Effects of Different Protein Supplements on Omasal Nutrient Flow and Microbial Protein Synthesis in Lactating Dairy Cows¹

A. F. Brito,^{*2} G. A. Broderick,^{†3} and S. M. Reynal^{*}

^{*}Department of Dairy Science, University of Wisconsin, and

[†]Agricultural Research Service, USDA, US Dairy Forage Research Center, 1925 Linden Drive West, Madison, Wisconsin 53706

ABSTRACT

Eight ruminally cannulated Holstein cows that were part of a larger lactation trial were used in 2 replicated 4 × 4 Latin squares to quantify effects of supplementing protein as urea, solvent soybean meal (SSBM), cottonseed meal (CSM), or canola meal (CM) on omasal nutrient flows and microbial protein synthesis. All diets contained (% of dry matter) 21% alfalfa silage and 35% corn silage plus 1) 2% urea plus 41% high-moisture shelled corn (HMSC), 2) 12% SSBM plus 31% HMSC, 3) 14% CSM plus 29% HMSC, or 4) 16% CM plus 27% HMSC. Crude protein was equal across diets, averaging 16.6%. The CSM diet supplied the least rumen-degraded protein and the most rumen-undegraded protein. Microbial nonammonia N flow was similar among the true protein supplements but was 14% lower in cows fed urea. In vivo ruminal passage rate, degradation rate, and estimated escape for the 3 true proteins were, respectively, 0.044/h, 0.105/h, and 29% for SSBM; 0.051/h, 0.050/h, and 51% for CSM; and 0.039/h, 0.081/h, and 34% for CM. This indicated that CSM protein was less degraded because of both a faster passage rate and slower degradation rate. Omasal flow of individual AA, branched-chain AA, essential AA, nonessential AA, and total AA all were lower in cows fed urea compared with one of the true protein supplements. Among the 3 diets supplemented with true protein, omasal flow of Arg was greatest on CSM, and omasal flow of His was greatest on CSM, intermediate on CM, and lowest on SSBM. Lower flows of AA and microbial nonammonia N explained lower yields of milk yield and milk components observed on the urea diet in the companion lactation trial. These results clearly showed that supplement-

tation with true protein was necessary to obtain sufficient microbial protein and rumen-undegraded protein to meet the metabolizable AA requirements of high-producing dairy cows.

Key words: nonprotein nitrogen, true protein, omasal flow, microbial protein synthesis

INTRODUCTION

Ammonia is the main N source for microbial protein synthesis (Nolan, 1975; Aharoni et al., 1991) and is essential for the growth of several species of ruminal bacteria (Allison, 1970; Bryant, 1973). However, a large number of studies have clearly established that preformed AA, either as free AA, peptides, or soluble proteins, increases microbial growth, fiber digestion, or both (Cotta and Russell, 1982; Chikunya et al., 1996; Griswold et al., 1996; Atasoglu et al., 1999; Carro and Miller, 1999). Thus, feeding true protein supplements rather than NPN may result in an increased supply of microbial NAN for the synthesis of milk protein in the mammary gland. Feed protein supplements differ substantially in ruminal degradability and they supply different amounts of RUP with varying AA compositions (NRC, 2001). Because microbial protein is the major source of metabolizable AA to the lactating cow, the most effective RUP sources have AA profiles that are complementary to microbial protein (Broderick, 1994).

We conducted a lactation trial in which dairy cows were fed diets with equal CP that were supplemented with urea or 1 of 3 true proteins sources: solvent soybean meal (**SSBM**), cottonseed meal (**CSM**), or canola meal (**CM**; Brito and Broderick, 2007). Intake increased about 10% but yield of milk and milk components was 20 to 35% greater when cows were supplemented with true protein. The objectives of this companion study were to investigate the effects of these different CP supplements on omasal nutrient flow and microbial protein synthesis in lactating dairy cows. It was of particular interest to measure in vivo rates of ruminal passage and degradation for each true protein source to quantify the RUP contributed by SSBM, CSM, and CM.

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²Current address: Agriculture and Agri-Food Canada, PO Box 90, 2000 Route 108 East, Lennoxville, QC, Canada.

³Corresponding author: gbroderi@wisc.edu

MATERIALS AND METHODS

Animals and Diets

Eight ruminally cannulated multiparous Holstein cows averaging (mean $\pm$ SD) 118 ± 15 DIM and 624 ± 38 kg of BW that were part of a larger trial investigating the effects of different protein supplements on milk production of lactating dairy cows (Brito and Broderick, 2007) were blocked by DIM, and within square, randomly assigned to treatment sequences in 2 replicated 4×4 Latin squares. Treatment sequences within Latin squares were balanced for carryover effects in subsequent periods. Each period lasted 28 d and consisted of 14 d for diet adaptation and 14 d for data and sample collection. All diets contained (% of DM) 21% alfalfa silage, 35% corn silage, 1.6% minerals and vitamins, and one of the following protein supplements: urea (1.9%), SSBM (12.1%), CSM (14.1%), or CM (16.1%). Rolled high-moisture shelled corn was decreased from 40.7% on the urea diet to 26.5% on the CM diet. Contents of dietary CP were equal, averaging 16.6% across diets. Care and handling of the animals, including ruminal cannulation, was conducted as outlined in the guidelines of the University of Wisconsin institutional animal care and use committee. Other details of the feeding trial, including feed and diet composition, are described in detail in the companion report (Brito and Broderick, 2007).

Omasal Sampling and Laboratory Analyses

Spot samples of digesta leaving the rumen were collected through the omasal orifice from the ruminally cannulated cows using the omasal sampling technique developed by Huhtanen et al. (1997) and Ahvenjärvi et al. (2000), as adapted by Reynal and Broderick (2005). The procedures used to quantify digesta flow were essentially those described in detail by Brito et al. (2006). This method used 3 digesta markers: indigestible NDF (Huhtanen et al., 1994) for the omasal large particle phase (**LP**), YbCl_3 (Siddons et al., 1985) for the omasal small particle phase (**SP**), and CoEDTA (Udén et al., 1980) for the omasal fluid phase (**FP**). The triple-marker technique of France and Siddons (1986) was used to determine the proportions with which to recombine these 3 phases to produce omasal true digesta (**OTD**). Before marker infusion began, whole ruminal contents were taken from each cow to determine the background ^{15}N abundance. Mean of the 32 observations for background ^{15}N abundance was 0.36815% of N. The marker solution containing YbCl_3 , CoEDTA, and $^{15}\text{NH}_4\text{SO}_4$ with 10 atom percent excess (**APE**) ^{15}N (Isotec, Miamisburg, OH) was prepared as described earlier (Reynal and Broderick, 2005). Each cow was

given a priming dose of 4 L of this marker solution via the ruminal cannula and was then continuously infused with marker at a constant rate of 2.62 L/d (providing 2.01 g of Co, 2.88 g of Yb, and 0.21 g of ^{15}N /d) from d 21 to 26 using 2 syringe pumps (model no. 33; Harvard Apparatus, Inc., Holliston, MA). After 64 h of infusion, omasal samples were collected at twelve 2-h intervals over a 3-d period to represent a 24-h day. Sampling protocols, including confirming that sample tubes were correctly positioned in the omasal canal, sampling times and volumes, sample processing, isolation of fluid-associated bacteria (**FAB**), particle-associated bacteria (**PAB**; bacteria associated with LP + SP) and protozoa, digesta marker analyses, and preparation of OTD were as described by Reynal and Broderick (2005) and Brito et al. (2006). In addition, 50 mL of omasal digesta was collected at each of the 12 sampling times and strained through 2 layers of cheesecloth; 10 mL of this filtrate was pipetted into a 250-mL flask, followed by the addition of 0.2 mL of concentrated H_2SO_4 , to obtain a composite omasal fluid sample from each cow over each sampling period for later analysis of ^{15}N enrichment of ammonia.

