Getting the Rumen to Work at its Optimum

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■ Take Home Messages

▸ Many dairy nutrition advisors are seeking to formulate dairy rations with increasing flexibility of carbohydrates with respect to ruminal digestibility and physical effectiveness to stimulate rumination. Ranging from low to high forage:concentrate and with varying starch:NDF in the concentrate, the rumen microbes must adapt efficiently for optimal digestibility and dry matter intake (DMI).

▸ Excess rumen-degraded starch or increased inclusion of fibrous co-products can promote associative effects in which the actual digestibility of multiple feeds is lower than the weighted average of those feeds had they been fed individually. Part of the negative association is from not considering that the fibrolytic microbes need rumen-degraded protein (RDP) from amino acids.

▸ With varying contributions of rumen-active fat, we need to be more careful with how forage and starch sources interact to influence milk fat depression.

▸ Sugar-based co-products (2.5 to 5.0% supplemental sugar), especially if in the liquid form, can help support rumen fibre digestibility directly or perhaps indirectly through reduced sorting behaviour, but moderate sugar shifts microbial populations or fermentation products (especially butyrate and valerate) to potentially increase ruminal pH and reduce the likelihood for milk fat depression.

▸ Models have improved but still do not model the complexity of the rumen microbial ecosystem, so fundamental knowledge will help troubleshoot unexpected responses.

■ Introduction

"When we feed the cow, we feed the rumen." This adage means different things to different people. It could mean that we need to apply appropriate ration formulation combined with thorough feed analyses, exercise

appropriate feedbunk management, and monitor cows carefully to prevent rumen acidosis; these efforts help nutritionists to reduce the likelihood of milk fat depression, improve feed efficiency, and reduce environmental impact. To me, 'feeding the rumen' means that we are providing different nutrients as substrates to trillions of microorganisms that are rather arbitrarily grouped into thousands of different 'species' representing three different domains of life. As our computer systems continue to improve our flexibility and success with different rations, there are still many different conditions that cannot yet be modelled consistently. Therefore, my paper will explain what we know and don't (yet) know about the rumen microbial ecosystem to aid nutrition advisors, veterinarians, and farmers to understand those diverse dietary conditions. My goal is to help you ask better questions when simulating new dietary combinations, troubleshooting different rations that look good on paper, or to explain why ration approaches vary among farms. Hopefully, this will help you to 'feed the rumen' better to use fibre more efficiently, reduce environmental footprint, and reduce variability on dairy farms.

■ Expanding Microbial Diversity While Diversifying Trade

Microbiologists have dethroned the traditional taxonomical division into 'kingdoms'. With the advent of genomics technologies about 20 years ago, microbiologists discovered that the bacterial 'kingdom' was two separate 'domains' (bacteria and methanogenic archaea). Since that time, various species have been renamed many times (more changes in nomenclature are still needed), and we now know that a 'species' is really an artificial distinction that is typically split into distributions that are 97% similar with respect to the sequence of the gene for the small subunit of the ribosome. These clusters of sequences are typically grouped into 'operational taxonomic units' (OTUs) because this 97% cutoff is very practical but not uniformly correct across what would be considered 'species' for higher forms of life. From sequence information deposited across the world, Ohio State researchers have estimated the number of rumen OTUs exceeds 3500 for bacteria, nearly 1000 for archaea, and several dozen for protozoa. The fungi appear to be much more phylogenetically similar, but their diversity has received less attention. In addition to the diversity already there, the rumen microbial ecosystem includes viruses that attack ruminal bacteria (bacteriophages) and is influenced by numerous proteins ('bacteriocins') released by ruminal bacteria to inhibit other bacteria, and there seem to be distinctive host effects resulting from compounds in the saliva, interactions with the rumen epithelium, or perhaps from physiological differences in ruminal motility, etc. Thus, predictable description of microbial population structure among animals fed the same diet or even on different days still hampers our ability to clearly predict dietary effects on microbial populations.

Along with the information on microbial diversity, there have been many studies assessing the rumen microbiome of different animals on different diets. The net result of gaining so much sequence-based information compared with not much more information on microbial physiology is that we now know 'less about more'. From a nutritional point of view, though, we want to know 'more about less' so that we can harness information to simulate, predict, or explain variation resulting in ruminal function. Therefore, there seem to be some generalizations that are helpful to discuss. A "core" of microbial groups seems to exist in humans and in other animals, including cattle. Microbiologists have named the primary degraders as "keystone" groups that are critical to proper degradation of feed. They generally have co-evolved with the host to such an extent that they provide useful products for the host, and the host recognizes them as beneficial and even provides nutrients for them. The secondary group has been termed "partners" but can be synergistic or antagonistic. In many ways, they provide growth factors, consume acids, and help to provide stability in other ways. These keystone groups and their synergistic partners appear to make up the core. Antagonistic groups are in the rumen because they *can* be, not necessarily because they *should* be. Because the rumen is an open system and there is so much diversity, we need to feed cattle to maintain a balanced microbial core.

