

BALANCING FOR RUMEN DEGRADABLE PROTEIN

C. J. Sniffen¹, W. H. Hoover², T. K. Miller-Webster², D. E. Putnam³ and S. M. Emanuele.
¹Fencrest, LLC, ² The Rumen Profiling Laboratory, West Virginia University, ³Balchem.

INTRODUCTION

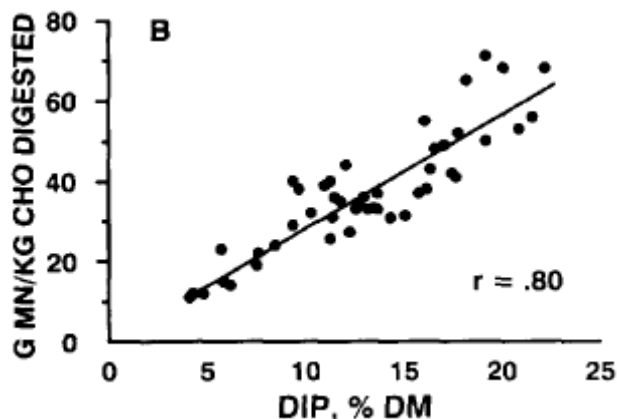
By now it is no mystery that in ruminant nutrition, protein is broken down into the rumen degradable (RDP) and rumen undegradable (RUP) fractions, and that it is the microbial protein created from RDP and carbohydrates digested in the rumen, along with RUP, that supplies metabolizable protein (MP) to the animal. Recently, in dairy nutrition, considerable attention has been given to the RUP fraction and the amino acid profile of the MP supplied to the cow. Deservedly so; however, in doing so, some significant contributions of RDP to animal performance may have been overlooked. This paper will attempt to highlight the important contributions of RDP in dairy nutrition, including discussions on amounts, sources and practical diet formulation guidelines.

ROLE OF RDP

The role of RDP is simple in some ways and complex in others. One of the earliest discussions on the importance of RDP was proposed by Hoover, 1987. RDP provides N to the rumen microorganisms in the correct form and at the correct time, to fuel their growth and key functions. In essence, this means to provide the N needed for the development of microbial protein and to support carbohydrate digestion and utilization in the rumen.

It has been typically thought that energy is most limiting for microbial growth, but research has shown that DIP or RDP as seen below can be equally limiting (Figure 1).

Figure 1. Digestible intake protein (DIP) and microbial efficiency.¹



¹Hoover & Stokes, 1991

Microbial protein production, a key result of microbial growth, is controlled by two factors; carbohydrate digested and microbial efficiency (g of microbial protein synthesized/g or kg of carbohydrate digested). A deficiency in RDP can limit digestion

of both structural and non-structural carbohydrates (Griswold et al., 2003). However, it also is notable that both *in vitro* and *in vivo* research has confirmed a linear relationship between RDP as a percent of DM and microbial efficiency (Hoover and Stokes, 1991). This is the net microbial protein appearing in the small intestine for a given amount of carbohydrate truly digested in the rumen.

The conversion of RDP into microbial protein creates the highest quality protein supplement we can feed to dairy cows. This is due to the advantage that microbial protein has over RUP supplements in the areas of amino acid profile, intestinal digestibility or both. Optimizing RDP nutrition, and hence, the production of microbial protein, is an important first step to meeting the MP and the amino acid and particularly the lysine and methionine requirements of dairy cows.

BALANCE OF RDP AND RUP

The use of RUP in dairy nutrition is clearly important and makes a significant contribution towards meeting the cow's overall MP requirement. However, its use has not always been as effective as desired. Santos et al. (1998) reviewed 12 years of published literature on RUP research in dairy cow nutrition and reported findings on rumen fermentation and animal performance.

With regards to rumen fermentation, in 29 comparisons from 15 trials in which SBM was replaced by high RUP supplements, essential amino acid supply increased only 20% of the time, with lysine only increased in 2 out of 26 comparisons and methionine in 1 of 26 comparisons. Although lysine as a percent of essential amino acids was similar between SBM and high RUP treatments, methionine as a percent of essential amino acids was depressed from 4.5% in the SBM diets to 4.0% in the high RUP diets. Similarly, when the author compared effects on microbial protein production, the high RUP diets significantly decreased microbial protein production in 76% of the treatment comparisons and numerically decreased microbial protein 93% of the time.

