

## Amino Acids Adequacy and Performance of Dairy Cattle

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### INTRODUCTION

The genetic code has codons for 20 amino acids (AA) that are used in protein synthesis in higher animals (Wikipedia, 2013). Livestock nutritionists know that nine of these 20 AA cannot be formed in tissue metabolism but must be absorbed from the gastro-intestinal tract. These nine are classified as the essential AA (EAA) and are abbreviated as His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val. Another AA, Arg, is synthesized in the urea cycle and, hence, is not strictly an essential nutrient; however, dietary Arg supplementation may improve protein efficiency in some non-ruminants species, notably carnivores, fish and poultry, in which tissue synthesis is often inadequate (Ball et al., 2007). Two additional AA, Cys and Tyr, are said to spare the requirement of Met and Phe because the two EAA are used to synthesize Cys (Met) and Tyr (Phe) when intestinal absorption is inadequate. These three AA, Arg, Cys and Tyr, are often referred to as semi-essential AA, while the remaining eight AA are non-essential. In this context, protein quality refers to EAA pattern, i.e., relative proportions of each EAA in a feed protein and how well these correspond to animal requirements. The microbial protein produced in the rumen is of better quality than many dietary ingredients commonly fed to domestic ruminants (Broderick, 1994; Schwab, 1996). In addition, microbial utilization of ammonia allows the feeding of some nonprotein N (NPN), such as urea, as well as capture of some recycled urea N that would otherwise be excreted in the urine.

Although lactating dairy cows are able to use poor quality proteins and NPN as sources of dietary crude protein (CP), they typically excrete three times more N in manure than they secrete in milk. One of our primary objectives in dairy nutrition and management is to improve N capture for milk production because inefficient protein utilization increases production costs and contributes to environmental pollution. Assuming 25% gross N efficiency (kg milk N/kg feed N-intake), a cow producing 10,000 kg of milk per lactation will excrete about 150 kg of manure N over the lactation curve. It is estimated that about 25% of dairy manure N is lost as ammonia under current U.S. practices (NRC, 2003). Similar losses likely occur in Brazil. Because milk prices have not kept pace with inflation, dairy operations around the world are increasing in size to obtain the financial economies of scale. Larger dairy farms import more feed and have elevated animal units per hectare; greater animal densities increase nutrient accumulation on farmland and the environmental impact of animal enterprises (NPM, 2006).

### OPTIMIZING FORMATION OF MICROBIAL PROTEIN IN THE RUMEN

The approach of most protein feeding models is to first match rumen-degradable protein (RDP) to rumen carbohydrate fermentation, and then meet any shortfall in metabolizable protein (MP) supply by providing rumen-undegraded protein (RUP). Reducing particle size and processing (e.g., steam-flaking) increases extent of rumen maize starch digestion much more

than total tract digestibility (Table 1; Owens et al., 1986). Grinding high moisture maize through a 1-cm screen optimized ammonia uptake in rumen in vitro; feeding cows high moisture maize ground to this extent (1.7 mm mean particle size) reduced rumen ammonia concentration in vivo and increased yield of milk (2.4 kg/d) and protein (120 g/d) compared to unground high moisture maize (4.3 mm mean particle size) (Ekinici and Broderick, 1997). Grinding dry shelled maize to reduce mean particle size from 3.5 to 0.6 mm increased rumen starch digestibility from 54 to 70% (Remond et al., 2004). Charbonneau et al. (2006) replaced cracked maize with finely ground maize and obtained 10% greater yield of milk and 14% greater yield of protein in lactating dairy cows.

Table 1. Effect of processing on digestibility of maize and barley starch (Owens et al., 1986)

Processing method	Proportion of Starch Digestion, %			Total tract
	Rumen	Small intestine	Large intestine	
Cracked maize	69	13	8	89
Ground maize	78	14	4	94
Steam-flaked maize	83	16	1	98
High moisture maize	86	6	1	95
Ground Barley	94	...	...	...

Maize silage is commonly fed throughout the world to provide high-energy “forage” and maize silage may be used to dilute the highly degradable protein in hay-crop silages. Dhiman and Satter (1997) replaced 1/3 or 2/3 of dietary lucerne silage with maize silage. Compared to 100% of the forage from lucerne, milk yield and N efficiency both were 6% higher over the whole lactation when dietary forage was 2/3 lucerne silage and 1/3 maize silage. Brito and Broderick (2006) made step-wise replacements of lucerne silage with maize silage. Greatest improvement in N efficiency, without loss of yield of milk, fat and protein, occurred with about half of the forage from lucerne silage and half from maize silage (Table 2). Optimizing dietary ratios of lucerne silage to maize silage resulted in similar or greater productivity at lower levels of supplementation of protein concentrate (Table 2). Part of these positive effects was due to improved microbial protein formation in the rumen (Brito et al., 2006; Table 2).

Metabolic problems associated with rumen acidosis limit feeding of readily fermented carbohydrates for stimulating microbial protein synthesis and it is important to know how much concentrate or processing will optimize performance without hurting the cow. Valadares et al. (2000) added high moisture maize to dilute dietary lucerne silage from (% lucerne silage/% maize-based concentrate, DM basis) 80/20 to 65/35, 50/50, and 35/65. The observed quadratic responses indicated that maximal DM intake and yield of fat-corrected milk (FCM) occurred at 51% concentrate (38% non-fiber carbohydrate; NFC) and maximal fat yield at 43% concentrate (34% NFC). However, milk and protein responses were linear rather than quadratic and both were still going up at 35% forage and 65% concentrate. Moreover, rumen protein formation, estimated from purine derivative excretion in the urine, also showed a linear response, despite low rumen pH and other signs of over-feeding of NFC (Valadares et al., 2000). Clearly, the lactating cow’s demand for energy is substantial and optimal dietary concentrate is dictated more by long-term rumen and animal health than by maximum milk production.

