	6.3	9.0	9.4	2.4	10.8	44.5	1

* As % of CP
[1] AA values (% of total EAA) obtained from NRC (2001)
[2] AA values (% of CP) obtained from O'Connor *et al* (1993)
[3] AA values (% of total EAA) obtained from Schwab *et al* (1976)
[4] Data (% of total EAA) from other sources (Av. of 441 bacterial samples from 35 exp) (Clark *et al*, 1992)
[5] AA values (% of AA) obtained from Evans (2003)
6 AA values (% of total EAA) obtained from Schwab (1995)

The rate of rumen microbial growth and protein synthesis are affected by a number of factors (Yang *et al*, 2001; Verbic, 2002) including:

→ Availability of rapidly fermentable carbohydrates: Energy supply in the rumen is usually first limiting for microbial growth and the rate of carbohydrate digestion in the rumen is the major factor controlling the amount of energy available (Hoover and Stokes, 1991). At sub-optimal energy input levels, microbial growth will increase with increased energy supply, but an oversupply does not result in extra growth, only reduced efficiency (Dijkstra *et al*, 1998) due to energy 'spilling' (Russell, 2007).

→ An adequate supply of N compounds: Peptides, AA and ammonia liberated from RDP are incorporated into MCP by rumen bacteria. A deficiency of RDP results in reduced MCP synthesis, fibre digestion, DMI and, ultimately, reduced milk production. Most feedstuffs contain some RDP, but feeds with relatively high RDP levels include soybeans, barley and urea (Rode and Vazquez-Anon, 2006).

→ A suitable rumen environment: Low or high pH values can be deleterious to microbial growth, reduce digestibility of fibre and divert energy in the rumen towards non-growth functions to maintain or correct the pH.

→ Rumen outflow (turnover) rate: High DMI increases rumen outflow rate, with microbes spending less time in the rumen. A faster turnover rate lowers maintenance costs due to less N recycling. Higher DMI therefore increases efficiency of MCP synthesis (Clark *et al*, 1992; Rodriquez *et al,* 2003) with improved N digestion in the rumen (Rode *et al,* 1985).

Dry matter intake has the biggest influence of all dietary factors on passage of microbial N to the small intestine, suggesting that CP in the diet should be determined relative to DMI. The CP content could therefore be reduced in the diet of a cow eating large amounts of DM without affecting microbial AA flow at the intestine or reducing milk yield (Clark *et al*, 1992).

It is well known that the major nutrients required by microbes for growth are proteins and carbohydrates, but the quantities, and most suitable sources needed for maximum growth have not been determined. With an oversupply of degradable N (i.e., RDP and NPN) or a lack of fermentable energy, the rate of ammonia release may exceed the ability of the microbes to utilize it, resulting in ammonia accumulation in the rumen. The maximum conversion rate of ammonia to MCP is approximately 30 - 32 g N/kg digestible OM consumed (Dugmore, 1995).

Various studies, focusing on improving efficiency of MCP synthesis by manipulating the diet (Herrera-Saldana *et al*, 1990; Aldrich *et al,* 1993a), proved it was possible to do so by synchronizing rapidly degraded energy and protein release in the rumen (Herrera-Saldana *et al*, 1990). Results also indicated, however, that synchronizing energy and N release is not necessarily enough, as it is also important to ensure a gradual release of fermentable energy and degradable N (Henning *et al*, 1993).

Microbial production is also influenced by minerals, such as sulfur and phosphorus, and nutrients such as ruminally unprotected fats, especially if unsaturated, can inhibit protozoal growth and fibre degradation in the rumen (Oldick and Firkins, 2000). Various fermentation products, such as yeast cultures, can improve the digestive capacity of rumen microbes.

Research in ruminal microbiology has had a major impact on improving and understanding ruminant nutrition. Manipulation of ruminal fermentation however has been restricted to a few antimicrobial feed additives, mostly ionophores, which are known for their effect on the rumen

bacterial populations and subsequent changes in fermentation stoichiometry, thereby improving protein flow from the rumen by reducing the rate of AA deamination. The rapid development of recombinant DNA technology has made the use of genetically engineered ruminal microorganisms a possibility (Wallace, 1994). Development of new, more effective and efficient, strains of ruminal bacteria could benefit the host animal tremendously.

2.3.3 *Rumen-undegradable crude protein*

Microbial CP has a very high quality AA profile but, alone, it is insufficient to supply adequate amounts of AA for optimum animal production (Rode and Vazquez-Anon, 2006). While RUP is a source of AA ready for digestion by the animal, rumen degradation of protein can be decreased by reducing the time it remains in the rumen. Factors influencing the rate of passage of digesta include DMI, specific gravity (**SG**), feed particle size and concentrate to forage ratio (Chalupa, 1975). An alternative is the use of feeds with naturally protected proteins that are relatively resistant to rumen degradation (Clark *et al,* 1992) or feeds that have been chemically or physically treated to reduce protein degradability and increase its RUP content.

Heat processing causes the carbonyl groups of sugars to combine with the amino groups of protein through the Maillard reaction, forming peptide links (i.e., protein-carbohydrate cross-linkages) that are more resistant to enzymatic hydrolysis (Rode and Vazquez-Anon, 2006). However, care should be taken during heat treatment since over-heating reduces intestinal digestibility of RUP and leads to the destruction of AA such as Cys, Arg and especially Lys.

Categories of chemical treatment include those that

→ Introduce cross-links by combining with proteins (e.g., aldehydes)

→ Alter protein structure through denaturation (e.g., acids, alkalis and ethanol)

→ Bind proteins without altering their structure (e.g., tannins)

However, the use of chemical treatments alone was accepted commercially, and lead to combined chemical and heat treatments, which has been more effective in increasing the amount of protein that escapes rumen degradation. One technique involves adding lignosulfonate (i.e., a by-product of the wood products industry) to oilseed meals before heat treatment (Borucki *et al*, 2007).

Most high quality grasses and legumes fed to lactating cows contain adequate amounts of RDP, but are deficient in RUP, moving the focus of protein supplementation to feedstuffs high in RUP (NRC, 2001). Common sources of RUP include animal and marine by-products such as fishmeal and blood meal, dried distillers grains (**DDG**), brewers dried grains and maize gluten meal (Rode and Vazquez-Anon, 2006). DDG are the solids that remain after fermentation of grains such as maize during the ethanol production process.

Two factors account for most of the variation in the AA profiles of duodenal protein, being the proportional contribution from RUP to total protein passage from the rumen (MP) and its AA profile (Rulquin *et al*, 1998). Methods to evaluate RUP content of feedstuffs include the polyester or nylon bag ruminal *in situ* technique and various *in vitro* techniques such as the Tilley and Terry method (1963), measuring gas production, using enzymes (which can be done independent of the animal), electrophoresis, near infrared reflectance spectroscopy (**NIR**) and others (Stern *et al*, 1997).

Use of the *in situ* technique led to the identification of the three N fractions (each with different solubility) namely, A (soluble), B (potentially degradable), and C (rumen undegradable) fractions. By knowing the content of each of these fractions in feedstuffs that are in the diet, computer programs can be used to estimate RUP levels reaching the duodenum (Ouellet *et al*, 2007). Another challenge in diet formulation is to optimize the level of RUP reaching the duodenum without reducing MCP synthesis, since low RDP levels have a potentially negative effect on microbial growth due to inadequate available N supplies (Clark *et al*, 1992; Ipharraguerre and Clark, 2005).

No single source of RUP provides a balance of EAA that matches the profile of milk, but proteins with the closest match are regarded as the highest quality with the best nutritive value. Animal by-products usually have the best AA profile, but are also the most expensive. In high forage and soybean hull-based diets, where RUP intake is low, or where animal-derived proteins make up most of the dietary RUP, Met is usually first limiting (Ahrar and Schingoethe, 1979; Schingoethe *et al*, 1988). In contrast, Lys has been identified as first limiting when maize and maize by-products provide most of the RUP in the diet (NRC, 2001). Recently, His has been identified as first limiting for milk production when grass silage and cereal (i.e., barley and oats)

based diets are fed (Vanhatalo *et al,* 1999). Microbial CP is low in Met, but relatively high in Lys, and the level of these AA is lower in most feedstuffs. A deficiency in one of these AA can therefore be exaggerated by feeding high levels of a single RUP source instead of combining several sources with complementary AA profiles (Ferguson *et al*, 2000; NRC, 2001).

Optimum productivity can be achieved with the minimum amount of dietary CP when rations are balanced to provide adequate amounts of RDP (i.e., to meet, not exceed, the N needs of microbes for maximum growth) and RUP sources with desired, complimentary, AA profiles (Clark *et al*, 1992; Ferguson *et al*, 2000; NRC, 2001). The efficiency with which MP is used for protein synthesis depends on the amount of EAA in it and how well the EAA profile in MP matches the AA profile required by animal metabolism (NRC, 2001). Reducing CP in diets balanced for AA supply could improve cost effectiveness and reduce environmental pollution but, even the most recent NRC (2001) recommendations have to be improved, and more detailed research is needed to provide reliable quantitative data on AA requirements (Ouellet *et al*, 2007).

Increased milk production and protein yields require an increase in feed CP intake and/or an improved postruminal supply of AA. Feedstuffs with low rumen degradability and/or high quality protein with a well balanced AA profile (such as fishmeal) can be used to increase postruminal AA supply, but they are expensive and legislatively forbidden in some countries. It is therefore becoming more difficult to formulate rations that will provide the desired AA concentrations and ratios in MP. Even achieving 90% of estimated requirements for Lys and Met is a major challenge when relying on available plant source feed protein supplements alone, thereby increasing the importance of adding individual AA to the diet.

2.4 Amino acid requirements

There are three main methods to estimate AA requirements of lactating dairy cows (Rulquin and Verite, 1993; Schwab 1995):

- *Factorial approach*

This is a mathematical approach that attempts to calculate and quantify absorbed AA requirements by separating requirements of different components (i.e., protein deposition in muscle

tissue and conceptus, secretion into milk protein and protein used for maintenance) and incorporating rates at which nutrients move through various digestive and metabolic pools. Quantifying requirements using this approach required knowledge of:

→ Net protein requirements for maintenance, growth, pregnancy and lactation,

→ Amino acid composition of the products formed, and

→ Efficiencies of use of absorbed AA for maintenance and production (O'Connor *et al*, 1993).

The CNCPS, for evaluating cattle diets, is the most dynamic of the AA factorial models (O'Connor *et al*, 1993), but the NRC subcommittee felt that there was not enough information available to develop a model to quantify AA requirements of dairy cattle. Even though there have been a few direct attempts to do this (Williams and Smith, 1974; Fenderson and Bergen, 1975; Titgemeyer *et al*, 1988), it is very difficult to supply graded amounts of specific limiting AA to ruminants at different production levels while measuring weight gains, AA flow to the small intestine and milk production (NRC, 2001).

- *Dose response approach*

This is a more direct approach to estimate the AA concentrations of MP required to ensure maximum efficiency of protein synthesis.

→ Direct dose-response approach involves graded infusions of AA into the abomasum or duodenum while measuring AA flow to the small intestine together with production responses. Various studies were conducted using graded levels of Lys with a constant amount of supplemental Met (Schwab *et al*, 1992b) or graded Met levels with constant Lys supplementation (King *et al,* 1991; Pisulewski *et al*, 1996).

→ The indirect-dose response approach involves a number of steps:

- predicting levels of AA in PDI for treatment and control groups
- using linear regressions to calculate reference production values for each production parameter at fixed AA concentrations in digesta
- calculating production responses of treatment and control groups relative to the reference production values (Schwab, 1995; NRC, 2001).

Integrating data from various studies in which Lys, Met, or a combination, were either infused into the abomasum or duodenum or fed in ruminally-inert forms, allowed development of a model to describe milk protein yield responses to a wide range of duodenal Lys and Met concentrations. According to this model, milk protein secretion is optimal when PDI contains 73 and 25 g/kg of intestinally digestible Lys and Met respectively (Rulquin and Verite, 1993), suggesting an optimum ratio of 3:1 for Lys and Met in MP. These values fall within the range of Lys (71 – 82 g/kg) and Met (24 – 26 g/kg) concentrations in milk protein (Table 2.1), supporting the concept of an ideal AA profile for optimum metabolic efficiency. Values from Rulquin and Verite (1993) were compared to data obtained from direct physiological and indirect factorial methods, with marked differences in values reported.

Animal requirements can be expressed in daily amounts (g/day) or as profiles (a proportion of total EAA). The latter seems preferred, since it is easier and more accurate to formulate a diet for a desired absorbable AA profile than for amount (Schwab, 1995). Requirements for Lys and Met for maximum milk protein content and yield can therefore also be expressed as 150 and 53 g/kg of total EAA in MP.

Recommendations for Lys and Met levels have so far been a function of the AA profile of milk, ignoring impacts that AA have on milk fat production, and other metabolic pathways. Prediction models usually estimate MP or digestible AA, predicting milk protein response and output using a fixed transfer coefficient of AA supply to milk output. This concept was recently challenged by Lapierre *et al* (2006), who demonstrated that requirements may actually be a function of the efficiency of AA utilization, determined by partitioning AA supply between milk protein production and catabolism. As protein supply increases, more AA are transferred to milk, but this is associated with increased catabolism in gut tissues and hepatic removal of some AA (i.e., the more AA flowing through the tissue bed, the greater its use) (Hanigan *et al,* 2004).

The AA truly available to the animal are those digested and absorbed in the small intestine (i.e., the difference between duodenal and ileal flows). Due to the difficulties of using ileal cannulae, AA digestibility has been determined as the difference between duodenal and fecal flows, which leads to either an overestimation of AA availability, since AA losses (oxidation to support the

needs of gut tissue and endogenous secretions in the duodenum and ileum) are unaccounted for, or an underestimation due to disregarded endogenous AA secretions and AA resulting from rectal MCP synthesis. Data from various studies suggest that certain AA, such as His and Phe, are only oxidized to a limited extent, while others, such as Lys and Met, are more extensively catabolized. Only some of these values have so far been documented (Lobley *et al*, 2003). Endogenous loss and oxidation of EAA averaged 300 – 550 g/kg of total digestible AA (Lapierre *et al*, 2006), indicating that AA supplied from MCP and RUP, that are available to the animal, is considerably altered by gut metabolism.

The AA absorbed into the portal vein seem to contribute to N used by the liver to synthesize urea (Bequette, 2002), suggesting that the liver changes the pattern of supply of AA to the mammary gland, and decreases the efficiency of transfer of absorbed AA into milk protein. This requires further investigation in order to develop a model to predict milk protein yield which includes parameters such as digestible AA supply and metabolism across the gut, liver and mammary gland (Lapierre *et al*, 2006). Since removal of AA across tissues is related to its inflow, DMI and abomasal infusion will increase luminal use of AA. Increased absorption of ammonia from the rumen increases the amount of AA used for urea synthesis (Bequette, 2002), suggesting that increased AA supply will result in decreased synthesis of milk protein (Hanigan, 2005).

According to Rode and Vazquez-Anon (2006), efficiency of Met utilization is controlled by the liver while the mammary gland controls efficiency of Lys utilization. This suggests that it might be time to evaluate Lys and Met requirements separately, rather than as a ratio.

Diet evaluations by NRC (2001) showed that most rations fed to high producing cows did not meet the optimum requirements for AA in MP, providing only 87-91% of Lys and 72-86% of Met requirements with a ratio of 3.41-3.66:1, considerably higher than the NRC suggested optimum. Methionine seemed to be the limiting AA in these diets and, by meeting Met requirements, other AA were supplied in excess of their ability to be used for milk protein production. Balancing rations for absorbable AA will reduce surplus AA and improve efficiency of MP utilization, unless there is a considerable surplus of MP due to overfeeding of RUP (Schwab and Boucher, 2007).

Providing required levels of the most limiting AA often leads to over-formulation of the CP portion in the diet, and excess N becomes a burden for the environment and the cow when she utilizes additional energy to convert excess N to urea for excretion in urine (Evans, 2003). However, when the diet fails to supply sufficient AA, and some AA necessary to synthesize specific milk or body proteins are not available, it leads to net catabolism of tissue and a decline in milk protein yield (Lapierre *et al*, 2002). These 'missing' AA are known as limiting AA.

2.5 Limiting amino acids

Limiting AA refer to EAA that are in shortest supply relative to requirements. They are important for the reasons outlined above. The limiting AA theory is best described by the barrel and stave example (Schwab and Boucher, 2007; Figure 2.1) in which the staves of a barrel are at different heights and the volume of liquid in the barrel is determined by the length of the shortest stave. The efficiency with which absorbed AA are utilized will likewise be determined by the supply of the first limiting AA.

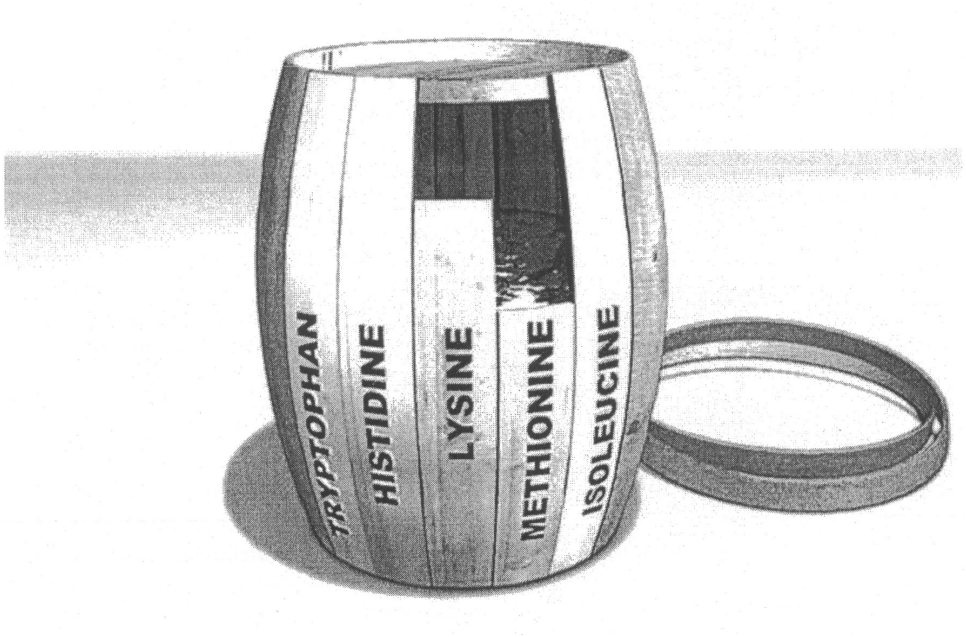


Figure 2. 1: Illustration of the limiting AA theory (barrel and stave example)

Methods to predict limiting AA in milk production include:

→ Calculating extraction and transfer efficiencies from measured arteriovenous differences, which is widely considered to be the most accurate indicator of AA use by

the mammary gland since no errors from estimates of blood flow occur (Nichols *et al,* 1998).

→ Infusions of individual AA into the duodenum or abomasum. Limiting EAA will not accumulate in the plasma until its requirements are met (Mitchell *et al,* 1968, Stockland *et al*, 1970).

→ Calculating milk protein scores, defined as the level of the most limiting AA in the protein supplement relative to that AA in milk protein, assuming that the AA composition of milk protein is indicative of the AA requirements for milk production (Chandler, 1989, as cited by Erasmus 1999).

→ Use of computer models such as CNCPS, CPM Dairy (the updated version 3 of the CNCPS), the Degussa model (Amino Cow) or the French PDI System for Lys and Met.

Methionine and Lys were identified, more than 30 years ago, as being the most limiting AA for milk production in dairy cattle (Schwab *et al*, 1976), growth in steers (Burris *et al*, 1976) and weaned dairy calves (Schwab *et al*, 1982), followed by Phe, Ile and Thr as most frequently limiting (Vik-Mo *et al,* 1974; Derrig *et al,* 1974; Nichols *et al*, 1998; Liu *et al*, 2000). These findings have been confirmed by many other infusion studies. His and Arg were recently identified as limiting when cows are fed grass-silage, barley and oat grain based diets (Vanhatalo *et al,* 1999; Huhtanen *et al*, 2002) and that His may be the third limiting AA in some maize-based rations.

The quantity and ratio of AA from MCP and dietary CP reaching the intestine may determine if animal production will respond to feeding rumen protected AA (**RPAA**). Due to the low Lys content in maize products (16.5 g Lys/kg CP), the contribution of Lys to AA passage to the intestine is reduced when higher levels of maize products are included in the diet (Rogers *et al*, 1989). It has been suggested that large amounts of maize in rations also reduces microbial growth, thereby reducing AA passage to the small intestine.

By supplementing, or infusing, various amounts of individual, or combinations, of EAA into the abomasum and duodenum, researchers measured the effects of AA supplementation on N

retention to try and increase milk protein production (Pisulewski *et al*, 1996; Huhtanen *et al*, 2002; Schei *et al*, 2007a,b).

2.6 Responses to amino acid infusions

Even though it is widely accepted that Lys and Met are the first limiting AA for milk production, results reported in postruminal infusion studies have been inconsistent, and Rulquin and Verite (1993) attributed the variation to the curvilinear pattern of milk protein responses to graded intestinal Lys and Met additions. Inconsistencies can also be attributed to the large variation in experimental conditions, since differences in stage of lactation and level of milk yield can change the ranking order of limiting AA profiles of control diets (Kim *et al,* 2000) and that most studies were short term, generally starting after peak production and missing the potentially most important stage of the lactation cycle (Erasmus, 1999). This was supported by Schei *et al* (2007b), who reported that the stage of lactation affects N metabolism and plasma hormone concentrations of cows, even though they received exactly the same AA dosages and were fed similar diets. Socha *et al* (2005) also suggested that the greatest responses to AA supplementation occur during early lactation when the need for absorbed AA is the highest and Polan *et al* (1991) reported that the magnitude of production differences between treatments declined with advancing stage of lactation, with similar milk production among treatments in late lactation.

Production responses of lactating dairy cattle to increased Lys and Met supplies (as both postruminal infusions and ruminally protected forms) include variable increases in milk protein yield, milk yield and feed intake (NRC, 2001). In an analysis of 121 studies, Rulquin *et al* (1992) reported that milk yield was mostly unaffected while milk protein yield and content increased. Milk fat proportion tended to be slightly reduced but increased fat proportions have been reported in some postruminal infusion studies.

The summative conclusion of most studies in which AA are supplemented (Clark, 1975; Schwab *et al*, 1976; Donkin *et al,* 1989; Chow *et al,* 1990; Pisulewski *et al,* 1996) are that:

→ Milk protein is more responsive to AA supplementations than milk yield.

→ Increases in milk protein proportion are independent of milk yield, and most predictable when all other AA are provided according to requirements.

→ Casein is the milk protein fraction mostly influenced by additional AA supplies.

→ Milk yield responses to these AA are more common during early lactation.

→ These responses are greatest with normal dietary CP levels of 140 to 180 g/kg.

Due to a lack of experimental data, it is not clear why milk protein concentrations respond differently to changed Lys and Met nutrition, and experiments rarely quantify lactational responses as a function of graded postruminal AA concentrations, limiting expression of results to dose-response relationships, which are needed to determine requirements and effects of over and underfeeding of these nutrients (Rulquin *et al*, 1993).

2.7 Amino acid supplementation

All AA exist as the isomers D and L which are chemically identical, the one being a mirror image of the other. However AA in plant and animal proteins, as well as some produced industrially, such as Lys, Thr and Trp, are in the L-form, while chemically synthesized Met is a mixture of the two (i.e., DL-Met). RPM fed in the D-form are absorbed into the plasma but needs to be converted to the L-isomer within tissues before it can be incorporated into animal proteins. Efficiency of conversion of commercial Met products from the D to L form has been of some concern, but studies have shown that the efficiency of use of D-Met, relative to L-Met, was 960 g/kg in growing steers (Campbell *et al*, 1996).

Free AA are very sensitive to microbial degradation in the rumen and, due to extensive deamination of hydrolyzed AA (Lewis and Emery, 1962), concentration of free AA in rumen fluid is low (Velle *et al,* 1997; Volden *et al*, 2001). Supplementing free (crystalline) AA to the diet has not been as effective in dairy cows as it has been in pigs and poultry, and studies with growing cattle have shown rapid degradation and minimal passage of free AA to the duodenum when it is introduced into the rumen (Campbell *et al,* 1997).

Many studies have been conducted to increase the amount of AA that escape from the rumen by protecting EAA such as Lys and Met using chemical alteration or physical protection.

2.7.1 *Rumen protected amino acids*

Significant progress has been made in developing technologies to increase availability and absorption of EAA by ruminants (NRC, 2001). The physical-chemical properties (i.e., high water solubility and reactivity) of Lys, however, makes its protection from degradation by rumen microbes extremely difficult and most technologies are currently only applied to Met, while research continues to find a method to successfully protect Lys.

2.7.1.1 *Methionine*

Protection methods currently used can be divided into three categories:

→ *Liquid sources of hydroxy analogs (chemically modified molecules)*

Analogs differ in chemical structure from their L-AA counterparts. They contain a hydroxyl instead of an amino group and are therefore recognized by rumen microbes as an organic acid (i.e., a fermentation end product) and not an AA. This aids in their rumen escape potential since they are usually relatively reduced acids (compared to lactate which is more oxidized) with only a selective group of microbes capable of extracting energy by fermenting it. The Met analog may be a free acid in an aqueous solution or a Ca salt in a dry solid (Koenig *et al,* 1999). As a liquid, it is easy to handle and can be incorporated into feed pellets. The most studied Met hydroxy analog is DL-2-hydroxy-4-Methylthiobutanoic acid (**HMB**) due to its successful use in monogastric animals. 'AliMet®', for example, is an 880 g/kg aqueous solution of dl-HMB and a source of L-Met. Rumen escape of HMB is a function of the passage rate of the liquid phase from the rumen, the extent of degradation by microorganisms and, to some extent, absorption of HMB across the rumen wall (Koenig *et al,* 2002). Absorption occurs by diffusion across the rumen (the portion degraded by microbes), omasum and intestinal wall (the portion escaping rumen degradation) into the blood stream. Productive tissues such as the mammary gland and muscle remove HMB from circulation and convert it to L-Met (Rode and Vazquez-Anon, 2006). Esterification of HMB to various alcohols, including the isopropyl ester of HMB (**HMBi**), decreases its rate and extent of rumen degradation (St-Pierre and Sylvester, 2005).

→ *Surface coating or matrices of saturated fatty acids and minerals*

Development of a lipid-protected product requires identification of a process, and a fat, to use for the matrix, or coating, that provides a reasonable degree of protection in the rumen while allowing adequate intestinal release (NRC, 2001). Products like 'Met-Plus^{TM}' rely on inert characteristics of saturated fats to attain insolubility and leave the rumen with the solids. Absorption only occurs through active transport at selective sites in the small intestine (Rode and Vazquez-Anon, 2006). Apparent bioavailability therefore depends on ruminal escape and intestinal release of AA. 'Met-Plus^{TM}' consist of 650 g/kg DL-Met in a matrix of Ca salts of long-chain fatty acids (**LCFA**), lauric acid, and a fatty acid (**FA**) preservative; butylated hydroxytoluene (**BHT**).

Carbohydrate-protected products, such as 'Mepron® M85', has a combination of coating materials applied to ensure slow degradation in the rumen and slow release of Met in the intestine. 'Mepron® M85' has a core, consisting of 850 g/kg DL-Met, coated with starch and several thin layers of stearic acid and ethylcellulose. The latter minimizes enzymatic digestion and so the release of Met depends on physical action and abrasion, wearing away the corners of the pellets (Lapierre *et al*, 2002).

→ *Surface coating with a fatty acid or pH-sensitive polymer mixture*

This system involves protection of an AA core by coating it with a lipid/pH-sensitive polymer as in 'Smartamine^{TM} M' (**SmM**). Release of AA do not depend on digestive enzymes, but rather a change in pH between the rumen (i.e., pH 6.2 $\pm$ 0.7), and the abomasum (i.e., pH 2.5 $\pm$ 0.3). SmM, a pellet with a core containing 750 g/kg DL-Met, is protected by a layer of ethylcellulose covered with a coating of stearic acid. It has improved resistance to rumen degradation due to the presence of a copolymer, poly (2-vinylpyridine-co-styrene), altering the steriochemistry of stearic acid. The copolymer solubilizes at low pH, rapidly releasing Met in the abomasum.

2.7.1.2 *Responses to rumen protected methionine*

Amino acid concentrations in plasma, measured at different time intervals after feeding the ruminally-protected product can be used to assess and compare AA availability among product, since there is a good relationship between the amount of Met escaping rumen degradation and the area under the curve (Bach and Marshall, 2000).

With the technology used for SmM, the product is inert in the rumen but quickly releases AA in the abomasum. Average bioavailability was estimated to be between 750 and 800 g/kg (Schwab *et al*, 1995), using a ruminal *in sacco* technique. In lactating dairy cows, availability was 750 to 970 g/kg using digestibility tests, and approximately 750 g/kg according to blood tests (Rulquin and Kowalczyk, 2003).

All other protection technologies allow a certain amount of AA to be released in the rumen, and the ruminal rate of passage will directly influence bioavailability. Rumen degradation can range from 220 g/kg (Overton *et al*, 1996) to 370 g/kg (Berthiaume *et al*, 2000), depending on rumen residence time.

Various *in vitro* (Vazquez-Anon *et al*, 2001) and *in vivo* (Koenig *et al,* 1999) studies showed HMB to be more resistant to rumen degradation than Met, but controversy exists about its rumen escape rate. Values range from as low as 10 g/kg of fed HMB recovered in the duodenum (Jones *et al,* 1988) to as high as 500 g/kg, based on serum Met concentrations (Koenig *et al,* 1999). This variation may be due to differences in dose levels, method of supplementation or physiological status of the cows (Rulquin *et al,* 2006). Animal responses to HMB has mainly been an increase in milk fat proportion, possibly due to its effect on rumen fermentation and VFA production, increasing acetate production (Noftsger *et al,* 2003), with less consistent responses in milk yield and none in milk protein (St-Pierre and Sylvester, 2005).

When examining these reports, it seems that HMB provides a rumen-protected form of Met while simultaneously improving efficiency of MCP synthesis (Vazquez-Anon *et al,* 2001), either by sparing Met precursors for more efficient protein synthesis or by shifting bacterial species. Regardless, Noftsger *et al* (2003) reported no difference in bacterial N flow from the rumen, concluding that HMB had no effect on microbial growth efficiency. More recent studies showed

that the main effects of HMB supplementation on milk production were to increase milk fat yield, suggesting that HMB doesn't meet Met requirements of dairy cows for milk protein synthesis (Rulquin *et al,* 2006a).

Approximately 500 g/kg of HMBi escapes rumen degradation, determined by using blood and milk true protein changes as bioavailability indicators (Noftsger *et al,* 2005), regardless of whether it is supplemented in liquid or dry form (St-Pierre and Sylvester, 2005). It is quickly absorbed in the small intestine, hydrolyzed into HMB and isopropyl and then converted to Met and acetone, delivering about 480 g/kg Met to the cow (Graulet *et al,* 2005).

Studies to quantify and compare ruminal effects, and production responses, from two Met hydroxy analogs, HMB and HMBi, indicated that HMBi is an improved rumen-protected form of Met. Effects produced by HMBi, but not HMB, include an increase in plasma free Met, improvements in N efficiency, increased milk production and milk true protein content. HMBi decreased milk urea N (**MUN**), but did not affect milk fat content, which was increased by HMB (Noftsger *et al,* 2005; St-Pierre and Sylvester, 2005; Rulquin *et al,* 2006a).

Due to the high rumen protection and intestinal release coefficient of AA in the pH sensitive products, it seems to be the most effective technology with the largest increases in blood AA concentrations, but according to Watanabe *et al* (2006), AA protected by a fat coating are just as capable of improving production performances as the pH sensitive polymer products.

2.7.1.3 *Lysine supplements and responses*

Limited information is available to describe effects of supplementing free L-Lys-HCL in lactating dairy cattle. Research with rumen- and abomasum-cannulated wethers indicated that only 62 g/kg of supplemental Lys from L-Lys-HCL (cows received 4.8 g of L-Lys from the 6 g of L-Lys-HCL fed) escaped rumen digestion, and did not increase the quantity of Lys reaching the abomasum. Also, 465-562 g/kg Lys was recovered in abomasal digesta when a Lys polymer (prepared by reacting L-Lys-HCL, urea and formaldehyde) was fed (4.52 g Lys was delivered from 15 g of polymer fed) (Amos and Evans, 1978). This indicates that some protection forms of Lys can reduce rumen degradation of free L-Lys-HCL. A more recent study in which AA, including L-

Lys-HCL, were administered intraruminally at four dosages (i.e., 75, 150, 300 and 600 mmol) to nonlactating cows, showed higher levels of Lys escaping rumen degradation (Velle *et al*, 1998). Velle *et al* (1998) also concluded that rumen escape of AA increased as dosages increased. However, based on duodenal concentrations in a study by Bernard *et al* (2003), none of the Lys, supplemented at a rate to provide 10 g/d Lys, escaped ruminal degradation. Studies in which Lys had positive effects on milk yield suggest that the response was due to increased MCP synthesis.

Numeral studies have been completed to determine effects of ruminal escape Lys and Met on milk yield and composition. However there are very few published articles involving supplementation of RPL without concurrent supplementation of RPM.

Rogers *et al* (1989) fed three amounts of RP L-Lys (5.9, 13.5, and 21.1 g/d) to 3 groups of cows at different stages of lactation and reported improved milk and milk protein production when cows were fed maize based diets, but not when soybean meal was added to the ration. Plasma concentrations of Met and Lys were also increased. Xu *et al* (1998) reported a positive response in milk yield and milk protein, that was consistent through different stages of lactation, and an increase in milk fat content during early lactation when RPL was supplemented to a ration limiting in metabolizable Lys and Met, to provide 27 and 40 g/d as available AA at the duodenum. Increased milk protein was attributed to increased milk casein N and not increased MUN.

Other responses observed include increased total milk N and casein N in high forage diets with the greater NPN content attributed to the addition of fat to the diet (Chow *et al,* 1990). A few studies also reported an increase in milk fat yield and percentage (Robinson *et al,* 1995; Socha *et al,* 2005).

However, responses have been inconsistent and some studies showed no benefits when RPAA were fed, especially when the ration contained soybean meal (Guillaume *et al,* 1991; Armentano *et al,* 1997). Some ascribe the lack of response to the control diet possibly meeting the AA requirements of the cows (Bremmer *et al,* 1997) or other factors, and/or other AA that might be more limiting than Lys or Met (Karunanandaa *et al,* 1994; Liu *et al,* 2000).

While Polan *et al* (1991) suggested that a positive result might have been detected in their study, had they fed only RPL rather than a combination of Lys and Met to a maize gluten meal

based diet, Robinson *et al* (1998) intended to separate the effects of RPL from RPM on animal performance by supplementing RPL to a ration calculated to be first limiting in Lys. However, post-experimental calculations suggested that the ration was first limiting in His, followed by Lys, thereby demonstrating that cows do not respond to enhanced Lys supplies when it is not limiting, unlike Met, which may enhance production of milk components even though it is not the limiting AA. In contrast, an RPL (15 g/d) increased the flow and percentage of Lys in duodenal digesta and plasma, and increased yields of milk and milk protein after being fed to cannulated and intact cows in early lactation (Blauwiekel *et al,* 1997).

