Effect of source of rumen-degraded protein on production and ruminal metabolism in lactating dairy cows¹

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ABSTRACT

Twenty-eight (8 with ruminal cannulas) lactating Holstein cows were assigned to seven 4 × 4 Latin squares in a 16-wk trial to study the effects on production and ruminal metabolism of feeding differing proportions of rumen-degraded protein (RDP) from soybean meal and urea. Diets contained [dry matter (DM) basis] 40% corn silage, 15% alfalfa silage, 28 to 30% high-moisture corn, plus varying levels of ground dry shelled corn, solvent- and lignosulfonate-treated soybean meal, and urea. Proportions of the soybean meals, urea, and dry corn were adjusted such that all diets contained 16.1% crude protein and 10.5% RDP, with urea providing 0, 1.2, 2.4, and 3.7% RDP (DM basis). As urea supplied greater proportions of RDP, there were linear decreases in DM intake, yield of milk, 3.5% fat-corrected milk, fat, protein, and solids-not-fat, and of weight gain. Milk contents of fat, protein, and solids-not-fat were not affected by source of RDP. Replacing soybean meal RDP with urea RDP resulted in several linear responses: increased excretion of urinary urea-N and concentration of milk urea-N, blood urea-N, and ruminal ammonia-N and decreased excretion of fecal N; there was also a trend for increased excretion of total urinary N. A linear increase in neutral detergent fiber (NDF) digestibility, probably due to digestion of NDF-N from lignosulfonate-treated soybean meal, was observed with greater urea intake. Omasal sampling revealed small but significant effects of N source on measured RDP supply, which averaged 11.0% (DM basis) across diets. Increasing the proportion of RDP from urea resulted in linear decrease in omasal flow of dietary nonammonia N (NAN) and microbial NAN and in microbial growth efficiency (microbial NAN/unit of organic matter truly digested in the rumen). These

changes were paralleled by large linear reductions in omasal flows of essential, nonessential, and total amino acids. Overall, these results indicated that replacing soybean meal RDP with that from urea reduced yield of milk and milk components, largely because of depressed microbial protein formation in the rumen and that RDP from nonprotein-N sources was not as effective as RDP provided by true protein.

Key words: rumen-degraded protein, nonprotein nitrogen, true protein, microbial protein synthesis

INTRODUCTION

Microbial protein synthesized in the rumen, RUP, and endogenous protein contribute metabolizable AA to the small intestine, with microbial protein accounting for the majority of the total AA (Clark et al., 1992). The NRC (2001) assumes that RDP from NPN sources such as urea are as effective as RDP from true protein for microbial protein formation. However, Hume (1970) observed about 10% greater microbial protein synthesis in sheep fed their entire dietary CP as true protein versus urea. Maeng and Baldwin (1976) found substantial increases in protein formation and efficiency with small supplements of protein AA to in vitro incubations of mixed ruminal organisms. Argyle and Baldwin (1989) showed that adding only 1 mg/L of a blend of protein AA plus 1 mg/L of peptides (from tryptic digests of casein) more than doubled in vitro cell yields of mixed ruminal organisms. They also found progressively lower responses to 10 and 100 times greater addition of AA and peptides. Hristov and Broderick (1994) observed that net microbial protein formation in vitro increased as the degradation rate of the added true proteins increased from 0.03 to 0.14/h, but changed little as degradation rate increased further to 0.68/h. Finally, Brito et al. (2007b) found that replacing supplemental urea N with true protein from solvent-extracted soybean meal, cottonseed meal, or canola meal increased microbial protein yield from 26.3 to a mean 29.4 g of NAN/kg of OM truly digested in the rumen (**OMTDR**). These results suggested that NPN sources were less effective than true protein for supplying RDP in vivo.

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The objectives of this trial were to determine the relative effectiveness of RDP from NPN (urea) versus RDP from true protein (soybean meal) for 1) production of milk and milk components; 2) nutrient utilization; and 3) microbial protein synthesis in lactating dairy cows.

