

MICROENCAPSULATION: OPPORTUNITIES TO IMPROVE ANIMAL PRODUCTS AND NUTRITION

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Microencapsulation is a technology that has increasing application in animal nutrition. This technology affords the protection of sensitive compounds through feed processing and storage conditions, yet maintaining high bioavailability and impact on the animal and tastemasking of unpalatable compounds. Time-release throughout the digestive tract gives the ability to allow for nutrients to be released when they can be utilized best. It also can deliver functional ingredients that can improve formulation and safety of certain finished products. Compounds to consider for microencapsulation include certain vitamins, amino acids, fatty acids, organic acids and live microorganisms. Understanding the interface between substrate, coating technology and environment is the key to successful application of microencapsulation in animal nutrition.

Microencapsulation Background

Microencapsulation (encapsulation) refers to a process of applying coatings to substrates to control their interaction with a defined environment. ‘Micro’ means that the particles tend to be small; indeed, typical particle sizes for the raw material and the finished encapsulated ingredient are under 1000 microns. Although there are many different types of encapsulation, the highest quality encapsulates are produced when individual particles are coated with great specificity, creating layers of coating that behave uniformly in a desired application.

Ingredients are encapsulated to control their interaction with the environment, however, it is important to recognize that the protection provided by the coating is not intended to last infinitely. Rather, the goal is to protect an ingredient and subsequently release the raw ingredient to perform its intended

function. The mechanism of release from the coating material can be manipulated by using different types and levels of coatings, and can be triggered by temperature, moisture, pH, digestion and others, depending upon the manner in which the ingredient is coated. For example with Reashure, it is desirable to protect a nutrient (choline) during feed processing, and have the coating be designed to be completely digestible so that the nutrient is available for absorption in the small intestine, compared to Nitroshure (encapsulated urea) which has release characteristics in the rumen to provide ammonia to the microbial population in a measured manner.

All encapsulated products are not created equal. Technical differences in manufacturing similar products can be extreme, placing an emphasis on the need for strong quality control. However, the right products used in the right applications can bring innovation in the form of functional performance, improved nutrient stability, increased shelf life and delivery of bioavailable compounds to the livestock industry.

Why Encapsulate Nutrients?

There are many reasons to use an encapsulated nutrient in the feed industry. Different reasons present different levels of complexity and require different levels of validation to expect performance. Too often, we ignore these differences and assume that any encapsulated product will meet any desirable objective. Reasons for using encapsulated nutrients include:

Product differentiation. Including an encapsulated product as part of a finished feed to present a marketing advantage to that finished feed is simple. It requires no assurance of performance of the product and can be met by using any encapsulation method. The cheapest encapsulated ingredient would be the product of choice for this use in this situation.

Taste masking. Taste masking is relatively simple to achieve with encapsulation. The considerations are: 1) can the cost of encapsulation be justified?; and 2) is there space in formulation for the additional DM? The encapsulate of choice will likely be the cheapest one available, although poor encapsulation may not effectively taste-mask some nutrients that have very strong tastes or are hygroscopic. Furthermore, some encapsulates may not survive feed processing conditions and will subsequently provide little taste masking in certain finished products.

Guaranteed content. High-quality encapsulated ingredient will lead to marked improvements in post-processing retention of nutrients through processes such as pelleting or extrusion. Furthermore, encapsulation can be an excellent approach to maintain desired nutrient contents of finished feeds throughout prolonged storage conditions.

Feed processing conditions can be harsh, and designing an appropriate encapsulate to protect nutrients during these conditions is technically challenging. The best encapsulate for stability may not effectively deliver bioavailable nutrients to the animal. Validating the post-processing content of the targeted nutrient is needed to ensure product performance. The best encapsulate to use is the one with the lowest cost per unit of nutrient retained in the finished product and delivers bioavailable nutrients to the animal.

Targeted enteric delivery. Protecting sensitive compounds from bacterial degradation in the rumen, the low pH in the gastric stomach and enzymatic activity in the duodenum are all reasons for using an encapsulated ingredient in animal nutrition. To achieve targeted enteric delivery requires sophisticated engineering of the coating. It also requires the coating to survive the feeding conditions it is exposed to (e.g., meal mixes, pelleting and extrusion) and maintain its coating integrity to achieve the desired targeted delivery.

