Improving Nitrogen Utilization in the Rumen of the Lactating Dairy Cow[1]

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Introduction

Ruminants make efficient use of diets that are poor in true protein content because microbes in the rumen are able to synthesize a large proportion of the animal's required protein. The amino acid (AA) pattern of this protein is of better quality than nearly all of the dietary ingredients commonly fed to domestic ruminants (Broderick, 1994; Schwab, 1996). In addition, ruminal microbial utilization of ammonia allows the feeding of nonprotein N (NPN) compounds, such as urea, as well as the capture of recycled urea N that would otherwise be excreted in the urine. Many studies have shown that lactating dairy cows use feed crude protein (CP; N x 6.25) more efficiently than other ruminant livestock. However, dairy cows still excrete 2-3 times more N in manure than they secrete in milk, even under conditions of optimal nutrition and management. Inefficient N utilization necessitates feeding supplemental protein, increasing milk production costs and contributing to environmental N pollution. One of our major objectives in protein nutrition of lactating ruminants must be to maximize ruminal formation of this high quality microbial protein and minimize feeding of costly protein supplements under all feeding regimes.

Microbial growth in the rumen is generally limited by energy rather than N supply (Russell et al., 1992). Therefore, improving energy availability from the lower quality forages available in the Southeast would be very beneficial. What are the best approaches for supplementing tropical forages with feeds high in non-fiber carbohydrates (NFC) to maximize microbial protein formation? Complex interactions between energy and N metabolism in the rumen influence the net flow of microbial protein to the abomasum. Are there N supplements that will maximize microbial protein formation on tropical forages? This paper will consider how dietary N and energy supply influence microbial protein formation in the rumen and will apply this information to lactating dairy cows, including those fed diets based on tropical forages.

Nitrogen Requirements of the Ruminal Microbes

That ruminants can utilize urea and other NPN sources has been known since

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early in the 20th Century (e.g., Armsby, 1911). Moreover, a number of experiments showed that ruminants could survive, even making modest weight gains (Loosli et al., 1949) and producing modest amounts of milk (Virtanen, 1966), on semi-purified diets in which virtually the entire CP was supplied from NPN. Bryant and Robinson (1962) studied the nutritional requirements of a large number of strains of bacteria freshly isolated from the rumen. In defined media containing branched-chain VFA, 56% of these isolates grew when all of their N was provided as either ammonia or enzymatic casein hydrolysate and 25% required ammonia but not casein hydrolysate. Only 6% required casein hydrolysate while the remaining 13% showed poor growth on the defined media used in this study. These and similar results (e.g., Allison et al., 1970) were taken as indicating that ammonia-N was relatively more important than N from AA and peptides for the growth of most ruminal bacteria.

It was generally assumed that rumen-degraded protein (RDP) from urea or other compounds that supplied only ammonia were equivalent to RDP from true protein until the experiments of Satter and Slyter (1974). These workers incubated mixed ruminal organisms in continuous culture fermenters that were fed diets in which CP was increased above 4% (DM basis) by adding only urea. In 3 such studies, ammonia concentrations remained at 1 mM or less, and microbial protein yield increased linearly, until dietary CP reached about 13% of DM. At that point, microbial protein outflow from the fermenters stopped increasing while ammonia began to climb rapidly. Overall, Satter and Slyter (1974) concluded that microbial protein yield did not increase with urea addition above an average dietary CP of 13.4% or a mean fermenter ammonia concentration of 2 mM. This latter value was adjusted up to about 3.6 mM (5 mg ammonia-N/dl) to provide a small safety margin. Schaefer et al. (1980) found that ammonia saturation constants—the ammonia concentrations giving 50% of maximal growth rate in ammonia-limited organisms—was 0.05 mM or less for 9 out of 10 pure cultures of ruminal bacteria they incubated in both batch and continuous culture. A growth rate equal to 95% of maximum is obtained with 20 times the saturation constant; therefore, 1 mM (1.4 mg/dl) or less was the ammonia-N concentration giving 95% of maximum growth for 9 of the 10 bacteria studied (Schaefer et al., 1980). Results from these two studies called into question the value of feeding urea or other NPN compounds in many situations.