2.7.1.4 *Factors affecting effectiveness of rumen protected amino acids*

The responsiveness of cows to supplementation of RPAA depends on the quantity and ratio of AA from dietary and MCP that reach the small intestine. The contribution of dietary Lys to AA that reaches the small intestine decreases as the level of maize products in the diet increase (Stern *et al*, 1983). Lucerne hay and soybean meal are high in Lys and may therefore deliver more Lys to the small intestine than products such as maize gluten meal. Effectiveness of any AA therefore depends on the ingredient composition of the ration fed.

Success of the pH-sensitive polymer coated RPAA products rely heavily on achieving a balance between protection of AA against rumen degradation and release thereof in the small intestine, aiming to minimize losses in the rumen and faeces while maximizing intestinal absorption of AA. Stability of these compounds is very important since pelleting and over-mixing can cause degradation of the protective coating and prolonged exposure to silages in a total mixed ration (**TMR**) may weaken the coatings if they are pH sensitive (Rode and Vazquez-Anon, 2006). No matter how effectively AA are protected from rumen degradation, protection will not last indefinitely. Release of Lys from fat coated products depends on its retention time in the rumen (i.e., mean retention time (**MRT**)). Its MRT, which is mainly affected by body size and level of feed intake of the cows, is therefore an important determinant of product effectiveness.

A number of factors affect rate of passage of foreign particles from the rumen. Density and size of particles, number of particles per unit of DM and physical characteristics of the particles, as well

as properties of the ruminal mat (hard or soft packed), relative rumen fill, and ruminal size can alter rates of appearance of material at the reticulo-omasal orifice (Welch, 1990). Properties of particles that determine whether they clear the rumen quickly or slowly have been studied using particle markers (King and Moore, 1957; Campling and Freer, 1962; Welch and Smith, 1978; desBordes and Welch, 1984).

The effect of SG on MRT of particles in the reticulo-rumen is due to the rate of separation of the particles out of the main digesta into the fluid layer passing to the omasum. Heavier particles separate rapidly and settle on the bottom of the reticulo-rumen, while lighter ones don't separate at all. These heavy and light weight particles are less readily transported in the liquid digesta leaving the rumen.

Maximal rate of passage of particles from the rumen occur at SG between 1.10 and 1.20 (King and Moore, 1957), or 1.17 and 1.42 (desBordes and Welch, 1984). Particles much lighter or heavier had a slower passage rate.

Campling and Freer (1962) reported that the MRT in the reticulo-rumen, when cows were fed hay or straw, was 73, 51, 36 and 28 h for particles with SG 1.02, 1.06, 1.12 and 1.21. Even though there was little difference in the effect of SG on the MRT in the reticulo-rumen between forage and concentrate diets, particles with SG 1.12 was retained much longer in cows fed a concentrate diet compared to the forage fed cows, and heavier particles (i.e., SG of 1.40 and 1.21 respectively) had the shortest retention times in these diets.

Optimum sizes for rapid rumen passage was determined to be 20 to 30 x 10^{-3} cm^3 by King and Moore (1957). Smaller particles tend to get trapped in the fibrous mat in the rumen, reducing their movement to the orifice for passage out of the rumen (Welch, 1982).

Chapter 3: Experiment 1. Identifying limiting amino acids in contemporary rations fed to high producing dairy cattle in California

3.1 Introduction

Over the past 10 years, there has been a huge increase in the number of ethanol distillation plants in the Midwestern US that, using maize grain as their feedstock, create vast quantities of maize distiller's by-products. California dairy rations have long depended upon maize based feedstuffs (i.e., maize grain, maize silage, maize gluten, as well as germ feeds and meals) and, with the widespread increase in use of maize DDG, it is not uncommon to find 30-40% of total CP in TMR's being from maize products.

It is not clear how many nutritionists on CA dairy farms use metabolic models such as CPM Dairy to formulate and/or evaluate their rations, but it is not high since many consulting nutritionists are not convinced of the biological accuracy of these metabolic models. Under many practical circumstances ration formulation is restricted to the type and amount of raw materials available on the farm and the performance of the cows, as well as their basic nutritional requirements, are used to formulate the ration. Maize proteins have long been recognized to have an AA profile that is poorly matched to that of the milk protein produced by dairy cows, raising concerns that increased CP levels in the ration, in order to meet animal requirements for limiting AA, might lead to an increase in the proportion of dietary CP that is excreted in urine and faeces. This is in direct opposition to recent efforts designed to minimize the negative impact of dairy cows on the environment.

Experts differ widely on which AA are limiting, but studies have suggested Lys and Met to be the most likely candidates (Burris *et al*, 1976; Schwab *et al*, 1976; 1982) followed by Phe, Ile, Thr (Vik-Mo *et al,* 1974; Derrig *et al,* 1974; Nichols *et al*, 1998; Piepenbrink and Schingoethe*,* 1998; Liu *et al*, 2000) His and Arg (Vanhatalo *et al,* 1999). More information is required regarding limiting AA, and the effect of supplementing them, in order to make ration formulation based on AA levels feasible.

This study involved a survey of management and feeding practices, collection of feed ingredients and TMR's and evaluation of dairy rations using three metabolic models in order to:

→ Predict AA profiles of intestinally delivered protein in California high group dairy cattle fed contemporary rations.

→ Determine the impact that the level of maize products in the ration has on animal productivity.

→ To identify limiting AA and determine if there is enough consistency in nutrient profiles of these rations to justify production of an RPAA complex to use as a supplement in California dairy rations. This might provide cows with the 'ideal' dietary AA profile with the potential to improve animal production and efficiency with the added environmental benefit of potentially reducing ammonia emissions from urine due to lower N excretion.

3.2 Materials and methods

3.2.1 *Farm, cows and management*

A group of 24 potential dairy farm co-operators were identified in Tulare and Kings County of California, USA. Dairies chosen for this initial list were judged, by two farm advisors, to be representative of dairy farms in the respective counties, willing to participate in the project and were milking more than 1000 cows. Of the 24 total dairies, 16 were finally chosen based on an assessment of factors including ration composition, organization and neatness of the dairy (i.e., accurate mixing and feeding records to determine amounts of feed mixed and TMR dropped at each pen), the use of a computerized herd record and management system (i.e., Dairy Comp 305 (**DC 305**), Valley Ag Software, Tulare, CA) and structural makeup and outlay of lactation pens. Each dairy had a consulting nutritionist in charge of formulating the ration, and care was taken during the selection process to select dairies with different nutritionists. A complete description of the 16 dairies can be seen in Appendix A3 (Table A3.1).

3.2.2 *Sample collection*

Three visits to each farm were scheduled in conjunction with their regular Dairy Herd Improvement Association (**DHIA**) milk test. During the first visit, dairies were appraised and the

farmers informed of procedures to follow. One of the high production multiparity pens was identified for use in the survey at each farm.

During the second visit, TMR preparation was observed before TMR samples were collected from the bunks as feed was being dropped at the specified pens. Six handfuls were collected at evenly spaced locations along the bunk-line, pooled and the entire sample quartered, keeping two opposite quarters for analysis. When TMR samples contained whole citrus pulp, large pieces were broken up by hand before quartering to ensure proper mixing.

Commodity feeds, mixed into the TMR, were identified and sampled by taking 4-5 handfuls of each. When sampling silages, more, smaller handfuls were collected due to high variation within the feed. A 'golf club' hay probe (Seifert Analytical, Lodi, CA, USA) was used to take 12 - 16 core samples from all hays, oat, wheat and rice straw.

A second TMR sample was collected (after preparation was observed again) prior to, or on, the day of regular DHIA milk testing, following the same procedures as above. Highly variable wet commodities, such as green lucerne chop, were also sampled a second time. As far as possible, the two sampling visits were scheduled at different feeding times.

All feed and TMR samples were stored in a cooler and later transferred to a freezer (-19 ^{o}C) until it was dried and sent for analysis. Chemical compositions obtained from previous studies were used for ingredients that were difficult to sample such as liquid whey, molasses and maize syrup, as well as commercially sold ingredients with standard or constant chemical compositions such as yeast cultures, ruminally inert fats and buffers.

Information on farm, cow and pen characteristics, mixing equipment, feeding sequences and any other anomalies were recorded for each dairy. The amount of TMR refused, and frequency of removal, was also recorded.

A DC 305 backup, with milk production and composition data from the most recent milk test (i.e., milk yield, protein and fat proportions, somatic cell counts (**SCC**), days in milk (**DIM**) and lactation numbers), was downloaded prior to the start of the project, and again after the DHIA milk test. In some cases, problems with the computer backup prevented data download and results for those milk tests were collected directly from DHIA.

Depending on the method used to monitor mixing and feeding, feed delivery records were collected for at least 5 days prior to the milk test from computerized programs (i.e., Feed Watch and EZfeed) or mixing sheets provided by the dairies. Mixing information was used to calculate daily DMI per pen.

3.2.3. *Analytical methods*

3.2.3.1 *Feed preparation and assays*

All the TMR samples, silages and other wet ingredients were weighed before being dried at 55°C for 48 hours. Some ingredients, especially pulps, were broken up and turned over after 24 h. All samples were removed and left to equilibrate for 24 h before the air dry samples were bagged, weighed and tagged for analysis.

Feed and TMR samples collected were analyzed at UC Davis service laboratory (**ANR**) for DM, Ash content, neutral detergent fibre (**NDF**), acid detergent fibre (**ADF**), lignin, starch, free sugars (soluble carbohydrates), CP, acid detergent insoluble N (**ADIN**), used to calculate acid detergent insoluble crude protein (**ADICP**), and trace minerals. Fat (**EE**), *in vitro* NDF digestibility after 30 h of rumen incubation (**dNDF$_{30}$**) and soluble CP (**SolCP**) were analyzed at Cumberland Valley laboratory in Maugansville, Maryland (USA).

All samples were ground to pass a 1mm screen on a model 4 Wiley Mill. The DM was determined through gravimetric loss of free water when heated to 105°C for 2 h in a forced air oven (Reuter *et al.*, 1986). Total N and ADIN were determined by the Leco method with a nitrogen gas analyzer using an induction furnace to ignite samples at 900°C and a thermal conductivity detector to determine the N content (Method 990.03, pp. 18-19, AOAC, 1997). The CP was calculated from the N content of the feed. The ADF and lignin were determined by the reflux method using sulfuric acid and heat to dissolve solubles, leaving a residue of lignin. The ADF was determined gravimetrically as the residue remaining after extraction (Method 973.18, AOAC, 1997) and NDF was determined by the reflux method using a sodium sulfite detergent and heat (Van Soest *et al.*, 1991). Heat stable amylase was added to samples with a high starch content to prevent filtering difficulties (i.e., amylase-treated NDF (**aNDF**)). The aNDFom and ADFom do not include ash. Ash determination was based on gravimetric loss by heating samples to 550°C for 8 hours. Soluble

carbohydrates (i.e., free sugars) were determined by high-performance liquid chromatography (**HPLC**) using a Phenomenex Luna NH_2 (250 mm x 4.6 mm) HPLC column at a flow rate of 2.75 ml/min, acetonitrile:water (78:22) (Johansen *et al.*, 1996). Starch was calculated as total glucose minus free glucose x 0.9. Total glucose was determined by enzymatically hydrolyzing the samples at 55°C with amyloglucosidase for 12 hours and then analyzing them by the same HPLC as above (Smith, 1969). Most minerals (i.e., P, S, Ca, Mg, Na, Zn, Mn, Fe, Cu, Co) were determined using a nitric acid/hydrogen peroxide microwave digestion/dissolution of samples and quantitative determination by atomic absorption spectrometry (**AAS**) and inductively coupled plasma atomic emission spectrometry (**ICP-AES**) (Meyer and Keliher, 1992). Total K was determined by atomic emission spectrometry (**AES**) and Cl by chloridometer after both minerals were extracted by 20 g/l acetic acid (Johnson and Ulrich, 1959). Total Se was extracted by nitric/perchloric acid digestion/dissolution and determined by vapour generation using inductively coupled plasma atomic emission spectroscopy (Tracy and Moeller, 1990).

Soluble N was determined by a borate phosphate buffer procedure (Krishnamoorthy *et al,* 1982) and EE was quantified using a standard Soxhlet extraction during which fat is dissolved in boiling ethyl ether and residues determined gravimetrically after drying (Method 2003.05, AOAC, 2006). The $dNDF_{30}$ was determined using the method described by Robinson *et al* (1999). Forage samples were weighed into individual bags and incubated in a DAISY® *in vitro* system (Ankom) at 39°C for 48 h after which the bags were removed and washed in cold water. The NDF was calculated as the residue in the bags after boiling the samples in ND solution with sodium sulfite and amylase for 1 h.

3.2.3.2 *Model evaluation*

Once all cow and feed assay information was collected and tabulated, the nutrient profiles of the 16 rations were evaluated by the metabolic models Amino Cow, CPM Dairy and Shield. Each model is shortly described in the list of products and programs used in the Appendix tables, but they are very similar, all largely empirical, but with different AA levels assigned to feeds and MCP. Even though it is possible that these models may provide inaccurate estimates of AA

requirements and availability, there is no other accepted AA evaluation model published that could evaluate these performance results in quantitative terms.

In all cases, cow information, calculated ingredient composition of the rations and the chemical composition of the feeds that were fed was entered into the models as required for each model. All default feed components were used with the exception of feed DM, CP, ADF, NDF and fat for Amino Cow, DM, CP, SolCP, ADICP, ADF, NDF, lignin, ash, fat, sugars and starch for CPM Dairy and DM, OM, fat, CP, SolCP, ADICP, NDF and $dNDF_{30}$ for Shield.

3.3 Results, calculations and discussion

3.3.1 *Ration evaluation*

Chemical compositions of commodity feeds are in Table 3.1. Where numerous samples of the same ingredient were collected, a subset of samples was pooled to obtain an average with a standard error (**SE**), except for maize distiller's grains where all samples were assayed. Average values were used during model evaluation. The composition of the ingredients was consistent among the dairies, with only minor differences in some nutrients.

Results for forages are reported separately by dairy (Table 3.2) due to greater variation amongst them. Averages and SE were calculated for groups with more samples, but individual values were used in model evaluations. Lucerne chop was sampled at both visits, since it is cut daily leading to compositional differences among days and because sampling is very difficult. The two samples were analyzed separately, but average values are reported in the table. Lucerne hay was divided into high or low quality (as designated by the dairy) when two sources were sampled, but there was little chemical difference between them. The forage composition was relatively consistent among dairies, with the possible exception of wheat silage and citrus pulp.

Table 3. 1: Chemical analysis (+ SE if enough samples were collected) of concentrate ingredients (% DM) used in TMR of the 16 dairies

Commodity	n	DM	OM	CP	ADICP[1]	SolCP[1]	aNDF[4]	$aNDF_{om}$[3]	$dNDF_{30}$[2]	ADF	ADF_{om}[3]	Lignin	Starch	Fat	Sugars
Almond hulls	5*	93.00 (1.344)	92.20 (0.453)	5.69 (0.172)	25.66 (2.040)	40.4 (1.55)	36.2 (1.68)	34.7 (1.71)	31.7 (3.73)	29.2 (1.17)	28.50 (1.11)	10.99 0.502)	1.72 (0.351)	2.45 (0.268)	17.81 (0.591)
Barley, rolled	1	91.00	97.19	12.19	2.56	22.3	22.6	21.5	55.7	8.5	7.8	2.00	50.70	1.74	1.30
Beet pulp shreds	2	94.10	93.93	9.52	3.94	43.0	33.1	32.3	86.4	20.3	20.2	0.83	6.43	0.74	18.80

Commodity	n	DM	OM	CP	ADICP[1]	SolCP[1]	aNDF[4]	aNDFom[3]	dNDF30[2]	ADF	ADFom[3]	Lignin	Starch	Fat	Sugars
Brandy pomace	1	30.94	89.96	10.75	37.79	30.8	43.4	39.7	31.2	46.8	43.9	21.70	0.50	2.50	0.20
Canola pellets	4*	91.15 (0.380)	91.59 (0.197)	42.72 (0.324)	6.09 (1.560)	33.4 (0.51)	26.1 (1.25)	24.4 (1.19)	45.4 (2.00)	18.7 (1.12)	18.6 (1.15)	7.63 (1.014)	2.83 0.782)	3.95 (0.108)	6.53 (0.312)
Carrot pulp	1	12.5	93.5	7.31	8.12	54.3	26.6	26.5	85.2	24.8	24.7	1.25	3.10	1.41	1.65
Maize gluten feed	2	91.80	91.91	23.53	1.85	51.6	32.2	30.6	61.8	10.6	9.6	1.00	14.50	3.37	1.00
Maize grain, flaked	3*	85.53 (0.384)	98.75 (0.062)	8.68 (0.554)	0.00 -	25.5 (3.08)	8.4 (0.18)	8.3 (0.23)	66.6 (2.69)	3.1 (0.19)	3.1 (0.15)	0.40 (0.100)	73.07 (2.335)	2.59 (0.704)	0.50 (0.153)
Cottonseed, whole linted	3*	93.17 (1.040)	95.69 (0.047)	21.34 (0.740)	7.39 (0.997)	23.0 (0.01)	44.8 (2.36)	43.2 (2.28)	9.3 (0.75)	33.9 (1.66)	33.8 (1.63)	9.80 (0.600)	0.50 -	20.18 (1.196)	0.67 -
Cottonseed, ground pima	3	93.30 (0.252)	95.17 (0.076)	23.34 (1.164)	6.76 (0.649)	25.3 (2.56)	38.5 (2.87)	36.9 (2.70)	31.4 (8.99)	28.5 (1.60)	28.4 (1.59)	10.22 (0.505)	0.53 (0.033)	22.49 (0.531)	0.52 (0.060)
Distillers grains, dried	6*	91.93 (0.400)	95.61 (0.025)	30.84 (0.550)	7.51 (1.804)	25.3 (2.40)	31.7 (1.09)	31.1 (1.10)	53.2 (2.90)	11.7 (0.81)	11.7 (0.79)	1.83 (0.475)	4.55 (1.136)	11.99 (0.583)	0.58 (0.149)
Distillers grains, wet	3	32.99 (0.708)	96.78 (0.162)	36.03 (0.883)	14.10 (1.132)	29.6 (3.37)	31.4 (0.79)	30.8 (0.78)	54.1 (2.93)	16.9 (0.96)	16.8 (0.98)	2.47 (0.203)	3.03 (0.318)	10.37 (0.183)	0.20 -
Linseed meal	1	91.80	92.03	43.70	2.79	28.2	48.6	-	68.5	16.1	14.6	5.75	2.55	2.04	2.45
Linseed pellets	1	91.90	92.39	35.19	3.73	25.8	34.6	32.6	36.6	25.6	24.4	7.10	2.60	2.22	4.50
Raisin tailings	1	92.20	90.28	8.20	24.01	41.9	24.7	21.1	40.7	28.5	24.9	10.65	0.50	0.39	26.20
Soybean meal	3	91.23 (0.260)	92.46 (0.128)	51.10 (0.749)	0.32 (0.317)	21.3 (0.05)	8.9 (0.75)	8.5 (0.70)	69.6 (0.24)	5.2 (0.39)	5.1 (0.38)	0.20 (0.058)	5.17 (0.491)	0.58 (0.106)	9.37 (0.617)
Wheat midds/millrun	3	90.58 (0.466)	94.57 (0.081)	18.48 (0.361)	2.28 (0.050)	38.5 (3.00)	37.6 (1.94)	37.3 (1.20)	45.9 (1.90)	11.6 (0.37)	11.5 (0.43)	2.78 (0.165)	24.13 (1.866)	3.22 (0.387)	2.60 (0.158)

[1] As a % of CP
[2] As a % of aNDF
[3] Fibre expressed exclusive of residual ash
[4] Amylase-treated NDF
* 1 less for SolCP, $dNDF_{30}$ and fat
** Unable to analyze sample due to clogging of the filter

Table 3. 2: Chemical analysis of forages (% DM) used in the TMR of the 16 dairies

Forage	Dairy	DM	OM	CP	APICP[1]	SolCP[1]	aNDF	aNDF om[3]	dNDF30[2]	ADF	ADF om[3]	Lignin	Starch	Fat	Sugars
Lucerne chop*	**5**	66.83	87.59	24.44	4.79	45.0	35.2	34.4	38.7	32.6	32.0	5.55	1.50	1.48	2.95
Lucerne chop	**8**	85.46	87.65	22.41	4.89	41.9	38.0	37.0	41.6	33.7	33.0	5.90	1.40	1.27	3.35
Lucerne chop	**10**	71.09	87.72	22.88	5.05	36.2	36.5	35.2	41.7	32.7	31.7	6.15	1.20	1.20	2.95
Lucerne chop	**11**	86.51	90.94	24.30	4.63	33.4	36.4	35.1	32.8	29.7	29.0	5.50	1.45	1.72	4.15
Lucerne chop	**13**	23.62	87.61	19.50	7.53	45.5	43.3	40.4	39.8	37.4	35.0	6.15	1.65	1.60	0.45
Lucerne chop	**14**	21.06	88.22	20.06	5.14	44.2	41.3	40.0	46.7	34.4	33.2	6.20	1.85	1.70	2.60
Lucerne chop	**15**	81.57	88.06	19.28	6.52	39.0	39.2	37.9	38.7	33.8	32.7	6.65	1.90	1.24	4.00
	Mean	**62.31**	**88.25**	**21.84**	**5.51**	**40.7**	**38.5**	**37.1**	**40.0**	**33.5**	**32.4**	**6.01**	**1.56**	**1.46**	**2.92**
	SE	**10.677**	**0.456**	**0.836**	**0.412**	**1.78**	**1.10**	**0.91**	**1.59**	**0.87**	**0.69**	**0.152**	**0.095**	**0.084**	**0.465**
Lucerne hay	**1**	93.80	90.05	18.60	6.38	38.4	40.1	39.4	34.9	32.5	31.4	6.20	2.60	1.24	4.70
Lucerne hay	**2**	91.30	88.48	23.13	4.05	31.3	31.9	29.9	34.1	25.1	23.6	4.80	1.80	2.11	5.30
Lucerne hay (L)**	**3**	92.90	87.46	20.50	6.40	37.0	37.9	37.3	33.2	31.6	31.3	5.80	1.50	1.15	3.50
Lucerne hay (S)**	**3**	92.50	89.97	21.80	5.16	33.8	38.3	37.6	37.7	29.8	29.4	4.80	1.50	1.18	3.40
Lucerne hay	**4**	91.90	88.89	17.75	6.34	34.0	41.8	40.8	35.8	35.7	35.0	7.00	1.40	1.65	3.70
Lucerne hay	**5**	90.80	91.08	18.44	5.76	37.1	40.3	39.5	47.1	32.8	32.7	6.40	1.70	1.64	4.50
Lucerne hay	**6**	91.90	89.61	21.13	5.03	37.9	39.9	38.5	35.0	33.2	32.3	6.30	1.80	1.80	3.80
Lucerne hay	**9**	92.70	86.78	24.60	5.08	36.5	34.8	33.3	37.6	28.0	26.6	5.00	2.10	1.45	3.60
Lucerne hay	**10**	93.10	89.68	20.22	4.95	34.0	38.9	37.3	34.3	33.6	32.5	6.10	2.40	1.23	5.15
Lucerne hay	**11**	91.50	89.08	25.60	4.88	36.5	29.9	29.8	44.9	23.6	23.6	3.30	1.20	1.42	3.20
Lucerne hay	**14**	92.40	89.25	19.94	4.39	37.4	32.4	31.1	41.3	25.9	25.4	4.60	2.60	1.88	5.80
	Mean	**92.25**	**89.12**	**21.06**	**5.31**	**35.8**	**36.9**	**35.9**	**37.8**	**30.2**	**29.4**	**5.48**	**1.87**	**1.52**	**4.24**
	SE	**0.264**	**0.365**	**0.764**	**0.243**	**0.67**	**1.21**	**1.23**	**1.40**	**1.20**	**1.20**	**0.324**	**0.147**	**0.096**	**0.268**

Forage	Dairy	DM	OM	CP	APICP[1]	SolCP[1]	aNDF	aNDF om[3]	dNDF30[2]	ADF	ADF om[3]	Lignin	Starch	Fat	Sugars
Lucerne hay HQ***	**12**	91.60	88.6	21.44	4.08	37.0	33.8	32.5	29.0	27.9	27.1	5.30	1.80	1.64	3.40
Lucerne hay HQ	**13**	92.70	90.2	23.10	5.95	35.4	38.3	36.9	34.8	31.0	30.1	5.90	1.80	1.46	4.10
Lucerne hay HQ	**16**	92.80	90.7	19.13	7.19	-	40.9	40.2	-	31.4	30.9	5.50	2.90	-	4.80
	Mean	**92.37**	**89.79**	**21.22**	**5.74**	**36.2**	**37.7**	**36.5**	**31.9**	**30.1**	**29.4**	**5.57**	**2.17**	**1.55**	**4.10**
	SE	**0.384**	**0.632**	**1.153**	**0.903**	**-**	**2.07**	**2.23**	**-**	**1.11**	**1.16**	**0.176**	**0.367**	**0.090**	**0.404**
Lucerne hay LQ***	**12**	92.00	87.1	23.63	4.50	39.2	35.6	33.7	35.3	29.7	28.5	5.60	1.60	1.02	4.00
Lucerne hay LQ	**13**	93.00	87.4	21.30	5.58	36.1	35.6	34.2	33.6	28.6	27.7	4.80	1.30	1.41	2.90
Lucerne hay LQ	**16**	91.80	86.1	23.31	8.04	-	41.1	38.9	-	34.4	32.8	6.20	0.70	-	2.00
	Mean	**92.27**	**86.84**	**22.75**	**6.04**	**37.6**	**37.4**	**35.6**	**34.5**	**30.9**	**29.7**	**5.53**	**1.20**	**1.22**	**2.97**
	SE	**0.371**	**0.389**	**0.729**	**1.049**	**-**	**1.83**	**1.66**	**-**	**1.78**	**1.58**	**0.406**	**0.265**	**-**	**0.578**
Lucerne silage	**2**	43.32	87.1	21.75	6.03	74.1	31.7	29.1	38.9	28.3	25.8	5.50	<0.5	2.60	1.20
Lucerne silage	**3**	54.80	84.9	25.90	5.55	62.1	34.7	31.2	43.5	29.6	26.9	5.50	<0.5	2.56	0.70
Lucerne silage	**11**	28.52	84.7	26.70	5.62	76.3	33.7	31.7	47.5	30.9	29.5	5.50	<0.5	3.87	0.00
Lucerne silage	**16**	46.70	88.0	27.63	5.20	-	33.3	31.5	-	26.9	25.3	5.10	<0.5	-	0.00
	Mean	**43.33**	**86.16**	**25.49**	**5.60**	**70.8**	**33.4**	**30.9**	**43.3**	**28.9**	**26.9**	**5.40**	**<0.5**	**3.01**	**0.48**
	SE	**5.494**	**0.814**	**1.297**	**0.171**	**4.42**	**0.62**	**0.60**	**2.49**	**0.86**	**0.94**	**0.100**	**-**	**0.430**	**0.293**
Citrus pulp	**1**	33.45	90.98	8.30	8.28	48.7	20.8	20.6	81.0	29.6	29.5	0.80	0.70	2.26	5.10
Citrus pulp	**12**	26.93	91.49	7.91	7.11	59.6	21.7	21.6	82.2	30.9	30.7	0.85	1.05	2.03	0.25
Citrus pulp	**15**	18.55	92.69	11.69	4.01	62.5	16.5	15.1	70.2	16.6	15.8	0.80	1.65	0.98	3.80
	Mean	**26.31**	**91.72**	**9.30**	**6.47**	**56.9**	**19.7**	**19.1**	**77.8**	**25.7**	**25.3**	**0.82**	**1.13**	**1.76**	**3.05**
	SE	**4.312**	**0.506**	**1.200**	**1.275**	**4.20**	**1.62**	**2.01**	**3.81**	**4.57**	**4.79**	**0.017**	**0.277**	**0.394**	**1.449**
Maize earlage	**9**	**60.08**	**97.24**	**8.35**	**37.43**	**66.2**	**21.7**	**21.2**	**64.3**	**11.3**	**10.6**	**1.00**	**53.10**	**3.09**	**0.90**
Maize silage	**1**	24.36	92.52	7.40	10.98	66.3	52.1	50.5	55.3	33.6	30.3	3.60	19.20	2.88	<0.2
Maize silage	**2**	30.79	93.95	8.50	7.35	70.0	44.7	43.4	52.5	31.0	28.4	3.30	23.30	2.87	0.4
Maize silage	**3**	31.94	93.45	7.10	7.92	70.7	40.7	39.3	50.3	26.8	23.9	2.50	31.10	3.13	<0.2
Maize silage	**5**	33.64	93.20	8.00	7.03	71.7	42.0	40.5	49.2	29.3	26.4	2.60	26.80	3.07	<0.2
Maize silage	**6**	31.52	93.28	8.00	9.38	67.6	45.7	44.5	56.6	31.5	28.6	3.40	21.70	3.26	<0.2
Maize silage	**7**	29.68	93.26	8.44	7.41	66.8	45.3	43.8	51.8	32.2	29.5	3.20	22.30	3.58	<0.2
Maize silage	**8**	33.25	92.91	7.69	8.94	68.7	44.4	42.9	51.7	31.0	27.4	3.10	23.70	3.21	<0.2
Maize silage	**9**	33.91	92.58	8.50	8.82	56.5	43.6	42.3	56.3	28.0	25.2	2.40	24.40	2.55	<0.2
Maize silage	**10**	30.62	92.76	8.81	10.64	60.5	43.5	41.7	46.1	33.3	29.8	4.10	22.60	5.11	<0.2
Maize silage	**11**	34.10	94.08	7.20	11.28	70.6	43.1	42.0	53.4	28.7	26.5	2.70	28.80	2.91	<0.2
Maize silage	**12**	34.50	90.52	7.75	7.26	69.3	40.1	35.9	42.5	28.5	23.0	2.70	27.30	2.71	<0.2
Maize silage	**13**	30.77	92.55	6.80	10.11	69.2	46.4	45.1	52.8	30.6	27.6	3.00	20.20	2.60	<0.2
Maize silage	**14**	34.14	92.23	7.19	7.83	67.4	43.0	40.2	47.4	29.9	25.4	2.40	27.00	3.55	<0.2
Maize silage	**15**	34.84	93.48	8.25	6.06	66.7	39.6	38.7	48.3	27.4	24.5	2.40	29.10	2.95	<0.2
Maize silage	**16**	29.06	92.02	8.97	11.15	-	48.3	46.0	-	31.5	27.8	2.70	22.45	-	<0.2
	Mean	**31.81**	**92.85**	**7.91**	**8.81**	**67.3**	**44.2**	**42.5**	**51.0**	**30.2**	**27.0**	**2.94**	**24.66**	**3.17**	**-**
	SE	**0.718**	**0.226**	**0.173**	**0.440**	**1.12**	**0.83**	**0.89**	**1.08**	**0.54**	**0.57**	**0.131**	**0.903**	**0.171**	**-**
Oat straw	**12**	**92.50**	**91.70**	**8.19**	**7.63**	**34.4**	**57.9**	**56.5**	**58.0**	**37.3**	**34.1**	**3.60**	**9.20**	**1.52**	**5.60**
Rice straw	**13**	**93.00**	**84.67**	**4.40**	**29.83**	**29.6**	**69.7**	**63.7**	**46.5**	**48.8**	**38.5**	**4.40**	**3.00**	**2.02**	**2.90**
Wheat silage	**4**	36.01	86.83	11.81	10.05	76.7	50.6	46.7	51.0	38.0	30.6	3.90	12.20	2.42	<0.2
Wheat silage	**7**	33.08	89.80	12.00	6.25	75.8	46.2	44.1	49.2	32.6	28.0	4.00	13.90	3.55	1.0
Wheat silage	**11**	27.85	85.39	12.20	10.25	70.7	56.3	52.4	54.4	40.8	34.2	4.40	4.00	2.48	<0.2
Wheat silage	**13**	31.22	88.64	9.10	13.05	76.8	57.2	53.1	57.6	40.2	33.0	4.50	4.90	2.26	1.5
Wheat silage	**14**	40.16	90.04	7.88	8.73	72.0	47.9	44.8	45.9	34.2	28.4	3.60	14.90	2.41	4.6
	Mean	**33.66**	**88.14**	**10.60**	**9.67**	**74.4**	**51.6**	**48.2**	**51.6**	**37.2**	**30.8**	**4.08**	**9.98**	**2.62**	**2.37**
	SE	**2.095**	**0.892**	**0.885**	**1.106**	**1.27**	**2.21**	**1.90**	**2.03**	**1.62**	**1.23**	**0.166**	**2.303**	**0.234**	**-**
Wheat straw	**5**	92.80	90.71	10.38	6.63	43.4	52.1	50.6	45.5	35.2	31.2	3.60	12.50	1.01	5.80
Wheat straw	**15**	92.70	90.39	6.63	13.21	26.0	66.2	63.5	46.0	44.2	38.3	4.60	7.30	0.96	3.30

[1] As a % of CP
[2] As a % of aNDF
[3] Fibre expressed exclusive of residual ash
* Lucerne chop were collected twice at 2 to 4 day intervals. Analyzed component values were averaged
** Large (L) and small (S) bales of lucerne were identified by the farm. It wasn't categorized according to quality
*** High (HQ) and Low (LQ) quality lucerne as specified by each farm

Table 3. 3: Chemical analysis (% DM) of high group TMR sampled at 16 dairies*

Farm number	1	2	3	4	5	6	7**	8**	9	10	11	12	13	14**	15	16	Avg	NRC***
DM	55.20	61.63	55.51	57.20	61.65	59.60	63.10	59.29	62.22	52.00	53.95	59.79	45.20	63.42	61.62	58.53	**58.12**	
OM	92.40	91.89	90.87	90.99	92.54	92.17	93.15	90.59	91.59	91.40	91.27	90.74	90.47	92.01	92.23	92.30	**91.66**	
CP	17.31	18.16	17.47	16.28	16.00	15.88	17.13	17.38	17.98	16.84	18.50	17.31	16.47	17.53	16.81	18.88	**17.25**	16.0 - 16.7
ADICP[1]	5.44	5.88	5.72	7.29	6.45	7.30	6.38	6.30	4.45	5.57	7.09	5.76	7.63	6.61	8.37	8.40	**6.61**	
SolCP[2]	39.42	38.39	37.31	37.92	35.70	39.68	34.82	40.39	40.92	39.16	41.87	34.53	39.50	34.77	40.42	36.86	**38.23**	
aNDF	27.85	32.95	28.95	30.15	32.80	29.85	32.10	31.10	32.43	30.10	33.50	27.80	34.03	31.85	30.85	32.75	**31.19**	25 - 33
aNDFom[3]	27.05	31.40	27.70	28.95	31.90	28.80	31.25	29.90	30.65	29.05	32.25	26.25	32.63	30.65	29.85	31.65	**30.00**	
dNDF30[4]	47.72	52.40	44.26	46.49	44.11	41.18	53.75	41.27	46.85	46.87	45.50	46.54	48.03	46.74	43.33	47.25	**46.39**	
ADF	19.30	21.25	20.70	23.05	21.60	20.65	20.60	22.05	21.00	21.45	21.90	20.20	24.40	21.65	21.30	22.80	**21.49**	17 - 21
ADFom[3]	18.30	20.00	19.10	21.25	20.75	19.60	19.65	20.80	19.30	20.15	20.70	18.35	22.10	20.35	20.60	21.70	**20.17**	
Lignin(sa)	3.50	4.80	2.75	4.95	4.85	4.90	4.40	4.75	3.43	4.25	4.60	3.40	4.40	4.80	5.90	4.35	**4.38**	
Starch	15.90	15.35	22.10	19.50	18.25	16.30	19.40	20.40	20.63	20.60	14.30	20.90	10.65	17.35	19.20	18.00	**18.05**	
Fat	5.47	5.39	4.68	4.84	5.14	5.19	5.95	5.04	5.09	5.09	7.03	7.06	6.20	5.84	5.30	7.62	**5.68**	
Sugars	4.90	3.25	2.95	3.25	4.55	4.90	3.55	3.25	2.03	1.60	2.65	1.55	3.18	3.35	4.15	2.50	**3.23**	
NE_L (MJ/kg)[5]	7.50	7.35	7.07	7.09	6.98	7.04	7.60	6.80	7.11	7.19	7.10	7.56	7.03	7.21	7.05	7.37	**7.19**	6.74
% DM																		
Ca	0.96	0.82	1.03	0.91	0.79	0.72	0.64	1.04	0.76	0.94	0.85	1.15	0.85	0.83	0.88	0.93	**0.88**	0.60 - 0.67
P	0.43	0.53	0.36	0.42	0.44	0.48	0.44	0.50	0.43	0.42	0.50	0.50	0.49	0.46	0.42	0.46	**0.46**	0.36 - 0.38
K	1.48	1.55	1.73	1.74	1.60	1.84	1.53	2.06	1.63	1.77	1.78	1.39	1.65	1.64	1.65	1.55	**1.66**	1.06 - 1.07
Mg	0.35	0.29	0.42	0.33	0.36	0.30	0.27	0.33	0.40	0.37	0.32	0.41	0.28	0.26	0.39	0.29	**0.34**	0.20
S	0.25	0.35	0.25	0.27	0.27	0.22	0.29	0.27	0.34	0.24	0.31	0.26	0.30	0.29	0.28	0.31	**0.28**	0.20
Na	0.35	0.36	0.43	0.52	0.26	0.38	0.21	0.41	0.32	0.50	0.58	0.51	0.58	0.38	0.30	0.38	**0.40**	0.22
Cl	0.57	0.54	0.65	0.73	0.59	0.58	0.48	0.83	0.64	0.46	0.60	0.37	0.77	0.42	0.41	0.50	**0.57**	0.28 - 0.29
ppm DM																		
Zn	59.5	63.0	78.5	103.5	73.5	43.0	52.5	103.0	71.5	67.5	74.5	110.0	84.5	57.0	78.0	72.5	**74.5**	52 - 55
Mn	40.5	46.0	77.5	69.0	69.0	32.0	38.5	95.5	62.8	58.5	75.5	73.5	54.8	55.0	81.0	58.0	**61.7**	13
Fe	282.0	313.5	410.0	272.5	164.5	304.5	188.5	286.0	385.3	193.5	269.0	506.5	295.8	260.0	170.5	266.0	**285.5**	17 - 18
Cu	10.3	21.4	18.2	24.3	12.6	8.0	6.8	25.7	12.2	23.2	13.8	22.7	14.9	12.8	12.4	10.0	**15.6**	11
Co	0.2	0.5	1.3	1.2	0.7	0.2	0.2	1.1	1.2	0.3	0.5	0.5	0.3	1.0	0.5	1.3	**0.7**	0.11
Se	0.35	0.44	0.39	0.46	0.52	0.25	0.25	0.60	0.44	0.22	0.47	0.60	0.63	0.44	0.53	0.34	**0.43**	0.30

* All Values represent average of two TMR samples

** Values only represent one TMR sample

*** NRC Values for 45 to 50 kg/day milk production

1 Acid detergent insoluble CP, an estimate of indigestible CP, as a % of CP

2 As a % of CP

3 Fibre expressed exclusive of residual ash

4 As a % of aNDF

5 Net energy available for lactation, calculated from equations utilizing chemical assays and *in vitro* determinations as described by Robinson *et al* (2004)

Chemical compositions of the two TMR samples from each dairy were analyzed separately and averaged (Table 3.3). Due to changes in the ration, or difficulties encountered during TMR mixing, only one of the TMR samples was analyzed for dairies 7, 8 and 14. Average values for all 16 dairies, and minimum NRC (2001) recommendations where appropriate, are listed for comparison. Almost all major nutrient requirements were met by the 16 TMR's, with no substantive undersupply. There is also high consistency among the dairies in the chemical composition of the TMR's and the energy available for lactation ($\mathbf{NE_L}$).