Table 2. Effect of replacing lucerne silage with maize silage in the diets of lactating dairy cows (Brito and Broderick, 2006; Brito et al., 2006)

Variable	Dietary Ratio of Lucerne Silage/Maize Silage				Probability
	100/0	74/26	47/53	21/79	
<u>Diet composition, % of DM</u>					
Lucerne silage	50.6	37.2	23.7	10.2	...
Maize silage	0	13.3	26.7	40.0	...
Soybean meal	3.0	7.3	11.7	16.1	...
Crude protein	17.2	16.9	16.6	16.3	...
<u>Production</u>					
DM intake, kg/d	26.8 <sup>a</sup>	26.5 <sup>a</sup>	25.4 <sup>b</sup>	23.7 <sup>c</sup>	< 0.01
Milk yield, kg/d	41.5 <sup>a</sup>	42.0 <sup>a</sup>	41.5 <sup>a</sup>	39.5 <sup>b</sup>	0.03
Milk/DM intake	1.53 <sup>b</sup>	1.58 <sup>b</sup>	1.64 <sup>a</sup>	1.67 <sup>a</sup>	< 0.01
Fat yield, kg/d	1.56 <sup>a</sup>	1.51 <sup>ab</sup>	1.40 <sup>bc</sup>	1.33 <sup>c</sup>	< 0.01
Protein yield, kg/d	1.26	1.32	1.30	1.25	0.13
Milk N/N Intake, %	26.5 <sup>d</sup>	28.5 <sup>c</sup>	30.0 <sup>b</sup>	31.7 <sup>a</sup>	< 0.01
Urinary N, g/d	217 <sup>a</sup>	215 <sup>a</sup>	201 <sup>b</sup>	188 <sup>b</sup>	< 0.01
<u>Rumen metabolism</u>					
pH	6.29	6.31	6.30	6.31	0.99
Ammonia-N, mg/dL	10.5 <sup>a</sup>	10.0 <sup>ab</sup>	8.7 <sup>b</sup>	6.2 <sup>c</sup>	< 0.01
RDP, g/d	3068 <sup>ab</sup>	3142 <sup>a</sup>	2809 <sup>b</sup>	2469 <sup>c</sup>	< 0.01
RUP, g/d	1459	1512	1630	1511	0.16
Microbial NAN, g/d	465 <sup>a</sup>	479 <sup>a</sup>	460 <sup>a</sup>	423 <sup>b</sup>	< 0.01
Microbial efficiency <sup>1</sup>	31	29	29	30	0.39

<sup>a-d</sup>Means in rows without common superscripts are different ( $P < 0.05$ ).

<sup>1</sup>Efficiency of microbial growth, g microbial NAN/kg organic matter truly digested in the rumen.

Most feeding systems assume that NPN is equivalent to true protein for supplying RDP to the rumen microbes. Indeed, supplementing with high RDP feeds such as soybean meal (SBM) or hay-crop silages generally cause large increases in rumen ammonia concentrations. But there may be confusion on the relative value of NPN and true protein as RDP sources because ammonia is formed largely from AA deamination and its production parallels release of peptides and free AA from protein degradation. Substantial evidence indicates that protein breakdown products other than ammonia that can stimulate microbial protein synthesis. Maeng and Baldwin (1976) observed increased protein formation and efficiency (protein formed per unit of carbohydrate fermented) by adding protein AA to incubations of mixed rumen microbes. Argyle and Baldwin (1989) showed that supplementing only 1 mg/L of a blend of protein AA plus 1 mg/L of peptides more than doubled in vitro microbial yield. In vivo results showed significant linear depression in yield of milk, fat and protein when RDP from urea replaced RDP from SBM; production effects were due largely to depressed rumen outflow of nonammonia N (NAN, equivalent to protein N) and EAA resulting from reduced formation of microbial protein (Table 3; Broderick and Reynal, 2009). Thus, under some conditions, NPN cannot provide all of the RDP and RDP from true protein increases microbial protein formation in vivo. The NPN content of the basal diet may also influence response to urea RDP: 20% of total CP was NPN (largely from lucerne silage) prior to dietary urea addition (Broderick and Reynal, 2009).

Table 3. Effect on production and rumen metabolism of replacing RDP from soybean meal with RDP from urea in diets fed lactating cows (Broderick and Reynal, 2009)

