

Can we improve the efficiency of nitrogen utilization in the lactating dairy cow?

G.A. Harrison¹ and T.P. Karnezos²

¹Alltech Biotechnology Centre, KY, USA, aharrison@alltech.com

²The Old Mill Troy, Inc., VT, USA

Summary

Ruminant protein nutrition has been a fertile research area over the past 25 years. Balancing diets for crude protein without consideration of protein quality or rumen degradability often led to overfeeding of nitrogen and less than optimal production. Recognition that rumen synthesis of bacterial protein was not sufficient to meet the needs of high-producing dairy cows led to the concept of bypass protein and a requirement for rumen undegradable protein. However, field and research responses to rumen undegradable protein were inconsistent. Model systems in use today are based on meeting metabolizable protein and amino acid requirements and consider both microbial and animal nitrogen requirements. This has led to improved performance and more efficient use of nitrogen. Future research efforts should focus on optimizing microbial protein synthesis and improving the efficiency of milk production.

Keywords: rumen, urea, protein degradation, microbial protein, milk yield

Introduction

Many excellent reviews are available on nitrogen requirements for ruminants and much has been written on this subject during the past twenty years. This review will discuss nitrogen requirements in relation to ration formulation for dairy cattle and focus on the role of rumen microbial protein synthesis in meeting these requirements, together with technological advances used to optimize microbial protein synthesis and nitrogen utilization.

Evolution of protein and nitrogen requirements

Crude protein recommendations

Many nutritionists were first exposed to nutrient requirements and feeding recommendations in an animal

nutrition course. A review of two of the more popular nutrition textbooks from 1978 shows that nutritional philosophies on protein feeding have evolved but some concepts remain sound.

In *Feeds and Nutrition*—complete, Ensminger and Olentine (1978) stated that the most important aspects in selecting protein sources were the amount of protein and its digestibility. In heifers, dry cows and low-producers, the quality of protein as measured by amino acids has no real meaning as rumen microbes produce the amino acids essential to the cow. However, in high-producing cows, bacterial synthesis of protein may not be sufficient to meet needs, and some dietary protein must escape rumen degradation for maximal production. This concept is the basis of a rumen undegraded protein requirement and the feeding of bypass protein. They also recommended 14% crude protein (CP) diets for most cows, although high-producing cows may require more. Their primary concern with feeding protein at levels exceeding protein needs was expense.

Kutches, in *Livestock Feeds and Feeding* (1978), recommended group feeding and feeding of protein according to milk production (< 44 lbs, 14–15% CP; 44–65 lbs, 15–16% CP; > 66 lbs, 16–17% CP). Although these production levels are low by today's standards, the protein levels are similar.

Microbial and animal requirements

The 1989 revision of *Nutrient Requirements for Dairy Cattle* (NRC 1989) took major steps in advancing concepts on protein nutrition and feeding practices. Requirements were published for both undegraded intake protein (UIP) and degraded intake protein (DIP) and the animal requirement was described as absorbed protein (amino acids absorbed from the small intestine). This factorial approach recognized three fates of dietary protein—fermentative digestion in the reticulo-rumen, hydrolytic/enzymatic digestion in the intestine, and passage of indigestible protein with faeces—and separated the requirements of rumen micro-organisms from those of the host animal. Limitations to this absorbed protein approach included the use of a fixed intestinal

digestibility for UIP (80%) with no consideration given to contribution of endogenous CP, amino acid composition of UIP, or prediction of microbial protein flow from net energy. This NRC publication also included a table with rumen protein degradabilities for common feedstuffs. However, more than 50% of these values were based on a single determination.

In the 1990s, researchers at Cornell University advanced the concepts set out by the 1989 NRC with the development of a new ration evaluation tool. The Cornell Net Carbohydrate Protein System (CNCPS) was designed to compare the adequacy of diets through modelling of digestion and nutrient metabolism. This system included a sub-model that compared rate of carbohydrate fermentation with rate of protein degradation and predicted rumen digestible OM, microbial protein synthesis, ammonia production and flow of undigested feed protein to the small intestine (Fox *et al.* 1998).

The work of Santos and co-workers (1998) shed new light on the practice of balancing diets according to protein degradability and explained some of the limitations of the 1989 NRC recommendations and the CNCPS. In a review of 88 lactation trials published from 1985 to 1997, they found that replacing soybean meal with high RUP sources (heated and chemically treated soybean meal, corn gluten meal, distillers' grains, brewers' grains, blood meal, meat and bone meal, feather meal, or blends of these sources) resulted in significantly higher milk yield in only 17% of the comparisons (Santos *et al.* 1998). Differences in responses (more positive responses with fish meal and treated SBM) suggested that the EAA content of RUP sources is critical. The authors cited the following as possible reasons for the lack of consistent responses to RUP: decreased microbial CP synthesis in the rumen, poor AA profile of RUP, low intestinal digestibility of RUP, or the inadequate RUP content of control diets. Replacing RDP with RUP without considering EAA content was not considered productive. They also stated that replacing soybean meal with higher RUP sources may limit microbial protein yield. Although they considered the current version of CNCPS to be an important step in the development of dynamic models, they concluded that the CNCPS overestimated the value of protein supplements high in RUP. Reviews of modelling approaches were in agreement with the conclusions of Santos and co-workers. Dijkstra *et al.* (1998) proposed that mechanistic models of rumen nitrogen metabolism could improve prediction of microbial protein synthesis. Hanigan *et al.* (1998) pointed out some of the shortcomings of the 1998 NRC system in relation to post absorptive protein and amino acid metabolism in the dairy cow.

