Manipulation of the Dietary N-Fractions to Improve Ruminal Microbial Synthesis and Yield[1]

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Introduction

Ruminants are efficient users of diets poor in amount and quality of crude protein (**CP**) because ruminal microbes synthesize a large proportion of the animal's needs for metabolizable protein and essential amino acids (**EAA**). In addition, microbial utilization of ammonia allows the feeding of non-protein N (**NPN**) compounds, such as urea, and enables ruminants to utilize urea-N that is recycled to the rumen (Lapierre & Lobley, 2001). This latter mechanism might be employed to capture some urea N that would otherwise be excreted in the urine. High secretion rates for milk protein are why lactating dairy cows have the highest EAA requirements among domestic ruminants. Although dairy cattle can utilize diets with relatively poor quality CP, they typically excrete 2 to 3 times more N in manure than they secrete in milk. This N inefficiency necessitates feeding large amounts of supplemental protein, which increases production costs and contributes to environmental pollution. Because of stagnant milk prices, U.S. dairy farms are increasing in size to capture the economies of scale and these larger operations are importing more feed and increasing animal units per hectare Greater animal densities result in increased nutrient accumulation on the farmland and in greater environmental impact from dairying.

"Optimizing" Microbial Protein Formation in the Rumen

The EAA pattern of microbial protein is of better quality than most dietary ingredients commonly fed to domestic ruminants (Broderick, 1994; Schwab, 1996). Notably, the proportion of Met and Lys in microbial protein formed in the rumen is very similar to that of lean tissue and milk (NRC, 2001). Although there is evidence that His might be limiting in lactating cows fed diets containing mostly rumen-degraded protein (**RDP**), and which depend largely on microbial protein synthesis (**MPS**) for their EAA (Korhonen et al., 2000), most abomasal infusion studies have indicated that there is little evidence that a single EAA is limiting under these conditions (Schwab et al., 2003). However, the total CP in ruminal microbial cells contains from 20% (NRC, 2001) to perhaps 33% (Clark et al., 1992) non-AA N (e.g., N in nucleic acids and cell-wall components). Thus, conversion of good quality feed proteins into microbial protein may

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actually impair N-utilization, despite an overall improvement in EAA pattern, because of a net reduction in EAA supply. There is evidence of linear increases in MPS in response to increasing dietary RDP (Broderick et al., 2010) while maximal milk protein secretion occurs at less than maximal MPS (Reynal and Broderick, 2005; Olmos Colmenero and Broderick, 2006). Hence, MPS should be “optimized” rather than “maximized”.

Value of NPN in Ruminant Diets

A number of experiments have shown that ruminants can be moderately productive on diets in which virtually all CP is supplied as NPN (e.g., Virtanen, 1966). Data from Bryant and Robinson (1962) indicated that ammonia-N was more important than N from amino acids (**AA**) and peptides for growth of many pure cultures of ruminal bacteria. However, Oltjen (1969) summarized results showing that replacing CP from the readily degradable isolated soy protein with urea CP in purified diets reduced gain, feed efficiency and N-retention an average 35% in beef steers and heifers. These effects may be partly attributed to ruminal escape of isolated soy protein but one may infer that much of the difference was due to depressed MPS on the urea-based diet. Satter and Slyter (1974) fed diets to mixed ruminal organisms in continuous culture fermenters in which CP content was increased above a basal level of 4% (dry matter (**DM**) basis) by adding only urea. In 3 experiments, ammonia concentrations remained at ~1 m*M*, and microbial protein yield increased linearly, until dietary CP reached ~13%. At that point, fermenter protein outflow stopped increasing and ammonia concentration climbed rapidly; microbial protein yield did not increase above a mean ammonia concentration of ~2 m*M* (Satter and Slyter, 1974). This value was adjusted to about 3.6 m*M* (5 mg ammonia-N/dL) for a safety margin. Schaefer et al. (1980) found that ≤1 m*M* ammonia (1.4 mg N/dL) gave 95% of maximum growth for 9 of 10 pure cultures of ruminal bacteria studied. Results reported in both studies called into question the value of feeding NPN in many situations. There has been much debate during the intervening years about whether 5 mg N/dL (“5 milligram percent”) was in fact the upper limit for ammonia utilization in the in vivo rumen. For example, Mehrez et al. (1977) infused urea into the sheep rumen and found that in situ barley DM digestion increased with increasing ammonia until reaching ~20 mg ammonia-N/dL. Odle and Schaefer (1987) conducted similar studies in cattle and observed maximal rates of in situ DM disappearance at 12 and 6 mg ammonia-N/dL for barley and corn, respectively. Ruminal concentration of diaminopimelic acid, now a rarely used marker for bacterial protein, increased with urea addition to a high-corn diet until ammonia-N reached 8.5 mg/dL (Kang-Meznarich and Broderick, 1980). Higher optimal ammonia concentrations may be required under some circumstances because the physical association of bacteria with particulate materials results in low ammonia levels in these localized niches (Odle and Schaefer, 1987). Moreover, ruminal ammonia varies greatly, so a mean 5 mg ammonia-N/dL over the day will be the resultant a wide a range of concentrations (Dixon, 1999).