Variable	Dietary RDP from urea, % of DM				Probability	
	0	1.2	2.4	3.7	Diet	Linear
<u>Diet composition, % of DM</u>						
Lucerne silage + maize silage	55.0	55.0	55.0	55.0	...	...
High moisture maize	29.6	28.7	28.5	27.8	...	...
Solvent soybean meal	14.0	9.8	5.0	0	...	...
Treated soybean meal	0	2.5	5.1	8.0	...	...
Urea	0	0.41	0.84	1.31	...	...
Crude protein	16.1	16.1	16.0	16.1	...	...
Total RDP (NRC, 2001)	10.6	10.5	10.4	10.5	...	...
<u>Production</u>						
DM intake, kg/d	23.6 <sup>a</sup>	23.2 <sup>a</sup>	23.0 <sup>ab</sup>	22.3 <sup>b</sup>	0.02	< 0.01
Milk yield, kg/d	39.3 <sup>a</sup>	38.6 <sup>a</sup>	38.5 <sup>a</sup>	36.0 <sup>b</sup>	< 0.01	< 0.01
Milk/DM intake	1.68	1.67	1.69	1.63	0.19	0.19
Fat, kg/d	1.20	1.19	1.10	1.11	0.07	0.02
Protein yield, kg/d	1.27 <sup>a</sup>	1.22 <sup>b</sup>	1.21 <sup>bc</sup>	1.17 <sup>c</sup>	< 0.01	< 0.01
Milk urea-N, mg/dL	6.8 <sup>b</sup>	7.5 <sup>b</sup>	8.1 <sup>b</sup>	9.1 <sup>a</sup>	< 0.01	< 0.01
Milk N/N Intake, %	33.2	32.1	32.4	32.1	0.38	0.19
<u>Rumen metabolism</u>						
Ammonia-N, mg/dL	8.2 <sup>b</sup>	9.3 <sup>ab</sup>	10.3 <sup>a</sup>	10.7 <sup>a</sup>	0.03	< 0.01
Total essential AA flow, g/d	1395 <sup>a</sup>	1259 <sup>b</sup>	1245 <sup>b</sup>	1082 <sup>c</sup>	< 0.01	< 0.01
Microbial NAN flow, g/d	440 <sup>a</sup>	363 <sup>b</sup>	374 <sup>b</sup>	342 <sup>b</sup>	< 0.01	< 0.01
Microbial efficiency <sup>1</sup>	29.3	28.6	24.4	24.5	0.23	0.05

<sup>a-c</sup>Means in rows without common superscripts are different ( $P < 0.05$ ).

<sup>1</sup>Efficiency of microbial growth, g microbial NAN/kg organic matter truly digested in the rumen.

Proportions of Met and Lys in rumen microbial protein are very similar to those of proteins in lean tissue and milk (NRC, 2001). Although there is evidence that His may be somewhat limited in microbial protein, overall its EAA pattern is of good quality. However, it must be emphasized that the total CP in rumen microbial cells contains from 20% (NRC, 2001) to as much as 33% (Clark et al., 1992) non-AA N (i.e., N in nucleic acids and cell-wall components). Moreover, intestinal digestibility of microbial protein also limits its utilization (NRC, 2001). Thus, conversion of good quality feed proteins into microbial protein may actually impair N-utilization, despite an overall improvement in EAA pattern, because of a net reduction in total EAA absorption. There is evidence that microbial protein synthesis will increase linearly over a wide range of supply of dietary RDP (Broderick et al., 2010) while maximal milk protein secretion will occur at substantially less than maximal microbial protein synthesis (Reynal and Broderick, 2005; Olmos Colmenero and Broderick, 2006b). Hence, microbial protein formation in the rumen should be “optimized” rather than “maximized”.

#### OPTIMIZING DAIRY DIETS FOR ESSENTIAL AMINO ACIDS

Experiments conducted in the middle of the last century showed that cattle could make small weight gains (Loosli et al., 1949) and produce modest amounts of milk (Virtanen, 1966) on diets with virtually all CP supplied as urea and ammonium salts. Although EAA were known to be required in the tissues of dairy cows (Kleiber et al., 1953) and presumably all ruminant livestock, findings on utilization of dietary NPN were consistent with prevailing dogma that the rumen microbes could provide most of the animal's protein. However, research at about the same time in the U.K. on treating protein to increase RUP content (e.g., Chalmers et al., 1954) and Australia on abomasal protein infusions (e.g., Reis and Schinckel, 1964) provided early evidence that microbial protein formation may not be adequate under all circumstances. Although, this work was largely done in sheep producing wool as their primary product and wool protein has a very different EAA composition than meat and milk, beef cattle (Richardson and Hatfield, 1978) and dairy cattle (Broderick et al., 1970) responded in much the same way. It soon became clear that it was necessary to quantify both microbial protein formation and dietary protein escape from the rumen in ration formulation and models applying quantitative estimates of both protein sources soon began to appear. The "urea-fermentation potential" model of Burroughs et al. (1974) was developed to predict whether urea could be effectively added to the diet, based on dietary supply of RDP and rumen-fermentable energy which drive microbial growth. Roffler and Satter (1975) proposed using rumen ammonia concentration as the index of whether NPN would be useful: if ammonia exceeded 3.5 mM (5 mg ammonia-N/dL), then urea or other NPN source would be deemed ineffective. These early models were very simple, applying static constants for microbial yield/unit rumen fermentable energy as well as RDP and RUP content of feedstuffs. However, it soon became obvious that protein digestion and metabolism was dynamic and complex, and the rationing models have evolved to keep pace with this greater understanding. Tedeschi (2013) reported on the use of nutritional models in cattle nutrition at this meeting.

Among nutritional models receiving widespread application are NRC (2001), the Cornell model (Tylutki, et al., 2008) and NorFor (2011). These models balance dairy rations for MP--the protein absorbed as AA from the small intestine. Metabolizable protein is also referred to as total AA (AAT) in NorFor, the French PDV system, the Dutch PDI model and several others and AAT may be used interchangeably with MP. Hanigan (2005) compared the NRC and Cornell models to three others and concluded that NRC (2001) was somewhat more accurate at predicting MP supply. A comparison of rumen protein flows, measured by omasal sampling, with NRC (2001) predictions indicated that NRC (2001) overestimated RUP, underestimated microbial protein, but gave a close approximation of total MP flow (Broderick et al., 2010). Predictions of milk protein yield, made using the NRC (2001) and NorFor models, were compared to milk protein yields observed in five American feeding studies in which 21 different diets were fed (Broderick and Åkerlind, 2012). Although both models estimated similar flows of MP, the NorFor model was found to give more reliable estimates of observed milk protein secretion. The NRC model underestimated yield at low MP supply. We speculate that NorFor proved more reliable mainly because it applies variable MP efficiency, which decreases as MP supply increases. Lapierre et al. (2007) also concluded that the NRC assumption of a constant 67% efficiency of MP utilization helped explain why the model's relatively accurate predictions of MP supply do not give rise to reliable predictions of milk and protein yield.