But the issue of ammonia utilization was far from settled. There has been much disagreement over the past 30 years regarding the appropriateness of 5 mg N/dl as the upper limit for ammonia utilization in the rumen. For example, Mehrez et al. (1977) infused urea into the sheep rumen and found that in situ digestion of barley DM increased with increasing ammonia, reaching maximum at about 20 mg ammonia-N/dl. Odle and Schaefer (1987) conducted similar studies in cattle and observed maximal rates of in situ DM disappearance at 12.5 and 6.1 mg ammonia-N/dl for barley and corn, respectively. Ruminal concentrations of diaminopimelic acid, a marker for bacterial protein, increased in a stepwise manner with urea addition to a high-corn diet until ruminal ammonia-N reached 8.5 mg/dl (Kang-Meznarich and Broderick, 1980). The relatively low ammonia levels required by ruminal organisms in vitro may have been due to their growing primarily on soluble substrates (e.g., Schaefer et al., 1980). It has been

speculated that higher optimal ammonia concentrations are required in situ and under some in vivo circumstances because the physical association of bacterial cells with particulate substrates results in localized niches with very low ammonia levels (Owens and Bergen, 1983; Odle and Schaefer, 1987).

Despite much research in the last 20 years, the question of what is the required concentration of ruminal ammonia, or even whether the question is even appropriate, remains unanswered. There are frequent reports of what appear to be conflicting results on this issue. Dixon (1999) reported that in situ DM digestion from 16 different roughages was increased from 24 to 87% with the addition of urea to a low protein diet. Ruminal ammonia concentration was less than 1 mM on the basal diet before urea supplementation. Dixon et al. (2003a) also found increased weight gain in sheep fed low quality hay when given isonitrogenous supplements from safflower meal, linseed meal, or a urea-barley mix, although the urea-barley mix reduced hay intake about 10%. Ruminal ammonia was 8.0 mg/dl on the basal diet in this trial. However, Dixon et al. (2003b) found that these same three N supplements gave comparable increases in roughage intake and digestibility, and allowed body weight to be maintained, in sheep fed only barley or oat straw. Ruminal ammonia-N averaged 1.0 and 2.0 mg/dl on the basal diets of only barley or oat straw, respectively, and neither straw alone allowed maintenance of body weight. These workers also observed that a urea supplement with added inorganic sulfur was equivalent to a fishmeal supplement and the urea-barley mix in their effects on stimulating intake and digestion of two different barley straws (Rafiq et al., 2003). Overall, urea supplementation appeared to be most effective for improving intake and digestion of low quality forages when ruminal ammonia concentrations were very low. Hamali et al. (2001) found no differences in microbial protein yields with either urea or casein supplementation in steers feed corn or hay-based diets, although corn diets had greater ruminal digestion of organic matter and NDF. However, Gressley (2005) recently reported that increasing dietary CP from 13.5 to 16.1% by adding urea to a diet based on corn and corn silage had no effect on yield of milk and milk components in lactating dairy cows (Table 1), despite the fact that the NRC (2001) indicated that milk yield should have been 6 kg/d greater with urea addition. It should be noted that in situ digestion of soy hull NDF was increased and there was a trend for elevated urinary excretion of purine derivatives (an indicator of microbial growth in the rumen) with urea supplementation in the Gressley (2005) trial. Thus, it appears that there are conditions where dietary addition of urea or other NPN sources will improve microbial digestion and/or microbial protein formation at ammonia-N concentrations in excess of 5 mg/dl and this may be related to the effective ammonia levels in specific niches occupied by particle-associated bacteria.

Part of the confusion regarding the ammonia “requirement” of ruminal microbes may stem from the confounding of ruminal ammonia with protein degradation. On practical diets, ammonia is formed largely from deamination of the AA produced from ruminal proteolysis and ammonia production will parallel formation of peptides and free AA. It has been known for some time that products of true protein degradation other than ammonia stimulate microbial protein synthesis in the rumen. Oltjen (1969) summarized a number of trials showing that, when all of the dietary CP was supplied by