Clearly, this type of delivery needs to be validated, and as above, the best encapsulate to use is one that has been proven to have the lowest cost per unit of nutrient retained in the finished product and delivers the desired target enteric delivery.

Bioavailability. Ideally, targeting an encapsulated ingredient to a specific enteric location will positively affect bioavailability. However, there can be notable exceptions to this rule. As will be discussed, bioavailability measures can be extremely difficult to perform, and achieving improved bioavailability can be even more difficult to accomplish.

Independent research should accompany any encapsulated product designed to deliver bioavailable nutrients, but unfortunately many products are commercially available that lack this type of rigor. The best encapsulate to use is one that has been validated to have the most cost-effective bioavailability.

Performance. For production agriculture, performance is the response of choice that each product must be independently validated to deliver. Performance may be milk yield, health, growth, etc. Keys to look for in the performance data behind a product are its repeatability and whether it logically fits the mode of action of the product as described.

Characteristics Of Encapsulates

According to Wu and Papas (1997), the criteria for a rumen-stable delivery system are as follows. It should have efficacy in providing rumen protection and post-ruminal bioavailability. The components used in the coating must meet approved food/feed safety standards. Finally, the delivery system must be cost effective. Although those comments were made in reference to a rumen-stable delivery system, they also fit the criteria that a high-quality, rumen-stable nutrient product should meet.

High-quality is also necessary for controlled release of an encapsulate. Products that require a measured release in the rumen to provide a steady constant availability to the microbial population need the same scrutiny as a product that escapes exposure to the microbial population.

Rumen stability. There are multiple validated means of measuring rumen stability, such as *in vitro* and *in vivo* methods. Correctly applying these tests to measure the rumen stability of encapsulated nutrients has often been a problem. Mistakes in the measuring of rumen stability of an encapsulated nutrient lead to significant misinterpretation of the quality of the product and its subsequent net cost in use and value to the end user.

It is helpful to understand the simple logic that goes into designing a rumen-stable encapsulated nutrient. Typically, the nutrient of interest is highly degradable or susceptible to modification in the rumen. Encapsulation technology is used to coat the nutrient to protect or isolate the nutrient from the ruminal environment in order to prevent losses of or modification to the nutrient in the rumen.

This encapsulation methodology can consist of coating a core nutrient with layers of a protective coating or combining the nutrient within a matrix of a rumen-stable compound. Successful encapsulation depends upon the use of a coating material resistant to modification and degradation in the rumen while providing release in the lower digestive tract.

Simply doing rumen dry matter disappearance can often give misleading results when testing these products because doing so ignores the question of from what fraction (coating versus nutrient) the dry matter disappearance occurred. Table 1 shows an example of some preliminary research conducted with two commercially available encapsulated choline products. One product uses a layered

coating, is 29% choline chloride and approximately 90% stable in the rumen. The coating of this product is almost completely inert in the rumen; the choline core fraction has approximately 10% disappearance in the rumen.

Table 1. Ruminal dry matter disappearance of various components of rumen-stable encapsulated choline products

Item	Product A	Product B
Choline chloride, %	29	40
Rumen stability – dry matter		
30 minutes	>95	59
6 hours	>95	55
12 hours	>95	58
24 hours	>95	54
Rumen stability – choline		
30 minutes	92	0
6 hours	91	0
12 hours	90	0
24 hours	96	0

The second product is a commercially available product designed for rumen stability with a rumen dry matter stability of approximately 60%. This product contains 40% choline chloride and 60% coating and other materials. Upon closer examination, the losses of dry matter in the rumen came almost entirely from the choline fraction, resulting in no stability of choline in the rumen with this product. This product achieved 60% ruminal dry matter stability of the coating material yet has no net delivery of choline to the animal.

An experiment was initiated to better understand the relationship between ruminal nutrient stability and ruminal dry matter stability and the differences in quality between commercially available rumen-stable choline products (Kung et al., 2003). In this work, products evaluated were product A (25% choline; U.S. manufactured and globally distributed), product B (13% choline; U.S. manufactured and distributed), product C (40% choline; Italian manufactured and North American distributed), product D (40% choline, Italian manufactured and Asian distributed) and product E (25% choline; Canadian manufactured and North American distributed).