Metabolizable protein and amino acids

The latest edition of Nutrient Requirements of Dairy Cattle (NRC 2001) built upon the foundation of the factorial approach to protein utilization that had been

laid by the previous edition. The 2001 NRC proposed a mechanistic system and made several changes to their previous recommendations. Absorbed protein was replaced with metabolizable protein (MP). DIP and UIP were replaced by RDP and RUP, respectively (RDP and RUP were already in use by the Journal of Dairy Science). Microbial protein flow was predicted from intake of total tract digestible organic matter instead of net energy. The regression equation approach replaced the factorial approach. RUP of feedstuffs was no longer considered static and, using this mechanistic system based on *in situ* data, RUP of ingredients was adjusted based on factors affecting rumen degradability. Regression equations predicted the content of each essential AA in total EAA of duodenal protein flow and digestible EAA contribution to MP. Inclusion of equations for predicting passage of EAA to the small intestine and RUP digestibility values should account for differences in nutritive value of RUP and improve the prediction of animal responses to substitution of protein sources.

Fate of protein (N sources) in the rumen

Rumen protein degradation

The sixth revised edition of Nutrient Requirements for Dairy Cattle (NRC 1989) recognized three fates of dietary protein: fermentative digestion in the reticulo-rumen, hydrolytic/enzymatic digestion in the intestine, and passage of indigestible protein with faeces. The latest edition of the NRC dairy publication (NRC 2001) expanded on these aspects. Degradation in the rumen of feed CP has a major effect on rumen fermentation and AA supply to dairy cattle. RDP and RUP are considered to have separate and distinct functions. Rumen degraded feed CP provides a mixture of peptides, free AA and ammonia for microbial growth and synthesis of microbial protein. Microbial protein typically supplies most of the AA passing to the small intestine. Rumen undegraded protein is the second most important source of absorbed AA for the animal. The most frequently used model to describe protein breakdown in the rumen divides feed CP into three fractions (A, B and C) based on *in situ* disappearance. Fraction A is the percentage of total CP that consists of NPN (rapidly degraded). Fraction C is the percentage of CP that is completely undegradable in the rumen, and 100% passes to the small intestine. Fraction B represents the rest of the CP and includes proteins that are potentially degradable. Only the B fraction is considered to be affected by passage rate. The CNCPS uses a more complex model and breaks the B fraction into 3 sub-fractions (Sniffen *et al.* 1992).

Protein degradation rates vary greatly and are affected by three-dimensional structure, intra- and inter-molecular bonding, inert barriers such as cell walls, and antinutritional factors. Differences in three-

dimensional structure and chemical bonding between protein molecules, between proteins, and carbohydrates are functions of both protein source and processing. Protein structure affects microbial access to proteins, the most important factor affecting the rate and extent of degradation in the rumen (NRC 2001). However, many other factors affect the rumen degradability of feed protein, including feed intake; forage-to-concentrate ratio; source, quality and amount of carbohydrate and CP in the diet; rumen fluid pH; associative effects of feeds; frequency of feeding; feed processing; forage conservation methods; micronutrient supply; feed additives; microbial proteolytic activity; environmental conditions (Clark and Davis 1983; NRC 1985; Sniffen and Robinson 1987; Broderick *et al.* 1991; Clark *et al.* 1992). Bateman *et al.* (2005) suggested that the variety of factors that affect rumen degradation of feed protein make it difficult to predict passage of feed protein to the small intestine with accuracy.

Microbial requirements and microbial protein synthesis

Peptides, AA and ammonia are nutrients for the growth of rumen bacteria, but protozoa cannot use ammonia (NRC 2001). Ammonia has long been considered the preferred nitrogen source of bacteria (Bryant 1974). However, Wallace (1997) noted that estimates of the contribution of ammonia vs. preformed AA to microbial protein synthesis by the mixed rumen population have been variable. Nitrogen source plays a role in ammonia use, and N availability affects the proportion of microbial N derived from ammonia (Salter *et al.* 1979; Wallace 1997). The minimum contribution to microbial N from ammonia is 26% when high concentrations of peptides and AA are present but increases to a potential maximum of 100% when ammonia is the sole N source (Wallace 1997).

Nolan (1975) and Leng and Nolan (1984) found that 50% or more of microbial N is derived from ammonia and the rest from peptides and AA. Cross-feeding among bacteria should meet AA requirements, and the mixed rumen microbial population is not considered to have an absolute requirement for AA (Virtanen 1966). However, improvements in microbial growth or efficiency have been noted when peptides or AA replaced ammonia or urea as the sole or major source of N (Cotta and Russell 1982; Russell and Sniffen 1984; Griswold *et al.* 1996).

Energy sources available to microbes and the degradability of these carbohydrates may influence N utilization by rumen micro-organisms and microbial growth. When rapidly degraded energy sources are available, AA and peptides stimulate microbial growth rate and yield of microbial protein (Russell *et al.* 1983; Chen *et al.* 1987; Argyle and Baldwin 1989; Cruz Soto *et al.* 1994). When slowly-degraded energy substrates are fermented, stimulation by peptides and AA may not occur (Chikunya *et al.* 1996). Rumen micro-organisms

that ferment non-structural carbohydrate obtain about two thirds of their N from AA or peptides (Russell *et al.* 1983); fibre digesting bacteria can derive all their N from ammonia (Bryant 1973). Species that degrade non-structural carbohydrate sources may need preformed AA (Russell *et al.* 1992). The work of Jones *et al.* (1998) suggested that with diets containing high levels of NSC, excessive peptide concentrations could depress protein digestion and ammonia concentrations, limit the growth of cellulolytic micro-organisms and reduce rumen fibre digestion and microbial protein production. Micro-organisms that ferment NSC produce and utilize peptides at the expense of ammonia production from protein and other N sources (Russell *et al.* 1992).

