Encapsulating Nutrients to Improve Reproduction and Nitrogen Utilization in Ruminants

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Introduction

Encapsulation involves surrounding food ingredients, nutrients, enzymes, bacteria or drugs with a small capsule. You can encapsulate solids, liquids or gases. It is the process of applying coatings to nutrients or substances to control their interaction with a specific environment. It creates a barrier between the active ingredient and the environment. This barrier is not intended to last indefinitely. It is designed to protect the active ingredient and then release it. Coating thickness and composition can control the rate and time of release. This makes it possible to deliver a nutrient to a specific site in the gastrointestinal tract. The food industry uses encapsulation technology to mask odors and taste, prevent oxidation and prevent enzymatic and microbial degradation of nutrients. The pharmaceutical industry uses encapsulation to deliver drugs to specific sites in the gastrointestinal tract. In the last 6 years, encapsulation technology has begun to be used in the animal feed industry. The present paper will focus on the use of microencapsulation for vitamins, urea and polyunsaturated fatty acids. Use of encapsulated ingredients in animal nutrition will be reviewed with emphasis on animal performance.

Why Encapsulate Nutrients in Livestock Feeds?

Encapsulation is an expensive process. It will increase the cost of the nutrient to be encapsulated. The reasons to consider encapsulation are:

1. **Guaranteed content** – protect sensitive nutrients from loss of activity due to feed processing or feed storage conditions. Nutrients that would benefit from encapsulation are ascorbic acid, folic acid, choline chloride and carotenoids
2. **Targeted enteric delivery** – Protect sensitive compounds from degradation in the rumen or the abomasum and deliver them to the small intestine. Targeted delivery via encapsulation would benefit choline chloride, niacin, lysine, methionine, CLA, probiotic bacteria, and omega-3 fatty acids
3. **Prevent nutrient interactions in the feed during storage** – keep reactive compounds from causing oxidation. Encapsulation would prevent oxidation in feed caused by ferrous sulfate, potassium chloride, choline chloride, and citric acid

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4. **Controlled-release in the rumen** – Urea and organic acids
5. **Taste-masking** – Encapsulation of anionic salts, or fish oil
6. **Performance-** Observe enhanced animal performance when the encapsulated nutrient is fed compared with feeding a raw form of the nutrient. Encapsulation results in greater animal performance for nutrients such as choline chloride, niacin, ascorbic acid, and probiotic bacteria

Any of these reasons to encapsulate can justify the additional cost of encapsulation. When dealing with livestock producers, the most important reasons to encapsulate are performance, targeted enteric delivery and prevention of nutrient interactions in the manufactured feed.

Encapsulated Vitamins

The most common reason to encapsulate vitamins is to extend their shelf life. This occurs because encapsulation prevents oxidation and prevents the vitamin from reacting with compounds in the feed. It also protects the vitamin from damage caused by heat and steam in the pelleting or extrusion process. The vitamins most susceptible to loss of activity during feed manufacturing are ascorbic acid (Vitamin C), folic acid and pyridoxine (Marchetti et al., 1999). A second reason to encapsulate vitamins is to prevent their degradation in the rumen by ruminal bacteria. Vitamins that are extensively metabolized in the rumen include choline, niacin, folic acid and riboflavin (Santschi et. al., 2005). Most research with encapsulated vitamins has focused on choline, and vitamin C. Vitamin C is sensitive to heat, moisture and light. During feed manufacturing and feed storage, the crystalline form of vitamin C undergoes extensive oxidation. The loss of vitamin C activity is 70 to 80% of the original activity. It is also extensively degraded in the rumen by ruminal bacteria. For these reasons, crystalline vitamin C is not supplemented to ruminant animals. Recent work with encapsulated vitamin C suggests that dairy and beef calves will respond to vitamin C supplementation (Cusack et al., 2005, Garrett et al., 2005a). When dairy calves received supplemental encapsulated vitamin C in their calf grain, average daily gain was increased 11%, calf starter intake was increased 9.3% and hip width increased by 8% compared to control calves that received the same starter grain but without additional vitamin C (Garrett et al., 2005a). Calf average daily gain was 1.16 lb/day for control calves and 1.3 lb/day for calves receiving supplemental encapsulated vitamin C ($P<0.09$). During the 56-day trial, encapsulated vitamin C intake was 1.09 grams per day for calves receiving 1000-ppm vitamin C in their starter grain. Additional work needs to be done in this area because when calves were supplemented with 1.25 – 2.5 grams per day of vitamin C, respiratory disease was reduced and infectious disease resistance was increased (Itze, 1984).