Table 3.4 shows the ingredient profiles (as a % of DM) of the ration mixed for the specified pen on each of the 16 dairies. This information was obtained using the computerized feed programs, which provided the actual weights of each ingredient added to the TMR during the week of the survey, or mix sheets which represent the theoretical TMR. Weights were converted to percentage using the analyzed DM for each ingredient. Average ingredient inclusion levels are also listed for easy comparison. In some cases, as in dairy 2, accurate information on the composition of added milk cow minerals were lacking. Some ingredients were used in more than 80% of the dairies while a few odd ingredients were only found in one or two of the rations. Maize products (mainly maize grain, DDG and maize silage with maize gluten feed in two and maize earlage in one of the dairies) make up 41% of the DM on average, ranging from 31 to 55 % of DM.

Weak relationships between the CP content of the TMR and both the inclusion level of maize products (r^2 = 0.21) and the maize CP contents of the TMR (r^2 = 0.26), suggest that neither increased inclusion of maize products in the TMR or increased contribution of maize CP to TMR DM had any significant impact on the total CP content of the TMR (Figure 3.1). The proportional contribution of CP from maize products to the total TMR CP did, however, increase with the level of maize in the ration (Figure 3.2).

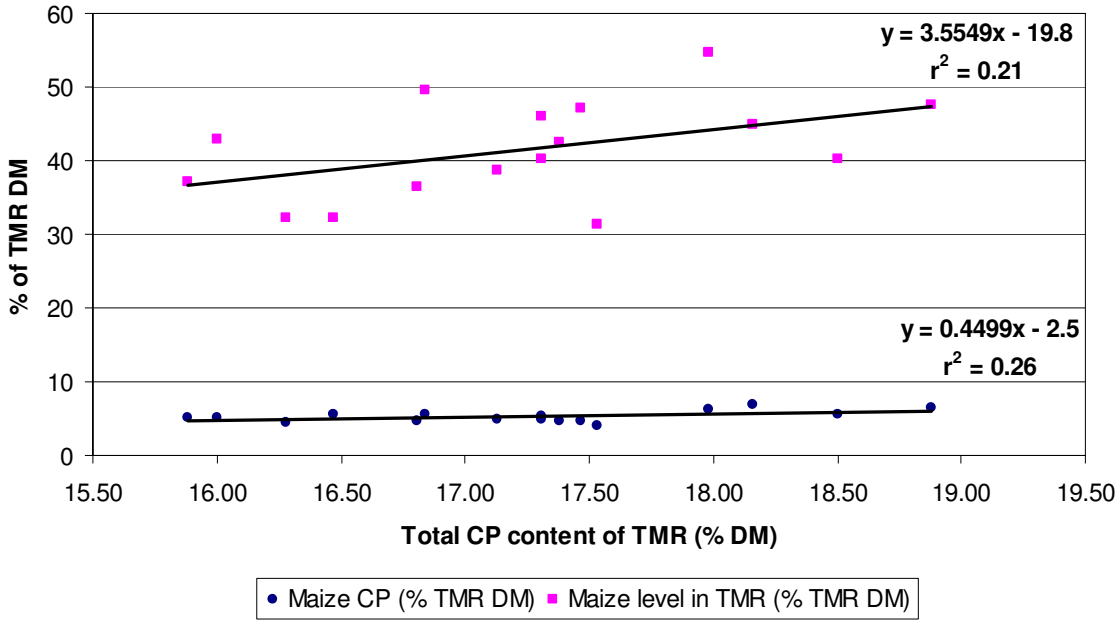


Figure 3.1: The effect of maize inclusion levels (upper) and maize CP (lower) on the CP contents of TMR

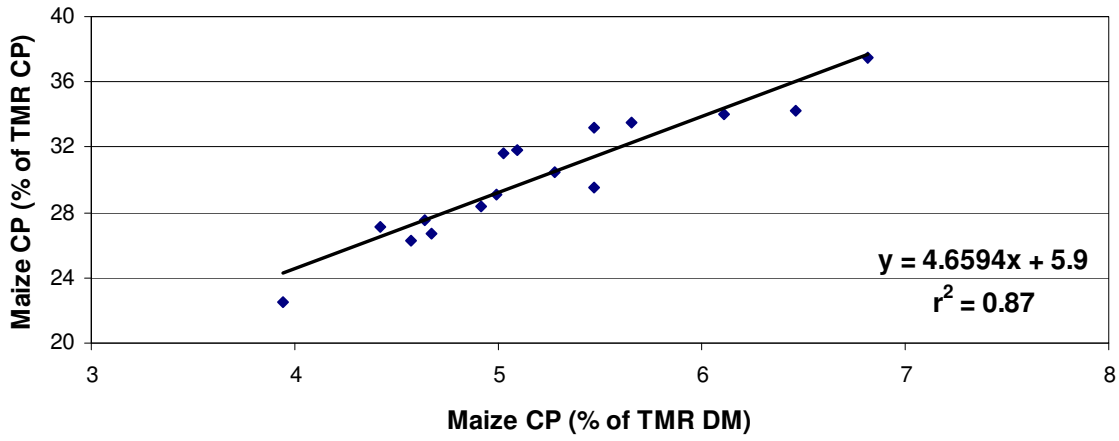


Figure 3.2: The contribution of maize CP to total TMR CP as a result of increased inclusion of maize products to the ration

This indicates that rations were being formulated according to the CP content of each ingredient rather than the inclusion level of the protein sources themselves, achieving reasonable TMR CP levels without increasing the amount of protein excreted in urine and faeces.

3.3.2 *Description of dairies*

The 16 dairies were characterized in terms of general farm management, milk production and composition, as well as intake levels and general characteristics of the cows in the specified high

group pens (Table 3.5). Production levels were used to assign dairy numbers starting with the lowest production of 32.7 kg/cow/day on dairy 1, increasing to 51.3 kg/cow/day on dairy 16 (Figure 3.3).

Milk yield was similar between dairies even though farms with a wide range of conditions were included. The survey included free stall and dry lot dairies, with 800 to 5000 cows milked 2 to 3 times a day in older 'flat barns' or a modern double 40 parallel milking parlour.

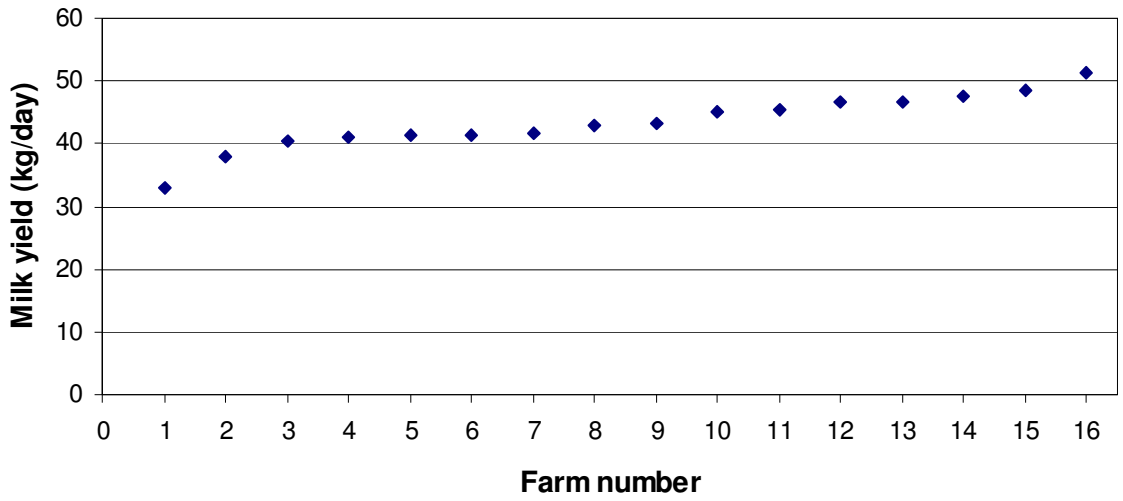


Figure 3.3: Average milk production (kg/d) across 16 dairies

Average DIM were calculated together with the 10th and 90th percentiles (10% less than highest and 10% higher than the lowest DIM) to exclude extreme values and give a better representation of DIM profiles of the cows in the pens. The number of cows in the high group pen represents only one pen, except where feed from one load were split between two relatively identical pens and uncertainties in the weight of TMR dropped at each pen necessitated combination of those pens for more accurate intake calculation. These dairies were numbers 7, 10, 14 and 15.

Intake levels were calculated from the amount of feed dropped, estimated or calculated refusals (orts) and cow numbers, together with analyzed TMR DM values, giving average intakes per cow. All other information was obtained using the dairy herd management programs and DHIA records and a complete description of the 16 dairies can be seen in Appendix A3 (Table A3.1)

Table 3. 4: Ingredient profiles (%DM) of high group TMR sampled at 16 dairies

Farm number	1	2*	3	4	5	6	7	8	9	10	11	12	13	14	15	16	*Average*
Forages:																	
Lucerne chop					9.73			24.17		20.52	2.1		3.76	5.72	20.48		12.35
Lucerne hay	22.62	16.85	19.27	18.9	8.18	21.75			23.82	5.13	7.67	20.6	10.31	20.1		14.38	16.12
Lucerne silage		7.24	4.69								3.93					7.76	5.91
Maize earlage									16.5								16.50
Maize silage	16.31	19.56	23.14		18.39	13.43	12.04	21.79	23.26	23.8	14.77	14.85	11.02	7.13	12.92	21.84	16.95
Oat straw/hay									1.65			1.59					1.62
Rice straw													1.85				1.85
Wheat silage				14.81			14.65				5.01		12.43	8.31			11.04
Wheat straw/hay					1.52										0.50		1.01
Plant products, grains and seeds:																	
Almond hulls	7.24	10.90	2.80	8.70	13.39	22.01	4.94	6.99			11.34		3.84	2.56	15.26	7.85	9.06
Barley, rolled							5.43										5.43
Beet pulp shreds							7.90							3.62			5.76
Brandy pomace													1.23				1.23
Canola pellets		6.10		4.54			7.42				3.78		8.22	7.46	8.30	7.94	6.72
Carrot pulp				4.66													4.66
Citrus pulp	3.37											9.27			3.01		5.22
Maize grain, flaked	15.02	10.03	18.43	24.77	17.64	15.18	18.69	15.70	8.40	19.81	16.68	26.50	8.49	17.90	17.87	15.33	16.65
Maize grain, ground			1.64														1.64
Maize gluten feed		6.12											3.65				4.89
Maize gluten meal			0.34														0.34
Cottonseed, whole linted	4.50		8.48	6.52	6.25	6.62		6.51	6.67	7.75	8.07	12.05	6.87		6.20	9.60	7.39
Cottonseed, ground pima		6.24					11.39							10.06			9.23
Cottonseed, meal										6.27							6.27
Distillers grains, dry	8.97	9.22	3.50	7.35	6.77	8.53	7.12	4.98	6.58	5.95	3.17	4.76	2.87	5.68		10.30	6.38
Distillers grains, wet											5.50		6.23		5.60		5.78
Linseed, meal									7.79								7.79
Linseed, pellets					6.76												6.76
Raisin tailings													2.76				2.76
Rice bran	2.64																2.64
Soy hulls			1.69														1.69
Soybean, meal	6.17		6.63	5.47		6.71		6.70				5.51					6.20
Soyplus			1.09					0.41									0.75
Wheat midds/millrun	5.28				8.15		7.61	4.27			7.65			8.23	6.00		6.74

Farm number	1	2*	3	4	5	6	7	8	9	10	11	12	13	14	15	16	*Average*
Miscellaneous:																	
Almond shells																0.23	0.23
Blood meal			0.72														0.72
Maize/distillers syrup							0.88							0.69			0.79
Fat (animal)	0.97												0.49				0.73
Fat (liquid)								0.69									0.69
Fat (rumen inert)			1.09	1.28			1.41	0.36	0.77	1.81			3.93	1.01	0.63	2.26	1.46
Fish meal								0.40									0.40
Generator D			0.0004								0.02						0.01
Millrun+tallow mix											3.68						3.68
Mineral mixes	0.69	7.73**	2.19	1.51	1.78	2.1	0.52	0.35	0.96	2.19	1.48	4.44**	2.13	1.53	1.87	0.58	1.42
Molasses			0.97		1.42			3.45	2.33						1.06		1.85
Prolac									0.69								0.69
Salt																0.57	0.57
Sodium Bicarbonate	0.49		0.74	0.99		0.73		0.45	0.59	0.92	0.77		0.90			0.57	0.72
Urea	0.29		0.14	0.46		0.60		0.37		0.56	0.33	0.25	0.26		0.28	0.39	0.36
Water			0.02	0.03	0.01												0.02
Whole Cottonseed replacer											1.84						1.84
Whey (liquid)	5.13		2.38			2.34		2.31		5.30	2.22		8.76				4.06
Yeast	0.3											0.19				0.4	0.30
Total amount of maize products used	40.30	44.93	47.05	32.12	42.80	37.14	38.73	42.47	54.74	49.56	40.12	46.11	32.26	31.40	36.39	47.47	41.47

* Accurate information on the composition of the milk cow mineral was not provided by the dairy
** Inclusion level of top mix/premix consisting of a mineral mix and other ingredients

Table 3. 5: Description of 16 dairies, cows and pens designated by the dairy as one of their high group multi-parity corrals

Farm number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
General information																
Total lactating cows	1000	1143	3000	1192	1809	2772	824	5000	1200	2648	2200	4100	5000	932	4400	1378
Milkings/day	2	2	2	2	2	3	2	2	3	2	2	3	3	2	2	3
Animals																
Cows in high group pen*	149	123	170	190	189	145	265**	408	158	513**	191	264	364	223**	587**	167
Days in milk, 10th %	84	46	57	99	29	22	112	36	89	94	63	91	31	35	37	42
Average	221	97	87	132	86	87	199	108	210	157	111	160	92	133	124	88
90th %	345	141	119	170	129	139	291	170	321	236	164	226	156	218	218	134
Parity (%) 1	5	22	0	0	15	1	0	2	1	2	0	4	1	0	0	1
2	62	53	49	30	58	52	39	11	59	47	44	46	48	16	0	37
> 3	33	25	51	70	27	47	61	87	40	51	56	50	51	84	100	62
Parity (maximum)	6	8	7	9	5	8	6	10	5	8	9	8	6	9	10	9
Production																
Milk yield (kg/d)	32.8	37.9	40.3	40.9	41.2	41.4	41.7	42.8	43.3	45.2	45.4	46.6	46.7	47.7	48.5	51.3
Milk yield (lb/d)	72.3	83.5	88.8	90.1	90.8	91.2	91.9	94.3	95.4	99.6	100.1	102.7	102.9	105.1	106.9	113.1
True Prot %	3.23	2.91	2.77	2.88	2.93	2.87	3.13	2.81	3.00	2.72	2.84	2.95	2.87	2.92	3.01	2.73
Fat %	3.32	3.49	3.19	3.67	3.14	3.49	3.54	3.08	3.68	3.04	3.32	3.54	3.19	3.49	3.45	3.79
SCC (,000)	739	270	75	187	70	122	262	264	219	163	132	95	375	438	416	364
Intakes																
As fed (kg/d)	43.9	34.7	48.3	43.5	46.2	37.8	45.0	44.9	40.2	52.5	48.2	45.8	53.0	41.1	48.8	49.9
As fed (lb/d)	96.8	76.5	106.3	95.9	101.8	83.3	99.2	98.9	88.6	115.7	106.1	101.0	116.9	90.5	107.6	110.0
DM basis (kg/d)	24.2	21.4	26.8	24.9	28.5	22.5	28.4	26.6	25.0	27.3	26.9	27.4	24.0	26.0	30.1	29.2
DM basis (lb/d)	53.4	47.1	59.0	54.9	62.8	49.6	62.6	58.6	55.1	60.2	59.4	60.4	52.9	57.4	66.3	64.4
TMR DM %	55.2	61.6	55.5	57.2	61.7	59.6	63.1	59.3	62.2	52.0	54.0	59.8	45.2	63.4	61.6	58.5

* Number of cows in the single high group pen used for the survey
** Number of cows in two, very similar pens, fed from the same truck, combined

3.3.3 *Animal performance*

To determine the possible impact of increased dietary maize protein levels on animal performance, correlations were drawn between various parameters to determine if there was any relationship between them.

3.3.3.1 *Effect of increased contribution of maize crude protein to total TMR crude protein on milk production*

Most of the rations had a CP level higher than NRC requirements (Table 3.3) and since 20 – 40% of total TMR CP come from maize products (Figure 3.2), a negative effect on milk production, due to the poorly balanced AA profile of maize proteins, might be expected. However, this does not seem to be the case since there is no relationship between the contribution of maize CP to the ration and milk production (Figure 3.4). A similar comparison was made between total maize inclusion level in the TMR and milk yield (not shown) with the same result.

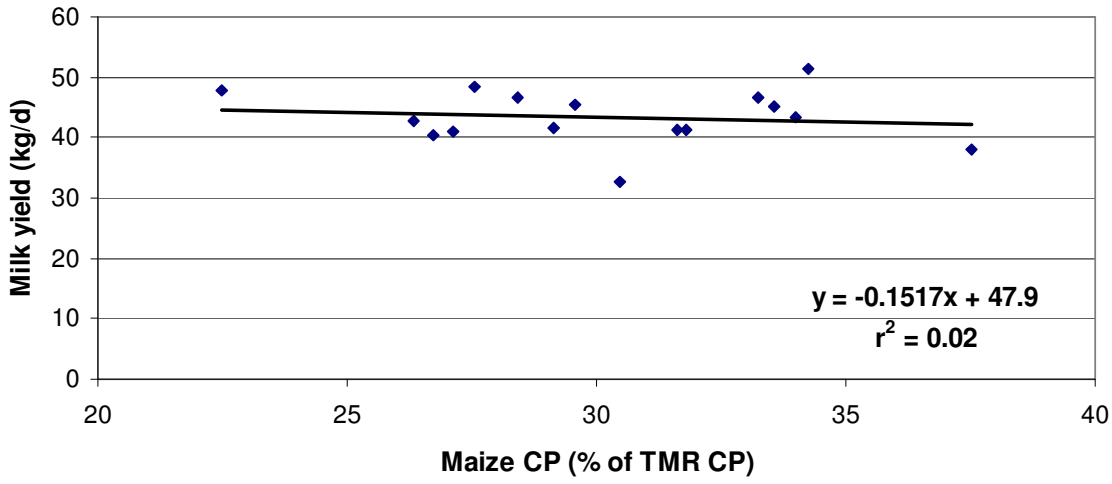


Figure 3.4: The response of milk yield (kg/d) to the increased contribution of maize CP to total TMR CP.

As discussed earlier, milk yield is usually less sensitive than milk components (i.e., protein and fat) to changes in AA profiles of intestinally delivered protein. Another comparison was therefore made between the maize CP content and percentages of protein and fat in milk (Figure 3.5). Once again there was no relationship between the two variables, indicating that total maize CP, as a percentage of TMR CP, had no effect on cow performance, suggesting that rations are being

balanced according to the nutrient requirements of the cows and, even though maize proteins make up a large proportion of total CP consumed, their unbalanced AA profile is offset by inclusion of other, possibly complementary, protein sources such as canola pellets, whole cottonseed, soybean meal and small amounts of animal protein sources (i.e., blood and fish meal).

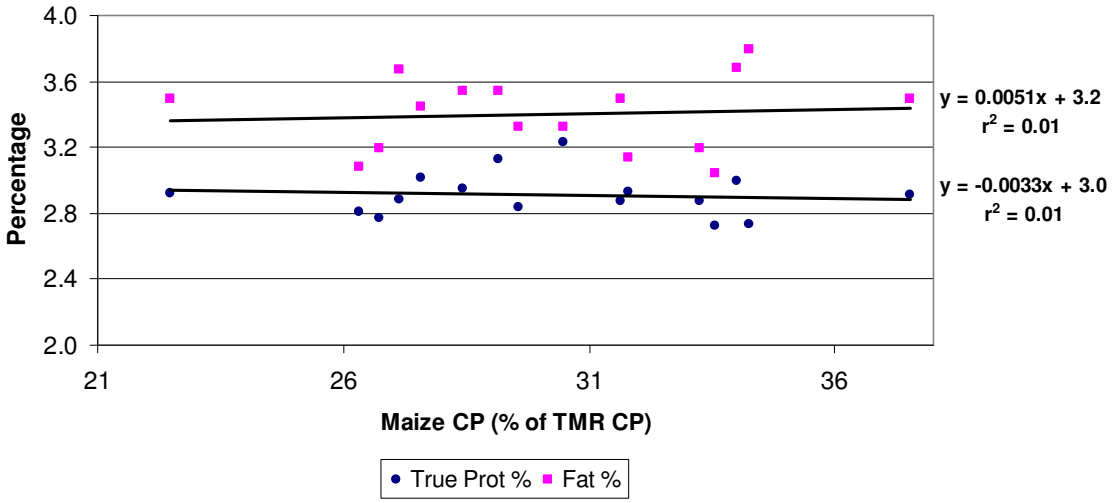


Figure 3.5: The response of milk true protein and fat percentage to the increased contribution of maize CP to total TMR CP.

3.3.4 *Model evaluation*

From the model evaluations, some predictions common to the models were tabulated. These included predicted DMI, MP delivery and balance (referred to in Shield as AP), as well as the delivery and balance of metabolizable Met, Lys, His, Ile, Leu, Val, Arg and Thr (Appendix A3; Table A3.2).

CPM Dairy estimated only 88% of the measured DMI while Amino Cow estimated 96% and Shield 102%. Average estimated delivery of MP was essentially the same between CPM Dairy and Shield (2960 vs. 2928 g/d) while Amino Cow estimated only 2594 g/d. The MP balances estimated were 122, 104 and 99% of requirements for Amino Cow, CPM Dairy and Shield respectively. However there was substantial variation among dairies within model.

Balances of average metabolizable AA were generally positive for all models, with the exception of 9 negative balances for Met within Amino Cow and 6 for Ile in CPM Dairy. Lys was the only AA for which negative balances were predicted at one or more of the dairies by all three models (Table A3.2). The average balance of metabolizable Met ranged from -1 (Amino Cow) to

18 g/d (Shield), while Lys ranged from 9 (Shield) to 26 g/d (CPM Dairy) (Figure 3.6). The ratio between Lys and Met was above 3 for Amino cow and CPM Dairy (3.29 and 3.24) while Shield predicted 2.61. The His balance was higher for CPM Dairy (18 g/d) vs. Amino Cow (8 g/d) and Shield (7 g/d). The Ile balance was much higher for Amino Cow (32 g/d) vs. CPM Dairy (6 g/d) and Shield (9 g/d). Leu balances varied dramatically among models from a low of 16 g/d (CPM Dairy) to 37 g/d (Amino Cow) and 69 g/d (Shield). The Val balance was lower for Shield (147 g/d) vs. Amino Cow (162 g/d) and CPM Dairy (172 g/d). Arg balance for CP Dairy was 191 g/d, which was much higher than values of 144 and 145 g/d for Amino Cow and Shield. The Thr balance was very similar among models, ranging from 137 to 143 g/d.

A summary of the AA balances (Figure 3.6) illustrates major differences among the predictions of the three models. Balances for Met and Leu varied dramatically among models, while Val and Thr balances were very similar. Amino Cow and Shield predicted similar values for Lys and His while Amino Cow and CPM Dairy corresponded in their Ile predictions. There is no visible or consistent pattern in the variation among the models.

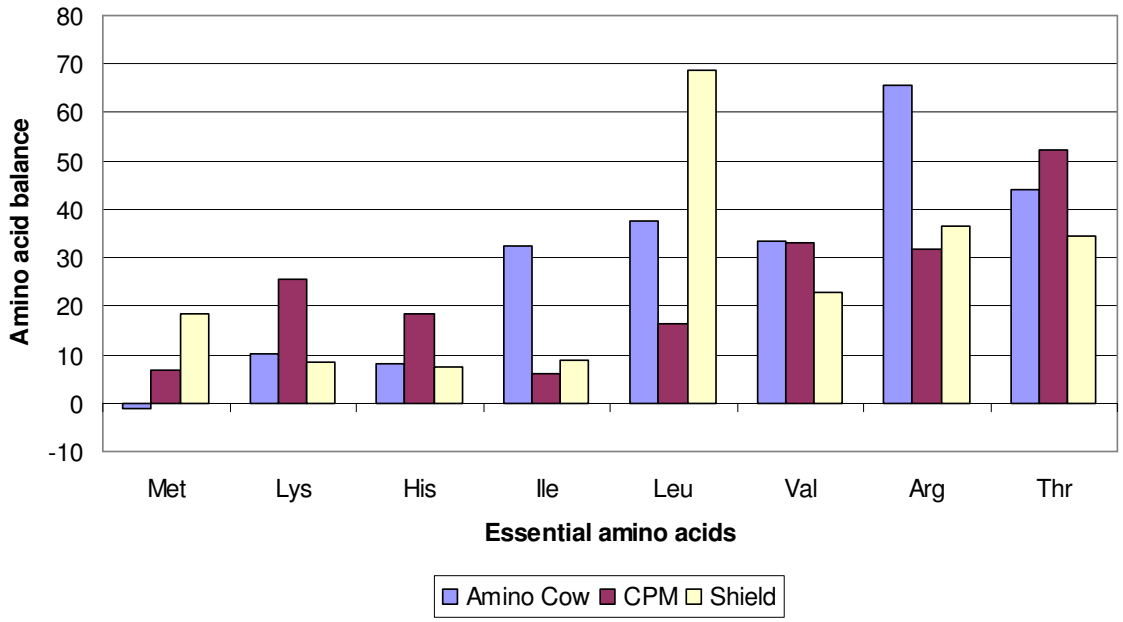


Figure 3.6: Balances of average metabolizable AA (difference between estimated AA requirement and delivery) for the 16 rations as estimated by Amino Cow, CPM Dairy and Shield.

3.3.4.1 *Effect of increased contribution of maize crude protein to total TMR crude protein on amino acid profile of metabolizable protein.*

Even though the models did not agree on the AA profiles of protein reaching the intestine, their predictions regarding the effect of increased maize levels in the diet on these AA profiles were very consistent. As might be expected due to the low level of Lys in maize proteins, all three models predicted the Lys to Met ratio in MP decreased as more maize protein was added to the TMR (Figure 3.7) even though the ratio itself differed sharply between the models.

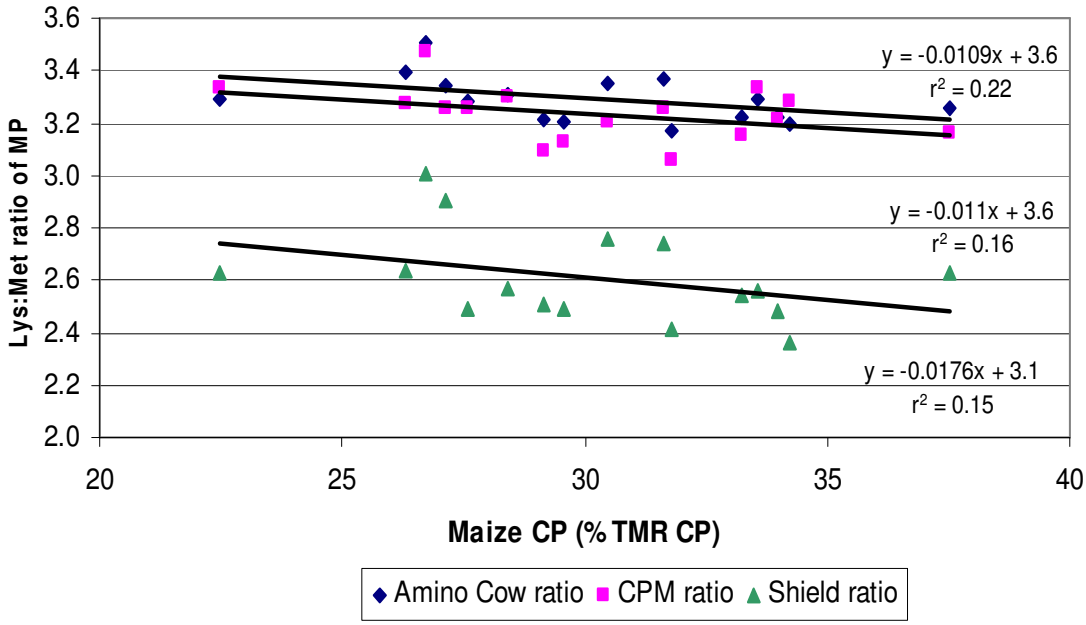


Figure 3.7: The predicted effect of maize CP in the TMR on the ratio of Lys to Met in MP according to Amino Cow, CPM Dairy and Shield.

The models also suggested a decrease in MP delivery with increased contribution of maize CP to total TMR CP (Figure 3.8) but none of them predicted any change in the percentage of Met or Lys in MP when maize CP in the TMR increased (Figure 3.9).

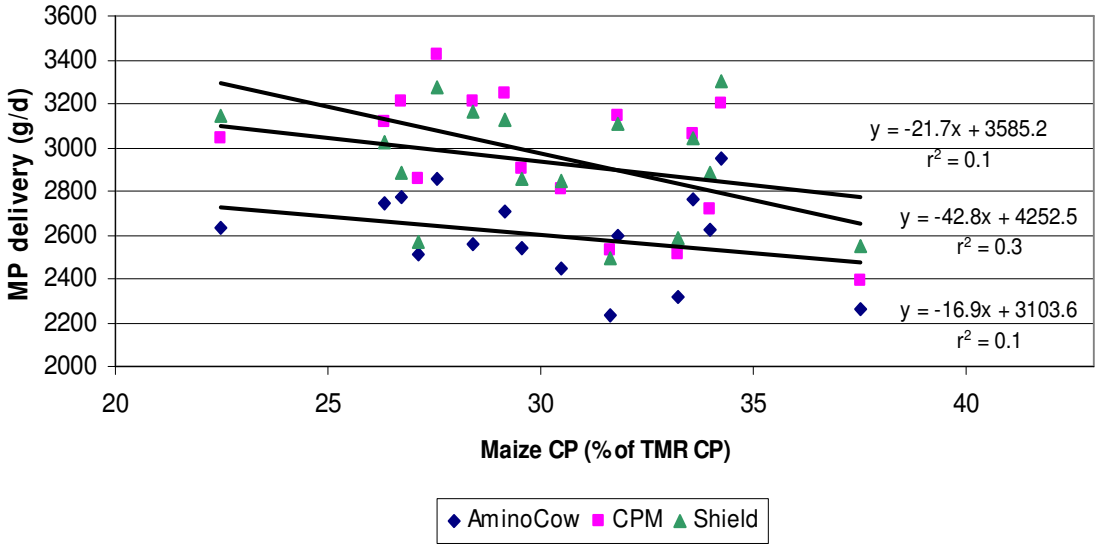


Figure 3.8: The predicted effect of maize CP in the TMR on MP delivery to the intestine according to Amino Cow, CPM Dairy and Shield.