The rumen ammonia level required to optimize microbial protein production has been debated over the years. The oft-cited rumen fermentor studies of Satter and Slyter (1974) found that microbial protein yield was maximized at rumen ammonia concentrations of 2–5 mg/dL. Other *in vivo* work found that microbial protein production was not maximized until rumen ammonia reached 10 mg/dL (Hume *et al.* 1970) or 29 mg/dL (Miller 1973). Huber and Kung (1981) suggested that although average levels of rumen ammonia may exceed the proposed optimum for microbial protein synthesis, there may still be periods when microbial protein synthesis is limited by an ammonia deficit. Oba and Allen (2003) reported that efficiency of microbial protein synthesis was not related to rumen ammonia levels.

Synchronization of nitrogen and carbohydrates

The growth of rumen micro-organisms, or microbial protein synthesis, is dependent upon a combination of chemical, physiological and nutritional components. Rumen fluid pH is the major chemical determinant, and turnover rate is the major physiological factor affecting microbial protein synthesis. Both factors are related to diet and dietary characteristics such as intake level, feeding management and particle length. Nitrogen (protein) and carbohydrates are the major nutrients that support microbial growth, but quantity and composition of these nutrients is hardly a constant. To optimize microbial protein synthesis, an understanding of the interaction of nitrogen and carbohydrates in the rumen is required (Hoover and Stokes 1991).

The concept of synchronization of nitrogen and carbohydrate degradation rates is based on the knowledge that both nutrients are required for microbial growth and matching the available supply of nitrogen and carbohydrates should result in more energetically efficient production of microbial protein. Russell and Hespell (1981) suggested that failure to synchronize energy and protein degradation rates in the rumen would result in less efficient growth and uncoupled utilization of substrate. Synchronization of energy and protein should result in more stable levels of nitrogen in the rumen (Newbold and Rust 1992; Henning *et al.* 1993).

Attempts at improving microbial yield or efficiency have met with mixed results. Aldrich *et al.* (1993) noted improvements in efficiency of bacterial N production when degradability of rumen protein matched that of rumen carbohydrates. However, these improvements did not result in measurable changes in milk production. Kolver *et al.* (1998) found that synchronizing rumen release of carbohydrates with nitrogen from pasture could improve capture of rumen nitrogen, but N status and milk production were not improved. Synchronizing energy and protein by altering feeding frequency had no benefit (Robinson and McQueen 1994). These authors suggested that soluble protein and/or peptide N could act as a pool to provide N needed for microbial growth when ammonia concentrations were low. Hoover and Stokes (1991), in their review of *in vitro* and *in vivo* literature, came to the conclusion that the major fact or controlling microbial growth was the rate of carbohydrate digestion. This is supported by the work of Henning *et al.* (1993), in which synchronizing rumen infusion of N and carbohydrates to sheep did not improve microbial yield. The authors concluded that dietary manipulations should be directed at obtaining a stable rumen energy supply and then providing the appropriate amount of rumen available nitrogen.

Stokes *et al.* (1991) demonstrated the importance of ensuring adequate levels of carbohydrates and RDP (using diets containing 31–39% NSC and 11.8–13.7% RDP on a DM basis) when synchronizing the degradation rates of these nutrient sources. Optimizing microbial protein synthesis resulted in increased VFA production in the rumen and increased microbial protein flow to the duodenum, which meant less RUP and energy being fed to meet the cow's production requirements.

Further advantages of synchronized rumen fermentation include improved fibre digestion and decreased absorption of ammonia (through utilization of ammonia by rumen bacteria). The challenge facing nutritionists in feeding RDP sources relates to the degradation rate and limiting the impact of excess RDP due to the mismatch in carbohydrate degradation rates. If ammonia from RDP is in excess in relation to carbon skeletons from carbohydrate digestion, blood urea nitrogen levels can increase. Blood urea nitrogen (BUN) concentrations greater than 20 mg/dL (Ferguson *et al.* 1988) and milk urea nitrogen (MUN) concentrations greater than 19 mg/dL have been linked to depressed reproductive performance (Butler *et al.* 1985). High levels of RDP are associated with altered ovarian and uterine physiology, resulting in luteal insufficiency and embryo loss when milk or plasma urea nitrogen concentrations exceed 190 mg/L (Sinclair *et al.* 2000). However, BUN levels above 20 mg/dL do not always result in decreased reproductive performance (Oldick and Ferkins 1996). Protein intake, BUN, and MUN concentration appear to play a role in reproductive performance, but management, energy balance, milk yield and health status also affect reproduction (NRC 2001).

Improving the efficiency of nitrogen utilization in the dairy cow

Optimizing microbial protein synthesis

As microbial fermentation is a major component of digestion in ruminants, optimizing microbial protein synthesis is very important in meeting the nutritional needs of ruminants. Rumen fermentation can account for 70–100% of the ruminant's AA supply (AFRC 1992). Microbial protein has the major impact on quantity and quality of metabolizable protein delivered to and absorbed from the small intestine and is highly digestible in the small intestine (O'Connor *et al.* 1993). Dietary protein sources with low rumen degradation may have lower digestibility than microbial protein in the small intestine and are more expensive than protein sources readily degraded in the rumen (Oba and Allen 2003). Therefore, formulating diets to maximize protein yield should result in high quality protein reaching the small intestine at a lower dietary cost. Maximizing microbial protein production and more efficient rumen utilization of the diet go hand-in-hand.

Santos and co-workers (1998) suggested that microbial protein could be considered the best available source of amino acids for milk protein synthesis. When the amino acid contents of protein sources are compared to the AA content of milk protein, microbial protein is the closest in AA content to milk protein (Table 1). When the utilization of each AA is considered, microbial protein has the highest score, followed by soybean meal. If lysine and methionine are the first two limiting AA for milk production and milk protein in most dairy diets (Schwab *et al.* 1992) and the ideal ratio of lysine to methionine (as a % of total essential AA) is 15:5 (Schwab 1994), then microbial protein has an excellent amino acid balance (Table 2).