Numerous dairy cattle trials with encapsulated choline have been published and are summarized in Table 1. Feeding encapsulated choline has a positive effect on reproduction, liver function and milk yield (Oelrichs et al., 2004; Piepenbrink and Overton, 2003; Pinotti et al., 2002). Choline plays a major role in lipid transport and this can explain the effect of choline on liver function. Choline is necessary for the transport of fat from the liver as lipoproteins (Pinotti et al., 2002). Choline appears to have a

Table 1. Impact of Encapsulated Choline on Milk Yield, Reproduction and Liver Function in Dairy Cattle

Stage of Lactation	Choline Chloride Fed g/day	Effects on Milk Yield and Milk Components	Effects on Liver Function	Effects on Reproduction	Reference
Dry period 45 – 60 days	15		Decreased NEFA and Liver Tri-glyceride		Grummer and Cooke (2005)
21 d prepartum	15	No Effect		Increased Conception Rate and Pregnancy Rate	Balchem Technical Research Report 2005:2
21 d prepartum to 70 DIM	15	Trend for greater milk yield	Decreased plasma NEFA and BHBA at parturition	Increased Conception Rate and Pregnancy Rate	Oelrichs et al. (2004)
21 d prepartum to 21 DIM	15	Trend for greater milk yield		Increased 1st service Conception Rate	Balchem Technical Research Report 2004:3
21 d prepartum to 63 DIM	11, 15 or 19	Increased 3.5% FCM Increased fat yield	Increased liver fatty acid metabolism and increased liver glycogen		Piepenbrink and Overton (2003)
14 d prepartum to 30 DIM	20	Increased milk and 3.5% FCM	Decreased NEFA at parturition		Pinotti et al. (2002)
28 d prepartum to 120 DIM	6 or 12	Increased milk through 56 DIM			Hartwell et al. (2000)
Early to Mid Lactation	10	Increased milk and milk fat percent Increased milk fat yield	Decreased plasma NEFA Increased plasma glucose and methionine		Bonomi et al. (1996)
20d prepartum to 100 DIM	5 to 45	No Effect			DiCostanzo and Spain (1995)
Early Lactation	33	Increased milk, 4% FCM and milk fat			Erdman (1994)
Mid-Lactation	20 to 58	Increased milk Increased milk protein			Erdman and Sharma (1991)

significant impact on reproduction (Oelrichs et al., 2004). This may be due to improved membrane integrity as choline is converted to phosphatidylcholine or sphingomyelin (Pinotti et al., 2002).

Feeding encapsulated choline does increase milk yield (Table 1). This is likely caused by improved liver metabolism due to less fat accumulation in the liver. This should lead to greater glucose production by the liver. Another possible explanation may be increased fat absorption from the small intestine. Phosphatidylcholine makes up 80% of the total lipid in ruminant bile (Moore and Christie, 1981). A research trial needs to be carried out to determine the effect of choline supplementation on fat absorption in dairy cattle.