Products were obtained through commercial distributors and stored at ambient temperature. Dry matter and choline stability were determined at 0.5, 6, 12 and 24 hours of incubation using an Ankom Dairy II Incubator. Triplicate samples were used for each time-point, corn silage was used as a standard. Post-incubation, each sample was dried for 24 hours at 65°C, with residues weighed and analyzed for choline content using a choline oxidase-based detection system. Rumen stability was calculated by subtracting the recovered dry matter or choline from the amount of dry matter or choline added to the bag originally.

Results are detailed in Table 2. All products had reasonable dry matter stability (63-98% at the 12-hour time point). However, choline stability varied considerably, with only one product (A) having choline stability after 12 hours of incubation. In agreement with the preliminary research mentioned above, considerable differences exist between commercially available, rumen-stable choline products.

Furthermore, dry matter stability was found to not be an acceptable method for accessing the quality of rumen-stable nutrients, as it correlated very poorly with the overall rumen stability of the nutrient fraction of the encapsulated products.

Bioavailability. Achieving rumen stability is only part of the problem in producing an effective product. Providing release of the protected nutrient at the proper point in the lower digestive tract for optimum absorption is the next critical step. To paraphrase Deuschler et al. (1998): Even if a nutrient is protected by ruminal degradation, it still must be released and subsequently absorbed.

As a general rule of thumb, there is an inverse relationship between stability in the rumen and digestibility/release in the small intestine. It is feasible that by the time enough coating is added to a nutrient to stabilize it, digestibility in the small intestine will be so low that the key nutrient will pass completely through the lower digestive tract.

At a minimum, a qualitative measure -- and ideally, a quantitative measure -- of bioavailability should be known about an encapsulated product to ensure that it will deliver the nutrient and have a benefit to the end user.

Admittedly, bioavailability can be difficult to define and even more difficult to measure. Ruminant animals, and particularly dairy cows, pose an enormous challenge for bioavailability measures. These challenges are due to their large feed intakes, difficulties in measuring/modeling the impact of ruminal fermentation on nutrient flow to the small intestine and the multitude of variables that can affect the primary productive response, milk yield.

Table 2. Comparison of rumen dry matter and choline stability of commercially available rumen-stable choline products.

Time (h)	Product A		Product B		Product C		Product D		Product E	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Rumen dry matter stability, %										
0.5	99.8	1.0	57.5	1.1	63.7	0.4	65.5	0.7	79.3	1.4
6	98.9	1.2	88.6	0.7	63.1	0.6	63.1	0.5	73.4	1.0
12	98.4	0.9	88.6	0.8	63.0	0.3	63.1	0.1	71.6	0.2
24	97.4	0.8	85.7	3.3	62.0	1.6	62.4	0.3	71.3	0.8
Rumen choline stability, %										
0.5	82.3	3.7	3.0	1.8	0.4	0.4	7.2	3.6	21.9	1.5
6	77.8	3.3	1.3	1.6	0.0	0.0	0.0	0.0	3.5	1.2
12	75.6	1.1	0.8	1	0.0	0.0	0.0	0.0	1.6	0.1
24	70.4	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Kung et al., 2003

Blood can be an attractive pool for bioavailability measures and has been used successfully for some nutrients such as amino acids. However, simple blood measures ignore the different pools in the body and the relative rate in which these pools turnover and recycle, making simple measures difficult to interpret for some nutrients.

Kinetic measures are very difficult and costly to obtain and are often limited to an experimental scenario that may not accurately depict the intended application. Furthermore, the metabolic clearance rate from the blood tissue can be too rapid for some nutrients such as choline (Sharma and Erdman, 1989) for blood to be a useful measure of bioavailability.

Hopefully, for the nutrient of interest, there is a logical pool to sample as a qualitative, and ideally, quantitative measure of bioavailability. Often, content or yield of a compound in milk can be a practical pool to measure. Examples of this include using milk protein yield as a measure of lysine or methionine bioavailability or using milk choline content as a measure of bioavailability of choline from rumen-stable choline.