urea, growth rate, feed efficiency and N-retention in ruminants were about 65% of that when the same diets contained CP from only isolated soy protein. If ruminal escape of isolated soy protein were discounted, then the about 50% (100/65) greater performance could be attributed to enhanced microbial protein yield from the AA and peptides provided by RDP from true protein versus that from only ammonia-N. Indeed, a number of in vitro studies indicated improved microbial growth with AA and peptide supplementation. Maeng and Baldwin (1976) observed substantial increases in protein formation and efficiency (protein formed per unit of carbohydrate fermented) with the addition of equal molar mixtures of protein AA in incubations of mixed ruminal organisms. Argyle and Baldwin (1989) showed that adding only 1 mg/L of a blend of all protein AA plus 1 mg/L of peptides (from the tryptic digest of casein) more than doubled cellular yields of mixed ruminal organisms. These workers found progressively lower responses to 10 and 100 times greater additions of AA and peptides and also observed that a complete mix of the protein AA was required to obtain the stimulation in microbial yields. Stokes et al. (1991) reported that microbial protein production in ruminal in vitro incubations increased linearly with RDP up to 20% of dietary DM. Moreover, Hristov and Broderick (1994) found that net microbial protein formation in vitro increased as degradation rate of the added true proteins increased up to 0.14/h but changed little as degradation increased up to 0.68/h. Furthermore, Chikunya et al (1996) observed that microbial yield, estimated in vivo from urinary purine derivative excretion, was increased more by casein than urea supplementation and was 10% greater in sheep fed a low energy hay but 80% greater in sheep fed a diet based on higher energy sugar beet pulp (Table 2). Finally, Kozloski et al. (2000) found that replacing dietary soybean meal with isonitrogenous amounts of urea depressed microbial protein synthesis in steers, despite a positive affect of urea addition on rumen cellulose digestion.

Branched-chain VFA, formed from catabolism of the branched-chain AA, are additional growth factors contributed by proteins but not by NPN sources. These compounds are used by certain ruminal bacteria to synthesize the branched chain AA that get incorporated into protein. There was some evidence that supplements of branched-chain VFA stimulated milk production of dairy cows fed corn silage (Felix et al., 1980). Interest in branched-chain VFA may have waned because the responses observed in large scale collaborative feeding studies, although usually positive, were much smaller than those reported in early trials.

Matching Energy Fermentation with Rumen-Degraded Protein

Microbial synthesis in the rumen provides most of the protein used by the lactating ruminant for maintenance and milk production, so increasing microbial protein formation is an ideal way to improve utilization of dietary CP. Matching ruminal carbohydrate fermentation with RDP availability should improve N efficiency. There are substantial differences among starch sources (Herrera-Saldana et al., 1990), and within grains due to processing, in the rates of energy release in the rumen. Effects of processing on extent of ruminal digestion of corn starch are much greater than on total tract digestibility (Table 3; Owens et al., 1986). Very similar results have been reported in more recent work (Owens et al., 1997). We found that grinding high moisture corn

through a through a 1-cm (3/8") screen, reducing mean particle size from 4.3 to 1.7 mm, greatly enhanced rates of in vitro ammonia uptake by ruminal microbes; finer grinds (using screens as small as 0.2-cm) did not further increase ammonia uptake (Ekinci and Broderick, 1997). Feeding this ground high moisture corn (with 1.7 mm mean particle size) to lactating cows increased milk and protein yield by 2.4 and 0.12 kg/day compared to unground high moisture corn. Much the same thing happens with dry corn, except at smaller particle sizes. Processing dry shelled corn to reduce mean particle size from 3.5 to 0.6 mm increased ruminal starch digestibility from 54 to 70% (Remond et al., 2004). Increasing ruminal fermentability of starch or other carbohydrate sources should be effective on diets based on tropical forages for increasing microbial protein formation and N utilization.

Ruminal acidosis and associated metabolic problems limit the amount of readily fermented carbohydrate that may be fed to stimulate microbial protein formation. There are "optimal" levels of carbohydrate and forage that will support maximal ruminal protein synthesis and milk production. A high fiber diet with 80% forage and 20% concentrate was diluted stepwise by increasing high moisture corn to (% alfalfa silage DM/% concentrate DM) 65/35, 50/50, and 35/65 in a reversal trial (Valadares et al., 2000). True protein and NPN, as proportions of total CP, were held constant by adding solvent soybean meal and urea as the silage was decreased. The observed quadratic responses indicated that DM intake and yield of FCM were maximal at 51% concentrate (38% NFC); fat yield was maximal at 43% concentrate (34% NFC). However, milk and protein responses were linear rather than quadratic--both were still going up at 35% forage and 65% concentrate. Moreover, purine derivative excretion in the urine, an indirect measure of ruminal protein formation, also showed a linear response, despite low ruminal pH and other signs of over-feeding NFC (Valadares et al., 1999). Clearly, the lactating cow's demand for energy is substantial but short-term reversal trials, such as the Valadares studies (1999, 2000), may be too short to observe the adverse effects on ruminal and animal health that may occur with long-term feeding of excessive concentrate.