Samples of OTD were analyzed for total N (Leco 2000; Leco Instruments, Inc., St. Joseph, MI), absolute DM, ash, and OM (AOAC, 1980), sequentially for NDF and ADF using heat-stable α -amylase and Na_2SO_3 (Hintz et al., 1995), and for neutral detergent insoluble nitrogen (**NDIN**) and ADIN. Extracts were prepared from OTD as follows: 10 mL of Na-citrate buffer (pH 2.2; 77.5 mM Na-citrate) was added to 0.5 g of freeze-dried OTD sample and then vortexed. After 30 min in a warm room (39°C), extracts were centrifuged ($15,000 \times g$, 15 min, 4°C) and supernatants stored at -20°C for later analysis of ammonia and total free AA (*o*-phthalaldehyde) using assays adapted to flow-injection (Broderick et al., 2004; Lachat Quik-Chem 8000 FIA; Lachat Instruments, Milwaukee, WI). True protein supplements were extracted with TCA (Licitra et al., 1996) and extracts were analyzed for N and NPN. Prior to AA analysis, OTD samples were hydrolyzed for 24 h at 110°C in sealed vials under a N_2 atmosphere using 6 N HCl with 0.1% wt/vol phenol (Mason et al., 1979). The ratio of sample N to acid was about 1 mg of total N per 5 mL of 6 N HCl. After hydrolysis, samples were cooled, HCl was removed by vacuum evaporation, and the residue was redissolved in pH 2.2-sample buffer containing norleucine as an internal standard. Analysis of individual AA was conducted using ion-exchange chromatography with ninhydrin detection (Beckman 6300 AA analyzer; Beckman Instruments, Inc., Palo Alto, CA).

Samples of FAB, PAB, protozoa, FP, and SP + LP were prepared for total NAN and ^{15}N analyses as follows: Approximately 100 μg of N from each sample was

weighed into tin capsules (Elemental Microanalysis Limited, Okehampton, UK) followed by addition of 50 μL of 72 mM K_2CO_3 . Capsules were placed in 96-well microtiter plates and dried in a 60°C oven overnight to volatilize ammonia. Samples of ruminal digesta collected prior to infusion for determination of background abundance of ^{15}N were freeze-dried, ground to pass through a 1-mm Wiley mill screen (Arthur H. Thomas Co., Philadelphia, PA) and then a 0.5-mm Udy mill screen (Udy Corporation, Fort Collins, CO). Samples were analyzed for total N and ^{15}N using a Carlo-Erba instrument interfaced to an isotope ratio mass spectrometer (University of California-Davis Stable Isotope Facility). Bacteria (FAB and PAB), protozoa, FP, and SP + LP also were analyzed for absolute DM, ash, and OM (AOAC, 1980). Enrichment of ^{15}N (^{15}N APE), flow of NAN and OM in FAB and PAB, nonammonia nonmicrobial N (NANMN) and RUP, OM truly digested in the rumen (OMTDR), and microbial efficiency (g of NAN/kg of OMTDR) were computed as described previously (Brito et al., 2006).

Ammonia for determination of ^{15}N enrichment was isolated by diffusion (Brooks et al., 1989) as follows. Composite omasal fluid samples were thawed, mixed well, and then centrifuged ($20,000 \times g$, 20 min, 4°C). With a paper punch, 7-mm-diameter disks of Whatman GD/D filter paper were cut and then pierced with 62-mm lengths of stainless-steel wire. Each filter paper disk was impregnated with 20 μL of 5 M H_2SO_4 and the wire with the acidified paper was suspended inside of a 100-mL specimen container containing 1 mL of the omasal fluid supernatant plus 1 mL of 10 N NaOH and then tightly capped. Specimen containers were left at room temperature for 6 d. After this period, filter paper disks were removed from the wire, put in separate wells of a 96-well microtiter plate, which was then placed inside a desiccator, and the disks were dried overnight over concentrated H_2SO_4 . Using tweezers, each filter disk was transferred to a tin capsule and sent for analysis of N and ^{15}N as described above.

The ruminal passage rates of SSBM, CSM, and CM were estimated from the decline in omasal concentration of La previously adsorbed on each protein. A 500-g sample of each protein supplement was soaked for 24 h at room temperature in 5 L of 0.1 N acetic acid solution containing 5 mg of La-acetate (Alfa Aesar, Ward Hill, MA) per mL. The labeled feed was then pressed through a 45- μm pore size Dacron mesh (Sefar America Inc., Depew, NY) and soaked in a 0.01 N acetic acid solution for 3 h while stirring every 20 min to remove the unbound or loosely bound La. Each feed was then washed with distilled water to remove the acetic acid, pressed as before, and dried at 60°C for about 48 h. On d 21 of each period, cows were pulse-dosed prefeeding through

the ruminal cannula with 500 g of La-labeled protein supplement. The labeled protein supplements were mixed thoroughly with ruminal contents and 200 mL of the omasal digesta samples were collected at 0 (pre-feeding), 2, 4, 6, 8, 12, 18, and 24 h postfeeding. Omasal samples were stored at -20°C, thawed, dried at 105°C for about 48 h, ground through a 1-mm Wiley mill screen, and analyzed for La concentration by direct current plasma emission spectroscopy as described by Reynal and Broderick (2005).

Omasal NANMN flow originating from the dietary ingredients was computed as

$$\begin{aligned} \text{total NANMN, g/d} &= \text{omasal NAN flow,} \\ &\text{g/d} - \text{total microbial NAN flow, g/d.} \end{aligned}$$

Except for supplemental CP, the urea diet contained the same basal ingredients as the other diets. Therefore, the proportion of NAN from basal ingredients escaping the rumen on the urea diet was computed as

$$\begin{aligned} \text{basal}_{\text{urea}}, \% \text{ of NAN intake} &= [\text{total NAN MN flow} \\ &(\text{urea diet}) \div \text{total NAN intake (urea diet)}] \\ &\times 100 = (74/375) \times 100 = 19.7\%. \end{aligned}$$

The DMI was 2.3 to 3.6 kg/d greater on diets containing true protein; however, the proportion of omasal NANMN originating from basal ingredients was assumed to be the same on all diets. Thus, omasal NANMN contributed by basal ingredients on true protein diets was computed as

$$\text{basal NANMN, g/d} = \text{basal NAN intake, g/d} \times 0.197.$$

Ruminal escape of NAN from each true protein supplement was computed as

$$\begin{aligned} \text{true protein NANMN, g/d} &= \text{total NANMN,} \\ &\text{g/d} - \text{basal NANMN, g/d;} \\ \text{true protein escape (RUP), \%} &= (\text{true protein NANMN,} \\ &\text{g/d} \div \text{true protein NAN intake, g/d}) \times 100. \end{aligned}$$

If the ruminal passage rate (k_p) and the proportion of in vivo ruminal escape are known for a protein, then in vivo ruminal degradation rate (k_d) may be computed using the equation (Reynal and Broderick, 2003)

$$k_d = [(\text{fraction B} \times k_p) \div (\text{RUP} - \text{fraction C})] - k_p,$$

where k_p was either the ruminal passage rate of La adsorbed onto each protein supplement or was set equal

to 0.06/h; fraction B is total N – NPN – ADIN for each protein supplement; fraction C is ADIN in each protein supplement; and fractions B and C and RUP are percents of total N.