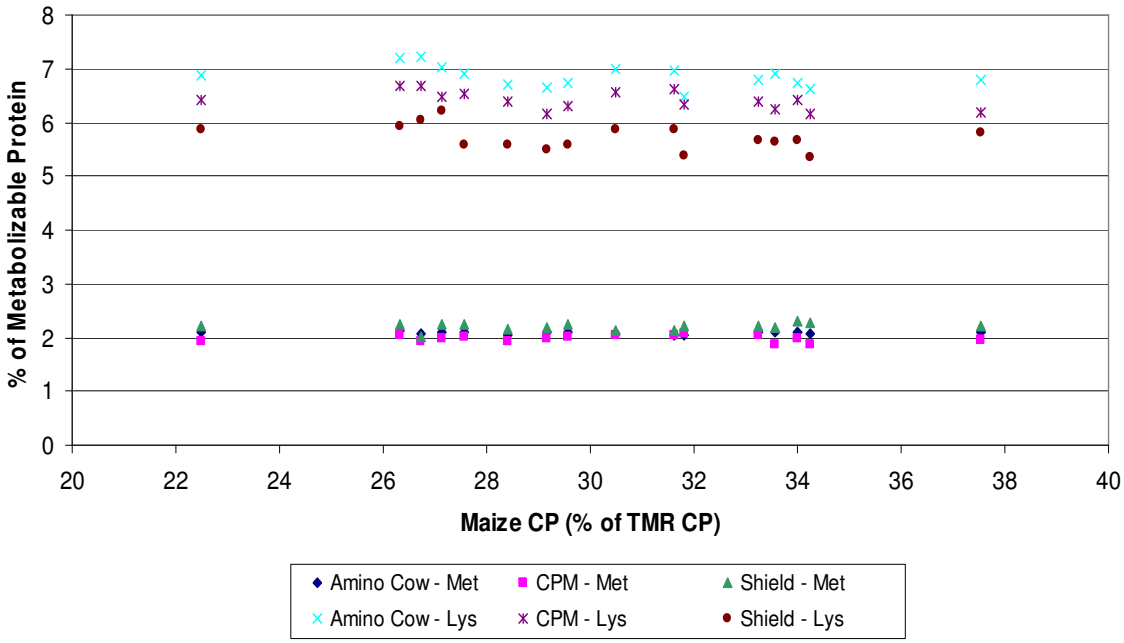


Figure 3.9: The predicted effect of maize CP in the TMR on percentages of Met and Lys in MP according to Amino Cow, CPM Dairy and Shield.

Most maize proteins are higher in RDP (~ 55%) than RUP, which could explain the predicted decrease of MP delivery when maize protein levels in the ration increased. That the AA levels in MP did not change, however, could be due to the increased proportional contribution of MCP (high in Lys and low in Met) to total MP, delivering a much better balance of AA to the intestine.

3.3.4.2 *Effect of increased contribution of maize crude protein to total TMR crude protein on milk composition.*

Even though there was no direct relationship between maize CP levels in the TMR and cow performance (Figure 3.4), it seemed to have caused a change in the ratio of EAA reaching the intestine which, in turn, might have impacted milk composition. However, neither the proportion nor the ratio of Lys and Met in MP had any affect on either milk fat (Figure 3.10) or milk true protein percentage (Figure 3.11). CPM Dairy was the only model that predicted, albeit to a very small extent, an increase in milk protein with a decrease in Lys to Met ratio (Figure 3.12), but this was due to increased delivery of Met, not a decrease in Lys, suggesting that the drop in Lys delivery to the intestine due to high inclusion levels of maize products was not large enough to have impacted milk protein, but the higher Met content of maize products possibly increased Met delivery, with a resulting impact on milk protein percentage.

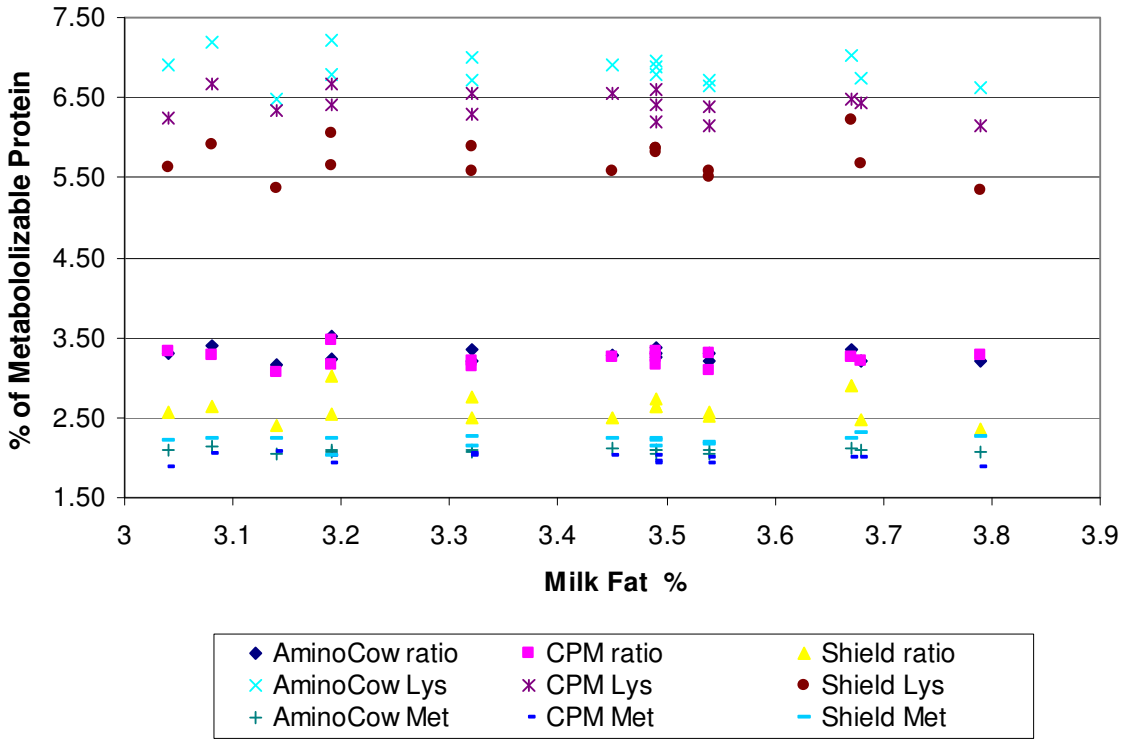


Figure 3.10: The response of milk fat percentage to changes in the predicted Lys to Met ratio and proportions of Lys and Met in MP according to Amino Cow, CPM Dairy and Shield.

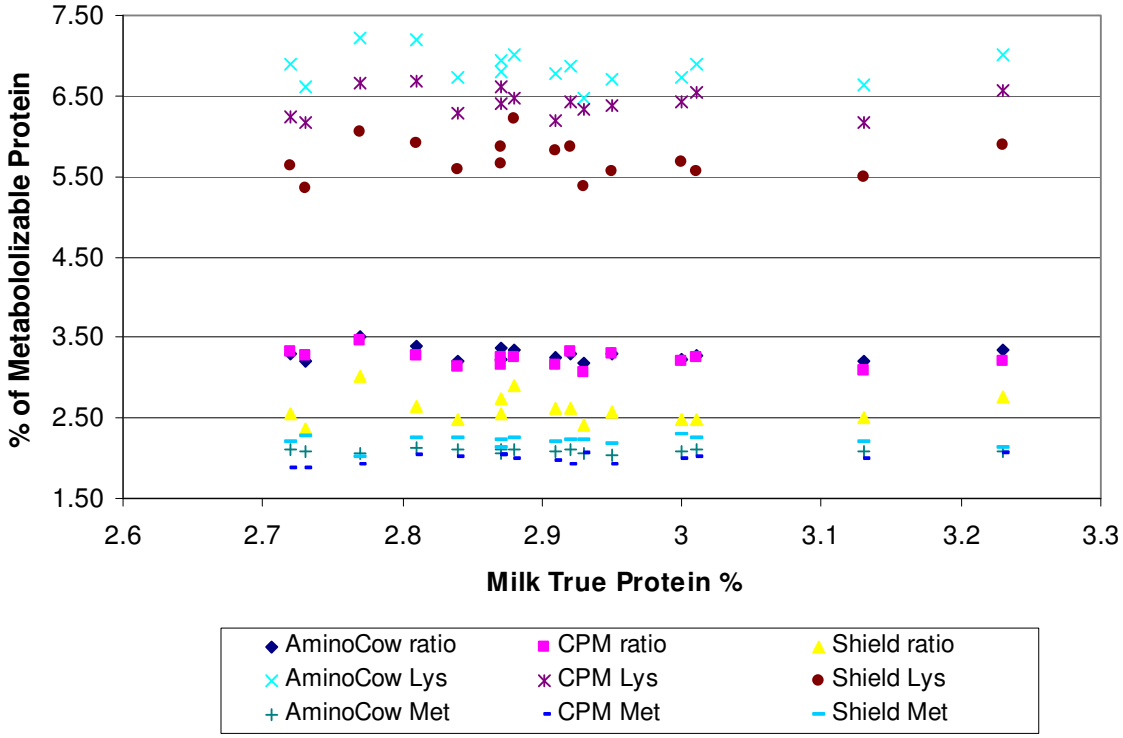


Figure 3.11: The response of milk true protein percentage to changes in the predicted Lys to Met ratio and proportions of Lys and Met in MP according to Amino Cow, CPM Dairy and Shield.

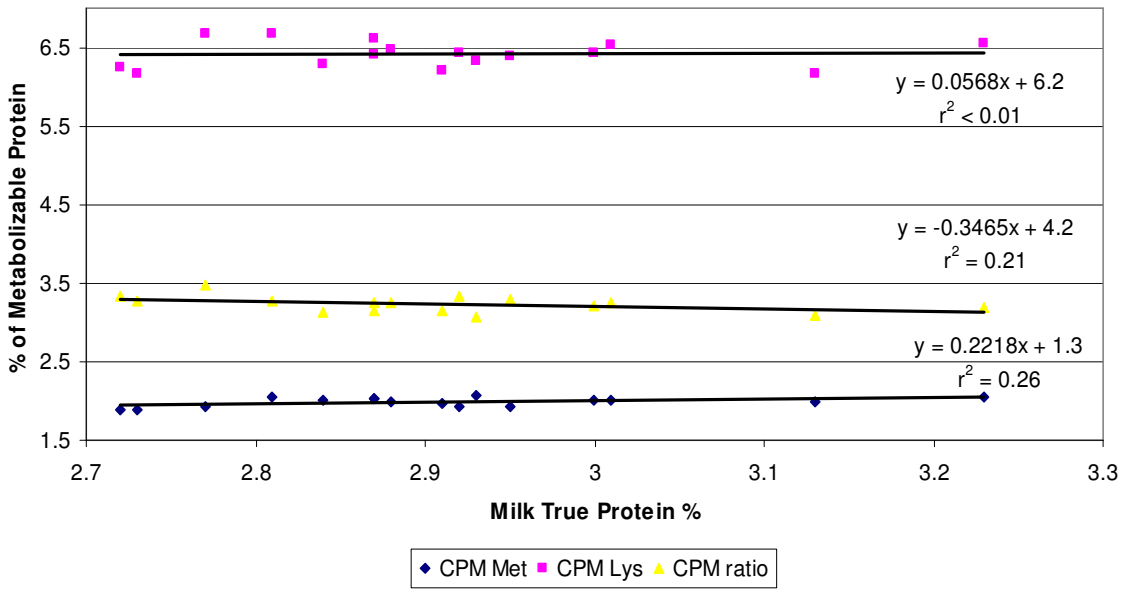


Figure 3.12: The response of milk true protein percentage to changes in the predicted Lys to Met ratio and proportions of Lys and Met in MP according to CPM Dairy.

However, the predicted increase in Met delivery (i.e., 1.88 to 2.07 g/d) only yielded half a percent increase in milk true protein, hardly a noticeable improvement. While CPM Dairy was the

only model to predict a correlation between AA and milk components (i.e., milk true protein), Shield was the only model to predict a correlation between AA and milk yield (Figure 3.13). Contrary to normal expectations, Shield predicted milk yield to increase when the ratio of Lys to Met decreased, due to higher Met and lower Lys proportions in MP (Figure 3.14), which corresponds with the AA levels in maize products.

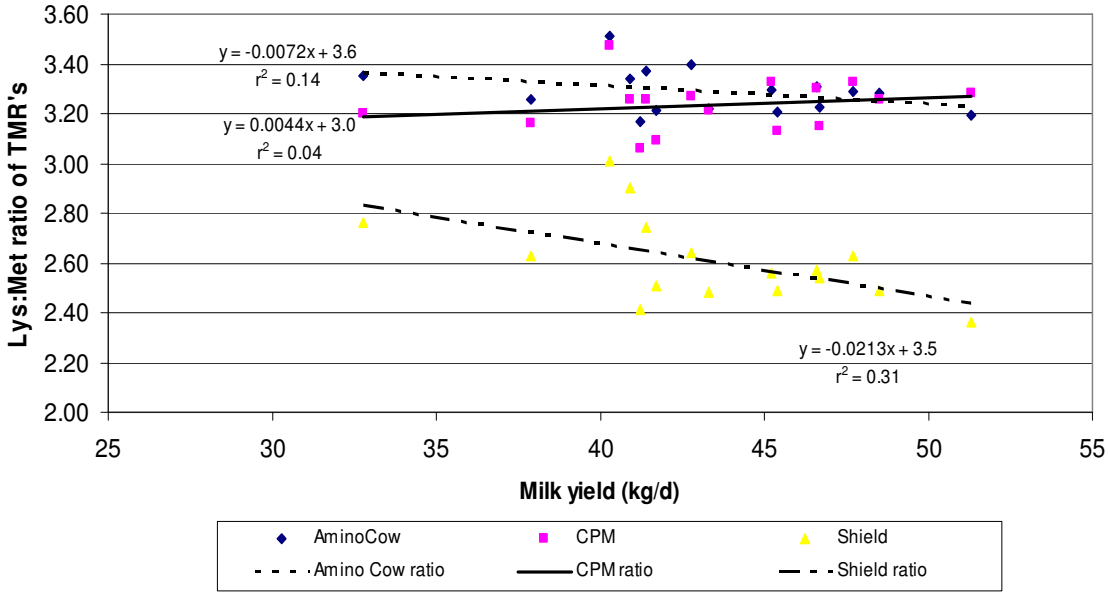


Figure 3.13: The response of milk yield (kg/d) to changes in the predicted Lys to Met ratio according to Amino Cow, CPM Dairy and Shield.

The yield increase of 18.5 kg/d, due to the changes in the ratio of Lys to Met (ranging from 3.01 to 2.36) predicted by Shield, is much more significant than the protein increase predicted by CPM Dairy. The possibility of increased dietary maize levels impacting milk yield therefore seems higher than for milk components.

Similar comparisons were made between other EAA and milk production (not shown), but no relationship was predicted by Amino Cow, CPM Dairy or Shield for either Ile, Leu or His.

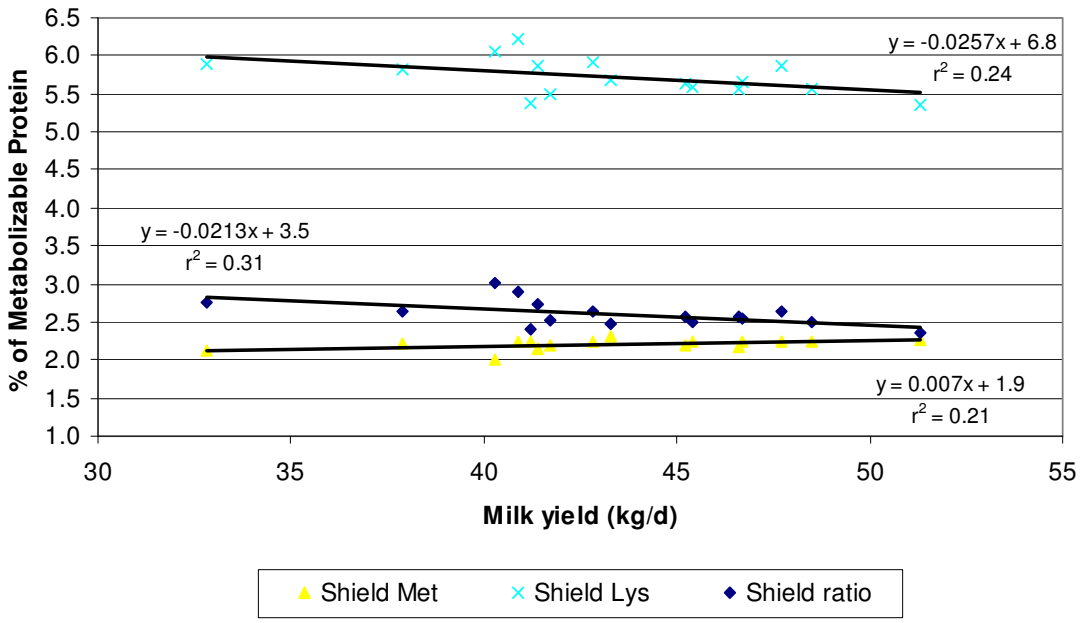


Figure 3.14: The response of milk yield (kg/d) to changes in the predicted Lys to Met ratio and proportions of Lys and Met in MP according to Shield.

3.3.4.3 *Predicted amino acid packages*

The sequence of AA limitation (Table 3.6) among dairies was the same within Amino Cow (i.e., Met, Lys, His, Leu, Val, Ile) and very similar within Shield (i.e., Lys, Ile, His, Val, Arg). In contrast, the sequence varied somewhat within CPM Dairy, although Ile and Leu were always (with one exception) either first or second limiting, Met and Lys were always third or fourth limiting followed by Arg, Val and His.

Based upon the evaluation of each ration by each model, average AA supplementation packages were calculated to bring model estimated AA deliveries to a minimum of 110, 120 and 130% of model estimated requirements (Table 3.7).

Table 3. 6: The sequence of amino acid limitation according to 'Amino Cow', 'CPM Dairy' and 'Shield'

Farm number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AA limitation sequence																
Amino Cow	Met	Met Lys His Leu	Met	Met Lys His	Met Lys His	Met Lys His Leu Val Ile	Met Lys His Leu	Met	Met Lys His Leu	Met Lys	Met Lys His Leu	Met Lys His Leu Val Ile	Met Lys His Leu Val Ile	Met Lys His Leu Val Ile	Met Lys His Leu	Met Lys His
CPM Dairy	Ile	Ile Leu Lys Met Arg Val		Ile Leu Met	Leu Ile	Ile Leu Met Lys Arg Val His	Ile Leu Lys Met	Ile Leu	Leu Ile Met Lys Arg Val His	Ile Leu Met Lys	Ile Leu Lys Met Arg Val	Ile Leu Met Lys	Ile Leu Lys Met Arg Val His	Ile Leu Met Lys Arg	Leu Ile Met Lys Arg	Ile Met Leu Lys Arg
Shield	Lys His	Lys Ile His	Ile		Lys Ile His	Lys Ile His Val	Lys Ile His Val Arg	Lys Ile His	Lys Ile His Val	Lys Ile	Lys Ile His Val	Lys Ile His Val	Lys Ile His Val Arg Thr	Lys Ile His Val	Lys Ile His Val	Lys Ile His

* Only AA predicted to be supplied below 120% of requirements are listed

Table 3. 7: Amino acid supplementation package sizes, and profiles, as predicted by 'Amino Cow', 'CPM Dairy' and 'Shield' to bring all amino acids to 130, 120 or 110% of estimated requirements.

	Package g/cow/d	Met %	Lys %	His %	Ile %	Leu %	Val %	Arg %	Thr %
To 130%									
Amino Cow	**86.4**	20.5	46.2	1.0	0.7	25.9	5.6	0.0	0.0
CPM Dairy	**143.6**	6.1	16.4	0.0	25.6	34.7	6.0	11.2	0.0
Shield	**85.2**	0.0	45.8	9.5	27.9	0.0	16.8	0.0	0.0
To 120%									
Amino Cow	**38.2**	32.0	60.7	0.8	0.0	6.4	0.0	0.0	0.0
CPM Dairy	**61.1**	5.9	11.7	0.0	36.8	45.4	0.0	0.2	0.0
Shield	**40.9**	0.0	56.6	7.1	31.6	0.0	4.7	0.0	0.0
To 110%									
Amino Cow	**13.2**	50.8	49.2	0.0	0.0	0.0	0.0	0.0	0.0
CPM Dairy	**13.9**	0.0	0.0	0.0	58.8	41.2	0.0	0.0	0.0
Shield	**9.3**	0.0	78.1	0.0	21.9	0.0	0.0	0.0	0.0
Amino Cow									
To 130%	**86.4**	20.5	46.2	1.0	0.7	25.9	5.6	0.0	0.0
To 120%	**38.2**	32.0	60.7	0.8	0.0	6.4	0.0	0.0	0.0
To 110%	**13.2**	50.8	49.2	0.0	0.0	0.0	0.0	0.0	0.0
CPM Dairy									
To 130%	**143.6**	6.1	16.4	0.0	25.6	34.7	6.0	11.2	0.0
To 120%	**61.1**	5.9	11.7	0.0	36.8	45.4	0.0	0.2	0.0
To 110%	**13.9**	0.0	0.0	0.0	58.8	41.2	0.0	0.0	0.0
Shield									
To 130%	**85.2**	0.0	45.8	9.5	27.9	0.0	16.8	0.0	0.0
To 120%	**40.9**	0.0	56.6	7.1	31.6	0.0	4.7	0.0	0.0
To 110%	**9.3**	0.0	78.1	0.0	21.9	0.0	0.0	0.0	0.0

Due to the differences among models in their predicted AA limitation sequences, the calculated amino acid supplementation packages varied sharply by model. In general, Amino Cow emphasized Met and Lys as being most limiting (Figure 3.15), CPM Dairy emphasized Ile and Leu (Figure 3.16), whereas Shield emphasized Lys and Ile (Figure 3.17). Only Thr appeared in no amino package, although Arg only appeared in CPM Dairy, and at low levels.

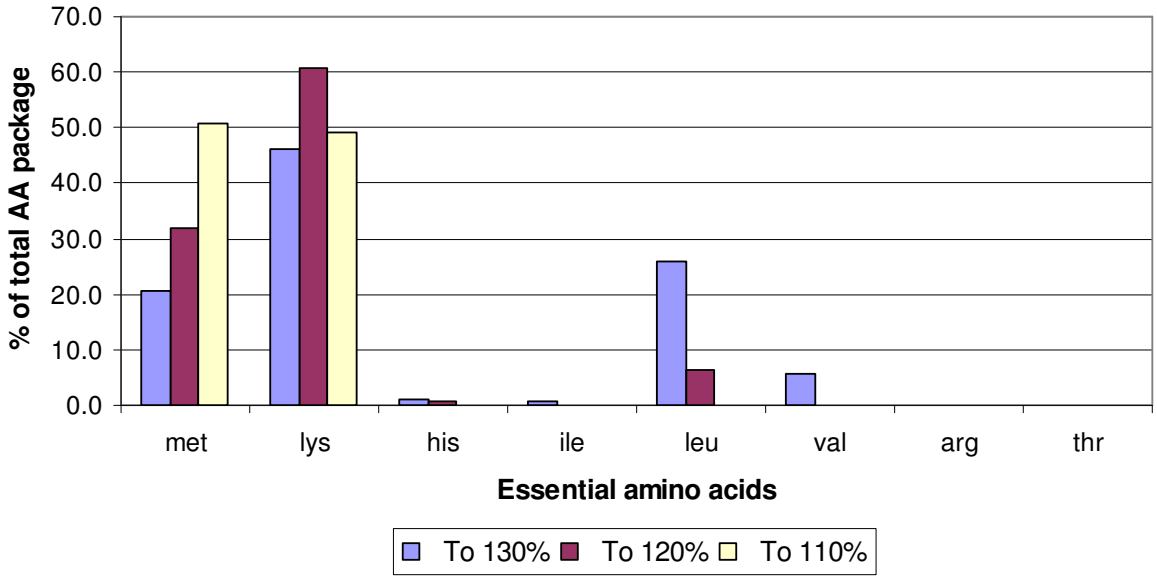


Figure 3.15: Supplementation packages for 130, 120 and 110% of requirements according to Amino Cow.

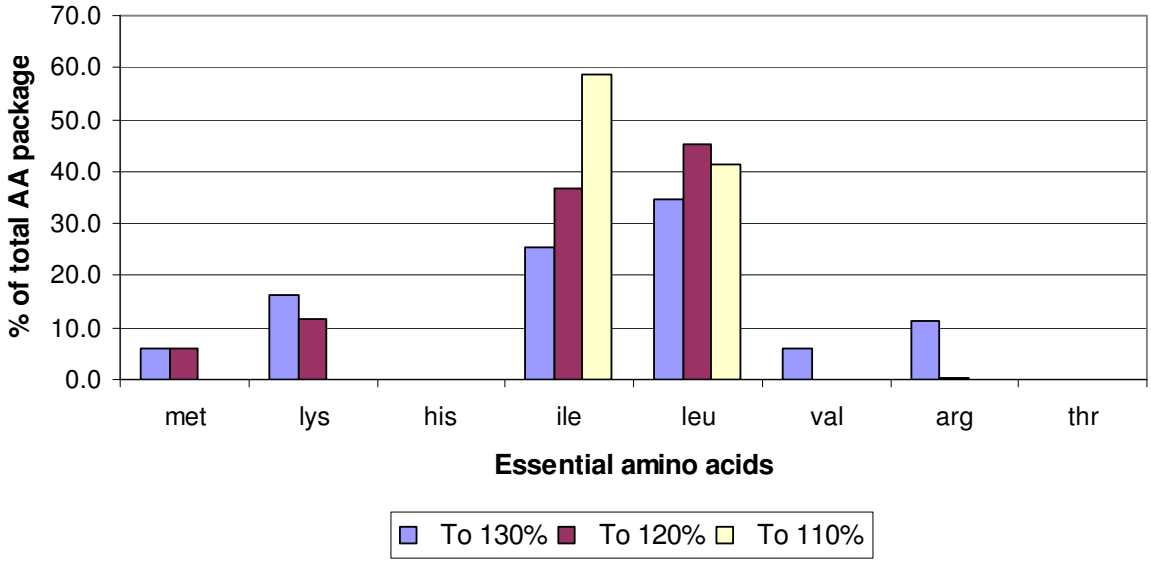


Figure 3.16: Supplementation packages for 130, 120 and 110% of requirements according to CPM Dairy.

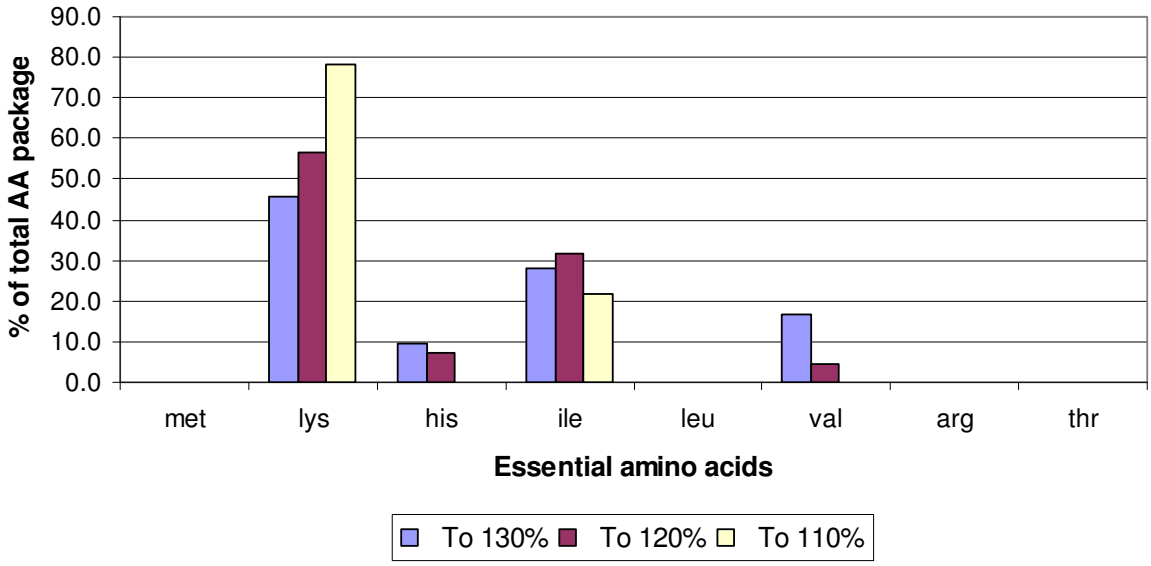


Figure 3.17: Supplementation packages for 130, 120 and 110% of requirements according to Shield.

Except at 110%, where the sizes of the AA packages were low (9 – 14 g/d), CPM Dairy required package sizes that were 50% (at 120%) to 70% (at 130%) higher than Amino Cow and Shield (Figure 3.18). This reflects the higher predicted animal requirements (g/d) for AA according to CPM Dairy.

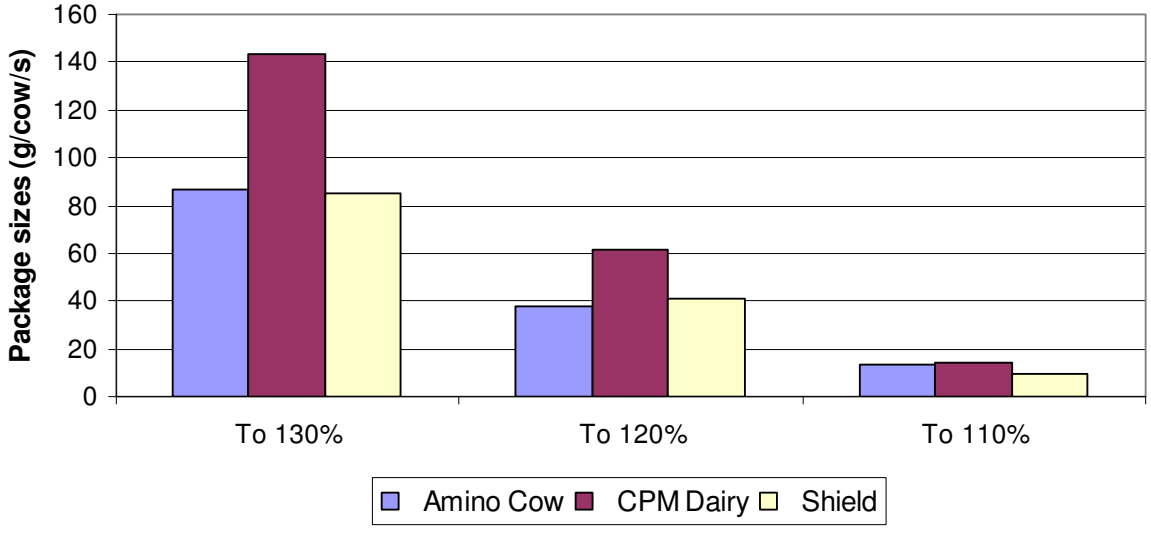


Figure 3.18: Package sizes of AA (g/cow/d) made up to 130, 120 and 110% of estimated requirements.

Variation in the predicted AA limitation sequences among models are likely due to differences in assigned AA levels of feed and MCP, and AA transfer coefficients on which each model based

predictions for efficiency of AA digestion, absorption and utilization. Likewise, predicted AA supply to the intestinal absorptive site depends on the default chemical composition of feed components in the individual ingredient libraries used to create the rations, as well as the assumed AA profiles of feed proteins escaping the rumen. Due to difficulties in measuring the flow of digesta and AA profile of protein reaching the intestine, the accuracy of these estimates are not known.

3.4 Conclusions

Production of vast quantities of DDG as a by-product of ethanol distillation plants in the Midwestern USA has lead to increased usage of DDG in California dairy rations. This study involving 16 dairy farms determined the inclusion levels of maize products (i.e., DDG and other common maize based feedstuffs) to be between 30 and 55% of TMR DM, with maize CP constituting 20 to 40% of the total CP in the TMR.

Commodities, both concentrates and forages, used in California rations were relatively consistent in terms of nutrient composition. TMR compositions were also relatively consistent, while meeting minimum nutrient requirements as suggested by the NRC (2001). A few ingredients (i.e., lucerne hay, maize silage, almond hulls, maize grain, whole cottonseed, DDG) were used in the TMR of most of the dairies, and suggest that a type of 'base' diet exists in these high group dairy rations.

Higher inclusion levels of maize products in dairy rations increased the contribution of maize CP to the total CP content of the TMR without affecting the CP levels in the ration, suggesting that rations are being formulated to balance the CP content, and that increased levels of maize protein are offset by decreased inclusion of other protein sources. The higher levels of maize in TMR had no impact on cow performance, probably because rations are being balanced to meet the nutrient requirements of the cows by considering the nutrient contents of each ingredient.

Even though the Lys to Met ratio decreased as more maize CP was included in the TMR, it did not have a major impact on the final predicted AA profile of MP. This could possibly be due to the superior AA profile, and large contribution, of MCP to MP. Regardless of the effect that maize CP had on AA entering the intestine, the changed AA ratios in MP did not have an impact on milk

component levels but, according to Shield, it had an effect on milk yield. However, relationships between these parameters were determined to show possible correlations among the variables even though it does not imply cause and effect. A relationship between the AA profile of MP and production was expected due to the importance of Lys in milk production, but it does not imply that maize CP levels will change cow production.

Identification of limiting AA proved to be difficult due to the differences amongst the three metabolic models. They suggested three dramatically different AA packages with Amino cow suggesting inclusion of Met and Lys, CPM Dairy suggesting Ile and Leu and Shield the inclusion of Lys and Ile to meet predicted requirements.

There appears to be a high degree of consistency within model in predicting the limiting AA sequence among dairies (Figure 3.19) even though there is a substantial variation in predicted AA and MP levels delivered by the rations among diaries. Other AA (i.e., Ile, Leu, His, Val, Arg and Thr) follow the same pattern as Met and, in the following figure, the only AA showing a slight variation among dairies is Lys.