One use of the newer rationing systems is to identify diets where lactating cows will respond to supplemental RUP. Dairy diets often contain high CP and high NPN (from hay-crop silages such as lucerne silage); frequently there are substantial responses to RUP supplements,

such as heat-treated soy protein (Broderick et al., 1990; Faldet and Satter, 1991) or fishmeal (Broderick, 1992) on such diets. Compared to an isonitrogenous diet containing urea, we found substantial responses to 3 true proteins differing in RUP (Brito and Broderick, 2007; Brito et al., 2007). Among the true proteins, flow of RUP and total protein from the rumen was greatest on cottonseed meal, intermediate on canola meal and lowest on solvent SBM; however, protein and fat yield both were highest on canola meal, intermediate on SBM, and lowest on cottonseed meal (Table 4). Lower protein and fat yields probably resulted from a poorer AA pattern in cottonseed meal RUP. Korhonen et al. (2002) took advantage of the relatively high RUP and Met contents of maize gluten meal to show that, when it replaced SBM, milk protein yield was increased and was equal to that observed on diets supplemented with fish meal. Reducing CP from 16.6% to 15.6% with added RUP from heat-treated SBM reduced milk yield 1.2 kg/d; however, milk and protein yield was similar on 2 diets with 16.6% CP (with or without SBM high in RUP) as was obtained on 17.6% CP (Olmos Colmenero and Broderick, 2006a). Low methionine in SBM RUP may have accounted for this lack of response; it would have been interesting if RPM had been added to the 15.6% CP containing expeller SBM (a heat-treated protein).

Table 4. Effect of supplementing urea or true proteins on production and omasal protein flow in dairy cows (all diets contained 16.5% CP; Brito and Broderick, 2007; Brito et al., 2007).

Item	Supplemental protein <sup>1</sup>				<i>P</i> > <i>F</i>
	Urea	SSBM	CSM	CM	
<u>Production, kg/d</u>					
DM intake	22.1 <sup>c</sup>	24.2 <sup>b</sup>	24.7 <sup>ab</sup>	24.9 <sup>a</sup>	< 0.01
Milk yield	32.9 <sup>b</sup>	40.0 <sup>a</sup>	40.5 <sup>a</sup>	41.1 <sup>a</sup>	< 0.01
Protein yield	0.92 <sup>c</sup>	1.23 <sup>ab</sup>	1.18 <sup>b</sup>	1.27 <sup>a</sup>	< 0.01
Fat yield	1.01 <sup>c</sup>	1.22 <sup>ab</sup>	1.18 <sup>b</sup>	1.29 <sup>a</sup>	< 0.01
<u>Omasal protein flow, g/d</u>					
Microbial protein	2344 <sup>b</sup>	2706 <sup>a</sup>	2706 <sup>a</sup>	2775 <sup>a</sup>	0.04
RUP	538 <sup>c</sup>	987 <sup>b</sup>	1348 <sup>a</sup>	1150 <sup>ab</sup>	< 0.01
Total protein	2882 <sup>c</sup>	3693 <sup>b</sup>	4054 <sup>a</sup>	3925 <sup>ab</sup>	< 0.01

<sup>1</sup>SSBM = solvent soybean meal; CSM = cottonseed meal; CM = canola meal.

<sup>a-c</sup>Means in rows without common superscripts are different (*P* < 0.05).

Of course, dairy cows require EAA rather than MP to synthesize proteins and the EAA most often cited as the limiting for lactating dairy cows are Met and Lys. This information derives largely from milk and protein yield responses to abomasal EAA infusion. For example, the early work of Schwab et al. (1976) observed that infusion of only Lys plus Met into cows fed a low CP diet based on maize silage plus grain gave on average 43% of the milk protein yield obtained with infusion of either casein or all nine EAA plus Arg. An assessment of research reported between 1976 and 1996 led Schwab (1996) to conclude that Met and Lys were the EAA most likely to be limiting on “conventional diets” based principally on maize and hay-crop silages, maize grain plus protein supplement from soybean or distillers grains. Much of this early research on abomasal infusion of EAA in lactating dairy cows was reviewed in NRC (2001).

Enhanced production with increased RUP in the trials just cited likely occurred because of better EAA pattern of the RUP supplied by fishmeal and canola meals, perhaps because they were more complementary to microbial protein (Broderick, 1994). Availability of rumen-

protected Met (RPM) and rumen-protected Lys (RPL) made it possible to supplement one or both of these potentially limiting AA with little N input. An early report of successful application of this approach was that of Donkin et al. (1989) who showed increased yield of milk protein and milk concentrations of total protein and  $\alpha$ -,  $\beta$ - and  $\kappa$ -caseins with supplementation of RPM plus RPL. A rather large literature has developed since the early studies on RPM and RPL. Thus far, responses to RPM have been more consistent than RPL, even though significant responses have been observed in a number of studies with abomasally infused Lys alone. Lower stability of early RPL preparations may partly explain the poor responses to RPL materials. However, differences in response to RPM and RPL may also be a matter the relative amounts Met and Lys required by the animal. Because the optimal Lys:Met ratio in MP approximates 3.0 (NRC, 2001), a shortfall of 5 g/d of Met supply corresponds to a deficiency of 15 g/d of Lys. Because of greater stability, Lys is marketed as the HCl salt; this increases the molecular mass from 146 to 183 g/mol, thus requiring 25% more mass to deliver the same amount of effective compound. Note that the racemic mixture, DL-Met serves about as well as all L-Met in the animal because of efficient conversion of the D- to L-isomer; 100% L-Lys must be provided because the D-Lys is not utilized for protein synthesis.