Amino-N Stimulates Microbial Protein Formation

Ammonia is formed partly from deamination of AA derived from ruminal protein degradation and ammonia production parallels formation of peptides and free AA. This confounds the explanation of whether MPS is responding to ammonia alone, or also to the peptides and AA that also gave rise to ammonia on natural diets. Work by Lee Baldwin's group at the University of California Davis shed considerable light in this area with a number of in vitro studies. Maeng et al. (1976) found that replacing 25% of urea-N with N from a mixture of 18% protein AA maximized the yield of microbial DM per unit carbohydrate fermented on glucose, starch and cellobiose; greater proportions of AA-N appeared to reduce microbial yields. Argyle and Baldwin (1989) showed that adding only 1 mg/L of a blend of protein AA plus 1 mg/L of peptides from trypticase (trypsin-digested casein) more than doubled in vitro cell yield of mixed ruminal organisms. They also found progressively lower response to 10 and 100 times greater addition of AA and peptides. Furthermore, Argyle and Baldwin (1989) found that mixtures of different classes of AA did not increase bacterial cell yield when added to a medium containing ammonia and peptides (trypticase); greater cell yield was only obtained with addition of a complete mixture of the protein AA. Other data indicated that in vitro MPS increased when degradation rate of protein sources in the medium increased from 0.01 to 0.14/h, but altered little with further increase in rate up to 0.54/h (Hristov and Broderick, 1994).

Dramatic responses to RDP supplementation also have been observed in vivo. Chikunya et al. (1996) found that replacing urea CP with casein CP in sheep fed a grass hay diet (with ruminal organic matter - **OM** - digestibility of ~32%) increased MPS 10%, but the same replacement in a sugar beet pulp diet (with ruminal OM digestibility of ~51%) increased MPS 82%. Kalscheur et al. (2003) held rumen-undegraded protein (**RUP**) supply constant but increased RDP by replacing treated soybean meal with solvent soybean meal; they observed significant increases in yield of milk, fat and protein at equal DM intake, although N efficiency declined. Recent in vivo results demonstrated significant linear depression in yield of milk, fat and protein when RDP from urea replaced RDP from soybean meal; these production effects appeared to be caused largely by depressed ruminal outflow of non-ammonia N (**NAN**), essential AA and total AA due to reduced efficiency of microbial protein synthesis (Broderick and Reynal, 2009). Kozloski et al. (2000) similarly observed a linear decline in MPS as more and more soybean meal CP was replaced by urea CP. On the other hand, Ahvenjarvi et al. (2002) attributed the increased flow of NAN to elevated RUP when they supplemented rapeseed meal to dairy cows fed a grass silage diet. But overall, at least under some conditions, NPN sources cannot provide all of the RDP and RDP from true protein required to optimize microbial protein formation in the in vivo rumen.

Stouthamer (1973) computed theoretical energetic efficiencies of microbial cell growth based on known metabolic pathways and estimated yields of 28.8 or 28.6 g OM/mol of ATP for organisms growing on media with N coming from, respectively, ammonia only or AA. However, Cruz Soto et al. (1994) reported that adding AA to the medium increased specific growth rates from 2x to 44x for a number of pure cultures of ruminal bacteria. Based on several in vitro studies, Russell et al. (1992) estimated that

addition of AA mixtures to ruminal incubations would improve cell yields by 18%, which seems more consistent with in vivo observations. Wallace's group at the Rowett Research Institute has explored possible mechanisms for these increased yields. Adding AA mixtures, and particularly mixed peptides (trypticase), resulted in substantial reduction in proportions of individual AA in microbial protein that derived from de novo synthesis of both the N-moieties (Atasoglu et al., 1999; Atasoglu et al., 2004) and the C-skeletons (Atasoglu et al., 2004). Deletion of single AA from the complete mix of protein AA slowed gas production when microbes were grown on a mixture soluble carbohydrates (Atasoglu et al., 2003) or xylan (Guliye et al., 2005), but did not alter protein yield in either study. It is not clear what causes the disparity between experimental observations and theoretical estimates but Russell (2007) speculated that AA and peptides reduced "energy spilling" during cell growth. The Wallace group (Cruz Soto et al., 1994) concluded that degree of stimulation by AA and peptides was related to carbohydrate fermentation rate, with greater responses occurring with more rapidly fermented substrates.