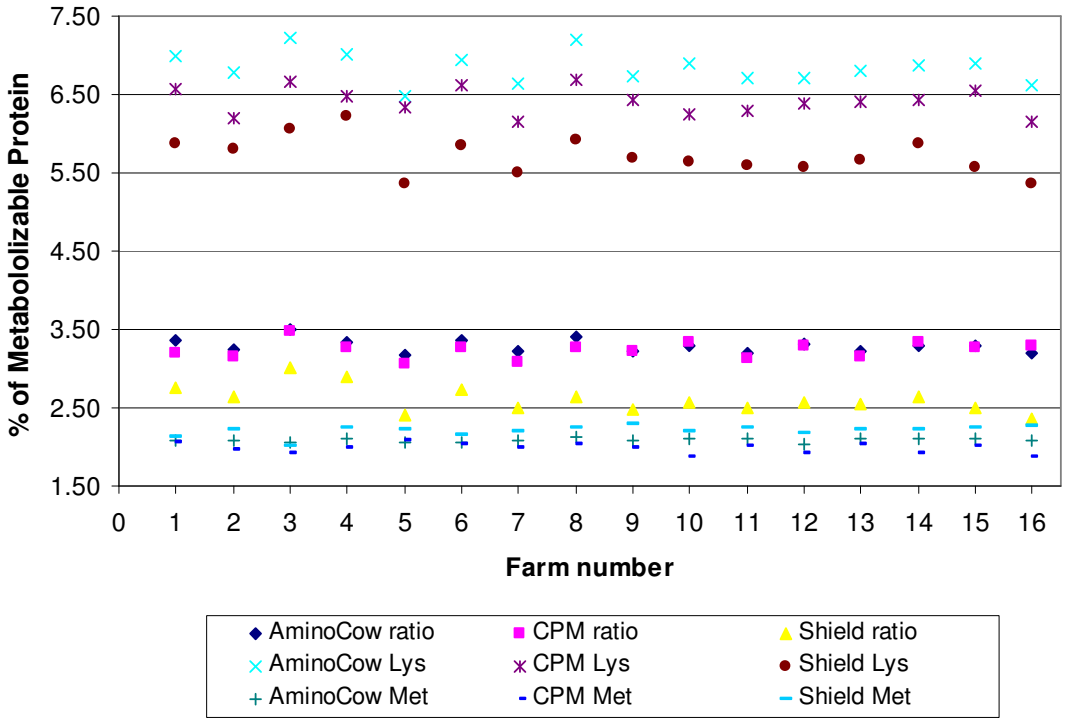


Figure 3.19: The ratio and proportion of Lys and Met in MP across the 16 dairies.

This suggests that there may be sufficient consistency in the nutrient profiles among rations to support a ruminally protected AA complex which could balance the model predicted AA profile,

thereby leading to increased animal productivity. However, there appears to be no good way to decide on which model is most correct without further research on animal responses to the packages. Despite the weak correlation ($r^2 = 0.30$) for the Shield predicted response of milk yield to Lys:Met ratios (Figure 3.13), this is a contrast to Amino Cow and CPM Dairy. All three models predicted the same relationship between milk yield and the MP Lys content (Figure 3.20), but differed dramatically in their predictions of the response of milk yield to different Met levels (Figure 3.21).

While CPM Dairy suggested a negative response, Amino Cow suggested none and Shield had the highest correlation among the models by predicting a positive relationship between the predicted Met in MP and milk yield.

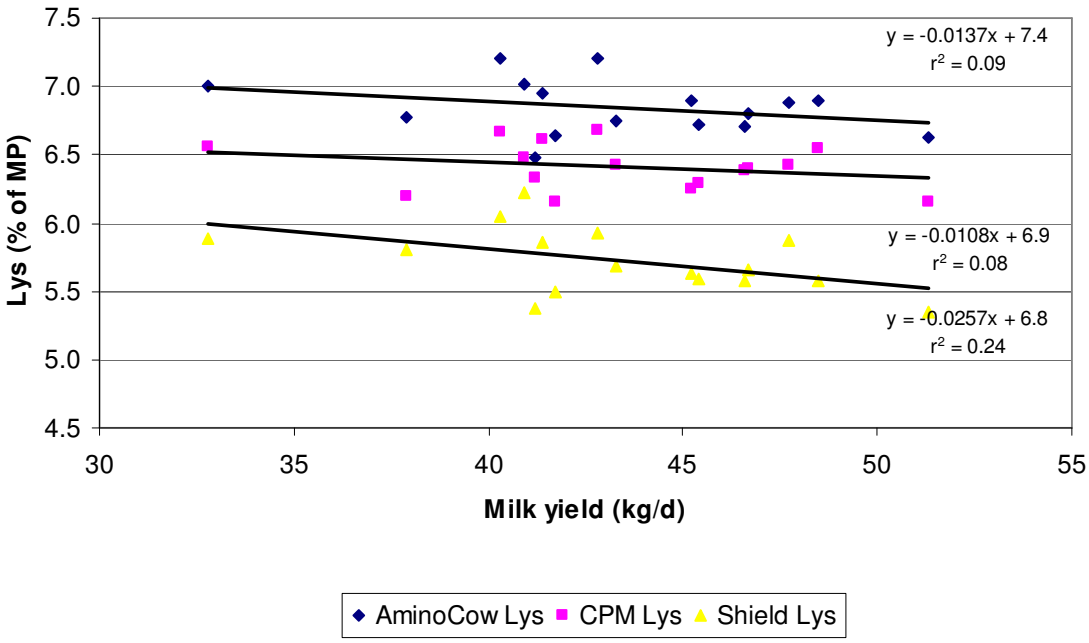


Figure 3.20: The response of milk yield (kg/d) to changes in the proportion of Lys in MP according to Amino Cow, CPM Dairy and Shield.

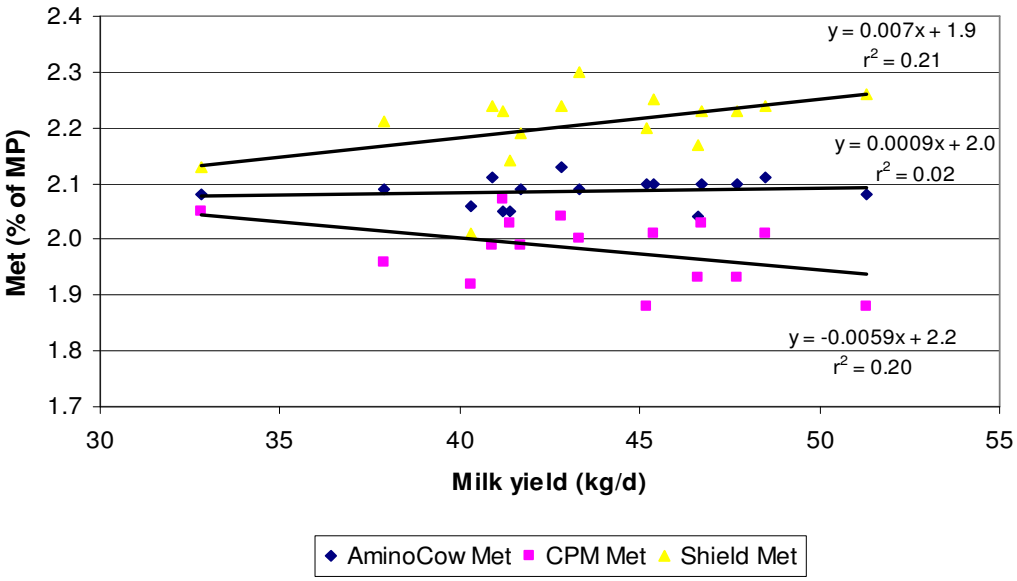


Figure 3.21: The response of milk yield (kg/d) to changes in the proportion of Lys in MP according to Amino Cow, CPM Dairy and Shield.

Overall, because Shield evaluations suggested a higher correlation between AA (both Lys and Met) and cow production, and predicted AA ratios with milk responses related to these ratios, while CPM Dairy and Amino Cow did not show such a relationship, it may be more appropriate to use the ruminally protected AA predicted by Shield in further research on the effectiveness and impacts of supplementing an AA package on milk production and component yields.

Chapter 4: Experiment 2. Impacts of feeding a ruminally protected lysine product on productivity of lactating dairy cows

4.1 Introduction

Increased milk production requires higher intakes of CP in the diet, and/or improved supply and ratios of AA delivered to the duodenum in order to meet animal needs for milk protein synthesis. Formulating rations to provide these AA concentrations are very difficult using currently available feed sources and metabolic models. Methionine and Lys have been suggested to be the most limiting AA for milk production when maize-based diets are fed, and maize levels in California dairy rations have been increasing due to the expanding Midwest ethanol industry that utilizes maize grain, thereby pushing more maize by-products into the dairy sector (Robinson *et al,* 2008). Increasing the CP level in the diet could result in reduced efficiencies, and higher N excretion in the urine, emphasizing the need for ruminally protected AA products to deliver specific AA to the duodenum (Clark *et al,* 1992; Ferguson *et al,* 2000).

Extensive research has been completed using currently available RPM products. There is, however, limited published information regarding RPL and there is currently no RPL product commercially available.

Using the information obtained from Experiment 1, a commercial dairy farm from that study, feeding a diet containing about 50% of DM as maize grain, maize silage and DDG and calculated to be first limiting in Lys, was identified and selected to conduct a further study in which a new RPL product was fed to the high producing dairy cows as a supplement to the diet.

The objectives of this study were to estimate the rumen escape potential of this RPL, estimating the amount of Lys reaching the small intestine, and to determine the effects, if any, of the Lys on milk lactose, fat and protein synthesis.

4.2 Materials and methods

The experiment was designed as a double (i.e., early and mid-lactation dairy cows) 2 x 2 factorial and consisted of two periods with two treatments (i.e., control and RPL) being reversed after 28 days. It was initiated in March 2007, with the treatments administered directly after the

dairy's regular DHIA milk test. One pen in each factorial received the control ration and one pen received the RPL supplemented ration in period 1, with the treatments reversed at the next milk test 28 days later. The experiment ended after the third DHIA test 56 days after the project started (i.e., early May 2007). All animals were cared for relative to applicable laws of the State of California and the USA.

4.2.1 *Farm, animals and management*

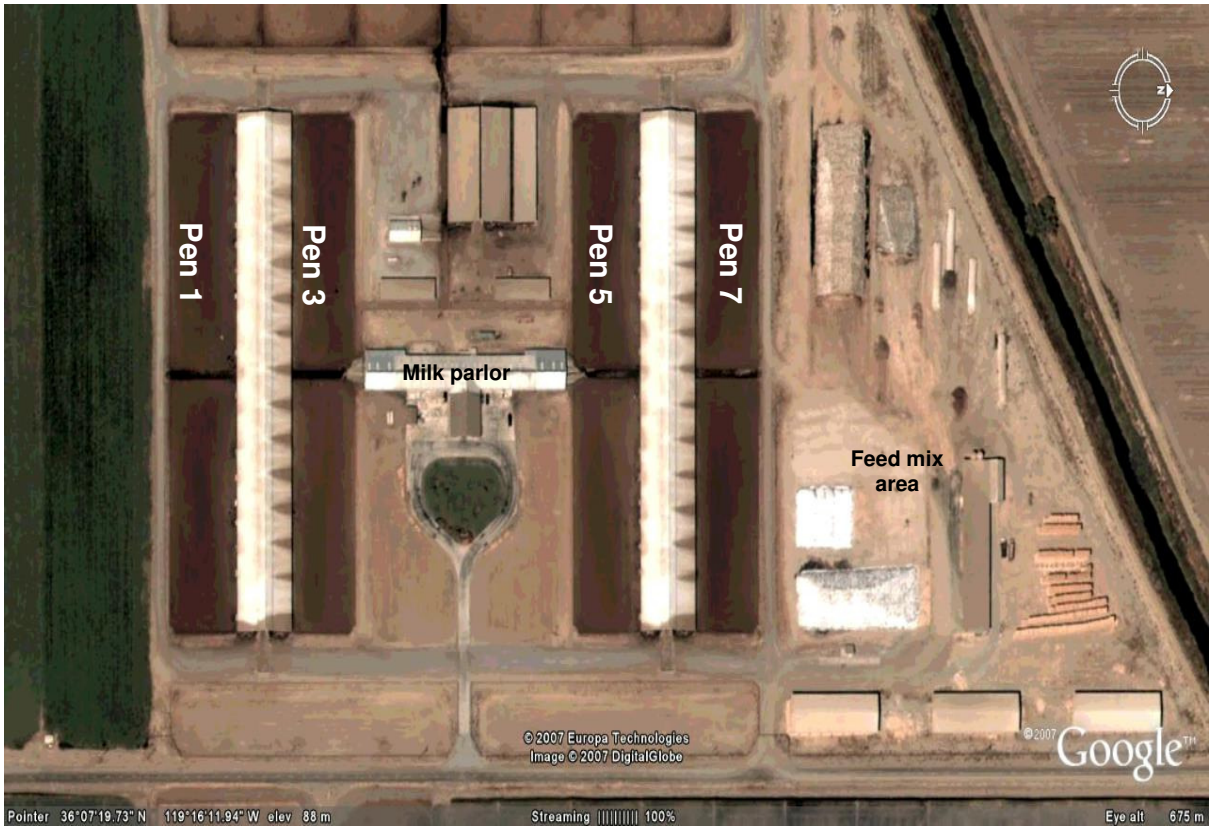
This dairy was selected from the group used in Experiment 1 based on the ingredient and chemical composition of the TMR, as well as the cow production data and dairy infrastructure.

The dairy selected is located near Tulare (CA) and milked approximately 1400 Holstein cows. It was chosen because of its high inclusion level of maize based feeds, and its high milk production (51.3 kg/d), and it had the lowest milk protein content (2.73% true protein) which suggests the possibility that RPL could increase milk protein synthesis. The ration consisted of the base diet ingredients, as shown in Experiment 1's dairy 16, removing effects of unconventional ingredients with poor nutrient default values (Table 3.4). It was predicted to supply all EAA at levels higher than 110% of requirements, except Lys, ensuring that no other AA would limit production. The CP concentration of 189 g/kg was desirable, since responsiveness of cows to supplemented AA depend on dietary CP levels (Socha *et al,* 2005) and a relatively high DMI (29.2 kg/d) ensures a rapid rate of passage of digesta, and the RPL, through the rumen. The dairy was ideally configured for the experiment due to the outlay and structural makeup in which four pens are located on each side of a central milking parlour (Figure 4.1). For the purpose of this study, only four pens were used, two situated North and two South of the milking parlour. These pens were mirror images of each other in all aspects except for the orientation of the dirt lots.

Cows were grouped according to production and DIM and randomly allocated to one of two pens, each holding ~ 180 cows. Pens with cows in early lactation (i.e., pens 3 and 5), were 77 $\pm$ 3.2 DIM with 48.2 $\pm$ 0.89 kg/day milk production and pens with mid lactation cows (i.e., pens 1 and 7), were 262 $\pm$ 6.7 DIM with 42.1 $\pm$ 0.56 kg/day of milk at the start of the study. The early and mid lactation pens were analyzed as separate experiments since the North side pens consisted of one early and one mid lactation pen, receiving the control diet during the first period. The same was

done for the South side pens, except that they received the RPL treatment during the first period. Each treatment therefore included one pen with early lactation and one pen with mid lactation cows. As the design of the study was a switch back, the treatments were reversed after four weeks.

Figure 4.1: Structural outlay of pens on the dairy used during Experiment 2



Pens were 110m long with 157 head gates (used to restrain cows during morning lockup for ~ 90 min for inspection and pregnancy checks) and 160 free stalls with dry manure solids as bedding. Flush feed aprons had rubber mats on the floor and the pens were fitted with fans and bunk-line misters automatically regulated by ambient temperatures. Cows had access to an outside exercise dirt lot.

Movement (~ 5% per week) of cows into and out of the pens was not restricted during the study and cows were moved to and from the hospital pens as needed for dairy operations. However, once a cow was moved, it was excluded from the sample group.

Cows were milked three times daily between 2 and 5 am, 10 am and 1 pm and again between 6 and 9 pm. The first Thursday of every month, Tulare DHIA record milk weights and collect samples for compositional analyses.

4.2.2 *Diets*

Cows were fed twice a day at 5:00 am and 10:30 am to appetite with free access to water. The TMR were mixed before each feeding in a horizontal stationary mixer (Mohrlang 820TMR, Brush, CO, USA) after which it was loaded into feeding trucks to be unloaded at the pens. One load was mixed and split evenly between the South side pens for every feeding, with the same for the North side pens.

The "Feed Watch" system monitored all feeding activities, keeping daily records of the total amount of TMR dropped at each pen and the actual ingredient profile of each batch of TMR that was mixed. Refusals were pushed out and weighed individually by pen every morning, thereby allowing calculation of average DMI per pen.

All pens received the same base ration formulated by Sierra Vista Consulting and estimated during the first experiment to be first limiting in Lys. The basal ration (Table 4.3) consisted of lucerne hay (a mixture of high and low quality hay at a ratio of 3:2), lucerne haylage, maize silage, steamed flaked maize, DDG, almond hulls, canola pellets and whole linted cottonseed with 17 kg/pen/day RPL (estimated to deliver approximately 15 – 19 g of intestinally absorbable Lys/cow/day) mixed into the TMR fed to RPL treatment pens.

The RPL product was created as a matrix, not an encapsulation, of which 80% is a mixture of Lys (L-Lys HCL) and ruminally protected fatty acids with a pH sensitive intestinal release mechanism (Ajinomoto Company, Tokyo, Japan). Once a week, all Lys bags were counted and the presence of RPL particles were confirmed to be in the TMR at the bunks of the RPL treated pens in order to verify mixing and feeding of the RPL to the correct pens.

4.2.3. *Sample collection*

Feed.

The TMR and feed ingredients were sampled twice during the last week of each experimental period using the same methods as described in section 3.2.2.

Urine and faeces.

Urine samples were collected on day 26 of each period from approximately 20 voluntarily urinating cows in each pen. The SG was determined immediately, using a handheld refractometer,

to calculate the urine volume (Brugos *et al,* 2005). One set of urine samples were cooled and sent to JL Analytical, Modesto (CA), for analysis of urea, ammonia and total N, while a duplicate set were frozen and kept at the UC Davis lab as a reserve. Faecal samples were collected on day 27 of each period from the first 15 cows identified out of the group of 20 from which urine were collected the previous day and frozen until analysis. Urine and faecal samples were collected to calculate feed digestibilities and milk N efficiency.

Blood.

Blood samples were not part of the original set of production parameters, but were included in the second period as visual inspection of the period 1 data suggested that the experimental outcome might not be as expected. Blood was sampled from the tail (coccygeal) vein of the same 15 cows from which faeces was collected using two blue top, 7 ml vacutainers (no additive). The AA concentrations in plasma from the coccygeal vein reflect those available for absorption by the mammary gland (Munneke *et al,* 1991). Samples were stored on ice for less than 30 min and taken to the Veterinary Medicine Teaching and Research Centre (**VMTRC**) near Tulare (CA) for centrifugation and freezing.

Milk.

For the purpose of this project, DHIA collected milk samples from all three milkings for all four pens at the end of each period. Milk weights were recorded using the Waikato milk yield proportioning device that retains a known small proportion of the milk (i.e., 25 g/kg) in a calibrated flask from which the total yield was read. A small representative sub-sample was drawn from the flask and preserved in 2-Bromo-nitropropane-1, 3-diol preservative, capable of preserving milk for up to three days, for subsequent analytical testing.

4.2.4 *Analytical methods*

4.2.4.1 Evaluation of the rumen protected lysine

Chemical composition.

Duplicate RPL samples were analyzed for chemical composition, including ash, N and fatty acids (including the FA profile). The DM, N and ash were determined by the same methods as described in section 3.2.3.1. To determine the FA profile, samples were directly methylated

utilizing a 10% methanolic HCL solution (Palmquist and Jenkins, 2003) and the composition of FA methyl esters were determined by separation in a Hewlett Packard 5890 gas chromatograph equipped with a 100 m capillary column (0.32 mm, 0.20 mm film thickness; Supelco 2560, Supelco Inc., Bellefonte, PA), utilizing hydrogen as the carrier gas. A detailed description of the gas chromatograph conditions and standards was reported by DePeters *et al* (2001).

Density.

The SG of the RPL was measured to allow estimation of the extent to which the RPL particles would pass from the rumen. The SG (g/cm^3) determination was completed at the University of California, Davis (CA), by placing the particles in combinations of water and salt, creating fluids with different SG (a higher salt content increases the SG), until equilibrium was reached and the particles remained suspended in the fluid. When the RPL particles were placed in double distilled de-ionized water (**$DDDH_2O$**) with SG = 1.00, all particles sank, indicative of a SG > 1.The highest possible SG using salt was 1.207, when 175 g of salt was dissolved in 500 ml of $DDDH_2O$. The SG was calculated as the weight of the fluid divided by the volume.

Rumen degradation.

Rumen protection properties of the RPL were confirmed using an *in situ* nylon-bag technique (Osuji *et al,* 1993; Nozière and Michalet-Doreau, 2000) and potential rumen escape of Lys was calculated. Three dairy cows were surgically fitted with 10 cm centre diameter rumen cannulae (Bar Diamond, Inc., Parma, ID, USA), and kept in separate grass pens on the experimental farm at the University of Pretoria, Pretoria, South Africa. They were fed a uniform TMR once a day at 8:30 am and water was freely available during the entire study. The nylon bags, containing 1.5 g RPL (15 mg/cm^2), were inserted into the rumen 48, 36, 24, 16, 8, 4, 2, 1 and 0.1 hours, prior to a common removal time after which bags were rinsed with cold water, dried at 55^oC for 48 hours and weighed. Contents of the bags were subjected to Dumas N analysis (AOAC, 2000) using the LecoFP 428. The experimental protocol for the cannulated study was approved by the University of Pretoria's Animal use and care committee. All surgical procedures were performed by registered veterinarians and relevant legislation and regulations were implemented where appropriate.

During a second degradation study done in Japan by Ajinomoto, a highly protected and completely undegradable L-Arg product (HP-Arg) was administered, together with the RPL

particles, into three ruminally and duodenally cannulated cows to serve as a control marker (Ajinomoto Company, Tokyo, Japan; personal communication). To ensure the same rumen escape kinetics, the marker had the same particle size and SG as the RPL particles. Every 3 h, 500 ml of duodenal digesta were collected from the three cows via a duodenal cannula. The RPL and HP-Arg particles were crushed to allow the Lys and Arg to dissolve, after which free Lys and Arg concentrations were measured using an AA analyzer. Changes in Lys and Arg concentrations were plotted to calculate the area under the curve (**AUC**). Rumen escape rate of the RPL is the ratio of Lys to Arg AUC when the escape of HP-Arg is considered to be 100%.

Durability.

The RPL durability was assessed to determine the degree to which the particles maintain conformation during mixing and feeding, as the size of the particle the cows actually consume may affect the rate at which Lys in the RPL escapes the rumen.

Replicated RPL samples were recovered from the TMR fed to treatment pens in both periods after a known amount of RPL particles were added to the TMR. Two TMR samples were collected directly after mixing before the TMR entered the feeding truck, two were collected from each of the treatment pens when the TMR was dropped in the bunks, and two after the TMR has been in the bunk for 5 h.

To facilitate recovery of the particles, the TMR samples were sifted through the Penn state particle separator, as a previous examination of a pure RPL had indicated that ~ 90% of the RPL particles were retained on the 0.05 cm sieve. The particles were removed from the TMR by hand and weighed to determine the percentage recovered.

4.2.4.2 Sample preparation and assays

Feed.

All the TMR samples, silages and other wet ingredients were weighed before being dried at 55^{o}C for 48 hours. Samples collected were analyzed at ANR Laboratory for DM, ash, NDF, ADF, lignin, starch, CP and ADICP. Sample preparation and analysis followed the same procedures as described in section 3.2.3.1. The TMR samples were also analyzed for a number of macro and trace minerals, free sugars (i.e., soluble carbohydrates), EE, $dNDF_{30}$ and SolCP. These, together with

OM, CP, ADICP and aNDFom, were used to estimate the net energy (**NE**) of the feed for high producing animals (i.e., 3x maintenance) as described by Robinson *et al* (2004).

Urine.

Total N was analyzed using a Kjeldahl procedure (AOAC, 1990) and urea concentration was measured by the diacetyl monoxime method (Marsh et al., 1957) using a Technicon analyzer (Technicon Instruments Corporation, Tarytown, NY).

Faeces.

Samples were dried at 55°C for 48 hours during which they were turned over once and broken in half to ensure proper drying. After 24 hours of equilibration, air dry samples were sent to be analyzed at the ANR Laboratory for DM, CP, lignin and NDF.

Blood.

Plasma was obtained by centrifugation with the Beckman Coulter Allegra™ 6R Centrifuge at 2060 *xg* for 15 min at 4°C, and transferred into replicate Ependorf tubes. A set of plasma samples was sent for analysis at AESCL Analytical Services (University of Missouri, Columbia, MO), for physiological AA (i.e., free plasma AA) levels, while another set was kept at UC Davis as a reserve.

Milk.

Fat, true protein and lactose contents, as well as SCC, were determined using NIR and MUN was determined at Southern Counties lab (Chino, CA) using the Bentley ChemSpec 150 milk urea analyzer.

4.2.5 *Calculations*

Average DMI/cow/day was determined at the end of both periods for each pen by subtracting refusals (orts) from the amount of feed (as is) dropped and multiplying it by the average DM content for the two TMR samples. This was divided by the average number of cows in the pen.

The Lys content of 42% in the RPL was calculated from 53% L-Lys HCl multiplied by 0.8 to give the amount of Lys alone.

The SG of RPL particles were calculated using solutions with different salt concentrations:

SG = Weight of salt solution / Volume

The N disappearance at each incubation time was calculated from the proportion of N remaining after incubation in the rumen as:

% N disapp = [(g N before incubation – g N after incubation) / g N before incubation] x 100

Where: g N before incubation = sample weight x g N in sample (DM basis)

g N after incubation = sample weight left in bag x g N in sample (DM basis)

Assuming that 17 kg of RPL was mixed into 18 000 lbs (i.e., 8 167 kg) of feed, the recovery of particles, without any breakdown, should be 2.082 g/kg. Particle recovery for the three different time frames was calculated as:

% Recovered = [weight of particles recovered / (weight of TMR sampled x 2.082)] x 100

Calculations used in SAS procedures include:

Intakes:

DMI (kg/d) = [As fed intake (AFI) x DM] / 100

OMI (kg/d) = [DMI x (100 – Ash)] / 100

CPI (kg/d) = (DMI x CP) / 100

NDFI (kg/d) = (DMI x NDF) / 100

Total N (g/d) = (CPI x 1000) / 6.25

Digestibility:

CPD (%) = (1 – (((Lignin$_{TMR}$ x 0.95*) / CP$_{TMR}$) / (Lignin$_{Faeces}$ / (N$_{Faeces}$ x 6.25)))) x 100

NDFD** (%) = (1 – (((Lignin$_{TMR}$ x 0.95*) / NDFom$_{TMR}$) / (Lignin$_{Faeces}$ / NDFom$_{Faeces}$))) x 100

** Assuming that 95% of lignin in the diet is indigestible and will appear in the faeces.*

*** NDFD predicted according to NRC 2001 requirements, using lignin as marker.*

Production:

Fat (kg/d) = (% Fat x Milk yield) / 100

Protein (kg/d) = (% Protein x Milk yield) / 100

Lactose (kg/d) = (% Lactose x Milk yield) / 100

MUN (g/d) = [(MUN[1]/1000) x 10] x Milk yield

MPN[2] (g/d) = (Protein yield / 6.38) x 1000

Milk energy (MJ/d) = Milk energy[3] x Milk yield

Total N (g/d)[4] = MUN output + MPN output

[1] *MUN (mg/dL)*

[2] *Milk protein N*

[3] *Milk energy (MJ/kg) was calculated using a prediction equation derived from multiple regression analysis by Tyrrell and Reid (1965) as:*

= ((((41.63 x % Fat) + (24.13 x % Protein) + (21.6 x % Lactose) – 11.72) x 2.204) / 1000) x 4.1855

[4] *Total N was calculated for the subset of 60 cows to be used in the partial N balance estimation.*

Urine:

Volume (L/d)* = [64104 x $(SG)^2$] – [133231 x SG] + 69242

Total N (g/d) = Total N (g/L) x Urine volume

Urea N (g/d) = Urea N (g/L) x Urine volume

Protein N (g/L) = Total N (g/L) – Ammonia N (g/L) – Urea N (g/L)**

** Urine volume was estimated using an equation derived by Burgos et al (2005) after measuring total daily output of urine (L/d) and relating it to the specific gravity (SG).*

*** Protein N was calculated for comparison to urea N (the contribution of ammonia N is insignificantly small) in order to determine change in the N fractions in urine.*

Faecal outputs:

Total N output (g/d) = [(CPI / 6.25) x 1000] x [(100 – CPD) / 100]

Partial N balance was calculated using the N outputs calculated for urine, faeces and milk. Individual cow intakes were not measured, making determination of total N balance impossible, but it can be estimated under the assumption that the cows in each pen consume the same amount of DM (i.e., average for the pen). Total N intake can then be calculated by dividing CPI by 6.25 x 1000 for each experiment.

4.2.6 *Statistical analysis*

Rate of digestion (kd or c) for the RPL particles were determined using the nonlinear regression (**NLIN**) procedure and the Gauss-Newton method of the statistical analysis software (SAS Institute Inc., 2000) with CP as the dependant variable.

EXCEL's descriptive statistics, with a 95% confidence level for the mean, was used to determine the mean and SE for rumen degradability, RPL durability and all feed ingredients.

Each experiment was statistically analyzed as a 2 x 2 design with pen as the experimental unit for DMI and cow as the experimental unit for all milk production parameters. Only cows that were in the same experimental pen throughout the entire experimental period were used for analysis.

Statistical analysis of ingredient profile and chemical composition of TMR, DMI and whole tract digestibility were conducted with period and treatment as factors, using the General linear model (**GLM**) procedure of SAS, by experiment.

Animal production, urine, faecal and blood parameters were statistically analyzed using the MIXED option of SAS, again by experiment. Cow within pen was included in the random statement with period, pen and treatment as factors. However plasma samples were only collected during the second period, and so period was not a factor in blood AA analysis.

Treatment differences were determined using the PDIFF option of SAS with significant differences accepted if $P \leq 0.05$ and tendencies to significance accepted if $0.05 < P \leq 0.10$.

4.3 Results and discussion

4.3.1 *Product evaluation*

The RPL product (Table 4.1) used in this study had a SG of 1.09 gm/cm^3 and a matrix comprising 42% Lys (53% L-Lys monohydrochloride) based on N assay, 42% fatty acids, 1% lecithin, 4% water, and < 1% ash.

Table 4. 1: Chemical composition (% DM), SG and other characteristics of the RPL product fed to high producing dairy cattle

Chemical composition of RPL	
Dry matter, %	96.8
Lys*	42.3
Fatty acids	41.7
Ash	0.15
Specific gravity	1.09
Rumen degradability of RPL (% N)	
Solubility	12.9 ± 1.76
24h residue	58.4 ± 3.25
36h residue	49.6 ± 3.37
48h residue	39.1 ± 2.55
Durability of RPL (% Particle recovery)	
after TMR mixing	49.01 ± 10.14
after delivery to the bunk	49.07 ± 5.97
after 5 h in the bunk	37.68 ± 1.29

* As total N x 5.219

Results from the *in situ* nylon-bag study indicated that 13% of the N was soluble in the rumen, while 58, 50 and 39% of N remained in the bags after 24, 36 and 48 h respectively (Figure 4.2). Using the marker method mentioned in section 4.2.4.1., using the HP-Arg marker, the average rumen escape rate was estimated to be 45%.

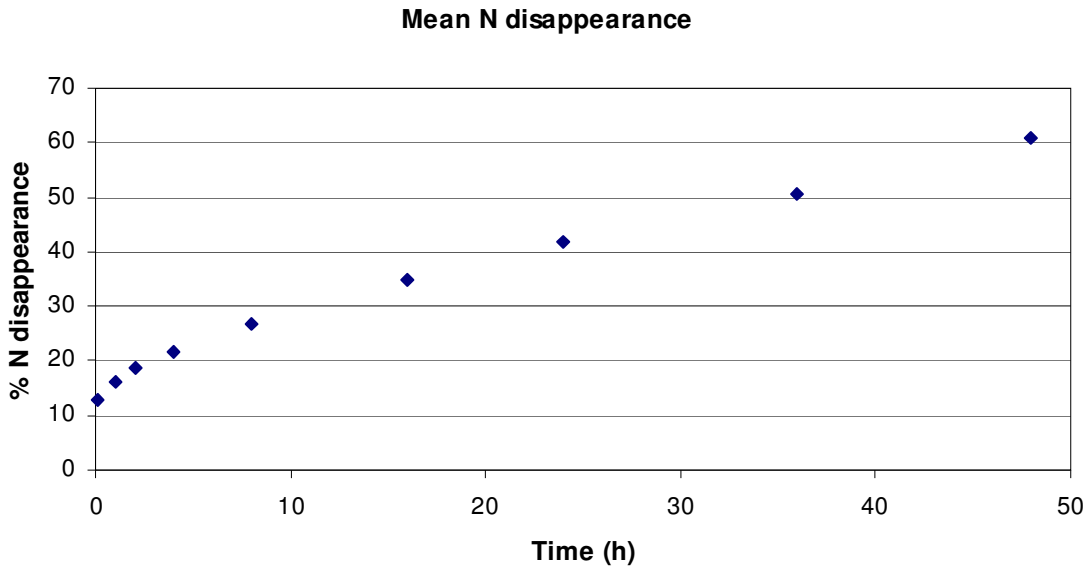


Figure 4.2: Average disappearance of N from RPL particles during 48 h of rumen incubation in three ruminally-cannulated cows.

After mixing the product into the TMR, 49% of particles were recovered intact and no additional break-up occurred until the TMR was unloaded at the pens. Only 38% of the particles were recovered after the TMR were in the bunk for a period of 5 h, suggesting further loss of particles due to feeding activity.

For Lys in the RPL to have an impact on milk protein synthesis, it needs to be delivered to the small intestine without being degraded in the rumen. To determine how much of the Lys escapes the rumen, it is necessary to consider the rate at which the particles are degraded and the amount of time that they spend in the rumen. Rumen degradability can then be calculated as Kp/(Kd + Kp) (McDonald *et al*, 2002) where Kp = rate of passage and Kd = rate of digestion. Using the results obtained from the degradation study on the RPL particles, the kd was 0.045/h. If a rate of passage of 0.05/h is assumed, which is the passage rate used for animals fed a diet between maintenance (0.02/h) and high production (0.08/h), 52.6% of the particles would have been delivered to the duodenum.

Rumen rate of passage (assumed here to be 0.05/h) can, however, be influenced by a number of nutritional factors, as well as particle characteristics (e.g., size and SG). Passage is faster for particles that are smaller, have an intermediate SG, are highly hydrated or partly digested. Higher DMI also increases the rate of passage of feed, and RPL, from the rumen. As the intake levels of the cows in this experiment were relatively high, it could increase the rate of passage to as high as 0.08/h, which could increase predicted escape to > 60% based on the rumen incubation study.

However, King and Moore (1957), Campling and Freer (1962), Welch and Smith (1978) and desBordes and Welch (1984) indicated that a SG of 1.20 and a particle size of 20 to 30 x 10^{-3} cm^3 would cause the most rapid rumen escape, with a ruminal MRT of 24 to 36 h. Assuming that MRT of the RPL particles fed during this experiment was 44 h, based on the findings of Campling and Freer (1962) and the calculated SG of 1.09, the proportion of RPL that was not degraded in the rumen may have been as low as 43%, which is close to the amount estimated during the HP-Arg marker method described in section 4.2.1.4.

According to King and Moore (1957), only 25% of particles with SG of 1.09 were recovered in faeces after 70 h, and almost all of them had been regurgitated and chewed which may be indicative that lighter particles were trapped in the fibrous mat in the rumen preventing outflow to the abomasum. Since the SG for RPL particles were measured to be lighter than anticipated, it is quite possible that it was retained in the rumen for a longer period, reducing the amount of Lys reaching the small intestine.