■ Carbohydrate Digestibility in the Rumen

Compared with older studies with non-lactating or low-producing cattle, high producing dairy cattle probably have 10 to 20% units lower starch digestibility because of faster passage from the rumen. Because increasing intake increases the amount of digestible starch, even if the digestibility coefficient decreases modestly, numerous studies have documented subacute rumen acidosis (SARA) resulting from volatile fatty acid (VFA) being produced faster than it can be absorbed. Thus, there is an understandable need to estimate the rumen digestibility of starch. In contrast with starch (which could theoretically be completely degraded in the rumen if given enough residence time), some fibre is indigestible even if it is retained in the rumen interminably (the "C" pool). Using Dacron bags allows us to estimate degradability among feeds. There is some potentially degradable starch or fibre that can wash out of the Dacron bag (the "A" pool), which is assumed completely degradable. Passage and degradation of the potentially degradable fraction of starch or fibre (the "B" pool; B = 100% - A - C) is usually assumed to be estimated using the first-order degradability function:

Rumen Degradability of a Nutrient = A + {(B) x [kd/(kd + kp)]}

For forage fibre, the potentially degradable B pool and its degradation rate (kd) are increased proportionately with lower maturity because the cellulose is more amorphous and there is less hemicellulose binding to lignin. Although it

might seem logical that lower degradability means more passage of fibre to the duodenum, the ruminal passage rate (kp) does not necessarily increase as a result. Passage rate is expressed as a proportion of the ruminal pool size, not of duodenal flow. Increasing ruminal fill tends to stimulate receptors in the rumen, thus stimulating rumination and kp. In contrast, increasing ruminal fill tends to decrease dry matter intake (DMI), and decreasing DMI tends to decrease kp. The net could be little change in kp.

In contrast with forage, non-forage fibre typically has little lignin (except cottonseed hulls). The B pool of most non-forage fibre sources is typically very high, and the C pool is very low. Although commonly misstated, most research has documented that the kd of non-forage fibre is relatively SLOW, and the kp can be relatively FAST. Although we often think about negative associative effects that depress fibre digestibility of forages, the microbes degrading non-forage fibre can be inhibited by low ruminal pH and other factors just the same as are those degrading forage fibre. Thus, non-forage fibre sources must be trapped and degraded in the rumen to make the best usage of their high B pool. When the diet has a really high concentration of non-forage fibre sources, there must be adequate effective fibre from forage to make a firm mat that prevents the non-forage fibre sources from sinking (they hydrate very rapidly) toward the reticulo-omasal orifice from which they pass to the omasum. There is a narrow window to have enough long forage but not too much to decrease DMI as a result of bulk fill (Khiaosa-ard and Zebeli, 2014). Using more grain by-products effectively means diluting starch to prevent these negative effects while also not diluting forage fibre too much such that the kp of the by-product increases dramatically.

In contrast with fibre, the kd of starch usually is much greater than its kp. Also, the surface area of starch from processed grains might actually exceed enzymatic capacity for microbial attack. Thus, the degradation of starch might not follow a first-order process, and using the equation above might not be appropriate. Such second-order kinetics might be particularly problematic when we use in vitro approaches. These laboratory tests most likely help to rank different grain sources, but using the rate constants in the first-order degradability function for an absolute value might be misleading. A meta-analysis from the French INRA group showed that estimation of ruminal starch digestibility in situ (using Dacron bags) overestimated ruminal digestibility of starch when it was high and underestimated starch digestibility when it was low. Consequently, differences in the animal might be ranked similar but not absolutely the same as those indicated by in vitro or in situ estimates.

When availability of starch is faster than the ability to use that energy for microbial growth processes, then the feed starch is degraded to glucose and resynthesized as microbial reserve carbohydrate (similar to glycogen storage in liver and muscle). Chemically indistinguishable from dietary starch,

microbial reserve carbohydrate requires the microbes to invest energy in the form of ATP. The liver and muscles of animals, including us, will have some cycling to keep the system in homeostasis under differing conditions, and microbes probably also cycle glycogen. Although the ATP cost is minor for aerobic cells that yield 30+ ATP per mole of glucose, anaerobic microbes yield only about 4 ATP per mole of glucose, so a single ATP used in synthesis or cycling of microbial glycogen is 25% of ATP yield. Therefore, excessive rumen-degraded carbohydrate is associated with decreasing efficiency of microbial protein synthesis (EMPS). The EMPS is usually expressed as g of microbial protein per kg of organic matter degraded, but its most correct representation would be g of microbial protein per kg of carbohydrate truly degraded. Despite this latter EMPS calculation being the basis for the Cornell or Cornell-Penn-Minor (CPM) models, digestible carbohydrate (sum of starch, sugars, and fibres) is rarely measured in animal studies. Because of inefficiency of reserve carbohydrate synthesis and cycling, the EMPS usually decreases with increasing starch degradability in the rumen. That is, high-moisture corn or barley will increase starch degradability in the rumen compared with rolled corn, but this increasing potential energy increases microbial protein flow to the duodenum at a decreasing rate (i.e., less than some models predict). Optimizing EMPS also depends on the provision of rumen-degraded protein (RDP) as preformed amino acids (AA), as follows.