In an example of the differences in bioavailability that can exist between different products designed to achieve the same endpoint, Robert et al. (1997) tested four commercially available products designed to deliver bioavailable methionine to ruminants. Using a single dose of the product followed by intensive blood sampling, blood plasma-free methionine concentrations (BPMC) and area-under-the-curve measurements were made. Considerable differences existed

between the four products (Table 3), with the relative bioavailability estimates indicating that product B had 25% bioavailability compared to product A, with products C and D having 3.2% bioavailability relative to A.

Currently, there is no perfect bioavailability measure, nor one unified approach that will fit all nutrients or compounds of interest. However, there is a need for rumen-stable encapsulated products to have at least qualitative measures of bioavailability. Ideally, this work should be done at an independent research institute or university with clear, publishable and repeatable methodologies.

Products that lack this information should be carefully considered for their true efficacy, as the degree of difficulty in achieving rumen-stability and post-ruminal availability is so great that most manufacturers have great difficulty in engineering products to deliver this performance. Furthermore, the evaluation of commercially available products has shown that there are several products available and in use that have little to no potential bioavailability.

Table 3. Bioavailability of methionine from different sources of methionine in dairy cows.

Item	Methionine source				SED	P<
	A	B	C	D		
Baseline BPMC	0.196	1.24	0.22	0.20	0.05	0.82
Maximum BPMC	4.15 ^a	1.04 ^b	0.39 ^b	0.36 ^b	0.42	0.003
Area under curve	51.9 ^a	13.0 ^b	2.1 ^c	1.2 ^d	2.22	0.001
Bioavailability relative to product A, %		25	3.2	3.2		

^{a,b,c,d} Means without common superscript differ at indicated P-value.

Cost effectiveness. Cost effectiveness for a rumen-stable encapsulated nutrient product is the same as for any nutrient or feed additive. The benefits derived from using the product must exceed the cost of its use. A typical industry rule of thumb has been that a product should provide at least a 3:1 ratio in benefit returned to cost of use. Consistency in providing those cost benefits is key to acceptability of any new product. Research by Siciliano-Jones and Putnam (2000) and Scheer et al. (2002) has shown that a validated rumen bypass choline product will provide that level of benefit-to-cost ratio.

Simply overfeeding a nutrient can, in some cases, provide an alternative to using a rumen bypass product. This methodology relies on enough of the nutrient flowing out of the rumen before the microbial population can digest or alter its structure. However, when deciding whether to use such a product, it is helpful to know how it compares to simply overfeeding the raw nutrient or a feed ingredient that contains the nutrient, and how different products compare to each other. Cost per unit delivered to the small intestine is also an important consideration (Table 4).

Table 4. Cost effectiveness: cost per unit delivered to the intestines

Item	Product A	Product B	Product C
Rumen stability, %	90	50	5
% nutrient	25	25	30
Delivery, g/100 g fed	22.5	10.8	1.5
Feeding rate needed to equal product A	1	2.1	15.0
Cost, relative to Product A	1.0	0.65	0.35
Cost per unit delivered	1.0	1.4	5.3

Handling effect. The characteristic of the nutrient or compound that is encapsulated has significant impact on the storage and handling of the final encapsulated product. Most feed processing and inventory conditions will negatively affect the stability of encapsulated products. It is irresponsible to assume otherwise, as any loss in stability during feed processing (mixing, pelleting, etc.) or storage either at the mill or on farm will result in a corresponding loss in rumen stability or lower the net delivery of the nutrient to the animal. Until recently, hydroscopic nutrients such as choline have produced fragile encapsulates that were susceptible to moisture breakdown during storage. Recent advancements in encapsulation technology has improved the storage stability of encapsulated choline (Reashure) that provides prolonged storage in meal feeds as well as improved stability during the pelleting process.

If proper feeding and handling care is not followed, the benefits to the end user for the product will greatly diminish.