With regards to performance, in 127 comparisons from 88 lactation trials, milk yield was higher for the high RUP diets in only 17% of the comparisons and milk protein was increased in 5% of the comparisons while being decreased in 22%. When comparing with only soy based RUP supplements, milk yield increased in 6 of 29 comparisons, but milk protein decreased in 8 of 29 comparisons.

So why was there a lack of consistent response to RUP in these studies? Certainly it should be acknowledged that this research was conducted prior to the 2001 NRC and much of it before computer models like CPM Dairy and the CNCPS were available. In many of the experiments, key RUP quality factors such as amino acid profile and digestibility were not considered. In addition, the cows used in some of the studies were not high in production, where the need to maximize microbial protein production is greatest. To this point, high quality RUP sources such as fish meal did stimulate milk yield, particularly in high producing cows. However, equally important is that in many of these experiments, either RUP was not limiting, or worse, when RUP was increased, it

came at the expense of RDP, either creating or exacerbating a deficiency in RDP in the diets. The deficiency of RDP has two impacts: first, there is a decrease in microbial yield and with it a decrease in quality protein and second, with the decrease in microbial growth comes a decrease in carbohydrate digestion, particularly fiber resulting in a reduction in ME supply and a potential decrease in intake. As stated by the authors, "The adequacy of RUP and RDP in diets should be considered independently, and it is illogical to increase RUP at the expense of RDP unless RDP is excessive."

HOW MUCH RDP SHOULD WE FEED?

The question then becomes, how much RDP should we feed? There is considerable research in this area to draw upon. Hoover and Stokes (1991) reported a linear relationship between bacterial N production and DIP (RDP) as a % of DM up to 19% of DM (Figure 1). Although this is clearly beyond where we would typically feed, it does point to the potential of rumen bacteria to respond to additional RDP. Stokes et al. (1991) found a similar linear relationship between level of RDP and NDF digestion. So clearly, from a rumen fermentation standpoint, more is RDP better.

From an animal performance standpoint, there are two relevant issues, what level of RDP maximizes productivity and what level of RDP is utilized effectively and efficiently by the bacteria and the animal. The 2001 NRC examined the relationship between RDP as a percent of DM and milk yield, and found a quadratic relationship, with milk yield maximized when RDP equaled 12.2% of DM. Additionally, this data set revealed a positive correlation between RDP and dry matter intake (a two percentage unit increase in RDP increased DMI by about 1.1 kg/d). In agreement with the NRC data set, previous reviews (e.g. Hoover and Miller, 1996) have suggested that a practical target for lactating dairy cow diets should be 11 to 12% of the DM.

Can this level of RDP be used efficiently in lactating dairy cow diets? Certainly much of this answer is going to be carbohydrate driven. Still, the fear of driving such items as milk urea nitrogen (MUN) to dangerously high levels is likely unfounded when 11 to 12% RDP diets are fed. In an example, a recent experiment (Ferguson et al., 2004) fed up to 11.8 % RDP diets to lactating cows, in which urea was fed at 0.4 lb per cow per day in the 11.8% RDP diet. Non-fiber carbohydrate levels were 40% for this treatment, while MUN was at 13.2 mg/dl, a very acceptable level. Similarly, a review by Hoover and Miller, 1991 summarized data from eight experiments and found that blood urea N concentrations were approximately 10 mg/dl or lower for diets containing 11 to 13% RDP as a percent of DM when non-structural carbohydrate (NSC) levels exceeded 35%. However, when NSC levels were less than 35%, BUN did elevate in response to increasing RDP.

TYPE OF RDP TO FEED

Different bacterial populations have different preferences or requirements for the form of N they receive. Structural carbohydrate fermenting bacteria have an absolute requirement for ammonia-N whereas NFC fermenting bacteria can use either ammonia

or amino-N in the form of peptides and amino acids as their source of N (Russell et al., 1992). The NFC fermenting bacteria derive 2/3 of their N from amino-N and 1/3 from ammonia N (Russell et al., 1983). Because of these differences, a blend of RDP sources can maximize microbial protein production and rumen carbohydrate digestibility.

We tend to think of sources of RDP like SBM as a gold standard for RDP, because it provides peptides and amino acids to the rumen bacteria, plus these amino-N sources can be deaminated to create ruminal ammonia. While SBM or other similar protein supplements are excellent RDP sources, it appears that there is a legitimate benefit to balancing a RDP source like SBM with a NPN source.