Feeding to minimize nitrogen excretion

While dairy cows utilize feed crude protein more efficiently than other ruminants, they still excrete up to 3-fold more N in manure than in milk. Inefficient N utilization results in increased feed costs and contributes to environmental N pollution (Broderick 2005). An average dairy cow producing 8200 kg of milk per lactation excretes about 20.9 tonnes of wet manure with about 109 kg of N (Van Horn *et al.* 1996). Since dietary nitrogen that is not utilized by the cow is excreted (primarily through urine), feeding protein above animal requirements adds even more N to the environment.

Researchers at the US Dairy Forage Research Centre in Wisconsin have examined production responses and nitrogen metabolism in high-producing dairy cows fed varying levels of crude protein in diets based on typical mid-western US diets (alfalfa haylage and corn silage forages). Broderick (2003) formulated diets containing 15.1, 16.7 and 18.4% CP and found that

milk and protein yield were not improved above 16.7% CP (Table 3). N excretion increased linearly and a higher percent of the extra N was found in urine. Nitrogen was also used more efficiently at lower dietary protein levels (milk N / N intake). This experiment was followed by a second trial with five levels of dietary CP ranging from 13.5–19.4% (Olmos Colmenero and Broderick 2003). The highest milk production was observed in the cows fed the 16.5% CP diet; increasing protein above 16.5% did not improve milk or protein yield (Table 4). Again, N excretion increased linearly and efficiency of N utilization for milk protein declined with increasing CP levels in the diet. Microbial protein yield was not improved above 16.5% CP with these diets. Brito and Broderick (2004) compared protein sources and found that diets balanced at 16.6% CP supported over 40 kg of milk when the primary supplemental protein source was soybean meal, cottonseed meal or canola meal (Table 5). Microbial protein flows were similar in cows fed each of these sources of vegetable protein; a urea-

based diet resulted in lower microbial protein flow to the lower gut.

Technological innovations to improve nitrogen utilization

Over the past 30 years, a number of technologies have been developed to synchronize rumen NPN release with carbohydrate degradation in the rumen and maximize rumen microbial yield. Many of these technologies have centred on controlling the NPN release from urea and include Starea (Bartley and Deyoe 1975), formaldehyde-treated urea (Prokop and Klopfenstein 1977), linseed oil-coated urea (Forero *et al.* 1980), isobutylidene monourea (Mathison *et al.* 1994), biuret (Löest *et al.* 2001) and polymer-coated urea (Galo *et al.* 2003). The efficacy of these slow release NPN sources varies from incomplete release of NPN to excessively fast release of NPN. One of the most promising of these technologies is a polymer-coated urea (Optigen 1200™,

Table 1 Amino acid composition of protein sources in relationship to milk protein.

Protein Source	His	Phe	Leu	Thr	Met	Arg	Val	Ile	Trp	Lys
Blood meal	100	100	93	86	45	33	70	10	76	91
Fish meal	77	69	58	68	100	59	59	47	71	80
Feather meal	11	59	66	59	23	32	38	32	29	13
Meat meal	67	65	46	59	49	76	51	36	39	58
Meat and bone meal	64	64	46	59	49	76	48	36	32	55
Corn gluten meal	67	100	100	60	100	36	48	40	30	18
Alfalfa meal, dehydrated	69	100	55	80	60	50	66	51	100	46
Brewers grain	56	100	83	65	78	53	65	74	87	34
Distillers grains w/solubles	74	84	72	63	81	42	53	38	45	24
Soybean meal	89	100	56	74	56	89	60	55	75	70
Microbes	90	97	54	100	97	79	66	61	99	100

¹Santos *et al.* (1998) adapted from Chandler (1991) and calculated as follows: (% of AA in feed protein/% of AA in milk protein) × 100.

A score of 100 is the maximum allowed for each amino acid value

Table 2 Ranking of protein sources in relationship to milk protein¹.

Protein source	Met	Lys	Met + Lys
Microbes	97	100	197
Fish meal	100	80	180
Blood meal	45	91	136
Soybean meal	56	70	126
Corn gluten meal	100	18	118
Brewers grain	78	34	112
Meat meal	49	58	107
Alfalfa meal, dehydrated	60	46	106
Distillers grains w/solubles	81	24	105
Meat and bone meal	49	55	104
Feather meal	23	13	36

¹Santos *et al.* (1998) adapted from Chandler (1991) and calculated as follows: (% of AA in feed protein/% of AA in milk protein) × 100.

A score of 100 is the maximum allowed for each amino acid value

Table 3 Effect of dietary CP content on milk production and composition, and on nitrogen metabolism¹.

Trait	Crude protein, % of DM				P ²		
	15.1	16.7	18.4	SE	CP	Linear	Quadratic
DM intake (kg/d)	21.2 ^c	22.1 ^b	22.6 ^a	0.3	<0.01	<0.01	0.30
N intake (kg/d)	0.512 ^c	0.593 ^b	0.666 ^a	0.007	<0.01	<0.01	0.49
Milk yield (kg/d)	33.0 ^b	34.1 ^a	34.1 ^a	0.6	0.01	0.01	0.14
Milk protein (%)	2.99 ^b	3.03 ^a	3.02 ^a	0.03	0.03	0.05	0.07
Milk protein yield (kg/d)	0.99 ^b	1.02 ^a	1.02 ^a	0.02	0.05	0.04	0.19
Milk urea N (mg/dL)	9.2 ^c	12.4 ^b	15.9 ^a	0.2	<0.01	<0.01	0.34
Milk N / N intake	0.303 ^a	0.270 ^b	0.239 ^c	0.004	<0.01	<0.01	0.72
Fecal N (g/d)	236 ^b	264 ^a	273 ^a	8	<0.01	<0.01	0.12
Urinary N (g/d)	140 ^c	193 ^b	236 ^a	5	<0.01	<0.01	0.20

¹Adapted from Broderick (2003)^{abc}Least squares means within the same row without a common superscript differ ($P < 0.05$)²Probability of a significant effect of CP or of a linear or quadratic effect of CP concentration in the diet**Table 4** Effect of dietary CP content on milk production and composition, and on nitrogen metabolism¹.