MATERIALS AND METHODS

Lactation Trial

Twelve multiparous Holstein cows (4 fitted with permanent ruminal cannulas), averaging parity of 2.8 (SD 1.8), 66 (SD 41) DIM, 46 (SD 9) kg of milk/d, and 634 (SD 47) kg of BW, and 16 primiparous Holstein cows (4 fitted with permanent ruminal cannulas), averaging 119 (SD 25) DIM, 39 (SD 3) kg of milk/d, and 589 (SD 37) kg of BW at the beginning of the study, were blocked by parity and DIM into seven 4 × 4 Latin squares (2 squares of cannulated cows). Cows were randomly assigned within squares to 4 balanced dietary treatment sequences (i.e., with each diet following every other diet once in each square over the trial). Diets were fed as TMR and contained (DM basis) 40% corn silage, 15% alfalfa silage, and 45% concentrate. Composition of the major feed ingredients were (DM basis): 23.2% CP, 38.3% NDF, and 29.4% ADF (alfalfa silage); 7.0% CP, 39.4% NDF, and 21.1% ADF (corn silage); 7.5% CP, 8.9% NDF, and 2.1% ADF (rolled high-moisture shelled corn); 8.0% CP, 7.8% NDF, and 2.8% ADF (ground shelled corn); 53.9% CP, 8.2% NDF, and 4.8% ADF (solvent-extracted soybean meal; **SSBM**); 50.5% CP, 22.5% NDF, and 7.0% ADF (lignosulfonate-treated soybean meal; **LSBM**; SoyPass, LignoTech USA Inc., Rothschild, WI); and 45.6% N (285% CP; urea). Dietary content of high-moisture shelled corn and **SSBM** were reduced, and that of ground shelled corn, **LSBM**, and urea were increased stepwise to maintain constant dietary CP and RDP, but to give increasing proportions of the RDP supplied from urea (Table 1). Measured CP and predicted (NRC, 2001) RUP and RDP averaged, respectively, 16.1 and 5.6 and 10.5% of dietary DM. All cows were injected every other week with bST (500 mg of Posilac, Monsanto, St. Louis, MO) from about 60 DIM; injections were synchronized such that animals received a full dose on d 1 and at 14-d intervals throughout the trial. Cows were housed in tie stalls and had free access to water during the trial. The Research Animal Resource Center of the University of Wisconsin-Madison approved all procedures involving animals.

Each experimental period lasted 28 d and consisted of 14 d for adaptation and 14 d for collection of intake and production data. Diets were offered once daily at 1000 h, except for the ruminally cannulated cows during omasal sampling when diets were offered twice daily

at 1000 and 2200 h. Orts were collected and weights recorded at 0900 h and feeding rate was adjusted daily to yield Orts of about 5 to 10% of intake. Weekly composites of corn silage, alfalfa silage, high-moisture shelled corn, TMR, and Orts were taken from daily samples of about 0.5 kg that were stored at -20°C. Weekly samples were also taken of urea, ground shelled corn, **SSBM**, and **LSBM** and stored at room temperature. The DM was determined in weekly composites of corn silage, alfalfa silage, and high-moisture shelled corn by drying at 60°C for 48 h and in weekly samples of urea, ground shelled corn, **SSBM**, and **LSBM** at 105°C (AOAC, 1980). Weekly samples of feed ingredients were also analyzed for total N using a combustion assay (Leco FP-2000 N Analyzer, Leco Instruments Inc., St. Joseph, MI). These DM and N contents were used to adjust dietary composition weekly to maintain similar levels of CP and RDP through the trial. Intake of DM was computed based on the 60°C DM determinations for TMR and Orts. After drying, ingredients and TMR were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA), and period composites were prepared by mixing equal DM from weekly samples. Composite samples were analyzed for total N, DM at 105°C, ash, and OM (AOAC, 1980), sequentially for NDF and ADF using heat-stable α -amylase and Na₂SO₃ (Van Soest et al., 1991; Hintz et al., 1995), and for indigestible ADF (ADF remaining after a 12-d in situ incubation; Huhtanen et al., 1994). Composite samples of TMR were also analyzed for starch (Hall et al., 1999; Rumen Profiling Lab, West Virginia Univ., Morgantown), NPN (Muck, 1987; Leco FP-2000 N Analyzer), and for 17 protein AA. In the AA analysis, TMR samples were hydrolyzed for 24 h at 110°C in sealed vials under a N₂ atmosphere using 6 N HCl containing 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 removed by vacuum evaporation, and the residue redissolved in pH 2.2-sample buffer containing norleucine as internal standard. Analysis of individual AA was conducted using ion exchange chromatography with ninhydrin detection (Beckman 6300 Amino Acid Analyzer, Beckman Instruments Inc., Palo Alto, CA). Tryptophan was not determined.