Readers are directed to several thorough reviews that summarize the many reports on supplementation of RPM and RPL to dairy cows (Rogers et al., 1989; Vyas and Erdman, 2009; Robinson, 2010). Patton (2010) conducted a meta-analysis of 37 different studies comparing responses to two commercial RPM products, Smartamine M and Mepron; the average response was an increase of 27 g/d of milk true protein to an estimated 10 g/d of absorbed Met. Milk contains 3.7% Cys plus Met (NRC, 2001); thus, average recovery of Met in milk protein was about 10%. Although Patton (2010) concluded Mepron was somewhat more effective, this conclusion has been disputed (C. G. Schwab, personal communication). We observed similar responses in milk protein yield to RPM provided as Mepron (Broderick et al., 2008; 2009), Smartamine M (Chen et al., 2011) and Meta-Smart (Chen et al., 2011). In Meta-Smart, Met is in the form of isopropyl-2-hydroxy-4-(methylthio)-butanoic acid; this compound depends on chemical protection of the Met precursor as the isopropyl ester. Supplementing cows with RPM and RPL in very early lactation has been shown to increase milk yield and milk protein content by, respectively, 6 kg/d and 2 g/kg (Xu et al., 1998), indicating that EAA are particularly limiting for milk production in early lactation when mobilization is at its highest. Wu and Papas (1997) have reviewed methods and applications for rumen-protection for AA and other materials.

There are several reports of His being first-limiting EAA that come mainly from Finnish trials in which lactating cows were fed diets based on grass silage plus cereal concentrates. In the first two studies, abomasal infusion of Met and Lys did not increase milk protein yield (Varvikko et al., 1999); however, abomasal infusion of His, either alone or in combination with Met and/or Lys, increased protein secretion by about 30 g/d (Vanhatalo et al., 1999). Moreover, this same group observed a linear response in milk protein secretion with abomasal infusion of 0 to 6 g/d of His in lactating cows on a similar dietary regime (Korhonen et al., 2000). Doelman et al. (2008) observed an increase in milk yield of 1.7 kg/d, and a tendency for increased protein yield, by including 2.5 g His/L in drinking water; cows drank 92 L/d, making a total His dose of 230 g/d. Although very little may escape to the intestine, post-rumen supplementation of very degradable, soluble substrates such as glucose (Osbourne et al., 2002) have been accomplished via drinking water; however, the milk and protein yield responses were obtained in the abomasal His infusions with only 6-8 g/d. Hadrova et al. (2012) found that duodenal His infusion in cows fed diets based on maize silage and lucerne hay increased both milk and milk protein secretion.

Relative to an MP-adequate control diet, Lee et al. (2012) compared diets that were MP inadequate without supplement, supplemented with RPM plus RPL, or supplemented with RPM, RPL plus an experimental preparation of rumen-protected His. Feeding the MP inadequate diet depressed yield of milk by 3.6 kg/d and protein by 120 g/d; however, supplementing RPM and RPL restored 90 g/d of protein yield and adding all 3 rumen-protected EAA gave milk and protein yield equivalent to the MP-adequate control diet. Feeding the 3 rumen-protected EAA allowed dietary CP to be reduced from 15.7 to 13.5%, reduced urinary N excretion from 143 to 97 g/d and increased apparent N efficiency (Milk N/N intake) from 29 to 34% (Lee et al., 2012). The results of Lee et al. (2012) illustrate what will likely be principal strategy used when balancing for EAA: supplementing with rumen-protected EAA or reformulating the diet to meet EAA requirements with reduced CP intakes. This approach will reduce urinary N excretion and, because urinary N is the most environmental labile form of excretory N (Misselbrook et al., 2005), will make dairy production more sustainable. The potential value for exploiting this strategy was also shown in German studies where supplementing RPM at 14.7% CP resulted in milk protein secretion equal to that at 17.5% CP, with 31 versus 27% conversion of dietary N to milk N (Kröber et al., 2000). We obtained similar protein yield, and even greater yield of milk and FCM, when RPM was fed in diets containing 17.3 and 16.1% CP versus an 18.6% CP diet without RPM (Table 5, Broderick et al., 2008). Moreover, production on 15.8% CP plus RPM was about equal to that on 17.1% CP without RPM in a later experiment (Broderick et al., 2009). Furthermore, we obtained similar improvement in a third trial in which the dietary treatments were not reversed (Broderick and Muck, 2009), giving us confidence that feeding RPM will correct the Met limitation occurring in typical production settings. However, there are a number of reports of no significant effect on production of supplementation lactating cows with RPM plus RPL (e.g., Piepenbrink et al., 1996) or RPM alone (e.g., Blum et al., 1999).