Results from the RPL durability study showed that only about 50% of the RPL particles were still intact after mixing and feeding of the TMR with an additional 10% breaking up while the feed was in the bunks, possibly due to the feeding activity of the cows. Disadvantages of broken particles relative to rumen escape of Lys include the possibility that some of them separated out of the feed to be removed with the feed refusals, thereby reducing the amount of Lys that was actually fed to the cows, and/or that those that were consumed may expose more Lys, which is rapidly degraded in the rumen, thereby reducing the amount of Lys delivered to the intestine. Breakdown of particles during mixing with the possibility of further size reduction during rumination may therefore result in longer rumen retention times and/or increased ruminal Lys degradation.

Surface characteristics of the RPL particles should also be considered. The plastic particles used in previously mentioned experiments had a much smoother surface than the particles fed to the cows during this study. Weight and mobility of the RPL particles may be adversely affected by rough edges and air bubbles trapped between them.

Since Lys escaping rumen degradation was estimated by various methods to be between 43 and 53%, and 55% of the particles broke down during mixing, the true amount of Lys escaping the rumen would be as low as 19 to 24%. As the RPL was added to the ration at a level of 97 g/cow/day, this suggests that between 7.9 and 10 g of intestinally absorbable Lys was delivered daily to each cow. This is considerably lower than the targeted 15 - 19 g upon which the level of feeding was based, due to a SG lower than anticipated and losses of product during TMR mixing and in the feed bunk.

Positive responses in milk yield, fat and protein were obtained by Rogers *et al,* (1987, 1989) when RPL was supplied at amounts as low as 5.9 and 7.8 g/d. Milk yield and protein percentage and yield increased when 15 g of Lys was fed to cows in early lactation (Blauwiekel *et al,* 1997), suggesting that the RPL product fed during this experiment delivered enough Lys to elicit a response.

As part of the second degradation study, intestinal absorption of RPL Lys was calculated by subtracting the faecal excretion of the product from its ruminal escape. Faecal excretion rate was evaluated by feeding the RPL to intact cows and collecting the faeces for 72 h. The average Lys excreted (g) was calculated from the concentration of Lys in the faecal slurry. Since the estimated faecal excretion of RPL Lys was approximately 4% (Ajinomoto, Tokyo, Japan; personal communication), this suggests that 96% of the RPL Lys that escaped rumen degradation was absorbed in the small intestine. The high absorption level suggests that the product was easily digested by intestinal enzymes. The total amount of RPL Lys delivered and absorbed can therefore be calculated as 41% (i.e., 45 – 4), which is very close to the amount of intestinally delivered Lys estimated from particle size, SG and N disappearance in the ruminal degradation study without considering possible particle fragmentation during mixing and feeding. However, neither of these calculations considered the significant net utilization of EAA by gut tissues. Indirect measurements

have suggested substantial losses of BCAA via oxidation (Lapierre *et al,* 2006), although studies on oxidation of Lys are limited.

4.3.2 *Ration evaluation*

The chemical composition of the forages and concentrates used in the TMR (Table 4.2) are generally similar to the nutrient composition of feeds analyzed and listed in NRC (2001), with a few exceptions such as the CP levels of lucerne silage, DDG and canola pellets, which are higher than NRC values, while ADICP tend to be lower. Canola pellets, almond hulls and DDG have lower NDF and ADF concentrations than suggested by NRC (2001).

Table 4. 2: Chemical analysis (+ SE) of ingredients used in TMR (g/kg DM) fed to high producing dairy cattle*

	Maize Silage	Lucerne silage	Lucerne hay HQ	Lucerne hay LQ	Maize flaked	WCS[1]	DDG[2]	Canola pellets	Almond hulls
105 °C DM (g/kg)									
Dry Matter	308	415	930	920	859	909	907	900	918
	(6.3)	(19.2)	(1.0)	(4.7)	(4.2)	(3.8)	(4.1)	(1.6)	(22.5)
Organic Matter	932	858	899	867	986	957	955	913	923
	(4.1)	(13.6)	(4.7)	(4.3)	(0.9)	(0.6)	(1.0)	(2.0)	(5.0)
Crude Protein	80.5	279	195	224	85.3	222	314	421	70.1
	(3.36)	(3.2)	(7.9)	(5.9)	(2.19)	(7.1)	(8.3)	(6.3)	(12.11)
ADICP[3]	118.7	52.1	61.6	67.6	0	89.0	62.1	71.1	189
	(19.91)	(1.49)	(5.50)	(6.70)	(-)	(2.96)	(18.27)	(12.36)	(27.3)
aNDF[4]	452	335	412	410	90.3	497	333	273	317
	(12.2)	(3.1)	(7.7)	(3.1)	(3.64)	(8.6)	(9.5)	(8.2)	(12.3)
aNDFom[5]	437	313	397	392	90.0	481	328	258	305
	(10.3)	(3.3)	(8.4)	(4.4)	(3.11)	(9.9)	(9.1)	(7.8)	(11.7)
ADF[6]	296	288	314	341	33.0	354	116	207	239
	(6.7)	(10.6)	(3.0)	(1.4)	(1.22)	(14.9)	(6.0)	(9.1)	(18.1)
ADFom[5]	268	270	302	328	32.3	353	115	205	235
	(4.0)	(9.4)	(3.1)	(0.9)	(1.11)	(15.5)	(6.6)	(8.5)	(16.5)
Lignin (sa)	26.8	51.0	50.3	61.8	4.50	109	18.5	83.8	81.6
	(0.63)	(-)	(1.89)	(0.44)	(0.289)	(3.5)	(4.84)	(7.09)	(10.14)
Starch	263	< 5	24.3	8.83	734	< 5	54.8	36.3	15.9
	(13.2)	(-)	(5.15)	(1.014)	(12.3)	(-)	(5.65)	(1.03)	(3.16)
Free glucose	< 2	< 2	6.75	3.83	3.25	< 2	5.50	< 2	92.1
	(-)	(-)	(0.479)	(0.441)	(0.629)	(-)	(1.258)	(-)	(1.98)

* Average values for a total of 4 samples collected, two during the last week of each period
[1] Whole linted cottonseed
[2] Dried distillers grains
[3] Acid detergent insoluble crude protein expressed as g/kg CP
[4] Neutral Detergent Fibre assayed with heat stable amylase
[5] aNDF expressed exclusive of residual ash
[6] Acid detergent fibre

The ingredient profiles of the TMR are generally consistent with typical California dairy rations (Table 4.3), except for a relatively high feeding level of DDG, although this is becoming more common. Although there were small differences in the TMR between treatments the numerical differences were judged not to be biologically important.

Compared to NRC (2001), the TMR met all minimum nutrient requirements with a slight oversupply of fat (76 g/kg vs. 65 g/kg). Mineral content of the rations either met or exceeded those recommended for lactating dairy cattle producing 45 – 50 l of milk/day (NRC, 2001).

Even though Ca and S are provided at 130% of requirements, the Ca:P ratio is 2:1, which is within NRC recommendations but the N:S ratio is 9:1, lower than the recommended 12:1 (Bouchard and Conrad, 1973).

Table 4. 3: Ingredient profile and chemical composition (g/kg DM) of TMR fed to high producing dairy cattle*

	Control	RPL	SE	*P*
g/kg DM				
Lucerne Hay	147.2	149.8	0.37	< 0.01
Maize grain, flaked	158.3	158.1	0.53	0.79
Whole cottonseed	95.6	92.8	0.20	< 0.01
Dried distillers grains	98.9	98.6	0.85	0.77
Canola pellets	82.4	81.6	0.56	0.35
Almond hulls	79.2	78.3	0.63	0.37
Energy II[1]	23.4	24.2	0.19	0.03
Lucerne silage	70.8	72.0	0.16	< 0.01
Maize silage	220	218	0.35	0.03
Yeast[2]	4.0	4.0	0.01	0.17
RPL product	0	3.2	0.01	< 0.01
Mineral premix[3]	20.6	19.4	0.28	0.03
NE_L (3xM) (MJ/kg)[4]	7.31	7.32	7.38	7.37
g/kg DM				
DM	595	604	3.0	0.06
OM	920	918	0.6	0.02
CP	181	180	1.9	0.62
SOLCP[5]	65.7	68.5	1.38	0.17
ADICP[6]	79.6	74.1	3.75	0.32
aNDF[7]	325	325	2.8	1.00
aNDFom[8]	314	312	2.9	0.65
$dNDF_{30}$[9]	467	468	5.2	0.78
ADF[10]	225	224	2.1	0.65
ADFom[8]	215	212	2.5	0.50
Fat	76.1	75.1	1.18	0.54
Lignin (sa)	45.6	46.0	1.19	0.83
Starch	185	183	4.3	0.69
Free sugars	24.1	26.6	0.95	0.09
Ca	8.91	9.10	0.106	0.23
P	4.44	4.40	0.031	0.40
K	16.2	16.0	0.19	0.46
Mg	3.16	3.25	0.114	0.60
S	3.10	3.09	0.115	0.94
Na	4.66	4.69	0.196	0.93

	Control	RPL	SE	*P*
Cl	5.20	5.30	0.266	0.79
mg/kg				
Zn	125	128	11.8	0.84
Mn	57.6	58.8	2.41	0.72
Fe	263	287	8.5	0.07
Cu	12.2	12.3	0.77	0.95
Co	2.36	2.29	0.227	0.85
Se	0.41	0.40	0.013	0.46

* Samples pooled by period (n=2), based on TMR samples collected twice for each pen, each period.
[1] Nutritech Solutions, Ltd. (Abbotsford, British Columbia, Canada)
[2] Diamond V Mills, Inc. (Cedar Rapids, IA)
[3] Premix (89.3%DM) contained 5.09% N, 0.75% P, 0.91% K, 9.22% S, 4.88% Ca, 6.70% Mg, 3.41% Zn, 1.79% Mn, 3.93% Fe and 0.30% Cu on DM basis
[4] Net energy requirements for lactation at 3 times maintenance
[5] Soluble crude protein expressed as g/kg DM
[6] Acid Detergent Insoluble Crude Protein expressed as g/kg CP
[7] Neutral Detergent Fibre assayed with heat stable amylase
[8] aNDF expressed exclusive of residual ash
[9] Estimated digestible NDF after 30 h of in vitro incubation
[10] Acid Detergent Fibre

The average unsupplemented ration was evaluated post-experimentally, using the metabolic model Shield (Appendix A4; Table A4.5) as it was judged to be the most likely to be accurate based upon results of Experiment 1, to examine potential oversupply of nutrients and determine potential limitations to performance. The ideal ration for measurement of the response to RPL should provide sufficient RDP to meet microbial N requirements and approximately 110% of calculated intestinally available RUP with all EAA, except those to be supplemented, provided at 110% of calculated requirements, thereby ensuring that performance is only limited by the intestinal availability of the supplemented AA (Robinson *et al,* 1998). However, retrospective evaluation of the ration revealed that, even though maize products still consisted 48% of TMR DM, the estimated RDP delivery was below requirements, possibly suppressing MCP synthesis (Verbic, 2002; Santos *et al,* 1998a), but the ingredients included in the ration provided enough RUP to ensure adequate AP delivery. Shield also suggested that free fat supplied by the diet was more than 5% of DM and high levels of fat might also inhibit MCP production (Oldick *et al*, 2000). Since rumen microbes are a very important source of, and contain more Lys than, most feedstuffs, reduced microbial flow to the intestine could explain why Lys was limiting in this ration. Even though MCP synthesis was suboptimal, the base diet still provided adequate amounts of absorbable AA, with Lys estimated at 103% and 105% of requirements (167 and 164 g/d of intestinally absorbable Lys) in early and mid lactation groups respectively (Table 4.4).

Table 4. 4: Protein evaluation and calculated intestinal AA balance according to Shield

	Early lactation		Mid lactation	
	Control	RPL	Control	RPL
Animal performance				
DMI (kg/d)	28.40	29.00	28.80	27.60
Milk Yield (kg/d)	53.24	54.00	41.84	40.90
Milk Fat %	3.50	3.29	3.74	3.54
Milk Crude Protein %[1]	2.92	2.91	3.28	3.29
Predicted BW Change (kg/d)	-2.21	-1.94	-1.24	-1.24
Protein and Energy status				
Absorbable protein[2]				
Required (g/d)	3112	3191	2948	2893
Delivered (g/d)	3569	3639 (3649)*	3599	3460 (3470)
Delivered/required	1.15	1.14 (1.14)	1.22	1.20 (1.20)
Degraded intake protein (RDP)				
Required (g/d)	3148	3205	3177	3066
Delivered (g/d)	2741	2784	2755	2672
Delivered/required	0.87	0.87	0.87	0.87
Digestible undegraded intake protein (RUP)				
Required (g/d)	1591	1666	1454	1418
Delivered (g/d)	1850	1909 (1919)	1901	1795 (1805)
Delivered/required	1.16	1.15 (1.15)	1.31	1.27 (1.27)
Amino acid status				
Lys				
Required (g/d)	167	172	164	160
Delivered (g/d)	172	175 (185)	172	168 (178)
Delivered/required	1.03	1.02 (1.08)	1.05	1.05 (1.11)
Met				
Required (g/d)	48	49	47	46
Delivered (g/d)	80	82	81	78
Delivered/required	1.66	1.65	1.70	1.68
Ile				
Required (g/d)	117	119	111	108
Delivered (g/d)	135	137	135	131
Delivered/required	1.16	1.15	1.22	1.21
Lys/Met ratio	2.15	2.14 (2.26)	2.13	2.16 (2.28)

[1] Calculated as true protein divided by 0.934

[2] Metabolizable protein

* Values in parenthesis include the estimate level of Lys delivered to the small intestine by the RPL

Even though the diet fed by Blauwiekel *et al* (1997) supplied 105% of Lys requirements, they still observed an increase in milk yield when RPL was fed. Due to uncertainties of predicting AA, any AA supplied below 110% of requirement is potentially limiting. To supplement Lys to a level of 110% of requirements, an additional 11.7 and 8.2 g of Lys needed to be delivered to the small intestine of the early and mid lactation cows respectively but, according to previous calculations, only 7.9 to 10 g of Lys was actually likely delivered by the RPL. This is enough to meet requirements of mid lactation cows but not those ones in early lactation. Shield also estimated that 10 g of Lys would suffice in raising Lys levels above 110% in the mid group cows, but not in the early group (Table 4.4).

Since Shield expresses energy surplus or shortage, provided by the ration, as either body weight gain or loss instead of NE_L, the NE_L levels of the feeds were calculated as suggested by Robinson *et al* (2004) at production level (i.e., 3x maintenance), using several chemical (i.e., OM, Fat, CP, ADICP and NDF) assays and one biological (i.e., $dNDF_{30}$) assay. There was no difference between the NE_L of the control and RPL rations in either lactation group (i.e., 7.32 vs. 7.38 MJ/kg DM). Both these NE_L values are higher than the recommended NRC requirements of 6.74 MJ/kg for high producing dairy cattle, and this may be due to the high fat content of the rations.

4.3.3 *Intake and digestibility*

Supplementation of RPL did not influence intakes of DM, OM, NDF, or CP (Table 4.5) in early lactation cows. In the mid lactating group, however, there was a tendency (P = 0.09) for lower DMI with addition of RPL. The CP digestibility decreased in the treatment group for early lactation cows, but there was no effect on NDF digestibility (**NDFD**) for either lactation group.

Table 4. 5: DMI (kg/d) and whole tract digestibility (g/kg) of early and mid lactation cows as influenced by RPL

	Control	RPL	SEM	*P*
Early lactation				
Intakes (kg/d)[1]				
DM	28.4	29.0	0.26	0.16
OM	26.1	26.6	0.22	0.20
CP	5.15	5.17	0.064	0.81
NDF	8.9	9.1	0.13	0.53
Total N (g/d)	824	827	10.3	0.81
Digestibility (g/kg)[2]				
CP	690	649	6.3	<0.01
NDF[3]	473	459	6.1	0.10
Mid Lactation				
Intakes (kg/d)				
DM	28.8	27.6	0.40	0.09
OM	26.5	25.3	0.38	0.08
CP	5.22	5.00	0.098	0.17
NDF	9.0	8.6	0.10	0.03
Total N (g/d)	836	800	15.7	0.17
Digestibility (g/kg)				
CP	684	681	10.8	0.80
NDF	479	485	6.5	0.56

[1] Data relate to all cows present in pens throughout the study
[2] Data relate to subgroup of 60 cows (30 cows per treatment) from which faecal samples were collected
[3] Potential NDFD determined for NDFom using NRC 2001 method

In most cases, DMI is not affected by RPAA supplementation (Rogers *et al,* 1989; Armentano *et al,* 1993; Christensen *et al,* 1994; Piepenbrink *et al,* 1996). Polan *et al* (1991) reported that DMI

was depressed by feeding RPM, but this depression was reversed when RPL was also supplemented. In contrast, RPL depressed DMI by 0.68 kg/d after being fed 16 g of lysine/day (Watanabe *et al,* 2006). Reductions in DMI might also be attributed to lower microbial activity, and therefore digestion in the rumen. However, analysis of the RPL didn't reveal any contaminants that could affect microbial growth, and digestibility of the diet in mid lactation cows showed an increase, rather than a decrease, when RPL was fed.

Reduced digestibility of diets fed to dairy cows are usually attributed to increased feed intake (Tyrrell and Moe, 1974) and increased rate of ruminal passage, but DMI was not, however, higher with RPL feeding. Calculation of CP digestibility is based on levels of N in the diet and faeces and its reduction could be due to the significant increase in faecal N (Appendix A4; Table A4.1) of early lactation cows when RPL was fed. Shield estimated that RDP supply was limiting, while RUP was supplied in excess of requirements, and higher RUP levels at the expense of RDP could cause a reduction in DMI (Olmos Colmenero and Broderick, 2006) and suppress microbial growth and protein synthesis which could negatively affect digestibility of feed protein and fibre (Santos *et al,* 1998).

During early lactation, cows in the treatment group consumed higher levels of NDF, but NDFD was lower. The opposite was true during mid lactation where the RPL diet had a higher NDFD with a lower NDF intake. Kauffman and St-Pierre (2001) reported that apparent NDFD was increased due to a higher NDF concentration in the diet, which was not the case during this experiment, but consistent with the suggestion that ruminal NDFD tends to decrease as more hay is fed to animals (Scholljegerdes *et al,* 2005), which may explain the decrease in NDFD during early lactation since the amount of lucerne hay fed to treatment groups were higher than the control groups. Changes in mixing procedures, times and equipment, which is difficult to determine, could also have affected digestibility.

Even though Robinson *et al* (2004) found little relationship between the lignin based NDFD calculation (NRC, 2001) and *in vitro* NDFD determination (**$dNDF_{30}$**), the similarity between the potential NDFD (Table 4.5) and $dNDF_{30}$ (Table 4.3) values in the control TMR fed in our experiment suggest that calculations using dietary lignin concentrations as a faecal marker are fairly accurate in estimating *in vivo* fibre digestibility. The $dNDF_{30}$ values estimate forestomach

digestion and are usually around 0.85 of NDFD values, which represent whole tract digestibility (Robinson *et al*, 1994), estimated using the lignin concentrations. Since there was no difference between $dNDF_{30}$ of the control and RPL TMR's (Table 4.3), but NDFD changed in both lactation groups when RPL was fed, it suggests that Lys possibly had an effect on cow metabolism and therefore lower tract digestion.

4.3.4 *Milk yield and composition*

Milk, true protein and lactose yields were not affected by RPL in early lactating cows. In the mid lactation group however, lactose tended (P = 0.07) to be lower while milk and true protein yields were reduced (41.8 vs. 40.9 kg/d and 1.27 vs. 1.25 kg/d; P = 0.05) (Table 4.6). Milk fat yield (1.86 vs. 1.77 kg/d in early and 1.56 vs. 1.43 kg/d in mid groups) and concentration (35.0 vs. 32.9 g/kg in early and 37.4 vs. 35.4 g/kg in mid groups) decreased (P < 0.01) in both lactation groups when RPL was fed. Addition of RPL increased MUN percentages in both lactation groups ($P \leq$ 0.01). The concentration of energy in the milk was reduced (P < 0.01) for both early and mid lactation groups. In early lactation cows, the true protein concentration also decreased with RPL treatment (P = 0.05), but this was not the case for cows in mid lactation. The SCC tended (P = 0.08) to increase in early lactation cows, but was not affected by RPL in mid lactation.

Table 4. 6: Production performance of early and mid lactation cows as influenced by RPL*

	Control	RPL	SEM	*P*
Early Lactation (n = 157)				
Yield (kg/d)				
Milk	53.2	54.0	0.85	0.23
Fat	1.86	1.77	0.033	< 0.01
True Protein	1.45	1.46	0.022	0.47
Lactose	2.53	2.57	0.042	0.22
Calculated energy (MJ)	148	146	2.4	0.21
MUN (g/d)	8.60	8.97	0.169	0.01
MPN (g/d)	227	229	3.4	0.47
UN:PN**	3.79	3.91	0.045	< 0.01
Components (g/kg)				
Fat	35.0	32.9	0.32	< 0.01
True Protein	27.3	27.2	0.15	0.05
Lactose	47.5	47.6	0.14	0.35
Calculated energy (MJ/kg)	2.79	2.71	0.014	< 0.01
MUN (mg/dL)	16.1	16.5	0.15	0.01
Ash	8.82	8.85	0.027	0.28
SCC	347	452	67.8	0.08

	Control	RPL	SEM	*P*
Mid Lactation (n = 230)				
Yield (kg/d)				
Milk	41.8	40.9	0.67	0.05
Fat	1.56	1.43	0.024	< 0.01
True Protein	1.27	1.25	0.019	0.05
Lactose	1.98	1.94	0.033	0.07
Energy (MJ)	123	117	1.9	< 0.01
MUN (g/d)	5.96	6.04	0.116	0.32
MPN (g/d)	199	195	2.9	0.05
UN:PN	2.99	3.08	0.029	< 0.01
Components (g/kg)				
Fat	37.4	35.4	0.29	< 0.01
True Protein	30.6	30.7	0.14	0.65
Lactose	47.3	47.3	0.14	0.92
Energy (MJ/kg)	2.95	2.88	0.013	< 0.01
MUN (mg/dL)	14.3	14.7	0.10	< 0.01
Ash	8.97	8.99	0.025	0.30
SCC	415	419	50.6	0.92

* Data for the group of cows, at the end of period one and two, that wasn't moved between pens during the study
** Ratio of urea N to protein N in the milk (MUN/MPN*100)

Studies reviewed by Robinson *et al* (1995) demonstrated that milk protein and fat production can be enhanced when RPL are supplemented in combination with RPM. Results from other studies also showed either unchanged (Donkin *et al,* 1989; Rogers *et al,* 1989; Canale *et al,* 1990; Guillaume *et al,* 1991; Armentano *et al,* 1993; Christensen *et al,* 1994) or increased (Rogers *et al,* 1987; Polan *et al,* 1991) milk yield, but in these cases the individual effects of RPL and RPM cannot be separated, and only a few studies have been published in which RPL was fed alone (Blauwiekel *et al,* 1997; Robinson *et al,* 1998; Misciattelli *et al,* 2003; Watanabe *et al,* 2006).

In Robinson *et al* (1998), cows failed to respond to RPL, probably because the basal diet was not limiting in Lys. Milk yield was not affected when 16 g/d of a fat coated RPL was fed to early lactating cows which correspond with our results (Watanabe *et al,* 2006). However, Blauwiekel *et al* (1997) reported higher milk yield, even though the diet supplied Lys at 105% of requirements to early lactating cows. Piepenbrink *et al* (1996) suggested that a reduction in milk yield could be due to detrimental effects on milk production from excessive amounts, and/or improper ratios, of absorbable Met and Lys and, by feeding RPL without RPM, milk yield could have been adversely affected. Shield estimated that the ratio of Lys to Met at the intestinal absorptive site increased from 2.16 to 2.28 in supplemented cows (Table 4.4), which seems too small to have had any substantive impact on milk yield, but the lower RUP content of the ration and estimated AP

delivered to the intestine of the treatment group may explain the reduction in milk yield for mid lactation cows.

The depression of milk fat percentage could potentially have reflected a negative effect induced by compounds used to protect the Lys. The RPL matrix, however, only consisted of Lys, lecithin, water (none of which could affect milk fat synthesis) and vegetable fat, and FA analysis did not reveal any fat components, such as conjugated linoleic acid (**CLA**), that would likely inhibit milk fat synthesis (Appendix A4; Table A4.2). In addition, its feeding level was very low.

Supplementation of Lys to a diet containing soybean meal (usually high in Lys but low in Met) reduced fat content and yield when Met was supplied at low levels. Once Met levels were increased, however, fat yields improved (Rogers *et al,* 1989). A linear trend to reduced milk fat content with increased Lys supplementation was reported by Polan *et al* (1991), who also fed soybean meal in the diet, but not when a maize gluten meal diet was fed. Methionine deficiencies lead to reduced milk fat synthesis, probably due to its involvement in transmethylation reactions of lipid synthesis (Campbell and Farrell, 2003), and their results suggest that an oversupply of Lys with a Met deficiency may amplify the fat depression.

Lysine is an important precursor for carnitine synthesis when modified to trimethyllysine using SAM (S-adenosylmethionine) as a methyl donor. Carnitine in turn is required for transport of FA from the cytosol into the mitochondria during lipid breakdown for the generation of metabolic energy. Methionine and Lys are therefore intricately connected in normal body functions and without adequate levels of Met, carnitine cannot be synthesized, leaving excess Lys to be excreted or used for other purposes. Methionine was not, however, likely to be limiting during this experiment.

Piepenbrink *et al* (1996) reported a quadratic effect of RPAA on milk fat yield and content. Responses were higher when 0 g/d of Met and 0 g/d of Lys or 33 g/d of Met plus 106 g/d of Lys were fed and were less when 11 g/d of Met plus 35 g/d of Lys or 22 g/d of Met plus 70 g/d of Lys were fed to cows consuming a ration with 14% CP. Thus fat synthesis was highest at 0 and 150% of absorbable AA requirements. It would appear that an oversupply of both Met and Lys (150% of estimated requirements) increased fat synthesis, while an intermediate supply did not. It is possible that AA limitations divert some of the excess AA, or whatever nutrient caused the increased fat

production, toward fat synthesis and that supplementation of limiting AA rectified the imbalance, increasing milk protein synthesis. With the oversupply of both Lys and Met however, excess AA was once again diverted and fat synthesis increased. This suggests that Lys was the limiting AA and that its supplementation diverted nutrients from milk fat synthesis, while the lack of protein response might suggest that the supplementation level was not high enough to allow the full effect on production to be seen.

Because predictability of AA delivery and absorption in dairy cows are unreliable, and since an undersupply of Lys was unlikely due to responses to feeding of the RPL (i.e., sharply reduced milk fat and increased MUN), it is possible that Lys supplementation could have been excessive, leading to toxic effects. In an attempt to determine possible toxic effects of feeding too much RPAA, Robinson *et al* (2000) infused Lys and Met into the abomasum of late-lactation Holstein cows at 135 to 160% of calculated intestinally absorbable requirements of the AA. Even though infusion of Lys alone only resulted in a numerical decline in DMI, the DMI dropped substantially when Met was infused with, or without Lys. Milk yield, lactose and protein production declined with infusion of either AA alone, while milk fat production was not influenced. Total energy output tended to be lower with Lys infusion, but was sharply lower when Met was infused alone. This was attributed to the negative energy balance of cows infused with Lys or Met and a decline in output of milk energy. Since milk energy is calculated from its fat, protein and lactose content, the drop in energy resulted primarily from lower lactose production while, in our experiment, it was due to lower fat production. It has been suggested that the cationic transport system used to absorb Lys uses energy (Baumrucker, 1985) and while the source of this energy is unknown, it is possible that a large increase in Lys absorption could reduce the amount of energy available for other purposes, such as milk fat synthesis.

Overall, Robinson *et al* (2000) showed that an oversupply of as little as 140 to 150% for either Met or Lys can negatively affect animal production and performance. While the negative impact of Met oversupply was much more severe than that of Lys, a simultaneous oversupply of both AA ameliorated the negative effects of either AA alone.

The amount of Met delivered to the intestine of the treatment groups during our experiment was much higher than during Met infusion in Robinson *et al* (2000) (78 to 82 g/d vs. 64 g/d), the

opposite was true for Lys delivery (178 to 185 g/d vs. 195 g/d). If Lys stimulates absorption of Met from the blood, the negative effects seen in milk production may not be due to increased absorption of Lys *per se*, but rather the extreme oversupply (> 160% of requirement) of Met. However, high Met levels may explain changes in milk yield and protein percentage, but not the decline in fat synthesis.

Acetate is an important ruminant metabolite which serves as precursor for milk fat synthesis (Black *et al,* 1957). Acetate levels increase with increased NDF concentrations in the diet (Scholljegerdes *et al,* 2005) and the lower NDF intake for the mid lactation treatment group, compared to the control, might have contributed to the drop in fat synthesis due to lower acetate production in the rumen. However, since VFA production in the rumen was not measured, there is no way to verify this and it is not clear whether the decline in NDF intake of 440 g/d was enough to affect ruminal VFA ratios. That NDF intake were higher in the early lactation group, but digestibility lower, may be the same effect as lower NDF intakes *per se*.

The decline in milk protein percentage with feeding of RPL in early lactating cows was very small (0.1 g/kg) and is not viewed as biologically significant. The drop in protein yield in mid lactation cows was primarily due to the decrease in milk yield as milk protein percentage was unaffected. The lack of response of milk protein to RPL supplementation suggests that the control diet provided adequate amounts of AA for milk protein syntheses and/or that other factors might have been limiting.

The relationship between protein nutrition and MUN in dairy cows was investigated by Hof *et al* (1997). Dietary CP degraded in the rumen is utilized for microbial growth, while excess ammonia N is absorbed from the rumen and converted to urea. Part of this is recycled back to the rumen and reused by microbes. When excess RDP in the diet increases, it increases the amount of ammonia N that has to be absorbed and less of the endogenous urea is used, thereby increasing blood urea N (**BUN**) (Roseler *et al,* 1993) and, since urea diffuses readily across cellular membranes into the urine, more urea will be excreted in urine. As milk is secreted in the mammary gland, urea diffuses into and out of the mammary gland to equilibrate with urea in blood, thereby increasing MUN (Jonker *et al,* 1998). The amount of urea in urine is therefore directly proportional to BUN, which in turn is proportional to MUN (Roseler *et al,* 1993). Thus MUN is an indicator of

the protein status and efficiency of protein utilization in cows (Roseler *et al,* 1993; Hof *et al,* 1997), and tends to increase as the protein to energy ratio in the diet increases (Oltner *et al,* 1983). The BUN did not differ between the control and RPL groups in our study (Table 4.7), suggesting that the ratio of protein to energy in the unsupplemented ration was adequate, even though MUN increased when RPL was fed, which agrees with results of Varvikko *et al* (1999) where excessive amounts of Lys increased MUN without affecting BUN, suggesting that urea synthesis in the mammary gland itself was increased. That BUN was only measured during the second period is recognized, but since CP intake for treatment groups was not higher, and RDP levels in the diet were likely below requirements for microbial growth, the additional urea in milk cannot be attributed to excess dietary N or higher BUN levels. The decline of ornithine and citrulline (i.e., urea cycle metabolites) in plasma of treatment groups also suggest lower ammonia production in the rumen and therefore less urea synthesized in the liver (Bergman and Heitmann, 1980). Decreased MUN, together with a reduction in N excreted in the urine and faeces, indicates that dietary CP is being utilized more efficiently (Chow *et al*, 1990; Bremmer *et al,* 1997). The increased levels of urea in milk and N in the faeces (Appendix A4; Table A4.1) in our study, without increased levels of CP in the ration or improved milk protein production, suggest reduced efficiency of N. However, milk N efficiency, calculated as the ratio between feed N and milk protein N, increased with RPL treatment in both groups (27.5 to 27.7% during early lactation and 23.8 to 24.4% in mid lactation), suggesting that N excreted in urine and faeces does not come from dietary CP but a different source, such as mobilization of body protein (i.e., negative N balance), possibly the same source as MUN. The partial N balance can be seen in Appendix A4 (Table A4.4) but total N balance was not calculated since N intake was not measured by cow.

Hindgut fermentation of slower-digesting, but potentially fermentable, carbohydrates (i.e., crystalline starches) escaping rumen degradation may also result in some MCP production which is excreted in the faeces if it is not absorbed, increasing its N content (Van Soest, 1994). This may be supported by the biggest increase in faecal N (during early lactation) being accompanied by reduced NDF digestibility. Linkages in hemicellulose, sensitive to weak acids, may be cleaved during peptic digestion in the stomach, making these fractions available for fermentation in the lower tract.

Average MUN concentrations can vary between 10.7 and 16.4 mg/dl (min = 0.5 and max = 19.8 mg/dl) due to large variations among individual cows that depend on many managerial and production factors such as milk fat percentage, body weight (**BW**) and parity and, since MUN values for our experiment still fall within the average concentration range, statistical differences in MUN yields between treatments may be considered to not be biologically important. However, Jonker *et al* (1998) and Hojman *et al* (2005) reported a negative correlation between BW and MUN concentrations at BW's of up to 650 kg (the BW assumed during our study, based on visual assessment since cows were not weighed) after which MUN tended to increase with higher BW's and MUN is also negatively correlated with milk fat yield (DePeters and Ferguson, 1992; Broderick and Clayton, 1997). Even though the methodology of these relationships is not known, it suggests that increased MUN can be expected due to BW loss (predicted by Shield) and a decline in milk fat synthesis in both lactation groups.

Varvikko *et al* (1999) infused 10 to 40 g/d Met and 15 to 60 g/d Lys continuously into the abomasums of five Ayrshire cows, and many of their results were similar to those during our experiment. Even though infusion of Lys had no affect on milk fat production, it resulted in a linear increase in propionic acid and a decrease in acetic acid in the rumen. It caused a linear increase in MUN and NPN, but had no affect on plasma urea concentrations.

4.3.5 *Blood plasma*

All plasma AA, except Lys and 3-methylhistidine (**3-MH**), decreased after RPL was fed to early lactating cows (Table 4.7), but none differed significantly. In the mid lactation group, however, the AA that had a tendency to decrease after feeding RPL included Gly ($P = 0.05$) and 3-MH ($P = 0.05$) while Leu ($P = 0.04$), Asn ($P = 0.01$), Glu ($P = 0.01$), Gln ($P < 0.01$) and Pro ($P < 0.01$) were reduced. Tryptophan concentrations increased in the treatment group ($P = 0.01$). Aside from the numerical increase in both early and mid lactation groups, Lys concentrations were not impacted (13.0 vs. 13.5 µg/mL and 13.6 vs. 14.1 µg/mL), although the Lys to Met ratio increased from 3.11 to 3.63 and 3.08 to 3.40 in early and mid lactation groups respectively. All other primary AA were unaffected by RPL treatment.