Statistical Analysis

Data were analyzed using the MIXED procedures of SAS (SAS Institute, 1999–2000) for a replicated 4 × 4 Latin square design according to the following model:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, S_i is the effect of square i , P_j is the effect of period j , $C_{k(i)}$ is the effect of cow k (within square i), T_l is the effect of treatment l , ST_{il} is the interaction between square i and treatment l , and E_{ijkl} is the residual error. All terms were considered fixed, except $C_{k(i)}$ and E_{ijkl} , which were considered random. The interaction term was removed from the model when $P > 0.25$. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. All reported values are least squares means. Passage rates were estimated by regressing the log of omasal concentration of La on time of sampling using a nonlinear method for determining slope (Littell et al., 1996; SAS Institute, 1999–2000); these slopes were equated to rates of passage (per hour) of each true protein supplement.

RESULTS AND DISCUSSION

Omasal Nutrient Flows and Ruminal Digestibilities

Intakes of DM ($P = 0.01$) and OM ($P = 0.03$) in cows fed urea were, respectively, 12 and 10% lower than the mean for cows fed true protein supplements (Table 1), which approximated the magnitude of the differences observed in the lactation study (Brito and Broderick, 2007). Cows fed CM consumed 1.3 kg/d more DM than those fed SSBM but, unlike when all 24 cows were used in the analysis (Brito and Broderick, 2007), this difference was not significant, reflecting the lower statistical power with fewer animals. Dry matter and OM entering the omasal canal paralleled intakes and also were lower on urea ($P < 0.01$) and did not differ among diets supplemented with true protein. Dry matter and OM apparently digested in the rumen were similar, averaging 9.3 and 10 kg/d across diets. However, DM and OM digestion as proportions of intake were both greater ($P < 0.01$) on the urea diet. Among diets supplemented with true protein, DM and OM apparently digested in the rumen were highest on SSBM, intermediate on CM, and lowest on CSM. No difference was observed for the amount of OMTDR, which averaged 14.7

kg/d (Table 1). However, OMTDR as a proportion of intake was 5 percentage units greater ($P < 0.01$) on the urea diet. Cows fed SSBM also had the highest proportion of OMTDR among diets supplemented with the true protein. Overall, OMTDR averaged 65% of OM intake in the present study and was within the expected range of 40 to 70% (Titgemeyer, 1997).

Intake of NDF was lower ($P < 0.01$; Table 1) on urea and SSBM than on CSM and CM, reflecting both DMI and dietary NDF content (Brito and Broderick, 2007). Omasal NDF flow paralleled intake, although it was significantly greater for CM than CSM. Apparent ruminal NDF digestibility, expressed either as the amount or proportion of NDF intake, did not differ and averaged, respectively, 1.8 kg/d and 32% across diets. Robinson et al. (1998) also observed no differences in forestomach NDF digestibility when comparing urea and SSBM as protein supplements for lactating dairy cows. Acid detergent fiber intake was significantly lower ($P < 0.01$) on the urea diet than on the SSBM, CSM, and CM diets (Table 1). Although ADF contents of the urea and SSBM diets were very similar (Brito and Broderick, 2007), cows fed SSBM consumed more ADF because of the greater DMI. Among the diets supplemented with true protein sources, cows offered either CSM or CM consumed 0.5 kg/d more ADF ($P < 0.01$) than those offered SSBM, as a result of the greater ADF content (Brito and Broderick, 2007) in CSM and CM. No significant differences in omasal ADF flows were observed between urea and SSBM, but ADF flow was significantly greater ($P < 0.01$) on CSM and CM. Acid detergent fiber apparently digested in the rumen, expressed either as the amount or percentage of ADF intake averaged, respectively, 1.0 kg/d and 31% and did not differ across diets.

Omasal Flow of Nitrogen Fractions and Microbial Protein Synthesis

Omasal flow of ammonia N was 18% higher ($P < 0.01$) on urea and CM than on SSBM and CSM (Table 2). That CM was similar to urea rather than SSBM and CSM was unexpected because the 3 true proteins had similar ruminal ammonia concentrations (Brito and Broderick, 2007); however, ammonia accounted for <2% to total omasal N flow. Total free AA N entering the omasal canal was greatest on SSBM, intermediate on CSM and CM, and lowest on the urea diet (Table 2). Free AA N contributed 10% of the total omasal N flow. Reynal et al. (2005b) reported that soluble AA accounted for 9 to 16% of total AA flow from the rumen. Omasal flows of both NDIN and ADIN reflected their dietary contents (Brito and Broderick, 2007) and were lower ($P < 0.01$) on urea and SSBM than on CSM and

Table 1. Effects of different protein supplements on intake, nutrient flow at the omasal canal, and ruminal digestibility

Item	Diet ¹				SED ²	<i>P</i> > <i>F</i> ³
	Urea	SSBM	CSM	CM		
DM						
Intake, kg/d	22.4 ^b	24.7 ^a	25.2 ^a	26.0 ^a	1.00	0.01
Omasal flow, kg/d	13.1 ^b	15.2 ^a	16.4 ^a	16.4 ^a	0.68	<0.01
Apparently digested in the rumen						
kg/d	9.27	9.43	8.79	9.59	0.48	0.41
% of DMI	41.7 ^a	38.3 ^b	34.7 ^c	36.7 ^{bc}	1.10	<0.01
OM						
Intake, kg/d	20.9 ^b	22.8 ^a	23.2 ^a	23.9 ^a	0.92	0.03
Omasal flow, kg/d	10.8 ^b	12.6 ^a	13.5 ^a	13.5 ^a	0.57	<0.01
Apparently digested in the rumen						
kg/d	10.1	10.3	9.7	10.4	0.50	0.58
% of OM intake	48.6 ^a	45.1 ^b	41.7 ^c	43.4 ^{bc}	1.04	<0.01
Truly digested in the rumen						
kg/d	14.2	14.9	14.6	15.1	0.67	0.56
% of OM intake	68.5 ^a	65.5 ^b	62.6 ^c	63.3 ^c	1.01	<0.01
NDF						
NDF intake, kg/d	5.05 ^b	5.56 ^b	6.10 ^a	6.48 ^a	0.25	<0.01
NDF flow, kg/d	3.40 ^c	3.59 ^c	4.20 ^b	4.63 ^a	0.21	<0.01
Apparently digested in the rumen						
kg/d	1.65	1.97	1.91	1.85	0.19	0.42
% of NDF intake	33.0	35.5	31.2	28.6	2.81	0.12
ADF						
ADF intake, kg/d	2.83 ^c	3.17 ^b	3.55 ^a	3.82 ^a	0.15	<0.01
ADF flow, kg/d	1.90 ^b	2.10 ^b	2.53 ^a	2.74 ^a	0.14	<0.01
Apparently digested in the rumen						
kg/d	0.93	1.07	1.02	1.08	0.13	0.64
% of ADF intake	32.7	34.2	29.0	28.3	3.27	0.25

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹SSBM = solvent soybean meal; CSM = cottonseed meal; CM = canola meal.