Encapsulated Urea and the Optimal Rumen Fermentation

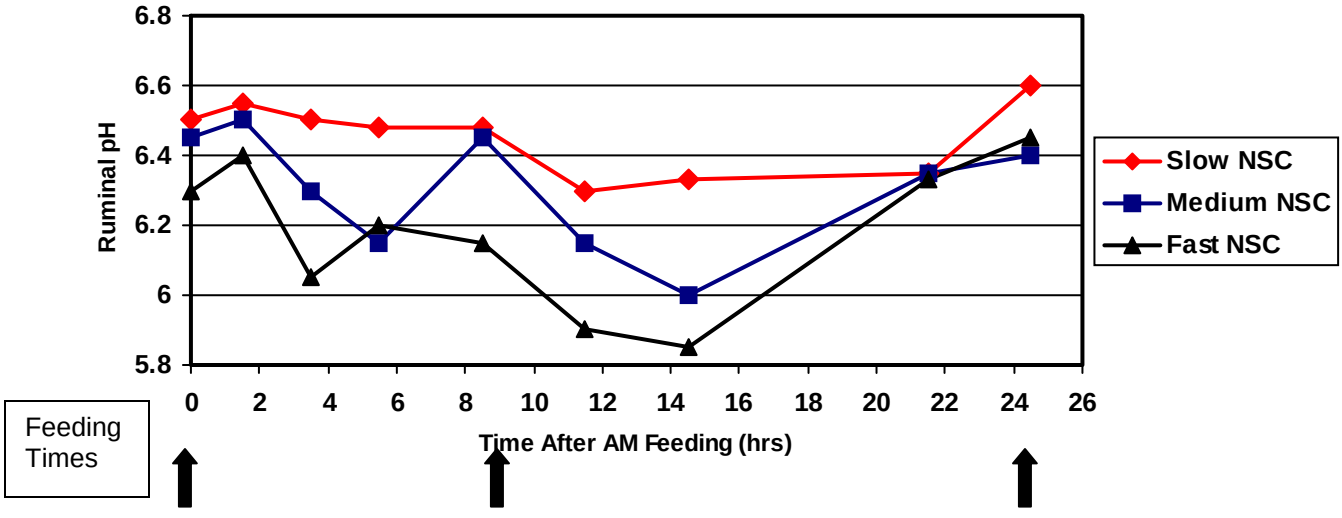
To achieve optimal rumen fermentation, we must recognize that the rumen is not a steady state fermentation vessel. It is really a batch fermentation vessel. Even when a TMR is fed to dairy cows, the variation in meal size, meal frequency and access to feed results in a batch type fermentation pattern. In a batch type fermentation there will be daily fluctuations in ruminal pH, ruminal ammonia concentration, VFA concentration, and microbial growth rates. (Lykos et al., 1997; Russell 2002). Lykos and coworkers (1997) attempted to minimize daily fluctuation in the rumen by feeding a TMR containing 55% forage, pushing up the feed 6 to 8 times per day, milking cows twice per day and feeding cows to achieve a 15% weigh back. These animals were fed and managed so that access to feed was not limited, and the time away from feed was minimized. The three diets fed in this trial had a minimum of 11.5% RDP as a % of DM and 33, 1% soluble protein as a % of crude protein (Table 2). These diets did not contain excess amounts of NSC. In fact the concentration of NSC in these diets was 32.1 – 33.7 % of DM (Table 2) which would be conservative compared with typical diets fed high producing cows. Based on the ingredient and chemical composition of these diets, ruminal acidosis would be unlikely and rumen ammonia should not limit microbial growth. These diets did differ in the rate of carbohydrate degradation and this was accomplished by replacing cracked corn with high moisture ear corn. Rumen pH was measured 9 times during a 24- hour cycle (Figure 1) and fluctuated with feeding time and the rate of carbohydrate degradability. When the TMR had a slow rate of carbohydrate fermentation, fluctuation in ruminal pH was 0.2 pH units during the 24-hour cycle. When the rate of carbohydrate fermentation was medium and fast, the fluctuation in ruminal pH was 0.4 units during the 24-hour cycle and did not represent steady-state conditions. Ruminal pH was lowest between 11.5 and 14 hours after the AM feeding. It is clear that the frequency of feeding influenced ruminal pH because the animals were fed twice per day and ruminal pH declined after each feeding. It took between 3.5 and 5.5 hours after feeding for ruminal pH to decline below 6.2.