Jones et al. (1998) compared a baseline diet containing 17.8% crude protein (CP) in which soy peptides were used to replace urea at incremental levels, in continuous culture fermenters (Table 1). Microbial protein production and digestion of DM and CP responded quadratically to peptide addition, indicating that adding soy peptides was beneficial to a point. Fiber digestibility however decreased in a linear fashion. Even though the diets were isonitrogenous, ammonia levels were not maintained by the peptide addition. The authors suggested that high levels of peptides resulted in a negative feedback on proteolysis, which resulted in depressed protein digestion and subsequent ammonia production, at least in high NSC diets.

Table 1. Effect of added soy peptides on ruminal parameters.¹

Item	Peptide added as soy peptide, % of total N			
	0	10	20	30
NH ₃ N, mg/100ml	8.38	7.08	5.14	3.94
Mic N, g/day	1.18	1.37	1.32	1.24
Mc N, G/kg DOM	25.0	26.8	26.6	25.8
VFA/MN, mmol/g	305.1	256.9	254.5	283.1
DOM, %	64.3	67.9	65.3	63.3
ADFD, %	39.6	39.1	36.3	32.1
NDFd, %	49.1	49.6	45.5	42.2

¹Jones et al, 1998

Similarly, Griswold et al. (2003) tested diets containing 8 or 11% RDP, with or without continuous infusion of urea, in culture (Table 2). Diets contained similar amounts of total CP; the high RDP diets substituted SBM for a combination of fish and blood meal. Digestibility of ADF, NDF, hemicellulose and starch and sugar increased in response to increasing RDP, while urea significantly increased digestibility of all parameters, although for hemicellulose, NSC and NDF the response was primarily limited to the low RDP diets. This suggested however, that with regards to ADF digestibility, NPN rather than simply RDP drives digestibility improvements. In addition, both RDP and urea increased microbial protein production, the efficiency of N capture in the rumen and the efficiency of microbial protein production. Collectively, these data suggest that both level of RDP and source of RDP are important considerations in diet formulation.

Table 2. Effect of rumen degraded protein and infused urea on microbial yield and fiber & starch & sugar digestion.¹

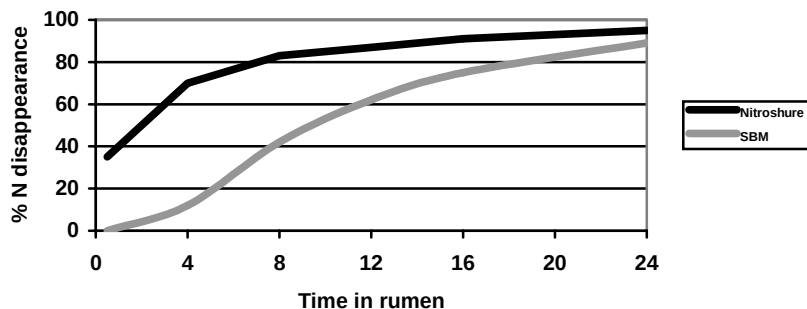
Variables	RDP, 8%DM		RDP, 11%DM	
	No Urea	Plus Urea	No Urea	Plus Urea
NH ₃ , mg/100ml	0.26	6.26	0.21	6.11
Microbial N, g/day	1.10	1.92	1.54	2.31
Microbial N, g/kg CHO dig	24.1	35.5	31.3	44.3
ADFD, %	38.7	46.0	41.1	46.8
NDFd, %	47.5	52.7	53.2	52.5
Hemicellulose Digestion, %	55.8	61.6	71.6	60.2
Starch & Sugar Digestion, %	73.6	92.5	83.4	93.0