■ Resilience to Unbalanced Microbial Consortia

After we realize that there are thousands of potential OTUs in any cow's rumen, we can reduce the mass of information to: 1) there are many fewer OTUs (perhaps several dozen to a few hundred) that constitute core structural components of a balanced consortium of microbes; 2) the ruminal microbes increase or decrease in abundance to meet their own population strategy, not necessarily the cow's; but 3) the cow's microbial populations have primarily evolved along with the cow. Therefore, the rumen microbial ecosystem should provide resilience against perturbed dietary conditions so long as we keep within some (as yet not fully defined) boundary of 'normal' microbial population structure. Using what we have learned from studies to mitigate methanogenesis, we first need to realize that methanogenic archaea *help* the cow by improving energy yield by important fibre-degrading bacteria, protozoa, and fungi. Thus, extreme efforts to suppress methane production can and do distort microbial populations and therefore potentially decrease fibre digestibility and/or feed intake within groups of cows. For example, rumen protozoa are often given a bad rap for wasting protein and enhancing methane production (which they do), whereas trying to over-suppress them removes their benefits from stabilizing fermentation or else simultaneously inhibits other bacteria (Firkins et al., 2007). Australian researchers have documented that source of sulfur can influence the ruminal fungi, which have an important role in degrading recalcitrant fibre both directly and indirectly by exposing more surface area for colonizing bacteria. Other studies with beef

cattle have documented that providing good quality forage with poor quality forage stimulates ruminal fibre degradability, with much of this benefit being a result of the RDP and fibre in higher quality forages. Thus, we need to work within the boundaries of the rumen ecosystem and provide better opportunity for balance and optimal efficiency of conversion of feed into milk components.

Negative associative effects (i.e., when fibre digestibility decreases as a result of excess starch in the diet) often have been explained through low ruminal pH, but low RDP and various other factors also can depress fibre digestibility as much or more than low pH (Firkins, 2010). In that paper, I explained why the core microbes must work together such that primary degraders partner with secondary degraders, which in turn provide amino acids and other growth factors to the primary degraders. Ruminal pH varies with ruminal location and fluctuates over time, so even cows with very low pH can maintain normal populations of cellulolytics (Palmonari et al., 2010). Experimentally induced acidosis from slug feeding of grain did not reduce the abundance of cellulolytic bacteria unless it progressed to severe acidosis (Khafipour et al., 2009). Thus, low pH is more likely an indicator than the cause of depressed fibre digestibility in more normal diets (that don't cause SARA). Rather than average pH, the time that pH is below a threshold of about 5.8 probably is the more critical response criterion relating to fibre digestibility (Firkins, 2010). If ruminal pH is not excessively low, though, the increased starch availability that promotes that low pH means that there is more energy by microbes that can be antagonistic to the primary degraders. They decrease the availability of AA or increase in abundance such that they are more likely to attach to newly ingested feed particles and thereby outcompete the fibrolytic specialists. Thus, the kd might decrease and result in more ruminal outflow of fibre that was otherwise potentially degradable.

■ Rumen Protozoa: Fibre Digestibility, Protein Degradation, and Methane Production

Rumen protozoa have been much discussed but incompletely studied because of various laboratory limitations, including the extreme challenge to separate them from bacteria (protozoa cannot live long-term without predating bacteria) and measurement of their biomass. Research in our laboratory has helped to cast doubt on some heuristic expectations that can be partially related to the conditions under which they were studied (Firkins et al., 2007). Clearly, protozoa predate bacteria for amino and nucleic acids, thus wasting bacterial protein that was previously assimilated using RDP and degraded carbohydrate. This wasteful cycle becomes self-limited, though, with increasing ruminal kp such as seen with high producing dairy cows. Moreover, the negative effect on EMPS must be considered under the microscope. Protozoa have fibrolytic enzymes, but probably their greatest effect on fibre digestibility is through indirect stimulation of bacteria, including

scavenging oxygen entering the rumen with new feed and also that the extra proteolysis can provide AA to prevent troughs of low AA or ammonia at the end of periods between meals. Research by my lab group and others has confirmed that protozoa consume significant proportions of dietary starch to cache as reserve carbohydrate (glycogen). Consequently, protozoa decrease the rate of starch degradation to VFA and indirectly help to 'buffer' the ruminal pH. Defaunation (elimination of protozoa) usually decreases fibre digestibility (Firkins et al., 2007). Depressed digestibility in the rumen also explains why there is a relatively consistent improvement in EMPS (decreasing the denominator of the equation) through defaunation that actually translates into only a modest benefit to the animal in the form of microbial protein supply to the duodenum while potentially being a detriment if total tract fibre digestibility is decreased.