Cows were milked twice daily at 0600 and 1700 h and milk yield was recorded at each milking in all experimental periods. Milk samples from a.m. and p.m. milkings were collected on d 18 and 25 of each period and analyzed for fat, true protein, lactose, and SNF by infrared analysis (AgSource, Verona, WI) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN) using AOAC (1990) method 972.16. For MUN determination, 5 mL of milk from both milkings was

Table 1. Composition of diets

Item	Total ¹ RDP (RDP from urea), % of DM			
	10.6 (0)	10.5 (1.2)	10.4 (2.4)	10.5 (3.7)
Ingredients, % of DM				
Corn silage	40.0	40.1	40.1	40.2
Alfalfa silage	15.0	14.9	14.9	14.8
Rolled high-moisture shelled corn	29.6	28.7	28.5	27.8
Ground shelled corn	0	2.04	4.06	6.40
Solvent-treated soybean meal	13.95	9.82	4.98	0
Lignosulfonate-treated soybean meal	0	2.52	5.12	8.00
Urea	0	0.41	0.84	1.31
Sodium bicarbonate	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30
Limestone	0.30	0.30	0.30	0.30
Dicalcium phosphate	0.20	0.20	0.20	0.20
Vitamin-mineral mix ²	0.20	0.20	0.20	0.20
Nutrient composition				
DM, %	49.6	49.3	49.4	48.3
CP, % of DM	16.1	16.1	16.0	16.1
Ash, % of DM	7.2	7.2	7.0	7.4
RDP, ¹ % of DM	10.6	10.5	10.4	10.5
RUP, ¹ % of DM	5.5	5.6	5.7	5.6
NE _L , ³ Mcal/kg of DM	1.58	1.58	1.58	1.57
NDF, % of DM	24.8	25.4	25.9	25.7
ADF, % of DM	14.1	14.2	14.4	13.9
Starch, % of DM	24.5	23.9	25.7	26.1
Neutral detergent insoluble N, % of total N	6.20	8.21	9.45	11.12
ADIN, % of total N	2.61	2.74	2.79	2.64
Fraction B3, ⁴ % of total N	3.59	5.47	6.66	8.48
Non-urea N, % of DM	2.58	2.37	2.13	1.92
NPN, ⁵ % of total N	20.3	27.8	35.5	43.6
True protein-N, ⁶ % of non-urea N	79.7	78.0	75.9	73.5

¹Predicted from the NRC (2001) model.

²Provided (/kg of DM): 56 mg of Zn, 46 mg of Mn, 22 mg of Fe, 12 mg of Cu, 0.9 mg of I, 0.4 mg of Co, 0.3 mg of Se, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

³Computed by discounting dietary energy based on actual DM intakes (NRC, 2001).

⁴Fraction B3 = neutral detergent insoluble N (% of total N) – ADIN (% of total N) (Fox et al., 2004).

⁵Proportion of total N soluble in 10% (wt/vol) TCA (Muck, 1987).

⁶Proportion of total N detected as 17 protein AA in acid-hydrolysates of each diet.

treated with 5 mL of 25% (wt/vol) TCA. Samples were vortexed and allowed to stand for 30 min at room temperature before filtering through Whatman no. 1 filter paper. Filtrates were stored at -20°C until MUN analysis by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow-injection (Lachat Quik-Chem 8000 FIA, Lachat Instruments, Milwaukee, WI). Concentrations and yields of fat, true protein, lactose, and SNF, and MUN concentration were computed as weighted means from a.m. and p.m. milk yields on each test day. Yield of 3.5% FCM (Sklan et al., 1992) was also computed. Efficiency of conversion of feed DM was calculated for each cow over the last 2 wk of each period by dividing mean yield of actual milk and FCM by mean DM intake. Apparent N efficiency (assuming no retention or mobilization of body N) was also calculated for each cow by dividing mean milk N output (milk true protein/6.38) by mean N intake. For computation of BW change, BW was measured on 3

consecutive days at the beginning of the experiment and at the end of each period.