¹Adapted from Griswold et al, 2003

ROLE FOR ENCAPSULATED UREA

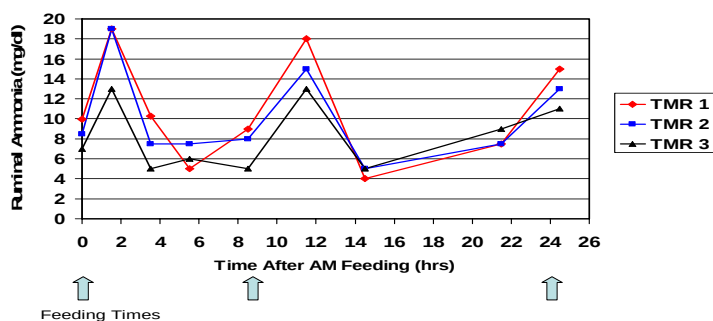
One key driver to the question on source of RDP is the need to maintain a minimum concentration of rumen ammonia at any given time to avoid limiting fiber and total carbohydrate digestibility and microbial growth. It has been widely referenced that 5 mg/100 ml rumen fluid is adequate to maintain normal microbial growth and carbohydrate digestibility. This is likely in fact too low, and that the real target is closer to a minimum of 8 to 10 mg/100 ml. Urea effectively increases ruminal ammonia, yet its effect is rapid and transient. True protein like soybean meal gradually supplies N to the rumen, yet it requires protein digestion and deamination to create rumen ammonia. Encapsulated urea offers an opportunity to fill the gap between urea and true protein in the RDP pool. Encapsulated urea is designed to make urea gradually available to the rumen microbes in order to generate an ammonia concentration curve with a shape more similar to that produced by SBM than to the curve produced by urea. This can be achieved, as shown in Figure 2. The benefit of this is two-fold. One is it directly provides NPN in balance to the RDP from true protein sources during the time post-feeding when the ammonia production from most NPN sources has already peaked and has fallen below 8 to 10 mg/100 ml. It is difficult to generate adequate ammonia during this period by feeding more SBM, as degraded protein must pass through the peptide pool to produce ammonia, and when SBM is fed in excess, the rate of peptide production is slowed due to the negative feedback mechanism. Encapsulated urea can create ammonia without the need to break down an excessive amount of true protein completely to ammonia and without creating an excess in the rumen peptide pool; this should increase the efficiency of N utilization. There is the additional problem that it is assumed that all of the peptide N will go through the NH₃ pool. Research by Jones et al, 1998 demonstrates, in fact that the bacteria might use the peptide directly and little may go through the NH₃ pool. This results in rumen NH₃ being, at times, below an optimum level to provide for adequate digestion of fiber. Secondly, the N density of encapsulated urea (255% CP) allows for the substitution of perhaps up to 2 lb of DM from a true protein source on an equal N basis, creating an opportunity to feed additional complementary carbohydrate in order to achieve an improvement in milk and or milk components on farm.

Figure 2. N disappearance from encapsulated urea versus soybean meal.



Frequently is it asked whether in a well managed, TMR situation, whether the combination of good management, good TMR and urea cycling can practically eliminate time periods of low rumen ammonia and overall daily ammonia fluctuations. Data from Lykos et al., 1997 give us insight into the answer to this question. In this work they examined the impact of the rate of rumen NSC degradability in diets containing 11.5 to 11.8% RDP, a level of RDP that most would agree is adequate for good rumen ammonia availability. Cows were housed in tie-stalls and fed a well balanced TMR, 2x per day. Feed was offered to a rate of 15% refusals, and pushed up 6x per day. So, a near perfect situation in order to optimize rumen ammonia concentrations throughout the day to minimize variation in concentrations and to prevent periods where ammonia would become too low for good rumen function. Plus, since this work was conducted in cows, urea cycling would be present to further balance out rumen ammonia concentrations. What was found, however, was that regardless of diet, 1) considerable diurnal variation still existed, and 2) there were several hours in the day that ammonia concentrations were at or below 8 mg/dl so that microbial growth and function may have been limited (Figure 3). This data shows that even in well managed situations with plenty of good quality RDP, that balancing RDP sources still offers an opportunity to drive further improvement in rumen function.

Figure 3. Diurnal ammonia variation in TMR fed cows.¹



Lykos et al., 1997

Recent research (Garrett et al., 2005) has shown that encapsulated urea can increase the efficiency and effectiveness of RDP use when balanced with other effective RDP sources (Table 3). In this experiment, encapsulated urea (Nitroshure™, Balchem Encapsulates, New Hampton, NY) was used on an isonitrogenous basis to substitute for urea, or in combination with corn and molasses, to substitute for SBM in a continuous culture experiment.