Protozoa have received considerable scrutiny because of international efforts to abate methane, which is about 25 times more potent than CO_2 to trap heat in the atmosphere (Hristov et al., 2013). Since the early studies based on microscopy documented a close association of the methanogenic archaea and protozoa, subsequent research has documented a rather important evolutionary co-relationship. Although suppression of protozoa should decrease methanogenesis, there are some potential side effects detected in some studies. As discussed previously, the protozoal hydrogenosome quenches traces of oxygen for the fastidiously anaerobic bacteria and methanogens. Some have estimated that 1/3 to 1/2 of the methane resulted from H_2 or formate produced by protozoa based on the estimate of up to 10,000 methanogens to associate with a single protozoan (Ushida, 2010). In contrast, these methanogens can be chemoattracted to H_2-producing bacteria and fungi that would displace protozoa if suppressed by dietary means. Moreover, many prior estimates inflate the mass of protozoa in the rumen of high producing dairy cattle (Firkins et al., 2007). Thus, efforts to abate methane should consider the potential benefits of protozoa on fibre degradation, and regressions based on how protozoal suppression decreases methanogenesis also should account for the likelihood of decreased fibre degradation (which would decrease H_2 or formate production). Protozoal suppression as a mechanism to decrease methane was therefore not recommended by an expert panel (Hristov et al., 2013).

Protozoa also are known for being active proteolytics, but some studies probably inflate their role by intentionally lysing protozoal preparations to assay proteolytic activity. Protozoa are often said to 'engulf' feed, but, rather, they form digestive vacuoles that degrade feed, bacteria, or even their own organelle remnants, so protease activity in intact cells is likely less than that of lysed cells. Our research suggests that estimates of bacterial consumption in vitro are likely over-estimated as a result of the assay conditions. Therefore, my consensus is that protozoal predation and lysis in the dairy cow contribute significantly to intraruminal protein recycling, but a potentially more important

aspect of these predators is that they impact the abundance of various bacterial populations and do interact with methanogens extensively (Firkins et al., 2008). Future research needs to sort out the negative ramifications from the positives for us to suppress them moderately without causing side effects.

■ Excessive Carbohydrate and Asynchronous Microbial Growth

We have all been taught in our ruminant nutrition classes that lactic acidosis is a spiral in which excess intake of rumen-degraded starch favors lactate-producing *Streptococcus bovis*, the "weed of the rumen". Although this species is usually at relatively low abundance, its rapid (even if inefficient) growth rate has been projected to allow this species to 'bloom' in population density. When it metabolizes glucose to lactate at one-half the ATP yield per molecule of glucose, it can produce more ATP per unit of time by consuming glucose at least 5 times faster (Nagaraja and Titgemeyer, 2007). Lactic acid is 10-fold more acidic than the VFA, so its tolerance to low pH allows *S. bovis* to produce lactate and outcompete the resident lactate consumers. Partway through the acute acidosis spiral, eventually even *S. bovis* is replaced by lactobacilli. Lactate is produced in L and D forms, but the conversion of the D to the L stereoisomer is slow in animal tissues, so the buildup of D-lactate in the blood causes acute systemic acidosis. Although Nagaraja and Titgemeyer (2007) document these findings for acute acidosis, they also explained why SARA hinders feedlot cattle even though lactate concentration in the rumen (and blood) remains only briefly increased and then only to < 5% of total organic acids (which is why the predominant form in the field is subacute). In fact, measurement of such a large bloom has been hard to document in lactating cow studies (Firkins and Yu, 2015). Probably a bigger potential problem is from spikes in enterotoxins released from lysed bacteria in the rumen or large intestine (Khiaosa-ard and Zebeli, 2014).

Organic acids, direct-fed microbials, and even residual fermentation extract in distillers byproducts can enhance the lactate-using populations to prevent a rapid pH decline from lactate produced in the rumen. Strains of *Megasphaera elsdenii* are probably the most well-known to ferment lactate to propionate or butyrate, so *S. bovis* or other lactate producers lose their competitive advantage (from low pH and acid tolerance) and rarely increase in abundance. For dairy cattle, there has been some discussion on whether or not *M. elsdenii* promotes *trans*-10 fatty acid production and thereby predispose cows to milk fat depression. In my interpretation of these studies, increasing *M. elsdenii* does not promote milk fat depression but, rather, is helping to prevent it. *M. elsdenii* probiotics have had mixed response on milk fat production but with no clear milk fat depression (Aikman et al., 2011; Zebeli et al., 2012b). More quantitative approaches are needed for confirmation, but potentially increased abundance of this bacterium might be

in response to increasing lactate production, which itself inhibits biohydrogenating bacteria (Maia et al., 2010). One could ask the question, how much worse would have been milk fat depression if lactate had accumulated because *M. elsdenii* had not increased in abundance? Protozoa also sequester unsaturated fatty acids from the biohydrogenation pool and can consume a significant proportion of the lactate produced. Their numbers typically are not measured or often decrease in dietary situations in which they could help buffer the ruminal pH (see prior discussion) and decrease accumulation of bioactive *trans* fatty acids.