Trait	Crude Protein, % of DM					P ²		
	13.5	15.0	16.5	17.9	19.4	SE	Linear	Quadratic
DM intake (kg/d)	21.6 ^b	21.8 ^{ab}	22.5 ^a	21.6 ^b	21.7 ^{ab}	0.4	<0.01	0.30
Milk yield (kg/d)	36.3 ^b	37.2 ^{ab}	38.3 ^a	36.6 ^b	37.0 ^{ab}	0.9	0.01	0.14
Milk protein (%)	3.09	3.15	3.09	3.18	3.16	0.04	0.05	0.07
Milk protein yield (kg/d)	1.10 ^b	1.15 ^{ab}	1.18 ^a	1.13 ^{ab}	1.15 ^{ab}	0.04	0.04	0.19
Milk N / N intake	0.367 ^a	0.344 ^b	0.307 ^c	0.279 ^d	0.255 ^e	0.006	<0.01	0.72
Urinary N excretion (g/d)	63.2 ^e	91.0 ^d	128.4 ^c	174.0 ^b	208.1 ^a	6.6	<0.01	0.12
Microbial CP flow (g/d)	993 ^b	1082 ^{ab}	1144 ^a	1127 ^a	1144 ^a	67	<0.01	0.20

¹Adapted from Olmos Colmenero and Broderick (2003)^{abcde}Least squares means within the same row without a common superscript differ ($P < 0.05$)²Probability of a significant effect of CP or of a linear or quadratic effect of CP concentration in the diet**Table 5** Effect of protein source on production and abomasal protein flows in lactating cows¹.

Trait	Urea	Soybean meal	Cottonseed meal	Canola meal	SE
Dietary CP (%)	16.5	16.5	16.6	16.6	
DM intake (kg/d)	22.1 ^c	24.2 ^b	24.7 ^{ab}	24.8 ^a	0.4
N intake (kg/d) ²	0.583	0.639	0.656	0.659	
Milk yield (kg/d)	32.9 ^b	40.0 ^a	40.5 ^a	41.1 ^a	1.0
Milk protein (%) ²	2.80	3.08	2.91	3.09	
Milk protein yield (kg/d)	0.92 ^c	1.23 ^{ab}	1.18 ^b	1.27 ^a	0.05
Milk N / N intake ²	0.252	0.308	0.288	0.308	
Omasal protein flows (g/d)					
Milk N / N intake	2344 ^b	2706 ^a	2706 ^a	2775 ^a	120
Fecal N (g/d)	538 ^c	987 ^b	1348 ^a	1150 ^{ab}	106
Urinary N (g/d)	2882 ^c	3693 ^b	4054 ^a	3925 ^{ab}	220

¹Adapted from Brito and Broderick (2003)^{abc}Least squares means within the same row without a common superscript differ ($P < 0.05$)²Calculated

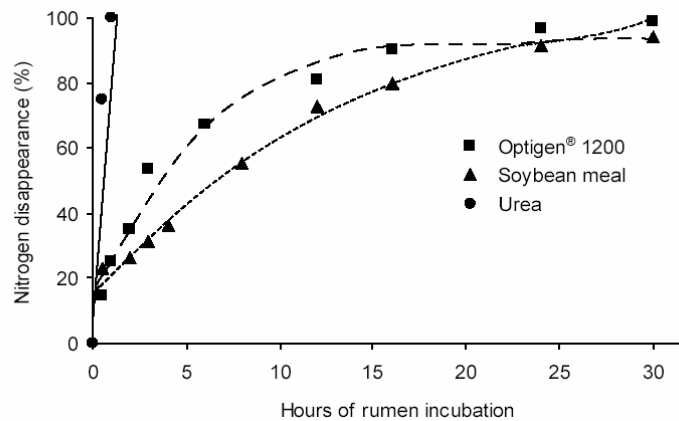


Figure 1 *In situ* nitrogen disappearance of Optigen® 1200, soybean meal and feed grade urea (Akay *et al.* 2004).

Alltech) that has been demonstrated to have a nitrogen disappearance rate similar to that of soybean meal (Figure 1).

The value of slow-release urea products extends beyond the potential impact of changing microbial protein synthesis and nutrient digestion. The practical value of some slow-release urea products is related to N density. For example, Optigen® 1200, a polymer-coated urea, has a crude protein value of 274% compared to 53% for soybean meal (DM basis). On a RDP basis, 1 g of Optigen® 1200 is equivalent to 6 g of soybean. The 6-fold increase in RDP nitrogen density between these two nitrogen sources results in creation of 'space' in the ration. For example, 170 g of Optigen replaces 1020 g of soybean meal, providing 850 grams of space in the diet. Utilization of this space provides nutritionists with significant flexibility in formulation. This formulation flexibility associated with utilizing the space created in the diet could be used in achieving one or more of the following objectives:

Increased milk production. Akay *et al.* (2004) conducted a study involving 220 dairy cows and comparing an Optigen® 1200 diet to a control diet with animal groups balanced for days in milk, parity and milk yield. In this experiment, 227 g of Optigen® 1200 allowed for a reduction of soybean meal, urea, Soy Plus® soy hulls and corn gluten meal. A net space of 1.1 kg was created and filled with 0.8 kg of wheat middlings and 0.3 kg of finely ground corn (DM basis). These changes increased RDP by 4.12% and starch content by 3.06%. The Optigen® 1200 treatment resulted in a 9.74% increase in milk yield, no change in milk fat yield, and an 8.9% increase in milk protein yield, even though RUP was reduced by 5.5%. Utilizing the space created by Optigen® 1200 by increasing the carbohydrate density may have improved microbial protein production as reflected in changes in performance.