On d 26 and 27 of each period, 2 spot urine and 2 spot fecal samples were collected from all 28 cows at 6 and 18 h after feeding. Fecal samples were dried in a forced draft oven (60°C ; 72 h) and then ground through a 1-mm screen (Wiley mill). Equal DM from each fecal subsample was mixed to obtain one composite sample for each cow in each period. Fecal samples were analyzed for total DM, ash, OM, N, NDF, ADF, and indigestible ADF as described earlier. Indigestible ADF was used as an internal marker to estimate both apparent nutrient digestibility and fecal output (Cochran et al., 1986). Urine samples were acidified immediately after collection by diluting 1 volume of urine with 4 volumes of 0.072 N H_2SO_4 and stored at -20°C . Later, urine samples were thawed at room temperature and filtered through Whatman no. 1 filter paper. Filtrates were analyzed for creatinine using a picric acid method

(Oser, 1965) adapted to flow-injection analysis (Lachat QuikChem 8000), for total N (Leco FP-2000 N Analyzer) and for urea with the colorimetric method also used for MUN. Daily urine volume and excretion of urea N and total N were computed from urinary creatinine concentration and BW assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

Ruminal Metabolism Trial

At the same time as the lactation trial, the 8 lactating Holstein cows fitted with ruminal cannulas were fed the same 4 experimental diets and housed and managed as described above. Omasal sampling was performed during the last week of each period using the techniques developed by Huhtanen et al. (1997) and Ahvenjärvi et al. (2000), as adapted by Reynal and Broderick (2005), to quantify digesta flow out of the rumen. Indigestible NDF (Huhtanen et al., 1994), YbCl₃ (Siddons et al., 1985), and Co-EDTA (Udén et al., 1980), which are mainly associated with, respectively, the large particle, small particle, and fluid phases of digesta, were used as flow markers at the omasal canal. The external microbial marker ¹⁵N was used to quantify microbial NAN flow from the rumen. The triple marker technique of France and Siddons (1986) was used to determine the proportions with which to recombine the 3 phases to produce omasal true digesta. Before marker infusion began, whole ruminal contents were taken from each cow to determine the background ¹⁵N abundance. Cobalt-EDTA, YbCl₃, and NH₄SO₄ containing 10% atom excess ¹⁵N (Isotec, Miamisburg, OH) were dissolved in distilled water and continuously infused into the rumen at rates of 2.01 g of Co, 2.88 g of Yb, and 210 mg of ¹⁵N per day in 2.62 L/d of solution. Markers were continuously infused for 158 h from 0800 h on d 20 to 2200 h on d 26 using 2 syringe pumps (Model 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 the 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 the small and large particle phases), digesta marker analyses, and preparation of omasal true digesta were as described by Reynal and Broderick (2005) and Brito et al. (2007b) except that ammonia and protozoa were not isolated for determination of ¹⁵N enrichment.

Samples of omasal true digesta were analyzed for total N, absolute DM, ash, OM, NDF, ADF, neutral detergent insoluble N, ADIN, and individual AA as

detailed above for feed samples. Samples of omasal true digesta and isolated bacteria were treated with K₂CO₃ (Brito et al., 2007b) to remove residual ammonia and analyzed for total N (equivalent to NAN) and for ¹⁵N abundance using a Costech 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA) interfaced to a Thermo-Finnigan Delta-Plus Advantage isotope ratio mass spectrometer (Thermo-Electron GmbH, Bremen, Germany). Equations used to compute flows of nutrients of dietary and microbial origin, and extents of ruminal digestion, were those detailed by Brito et al. (2007b).

On d 27 of each period, about 100 to 200 mL of digesta was collected from 3 locations in the ventral rumen at 0 (just before feeding), 1, 2, 4, 6, 8, 12, 18, and 24 h after feeding, strained through 2 layers of cheesecloth, and pH measured immediately using a glass electrode. Two 10-mL samples of ruminal fluid were then preserved in scintillation vials by addition of 0.2 mL of 50% H₂SO₄ and stored at -20°C. Just before analysis, samples were thawed and centrifuged (15,300 × *g* for 20 min at 4°C) and flow-injection analyses (Lachat QuikChem 8000) were applied to supernatants to determine ammonia, using a phenol-hypochlorite method (Lachat Method 18-107-06-1-A), and total AA using a fluorimetric procedure based on the reaction with *o*-phthalaldehyde (Roth, 1971). Leucine was the standard in the *o*-phthalaldehyde assay and total AA are reported in leucine equivalents. Samples also were thawed and centrifuged (28,000 × *g* for 30 min at 4°C) for determination of individual and total ruminal VFA using a modification of the GLC method for free fatty acids described in Supelco Bulletin 855B (Supelco Inc., Supelco Park, Bellefonte, PA) with flame-ionization detection. Standards or supernatants (0.5 or 1 µL) were injected onto a ZB-FFAP capillary column (30 m × 0.53 mm × 1.0 µm; no. 7HK-G009-22; Phenomenex Inc., Torrance, CA) with helium carrier gas at 100 kPa and a flow rate of 20 mL/min. Column oven temperature was 100°C at injection; after 2 min, the temperature was increased to 130°C at 10°C/min. Injector and detector temperatures were 230°C and 250°C. The method did not resolve isovalerate and 2-methylbutyrate.