When used as simply a replacement for urea, it appears that the provision of urea from the encapsulated urea was too slow to support maximum nutrient digestion. Still, the efficiency of microbial protein production did significantly improve (14%). With regards to most parameters, the best treatment was one using encapsulated urea up to 0.68% of DM (0.34 lb/cow per day for a cow consuming 50 lb. of DM) plus additional corn (2.56% of DM; 1.28 lb on 50 lb DMI basis) and molasses (0.45%; 0.23 lb. on 50 lb. DMI basis), in replacement of 3.65% of DM from SBM (1.83 lb. on 50 lb. DMI basis). This diet still contained both urea and SBM, yet used encapsulated urea to “balance” out the gap between those two RDP sources. This treatment maintained a similar total protein supply past rumen fermentation, yet shifted the composition of this from 75.7% microbial protein to 83.3% with Nitroshure (10% improvement). This combination also maintained a rumen NH₃ N level above what has been recommended (Figure 4) as the minimal level of 5 mg/100 ml and it suggested that the more appropriate level needs to be over 10 mg/100 ml. One of the most sensitive measurements is the TVFA/kg Mic N. This measurement can be made in continuous culture because the total volatile fatty acids that the bacteria produce can be measured. We talk about this ratio in terms of coupled and uncoupled fermentation. Above you will notice that the bacteria produced 189 mmoles of VFA/kg Mic N on the 0 Nitroshure ration. When you compare this to the ration where Nitroshure was 0.68% DM and urea was 0.55% DM there was an 11% improvement in coupled fermentation. This means that the RDP mix was more balanced with the fermentation requirement and more nutrients was going into making more microbial mass and less into waste products – VFA.

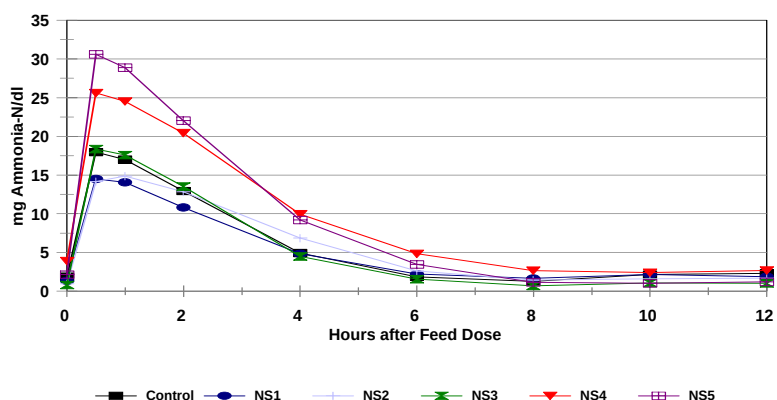
Table 3. Effects of Nitroshure on ruminal fermentation parameters.¹

ITEM	Combinations of SBM (S), Nitroshure (N), and urea (u), %DM					
	S: 12.3 N: 0 U: .65	S: 12.3 N: .46 U: .18	S: 12.3 N: .65 U: 0	S: 10.8 N: .28 U: .55	S: 8.65 N: .68 U: .55	S: 6.38 N: 1.12 U: .55
Ammonia N, mg/dl	6.13 ^{a,b}	5.30 ^b	5.32 ^b	4.66 ^b	7.58 ^{a,b}	9.23 ^a
Microbial N, g/d	2.18	2.08	2.33	2.23	2.39	2.37
Mic. N/kg CHOD ³	46.9 ^b	45.3 ^b	55.0 ^a	50.6 ^{a,b}	47.2 ^b	49.3 ^{a,b}
TVFA/kg Mic N ⁶	189 ^{a,b}	194 ^a	172 ^{a,b}	174 ^{a,b}	169 ^{a,b}	162 ^b
NDF	53.7 ^{a,b}	54.6 ^{a,b}	49.4 ^b	48.9 ^b	59.4 ^a	53.0 ^{a,b}
ADF	52.4	51.4	51.2	48.8	55.3	54.8
Total Carbohydrate ² , g/d	46.6 ^{a,b,c}	45.9 ^{a,b,c}	42.6 ^c	44.3 ^{b,c}	50.7 ^a	48.0 ^{a,b}

^{a,b,c} Values with different superscripts differ, P<.05

¹Garrett et al, 2005

Figure 4. Effect of encapsulated urea on rumen ammonia concentration.¹



¹Garrett et al, 2005

PUTTING RDP NUTRITION INTO PRACTICE

If we agree initially, that we need an RDP of a minimum of 11% DM then the question becomes one of how do we best partition the RDP into providing peptides and NH_3 ? The challenge starts with the carbohydrate fraction pool sizes and then the rates of fermentation of the pools. With this defined, it is then the challenge of providing the right rumen degradable pools of protein.