In contrast to an expectation for small amounts of sugars (2 to 5%) to decrease ruminal pH and potentially inhibit ruminal NDF digestibility, the opposite results tend to occur (Oba, 2011). Besides potentially decreasing sorting behavior, sugars could help to stimulate fermentation of lactate to propionate and butyrate, thus maintaining a population of lactate-using bacteria or protozoa to buffer against lactate accumulation. Studies from Alberta, Kansas State and Ohio State have documented increased *trans*-11 18:1 and/or decreases in *trans*-10 18:1 (the indicator of milk fat depression) in milk of cows fed moderate amounts of sugars. Another mechanism is that increasing butyrate production might increase absorption rate of VFA and help buffer the rumen pH as a result of lactate conversion or improved VFA absorption rate (see later discussion).

Both depressed pH and increasing concentrate inclusion are well known to potentially depress milk fat, sometimes sporadically among different cows fed the same diet. Although shifts in populations of biohydrogenating bacteria have been detected, there are other reasons why some cows have depressed milk fat. Rates of lipolysis can influence the availability of free fatty acids (only free fatty acids can be biohydrogenated), and other dietary factors influence the rate of biohydrogenation (Jenkins et al., 2008). Increasing lactate concentration from excessive starch digestibility in the rumen probably increases the toxicity of linoleic acid to biohydrogenating bacteria (Maia et al., 2010). In addition to helping to metabolize lactate (probably to butyrate), rumen protozoa preferentially incorporate unsaturated fatty acids or biohydrogenation intermediates such as conjugated linoleic acids into their membranes, potentially reducing the risk for milk fat depression (Firkins et al., 2008). Kevin Harvatine and colleagues at Pennsylvania State University have shown that some cows are more responsive to recovery from a bout of milk fat depression than are other cows.

Ionophores are commonly fed to beef and dairy cattle, and there has been no study suggesting a linkage to antibiotic resistance as shown for other feed-grade antibiotics. Ionophores are often expected to decrease the acetate:propionate ratio in beef cattle, but many dairy studies did not detect such responses, perhaps because of the generally lower dosage relative to feed intake in dairy versus beef studies (Firkins and Yu, 2015). As we

described in that report, a shift within phylum is more likely than a shift among gram-positive versus gram-negative bacteria (as often proposed). Our studies and analyses have shown that protozoa adjust to monensin, and effects on methanogenesis are varied. However, monensin probably will decrease methane when expressed per unit of milk production because of improved feed efficiency (Hristov et al., 2013). Similarly, monensin should have minimal effect on milk fat production unless unsaturated fat is higher than it should be. For example, most of the studies evaluating dose response of distillers grains (with ~10% free oil) have not resulted in milk fat depression, but they also generally did not include monensin in the diet.

■ Rumen-Degraded Protein, Ruminal Fibre Digestibility, and Dry Matter Intake

The Cornell model and its derivations have at its core the concept that the fibre-degrading bacteria only require ammonia, which could come from AA in RDP, cheaper sources of non-protein nitrogen such as urea, or even the 'free' supply of blood urea nitrogen (BUN) that recycles to the rumen. In that model's structure, only the nonstructural carbohydrate-using bacteria benefit from preformed AA. Such an approach is understandable because the three well characterized cellulolytic bacterial isolates all require ammonia as the principal nitrogen sources plus branched chain VFA, the latter of which should not be limiting their growth in dairy cows fed appropriately. Research from the Rowett Research Institute in Scotland supports the stimulation of fibre digestibility by addition of preformed AA. Previous contentions were that growth by cellulolytic bacterial cultures actually only is directly stimulated by preformed AA when isolates were fed cellobiose (the disaccharide repeating unit of cellulose) as an energy source (which is not typical of the rumen); thus, the benefit to fibre degradation might be indirectly from stimulating the synergistic non-cellulolytic partners. In contrast, the ruminococci (a major group of cellulolytic bacteria) have a clear and critical requirement for preformed phenylalanine needed for adhesion to cellulose but not hemicellulose. Moreover, other research from Japan and my lab at Ohio State supports an interaction of phenylalanine within the other aromatic AA, within the branched chain AA, and between the branched chain AA and methionine. Thus, it seems likely that RDP quality can affect EMPS.

Our desire to provide adequate RDP plus a safety margin to optimize fibre digestibility and DMI is in competition with our desire to decrease the emissions of ammonia and nitrous oxide from dairy farms. Unfortunately, many have over-extended results from ratios expressing N excretion per unit of N intake. Although logical, an improvement in this ratio from depressed N intake resulting from decreased DMI can decrease milk production per cow and, require more cows and their replacements to support the same amount of milk in a milk market, and negate the benefit. Thus, we need to better

understand the control points regarding how rumen bacteria use ammonia and why these control points can vary under different dietary conditions.

Ruminants have a tremendous ability to convert BUN into microbial AA synthesis and supply to the animal. In fact, the amount of BUN transferring into the rumen can exceed 50% of the nitrogen consumed in the diet. The BUN transfer efficiency (BUN trapped as microbial N divided by BUN transferring into the rumen) is increased as the dietary N is decreased, apparently because of an up-regulation of urea transporters. Research has proven that the ruminant has evolved to take advantage of BUN recycling with decreasing N intake. However, the more that the animal relies on transferred BUN for microbial AA assimilation, the more limiting will be amino sources of N that stimulate microbial growth rate. That is, if a preformed AA limits rate of microbial protein synthesis, adequate AA from RDP can actually help increase the transfer efficiency of BUN into microbial protein. There must be a moderate optimum to decrease RDP to decrease N excretion in a system that is aggregated at the farm or region levels.