Increased efficiency of milk production. In a second lactation trial (Akay *et al.* 2004), crude protein and net energy level of the control and Optigen diets were similar. Milk, protein and fat yields were similar across treatments, but DMI for the Optigen® 1200 group

was 3.73% lower. The net result was an improvement in the efficiency of milk production (kg milk per kg DMI) of 4.2% for the Optigen® 1200 treatment. In this study, the inclusion of 159 g of Optigen allowed a portion of the soybean meal, urea and cottonseed to be substituted for forage and corn. The cows fed Optigen® 1200 consumed 7.89% more RUP per day than the controls, suggesting that the improvement in efficiency may have been due to increased microbial protein production.

Increased nutrient density of the diet. Increasing nutrient density may be advantageous during times when intake is depressed: during heat stress, for close-up dry cows, during early lactation, for animals that are off feed or in the hospital pen. Heat stress in dairy cattle is a function of environmental temperature, relative humidity and the duration of the conditions. Dry matter intake can be reduced by as much as 7–10% during heat stress. Increasing the density of the diet provides an opportunity to feed less DM and still meet the nutrient needs of the animal. The extra space provided by concentrating the RDP source can be filled with energy, RUP and buffer while maintaining the forage-to-grain ratio. When increasing nutrient density during times of depressed intake, it is important to continue to provide adequate forage and effective fibre to insure normal, healthy rumen function.

Reduced ration cost while maintaining milk production. The space created by a concentrated slow-release RDP source can be filled with high quality forage or soluble fibre that could reduce the cost of feeding while maintaining levels of production.

Enhanced rumen health. Extra space also provides opportunities to raise forage and fibre levels if decreased soluble carbohydrate load in the rumen is desired. To maintain rumen health, fibre and carbohydrate levels need to be considered in relation to one another. Starch or NSC concentrations can be adjusted based on the forage NDF level in the diet. Cows fed diets higher levels of forage NDF can better handle diets with high starch contents. However, cows consuming low-forage-NDF diets should be fed less starch.

Diet formulation and nitrogen utilization

Using the knowledge gained from the research available, can we formulate diets that support high milk production and improve the efficiency of nitrogen utilization in the dairy cow?

Dietary formulation must first take into account the nutrient needs of the rumen microbial population. Ammonia may be the preferred source of nitrogen for bacteria, but NPN alone will not maximize microbial growth or efficiency. Although many studies have shown stimulatory effects of amino acids and peptides on microbial growth, their exact role or contribution is debatable. However, the practice of formulating with multiple protein sources is an attempt to better meet the needs of both the rumen microbes and the cow. Energy sources and carbohydrate degradability also influence N utilization by rumen microbes.

Synchronization of nitrogen and carbohydrate degradation rates should, in theory, result in energetically efficient production of microbial protein. Researchers have had success in improving capture of rumen N by synchronizing release of N and carbohydrates. However, attempts at demonstrating measurable improvements in performance have not met with a great deal of success. Hoover and Stokes (1991) stated that dietary manipulations would be aimed at obtaining a stable rumen energy supply and then providing the appropriate amount of rumen available nitrogen. This suggests that synchronization cannot be achieved without first ensuring stable rumen fermentation.

Formulating diets at lower CP levels can certainly reduce N excretion and improve efficiency of N utilization. Research has shown that high production can be achieved with diets containing 16–17% CP, although nutritionists continue to balance rations at higher levels. Model formulation systems using linear optimization may not be perfect, but they have the potential to improve both performance and efficiency.

Conclusions

The knowledge gained through research efforts over the past 25 years has certainly advanced the science of ruminant protein nutrition. Balancing diets according to crude protein content often led to overfeeding and did not always optimize performance. Formulating rations to meet rumen degradable and undegradable protein was a step in the right direction but did not always increase microbial protein flow to the small intestine. Model systems in use today are based on meeting metabolizable protein and amino acid requirements and consider both microbial and animal nitrogen requirements. This has led to improved performance and more efficient use of nitrogen. Future research efforts should focus on optimizing microbial protein synthesis and improving the efficiency of milk production.

References

- Agricultural Research Council (1980). *The Nutrient Requirements of Ruminant Livestock*. Commonwealth Bureaux, Slough, UK.
- Agricultural and Food Research Council (1992). Technical Committee on Responses to Nutrients, Rep. No. 9. Nutritive Requirements of Ruminant Animals: Protein. *Nutrition Abstracts and Reviews, Series B, Livestock Feeds and Feeding* 62, 787–835.
- Akay, V., Tikofsky, J., Holtz, C. and Dawson, K.A. (2004). Optigen^{®1200}: Controlled release of non-protein nitrogen in the rumen. *Proceedings of the 20th Alltech Symposium*, pp. 179–185.
- Aldrich, J.M., Muller, L.D. and Varga, G.A. (1993). Non-structural carbohydrate and protein effects on rumen fermentation, nutrient flow, and performance in dairy cows. *Journal of Dairy Science* 76, 1091–1105.
- Argyle, J.L. and Baldwin, R.L. (1989). Effects of amino acids and peptides on rumen microbial growth yields. *Journal of Dairy Science* 72, 2017–2027.
- Bartley, E.E. and Deyoe, C.W. (1975). Starea as a protein replacer for ruminants. A review of 10 years of research. *Feedstuffs* 47, 42–44.
- Bateman, H.G., Clark, J.H. and Murphy, M.R. (2005). Development of a system to predict feed protein flow to the small intestine of cattle. *Journal of Dairy Science* 88, 282–295.
- Brito, A.F. and Broderick, G.A. (2004). Effects of different protein supplements on nitrogen utilization in dairy cows. I. Lactation performance and rumen metabolism. *Journal of Dairy Science* 87 (Suppl. 1), 161 (Abstr.).
- Broderick, G.A. (2003). Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *Journal of Dairy Science* 86, 1370–1381.
- Broderick, G.A. (2005). Feeding dairy cows to minimize nitrogen excretion. *Proceedings of Tri-State Nutrition Conference*, Ft. Wayne, IN., The Ohio State University, pp. 137–152.
- Broderick, G.A., Wallace, R.J. and Orskov, E.R. (1991). Control of rate and extent of protein degradation. In: *Physiological Aspects of Digestion and Metabolism in Ruminants*, pp. 541–592 (eds. T. Tsuda, Y. Sasaki and R. Kawashima). Academic Press, Orlando, FL, USA.
- Bryant, M.P. (1973). Nutritional requirements of the predominant rumen cellulolytic bacteria. *Federation Proceedings* 32, 1809.
- Bryant, M.P. (1974). Nutritional features and ecology of predominant anaerobic bacteria in the intestinal tract. *Journal of Dairy Science* 27, 1313.
- Butler, W.R., Calaman, J.J. and Beam, S.W. (1985). Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cows. *Journal of Animal Science* 74, 858–865.
- Chandler, P.T. (1989). Achievement of optimum amino acid balance possible. *Feedstuffs* 61, pp. 14, 25.
- Chen, G., Sniffen, C.J. and Russell, J.B. (1987). Concentration and estimated flow of peptides from