Table 5. Effect on production and N excretion of lactating dairy cows of reducing dietary CP and feeding rumen-protected methionine (RPM, fed as Mepron®) (Broderick et al., 2008)

Item	RPM <sup>1</sup> , g/d	Dietary CP, %				Probability
		18.6 0	17.3 5	16.1 10	14.8 15	
<u>Production</u>						
DM intake, kg/d		23.4	23.4	23.8	23.7	0.85
Milk yield, kg/d		39.7 <sup>b</sup>	41.6 <sup>a</sup>	41.6 <sup>a</sup>	39.7 <sup>b</sup>	0.05
Milk/DM intake		1.72 <sup>ab</sup>	1.80 <sup>a</sup>	1.77 <sup>ab</sup>	1.69 <sup>b</sup>	0.07
Fat yield, kg/d		1.37 <sup>ab</sup>	1.49 <sup>a</sup>	1.43 <sup>ab</sup>	1.32 <sup>b</sup>	0.09
Protein yield, kg/d		1.15 <sup>b</sup>	1.23 <sup>a</sup>	1.23 <sup>a</sup>	1.20 <sup>ab</sup>	0.16
Milk urea N, mg/dL		14.5 <sup>a</sup>	11.8 <sup>b</sup>	9.5 <sup>c</sup>	7.9 <sup>d</sup>	< 0.01
Milk-N/N-intake, %		26.2 <sup>c</sup>	29.9 <sup>b</sup>	31.7 <sup>b</sup>	34.0 <sup>a</sup>	< 0.01
<u>Excretion</u>						
Urinary-N, g/d		260 <sup>a</sup>	207 <sup>b</sup>	188 <sup>c</sup>	150 <sup>d</sup>	< 0.01
Fecal N, g/d		250	246	259	237	0.20
Total manure-N, g/d		510 <sup>a</sup>	453 <sup>b</sup>	447 <sup>b</sup>	387 <sup>c</sup>	< 0.01
Computed N-balance, g/d		28	25	14	-7	0.01

<sup>a-d</sup>Least squares means with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Metabolizable Met assuming 60% absorption of the DL-Met in Mepron.



## SUMMARY

All animals, including dairy cattle and other ruminants, cannot synthesize nine EAA in their tissues and must absorb these from the gastro-intestinal tract. Microbial protein synthesis in the rumen converts NPN into good quality protein. However, NPN can replace only part of the dietary RDP because RDP in the form of peptides and AA stimulates protein production by the rumen microbes. Rumen microbial protein formation should be “optimized” rather than “maximized”. Reducing particle size and heat processing increase rumen digestion of grain starch and increase microbial protein formation, as long as rumen pH is not depressed. Ration formulation models help predict when lactating cows will respond to supplementation of MP in the form of RUP. Models can also be used to identify limiting EAA. Results indicate that Met and Lys are most often the first-limiting EAA on typical diets. Data from trials conducted largely with lactating cows fed grass silage-based diets indicate the His may be first-limiting under some circumstances. Altering dietary sources of RUP or supplementing with rumen-protected Met and Lys often can be used to maintain, or even improve, milk and protein yield of lactating cows fed lower CP diets. This approach increases protein efficiency and reduces urinary N excretion and the environmental impact of dairy production. Therefore, this feeding strategy is likely to be implemented in the future to reduce the environmental impact resulting from increased herd sizes.

## REFERENCES

- Argyle, J. L. and R. L. Baldwin. 1989. Effects of amino acids and peptides on rumen microbial yields. *J. Dairy Sci.* 72:2017-2027.
- Ball, R. O., K. L. Urschel, and P. B. Pencharz. 2007. Nutritional consequences of interspecies differences in arginine and lysine metabolism. 2007. *J. Nutr.* 137:1626S-1641S.
- Blum, J. W., R. M. Bruckmaier, and F. Jans. 1999. Rumen-protected methionine fed to dairy cows: bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. *J. Dairy Sci.* 82:1991–1998.
- Brito, A. F., and G. A. Broderick. 2006. Effect of varying dietary ratios of alfalfa silage to corn silage on production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 89:3924-3938.
- Brito, A. F., and G. A. Broderick. 2007. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. *J. Dairy Sci.* 90:1816-1827.
- Brito, A. F., G. A. Broderick, and R. M. Reynal. 2006. Effect of varying dietary ratios of alfalfa silage to corn silage on omasal flow and microbial protein synthesis in dairy cows. *J. Dairy Sci.* 89:3939-3953.
- Brito, A. F., G. A. Broderick, and R. M. Reynal. 2007. Effects of different protein supplements on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. *J. Dairy Sci.* 90:1828-1841.
- Broderick, G. A. 1992. Relative value of fish meal versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. *J. Dairy Sci.* 75:174-183.
- Broderick, G. A. 1994. Quantifying forage protein quality. In G. C. Fahey, Jr., M. D. Collins, D. R. Mertens and L. E. Moser (ed.) *Forage Quality, Evaluation, and Utilization*. American Soc. Agron., Madison, WI, pp. 200-228.
- Broderick, G. A., P. Huhtanen, S. Ahvenjärvi, S. M. Reynal, and K. J. Shingfield. 2010.

- Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle—A meta-analysis. *J. Dairy Sci.* 93:3216-3230.
- Broderick, G. A., T. Kowalczyk, and L. D. Satter. 1970. Milk production response to supplementation with encapsulated methionine per os or casein per abomasum. *J. Dairy Sci.* 53:1714-1721.
- Broderick, G. A., and R. E. Muck. 2009. Effect of dietary crude protein, rumen-undegraded protein and rumen-protected methionine on milk production of lactating dairy cows. *J. Dairy Sci.* 92:1281-1289.
- Broderick, G. A., and S. M. Reynal. 2009. Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows. *J. Dairy Sci.* 92: 2719-2728.
- Broderick, G. A., D. B. Ricker, and L. S. Driver. 1990. Expeller soybean meal and corn by products versus solvent soybean meal for lactating dairy cows. *J. Dairy Sci.* 73:453- 462.
- Broderick, G. A., M. J. Stevenson, R. A. Patton, N. E. Lobos, and J. J. Olmos Colmenero. 2008. Supplementing rumen-protected methionine to reduce dietary crude protein in dairy cows. *J. Dairy Sci.* 91:1092-1102
- Broderick, G. A., M. J. Stevenson, and R. A. Patton. 2009. Effect of dietary crude protein, rumen-undegraded protein and rumen-protected methionine on milk production of lactating dairy cows. *J. Dairy Sci.* 92:2719-2728.
- Broderick, G. A., and M. Åkerlind. 2012. A comparison of dietary protein evaluations by the NRC-2001 and Nordic Feed Evaluation systems. Pages 128-134 in *Proceedings 3<sup>rd</sup> Nordic Feed Sci. Conf.*  
<http://www.slu.se/PageFiles/66523/NFSC%20Proceedings%20version%203.pdf>
- Burroughs, W. A., A. H. Trenkle, and R. L. Vetter. 1974. A system of protein evaluation for cattle and sheep involving metabolizable protein (amino acids) and urea fermentation potential of feedstuffs. *Vet Med. Small Anim. Clin.* 69:713-722.
- Chalmers, M. I., D. F. Cuthbertson, and R.L.M. Syngé. 1954. Ruminal ammonia formation in relation to the protein requirement of sheep. 1. Duodenal administration and heat processing as factors influencing fate of casein supplements. *J. Agric. Sci. (Camb.)* 44:254-262.
- Charbonneau, E., P. Y. Chouinard, G. Allard, H. Lapierre, and D. Pellerin. 2006. Milk from forage as affected by carbohydrate source and degradability with alfalfa silage-based diets. *J. Dairy Sci.* 89:283-293.
- Chen, Z., G. A. Broderick, N. D. Luchini, B. K. Sloan, and E. Devillard. 2011. Effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows. *J. Dairy Sci.* 94:1978-1988.
- Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Symposium: Nitrogen metabolism and amino acid nutrition in dairy cattle microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *J. Dairy Sci.* 75:2304-2323.
- Dhiman, T. R., and L. D. Satter. 1997. Yield response of dairy cows fed different proportions of alfalfa silage and corn silage. *J. Dairy Sci.* 80:2069-2082.
- Doelman, J., N. G. Purdie, V. R. Osborne, and J. P. Cant. 2008. Short communication: The effects of histidine-supplemented drinking water on the performance of lactating dairy cows. *J. Dairy Sci.* 91:3998-4001.
- Donkin, S. S., G. A. Varga, T. F. Sweeney, and L. D. Muller. 1989. Rumen-protected methionine and lysine: effects on animal performance, milk protein yield, and physiological measures. *J. Dairy Sci.* 72:1484-1491.

- Ekinci, C., and G. A. Broderick. 1997. Effect of processing high moisture corn on ruminal fermentation and milk production. *J. Dairy Sci.* 80:3298-3307.
- Faldet, M. A., and L. D. Satter. 1991. Feeding heat-treated full fat soybeans to cows in early lactation. *J. Dairy Sci.* 74:3047-3054.
- Hadrova, S., L. Krizova, M. Richter, J. Trinacty, and M. Drackova. 2012. The effect of duodenal infusion of histidine on milk yield, milk composition, and plasma amino acids in dairy cows. *J. Anim. Feed Sci.* 21:555-565.
- Hanigan, M. D. 2005. Quantitative aspects of ruminant splanchnic metabolism as related to predicting animal performance. *Anim. Sci.* 80:23-32.
- Kleiber, M., A. H. Smith, A. L. Black, M. A. Brown, and B. M. Tolbert. 1952. Acetate as a precursor of milk constituents in the intact dairy cow. *J. Bio. Chem.* 197:371-379.
- Korhonen, M., A. Vanhatalo, T. Varvikko, and P. Huhtanen. 2000. Responses to graded post-ruminal doses of histidine in dairy cows fed grass silage diets. *J. Dairy Sci.* 83:2596-2608.
- Korhonen, M., A. Vanhatalo, T. Varvikko, and P. Huhtanen. 2002. Effect of protein source on amino acid supply, milk production, and metabolism of plasma nutrients in dairy cows fed grass silages. *J. Dairy Sci.* 85:3336-3351.
- Kröber, T. F., D. R. Külling, H. Menzi, F. Sutter, and M. Kreuzer. 2000. Quantitative Effects of Feed Protein Reduction and Methionine on Nitrogen Use by Cows and Nitrogen Emission from Slurry. *J. Dairy Sci.* 83:2941-2951.
- Lapierre, H., G. E. Lobley, D. R. Ouellette, L. Doepel, and D. Pacheco. 2007. Amino acid requirements for lactating dairy cows: Reconciling predictive models and biology. Pages 39-59 in *Proceedings 2007 Cornell Nutr. Conf. Feed Manufacturers.*
- Lee, C., A. N. Hristov, K. S. Heyler, T. W. Cassidy, H. Lapierre, G. A. Varga, and C. Parys. 2012. Effects of metabolizable protein supply and amino acid supplementation on nitrogen utilization, milk production, and ammonia emissions from manure in dairy cows. *J. Dairy Sci.* 95:5253-5268.
- Loosli, J. K., H. H. Williams, W. E. Thomas, F. H. Ferris, and L. A. Maynard. 1949. Synthesis of amino acids in the rumen. *Science* 110:144-145.
- Maeng, W. J., and R. L. Baldwin. 1976. Factors influencing rumen microbial growth rates and yields: Effect of amino acid additions to a purified diet with nitrogen from urea. *J. Dairy Sci.* 59:648-655.
- Misselbrook, T. M., J. M. Powell, G. A. Broderick, and J. H. Grabber. 2005. Dietary Manipulation in Dairy Cattle: Laboratory Experiments to Assess the Influence on Ammonia Emissions. *J. Dairy Sci.* 88:1765-1777.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle.* 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- National Research Council. 2003. *Air emissions from animal feeding operations. Current knowledge, future needs.* The National Academic Press. Washington DC. pp 263.
- NorFor-The Nordic feed evaluation system. 2011. EAAP publication no. 130, H. Volden, Ed. Wageningen Academic Publishers, the Netherlands.
- NPM. 2006. On farmers' ground. Available at [http://ipcm.wisc.edu/pubs/nutrient\\_ref.htm](http://ipcm.wisc.edu/pubs/nutrient_ref.htm) (accessed 12 Apr. 2006; verified 31 Oct. 2006). Nutrient and Pest Manage. Program, Univ. of Wisconsin Coop. Ext., Madison.
- Olmos Colmenero, J. J., and G. A. Broderick. 2006a. Effect of amount and ruminal degradability of soybean meal protein on performance of lactating dairy cows. *J. Dairy Sci.* 89:1635-1643.