Reducing NPN in Forage Improves N-Utilization

Formic acid addition to direct-cut, hay-crop silages is used widely in Europe but is thought unnecessary for forages wilted to greater than 35% DM, such as those commonly harvested in North America. However, Nagel and Broderick (1992) found that applying formic acid to alfalfa wilted to 40% DM reduced silage NPN by one-third and increased milk yield 3.4 kg/day and protein yield 110 g/day when fed to cows in early lactation. Although there are concerns about cost, corrosion of machinery, and farmer safety, formic acid may be a practical way to reduce silage NPN. Rather than applying formic acid, Charmley and Veira (1990) suppressed proteolysis in ensiled alfalfa using a 2-min steam-treatment. The treatment did not alter neutral detergent fiber (**NDF**) or acid detergent insoluble N (**ADIN**) but reduced silage NPN from 65 to 40% of total N. When fed to sheep, flow of protein N from the rumen was increased from 22 to 27 g/d; about 60% of this increase was due to greater microbial protein. Charmley and Veira (1990) also reported that growth efficiency increased from 22 to 37 g microbial NAN/kg OM apparently digested in the rumen. This suggests the improved production observed by Nagel and Broderick (1992) was at least partly caused by improved microbial protein formation in the rumen. Alfalfa harvested in 3 trials from alternate windrows as either 40% DM silage or hay in small rectangular bales averaged 2.5 percentage units less CP (20.6 versus 18.1%) when harvested as hay (Broderick, 1995; Vagnoni and Broderick, 1997). However, despite these protein losses, feeding forage as hay rather than silage resulted in greater milk protein secretion and little response to supplementing with additional protein. In vitro studies conducted with samples of forages collected during these 3 in vivo trials indicated that alfalfa hay and silage had similar RDP and RUP contents but that hay gave rise to greater yields of microbial protein (Peltekova and Broderick, 1996).

Slow-Release Urea Compounds

In a feeding trial studying the effects of hammer-milling high moisture corn fed to lactating dairy cows, we found that grinding corn increased yield of milk (2.4 kg/d) and protein (120 g/d) (Ekinci and Broderick, 1997). Although grain processing reduced ruminal ammonia, maximal concentration still reached 21 mg ammonia-N/dL, greatly in excess of useful levels. Dixon (1999) observed that ruminal ammonia-N was usually <1.0 mg/dL over the 24-h day when sheep were fed oat hay with ~7% CP. Supplementing urea to increase this diet to ~14% CP increased maximum ammonia to 40 mg/dL, but ammonia was still <5 mg/dL for ~8 h/day. Hettiarachchi et al. (1999) infused urea into the rumen throughout the day and observed increased ruminal NDF digestion when sheep were fed NaOH-treated straw, but not when fed untreated straw or untreated straw plus barley. Cabrita et al. (2003) observed a 2 kg/d increase in milk yield and a 50 g/d increase in protein yield when lactating cows were given a their protein supplement in 2 meals/d versus 1 meal/d; feeding protein twice/d was as effective as providing the supplement in a total mixed ration. These findings are just some of the evidence that has renewed interest in slow-release urea products in the last 10 years (Galo et al., 2003). Although Inostroza et al. (2009) reported an economic advantage for feeding slow-release urea to lactating cows, many reports are less favorable. For example, Taylor-Edwards et al. (2007b) observed substantial reduction in ruminal ammonia concentrations and ammonia absorption from the gastrointestinal tract when a slow-release urea product replaced conventional urea in beef cattle diets based on corn silage; however, weight gain and feed efficiency were not improved by feeding slow-release urea (Taylor-Edwards et al., 2007a). Providing RDP in the form of true protein may be more effective in this and perhaps most settings.

Summary and Conclusion

Dairy cows excrete substantially more N in manure than they secrete in milk, which increases milk production costs and environmental N pollution. Although the AA pattern of microbial protein produced in the rumen is of good quality, 20 to 33% of the CP equivalent in microbial cells is in the form of non-AA N (such as nucleic acids). Optimizing (as opposed to maximizing) microbial protein formation, by providing the appropriate N compounds in the rumen, will improve the protein status of the lactating cows and other productive ruminants. Non-protein N can replace only part of the dietary RDP because RDP from peptides and AA stimulates microbial protein synthesis (both in amount and efficiency of protein formed per unit of energy fermentation). The effects of providing RDP in the form of AA and/or peptides are complex and the mechanism of these improvements in protein yield is not clear. Reducing dietary NPN by suppressing protein degradation in the silo also improves efficiency of microbial protein formation. Timeliness of provision of RDP in the rumen appears to be important; however, slow-release urea compounds have proven no more effective than conventional urea from the stand point of animal performance. At this time, it appears that supplying RDP as true protein supplements will usually result in more efficient microbial growth in the rumen and greater productivity of the animal.

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