Table 4.7: Free AA concentrations (µg/mL) and urea in plasma of early and mid lactation cows as influenced by RPL*

	Control	RPL	SE	*P*	Control	RPL	SE	*P*
	Early lactation				Mid lactation			
EEA								
Thr	13.4	10.6	0.98	0.05	11.1	10.8	0.47	0.58
Val	36.1	35.3	1.79	0.74	35.0	30.1	2.19	0.13
Met	4.2	3.7	0.19	0.11	4.4	4.2	0.16	0.27
Ile	17.9	17.3	1.08	0.72	16.3	17.2	0.64	0.32
Leu	29.9	28.4	1.49	0.50	27.8	22.6	1.72	0.04
Phe	9.4	8.4	0.54	0.20	8.2	8.0	0.24	0.47
Trp	8.0	7.3	0.68	0.48	6.8	8.5	0.41	0.01
Lys	13.0	13.5	0.72	0.61	13.6	14.1	0.78	0.71
Arg	23.3	23.2	1.10	0.93	26.8	26.9	0.83	0.93
His	7.8	7.7	0.38	0.86	9.2	8.7	0.57	0.49
NEAA								
Cystine[1]	0	0	-	-	0	0	-	-
Aspartic Acid	1.2	1.1	0.10	0.35	1.1	1.0	0.14	0.57
Serine	8.2	8.0	0.52	0.87	8.6	8.0	0.32	0.21
Asparagine	6.2	5.9	0.35	0.50	6.0	5.0	0.29	0.01
Glutamic Acid	7.9	7.0	0.37	0.10	9.6	8.0	0.40	0.01
Glutamine	37.3	36.0	1.90	0.63	48.0	37.6	2.24	<0.01
Proline	11.8	10.3	0.64	0.10	10.1	8.3	0.37	<0.01
Glycine	22.9	19.8	2.49	0.39	23.4	19.5	1.34	0.05
Ala	27.5	24.7	1.35	0.15	24.8	23.0	0.96	0.19
Tyrosine	13.1	11.4	1.07	0.26	11.6	11.1	0.48	0.48
3-MH	0.49	0.53	0.038	0.56	0.56	0.39	0.060	0.05
Urea	308	288	19.1	0.46	289	284	8.7	0.67

* Plasma samples only collected during second period
[1] Cystine was below the method detection limit (i.e., levels too low to measure accurately)

Increased supply to the small intestine of any AA is expected to change its concentrations in the blood and, possibly, improve availability of that AA for milk protein synthesis in the mammary gland. This was demonstrated by Blauwiekel *et al* (1997) in an experiment in which Lys supplementation increased flow to the duodenum, as well as the concentration of Lys (and all other EAA except Phe) in plasma, leading to an increase in milk and milk protein yield. Blauwiekel attributed this increase to higher N intakes and/or MCP yields, neither of which was true for our experiment during which plasma concentrations of most EAA (except Lys in the early, and Ile, Trp and Lys in mid lactation group) and all NEAA (except 3MH in the early lactation group) decreased after RPL was fed. Decreased plasma AA concentrations can either be attributed to a reduction of AA absorption from the small intestine or improved absorption and utilization by body tissues.

The plasma membrane of absorptive cells in the intestine has at least four sodium-dependent AA transport systems; one for acidic (Asp, Glu), basic (Lys, Arg, His), polar (Gly, Asn, Ser, Gln, Thr, Tyr, Cys) and non-polar (hydrophobic) AA. The membranes of enterocytes and tissue cells

contain additional transporters, not dependent on a sodium gradient, which export AA from intestinal cells into the blood and from there into other body cells.

Baumrucker (1985) explained that the transport systems for absorption of AA depend on transport specificity and competition between AA. In theory, feeding RPL provides more Lys to cells with a Y^+ (cationic) transport system and uptake of AA sharing the same transport system (i.e., Arg and ornithine) may be reduced through competitive inhibition by Lys. It is unlikely, however, that the level of Lys in the small intestine reached levels high enough to saturate the transport system and limit uptake of other AA, creating other limitations to production, unless the amount of Lys delivered to the intestine was much higher than expected, and the concentration of Lys in the plasma was not high enough to impact Arg absorption by cells. To prevent inhibition of other AA, all AA should be increased proportionally, which supports other studies suggesting that supplementation of a combination of AA would elicit a larger response than any AA alone.

Another transport system, the L system, was identified in bovine mammary tissue. This system is thought to function by exchanging AA, principally BCAA (i.e., Phe, Met and Trp). The increase in the concentration of Trp in the plasma of the mid lactations group with feeding of RPL suggests that Trp absorption from the blood might have been inhibited by increased absorption of other AA using this system. This is consistent with the previously discussed possibility that Lys may have stimulated absorption of AA such as Met. Tryptophan is one of the ketogenic AA involved in FA synthesis and, together with Ile (the only other EAA that wasn't reduced); it provides acetyl-CoA to the citric acid cycle (Campbell and Farrell, 2003) for AA biosynthesis and degradation. If absorption of these two AA were inhibited in some way, it might have had a crippling effect on energy metabolism and possibly fat synthesis.

Endocrine regulation of metabolism is very important in ruminants, but the extent to which AA elicit hormone responses in lactating cows is not known. It has been shown that AA infusions increase serum concentrations of glucagon, insulin and growth hormone in sheep (Bassett, 1971) and that these factors, among others, may have a stimulatory effect on AA transport systems (Kilberg, 1982). More research is needed to determine relationships between AA and hormones, and the amount of AA needed to increase serum hormone concentrations is most likely much more than the amount of Lys that was available after RPL supplementation in our study.

Since Lys was the AA supplemented, an increase in plasma Lys concentration was expected once animal requirements for Lys was met, but because mammary uptake of Lys usually exceeds its requirements for milk production (Lapierre *et al,* 2005a; Rulquin and Pisulewski, 2006), the small numerical increase in plasma concentration may suggest that absorbed Lys was used for other purposes. Since excess Lys is not extracted by the liver, it is probably deaminated in other parts of the body, such as the mammary gland, after which the N is either returned to the liver for excretion as urea or used to synthesize NEAA (Lobley and Lapierre, 2003); Lys is a known precursor for *de novo* Arg and Pro synthesis (Bequette, 2002). If excess Lys was metabolized in the liver or mammary gland, an increase in plasma Arg concentrations or, due to metabolism of Arg, an increase in ornithine concentrations would be likely (Clark, 1975; King *et al*, 1991). That Arg was seemingly unaffected by RPL feeding in our study, may support this hypothesis.

The decrease in concentrations of essential and non-essential AA in plasma after RPL was fed suggests that Lys was the limiting AA and that its supplementation led to improved absorption and rapid utilization of other AA (Clark, 1975). Graded doses of Lys had an effect on extraction rates and metabolism of AA in the mammary gland (Varvikko *et al,* 1999), but it appeared to facilitate absorption of BCAA into the blood while suppressing its uptake and utilization by mammary tissue. There was no increase in milk protein production during our study and because the decrease of NEAA in plasma of treatment groups was larger than EAA, this suggests that EAA were not utilized to synthesize NEAA or milk protein. Since NEAA are extensively utilized for hepatic glucose synthesis (Wolff and Bergman, 1972) and extraction of NEAA (i.e., Pro, Glu, and Asp) are much lower than the amounts required for milk protein synthesis, it is unlikely that the substantial drop in their plasma concentrations were due to absorption by the mammary gland. This is supported by Varvikko *et al* (1999), who suggested that increased availability of Lys decreased uptake of NEAA by, and use of, BCAA and Arg for NEAA synthesis in the mammary gland.

Used AA for synthesis of milk and body protein were not estimated by measuring body condition scores (**BCS**) or BW in this study, eliminating the use of BW changes as an indication of synthesis or mobilization of body protein or fat. However, urinary output of 3-MH provides a reliable index for myofibrillar protein degradation (Harris, 1981) and, since it is released into blood during degradation of actin and myosin in skeletal muscles (Young and Munro, 1978; Blum *et al*,

1984), plasma 3-MH concentrations can be used to judge if body protein was synthesized or degraded. Studies to determine the role of Leu in protein metabolism showed that high Leu concentrations can stimulate muscle protein synthesis by enhancing the sensitivity of muscle to insulin (Garlick, 2005; Carvalho *et al,* 2006). Bequette *et al* (2002) suggested that for goats in mid lactation, leg tissues were more sensitive to AA supply than the mammary gland and that the decreased AA concentrations in the blood were due to enhanced muscle protein synthesis. The decreased concentration of 3-MH in plasma when RPL was fed to mid lactation cows could be due to Lys increasing absorption of Leu ($P = 0.04$) from the blood, thereby stimulating muscle protein synthesis and reducing protein degradation. Rulquin and Pisulewski (2006) showed that the continuous infusion of Leu into the duodenum of lactating cows resulted in a reduction of lactose and fat secretion in milk which may, explain the reduction in milk fat synthesis during our experiment. They were not, however, able to explain why this occurred.

Skeletal muscle breakdown and mobilization of AA is higher in early lactation cows due to a larger protein and energy deficiency (Botts *et al,* 1979), which corresponds with the higher predicted BW loss in these groups (Table 4.4). Blum *et al* (1985) showed that peak 3-MH concentrations were negatively related to energy (NE_L) and protein (AP) intakes and closely related to milk protein yields. This is contrary to what happened in our study since increased 3-MH concentrations in early lactation pens were associated with higher energy (calculated as DMI x NE_L or ME) and CP intakes (even though it was not statistically significant), as well as increased AP delivery, and decreased 3-MH concentrations during mid lactation was associated with lower DMI.

Blum *et al* (1985) also demonstrated that enhanced degradation of actin and mobilization of body fat was relatively closely related to each other, even though the reason for this is still unclear. Increased muscle protein synthesis might be indicative of increased body fat synthesis, which could explain why energy was diverted away from milk fat synthesis.

Since higher protein and energy intakes did not prevent mobilization of body tissue in early lactation cows (judging on the blood 3-MH content) while mobilization was reduced in mid lactation cows regardless of decreased protein and energy intakes, it is evident that Lys had some effect, whether directly or indirectly, on muscle protein turnover and energy metabolism, significantly impacting intakes, metabolism and absorption of AA and milk production in mid

lactation cows. This is consistent with Robinson *et al* (2000), who suggested that infusing Lys and/or Met into late lactation cows possibly changed animal metabolism, reducing performance due to possible AA imbalances or toxicities. However, Lys had no major impact on early lactation cows.

4.4 Conclusions

Increased milk production and higher milk protein yields generally require increased dietary CP levels. Since large amounts of maize distiller's by-products are entering the California dairy sector from ethanol distillation plants, the proportion of total dietary CP coming from low Lys maize products are increasing. This has created a potential market for RPAA products, such as RPL, since there is no such product currently available.

Such an RPL product was evaluated through chemical analysis and measurement of density, rumen degradation (based on ruminal *in situ* incubation) and durability, revealing:

→ a lysine content of 42%,

→ estimated rumen escape of 43 to 54% depending upon assumptions of rumen rate of passage,

→ intestinal Lys delivery 19 to 24% due to particle degradation during TMR mixing and time in the feed bunk,

→ final intestinal absorption of 18 to 23% based on a post-ruminal digestion of 96%.

Based upon the RPL rumen degradation and intestinal disappearance studies, there appears to be little doubt that this RPL product resulted in increased intestinal absorption of Lys. However, depending upon assumptions of its rumen degradation rate and rumen passage rate, and the impact of physical breakage of the RPL during mixing on rumen degradation of the RPL, the likely range in the increase in intestinal absorption of Lys was between 8 and 22 g/d. The sharp decline in milk fat synthesis in both the early and mid lactation cows with RPL feeding, and changes in concentrations of several major AA in blood plasma, is also strong evidence that this RPL did increase intestinal absorption of Lys, and the lack of difference in plasma Lys concentrations with RPL feeding suggests that this Lys, at whatever level, was metabolized.

All our data seem to suggest differences in the level and ratio of Lys and Met in AP are the likely cause of differences in cow production during this study. Results from this, and other, studies indicate that excessive amounts of either AA alone have detrimental effects on milk yield due to a disturbance in the balance between them. Even though the predicted ratio of Lys to Met in AP increased in both lactation groups, the mid lactation cows had a much larger negative response to RPL feeding relative to milk, fat and protein yields, and RPL supplementation was associated with increased absorption of most AA, especially Leu, from the plasma but lacked a positive response in milk protein production, suggesting that something other than the Lys:Met ratio (e.g., stage of lactation) is important. The RPL delivered Lys and it impacted metabolism, but the benefits can not be seen using the parameters recorded during this experiment. It is possible, however, that the benefits were elsewhere, such as an increase in body protein turnover.

Studies have shown that postruminal Lys supplementation increase absorption of other AA, but reduce their utilization by the mammary gland which suggests, due to the lack of additional N in the urine, that these AA are utilized elsewhere. Leucine is known to act as an insulin secretagogue and, together with the decreased concentration of 3-MH in the plasma, it could indicate increased muscle protein synthesis. Other AA involved, to various extents, in muscle protein synthesis include Pro and Arg, both derived from Lys catabolism and Gln. All of these AA were reduced in the plasma of RPL fed cows, and the lack of change in Arg concentrations may be due to biosynthesis from excess Lys.

The AA's that regulate energy and fat metabolism include Met, supplied at > 160% of requirements which could impact milk yield and protein if absorption was stimulated even further, Ile, Thr and Trp. Plasma levels of these AA were not reduced, suggesting that they might not have been absorbed and/or utilized, which may explain why milk fat synthesis were reduced.

The close relationship between body protein and fat turnover may suggest diversion of AA and energy away from milk production towards body tissue synthesis. The early lactation cows showed a trend towards the same responses as the mid lactation cows, even though intakes and plasma AA concentrations were not impacted. Shield predicted the need for an additional 11.7 g intestinally available Lys per day to bring the Lys level to 110% of requirements for early lactating cows. The estimated 10 g delivered during this study may be sufficient for mid lactations cows, but it leaves

Lys supply below requirements for the early group cows and since protein mobilization is higher during early lactation, it may take higher levels of Lys to induce the same effects as seen in mid lactating cows.

Chapter 5: General discussion

5.1 Conclusion and implications

Opinions differ widely on which AA are truly limiting in lactating dairy cows, but studies have suggested Lys and Met to be the most likely, followed by others such as Ile. The inconsistencies in results obtained in other studies during which an AA initially predicted to be limiting was fed, could be attributed to changes in the ranking order of limiting AA. This may be caused by differences in the stage of lactation and the level of milk yield, despite the use of similar basal diets (Kim *et al,* 200). More information needs to be gathered regarding AA limitations, and the effect of supplementing them, in order to make ration formulation according to AA levels feasible.

Evaluation of contemporary rations fed to high producing dairy cattle throughout the largest milk producing counties of CA, showed that maize products contribute as much as 55% to TMR DM with maize CP making up ~ 30 % of total CP. However, even at these high inclusion levels it did not have any detrimental effect on milk production. While there are many other factors influencing animal performance, current management and feeding strategies were judged to be adequate in avoiding expected/predicted negative consequences of increasing maize and DDG levels in the ration. The nutritionists appear to be formulating rations to maintain CP levels, while considering nutrient profiles of individual ingredients.

Even though the high contribution of maize CP to total TMR CP did not have any direct effect on milk production, it changed the ratio of Lys to Met in MP, due to lower predicted Lys delivery to the intestine, as well as reducing the amount of MP reaching the intestine. The continued high milk yield suggests that the contribution of MCP to MP was underestimated, and that MCP is a powerful preventor of AA imbalances at the intestinal absorptive site.

Effects of maize CP are minor as long as maize levels are low, but when maize CP rise above 30% of TMR CP the decline in MP increases dramatically. The levels of maize products in rations have been consistently increasing in California with no noticeable problems in production of the cows. However, continuation of this trend might cause a change in MP, whether it is the amount or profile of AA delivered to the intestine, to an extent that is beyond the ability of rumen MCP to rectify and, since there is a positive correlation between milk yield and MP delivery, this may have negative effects on future milk production. If maize inclusion levels continue to increase,

nutritionists will have to take better care in formulating rations to balance nutrients to improve MCP synthesis and maintain MP delivery. Clearly MCP is a major source of metabolizable AA and enhancing rumen microbial populations, instead of supplementing individual AA, may be the best option.

Our attempt to identify limiting AA in dairy rations proved to be difficult due to the variation among metabolic models. Met, Ile and Lys were predicted to be most limiting according to Amino Cow, CPM Dairy and Shield respectively. Even though this corresponds with what we already know from previous studies, it is difficult to define a suitable AA package. However, within model variation was small enough to conclude that there is enough consistency among dairy rations to validate production of an AA supplementation package. However, even though the nutrient compositions of TMR are relatively consistent among dairies in California, creating such a package will not be possible unless a method is developed to identify the most accurate model. Once that is done, an RPAA package, consisting of the most limiting AA, can be formulated and manufactured.

The one AA that was consistently emphasized throughout all dairies and models was Lys, suggesting that development and supplementation of a ruminally protected Lys product may be beneficial to dairy farmers. The general expectation of inclusion of RPL in the ration was that milk yield and/or milk protein content and yield would increase but, feeding of the product to one group of high producing dairy cattle, showed that production of cows in early lactation was slightly negatively affected by the level of RPL fed, while increased absorption of most AA in mid lactation cows were associated with even more negative responses in terms of milk, fat and protein yield.

There was enough supportive evidence to conclude that Lys from the RPL was delivered to the intestine, absorbed and metabolized. If the RPL failed to deliver any Lys to the small intestine due to degradation in the rumen, micro organisms would have used Lys to synthesize MCP without affecting any of the production parameters and/or if the Lys was not absorbed into the blood, there should not have been any change in other AA concentrations.

It is clear that supplementation of Lys alone did not have the expected response in terms of milk production. Since Lys was still the limiting AA after supplementation (108% and 111% of requirements; Table 4.4), the lack of response cannot be attributed to another AA becoming more

limiting. Since body tissues are more sensitive to AA changes and milk protein synthesis was not negatively affected by RPL supplementation, the milk response might have become positive if more Lys was supplemented and enough AA were available to increase muscle and milk protein simultaneously. However, results from the survey of 16 commercial dairies indicated that milk yield decreased when the Lys to Met ratio increased. Korhonen *et al* (2002) reported that the efficiency of conversion of AA into milk protein could be improved if the ration is supplemented with the first and second limiting AA. This agrees with other studies (Clark, 1975; Schwab *et al,* 1976) suggesting that cows respond more favorably to supplementation of several AA and feeding a number of AA, combined in an RPAA package, may be the solution.

5.2 Future research and critical evaluation

Results of Experiment 1 suggest that feeding a combination of most limiting AA may be the most advantageous course of action to improve efficiency of dairy cattle production. However due to variations among metabolic models in their suggested AA combinations, the key step is to determine which AA's to combine. Ideally, all three AA packages could be created to determine responses in milk production to each one in a feeding study, but manufacturing some of these AA are costly and difficult, and further studies using AA infusions may be required to justify their production. Since Shield was judged to be the most reliable model in terms of predicting absorbable AA's and correlating them with production responses, development of the RPAA package suggested by Shield should take priority.

More research is needed to determine effects of feeding higher levels of Lys and Met, as well as their interactions on animal metabolism. The possibility of another AA being limiting should also be investigated. Experiments using intestinally cannulated cows can aid in determining effects of:

→ Lysine on:

- ❖ Nitrogen balance, by measuring individual intakes and milk N.
- ❖ Rumen fermentation products and milk fat synthesis when VFA concentrations are measured.

- ❖ Milk production and components by feeding different levels of RPL and taking daily milk samples.
- ❖ AA metabolism, absorption and utilization by taking daily blood samples and analyzing plasma AA concentrations.

→ Methionine and its toxicities on animal performance.

→ Isoleucine on animal performance to determine whether it is the next limiting AA.

→ A combination of Lys and other AA on animal production.

If another study, very similar to Experiment 2, is to be done, the RPL should be included in the ration at higher and/or multiple levels for both early and mid lactation cows and production parameters should include feed analysis, urine, fecal and milk samples, as before, however additional parameters could be included. This should include weighing individual cows to determine BW changes but, as this is often not feasible in large groups of cows, the BW changes could be estimated using BCS. Shield predicted BW changes based on animal production, dietary intake and other physiological predictions, but cannot specify whether changes are due to body protein or fat turnover. Non-esterified fatty acids (**NEFA**) concentrations could give an indication of the energy status of the cow, and should be included to determine whether BW/BCS changes are due to body protein or fat turnover, since increased concentrations of NEFA in the blood indicate that body fat is being mobilized. Plasma AA concentrations are a useful indicator of AA absorption from the intestine, and the RPL's success in delivering Lys to the intestinal absorptive site. It also aids in determining whether AA were absorbed from the blood and should be included as a production parameter in both periods. Analyzing the FA profile of milk may indicate which FA is most affected when RPL is fed. Collecting more frequent milk samples (e.g., every 1 to 2 weeks) could eliminate the effect of fluctuating milk component yields on results and reduce variation.

In order to determine the most effective level of RPL to use in a study resembling Experiment 2, as described above, a number of *in situ* studies, using graded levels of RPL introduced directly into the rumen, could be carried out. This could indicate the level at which RPL starts affecting cow production, as well as the sequence in which parameters are affected. However, it is not possible to recreate the diets that are used on commercial dairies for such small groups of cows and

among cow variation could obscure infusion results while interaction between cow and treatment makes it hard to interpret these results.

Based on results from this and previous studies there are a few options to follow up on our study. In my opinion, the best option regarding research following our two experiments would be to feed the Shield predicted AA complex vs. a control on a commercial dairy, and to do a study, similar to Experiment 2, on a commercial dairy with 3 groups of cows fed different levels of lysine.

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Appendix A2 (Chapter 2)

Table A2. 1: Description of a few known functions of major AA in animals and humans

Amino Acid	Description and Function
Arginine (Arg, R)	Abundant in protamines and histones (proteins associated with nucleic acids) Enhances the immune system Assists in neutralizing ammonia in the liver Involved in the skin and connective tissue Assists in maintaining nitrogen balance in muscle metabolism Synthesized from lysine Helps with weight control (facilitates increase of muscle mass while reducing body fat)
Histidine (His, H)	Precursor of histamine (released by immune system during allergic reaction) Needed for growth and repair of tissue Maintenance of myelin sheaths (act as protector for nerve cells)
Isoleucine (Ile, I)	Promotes muscle recovery Involved in blood-clot formation Provides acetyl-CoA to the citric acid cycle
Leucine (Leu, L)	Regulation of blood-sugar levels Growth and repair of muscle tissue (prevents degradation of muscle protein by increasing sensitivity of muscles to insulin) Involved in energy metabolism.
Lysine (Lys, K)	Assists in calcium absorption Maintains appropriate nitrogen balance in the body Needed to produce antibodies, hormones, enzymes and collagen formation Repair of tissue and building of muscle protein Precursor for glutamine, arginine and proline synthesis
Methionine (Met, M)	Lipotropics (assist in the breakdown of fats) Intermediate in transmethylation reactions : donates methyl group to synthesize creatine (essential for energy production and muscle building) Donates methyl group to synthesize carnitine (for lipid metabolism and FA mobilization) Precursor for cysteine
Phenylalanine (Phe, F)	Converted into tyrosine (essential for making proteins, certain brain chemicals and thyroid hormones)
Threonine (Thr, T)	Assists in the formation of collagen and elastin in the skin Involved in liver functioning (including fighting fatty liver) Lipotropic when combined with aspartic acid and methionine Precursor for isoleucine
Tryptophan (Trp, W)	Ketogenic AA involved in FA synthesis, it helps with weight loss and reducing appetite while providing acetyl-CoA to the citric acid cycle
Valine (Val, V)	Muscle metabolism, repair and growth of tissue and maintaining the nitrogen balance in the body Used as an energy source in the muscles, and in doing so preserves the use of glucose
Alanine (Ala, A)	AA most widely used by the body to build protein Glucogenic AA required for metabolism of glucose and Trp Constituent of vitamin B5 (pantothenic acid) and Coenzyme A Demonstrated a cholesterol-reducing effect in rats
Asparagine (Asn, N)	There is no suggested need for asparagine supplementation presently available in the literature Interrelated with the amino acid aspartic acid Low levels indicate poor metabolism or synthesis of aspartic acid, which can result in inability to synthesize and excrete urea
Aspartic acid (Asp, D)	Glucogenic AA involved in construction of AA and biochemicals in the citric acid cycle Synthesizes of asparagine, Arg, Lys, Met, Thr, Ile Assists liver by removing excess ammonia and toxins from bloodstream
Glutamic acid (Glu, E)	Synthesized from ornithine and Arg Functions as excitatory neurotransmitter Glucogenic AA it involved in metabolism of sugars and fats
Glutamine (Gln, Q)	Synthesis of muscle proteins Source of fuel for cells lining the intestines Used by white blood cells for immune function
Glycine (Gly, G)	Synthesis of nucleic acids Aid in absorption of calcium Muscle metabolism (helps to supply extra creatine in the body) One of the glucogenic AA

Amino Acid	Description and Function
Proline (Pro, P)	Thought to be important in the maintenance of muscles, joints and tendons Synthesized from Lys
Serine (Ser, S)	Metabolism of fat (glucogenic AA), muscle synthesis and the immune system
Tyrosine (Tyr, Y)	Known for its affect on neurotransmitters and growth hormone stimulation Production of melanin (pigment responsible for hair and skin color) Involved in functions of adrenal, thryroid, and pituitary glands
Cysteine (Cys, C)	Sulfur containing AA, main protein in nails, skin and hair Important in collagen production, assists in skin elasticity and texture Critical in metabolism of Coenzyme A, heparin, biotin, lipoid acid, and glutathione (component of the protective antioxidant systems in body)

Appendix A3 (Chapter 3)

Table A3. 1: Complete description of the 16 dairies, cows and pens designated by the dairy as one of their high group multi-parity corrals

Farm number	1	2	3	4	5	6	7	8
General management								
Total lactating cows	1000	1143	3000	1192	1809	2772	824	5000
TMR Mix and drop monitors	Printouts	Printouts	Feedwatch	Printouts	Feedwatch	Printouts	EZ Feed	Printouts
DC 305	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nr of milkings/day	2	2	2	2	2	3	2	2
DHIA test proc	Weight and components of one milking	Weight and components of one milking	Weight and components of one milking	Weigh both, components of one, SCC every 2nd test	Weight and components of one milking	Weigh 2, components of one milking	Weight and components of both milkings	Weight and components of both milkings
Pen info	Nr 2	Nr 4	Nr 3	Nr 7	Nr 9	Nr 24	Nr 1 & 2 comb	Nr 9
Head gates	140	110	218	195	199	150	300	450
Free stalls	124	-	208	178	204	-	272	390
Bedding	Dry manure solids	Dry lot dairy	Dry manure solids	Dry manure solids	Dry manure solids	Dry lot dairy	Dry manure solids	Dry manure solids
Fans	x	-	x	-	x	-	x	-
Bunk line misters	x	x	x	x	x	x	x	x
Flush feed apron with rubber mat	x	-	x	x	-	x	x	x
Outside dirt lot	-	-	x	x	x	-	x	x
BST	x	-	-	-	-	-	-	-
Animal information								
Nr of cows in high group pen	149	123	170	190	189	145	265	408
DIM 10% P	84	46	57	99	29	22	112	36
Average	221	97	87	132	86	87	199	108
90% P	345	141	119	170	129	139	291	170
Lact (%) 1	5	22	0	0	15	1	0	2
2	62	53	49	30	58	52	39	11
> 3	33	25	51	70	27	47	61	87
Lact (max)	6	8	7	9	5	8	6	10
Average Lactation	2.4	2.2	2.9	3.6	2.2	2.8	3	3.7
Outlier cows removed	11	8	5	4	4	0	1	10

Farm number	1	2	3	4	5	6	7	8
Production information								
Date of milk test	6/29/07	06/14/07	06/22/07	05/30/07	06/06/07	06/04/07	06/06/07	06/12/07
Milk yield (kg)	32.8	37.9	40.3	40.9	41.2	41.4	41.7	42.8
True Prot %	3.23	2.91	2.77	2.88	2.93	2.87	3.13	2.81
Fat %	3.32	3.49	3.19	3.67	3.14	3.49	3.54	3.08
SCC	739	270	75	187	70	122	262	264
Feed information								
Feeding times	5:30am 12:30pm	4:30am 2:00pm	1:30am 4:30am 10:00am 12:00pm	5:00am 12:00pm	6:00am 11:30am	2:00am (no whey) 4:00am (whey) 10:00am (whey) 1:30pm (no whey)	4:00am 3:30pm	7:00am 12:30pm
Sampling dates and times	06/20/07 1:00pm 06/27/07 6:00am	06/12/07 2:30pm 06/13/07 2:30pm	06/19/07 9:30am 06/21/07 4:15am	06/01/07 12:00pm 06/05/07 12:00pm	06/01/07 11:30am 06/05/07 5:30am	05/31/07 10:00am 06/01/07 2:00pm	06/05/07 4:00pm 06/07/07 4:00pm	06/06/07 12:30pm 06/12/07 12:00pm
Type of mixer	Horizontal Kirby 800 Aggressor truck (red)	Vertical, 2 screw, tractor&trailer (red), 900T Supreme feed processor	Stationary horizontal Laird 1500 & Vertical, 2 screw, Supreme truck (red)	Vertical, 2 screw, tractor & trailer (red), 1100 CF Peecon	Vertical, 2 screw, tractor & trailer (red)- Peecon	Horizontal truck (white) - Harsh	Horizontal tractor & trailer (white) - Laird/R.M.H.	Vertical, 2 screw, truck & trailer (red) and Horizontal 920 Dairy special (yellow) truck
Refusals	1% (written)	1% (written)	3% (Feedwatch)	2% (estimated)	1% (estimated)	1% (estimate)	3% (estimated)	1% (written)
Rumensin	-	Yes	Yes	Yes	Yes	-	-	Yes
Intakes								
As fed (kg/d)	43.92	34.69	48.25	43.53	46.19	37.79	45	44.86
DM basis (kg/d)	24.24	21.38	26.78	24.9	28.48	22.52	28.4	26.6
TMR DM %	55.2	61.63	55.51	57.2	61.65	59.6	63.1	59.29

Table A3. 1 (Continued)

Farm number	9	10	11	12	13	14	15	16
General management								
Total lactating cows	1200	2648	2200	4100	5000	932	4400	1378
TMR Mix and drop monitors	Printouts	Printouts	Feedwatch	Printouts	Printouts	EZ Feed	Feedwatch	Feedwatch
DC 305	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Nr of milkings/day	3	2	2	3	3	2	2	3
DHIA test proc	Take own weights, DHIA test compo-nents of one milking	Weight and components on AM milking	Own weights, DHIA do components on one milking	Weight of two, components of one milking	Weight of two, components of one milking	Own weights, DHIA do components on one milking	Weight of two, components of one milking	Weight of two, components of one milking
Pen info	Nr 1	Nr 1 & 2 comb	Nr 4	Nr 1	Nr 6	Nr 7 & 8 comb	Nr 11 & 12 comb	Nr 3
Headwaters	200	594	208	260	355	250	604	157
Free stalls	180	532	188	238	322	208	572	160
Bedding	Dry manure solids	Dry manure solids	Dry manure solids	Sand	Dry manure solids	Dry manure solids	Dry manure solids	Dry manure solids
Fans	x	x	x	-	x	x (half)	x	x
Bunk line misters	x	-	x	x	x	x (half)	-	x
Flush feed apron with rubber mat	x	-	x	-	x	-	-	x
Outside dirt lot	-	x	x	x	x	x	x	x
BST	x	-	-	x	-	-	x	x
Animal information								
Nr of cows in high group pen	158	513	191	264	364	223	587	167
DIM 10% P	89	94	63	91	31	35	37	42
Average	210	157	111	160	92	133	124	88
90% P	321	236	164	226	156	218	218	134
Lact (%) 1	1	2	0	4	1	0	0	1
2	59	47	44	46	48	16	0	37
> 3	40	51	56	50	51	84	100	62
Lact (max)	5	8	9	8	6	9	10	9
Average Lactation	2.5	2.9	3.1	2.8	2.5	3.7	4.1	3.2
Outlier cows removed	3	0	0	3	1	18	3	8

Farm number	9	10	11	12	13	14	15	16
Production information								
Date of milk test	06/27/07	06/04/07	06/19/07	06/11/07	06/27/07	06/11/07	06/12/07	05/03/07
Milk yield (kg)	43.3	45.2	45.4	46.6	46.7	47.7	48.5	51.3
True Prot %	3.00	2.72	2.84	2.95	2.87	2.92	3.01	2.73
Fat %	3.68	3.04	3.32	3.54	3.19	3.49	3.45	3.79
SCC	219	163	132	95	375	438	416	364
Feed information								
Feeding times	4:30am 10:30am 3:30pm	5:00am (full load) 9:00am (split load)	5:30am 3:30pm	4:30am 10:00am	5:30am 1:00pm	6:00am 1:00pm	4:30am 10:30am	5:00am 10:30am
Sampling dates and times	06/21/07 10:30am 06/26/07 3:00pm	05/31/07 4:30am 06/07/07 8:40am	06/19/07 6:00am 06/20/07 3:30pm	06/07/07 4:00am 06/12/07 4:00am	06/21/07 12:45pm 06/27/07 12:00pm	06/07/07 6:00am 06/12/07 6:00am	06/05/07 10:30am 06/07/07 10:30am	04/26/07 10:30am 05/01/07 10:30am
Type of mixer	Horizontal Kirby 705 tractor & trailer (red)	Vertical, 2 screw, truck (white) Laird VT 1200 Hydrostatic	Vertical, 3 screw, Tractor & trailer (white), Kuhn Knight 51100	Vertical, 2 screw, 1200 Supreme truck (red)	Vertical, 2 screw, tractor & trailer (red) Supreme 900T	Horizontal truck (red), Aggressor 920	Vertical, 2 screw, tractor & trailer (red) - Peecon	Horizontal, stationary - Mohrlang (820TMR) mixer feeder
Refusals	1% (written)	1% (estimated)	1% (estimated)	3% (estim/written)	2% (written)	3% (estimated)	3% (estimated)	3% (weights)
Rumensin	-	-	Yes	Yes	-	-	Yes	-
Intakes								
As fed (kg/d)	40.2	52.51	48.16	45.84	53.04	41.06	48.82	49.93
DM basis (kg/d)	25.01	27.3	26.93	27.41	24	26.04	30.08	29.22
TMR DM %	62.22	52	53.95	59.79	45.2	63.42	61.62	58.53

x Present in the high group pens

- Not present in the high group pens

Table A3. 2: Protein and AA status of the high group rations according to 'Amino Cow', 'CPM Dairy' and 'Shield'