- Olmos Colmenero, J. J., and G. A. Broderick. 2006b. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *J. Dairy Sci.* 89:1704-1712.
- Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. *J. Anim. Sci.* 63:1634-1648.
- Osbourne, V. R., K. E. Leslie, and B. W. McBride. 2002. Effect of supplementing glucose in drinking water on the energy and nitrogen status of the transition dairy cow. *Can. J. Anim. Sci.* 82:427-433.
- Patton, R. A. 2010. Effect of rumen-protected methionine on feed intake, milk production, true milk protein concentration, and true milk protein yield, and the factors that influence these effects: A meta-analysis. *J. Dairy Sci.* 93:2105-2118.
- Piepenbrink, M. S., T. R. Overton, and J. H. Clark. 1996. Response of cows fed a low crude protein diet to ruminally protected methionine and lysine. *J. Dairy Sci.* 79:1636-1646.
- Reis, P. J., and P. G. Schinckel. 1964. The growth and composition of wool. The effect of casein gelatin and sulphur-containing amino acids given per abomasum. *Aust. J. Biol. Sci.* 17:532-547.
- Remond, D., J. I. Cabrera-Estrada, M. Champion, B. Chauveau, R. Coudure, and C. Poncet. 2004. Effect of corn particle size on site and extent of starch digestion in lactating dairy cows. *J. Dairy Sci.* 87:1389-1399.
- Reynal, S.M., and Broderick, G.A. 2005. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. *J. Dairy Sci.* 88:4045-4064.
- Richardson, C. R., and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. *J. Anim. Sci.* 46:740-745.
- Roffler, R. E., and L. D. Satter. 1975. Relationship between ruminal ammonia and nonprotein nitrogen utilization by ruminants. I. Development of a model for predicting nonprotein nitrogen utilization by cattle. *J. Dairy Sci.* 58:1880-1888.
- Rogers, J. A., W. A. Clark, D. N. Ferraro, A. C. Spearow, V. E. Blumer, C. H. Dougherty, Jr., R. M. Cook, A. A. Jimenez, D. D. Dildey, and L. E. Armentano. 1989. Milk production of dairy cows fed ammonium and calcium salts of volatile fatty acids on 43 commercial dairy farms. *J. Dairy Sci.* 72:270-283.
- Robinson, P. H. 2010. Impacts of manipulating ration metabolizable lysine and methionine levels on the performance of lactating dairy cows: A systematic review of the literature. *Anim. Feed Sci. Technol.* 127:115-126.
- Rulquin, H., B. Graulet, L. Delaby, and J. C. Robert. 2006. Effect of different forms of methionine on lactational performance of dairy cows. *J. Dairy Sci.* 89:4387-4394.
- Schwab, C. G. 1996. Rumen-protected amino acids for dairy cattle: Progress towards determining lysine and methionine requirements. *Anim. Feed Sci. Tech.* 59:87-101.
- Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. *J. Dairy Sci.* 59:1254-1270.
- Tedeschi, L. O. 2013. Protein models and amino acids requirements for cattle. This meeting.
- Tylutki, T. P., D. G. Fox, V. M. Durbal, L. O. Tedeschi, J. B. Russell, M. E. Van Amburgh, T. R. Overton, L. E. Chase, and A. N. Pell. 2008. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. *Anim. Feed Sci. Technol.* 143:174-202.
- Virtanen, A. I. 1966. Milk production of cows on protein-free feed. *Science* 153:1603-14.
- Valadares Filho, S. C., G. A. Broderick, R.F.D. Valadares, and M. K. Clayton. 2000. Effect of replacing alfalfa silage with high moisture corn on nutrient utilization and milk production. *J.*

- Dairy Sci. 83:106-114.
- Vanhatalo, A., P. Huhtanen, V. Toivonen, and T. Varvikko. 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. *J. Dairy Sci.* 82:2674-2685.
- Varvikko, T., A. Vanhatalo, T. Jalava, and P. Huhtanen. 1999. Lactation and metabolic responses to graded abomasal doses of methionine and lysine in cows fed grass silage diets. *J. Dairy Sci.* 82:2659-2673.
- Vyas, D., and R. A. Erdman, 2009. Meta-analysis of milk protein yield responses to lysine and methionine supplementation. *J. Dairy Sci.* 92:5011-5018.
- Wikipedia, 2013. Genetic code. [http://en.wikipedia.org/wiki/Genetic\\_code](http://en.wikipedia.org/wiki/Genetic_code). Accessed May 6, 2013.
- Wu, S.H.W., and A. Papas. 1997. Rumen-stable delivery systems. *Adv. Drug Delivery Rev.* 28:323-334.
- Xu, S., J. H. Harrison, W. Chalupa, C. Sniffen, W. Julien, H. Sato, T. Fujieda, K. Watanabe, T. Ueda, and H. Suzuki. 1998. The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. *J. Dairy Sci.* 81:1062–1077.