Encapsulated Products

Encapsulated products are not unique to the livestock industry. During the last half century refinements in encapsulating nutrients and compounds has been important in innovation in the food industry. Encapsulation of sodium bicarbonate, salt, acidulants, vitamins, probiotics and other nutrients has been important in improving shelf life, flavor and cooking quality of frozen foods, candies and products of the health-food and fast-food industry. Applying this technology to the livestock industry has the same potential to make marked improvements in feeding programs and animal performance.

Encapsulated choline. Choline is a nutrient that has been identified as essential for maintenance of optimum growth and performance in non-ruminant species. Until recently, the rumen microbial population has discouraged the supplementation of choline for ruminants due to the high degradation of the choline molecule. With the development of rumen-stable encapsulation, ruminants can now be supplemented with choline during periods of choline deficiency. Recent research from the University of Wisconsin (Cooke et al., 2004) showed that during periods of low dry matter intake, insufficient choline or choline precursors available to the dairy cow resulted in greater fat infiltration into the liver and slower clearance of that fat from the liver (Table 5). This is the typical symptom of choline deficiency that has been demonstrated in non-ruminants such as swine, rats and poultry. Additional research has shown that providing encapsulated choline during these deficiency periods results in improvement in overall animal performance and reproduction (Oelrichs et al., 2004).

Table 5. Influence of encapsulated choline on fatty liver.

Item	Control	Encapsulated choline
Induced fatty liver		
NEFA, $\mu\text{Eq/L}$	703	562*
Liver TG, $\mu\text{g}/\mu\text{g DNA}$	16.7	9.3*
Recovery from fatty liver		
Day 3, % of prior	60.4	52.2
Day 6, % of prior	48.5	29.9
Cooke, et al. 2004		*P<0.05

Encapsulated niacin. Niacin is another nutrient that has been shown to be deficient in certain situations, requiring supplementation to improve ruminant health and performance. Once again, niacin is susceptible to major degradation

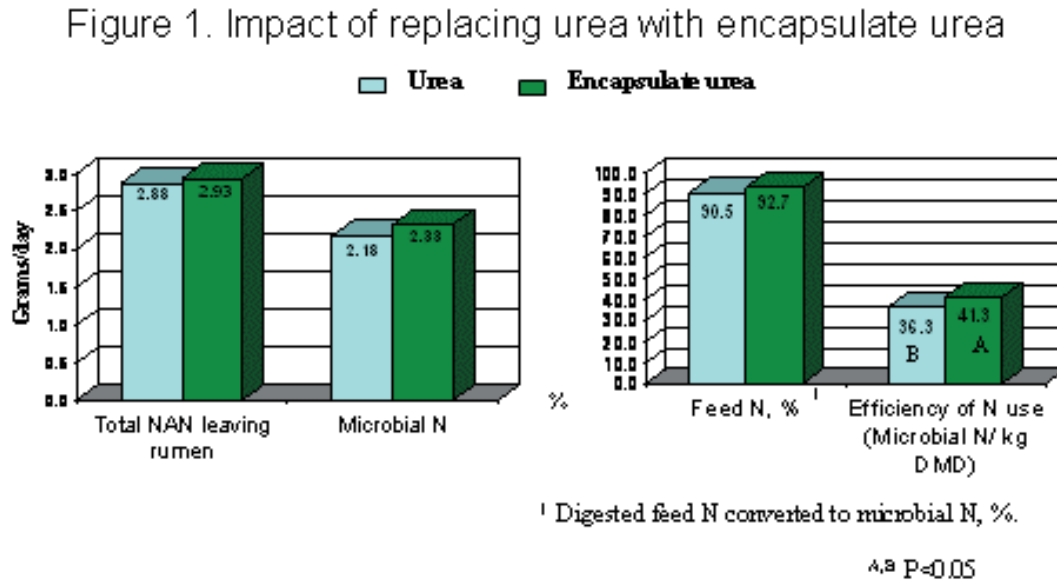
by the rumen microbial population. Currently, the method of overcoming the high degree of degradation of niacin in the rumen has been to feed excessive levels to ruminants. With overfeeding of niacin, there is the potential for escape from microbial degradation and passing into the lower digestive tract for absorption. Encapsulation of niacin provides a means of reducing the overfeeding and delivering a measurement amount for absorption in the small intestines. In this situation, the use of encapsulation, because of the high degree of niacin breakdown in the rumen, can provide an economically beneficial scenario before animal performance is considered.