Can the mechanistic research be reconciled with lactation studies and industry efforts to decrease N excretion sustainably? I think we need to look beyond individual research studies supporting exceptionally low RDP required to maintain milk protein synthesis for several reasons. First, university studies maintain more control over dietary protein percentage and cow variability than would be seen on most farms. For example, individualized feeding is not typical, so sorting in the field might be more common. Second, decisions need to be based not just on average responses from several studies - decisions also are based on variation around an average response and subsequent risk for lost milk and lost clients. Third, there is still unknown biological variation, which obligates a certain amount of randomness to simulated dietary response and therefore needing trial feeding on the farm.

Numerous studies have documented processes of proteolysis, deamination, and ammonia uptake (Walker et al., 2005). However, if RDP does not provide enough AA to microbes to optimize their growth, then the microbes become less efficient and supply less microbial protein to the small intestine. RDP limitation would be increasingly likely as we improve carbohydrate digestibility through grain processing, more digestible forages, and better feedbunk management (i.e., more consistent consumption of the diet by more cows). That is, the more we can provide a consistent availability of substrate for the rumen microbes, the more critical it is for an increased supply of steadily available AA from RDP because the intracellular concentration should not then rate-limit protein synthesis. A more synchronous supply route would decrease the need for inflated RDP safety factors and allow more ration space for other ingredients.

Another reason why there must be a safety factor to allow for variability is that there is a group of bacteria in the rumen that can use AA for protein synthesis but also degrade them very rapidly and extensively for energy (Walker et al., 2005). This group, sometimes termed "hyperammonia producers" is in very low numbers and has to deaminate many AA to gain enough energy to grow because they do not competitively use carbohydrate as an energy source. The hyperammonia producers (AA deaminators) could periodically spike in their numbers and deplete the concentration of AA that would stimulate the growth and fibrolytic capacity of the consortium of bacteria that breaks down fibre, particularly as we try to decrease the RDP safety factor. The literature would support the use of an ionophore or perhaps other dietary methods (some bioactive plant additives that are being researched currently?) to inhibit these AA-fermenting bacteria, whereas another effective strategy might be to stimulate the growth of sugar-using bacteria that can effectively compete for AA against the hyperammonia producers (Firkins, 2010).

The Dairy NRC took a big step to use methodology and data from the literature to provide a system while also providing a library for most feeds. Firkins et al. (2006) extended results from the NRC and noted that milk protein was related to rumen-undegraded protein (RUP, % of DM) and rumen-degraded protein (RDP, % of DM), with the RDP effect primarily a result of increased DMI:

Milk protein (kg/d) = 0.703 + 0.0202(RUP) + (0.0160)(RDP) - (0.000360)(RDP^2)

This equation documents a modest but important role for RDP, particularly to maintain DMI (embedded with RDP).

The Dairy NRC has a limitation in that it predicts microbial protein flow very empirically. While being reasonably robust, on average (St-Pierre, 2003), situations deviating from average would have little predictive power. In contrast, the Cornell model (or its derivations) should be more flexible because it predicts EMPS while also predicting nutrient digestibility in the rumen. However, CNCPS predicts microbial protein production to be higher than do the other systems (Pacheco et al., 2012). The mathematical product of the predicted EMPS and the predicted rumen digestibility is microbial protein supply to the duodenum for the cow's uses for metabolism (i.e., metabolisable protein). This system also probably has an advantage in a much more extensive library and experience by various users. In both systems, the prediction of microbial protein supply is used to predict the requirement of RUP by estimating the metabolizable protein requirement and subtracting the microbial protein supply after accounting for intestinal absorption. Clearly, the more times a predicted output is used for input into a subsequent equation, the more likely you can balloon variation and have large deviations of predicted protein required relative to actual supply to the cow.

For this reason, I think some caution must be exercised as use model simulations to decrease protein intake in cows to reduce feed costs or environmental concerns.

We have long known that anything that improves DMI should increase microbial protein synthesis (Oldick et al., 1999) but with limiting returns (i.e., an asymptotic response). Thus, if RDP stimulates fibre digestibility and DMI, both of these responses (or a positive interaction) could enhance efficiency of microbial growth. Any managerial aspect that enhances DMI should also simultaneously enhance microbial growth. Second, in some diets with high amounts of fibrous co-products, the NEL density is decreased such that cows need to eat more to meet their energy demands so long as bulk fill does not limit DMI. Some models predict that unsaturated fat replaces carbohydrate that is needed for microbial growth; consequently, fat is predicted to depress microbial protein. In contrast, most studies refute this concept probably because fat decreases intraruminal nitrogen recycling by depressing protozoa or else that feeding fat and subsequent transport into microbes spares energy that would otherwise have been used by microbes for biosynthesis. Enhanced DMI in these situations could stimulate microbial growth while also allowing a lower percentage of RDP to meet the same RDP requirement (grams/day) compared with more traditional diets. Meta-analyses have confirmed that fat should not affect microbial protein synthesis unless DMI is depressed.