The Cornell model (Sniffen et al., 1992) predicts reduced formation of microbial protein per unit of fermented energy when ruminal pH falls below 6.2. This effect would be particularly problematic in dairy cows fed diets based on high-fiber tropical forages. Ruminal pH in high producing dairy cows fed large amounts of fermentable concentrate may remain below 6.0 for much of the day, often averaging well below 6.2 overall (Ekinci and Broderick, 1997; Weimer et al., 1999). De Veth and Kolver (2001) reported that, when pH in rumen continuous culture fermenters was reduced from 6.3 to 5.4 for periods of 4, 8, and 12 hours per day, there was a negative linear relationship between microbial protein yield and the length of time pH was at 5.4. It is important to confirm in vivo the pH at which high concentrate feeding begins to reduce utilization of dietary NPN and otherwise depresses microbial protein formation and to quantify the magnitude of these effects. Depression of fiber digestion at low ruminal pH can aggravate the problem of depressed milk fat yield by reducing formation of acetate (e.g., Dixon and Stockdale, 1999), a major milk fat precursor.

A number of years ago, considerable research were directed toward synchronizing carbohydrate fermentation with N release in the rumen to improve microbial capture of RDP. Ruminal organisms fermenting NFC, particularly soluble sugars and pectins, were thought to make greater contribution to microbial protein synthesis per unit of fermented carbohydrate (Russell et al., 1992). Chamberlain et al. (1993) found that sugar supplements were more effective than starch in grass silage diets for increasing urinary excretion of purine derivatives in sheep; the order of carbohydrate effectiveness was sucrose > lactose > fructose > xylose > wheat starch. Later Scottish research indicated that ruminal infusions of sucrose (Kim et al., 1999a) or maltodextrin (partially digested starch; Kim et al., 1999b), to supplement of grass silage diets in dairy cows, stimulated microbial protein synthesis in the rumen. Trevaskis et al. (2001) reported that sucrose infusion into the rumen was more effective for stimulating microbial protein formation when it was synchronized with the ammonia peak occurring 1-2 hours after feeding. In the Scottish work, effect of maltodextrin supplementation was greater in one case when synchronized with ruminal ammonia (Kim et al., 1999b), but not in the sucrose trial (Kim et al., 1999a). Korean research (Kim et al., 2000) also showed a positive effect of sucrose infusion into the rumen but no advantage of synchrony with ruminal ammonia. Molasses and a number of other byproduct feeds can serve as economical sugar sources. Corn starch was replaced with sucrose (Broderick et al., 2000), or dried molasses or liquid molasses (Broderick and Radloff, 2004), in 3 feeding studies in which the basal diets contained 2.6% total sugars. An overall analysis of the data from these trials indicated maximum feed intake at (DM basis) 6.8% total sugars and maximum milk protein yield at 4.8% total sugars. However, the positive production effects of sugar feeding in these trials were at least partly driven by increased feed intake. Harvesting forages in late afternoon, just after maximal photosynthetic activity, increases forage sugar and NFC contents (Owens et al., 1999). Trevaskis et al. (2004) reported that managing grazing cows such that they consumed foliage largely in late-afternoon was effective for improving milk production. Kim et al. (2005) observed a linear increase in microbial protein yield with increased ruminal infusions of sucrose-urea mixtures in sheep but there was a linear decline in microbial efficiency (g microbial protein/kg ruminal fermented organic matter). However, most evidence, including that from recent studies (Cabrita et al., 2003; Richardson et al., 2003; Trevaskis et al., 2004) show little or no production benefit from direct manipulations to synchronize protein degradation and energy fermentation in the rumen. Nevertheless, none of this research was attempted at minimal CP intake. We speculate that, when feeding reduced CP diets, there would be more and longer periods of the day when RDP will be limiting and microbial protein formation might be improved by synchronizing energy fermentation with N release in the rumen.