- the rumen of dairy cattle: Effects of protein solubility and feeding frequency. *Journal of Dairy Science* 70, 983–992.
- Chikunya, S., Newbold, C.J., Rode, L., Chen X.B. and Wallace, R.J. (1996). The influence of non protein nitrogen, preformed amino acids and protein on microbial activity in the rumen of sheep receiving diets containing rapidly and slowly degraded fibre sources. *Animal Feed Science Technology* 63, 333–340.
- Church, D.C. (1978). *Livestock Feeds and Feeding*. First Edition. Oxford Press, Portland, OR, USA.
- Clark, J.H. and Davis, C.L. (1983). Future improvement of milk production; potential for nutritional improvement. *Journal of Animal Science* 57, 750–764.
- Clark, J.H., Klusmeyer, T.H. and Cameron, M.A. (1992). Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *Journal of Dairy Science* 75, 2304–2323.
- Cotta, M.A. and Russell, J.B. (1982). Effect of peptides and amino acids on efficiency of rumen bacterial protein synthesis in continuous culture. *Journal of Dairy Science* 65, 226–234.
- Cruz Soto, R., Muhammand S.A., Newbold, C.J., Stewart, C.S. and Wallace, R.J. (1994). Influence of peptides, amino acids and urea on microbial activity in the rumen of sheep receiving grass hay and on the growth of rumen bacteria in vitro. *Animal Feed Science Technology* 49, 151–161.
- Dewhurst, R.J., Webster, A.J.F., Wainman, F.W. and Dewey, P.J.S. (1986). Prediction of the true metabolizable energy concentration in forages for ruminants. *Animal Production* 43, 183–194.
- Dijkstra, J., France, J. and Davies, D.R. (1998). Different mathematical approaches to estimating microbial protein supply in ruminants. *Journal of Dairy Science* 81, 3370–3384.
- Ensminger, M.E. and Olentine, C.G. (1978). *Feeds and Nutrition—complete*. First Edition. The Ensminger Publishing Company, Clovis, CA, USA.
- Maynard, L.A. and Loosli, J.K. 1969. *Animal Nutrition*. Sixth Edition. McGraw–Hill Book Company, New York, NY, USA.
- Ferguson, J.D., Blanchard, T., Galligan, D.T., Hoshall, D.C. and Chalupa, W. (1988). Infertility in dairy cattle fed a high percentage of protein degradable in the rumen. *Journal of the American Veterinary Association* 192, 659–662.
- Forero, O., Owens, F.N. and Lusby, K.S. (1980). Evaluation of slow–release urea for winter supplementation of lactating range cows. *Journal of Animal Science* 50, 532–538.
- Fox, D.G., Sniffen, C.J., O'Connor, J.D., Russell, J.B. and Van Soest, P.J. (1992). A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. *Journal of Animal Science* 70, 3578–3596.
- Galo, E., Emanuele, S.M., Sniffen, C.J., White, J.H. and Knapp J.R. (2003). Effects of a polymer–coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. *Journal of Dairy Science* 86, 2154–2162.
- Griswold, K.E., Hoover, W.H., Miller T.K. and Thayne, W.V. (1996). Effect of form of nitrogen on growth of rumen microbes in continuous culture. *Journal of Animal Science* 74, 483–491.
- Henning, P.H., Steyn, D.G. and Meissner, H.H. (1993). Effect of synchronization of energy and nitrogen supply on rumen characteristics and microbial growth. *Journal of Animal Science* 71, 2516–2528.
- Hoover, W.H. and Stokes, S.R. (1991). Balancing carbohydrates and proteins for optimum rumen microbial yield. *Journal of Dairy Science* 74, 3630–3644.
- Huber, J.T. (1975). Protein and nonprotein nitrogen utilization in practical dairy ration. *Journal of Animal Science* 41, 954.
- Huber, J.T. and Kung, L., Jr. (1981). Protein and nonprotein nitrogen utilization in dairy cattle. *Journal of Animal Science* 64, 1170–1195.
- Hume, I.D., Moir, R.J. and Somers, M. (1970). Synthesis of microbial protein in the rumen. I. Influence of level of nitrogen intake. *Australian Journal of Agricultural Research* 21, 283.
- Jones, D.F., Hoover W.H. and Miller–Webster, T.K. (1998). Effects of concentrations of peptides on microbial metabolism in continuous culture. *Journal of Animal Science* 76, 611–616.
- Kolver, E., Muller L.D., Varga, G.A. and Cassidy, T.J. (1998). Synchronization of rumen degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *Journal of Animal Science* 81, 2017–2028.
- Kutches, A.J. (1978). Feeding dairy cattle. In: *Livestock Feeds and Feeding*. First Edition, (ed. D.C. Church). Oxford Press, Portland, OR, USA.
- Leng, R.A. and Nolan, J.V. (1984). Nitrogen metabolism in the ruminant. *Journal of Animal Science* 67, 1072–1089.
- Löest, C.A., Titgemeyer, E.C., Drouillard, J.S., Lambert, B.D. and Trater, A.M. (2001). Urea and biuret as non–protein nitrogen sources in cooked molasses blocks for steers fed prairie hay. *Animal Feed Science Technology* 94, 115–126.
- Mathison, G.W., Soofi, S.R. and Woosley, M. (1994). The potential of isobutyraldehyde monourea (propanal, 2–methyl–monourea) as a non–protein nitrogen sources for ruminant animals. *Journal of Animal Science* 74, 665–674.
- Miller, E.L. (1973). Symposium on nitrogen utilization by the ruminant. Evaluation of foods as sources of nitrogen and amino acids. *Proceedings of the Nutrition Society* 32, 79.
- National Research Council (1985). *Ruminant Nitrogen Usage*. National Academy Press, Washington, DC, USA.
- National Research Council (1989). *Nutrient Requirements of Dairy Cattle*. Sixth Revised Edition. National Academy Press, Washington, DC, USA.