Table 2. Ingredient and Chemical Composition of Dairy TMR

Ingredient, % of DM	Slow NSC Degradability	Medium NSC Degradability	Fast NSC Degradability
Alfalfa Silage	23.2	19.7	15.7
Corn Silage	27.1	30.9	35.9
Alfalfa Hay, chopped	4.1	4.1	4.2
Cracked Corn	24.6	12.1	0
High Moisture Corn, Gr.	0	10.7	20.5
Soybean Meal, 48% CP	3.2	3.8	3.9
Roasted Soybeans	14.3	14.9	16.2
Minerals. Vitamins, Buffer	3.6	3.9	3.6
Chemical Composition			
CP, % of DM	17.2	16.7	16.6
RDP, % of DM	11.5	11.5	11.75
Soluble CP, % of CP	33.1	35.4	33.2
NSC, % of DM	32.4	32.1	33.7
Net Energy, mcal/lb.	0.71	0.73	0.74
NSC Degradation Rate, %h	6.0	7.0	8.0
CP Degradation Rates, %h	5.6	5.5	5.4

Adapted from Lykos et al., J. Dairy Sci. 80:3341-3355

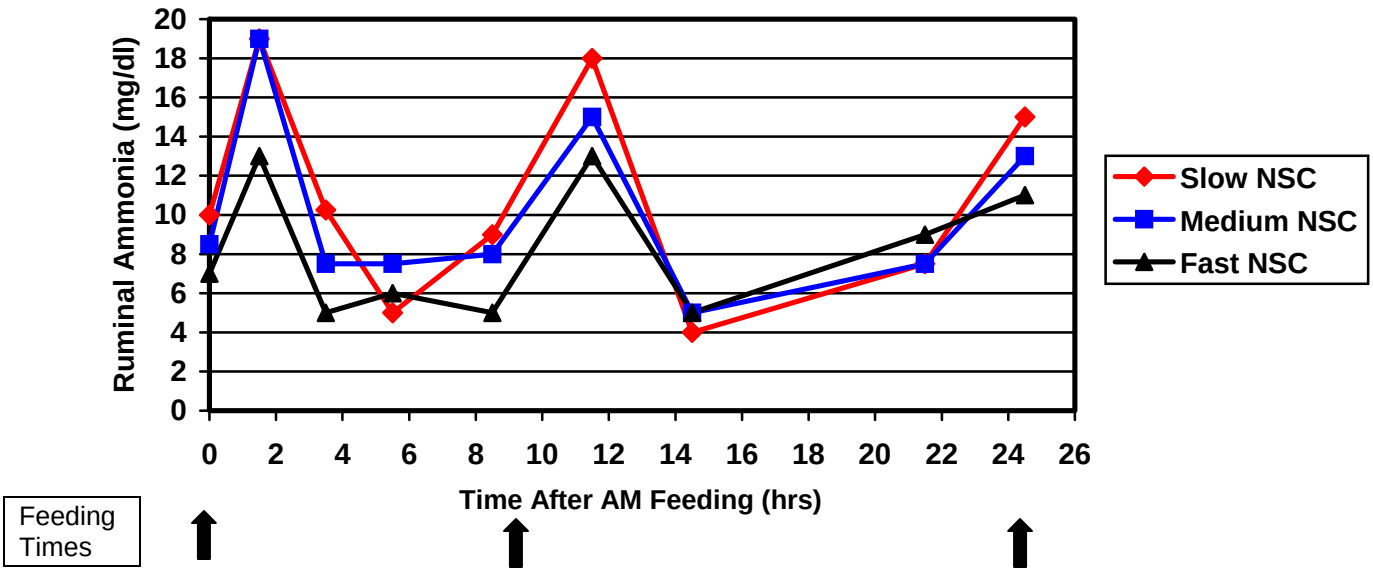
Figure 1. Ruminal pH when cows fed a TMR twice/day



Redrawn from Lykos et al., J. Dairy Sci. 80:341-3355

A similar pattern was observed in ruminal ammonia concentration (Figure 2). Ruminal ammonia concentration peaked approximately 1.5 hours after feeding but declined rapidly and reached its lowest concentration approximately 3.5 hours after feeding (Lykos et al. 1997). The ammonia concentration curves follow a batch type fermentation pattern. The protein sources in the diets were very degradable in the rumen, which explain the peaks in ammonia concentration occurring approximately 1.5 hours after feeding. (Lykos et al., 1997). The rapid decline in ruminal ammonia concentration would suggest either rapid microbial growth with incorporation of the ammonia nitrogen into microbial protein or rapid absorption of ammonia across the rumen wall. The flux in ruminal ammonia concentration indicates that a steady state does not exist in the rumen.