I also performed a similar analysis using data from three research trials that used the NRC approach to estimate RDP for all feeds to get a cumulative dietary RDP and which only changed RDP supply (Figure 1). I noted a quadratic relationship between RDP (% of DM, X) and milk protein (kg/d, Y variable): $Y = 0.14 + 0.194X - 0.0089X^2$. I also verified that much of this response is indeed a result of the relationship between RDP and DMI. What this quadratic relationship shows is that, as RDP is progressively decreased, you would expect a progressively decreased production of milk protein. For example, dropping from 10.0 to 9.0% RDP was predicted to lose 25 grams/day of milk protein/cow/day; and from 9.0 to 8.0, an additional 43 grams/day. In contrast, another meta-analysis predicted very little response from RDP (Huhtanen and Hristov, 2009). Because this analysis with controlled studies ignores variability in the field and because most of the studies just used book values for RDP rather than actually calculating it, I think we need to reconsider uncertainty. A mean follows a bell-shaped curve in which there should be little, if any, positive response if actual RDP is randomly greater than the expectation based on diet formulation; however, when actual RDP is randomly lower than expected, then a farm could have a worse response than the average shown from a regression. My colleagues, Bill Weiss and Normand St-Pierre, have shown that there is resilience to fluctuation in RDP supplies within or among days to support milk protein production in university studies, yet they also have shown that there is plenty of risk for lower RDP in diets on actual farms than on the ration sheets.

Therefore, if you can't actually measure RDP of feeds or if the cost of its measurement is prohibitive, the natural inclination will continue to be to provide a RDP safety factor.
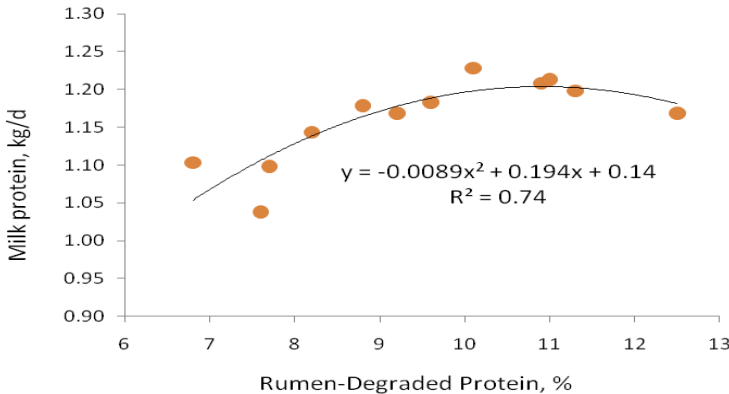

Figure 1. Data from three trials were adjusted to the average effect of trial (i.e., the mean milk protein production within a trial was adjusted to be equivalent to the mean milk protein from all three trials). Each study had four concentrations of RDP (% of DM) from all feeds determined as by the NRC) while all other aspects of the diet were constant except RDP. Trial adjustment reduced variation while combining trials increased observations to 12. Thus, the regression was more robust and provided a fit that was quadratic, not linear.

Work from Mark Hanigan (Virginia Tech), Helene Lapierre (Agriculture and Agri-Food Canada) and others has shown that decreasing metabolizable protein supply increases the efficiency of AA absorbed from the intestine, transported into the mammary glands, and converted into milk protein. This increased transfer efficiency explains why models with constant efficiencies overestimate responses from changing metabolizable protein. This increasing efficiency with decreasing metabolizable protein supply probably helps soften the potential problem of RDP potentially limiting microbial protein supply, but again those results are all from situations in which DMI was known and for which the lower RDP or metabolizable AA treatments were controlled with more precision than would be expected in the field. Because of greater variation within and among farms, I think we need to be very cautious in pushing extremely low RDP diets because a limitation in metabolizable protein supply is less severe than a limitation in DMI, which affects intake of *every* nutrient.

■ Strategies to More Efficiently Convert AA from RDP into Microbial Protein

Synchronizing carbohydrate and RDP provides inconsistent benefits in the field (Hall and Huntington, 2007). Although often discussed, matching fast sources of RDP with fast sources of rumen-degraded carbohydrate is somewhat over-simplistic, I think, because the cow's feeding pattern probably is the more important mediator of synchronicity of carbohydrate and RDP. I do think that a small amount of sugars is likely to help to provide a basal population of lactate-consuming bacteria that also use AA and can outcompete the hyperammonia-producing bacteria (Firkins, 2010). As discussed previously, increasing starch digestibility probably decreases the efficiency of microbial protein synthesis (i.e., the amount of microbial protein produced per unit of carbohydrate degraded in the rumen) such that the amount of microbial protein supply (i.e., grams/day) to the cow is increased less than expected or perhaps not at all. Therefore, with increasing starch fermentability, RDP probably does become more important, so the CNCPS or CPM models (which would suggest more RDP under these circumstances) should be useful to better formulate diets or simulate diet scenarios (e.g., for troubleshooting).