In CPM there are A, B1, B2 and B3 fractions. The A and B1 fractions are a division of the soluble protein fraction. This is the protein or N that goes into solution in rumen fluid within one hour. We measure the B1 by precipitating the protein in the soluble protein fraction, historically, using a 10% trichloroacetic acid (TCA) and now more recently tungstic acid (TA). TA precipitates out the true protein and peptides that have a peptide length greater than 3 amino acids in length. This results in an A fraction that has a few small peptides, AA, NH_3 , NO_3 , and other nitrogenous compounds. This fraction breaks down in the rumen very rapidly. The B1 fraction supplies a significant amount of peptide and the B2 fraction also supplies a significant amount of peptide, depending on its rate of degradation. The B3 pool provides little peptide.

Table 4 is a screen from CPM with the protein pools from the study done at West Virginia as discussed above (Table 3). This was an 18 % ration with a significant part of the ration protein in the A fraction. Note that 26% of the A comes from the forages. This will become rapidly available when consumed. It is hard to change this – we need the effective fiber. You will also note that the hay has 50% of the soluble protein in the B1 fraction and the ensiled forages 0%. These are book values and not measured. In fact we should have the capability for measurement of the B1 in the near future and as we move ahead this could become more important. The reason for this is because this will help define the peptides that provide the AA required by the starch and sugar bacteria as well as the protein digesting bacteria. Also the isoacids needed by the fiber bacteria are met through the microbial metabolism of the peptides and AA. We

discussed earlier Russell's microbial model. This suggests that most of the N requirement of the microbial mass in the rumen needs to be met with NH₃ (2/3 of the N for the NFC bacteria and 100% for the fiber bacteria). Note that B2 is the next biggest pool. Most of this B2 pool comes from soy. About 50 to 60 % of this protein is degraded in the rumen and goes through the peptide pool. This provides the peptides required but also provides a slow release form of NH₃.

This form of NH₃ is on the one hand a slow release and that is positive in terms of carbohydrate fermentation but on the other hand is energetically an expensive means of meeting the NH₃ requirement. Going back to the A fraction it will be noted that Nitroshure supplies 30% of the A fraction. If this were all straight urea, we would have potential of high inefficiencies and high MUN. The fact is the urea is supplying NH₃ in a slow release manner and is replacing some of the NH₃ that we get from SBM. It also should be noted that of the total N coming from urea, about 47% is coming from straight urea and the remaining from Nitroshure. If we do not have a product like Nitroshure, we need a protein with the degradation characteristics such as from soy that provides a continuous supply of NH₃ for the starch and fiber fermentation, recognizing that this is not efficient. Urea will not work to meet this requirement solely.

This, then brings us to formulation. If we are using Nitroshure, it is suggested in CPM that we balance to 101 to 108% of the peptide requirement and then balance the NH₃ requirement to meet the RDP requirement of minimum of 11% DM (the ration above was balanced to 106% of the peptide requirement). This latter usually comes out to be around 122 to 130% of requirement. If there are higher amounts of added sugar in the ration, it might be wise to replace some of the Nitroshure with urea. Rapidly fermented starch and fiber might also influence this.

Table 4. Protein Pools – CPM Dairy

Degradation Rates Ingredient	Protein Pools - Amount (lb/d)					
	----> Total	Fast A	Fast B1	Med B2	Slow B3	Unavail C
Alfalfa Hay	1.18	0.20	0.20	0.56	0.18	0.05
Mixed Haylage	0.16	0.07	0.00	0.03	0.01	0.04
CrnSilUp30Dm49NdfMed	1.05	0.57	0.00	0.29	0.17	0.02
SoybeanHullsPellet	0.40	0.06	0.02	0.18	0.10	0.04
BarleyGrnFlkd	0.23	0.05	0.01	0.14	0.01	0.01
CornGrainGrndFine	1.43	0.22	0.05	1.01	0.07	0.07
MolassesCane	0.07	0.07	0.00	0.00	0.00	0.00
CornGlutMeal60%	0.36	0.00	0.02	0.31	0.02	0.01
SoybeanML47.5Solv	2.57	0.28	0.24	1.98	0.03	0.05
CottonseedWhlwLint	0.52	0.00	0.13	0.33	0.01	0.04
Megalac	0.00	0.00	0.00	0.00	0.00	0.00
Nitroshure	0.94	0.93	0.00	0.01	0.00	0.00
Urea281CP	0.84	0.84	0.00	0.00	0.00	0.00
Min Vit	0.00	0.00	0.00	0.00	0.00	0.00
Ration	9.75	3.28	0.68	4.84	0.60	0.35