Figure 2. Ruminal Ammonia Concentration for Cows Fed a TMR



Redrawn from Lykos et al. J. Dairy Sci. 80:3341-3355

Minimum ruminal ammonia concentration was 4 – 5 mg/dl, while maximum concentration was 19 mg/dl. This is a swing in ruminal ammonia of 79 percent. With the large daily fluctuation in ruminal ammonia concentration, one has to ask the question, how can we minimize periods when we have a great excess of ruminal ammonia and still have enough ammonia for optimal microbial growth.

How Much Rumen Degradable Protein Should We Feed?

Hoover and Stokes (1991) reported a linear relationship between bacterial N production and DIP (RDP) as a % of DM up to 19% of DM. This is clearly beyond where we would typically feed, but it does point to the potential of rumen bacteria to respond to additional RDP. Stokes et al. (1991), observed a similar linear relationship between level of RDP and NDF digestion. So clearly, from a rumen fermentation standpoint, more RDP is 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 efficiency 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. 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.

The amount of RDP that can be used in a dairy cattle diet will depend on the amount of rumen fermentable carbohydrate in the diet. When the diet contains carbohydrates with a fast rate of fermentation, the need for RDP will be greater. In a recent experiment (Ferguson et al., 2004) fed diets containing up to 11.8 % RDP to lactating cows, in which urea was fed at 0.4 lb per cow per day. 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%. From the data reported by Lykos et al. (1997), even in diets with less than 35% NSC, RDP needs to be greater than 11% of diet DM.

For optimal rumen fermentation it is necessary to maintain a minimum concentration of rumen ammonia at any given time to avoid limiting fiber and total carbohydrate digestibility. It has been widely referenced that 5-mg/100 ml rumen fluid is adequate to maintain normal microbial growth and carbohydrate digestibility. This is in fact too low, and that the real target is closer to a minimum of 8-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. The benefit of this gradual NPN delivery is that it provides NPN in balance to the RDP from true protein sources during the time post-feeding when ruminal ammonia concentration decreases to below 8 to 10 mg/100 ml. Encapsulated urea can supply ammonia without the need to break down an excessive amount of true protein completely to ammonia. This should increase the efficiency of nitrogen utilization. There is the additional problem that it is assumed that all of the peptide nitrogen will go through the NH_3 pool. Research by Jones et al, (1998) demonstrates, in fact that the bacteria might use the peptide directly and little may go through the NH_3 pool. This results in rumen NH_3 being, at times, below an optimum level to provide for adequate digestion of fiber.

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 soybean meal 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 (0.68% of DM; 0.34 lb/cow on a 50 lb DMI basis), and soybean meal (8.65% of DM) plus additional corn (2.56% of DM) and molasses (0.45% of DM), 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 combination maintained a rumen NH_3 N level above what has been recommended as the minimal level of 5 mg/100ml. In this experiment, ADF, NDF and total carbohydrate fermented was maximized when ammonia concentration exceeded 5-mg/dl. This data would suggest that the more appropriate concentration for ruminal ammonia needs to be 8-mg/100 ml for optimal ruminal fermentation.

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

ITEM	Combinations of SBM (S), Nitroshure (N), and urea (U) % of Diet 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
Microbial. N/kg CHOD[2]	46.9[b]	45.3[b]	55.0[a]	50.6[a,b]	47.2[b]	49.3[a,b]
TVFA/kg Microbial N	189[a,b]	194[a]	172[a,b]	174[a,b]	169[a,b]	162[b]
NDF Digestibility, %	53.7[a,b]	54.6[a,b]	49.4[b]	48.9[b]	59.4[a]	53.0[a,b]
ADF Digestibility, %	52.4	51.4	51.2	48.8	55.3	54.8
Total Carbohydrate Fermented 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

[1]Garrett et al, 2005

[2]CHOD = carbohydrate digested

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 the dairy cow 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. 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 only 5% of the comparisons while being decreased in 22%, of the 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, when high quality RUP sources such as fishmeal were fed, milk yield was stimulated. 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. The decrease in carbohydrate digestion will result in a reduction in ME supply and a potential decrease in intake.