In North America, corn silage is commonly fed as a major portion of the ration. This high energy “forage” is low in CP and, thus, can be fed to dilute hay-crop forages with highly degradable protein (such as legume or grass silages). Dhiman and Satter (1997) replaced 1/3 or 2/3 of the dietary alfalfa silage with corn silage. Compared to 100% of the forage from alfalfa, milk yield was 6% higher over the whole lactation when 2/3 of the dietary forage was alfalfa silage and 1/3 was corn silage; there also were comparable improvements in N efficiency. Feeding forage as 1/3 alfalfa silage and 2/3

corn silage slightly reduced production and substantially increased the need for dietary protein concentrate. Brito and Broderick (2003) assessed the effects of step-wise replacement of alfalfa silage with corn silage. The greatest improvement in N efficiency, without loss of production of milk, fat and protein, occurred at about 50% of the forage from alfalfa silage and 50% from corn silage, mainly because intake declined when alfalfa was reduced (Table 4).

Using Nutritional Models in Ration Formulation

The value of applying nutritional models, such as the NRC (2001) or the Cornell systems (e.g., O'Connor et al., 1993), to formulation of dairy cow rations does not need extensive elaboration. Hanigan (2005) recently compared these two models with three others and concluded that the NRC (2001) model was somewhat more accurate at predicting metabolizable protein supply. Both the NRC (2001) and Cornell protein models are valuable but both require accurate characterization of feedstuffs, not only chemical composition but also ruminal and intestinal degradation and digestion. The tabulated values for rumen-undegraded protein (RUP) contents in NRC (2001) tables illustrate this problem. Although based on a simple, single compartment in situ model, many RUP estimates derive from very few in situ measurements. Data on solvent soybean meal came from 14 determinations but only three values contributed to the mean for corn gluten meal. Moreover, we found that microbial non-ammonia N (NAN) predicted using the NRC (2001) equation yielded a slope of only 38% when regressed on microbial NAN flows measured at the omasum in 6 recent experiments (S. M. Reynal, personal communication). However, the NRC (2001) model was much more reliable for predicting the RDP, RUP and total protein flows measured in these same trials, indicating that the model probably over-predicted RUP, yielding overall estimates of protein flow that were more nearly correct. Furthermore, the NRC (2001) protein model was more effective at predicting milk and protein production than at predicting protein flow from the rumen. This suggests that compensating factors within the model correct many of its errors and illustrate that imperfect models can still be very useful. Similar results have been found using the Cornell system (D. G. Fox, personal communication). The equating of RDP from NPN and true protein in the NRC (2001) and Cornel models (and probably most other systems currently in use) may lead to erroneous conclusions about when urea supplementation is appropriate. For example, we found that, compared to an isonitrogenous diet containing urea, there were 7 to 8 kg/d more milk, 260 to 350 g/d more milk protein, 170 to 280 g/d more milk fat, and greater microbial protein yield, in dairy cows supplemented with one of three true proteins that differed in RUP and AA pattern (Table 5; Brito and Broderick, 2004). Flow of RUP and total protein (NAN x 6.25) from the rumen was greatest on cottonseed meal, intermediate on canola meal and lowest on solvent soybean meal. However, milk and protein yield were highest on canola, intermediate on soybean, and lowest on cottonseed meal (Table 5). Although urea can be effective under situations where ruminal ammonia is limiting, there may be substantially better performance with RDP supplied from true protein in high producing animals.

Summary

Inefficient utilization of the CP in ruminant diets, and the feeding of large amounts of protein supplements that this inefficiency necessitates, leads to excessive feed costs and environmental N losses in dairy production. Maximizing microbial protein formation in the rumen is the most effective way to improve the protein status of the lactating cow. Only part of the dietary protein can be replaced by urea or other NPN sources because of a limitation in the ability of ruminal microbes to utilize the resulting ammonia as their sole source of RDP. Ammonia is used best on diets with greater amounts of NFC and/or higher digestible fiber; thus, enhancing the value of NPN supplementation on diets based on tropical forages is problematic. Feeding more extensively processed concentrate, if adequate effective fiber is maintained in the diet, will help maximize utilization of dietary NPN for microbial protein formation in the rumen. Replacing some dietary NFC with sugars or high-pectin feeds may be advantageous for improving ruminal microbial protein formation. As well as supplying ruminal energy, adding molasses to the diet stimulates intake and, thus, helps improve production. The NRC (2001) model can also be used to match RDP with carbohydrate fermentation. Future research should be directed toward feeding only the CP required so as to minimize N excretion without losing animal productivity.