²SED = standard error of the least squares means difference.

³Probability of a significant effect of diet.

CM (Table 2). The flow of fraction B₃ (NDIN – ADIN) of the Cornell Net Carbohydrate and Protein System model (Sniffen et al., 1992) did not differ and averaged 11 g/d across diets (Table 2).

Nonammonia N entering the omasal canal averaged 27% less ($P < 0.01$) on the urea diet than on the SSBM, CSM, and CM diets (Table 2). This difference was expected because true protein intake and flow of RUP and microbial protein all were greater in cows supplemented with true protein (Table 2). Omasal flow of NANMN was 59% lower ($P < 0.01$) on urea compared with SSBM, CSM, and CM (Table 2), indicating that the true protein sources accounted for most of the RUP. Among the diets supplemented with true protein, NANMN flow was 28 and 17% greater ($P < 0.01$) in cows fed CSM than in cows fed SSBM and CM. As proportions of N intake and NAN flow, NANMN flow was lowest on urea, intermediate on SSBM and CM, and greatest on CSM (Table 2). These data indicated that CSM supplied more RUP than the other true proteins.

Omasal flows of microbial NAN contributed by FAB, PAB, and FAB plus PAB (total microbial flow) were

quantified using ¹⁵N enrichment and are reported in Table 2. The flow of FAB NAN was highest ($P < 0.01$) on CM, intermediate on SSBM, and lowest on urea and CSM, possibly because of a shortage of amino N (AA, peptides, and soluble protein). Urea, of course, gives rise only to ammonia, and the CSM diet contributed the least RDP (Table 2). Reynal and Broderick (2005) observed a linear reduction in FAB NAN flow when the RDP supply decreased from 3,076 to 2,403 g/d. Omasal flow of PAB NAN was 17% lower ($P = 0.03$) on urea than on SSBM, CSM, and CM but not different among the true proteins (Table 2). Supplying RDP as free AA and peptides has been shown to improve microbial growth and efficiency in a large number of reports (Cotta and Russell, 1982; Chikunya et al., 1996; Griswold et al., 1996; Atasoglu et al., 1999; Carro and Miller, 1999). Across diets, FAB and PAB NAN contributed, respectively, 45 and 55% of total microbial NAN flow (Table 2). Somewhat greater proportions of PAB NAN in total microbial NAN flow were found in our earlier work (Reynal and Broderick, 2005; Brito et al., 2006; Brito et al., 2007), which was consistent with the observation that 70 to 80% of microbial OM in whole ruminal

Table 2. Effects of different protein supplements on omasal flow of nitrogen fractions

Item ¹	Diet ²				SED ³	<i>P</i> > <i>F</i> ⁴
	Urea	SSBM	CSM	CM		
N intake, g/d	590 ^b	653 ^a	671 ^a	695 ^a	27	<0.01
RDP supply ⁵						
g/d	3,148 ^a	3,062 ^{ab}	2,845 ^b	3,192 ^a	129	0.05
% of DMI	14.0 ^a	12.2 ^b	11.2 ^c	12.3 ^b	0.35	<0.01
Flow at omasal canal						
Total N, g/d	461 ^b	598 ^a	649 ^a	628 ^a	35	<0.01
Total N, % of N intake	78.2 ^c	91.5 ^b	97.6 ^a	90.6 ^b	2.17	<0.01
Ammonia N, g/d	12.1 ^a	10.4 ^b	9.40 ^b	12.1 ^a	0.71	<0.01
Total free AA N, g/d	49.4 ^b	61.9 ^a	53.7 ^{ab}	57.8 ^{ab}	4.10	0.04
NDIN, g/d	14.3 ^c	14.4 ^c	21.4 ^b	27.7 ^a	1.78	<0.01
ADIN, g/d	4.02 ^c	3.57 ^c	9.40 ^b	17.7 ^a	1.09	<0.01
B ₃ , ⁶ g/d	10.2	10.8	12.0	10.0	1.59	0.61
NAN, g/d	449 ^b	587 ^a	639 ^a	616 ^a	35	<0.01
NAN, % of N intake	76.1 ^c	89.9 ^b	96.2 ^a	88.8 ^b	2.15	<0.01
NANMN, g/d	73.9 ^c	159 ^b	206 ^a	172 ^b	17	<0.01
NANMN, % of NAN	16.7 ^c	25.3 ^b	32.6 ^a	27.6 ^b	2.0	<0.01
NANMN, % of N intake	12.7 ^c	24.5 ^b	31.3 ^a	24.5 ^b	1.92	<0.01
RUP ⁵						
g/d	538 ^c	1,061 ^b	1,348 ^a	1,150 ^{ab}	106	<0.01
% of DMI	2.42 ^c	4.31 ^b	5.39 ^a	4.40 ^b	0.32	<0.01
FAB NAN						
g/d	174 ^b	195 ^{ab}	181 ^b	206 ^a	11	0.04
% of total microbial NAN	46.6	45.2	42.2	46.1	1.80	0.10
PAB NAN						
g/d	202 ^b	238 ^a	253 ^a	238 ^a	18	0.03
% of total microbial NAN	53.4	54.8	57.8	53.9	1.80	0.10
Total microbial NAN						
g/d	375 ^b	433 ^a	433 ^a	444 ^a	21	0.02
% of total NAN	83.3 ^a	74.7 ^b	67.4 ^c	72.4 ^b	2.01	<0.01
Microbial N flow, ⁷ g/d	238	271	300	219	31	0.08
Microbial efficiency						
g of NAN/kg of OMTDR	26.3 ^b	29.0 ^a	29.7 ^a	29.5 ^a	0.76	<0.01

^{a-c}Means within a row with different superscripts differ (*P* < 0.05).

¹FAB = fluid-associated bacteria, NANMN = nonammonia nonmicrobial N, NDIN = neutral detergent insoluble N, OMTDR = organic matter truly digested in the rumen, PAB = particle-associated bacteria.

²SSBM = solvent soybean meal; CSM = cottonseed meal; CM = canola meal.

³SED = standard error of the least squares means difference.

⁴Probability of a significant effect of diet.

⁵Estimated in vivo using omasal sampling.

⁶B₃ = NDIN flow (g/d) – ADIN flow (g/d).

⁷Estimated from urinary excretion of purine derivatives according to Vagnoni et al. (1997).

contents was associated with the particulate phase (Craig et al., 1987). However, Hristov and Broderick (1996) reported similar contributions from PAB and FAB, despite a greater ruminal pool of PAB, because of 3 times more rapid outflow of the fluid phase. Olmos Colmenero and Broderick (2006) observed a slightly greater contribution from FAB to total microbial NAN flow.