If using a linear program rather than CPM, we can still do an excellent job with RDP based ration balancing. First, maintain at least 3 lb/cow per day of SBM or a similar protein source like canola to provide a base of peptides for the rumen bacteria. Set your RDP target at somewhere between 11 to 12% of DM. Set your target NFC to be 3.5 times your RDP target, provided that you have enough effective fiber in the diet and are not overfeeding fat, particularly unsaturated fat sources. Set your minimum sugar target at 5%. Initially, use urea up to .15 lb/cow per day and Nitroshure at .25 lb/cow per day. Formulate the remainder of the diet and evaluate how RDP, NFC and other key specifications of the diet are being met. Adjust Nitroshure upward or downwards depending upon the adequacy of the diet relative to targets. Table 5 illustrates these basic recommendations.

Table 5. Recommendations for Rumen Degradable Protein balancing

Item	% DM	Lbs per day (based on 50 lbs DMI)
RDP	11 to 12	5.5 to 6.0
Rapidly degraded N from feeds	2.5 to 3.5	0.14 to 0.21
Urea	0.2 to 0.55	0.1 to 0.27
Nitroshure	0.3 to 0.8	0.2 to 0.4

CONCLUSIONS

Balancing for the amount of RDP in the rations has now been shown, through many controlled studies to be very important. The research now is showing that the blend of the N sources that are in the RDP is also important. We do need further research to fine tune these recommendations for the mixture of rumen fermentable carbohydrates, however, it would appear that if 15 to 17% of the Urea N be in the form of urea and the rest in the form of Nitroshure and that this combination be used to meet the RDP of 11 to 12% DM, we should see a improvement in animal performance.

REFERENCES

- Ferguson, S. E., R. S. Ordway, N. L. Whitehouse, P. J. Kononoff and C. G. Schwab. 2004. Urea supplementation increased rumen function in lactating dairy cows fed a corn silage based diet deficient in rumen-degradable feed protein (RDP). J. Dairy Sci. 87:(Suppl. 1)127.
- Garrett, J. E., T. K. Miller-Webster, W. H. Hoover, C. J. Sniffen and D. E. Putnam. 2005. Addition of encapsulated slow release urea to lactating dairy cow diets impacts microbial efficiency and metabolism in continuous culture. J. Dairy Sci. 88:(Suppl. 1)344.
- Griswold, K. E., G. A. Apgar, J. Bouton and J. L. Firkins. 2003. Effect of urea infusion and rumen-degradable protein concentration on microbial growth, digestibility and fermentation in continuous culture. J. Anim. Sci. 81:329-336.
- Hoover, W. H. 1987. Potential for Managing Rumen Fermentation. Proceedings Cornell Nutrition Conference for Feed Manufacturers. 53-60.

- Hoover, W. H., and T. K. Miller. 1991. Carbohydrate-protein considerations in ration formulation. *In* Proceedings Large Dairy Herd Management Conference. Cornell Cooperative Extension.
- Hoover W. H. and S. R. Stokes. 1991. Balancing Carbohydrates and Proteins for Optimum Rumen Microbial Yield. *J. Dairy Sci.* 74: 3630-3644.
- Jones, D. F., W. H. Hoover and T. K. Miller-Webster. 1998. Effect of concentrations of peptides on microbial metabolism in continuous culture. *J. Anim. Sci.* 76:611-616.
- Lykos, T., G. A. Varga and D. Casper. 1997. Varying degradation rates of total non-structural carbohydrates: Effects on ruminal fermentation, blood metabolites and milk production and composition in high producing Holstein cows. *J. Dairy Sci.* 80:3341-3355.
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 75:3551-3561.
- Russell, J. B., C. J. Sniffen and P. J. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed ruminal bacteria. *J. Dairy Sci.* 66:783.
- Santos, F.A.P., J.A.P. Santos, C. B. Theurer and J. T. Huber. 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12 year literature review. *J. Dairy Sci.* 81:3182-3213.
- Stokes, S. R., W. H. Hoover, T. K. Miller and R. P. Manski. 1991. Impact of carbohydrate and protein levels on bacterial metabolism in continuous culture. *J. Dairy Sci.* 74:860-870.