Farm number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Average
DMI (kg/d)																	
Measured	24.2	21.4	26.8	24.9	28.5	22.5	28.4	26.6	25.0	27.3	24.5	27.4	24.0	26.0	30.1	29.2	**26.1**
Predicted																	
Amino Cow	22.2	23.8	23.6	25.0	23.7	24.4	25.0	24.4	25.7	25.0	25.6	26.4	25.4	26.6	26.7	27.9	**25.1**
CPM	20.1	21.6	21.2	22.9	21.8	22.0	22.4	22.2	23.1	22.4	23.4	24.2	23.4	24.3	24.5	25.8	**22.8**
Shield	22.7	25.9	28.5	30.5	24.9	25.4	23.1	25.9	26.7	27.9	27.4	24.5	27.2	28.3	27.0	28.1	**26.5**
MP Delivery (g/d)																	
Amino Cow	2447	2261	2769	2516	2592	2236	2704	2740	2622	2764	2537	2560	2317	2631	2858	2948	**2594**
CPM	2805	2389	3212	2853	3143	2527	3246	3119	2712	3059	2903	3207	2512	3045	3422	3200	**2960**
Shield	2844	2545	2884	2566	3104	2495	3130	3026	2886	3043	2855	3160	2587	3140	3279	3299	**2928**
MP bal (g/d)																	
Amino Cow	727	457	974	581	644	279	616	782	521	769	470	376	129	399	523	703	**559**
CPM	433	-7	608	195	343	-135	277	393	-165	221	-9	228	-419	21	122	20	**133**
Shield	148	-193	398	297	306	-284	-228	109	-227	169	-200	-95	-653	-275	0	92	**-40**
mMet g*																	
Amino Cow	51	47	57	53	53	46	56	58	55	58	53	52	49	55	60	61	**54**
CPM	57	47	62	57	65	51	65	64	54	57	58	62	51	59	69	60	**59**
Shield	61	56	58	55	69	53	68	68	66	67	64	69	58	70	74	75	**64**
mMet bal**																	
Amino Cow	4	-2	8	1	0	-7	-1	5	-2	4	-3	-7	-10	-6	-3	0	**-1**
CPM	14	3	14	8	14	2	10	14	2	5	5	7	-3	3	8	2	**7**
Shield	18	16	16	18	24	12	17	22	19	21	17	19	10	19	22	25	**18**
mMet %MP***																	
Amino Cow	2.08	2.09	2.06	2.11	2.05	2.05	2.09	2.13	2.09	2.1	2.1	2.04	2.1	2.1	2.11	2.08	**2.09**
CPM	2.05	1.96	1.92	1.99	2.07	2.03	1.99	2.04	2	1.88	2.01	1.93	2.03	1.93	2.01	1.88	**1.98**
Shield	2.13	2.21	2.01	2.24	2.23	2.14	2.19	2.24	2.3	2.2	2.25	2.17	2.23	2.23	2.24	2.26	**2.20**
mLys g																	
Amino Cow	171	153	200	177	168	155	180	197	177	191	170	172	158	181	197	195	**178**
CPM	184	148	214	185	199	167	200	209	174	191	183	205	161	195	224	197	**190**
Shield	167	148	174	160	167	146	172	179	164	171	160	176	147	184	183	176	**167**
mLys bal																	
Amino Cow	30	5	52	18	8	-6	8	36	4	27	0	-8	-22	-3	5	10	**10**
CPM	47	10	63	31	36	13	27	50	7	26	14	32	-9	20	32	12	**26**
Shield	22	8	32	29	10	3	-6	22	2	14	-3	4	-18	9	5	4	**9**
mLys %MP																	
Amino Cow	7	6.78	7.21	7.02	6.48	6.95	6.64	7.2	6.74	6.9	6.72	6.71	6.8	6.88	6.9	6.62	**6.85**
CPM	6.56	6.2	6.67	6.48	6.33	6.61	6.16	6.68	6.43	6.25	6.29	6.38	6.4	6.42	6.54	6.16	**6.41**
Shield	5.88	5.81	6.05	6.22	5.37	5.86	5.5	5.92	5.68	5.63	5.59	5.57	5.66	5.87	5.57	5.35	**5.72**

Farm number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Average
Lys:Met																	
Amino Cow	3.35	3.26	3.51	3.34	3.17	3.37	3.21	3.40	3.22	3.29	3.21	3.31	3.22	3.29	3.28	3.20	**3.29**
CPM	3.2	3.16	3.47	3.26	3.06	3.26	3.09	3.27	3.21	3.33	3.13	3.3	3.15	3.33	3.26	3.28	**3.24**
Shield	2.76	2.63	3.01	2.9	2.41	2.74	2.51	2.64	2.48	2.56	2.49	2.57	2.54	2.63	2.49	2.36	**2.61**
mHis g																	
Amino Cow	61	56	73	63	63	55	68	68	64	69	63	64	57	66	71	73	**65**
CPM	72	61	90	75	80	65	87	81	70	80	76	82	67	83	93	85	**78**
Shield	57	50	67	52	59	49	64	61	54	64	56	63	51	62	66	66	**59**
mHis bal																	
Amino Cow	13	6	23	9	9	1	10	14	6	14	6	3	-4	4	6	11	**8**
CPM	22	11	35	19	20	9	24	23	9	20	14	20	6	19	23	18	**18**
Shield	9	4	21	11	8	2	5	10	2	13	3	7	-2	6	9	10	**7**
mHis %MP																	
Amino Cow	2.49	2.47	2.64	2.51	2.43	2.47	2.53	2.5	2.46	2.5	2.48	2.48	2.47	2.51	2.48	2.46	**2.49**
CPM	2.55	2.57	2.8	2.63	2.54	2.56	2.67	2.58	2.57	2.62	2.61	2.56	2.68	2.71	2.71	2.66	**2.63**
Shield	2	1.96	2.34	2.02	1.89	1.95	2.03	2.02	1.87	2.12	1.97	1.99	1.99	1.98	2.01	1.99	**2.01**
mIle g																	
Amino Cow	137	124	153	141	138	124	143	156	144	153	138	138	128	143	157	158	**142**
CPM	142	120	158	143	161	129	156	159	142	154	145	161	128	155	175	158	**149**
Shield	117	104	112	111	116	102	123	121	112	121	114	123	108	133	132	130	**117**
mIle bal																	
Amino Cow	44	27	56	37	33	19	30	50	31	45	26	20	10	22	31	37	**32**
CPM	22	-3	28	9	21	-6	7	22	-5	12	-2	8	-22	0	9	-2	**6**
Shield	20	8	15	19	10	3	3	15	1	14	3	5	-6	10	9	12	**9**
mIle %MP																	
Amino Cow	5.62	5.48	5.53	5.59	5.32	5.57	5.29	5.69	5.5	5.53	5.43	5.38	5.5	5.45	5.51	5.36	**5.48**
CPM	5.05	5.01	4.92	5.01	5.11	5.1	4.82	5.11	5.22	5.03	4.98	5.01	5.1	5.09	5.12	4.95	**5.04**
Shield	4.13	4.09	3.87	4.31	3.73	4.11	3.93	4.01	3.9	3.96	4.01	3.9	4.17	4.14	4.01	3.93	**4.01**
mLeu g																	
Amino Cow	227	207	262	233	230	205	240	252	240	253	232	232	215	235	260	268	**237**
CPM	224	192	269	233	246	203	252	250	214	242	230	257	206	241	271	256	**237**
Shield	248	216	256	214	260	216	267	259	232	259	251	275	232	269	284	286	**252**
mLeu bal																	
Amino Cow	59	31	86	43	39	13	35	60	34	57	29	18	0	16	31	48	**37**
CPM	39	4	67	26	29	-4	21	38	-11	22	3	24	-24	4	14	9	**16**
Shield	81	54	93	65	80	50	60	78	44	79	63	76	42	66	79	88	**69**
mLeu %MP																	
Amino Cow	9.29	9.15	9.45	9.27	8.86	9.19	8.88	9.2	9.16	9.16	9.14	9.07	9.28	8.94	9.11	9.11	**9.14**
CPM	7.99	8.03	8.38	8.16	7.82	8.04	7.77	8.01	7.87	7.91	7.93	8.02	8.18	7.92	7.91	8	**8.00**
Shield	8.74	8.48	8.89	8.33	8.37	8.68	8.52	8.57	8.02	8.53	8.8	8.71	8.97	8.55	8.66	8.68	**8.59**

Farm number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Average
mVal g																	
Amino Cow	154	141	177	157	158	139	165	173	165	173	157	156	146	164	178	181	**162**
CPM	160	138	189	164	183	146	184	179	163	178	168	180	148	180	201	188	**172**
Shield	142	133	143	133	148	124	160	149	139	154	147	155	132	164	167	165	**147**
mVal bal																	
Amino Cow	6	28	64	35	36	16	34	50	33	48	27	18	8	23	31	40	**34**
CPM	44	20	61	33	46	15	39	46	21	39	25	33	4	31	39	32	**33**
Shield	29	23	32	32	26	10	21	26	12	31	19	20	2	26	28	30	**23**
mVal %MP																	
Amino Cow	6.27	6.24	6.38	6.25	6.08	6.2	6.09	6.31	6.3	6.27	6.19	6.11	6.29	6.22	6.24	6.14	**6.22**
CPM	5.72	5.78	5.87	5.73	5.82	5.78	5.67	5.75	6	5.82	5.79	5.62	5.9	5.92	5.88	5.86	**5.81**
Shield	5	5.22	4.95	5.2	4.78	4.96	5.12	4.91	4.83	5.05	5.12	4.91	5.11	5.22	5.1	5	**5.03**
mArg g																	
Amino Cow	134	119	156	139	144	122	151	154	148	162	139	146	124	148	155	157	**144**
CPM	181	146	207	181	206	164	207	204	184	204	183	207	158	198	218	200	**191**
Shield	141	126	148	131	149	120	151	154	142	168	137	154	122	157	160	152	**145**
mArg bal																	
Amino Cow	67	49	86	64	69	47	71	79	68	85	60	63	41	63	67	72	**66**
CPM	48	12	60	32	48	14	39	51	23	44	19	40	-6	29	32	21	**32**
Shield	37	30	52	53	41	25	23	45	32	60	25	37	12	37	42	36	**37**
mArg %MP																	
Amino Cow	5.47	5.25	5.63	5.51	5.56	5.44	5.58	5.61	5.66	5.85	5.47	5.7	5.33	5.64	5.44	5.33	**5.53**
CPM	6.44	6.1	6.44	6.35	6.57	6.47	6.37	6.54	6.79	6.67	6.3	6.44	6.3	6.5	6.36	6.26	**6.43**
Shield	4.97	4.95	5.13	5.12	4.82	4.83	4.83	5.1	4.92	5.52	4.78	4.86	4.73	4.98	4.87	4.6	**4.94**
mThr g																	
Amino Cow	134	123	152	138	137	122	143	152	142	151	137	136	126	143	156	157	**141**
CPM	138	113	155	139	151	125	151	154	132	145	138	154	121	147	167	151	**143**
Shield	135	121	131	127	140	118	143	141	136	138	134	144	123	152	154	152	**137**
mThr bal																	
Amino Cow	53	38	67	46	45	29	44	59	42	57	39	33	22	37	45	51	**44**
CPM	62	37	71	54	62	40	56	67	40	54	45	60	28	51	62	49	**52**
Shield	42	31	39	43	39	25	28	40	31	36	28	33	17	39	39	41	**34**
mThr %MP																	
Amino Cow	5.49	5.43	5.49	5.48	5.28	5.44	5.29	5.53	5.41	5.46	5.39	5.33	5.44	5.42	5.46	5.34	**5.42**
CPM	4.91	4.71	4.82	4.87	4.82	4.96	4.66	4.93	4.86	4.73	4.76	4.81	4.83	4.82	4.89	4.73	**4.82**
Shield	4.76	4.75	4.55	4.94	4.51	4.72	4.57	4.67	4.7	4.53	4.68	4.56	4.74	4.86	4.69	4.6	**4.68**

* Estimated delivery (g/d) of amino acids to the small intestine
** Balance between estimated requirements and delivery of amino acids at the small intestine
*** Metabolizable amino acids expressed as a percentage of Metabolizable CP

Appendix A4 (Chapter 4)

Table A4. 1: Chemical analysis of faeces for early and mid lactation cows as influenced by RPL*

	Control	RPL	SEM	*P*
Early Lactation				
g/kg				
Total N	27.7	29.1	0.40	0.01
NDFom	515	501	5.3	0.08
Lignin	134	131	1.9	0.27
Total N output (g/d)	255	289	6.5	0.01
Ratios				
Lignin : CP	0.777	0.726	0.0131	0.01
Lignin : NDF	0.260	0.262	0.0032	0.78
Mid Lactation				
g/kg				
Total N	28.4	29.0	0.55	0.33
NDFom	508	510	9.0	0.84
Lignin	134	137	2.1	0.43
Total N output (g/d)	271	263	11.1	0.63
Ratios				
Lignin : CP	0.764	0.769	0.0236	0.84
Lignin : NDF	0.269	0.273	0.0034	0.43

* Data relate to the subgroup of 60 cows (30 cows per treatment) from which urine and faecal samples were taken

Table A4. 2: Fatty acid composition (g/100g) and profile of the RPL product*

Fatty acid	g/100g of sample	% of fatty acids
C14	0.053	0.127
C15	0.021	0.051
C16	4.796	11.497
C17	0.096	0.231
C18	35.59	85.331
C18:1 cis 9&10	0.152	0.365
C18:2	0.411	0.985
C20	0.257	0.616
C18:3 n3	0.047	0.112
C24:1	0.048	0.115
Unknown	0.237	0.568

* Values averaged from replicate analysis

Table A4. 3: Specific Gravity and N concentrations in urine of early and mid lactation cows as influenced by RPL*

	Control	RPL	SEM	*P*
Early Lactation				
Specific gravity	1.021	1.019	0.0007	0.19
Components (g/L)				
Total N	7.00	6.65	0.304	0.42
Urea N	5.42	5.25	0.248	0.62
Ammonia N	0.145	0.138	0.0127	0.62
Protein N[1]	1.45	1.28	0.081	0.14
Outputs				
Urine volume (L/d)	39.5	42.7	1.78	0.21
Total N (g/d)	264	272	5.5	0.30

	Control	RPL	SEM	*P*
Urea N (g/d)	206	211	5.1	0.48
UN:PN	3.89	4.44	0.273	0.16
Mid Lactation				
Specific gravity	1.021	1.020	0.0014	0.74
Components (g/L)				
Total N	6.85	6.94	0.491	0.86
Urea N	5.30	5.40	0.409	0.81
Ammonia N	0.11	0.15	0.012	0.05
Protein N	1.41	1.36	0.093	0.74
Outputs				
Urine volume (L/d)	41.2	42.8	3.87	0.71
Total N (g/d)	246	254	5.4	0.34
Urea N (g/d)	195	203	4.9	0.26
UN:TN	5.19	4.75	0.616	0.61

* Data relate to the subgroup of 60 cows (30 cows per treatment) from which urine and fecal samples were taken

[1] Protein N = Total N - urea N - ammonia N

Table A4. 4: Partial N balance (g/d) for early and mid lactation cows as influenced by RPL*

	Control	RPL	SEM	*P*
Early Lactation				
Urine	265	267	6.1	0.75
Faeces	255	289	6.5	0.01
Milk	248	245	10.3	0.84
Mid Lactation				
Urine	246	254	5.4	0.34
Faeces	271	263	11.1	0.63
Milk	213	188	9.8	0.08

* Data for subset of 60 cows (30 per treatment) from which urine and fecal samples were collected.

Table A4. 5: Shield output for the rations fed to the four treatment groups

Shield output for early lactation cows on control diet

ANIMAL INPUTS			*ENVIRONMENTAL INPUTS*			**Predicted Parameters (Misc.)**		
Milk yield	53.24	kg/d	Temp, max	**28.0**	oC	BW adjust to BCS=3	650	kg
Milk fat	3.50	%	, min	**9.0**	oC	Intake : as fed	47.46	kg/d
Milk crude protein	2.92	%	Hum, max tp	**40.0**	%	: DM	4.37	% of BW
Body weight (BW)	650	kg	, at min tp	**60.0**	%	: NEl	3.51	xM
Body condition (BCS)	3.00	units	Humidex	74.4	units	Rumen : pH	6.24	units
Body locomotion (BLS)	1.10	units				: ammonia N	156	mg/L
Daily walking	2.0	km/d	**Dry Matter Intake Predictors**			: peptide N	135	mg/L
Lactation	3.1	number	Max DMI	25.06	kg/d	: total VFA	106	mM/L
Days in milk (DIM; avg)	88	days	DIM adj	1.001	factor	Bact'l CP : max	2305	g/d
Days in milk (minimum)	51	days	DP adj	1.000	factor	: RDP adj	1515	g/d
Days pregnant (DP)	0	days	BLS adj	0.999	factor	: relative	1.000	factor
Expected calf birth weight	42	kg	Humidex adj	0.972	factor	: digestible	909	g/d
Potential milk yield	14,614	kg/305d	Diet DM adj	1.000	factor	Prot'l CP : actual	252	g/d
Dry Matter (DM) Intake	28.40	kg/d	Diet fat adj	0.916	factor	: digestible	201	g/d
Maternal BW gain	0.00	kg/d	Pot'l yield adj	1.308	factor	Milk : NPN	6.5	% of CP
Maternal BW loss	2.21	kg/d				: casein	72.4	% of CP
Maternal growth	0.04	kg/d	Pred'd DMI	29.18	kg/d	: whey	21.1	% of CP
Fetal growth	0.00	kg/d	***(maximum)***	4.49	% of BW	: urea N (MUN)	160.7	mg/L
Net Maternal BW change	-2.21	kg/d				Urine: pH	8.20	pH units

Protein/Energy Requirements Summary

	Delivered	Required	Net	Units
Crude protein	4997	5088	-91	g/d
RDP, total	2741	3148	-407	g/d
, soluble (total)	1725	1249	477	g/d
, soluble (true)	455	-	-	g/d
, insoluble	1016	1899	-883	g/d
RUP, digestible	1850	1591	259	g/d
, indigestible	407	0	-	g/d
Rumen escape peptide	410	-	-	g/d
Rumen escape true SP	198	-	-	g/d
Absorbable protein	3569	3112	457	g/d
NEl	37.70	37.70	0.01	Mcal/d
NDF	9.59	7.67	1.92	kg/d

Diet Summary

	Actual	Guide	Units
Dry matter	59.8	45->85	%
Fat (free)	5.4	2->5	% DM
Fat (rumen inert)	2.5	< 4	% DM
Crude protein	17.6	14->17	% DM
Soluble CP	34.5	30->35	% CP
NFC (total)	32.1	37->40	% DM
NFC (rumen ferm'ble)	88.8	>80	%NSC
NEl (total)	1.33	1.5->1.7	Mcal/kg
NDF (total)	33.8	25->35	% DM
NDF (rumen dig)	51.3	> 50%	% NDF
NDF (phys eff've)	26.3	> 19%	% DM
Energy Discount	-4.39	< 6%	%unitM

Intestinally Absorbable Amino Acid Balance

	Total Delivered (g/d)	Absorbable Delivered (g/d)	Absorbable Delivered % AP	Absorbable Required (g/d)	Difference (g/d)	Difference % req
Methionine	99	80	2.25	48	32	166
Lysine	228	172	4.83	167	5	103
Threonine	206	158	4.43	108	50	147
Leucine	377	303	8.50	193	110	157
Isoleucine	176	135	3.79	117	19	116
Valine	219	171	4.79	132	39	130
Histidine	85	68	1.91	53	15	128
Arginine	204	157	4.39	107	49	146
Lys/Met	2.30	2.15	2.15	3.47	-1.32	62

Absorbable Protein Balance

Delivery		Units
Digestible RUP	1850	g/d
Microbes	1111	g/d
Soluble true CP	198	g/d
Peptides	410	g/d
Total delivery	3569	g/d
Requirement		
Maintenance	226	g/d
Milk	3242	g/d
BW Gain	0	g/d
BW Loss	-368	g/d
Maternal growth	12	g/d
Fetal growth	0	g/d
Total requirement	3112	g/d
	115	% Req

Net Energy Balance

Intake	37.70	Mcal/d
Requirement		
Milk	36.55	Mcal/d
Maintenance		
Base	10.30	Mcal/d
Exercise	0.38	Mcal/d
Heat loss	0.04	Mcal/d
Urea excretion	0.03	Mcal/d
Intake	1.08	Mcal/d
Total maintenance	11.83	Mcal/d
BW Change		
Maternal growth	0.19	Mcal/d
BW Gain	0.00	Mcal/d
BW Loss	-10.87	Mcal/d
Fetal growth	0.00	Mcal/d
Total growth	-10.68	Mcal/d
Total requirement	37.70	Mcal/d
	100	% Req

Shield output for early lactation cows on supplemented diet

ANIMAL INPUTS

Milk yield	54.00	kg/d
Milk fat	3.29	%
Milk crude protein	2.91	%
Body weight (BW)	650	kg
Body condition (BCS)	3.00	units
Body locomotion (BLS)	1.10	units
Daily walking	2.0	km/d
Lactation	3.1	number
Days in milk (DIM; avg)	88	days
Days in milk (minimum)	51	days
Days pregnant (DP)	0	days
Expected calf birth weight	42	kg
Potential milk yield	14,823	kg/305d
Dry Matter (DM) Intake	29.00	kg/d
Maternal BW gain	0.00	kg/d
Maternal BW loss	1.94	kg/d
Maternal growth	0.04	kg/d
Fetal growth	0.00	kg/d
Net Maternal BW change	-1.94	kg/d

ENVIRONMENTAL INPUTS

Temp, max	**28.0**	oC
, min	**9.0**	oC
Hum, max tp	**40.0**	%
, at min tp	**60.0**	%
Humidex	74.4	units

Dry Matter Intake Predictors

Max DMI	25.10	kg/d
DIM adj	1.001	factor
DP adj	1.000	factor
BLS adj	0.999	factor
Humidex adj	0.972	factor
Diet DM adj	1.000	factor
Diet fat adj	0.916	factor
Pot'l yield adj	1.322	factor
Pred'd DMI	29.53	kg/d
(maximum)	4.54	% of BW

Predicted Parameters (Misc.)

BW adjust to BCS=3	650	kg
Intake : as fed	48.46	kg/d
: DM	4.46	% of BW
: NEl	3.59	xM
Rumen : pH	6.25	units
: ammonia N	161	mg/L
: peptide N	136	mg/L
: total VFA	105	mM/L
Bact'l CP : max	2334	g/d
: RDP adj	1501	g/d
: relative	1.000	factor
: digestible	901	g/d
Prot'l CP : actual	253	g/d
: digestible	203	g/d
Milk : NPN	6.5	% of CP
: casein	72.4	% of CP
: whey	21.1	% of CP
: urea N (MUN)	158.6	mg/L
Urine: pH	8.20	pH units

Protein/Energy Requirements Summary

	Delivered	Required	Net	Units
Crude protein	5108	5233	-124	g/d
RDP, total	2784	3205	-421	g/d
, soluble (total)	1768	1253	515	g/d
, soluble (true)	463	-	-	g/d
, insoluble	1015	1952	-936	g/d
RUP, digestible	1909	1666	243	g/d
, indigestible	415	0	-	g/d
Rumen escape peptide	422	-	-	g/d
Rumen escape true SP	205	-	-	g/d
Absorbable protein	3639	3191	448	g/d
NEl	38.50	38.52	-0.01	Mcal/d
NDF	9.78	7.83	1.95	kg/d

Diet Summary

	Actual	Guide	Units
Dry matter	59.8	45->85	%
Fat (free)	5.3	2->5	% DM
Fat (rumen inert)	2.5	< 4	% DM
Crude protein	17.6	14->17	% DM
Soluble CP	34.6	30->35	% CP
NFC (total)	32.3	37->40	% DM
NFC (rumen ferm'ble)	88.9	>80	%NSC
NEl (total)	1.33	1.5->1.7	Mcal/kg
NDF (total)	33.7	25->35	% DM
NDF (rumen dig)	51.4	> 50%	% NDF
NDF (phys eff've)	26.3	> 19%	% DM
Energy Discount	-4.56	< 6%	%unitM

Intestinally Absorbable Amino Acid Balance

	Total Delivered (g/d)	Absorbable Delivered (g/d)	% AP	Required (g/d)	Difference (g/d)	% req
Methionine	100	82	2.25	49	32	165
Lysine	229	175	4.80	172	3	102
Threonine	208	161	4.41	110	50	145
Leucine	382	309	8.49	198	111	156
Isoleucine	177	137	3.77	119	18	115
Valine	222	174	4.78	135	39	129
Histidine	86	70	1.91	55	15	127
Arginine	205	159	4.37	111	48	143
Lys/Met	2.29	2.14	2.14	3.47	-1.33	62

Absorbable Protein Balance

Delivery		Units
Digestible RUP	1909	g/d
Microbes	1103	g/d
Soluble true CP	205	g/d
Peptides	422	g/d
Total delivery	3639	g/d
Requirement		
Maintenance	226	g/d
Milk	3276	g/d
BW Gain	0	g/d
BW Loss	-323	g/d
Maternal growth	12	g/d
Fetal growth	0	g/d
Total requirement	3191	g/d
	114	% Req

Net Energy Balance

Intake	38.50	Mcal/d
Requirement		
Milk	36.03	Mcal/d
Maintenance		
Base	10.30	Mcal/d
Exercise	0.38	Mcal/d
Heat loss	0.04	Mcal/d
Urea excretion	0.02	Mcal/d
Intake	1.11	Mcal/d
Total maintenance	11.85	Mcal/d
BW Change		
Maternal growth	0.19	Mcal/d
BW Gain	0.00	Mcal/d
BW Loss	-9.54	Mcal/d
Fetal growth	0.00	Mcal/d
Total growth	-9.36	Mcal/d
Total requirement	38.52	Mcal/d
	100	% Req

Shield output for mid lactation cows on control diet

ANIMAL INPUTS

Milk yield	41.84	kg/d
Milk fat	3.74	%
Milk crude protein	3.28	%
Body weight (BW)	650	kg
Body condition (BCS)	3.25	units
Body locomotion (BLS)	1.10	units
Daily walking	2.0	km/d
Lactation	2.3	number
Days in milk (DIM; avg)	284	days
Days in milk (minimum)	257	days
Days pregnant (DP)	147	days
Expected calf birth weight	42	kg
Potential milk yield	11,485	kg/305d
Dry Matter (DM) Intake	28.80	kg/d
Maternal BW gain	0.00	kg/d
Maternal BW loss	1.24	kg/d
Maternal growth	0.08	kg/d
Fetal growth	0.29	kg/d
Net Maternal BW change	-1.24	kg/d

ENVIRONMENTAL INPUTS

Temp, max	**28.0**	oC
, min	**9.0**	oC
Hum, max tp	**40.0**	%
, at min tp	**60.0**	%
Humidex	74.4	units

Dry Matter Intake Predictors

Max DMI	24.57	kg/d
DIM adj	1.001	factor
DP adj	0.919	factor
BLS adj	0.999	factor
Humidex adj	0.972	factor
Diet DM adj	1.000	factor
Diet fat adj	0.916	factor
Pot'l yield adj	1.099	factor
Pred'd DMI	22.12	kg/d
(maximum)	3.40	% of BW

Predicted Parameters (Misc.)

BW adjust to BCS=3	637	kg
Intake : as fed	48.13	kg/d
: DM	4.43	% of BW
: NEl	3.54	xM
Rumen : pH	6.33	units
: ammonia N	163	mg/L
: peptide N	139	mg/L
: total VFA	100	mM/L
Bact'l CP : max	2314	g/d
: RDP adj	1477	g/d
: relative	1.000	factor
: digestible	886	g/d
Prot'l CP : actual	250	g/d
: digestible	200	g/d
Milk : NPN	6.5	% of CP
: casein	71.4	% of CP
: whey	22.1	% of CP
: urea N (MUN)	160.6	mg/L
Urine: pH	8.20	pH units

Protein/Energy Requirements Summary

	Delivered	Required	Net	Units
Crude protein	5068	4946	122	g/d
RDP, total	2755	3177	-422	g/d
, soluble (total)	1750	1227	523	g/d
, soluble (true)	462	-	-	g/d
, insoluble	1005	1949	-944	g/d
RUP, digestible	1901	1454	447	g/d
, indigestible	412	0	-	g/d
Rumen escape peptide	408	-	-	g/d
Rumen escape true SP	204	-	-	g/d
Absorbable protein	3599	2948	651	g/d
NEl	38.12	38.09	0.03	Mcal/d
NDF	9.73	7.78	1.95	kg/d

Diet Summary

	Actual	Guide	Units
Dry matter	59.8	45->85	%
Fat (free)	5.4	2->5	% DM
Fat (rumen inert)	2.5	< 4	% DM
Crude protein	17.6	14->17	% DM
Soluble CP	34.5	30->35	% CP
NFC (total)	32.1	37->40	% DM
NFC (rumen ferm'ble)	88.8	>80	%NSC
NEl (total)	1.32	1.5->1.7	Mcal/kg
NDF (total)	33.8	25->35	% DM
NDF (rumen dig)	51.3	> 50%	% NDF
NDF (phys eff've)	26.3	> 19%	% DM
Energy Discount	-4.28	< 6%	%unitM

Intestinally Absorbable Amino Acid Balance

	Total Delivered (g/d)	Absorbable Delivered (g/d)	% AP	Required (g/d)	Difference (g/d)	% req
Methionine	99	81	2.25	47	33	170
Lysine	226	172	4.78	164	8	105
Threonine	205	158	4.40	106	53	150
Leucine	377	305	8.48	189	116	161
Isoleucine	175	135	3.76	111	24	122
Valine	219	172	4.77	128	44	134
Histidine	85	69	1.91	53	16	129
Arginine	203	157	4.36	113	44	139
Lys/Met (3:1)	2.28	2.13	2.13	3.45	-1.32	62

Absorbable Protein Balance

Delivery		Units
Digestible RUP	1901	g/d
Microbes	1086	g/d
Soluble true CP	204	g/d
Peptides	408	g/d
Total delivery	3599	g/d
Requirement		
Maintenance	226	g/d
Milk	2856	g/d
BW Gain	0	g/d
BW Loss	-207	g/d
Maternal growth	25	g/d
Fetal growth	48	g/d
Total requirement	2948	g/d
	122	% Req

Net Energy Balance

Intake	38.12	Mcal/d
Requirement		
Milk	30.36	Mcal/d
Maintenance		
Base	10.30	Mcal/d
Exercise	0.38	Mcal/d
Heat loss	0.04	Mcal/d
Urea excretion	0.16	Mcal/d
Intake	1.10	Mcal/d
Total maintenance	11.98	Mcal/d
BW Change		
Maternal growth	0.39	Mcal/d
BW Gain	0.00	Mcal/d
BW Loss	-6.10	Mcal/d
Fetal growth	1.46	Mcal/d
Total growth	-4.25	Mcal/d
Total requirement	38.09	Mcal/d
	100	% Req

Shield output for mid lactation cows on supplemented diet

ANIMAL INPUTS

Milk yield	40.90	kg/d
Milk fat	3.54	%
Milk crude protein	3.29	%
Body weight (BW)	650	kg
Body condition (BCS)	3.25	units
Body locomotion (BLS)	1.10	units
Daily walking	2.0	km/d
Lactation	2.3	number
Days in milk (DIM; avg)	284	days
Days in milk (minimum)	257	days
Days pregnant (DP)	147	days
Expected calf birth weight	42	kg
Potential milk yield	11,227	kg/305d
Dry Matter (DM) Intake	27.60	kg/d
Maternal BW gain	0.00	kg/d
Maternal BW loss	1.24	kg/d
Maternal growth	0.08	kg/d
Fetal growth	0.29	kg/d
Net Maternal BW change	-1.24	kg/d

ENVIRONMENTAL INPUTS

Temp, max	**28.0**	oC
, min	**9.0**	oC
Hum, max tp	**40.0**	%
, at min tp	**60.0**	%
Humidex	74.4	units

Dry Matter Intake Predictors

Max DMI	24.61	kg/d
DIM adj	1.001	factor
DP adj	0.919	factor
BLS adj	0.999	factor
Humidex adj	0.972	factor
Diet DM adj	1.000	factor
Diet fat adj	0.916	factor
Pot'l yield adj	1.082	factor
Pred'd DMI	21.79	kg/d
(maximum)	3.35	% of BW

Predicted Parameters (Misc.)

BW adjust to BCS=3	637	kg
Intake : as fed	46.12	kg/d
: DM	4.25	% of BW
: NEl	3.40	xM
Rumen : pH	6.30	units
: ammonia N	156	mg/L
: peptide N	137	mg/L
: total VFA	102	mM/L
Bact'l CP : max	2254	g/d
: RDP adj	1487	g/d
: relative	1.000	factor
: digestible	892	g/d
Prot'l CP : actual	247	g/d
: digestible	197	g/d
Milk : NPN	6.5	% of CP
: casein	71.4	% of CP
: whey	22.1	% of CP
: urea N (MUN)	158.9	mg/L
Urine: pH	8.20	pH units

Protein/Energy Requirements Summary

	Delivered	Required	Net	Units
Crude protein	4862	4796	65	g/d
RDP, total	2672	3066	-394	g/d
, soluble (total)	1683	1219	464	g/d
, soluble (true)	440	-	-	g/d
, insoluble	989	1847	-858	g/d
RUP, digestible	1795	1418	376	g/d
, indigestible	395	0	-	g/d
Rumen escape peptide	385	-	-	g/d
Rumen escape true SP	191	-	-	g/d
Absorbable protein	3460	2893	567	g/d
NEl	36.58	36.58	0.00	Mcal/d
NDF	9.31	7.45	1.86	kg/d

Diet Summary

	Actual	Guide	Units
Dry matter	59.8	45->85	%
Fat (free)	5.3	2->5	% DM
Fat (rumen inert)	2.5	< 4	% DM
Crude protein	17.6	14->17	% DM
Soluble CP	34.6	30->35	% CP
NFC (total)	32.3	37->40	% DM
NFC (rumen ferm'ble)	88.9	>80	%NSC
NEl (total)	1.33	1.5->1.7	Mcal/kg
NDF (total)	33.7	25->35	% DM
NDF (rumen dig)	51.4	> 50%	% NDF
NDF (phys eff've)	26.3	> 19%	% DM
Energy Discount	-4.45	< 6%	%unitM

Intestinally Absorbable Amino Acid Balance

	Total Delivered (g/d)	Absorbable Delivered (g/d)	Absorbable Delivered % AP	Absorbable Required (g/d)	Difference (g/d)	Difference % req
Methionine	96	78	2.25	46	32	168
Lysine	222	168	4.84	160	8	105
Threonine	201	154	4.44	103	51	149
Leucine	367	294	8.51	184	110	160
Isoleucine	171	131	3.80	108	23	121
Valine	213	166	4.79	125	41	133
Histidine	82	66	1.91	52	14	128
Arginine	198	152	4.39	110	42	139
Lys/Met (3:1)	2.31	2.16	2.16	3.45	-1.30	62

Absorbable Protein Balance

Delivery		Units
Digestible RUP	1795	g/d
Microbes	1089	g/d
Soluble true CP	191	g/d
Peptides	385	g/d
Total delivery	3460	g/d
Requirement		
Maintenance	226	g/d
Milk	2801	g/d
BW Gain	0	g/d
BW Loss	-207	g/d
Maternal growth	25	g/d
Fetal growth	48	g/d
Total requirement	2893	g/d
	120	% Req

Net Energy Balance

Intake	36.58	Mcal/d
Requirement		
Milk	28.97	Mcal/d
Maintenance		
Base	10.30	Mcal/d
Exercise	0.38	Mcal/d
Heat loss	0.04	Mcal/d
Urea excretion	0.12	Mcal/d
Intake	1.03	Mcal/d
Total maintenance	11.86	Mcal/d
BW Change		
Maternal growth	0.39	Mcal/d
BW Gain	0.00	Mcal/d
BW Loss	-6.10	Mcal/d
Fetal growth	1.46	Mcal/d
Total growth	-4.25	Mcal/d
Total requirement	36.58	Mcal/d
	100	% Req