Total microbial NAN flow at the omasum was similar on the true proteins, averaging 437 g/d, which was 62 g/d greater (*P* = 0.02) than on urea (Table 2). Reynal and Broderick (2005) observed a linear increase (*P* < 0.01) in microbial NAN flow when RDP supply from true protein increased from 10.6 to 13.2% of dietary DM. Brito et al. (2006, 2007) found that a greater RDP

supply was associated with increased microbial NAN flow. Branched-chain VFA (isobutyrate, isovalerate, and 2-methyl butyrate) are important growth factors for cellulolytic bacteria (Russell and Sniffen, 1984; Hoover, 1986). Although the urea diet supplied 14% of RDP, ruminal isobutyrate was depressed relative to the SSBM and CM diets (Brito and Broderick, 2007). Brito et al. (2007) observed that the higher microbial NAN flow on alfalfa silage vs. red clover silage diets was associated with greater ruminal concentrations of isobutyrate and isovalerate. Moreover, a linear decrease in microbial NAN flow when corn silage incrementally replaced alfalfa silage (Brito et al., 2006) was accompanied by a linear decline in ruminal isobutyrate concentrations (Brito and Broderick, 2006). Greater (*P* < 0.01;

Table 2) RDP supply on SSBM and CM than on CSM did not increase microbial NAN flow from the rumen, indicating that 11.2% RDP in diets supplemented with true protein was adequate to support ruminal microbial growth.

Tagari et al. (1995) reported no significant difference in microbial N flow from the rumen among urea, SSBM, and CSM. Robinson et al. (1998) observed similar flows of bacterial NAN in cows fed either urea or SSBM, which averaged, respectively, 179 and 174 g/d; duodenal flow of protozoal NAN also did not differ. However, it is important to note that a lack of significance in microbial flows (bacteria plus protozoa) in research reported by Robinson et al. (1998) may have been related to use of diaminopimelic acid as a bacterial marker and phosphatidylcholine as a protozoal marker. The principal shortcoming of diaminopimelic acid is that much of it is associated not only with intact bacteria in the rumen but also with bacterial cell wall fragments, peptides, and as a free compound (Broderick and Merchen, 1992). Phosphatidylcholine was suggested by John and Ulyatt (1984) as a protozoal marker because it is widely distributed in protozoa and was not detected in ruminal bacteria. However, interference from dietary (Galliard, 1973; Neill et al., 1979) and endogenous phosphatidylcholine (Dawson et al., 1981) may preclude its use. Song and Kennelly (1989), assuming a constant ratio of microbial N:RNA, reported lower duodenal flow of microbial N when lactating cows were fed barley silage supplemented with CM rather than urea. However, this ratio is unlikely to be constant under widely varying conditions and in the different pools of ruminal microbes. Therefore, the discrepancy between literature reports and the current study is at least partly related to the methodology used to quantify microbial protein synthesis in the rumen.

In cows fed urea, 83% of the total NAN flow was derived from total microbial NAN, a greater ($P < 0.01$) proportion than found in cows fed true protein supplements; this was expected because RUP flow was 648 g/d lower on urea (Table 2). The proportion of microbial NAN in total NAN on those 3 true protein diets ranged from 67% (CSM) to 75% (SSBM) and was significantly lower in cows fed CSM, the diet with the greatest amount of RUP. Robinson et al. (1998) reported that microbial N provided 67 or 61% of total NAN on diets supplemented with urea or SSBM, whereas data from Song and Kennelly (1989) indicated that 50 or 54% of the total NAN was derived from microbial N when cows were supplemented with CM or urea on barley silage diets. As discussed, microbial protein synthesis may have been underestimated in both studies. Moreover, lower intake of fermentable energy in these 2 trials

than in the current study may have restricted microbial protein synthesis and its contribution to total NAN flow.

Microbial efficiency (microbial NAN/OMTDR) was 11% lower ($P < 0.01$) on urea than on the SSBM, CSM, and CM diets (Table 2). Lower microbial NAN and lower growth efficiency were associated with depressed conversion of feed N to milk N when cows were fed urea (Brito and Broderick, 2007). This was expected because any excess ammonia produced from urea hydrolysis that is not captured by ruminal microbes is absorbed from the stomach and largely excreted in urine. No differences were observed in microbial efficiency among the true protein sources; however, cows fed CSM had lower conversion of dietary N to milk N than cows fed SSBM (Brito and Broderick, 2007). This might have been due to the AA profile of RUP from CSM not being complementary to that of microbial protein, resulting in lower milk and protein yields, as was discussed in our companion report (Brito and Broderick, 2007).

Omasal AA flows are presented in Table 3. Lower flows of individual AA, branched-chain AA, essential AA, nonessential AA, and total AA ($P < 0.01$), and Cys ($P = 0.06$), in cows fed urea compared with the true proteins, explained the depressed yields of milk and milk components on that diet (Brito and Broderick, 2007). Omasal flow of most essential AA did not differ among the SSBM, CSM, and CM diets. However, omasal flow of His was greatest ($P < 0.01$) on CSM, intermediate on CM, and lowest on SSBM, whereas Arg flow was greater ($P < 0.01$) on CSM than on the remaining 2 diets supplemented with true protein. Literature reports (Clark et al., 1987; Coppock et al., 1987; Calhoun et al., 1995; Blackwelder et al., 1998) have indicated that low Lys content and availability might compromise use of CSM for lactating dairy cows. In fact, we observed that cows fed CSM had lower milk protein content than those fed SSBM and CM and had lower milk protein yield than cows fed CM (Brito and Broderick, 2007). However, omasal Lys flow and the Lys:Met ratio did not differ, averaging 197 g/d and 2.79, respectively, on diets supplemented with true protein (Table 3). Reaction of gossypol with Lys residues in CSM protein may limit intestinal Lys absorption (Craig and Broderick, 1981; Calhoun et al., 1995), and omasal Lys flow may not reflect its actual contribution to metabolizable Lys. Although we speculated that the gossypol-Lys interaction was not a major factor accounting for the lower protein yield on CSM in our lactation study (Brito and Broderick, 2007), a Lys:Met ratio of <3.0 suggested that Lys rather than Met could have been the limiting AA.

NRC (2001) Model Predictions

Microbial protein synthesis among diets predicted by the NRC (2001) model averaged 330 g/d, 22% lower

Table 3. Effects of different protein supplements on omasal flow of individual AA and branched-chain, essential, nonessential, and total AA