Encapsulated ascorbic acid. Ascorbic acid is rarely supplemented to ruminants because of the reducing environment in the rumen, resulting in its antioxidant activity being destroyed before any can pass to the lower digestive tract for absorption. Recent research with ascorbic acid has indicated a potential application it might have during periods of stress or health challenge in ruminants. Calves going through the weaning process experience stress and have been shown to respond to inclusion of encapsulated ascorbic acid in their ration (Garrett et al., 2005a). Other research indicates that dairy cows under stress conditions such as during calving or health challenges such as mastitis, may benefit from supplementation with encapsulated ascorbic acid (unpublished research).

Encapsulated urea. Controlled release is also an attribute of encapsulation. The rapid availability of urea, unless formulated precisely with availability of carbon chains for production of amino acids, results in a loss of the ammonia through rumen absorption or passage to the lower digestive tract. By encapsulating urea, the release rate of ammonia can be measured out such that it matches well with the availability of carbon skeletons from carbohydrate digestion to enhance microbial efficiency of protein production. Recent research has shown that microbial protein production is improved by greater than 14% when the release of ammonia from urea is metered out to better match carbohydrate digestion (Garrett et al., 2005b; Fig. 1)

Encapsulated CLA. One area of recent attention has been the use of encapsulation to deliver targeted fatty acids, particularly specialty unsaturated fatty acids. In this situation, encapsulation, like it often does, serves two purposes. It serves to stabilize the fatty acid against oxidation during storage and handling and it protects it against biohydrogenation in the rumen so that the fatty acid of interest is delivered into the animal. A good example of this is CLA. Recent work has shown that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid is a potent inhibitor of milk fat synthesis. This offers a unique opportunity to control milk fat output, which is of great economic benefit under certain quota systems. High quality microencapsulation has been shown to have great efficacy

in delivering this fatty acid for precise control of milk fat content (Lock et al., 2005).



Intangibles

There are other factors that should be considered when using encapsulated products, as their cost demands that these products work and deliver value to the end-user on a continual basis. Independent review and research of the encapsulated product is extremely important. It should be expected that these products have been specifically tested under independent third-party conditions to validate their performance.

For example, it is clear that all rumen-stable/bypass products are not equal in performance or validation. To evaluate the effects of rumen-bypass amino acids without taking into account the differences in the performance of the different products tested over the last 15 years would lead to erroneous conclusions regarding the potential for success for this concept. Published results for rumen-bypass choline vary for the type of rumen-bypass choline product used and how those products were handled during a given experiment.

Not all products are alike and each requires individual evaluation of their benefits to the end user. It is important not to assume that if one product works, all similar type products will provide the same benefits.

In vivo performance data is equally, if not more, important than the basic rumen stability and bioavailability data. Being able to work with expensive, low-inclusion encapsulated products and convert their use into a positive return on investment is not a given. Responses in these trials should be consistent with the expected mode of action of the nutrient fed.

Choline's classic deficiency symptom, noted across many species, is fatty liver. Work at Cornell University by Piepenbrink and Overton (2000) confirmed that using rumen-protected choline in transition dairy cows significantly reduced the rate of fat accumulation in the liver, resulting in a corresponding change in glucose synthetic capacity and productivity in early lactation. However, it should not be assumed that all rumen bypass choline products would deliver the same results. Individual validation is necessary.

Implications

The development of encapsulation technology has provided a valuable tool to ruminant nutritionist to deliver specific nutrients to the site of absorption in the small intestines. With additional advancements in encapsulation technology, more and more nutrients will become available to the industry.

These new products will become an indispensable tool for formulating rations for specific phases of production. The key to the ultimate success of encapsulation technology will be verification of delivery of the nutrient to the proper site of absorption.

If purchasing a product that claims rumen bypass characteristics, always request independent verification of its results and response. Without such verification, failure to accept valid effective products will leave us without a valuable tool to maximize our ability to deliver necessary nutrients to our ever-increasing, high-producing dairy cows.

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