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? If using a linear ration formulation program rather than a modeling program like CPM, you can still do 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. These suggested targets are listed in Table 4.

Table 4. Recommendations for Balancing Dairy Diets for RDP When Using Encapsulated Urea

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 0.40	0.10 to 0.20
Nitroshure	0.3 to 0.8	0.20 to 0.40

Encapsulated Polyunsaturated Fatty Acids

Due to the health benefits of consuming a diet rich in omega-3 fatty acids, food manufacturers are looking for a way to fortify foods with these fatty acids (Schrooyen et al., 2001). Encapsulation of these fatty acids prevents oxidation, improves palatability and enables you to deliver them to the intestines for absorption. Microencapsulated fish oils or esters of polyunsaturated fatty acids are currently marketed in the U.S., Europe, Israel and Australia (Ackman, 2006). It may be possible to protect these fatty acids from hydrogenation in the rumen and deliver them to the intestines. Protecting fatty acids from hydrogenation in the rumen has already been accomplished through lipid encapsulation of conjugated linoleic acid (CLA). Encapsulation was used to get the CLA through the rumen without undergoing hydrogenation (Perfield et al. 2004). In this trial there were three treatments; control- no supplemental CLA; amide-protected CLA (AP-CLA) and lipid-encapsulated CLA (LE-CLA). When 10 grams per day of lipid-encapsulated *trans*-10, *cis* 12 CLA or amide-protected CLA was fed to dairy cows, milk fat percent and yield were depressed ($p<0.001$) compared to cows not receiving the CLA supplement (Table 5.). Dry matter intake and milk yield was not different between the treatments. Both the amide-protected CLA and lipid-encapsulated CLA were equally effective at depressing milk fat percent. This would imply that these technologies protected the CLA from hydrogenation by ruminal bacteria. The CLA content of milk fat was increased significantly ($p<0.001$) compared to the control group when cows were fed rumen-protected CLA supplements (Table 5). Research needs to be done to determine if these technologies can be used to protect omega-3 fatty acids from hydrogenation in the rumen.

Table 5. Performance of lactating cows receiving rumen-protected supplements of CLA

Variable	Control	AP-CLA	LE-CLA	SEM	Probability
DMI, kg/d	30.6	31.6	30.4	0.9	0.50
Milk, kg/d	40.5	42.6	42.7	3.5	0.32
Milk fat %	3.23[a]	2.37[b]	2.34[b]	0.15	<0.001
Milk fat yield, kg/d	1.27[a]	1.00[b]	0.99[b]	0.08	<0.001
Milk protein yield, kg/d	1.00[b]	1.06[a]	1.09[a]	0.02	<0.02
10, 12 CLA in milk fat, %	<0.01[b]	0.08[a]	0.09[a]	0.01	<0.001
9, 11 CLA in milk fat %	0.57[b]	0.83[a]	0.80[a]	0.05	<0.001

Source: Perfield et al., 2004, J. Dairy Sci. 87:3010-3016

Conclusion

Encapsulation of nutrients now makes it possible to deliver nutrients to specific sites in the intestinal tract. Encapsulation of vitamin C and choline make it possible to supplement these nutrients into the diet of ruminants. The supplementation of dairy cattle diets with encapsulated choline has improved liver function, reproduction and milk yield. By encapsulating vitamin C, it is now possible to examine the effect of vitamin C on calf health and performance. The encapsulation of urea, may improve the utilization of nitrogen in ruminant diets by optimizing ruminal fermentation. It is possible to

encapsulate CLA and omega-3 fatty acids. Use of encapsulation may make it possible to produce milk with enhanced amounts of CLA or Omega-3 fatty acids in the milk fat. Encapsulation will be used to improve the efficiency of reproduction and nitrogen utilization in ruminants.

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