Item	Diet ¹				SED ²	<i>P</i> > <i>F</i> ³
	Urea	SSBM	CSM	CM		
AA, g/d						
Asp	260 ^b	366 ^a	370 ^a	373 ^a	17.9	<0.01
Thr	120 ^b	164 ^a	167 ^a	177 ^a	8.38	<0.01
Ser	110 ^b	161 ^a	166 ^a	172 ^a	9.17	<0.01
Glu	366 ^c	521 ^b	576 ^a	548 ^{ab}	28.2	<0.01
Pro	97.7 ^c	132 ^b	136 ^b	153 ^a	7.52	<0.01
Gly	122 ^b	166 ^a	171 ^a	182 ^a	8.50	<0.01
Ala	165 ^b	218 ^a	219 ^a	234 ^a	10.6	<0.01
Cys	20.6	36.2	52.1	38.8	11.1	0.06
Val	138 ^b	188 ^a	188 ^a	199 ^a	11.4	<0.01
Met	50.9 ^b	67.9 ^a	70.3 ^a	73.9 ^a	3.66	<0.01
Ile	111 ^b	149 ^a	147 ^a	152 ^a	8.25	<0.01
Leu	200 ^b	274 ^a	273 ^a	290 ^a	14.0	<0.01
Tyr	114 ^b	145 ^a	147 ^a	151 ^a	8.63	<0.01
Phe	121 ^b	165 ^a	173 ^a	173 ^a	9.53	<0.01
His	44.0 ^c	62.1 ^b	68.4 ^a	67.5 ^{ab}	3.14	<0.01
Lys	147 ^b	194 ^a	196 ^a	201 ^a	10.4	<0.01
Arg	104 ^c	154 ^b	192 ^a	163 ^b	10.5	<0.01
Lys:Met	2.85	2.85	2.78	2.74	0.08	0.47
BCAA ⁴	448 ^b	611 ^a	608 ^a	640 ^a	32.4	<0.01
EAA ⁵	1,035 ^b	1,418 ^a	1,474 ^a	1,496 ^a	74.7	<0.01
NEAA ⁶	1,251 ^b	1,745 ^a	1,836 ^a	1,848 ^a	88.8	<0.01
TAA ⁷	2,286 ^b	3,163 ^a	3,310 ^a	3,343 ^a	161	<0.01

^{a-c}Means within a row with different superscripts differ (*P* < 0.05).

¹SSBM = solvent soybean meal; CSM = cottonseed meal; CM = canola meal.

²SED = standard error of the least squares means difference.

³Probability of a significant effect of diet.

⁴BCAA = branched-chain AA (Val, Ile, and Leu).

⁵EAA = essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val).

⁶NEAA = nonessential AA (Ala, Asp, Cys, Glu, Gly, Pro, Ser, and Tyr).

⁷TAA = total AA.

than the mean of 421 g/d measured using ¹⁵N (Table 4). However, the NRC (2001) predictions paralleled in vivo measurements, indicating a 10% reduction in microbial protein on urea, compared with the 14% reduction that was measured in vivo on the true protein diets. Underprediction of microbial protein synthesis by the NRC model may be related to total purines (Zinn and Owens, 1986) being the primary microbial marker used for the microbial protein measurements in the NRC database. Low purine recoveries (Makkar and Becker, 1999; Obispo and Dehority, 1999) may have contributed to underestimates of microbial protein in many of the studies used in the NRC database. Microbial protein synthesis also was underpredicted by the NRC (2001) model in previous studies in which the same methods were used as in the current trial (Reynal et al., 2005a; Brito et al., 2006, 2007). Furthermore, many factors that influence microbial protein synthesis, such as form of N available in the rumen, source and amount of carbohydrate, amount and source of dietary fat, and ruminal pH and dilution rate, are not considered in the

NRC (2001) model and can account for discrepancies between predicted and in vivo observations.

Estimates of microbial NAN flow based on urinary excretion of purine derivatives (PD) averaged 39% lower than those measured using ¹⁵N and appeared to be unreliable in the present study. For example, PD excretion suggested that microbial protein supply on the urea diet was numerically greater than that on the CM diet (Table 4). Perez et al. (1997) reported that urinary PD excretion consistently underestimated microbial N flow compared with using purines or ¹⁵N as microbial markers in duodenal digesta. Despite underestimation, regressions of PD on ¹⁵N measurements reported in the literature indicated good relationships among methods, indicating that PD excretion was sensitive to significant dietary effects on microbial protein synthesis in the rumen (Perez et al., 1996; Reynal et al., 2005a). It was not apparent why PD excretion was unreliable in the present trial.

The NRC (2001) model underpredicted RDP supply and overpredicted RUP flow by 7 and 17%, respectively,

Table 4. Comparisons of flows of nitrogen fractions measured in vivo with those predicted by the NRC (2001) model

Item	Diet ¹			
	Urea	SSBM	CSM	CM
Microbial NAN flow, g/d				
Measured (using ¹⁵ N)	375	433	433	444
Estimated (using PD ²)	238	271	300	219
Predicted ³	305	334	336	345
RDP supply, g/d				
Measured (using ¹⁵ N)	3,148 ^a	3,062 ^{ab}	2,845 ^b	3,192 ^a
Predicted	2,936	2,720	2,700	2,975
RUP flow, g/d				
Measured (using ¹⁵ N)	538 ^c	1,061 ^b	1,348 ^a	1,150 ^{ab}
Predicted	708	1,369	1,524	1,345
Lys flow, g/d				
Measured (using ¹⁵ N)	147 ^b	194 ^a	196 ^a	201 ^a
Predicted	158	208	202	208
Met flow, g/d				
Measured (using ¹⁵ N)	50.9 ^b	67.9 ^a	70.3 ^a	73.9 ^a
Predicted	50.0	59.0	62.0	63.0
Metabolizable Lys, g/d				
Calculated ⁴	111	159	159	162
Predicted	126	171	164	164
Metabolizable Met, g/d				
Calculated	39.0	55.0	58.0	59.0
Predicted	40.0	48.0	51.0	49.0
Metabolizable Lys:Met ratio				
Calculated	2.85	2.87	2.76	2.78
Predicted	3.18	3.56	3.23	3.33

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹SSBM = solvent soybean meal; CSM = cottonseed meal; CM = canola meal.

²Microbial NAN flow estimated from urinary excretion of purine derivatives (PD) according to Vagnoni et al. (1997).

³Predicted from the NRC (2001) model.

⁴Calculated from in vivo omasal flow of Lys and Met using the intestinal digestibility coefficients of the NRC (2001) model.

with the greatest discrepancy found for the SSBM diet (Table 4). Duodenal Lys flows predicted by the NRC (2001) model differed by only 5% from omasal Lys flows measured in vivo, and values were more similar on the CSM and CM diets, which had greater protein flows. However, the relationship was not as good for Met. Although predicted and measured Met flows differed by only 1 g/d on the urea diet, discrepancies ranged from 8 to 11 g/d, underestimates of 12 to 15%, on the 3 diets supplemented with true protein. According to the NRC (2001) model, optimal protein utilization for maintenance and milk protein secretion requires Lys and Met to contribute, respectively, 7.2 and 2.4% of the MP absorbed at the intestine, a Lys:Met ratio of 3:1. The ratio of Lys:Met in MP computed from omasal flow measurements averaged 2.8:1, whereas the mean predicted by the NRC (2001) model was 3.3:1. Thus, omasal flow data suggested that Lys was the limiting AA, whereas Met appeared to be limiting based on NRC (2001) predictions.

Rates of Protein Degradation

Extent of protein degradation is the resultant of the rates of passage and degradation, and both were determined in vivo (Table 5). Fractions A (NPN) and C (ADIN) were small in all 3 true proteins, and fraction B, representing the proportion of degradable true protein, varied from 94.5 (CM) to 98.6% (SSBM) of total N. No significant differences were observed in total NAN intake (NAN intake from basal ingredients plus the true protein supplement) among the SSBM, CSM, and CM diets. Nonammonia N intake from the basal ingredients also did not differ and averaged 383 g/d across diets. However, NAN intake contributed by the true protein supplement differed; cows on CM consumed 24 g/d more NAN ($P = 0.05$) than those fed SSBM or CSM.