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Table 1. Effect of urea supplementation of a corn and corn-silage diet on milk yield and ruminal metabolism (Gressley, 2005).

	Diet		
Item	Low RDP	High RDP	Probability
Dietary CP, %	13.5	16.1	. . .
Dietary urea, %	0.0	1.0	. . .
Response			
Milk, kg/d	30.5	29.9	0.25
Protein, kg/d	0.98	0.96	0.25
Fat, kg/d	1.09	1.07	0.29
Rumen NH_3-N, mg/dl	3.2	7.9	0.001
Urinary PD,[1] mmol/d	469	491	0.11
In situ NDF,[2] %	25.0	27.5	0.02

[1]PD = Purine derivatives (allantoin plus uric acid).
[2]In situ digestion of soy hulls NDF in 24-h.

Table 2. Effect on ruminal microbial activity of supplementing two different forages—low energy hay and higher energy sugar beet pulp—with equal N from either urea or casein (Chikunya et al., 1996).

	Hay[1]		Sugar beet pulp[1]		Contrasts		
	Urea	Casein	Urea	Casein	Forage	N	F x N
Viable bacteria (x 10^8/ml)	1.35	1.27	7.41	16.91	< 0.001	< 0.01	< 0.01
Cellulolytic bacteria (x 10^7/ml)	4.14	5.15	4.70	4.83	NS	NS	NS
Protozoa (x 10^5/ml)	1.89	1.89	1.07	1.54	NS	NS	NS
Microbial N yield,[2] g/d	5.88	6.51	7.41	13.46	< 0.01	< 0.05	< 0.05

[1]Organic matter digestibilities estimated in situ were 32 and 51% for hay and sugar beet pulp. N contents of supplemented forages were 1.8 and 2.5% of DM for hay and sugar beet pulp.
[2]Computed from urinary excretion of purine derivatives (allantoin plus uric acid).

Table 3. Effect of processing on digestibility of corn and barley starch (Owens et al., 1986).

Processing Method	Proportion of Starch Digestion, % Rumen	Small Intestine	Large Intestine	Total tract
Cracked Corn	69	13	8	89
Ground Corn	78	14	4	94
Steam-Flaked Corn	83	16	1	98
High Moisture Corn	86	6	1	95
Ground Barley	94	. . .	. . .	. . .

Table 4. Effect of replacing alfalfa silage with corn silage in the diets of lactating dairy cows (Brito and Broderick, 2003).

Item	Alfalfa Silage/Corn Silage 100/0	74/26	47/53	21/79
Composition (% of DM)				
Alfalfa Silage	50.6	37.2	23.7	10.2
Corn Silage	0	13.3	26.7	40.0
Crude Protein	17.2	16.9	16.6	16.2
Production				
DM Intake (kg/d)	26.5[a]	25.9[a]	25.0[b]	23.2[c]
Milk Yield (kg/d)	41.5[a]	42.0[a]	41.5[a]	39.5[b]
Ruminal ammonia-N (mg/dl)	10.3[a]	10.0[a]	8.0[b]	4.7[c]

[a,b,c]Means in rows without common superscripts are different ($P < 0.05$).

Table 5. Effect of supplementing with urea or different sources of true protein on production and omasal protein flows in lactating dairy cows. Diets were composed principally of alfalfa and corn silages plus high moisture corn (Brito and Broderick, 2004).

	Supplemental protein[1]				
Item	Urea	SSBM	CSM	CM	*P* > F
CP, % of DM	16.5	16.5	16.6	16.6	
Production (kg/d)					
DM intake	22.1[c]	24.2[b]	24.7[ab]	24.9[a]	< 0.01
Milk	32.9[b]	40.0[a]	40.5[a]	41.1[a]	< 0.01
Milk protein	0.92[c]	1.23[ab]	1.18[b]	1.27[a]	< 0.01
Milk fat	1.01[c]	1.22[ab]	1.18[b]	1.29[a]	< 0.01
Omasal protein flows (g/d)					
Microbial protein	2344[b]	2706[a]	2706[a]	2775[a]	0.04
RUP	538[c]	987[b]	1348[a]	1150[ab]	<0.01
Total protein	2882[c]	3693[b]	4054[a]	3925[ab]	<0.01

[1]CM = canola meal; CSM = cottonseed meal; SSBM = solvent soybean meal.
[a,b,c]Means in rows without common superscripts are different (P < 0.05).