How do we properly formulate diets for structural and non-structural carbohydrates for optimal rumen function? The Dairy NRC has made the first step in providing a sliding scale in integrating these two feed fractions i.e., the lower the forage NDF, the lower should be the non-fibre carbohydrate. Improvements in effective NDF values and chemical measurements of starch and sugar help refine this system. We should be formulating diets that have a proper ratio of rumen-degraded carbohydrate relative to effective fibre (Zebeli et al., 2010) and then fine-tuning this concept according to different farms' forage and grain sources and managerial capacities. In particular, the window for optimizing DMI (Zebeli et al., 2012a) should also optimize microbial protein production. TMR feeding and enhancing multiple meals per day through multiple feedings or pushups should help maintain balance in the rumen and improve fibre digestibility. Finally, decision-making can be enhanced by the usage of in vitro procedures by feed testing labs to rank starch degradation characteristics or forage quality, particularly if these values are used repeatedly from the same farm for baseline values and for comparison to production measurements.

■ Revisiting the Rumen Epithelium

The bacteria associating with the rumen epithelium were first characterized about thirty years ago (before advances in genomics). One finding was that many bacteria had high ureolytic activities to provide ammonia that would diffuse into rumen fluid and be used by the predominant microbes associating

with ruminal fluid and particulate matter. Since that time, researchers have noted a greater diversity of these epimural bacteria but also, surprisingly, a higher-than-expected abundance of methanogens. As described previously, the isotrichid protozoa were noted to sink to the bottom of the reticulum and rumen, buried in the epithelium, to sequester until their reserve carbohydrate supply decreases and they migrate dorsally to search for sugars from ingested feed.

One of the surprising findings was that, although in low abundance, certain strains of ruminal *Escherichia coli* can have important systemic responses that affect milk fat production. Small bursts of serum virulence factors from *E. coli* were noted in dairy cattle with grain-induced SARA (Khafipour et al., 2011). If enterotoxins increase, the animal mounts an immune response that might partition energy away from the mammary gland (lowering milk fat secretion) for that energy instead to be used by immune cells. Moreover, when diets have excessive carbohydrate availability in the rumen, starch can spill into the intestine because of the cow's fast passage rate. After the abomasum, the intestinal chyme is buffered by pancreatic secretions and passed VFA are absorbed, so the pH becomes neutral or slightly basic. When starch is degraded in the large intestine, there is little buffering, so blooms and lysis of Gram-negative bacteria that can lead to a host immune response (Khiaosa-ard and Zebeli, 2014).

Greg Penner and colleagues from Saskatchewan and Germany have documented that some animals improve VFA absorption rate for a more buffered environment compared with others. More extensive VFA absorption concomitantly either absorbs the proton accompanying that VFA or substitutes a bicarbonate anion to neutralize a proton. Production and absorption of butyrate or valerate could stimulate rumen papillae surface area and thereby increase the rates of acetate and propionate absorption into blood, thus removing these acids from the rumen more quickly. Increasing the inclusion rate of molasses increased ruminal pH, which was attributed to increasing butyrate increasing the flow rate of blood draining the rumen to pull more VFA absorption into blood (Martel et al., 2011). This premise was supported by butyrate infusion and blood flow measurements used to build a VFA absorption model (Storm et al., 2011). Those workers also noted that greater motility in the rumen allowed faster diffusion of VFA to the epithelium. Therefore, focusing on effective fibre to stimulate secretion of salivary buffers ignores that excessive long forage might have a diminishing effect on rumination activity for neutralization but also might decrease transfer rate of VFA from the rumen mat to the epithelium for VFA absorption. We focus on salivary or dietary buffers, but another way to increase ruminal pH is to speed up the removal of the VFA and its accompanying proton from the rumen.

If we presume that increasing butyrate and valerate are good for the animal, what factors increase butyrate and valerate production by microbes? Studies

with ruminal or intestinal bacteria that use lactate for fuel have consistently documented shifts in fermentation toward higher chain VFA often by taking up previously produced acetate (to make butyrate) or propionate (to make valerate). Many, but not all, studies with sugars show increased molar proportion of butyrate or valerate (Oba, 2011). Therefore ruminal pH is typically increased either by these longer chain VFA stimulating absorption rate but also because of condensation of two VFA into one.

■ Conclusions

Dairy rations between 20 and 25% starch provide good opportunities for dietary inclusion of fibrous co-products and sugars of varying physical and chemical composition. These conditions, combined with variability in forage quality and physical effectiveness, enhance the need to understand the rumen microbial ecosystem to optimize feed efficiency. I have described the microbial populations and their roles in a balanced consortium that requires adequate RDP but does not waste that RDP, biohydrogenates unsaturated fat without causing milk fat depression, and can reduce methane production without depressing fibre digestibility. Integration of microbiology with dairy nutrition will lead to enhanced future opportunities to derive quantitative statistical prediction of an optimal microbial consortium to use enhance feed efficiency with less trial feeding and with less environmental impact.

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