In vivo passage rates were measured from ruminal disappearance of the external marker La that was adsorbed on the true protein supplements. No significant difference in passage rate was observed for these proteins in the present trial (mean $k_p = 0.044$), although CSM had numerically the most rapid rate (Table 5). Reynal and Broderick (2003) estimated SP passage rates in vivo from the disappearance of Yb infused directly into the rumen to mark small particles and observed rates that ranged from 0.12 to 0.14/h. These authors expected that protein supplements would flow with the SP. However, they observed mean passage rates for the liquid marker CoEDTA of 0.14/h compared with 0.13/h for Yb, suggesting that both FP and SP leave the rumen at similar rates. That La adsorbed directly onto protein supplements in the present trial left the rumen at only one-third the rate of Yb suggested that SP passage did not describe ruminal outflow of the protein supplements and would lead to overestimation of protein escape.

Escape of protein supplements averaged 29, 51, and 34% for SSBM, CSM, and CM, respectively (Table 5). These results show that both SSBM and CM were extensively degraded in the rumen, whereas more than half of the CSM protein escaped degradation. Reynal and Broderick (2003) estimated a 27% ruminal escape for SSBM protein. Satter (1986) reported in vivo estimates of ruminal protein escape for SSBM, solvent-extracted CSM, and CM of, respectively, 27% (10 estimates; range 10 to 61%), 41% (3 estimates; range 24 to 61%), and 23% (1 study). Although these reports agree with our result for SSBM, differences of 10 and 11 percentage units for CSM and CM may be related to the processing methods used in manufacturing these meals. Solvent-extracted CSM is more extensively degraded than prepress solvent or expeller CSM (Broderick and Craig, 1980), and our estimate for CSM falls within the range reported by Satter (1986). According

Table 5. Rate and extent of ruminal degradation of protein supplements determined in vivo

Item	Diet ¹				SED ²	P > F ³
	Urea	SSBM	CSM	CM		
Protein fraction, % of total N						
Fraction A (NPN)	(100)	1.27	1.04	1.50	—	—
Fraction B	—	98.6	96.9	94.5	—	—
Fraction C (ADIN)	—	0.14	2.09	3.97	—	—
NAN intake and flow, g/d						
Total intake	376 ^b	632 ^a	641 ^a	666 ^a	25	<0.01
True protein intake	—	252 ^b	255 ^b	277 ^a	10	0.05
Basal intake	376	381	386	389	16	0.86
Omasal flow	449 ^b	581 ^a	639 ^a	616 ^a	33	<0.01
Microbial flow	375 ^b	433 ^a	433 ^a	444 ^a	21	0.02
Omasal nonammonia nonmicrobial N						
Total, ⁴ g/d	74 ^c	148 ^b	206 ^a	172 ^b	17	<0.01
Basal _{urea} , ⁵ % of NAN intake	19.7	—	—	—	—	—
Basal, ⁶ g/d	(74)	75	76	77	—	—
True protein, ⁷ g/d	—	73	130	95	—	—
Degradation estimates						
Ruminal escape, ⁸ %	—	29	51	34	—	—
Passage rate (k _p), /h	—	0.044	0.051	0.039	0.69	0.23
Degradation rate (in vivo), ⁹ /h	—	0.105	0.050	0.081	—	—
Degradation rate (0.06), ⁹ /h	—	0.145	0.054	0.105	—	—

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹SSBM = solvent soybean meal; CSM = cottonseed meal; CM = canola meal.

²SED = standard error of the least squares means difference.

³Probability of a significant effect of diet.

⁴Total nonammonia nonmicrobial N (NANMN) flow, g/d = omasal NAN flow, g/d - microbial NAN flow, g/d.

⁵Basal_{urea}, % of intake = $[\text{RUP}_{\text{Total}}, \text{g/d (urea)} + \text{NAN intake}_{\text{Basal}}, \text{g/d (urea)}] \times 100 = 19.7\%$.

⁶Basal NANMN, g/d = basal NAN intake, g/d $\times$ 0.197. Value in parentheses is the NANMN flow observed on the urea diet.

⁷True protein NANMN, g/d = total NANMN, g/d - basal NANMN, g/d.

⁸Ruminal escape, % = $(\text{true protein NAN, g/d} + \text{true protein NAN intake}) \times 100$.

⁹Ruminal degradation rate = $[(\text{fraction B} \times k_p) + (\text{escape, \%} - \text{fraction C, \%})] - k_p$, where k_p = either the passage rate estimated in vivo or 0.06/h (Reynal and Broderick, 2003).

to Satter (1986), the wide variation in estimates of in vivo protein degradation also was associated with errors in measuring flow when sampling abomasal or duodenal digesta and differences in type of animal (sheep, steer, or dairy cow), diet, and level of intake.

The degradation rate observed (Table 5) for SSBM was 29% higher, and those observed for CSM and CM were 26 and 22% lower, than the rates reported by the NRC (2001). However, NRC (2001) ruminal degradation rates were estimated with the in situ procedure and differences between in vivo vs. in situ results are to be expected. When Reynal and Broderick (2003) used an assumed passage rate of 0.06/h rather than the SP passage rate (0.14/h), the degradation rate estimated for SSBM was 0.179/h, which was similar to the 0.145/h determined in the present study.

Microbial Composition and Enrichment

No differences were observed for bacterial OM content, which averaged 77% for FAB and 84% for PAB

across diets (Table 6). However, NAN content of FAB was lower ($P < 0.01$) on urea and CSM than on SSBM and CM, and PAB NAN content was lowest on CSM. Compared with the other 3 diets, ¹⁵N enrichment of both FAB and PAB was greater ($P < 0.01$) when cows were fed CSM (Table 6). Greater ¹⁵N enrichment of ruminal microbes probably derived from both reduced dilution of the ¹⁵N ammonia pool and increased ammonia uptake, because of the lower supply of preformed AA resulting from the lower RDP contribution on CSM (Table 2). Ammonia concentration was greater (Brito and Broderick, 2007), and ¹⁵N ammonia was substantially more diluted, on the urea diet vs. the 3 diets supplemented with true protein (Table 6). Although not different from CM, ruminal free AA concentration was significantly lower on CSM than SSBM (Brito and Broderick, 2007), suggesting reduced availability of amino N in the rumen of cows fed CSM rather than SSBM. Replacing alfalfa silage plus high-moisture shelled corn with corn silage plus SSBM resulted in a linear decrease in degradability of dietary CP and a linear increase in

Table 6. Effects of different protein supplements on composition and isotopic enrichment of ruminal microorganisms