- National Research Council (2001). *Nutrient Requirements of Dairy Cattle*. Seventh Revised Edition. National Academy Press, Washington, DC, USA.
- Nolan, J.V. (1975). *Quantitative models of nitrogen metabolism in sheep*. In: *Digestion and Metabolism in the Ruminant* (eds. I.W. McDonald and A.C.I. Warner), pp. 416–431. University of New England Publishing Unit, Armidale, Australia.
- Oba, M. and Allen, M.S. (2003). Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. *Journal of Dairy Science* 86, 195–207.
- O'Connor, J.D., Sniffen, C.J., Fox, D.G. and Chalupa, W. (1993). A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. *Journal of Animal Science* 71, 1298–1311.
- Oldick, B.S. and Ferkins, J.L. (1996). Imbalanced, inadequate diets effect reproduction performance. *Feedstuffs* 51, 12–14, 25.
- Olmos Colmenero, J.J. and Broderick, G.A. (2003). Effect of different crude protein level on milk yield and rumen metabolism of lactating dairy cows. *Journal of Dairy Science* 86 (Suppl. 1), 273 (Abstr.).
- Prokop, M.J. and Klopfenstein, T.J. (1977). Slow ammonia release urea. Nebraska Beef Cattle Report No. EC 77, p.218.
- Russell, J.B. and Hespell, R.B. (1981). Microbial rumen fermentation. *Journal of Animal Science* 64, 1153.
- Russell, J.B., Sniffen, C.J. and Van Soest, P.J. (1983). Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *Journal of Animal Science* 66, 763.
- Russell, J.B. and Sniffen, C.J. (1984). Effect of carbon-4 and carbon-5 volatile fatty acids on growth of mixed rumen bacteria in vitro *Journal of Animal Science* 67, 987.
- Russell, J.B., O'Connor, J.D., Fox, D.G., Van Soest, P.J. and Sniffen, C.J. (1992). A net carbohydrate and protein system for evaluating cattle diets: I. Rumen fermentation. *Journal of Animal Science* 70, 3551–3561.
- Salter, D.N., Daneshaver, K. and Smith, R.H. (1979). The origin of nitrogen incorporated into compounds in the rumen bacteria of steers given protein- and urea-containing diets. *British Journal of Nutrition* 41, 197–209.
- Satter, L.D. and Slyter, L.L. (1974). Effect of ammonia concentration on rumen microbial protein production in vitro. *British Journal of Nutrition* 32, 199.
- Santos, F.A.P., Santos, J.E.P., Theurer, C.B. and Huber, J.T. (1998). Effects of rumen-undegradable protein on dairy cow performance: a 12-year literature review. *Journal of Dairy Science* 81, 3182–3213.
- Schwab, C.G. (1994). Optimizing amino acid nutrition for optimum yields of milk and milk protein. *Proceedings of the Southwest Nutrition Management Conference*, pp. 114–129.
- Schwab, C.G., Bozak, C.K., Whitehouse, N.L. and Mesbah, M.M.A. (1992). Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. *Journal of Dairy Science* 75, 3486–3502.
- Sinclair, K.D., Sinclair, L.A. and Robinson, J.J. (2000). Nitrogen metabolism and fertility in cattle: I. Adaptive changes in intake and metabolism to diets differing in their rate of energy and nitrogen release in the rumen. *Journal of Animal Science* 78, 2659–2669.
- Sniffen, C.J. and Robinson, P.H. (1987). Microbial growth and flow as influenced by dietary manipulations. *Journal of Dairy Science* 70, 425–441.
- Sniffen, C.J., O'Connor, J.D., Van Soest, P.J., Fox, D.G. and Russell, J.B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science* 70, 3562–3577.
- Stokes, S.R., Hoover, W.H., Miller, T.K. and Blauweikel, R. (1991). Rumen digestion and microbial utilization of diets varying in type of carbohydrate and protein. *Journal of Dairy Science* 74, 871–881.
- Van Kessel, J.S. and Russell, J.B. (1996). The effect of amino nitrogen on the energetics of rumen bacteria and its impact on energy spilling. *Journal of Animal Science* 79, 1237–1243.
- Virtanen, A.I. (1966). Milk production of cows on protein-free feed. *Science* 153, 1603–1614.
- Wallace, R.J. (1997). Peptide metabolism and its efficiency in ruminant production. In: *Rumen Microbes and Digestive Physiology in Ruminants* (ed. R. Onodera), pp. 95–105. Japan Scientific Society Press, Tokyo/S Karger, Basel.