Item ¹	Diet ²				SED ³	P > F ⁴
	Urea	SSBM	CSM	CM		
¹⁵ NH ₃ APE	0.049 ^b	0.063 ^a	0.061 ^a	0.060 ^a	0.004	<0.01
FAB						
OM, % of DM	76.8	77.1	76.6	76.8	0.64	0.93
NAN, % of DM	6.84 ^b	7.13 ^a	6.84 ^b	7.24 ^a	0.10	<0.01
¹⁵ N APE	0.040 ^b	0.039 ^b	0.046 ^a	0.041 ^b	0.002	<0.01
¹⁵ N from ¹⁵ NH ₃ , ⁵ %	83.3 ^a	64.2 ^c	76.5 ^{ab}	69.0 ^{bc}	4.3	<0.01
¹⁵ N from non- ¹⁵ NH ₃ , ⁶ %	16.7 ^a	35.8 ^c	23.5 ^{ab}	31.0 ^{bc}	4.3	<0.01
PAB						
OM, % of DM	83.6	83.7	83.0	83.7	0.51	0.49
NAN, % of DM	7.68 ^a	7.73 ^a	7.42 ^b	7.77 ^a	0.12	0.03
¹⁵ N APE	0.037 ^b	0.037 ^b	0.043 ^a	0.039 ^b	0.002	<0.01
¹⁵ N from ¹⁵ NH ₃ , %	77.6 ^a	59.8 ^c	71.3 ^{ab}	64.7 ^{bc}	3.6	<0.01
¹⁵ N from non- ¹⁵ NH ₃ , %	22.4 ^a	40.2 ^c	28.7 ^{ab}	35.3 ^{bc}	3.6	<0.01
Total bacteria						
¹⁵ N from ¹⁵ NH ₃ , ⁷ %	80.2 ^a	61.7 ^c	73.4 ^{ab}	66.6 ^{bc}	3.9	<0.01
¹⁵ N from non- ¹⁵ NH ₃ , %	19.8 ^a	38.3 ^c	26.6 ^{ab}	33.4 ^{bc}	3.9	<0.01
Protozoa						
OM, % of DM	96.2	96.1	96.7	96.3	0.98	0.91
NAN, % of DM	2.72 ^a	2.76 ^a	2.51 ^{ab}	2.39 ^b	0.14	0.04
¹⁵ N APE	0.037 ^b	0.038 ^b	0.046 ^a	0.041 ^{ab}	0.003	0.01

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹APE = atom % excess; FAB = fluid-associated bacteria; PAB = particle-associated bacteria.

²SSBM = solvent soybean meal; CSM = cottonseed meal; CM = canola meal.

³SED = standard error of the least squares means difference.

⁴Probability of a significant effect of diet

⁵(¹⁵N APE in FAB or PAB ÷ ¹⁵NH₃ APE) – 100.

⁶(100 – % of ¹⁵N from ¹⁵NH₃).

⁷[(¹⁵N APE in FAB × % of FAB in total NAN flow) + (¹⁵N APE in PAB × % of PAB in total NAN flow)] ÷ ¹⁵NH₃ APE.

¹⁵N enrichment of both FAB and PAB (Brito et al., 2006). Feeding low-NPN red clover silage also gave rise to greater ¹⁵N enrichment of FAB and PAB compared with feeding high-NPN alfalfa silage (Brito et al., 2007). Mean ¹⁵N enrichment of FAB was 7% greater than PAB (Table 6), which agreed with literature reports (Reynal et al., 2005a; Brito et al., 2006; Brito et al., 2007). This difference can be attributed to greater availability of free ammonia in the FP to FAB than to the PAB. Microbes associated with particulate matter probably utilized greater amounts of N from AA and peptides, thus diluting the ¹⁵N from ammonia. Because this finding has been consistent across several studies, the differential isotopic enrichments of FAB and PAB must be accounted for when estimating microbial NAN flow from the rumen.

The proportion of N in FAB, PAB, and total bacteria incorporated from the ammonia pool was greatest on urea, intermediate on CSM and CM, and lowest on SSBM (Table 6), results that were consistent with the ruminal degradabilities observed for these CP supplements (Table 5). Among the true proteins, ammonia N incorporation was greatest on CSM (51% degraded), intermediate on CM (34% degraded), and lowest on

SSBM (29% degraded). Literature estimates of N contribution from ammonia vs. preformed AA in ruminal microbes have been highly variable, ranging from 18 to 100% (Salter et al., 1979), 50% or more (Nolan, 1975; Leng and Nolan, 1984), and 26 to 100% (Wallace, 1997). This variation has been attributed to differences in N availability from different N sources (Salter et al., 1979; Wallace, 1997).

Organic matter content of protozoa did not differ and averaged 96% across diets (Table 6). Conversely, protozoal NAN content was greatest on urea and SSBM, intermediate on CSM, and lowest on CM. However, NAN content of protozoa was much lower than that of bacteria in the current study. Ahvenjärvi et al. (2002) reported a greater N content of PAB compared with protozoa but no difference between FAB and protozoa. According to these authors, protozoal pellets were always contaminated with fine feed particles. Therefore, lower protozoal NAN in the present study might have been the result of contamination with feed particles plus dilution from glycogen accumulation because of the use of glucose during isolation of protozoa. Ahvenjärvi et al. (2002) used saline to wash the protozoal pellets through a polyester fabric, which may have re-

duced N dilution from feed residues. Reynal et al. (2005a) observed an average of 3.1% NAN in protozoal samples isolated using the same method as the present study. According to Reynal et al. (2005a), contamination with feed N did not explain the low NAN content of the protozoal isolates because ^{15}N APE of protozoa was only slightly lower than that of FAB and PAB; they concluded that contamination was from nonnitrogenous sources. Sylvester et al. (2005) reported that the N content of sedimented protozoa (3.82% N) was 45% lower than that of filtrated protozoa (6.95% N), suggesting that this discrepancy was caused by an extensive contamination with low-N plant material.

Isotopic enrichment of protozoa was greatest on CSM, intermediate on CM, and lowest on urea and SSBM (Table 6). This was expected because the greatest ^{15}N enrichment of both PAB and FAB occurred on CSM, and protozoa become labeled indirectly through bacterial predation. Literature reports (Firkins et al., 1987; Hristov and Broderick, 1996; Ahvenjärvi et al., 2002) have shown that ^{15}N enrichment of protozoa generally is somewhat lower than that of bacteria, possibly because of direct utilization of feed protein N diluting the ^{15}N below bacterial enrichment. In the present trial, mean protozoal:FAB and mean protozoal:PAB ^{15}N enrichment ratios were, respectively, 0.98 and 1.04; the greater protozoal:bacterial ratios compared with literature data were possibly caused by bacterial contamination of the protozoal pellets. Sylvester et al. (2005) reported a 33-fold reduction in bacterial contamination of the protozoa samples using filtration rather than a sedimentation technique to separate protozoal cells.

CONCLUSIONS

Cows were fed diets based on alfalfa silage, corn silage, and high-moisture corn, supplemented to 16.6% of CP with urea, SSBM, CSM, or CM. In vivo RUP estimates for the true proteins were 29% (SSBM), 34% (CM), and 51% (CSM). Microbial efficiency and NAN flow were similar in cows fed true protein supplements but were significantly lower when fed urea, probably because of an inadequate amino N supply in the RDP. The true proteins had similar rates of passage; ruminal degradation rates, computed from RUP and ruminal passage rates, were 0.105/h (SSBM), 0.081/h (CM), and 0.050/h (CSM). Omasal flow of individual AA, branched-chain AA, essential AA, nonessential AA, and total AA all were lower in cows fed urea. Lower flows of microbial NAN and AA explained depressed yields of milk and milk components on the urea diet, compared with true protein, in the companion lactation trial (Brito and Broderick, 2007). These results showed that supplementation with true protein was necessary to obtain

sufficient microbial growth plus RUP to meet the metabolizable AA requirements of high-producing dairy cows.

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