Statistical Analysis

Results were analyzed using the mixed procedures of SAS (SAS Institute, 1999–2000). The following model was used to fit data from the lactation trial and omasal flow measurements to assess effects of dietary treatments:

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

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = effect of square i , P_j = effect of period j , $C_{k(i)}$ = effect of cow k (within square i), T_l = effect of treatment l , ST_{il} = interaction between square i and treatment l , and E_{ijkl} = residual error. All terms were considered fixed, except for $C_{k(i)}$ and E_{ijkl} , which were considered random. The following model was used for ruminal variables for which there were repeated measurements over time (pH, ammonia, total free AA, and individual and total VFA):

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

where Y_{ijklm} = dependent variable, μ = overall mean, S_i = effect of square i , P_j = effect of period j , $C_{k(i)}$ = effect of cow k (within square i), T_l = effect of treatment l , ST_{il} = interaction between square i and treatment l , E_{ijkl} = whole plot error, Z_m = effect of time m , ZT_{ml} = interaction between time m and treatment l , and ω_{ijklm} = subplot error. The spatial covariance structure SP(POW) was used for estimating covariance and the subject of the repeated measurements was defined as cow(square $\times$ period $\times$ treatment). All terms were considered fixed, except for $C_{k(i)}$, E_{ijkl} , and ω_{ijklm} , which were considered random. For all models used, the interaction term ST_{il} or ST_{im} was removed when $P > 0.25$. Linear, quadratic, and cubic effects of urea concentration were tested by partitioning degrees of freedom for

diet into single degree of freedom variables corresponding to linear, quadratic, and cubic effects. Cubic effects were not statistically significant ($P \geq 0.11$) for any of the variables and are not reported. Differences between least squares means are reported only if the F -test for dietary treatment was significant at $\alpha = 0.05$. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Lactation Trial

Cows in this trial were fed high-energy diets containing 25 to 26% NDF, 1.58 mg of NE_L/kg of DM, and about 25% total starch (Table 1). Diets were originally formulated for 28% NDF but the alfalfa and corn silages declined from an average of about 44% NDF before the trial to only 38 to 39% NDF; low effective fiber probably accounted in part for milk fat content averaging 3.05%. However, we achieved our goal of maintaining constant CP and predicted RDP across diets, as the proportion of RDP supplied from urea was increased from 0 to 3.7% of DM. As designed, non-urea N, true protein N (Table 1), and AA-N (Tables 1 and 2) all declined and NPN content increased (Table 1) with increasing dietary RDP from urea. Therefore, the diets fed in this trial were probably satisfactory for our objectives despite being relatively low in fiber.

Although no quadratic contrasts for production traits were significant ($P \geq 0.12$), several linear effects were

Table 2. Amino acid composition of diets

Item	Total ¹ RDP (RDP from urea), % of DM			
	10.6 (0)	10.5 (1.2)	10.4 (2.4)	10.5 (3.7)
Essential AA	(% of CP)			
Arg	3.59	3.45	3.42	2.87
His	1.86	1.82	1.82	1.66
Ile	3.43	3.37	3.33	3.12
Leu	7.89	7.83	7.86	7.38
Lys	3.41	3.31	3.21	2.80
Met	1.13	1.16	1.13	1.05
Phe	4.14	3.65	3.70	3.38
Thr	3.48	3.37	3.36	3.19
Val	4.30	4.21	4.25	4.04
Nonessential AA				
Ala	6.39	6.52	6.65	6.36
Asp	8.30	8.05	7.90	6.99
Cys	1.38	1.35	1.41	1.16
Glu	14.6	14.3	14.2	12.6
Gly	3.89	3.80	3.82	3.67
Pro	5.78	6.01	6.18	5.61
Ser	3.73	3.67	3.69	3.32
Tyr	2.55	2.53	2.54	2.39
Essential AA	33.2	32.2	32.1	29.5
Nonessential AA	46.6	46.2	46.4	42.1
Total AA	79.8	78.4	78.5	71.6

¹Predicted from the NRC (2001) model.

detected as urea replaced RDP from SSBM (Table 3). There were linear declines ($P \leq 0.04$) in DM intake, BW gain, yield of milk, FCM, fat, true protein, lactose, and SNF, and milk lactose content, as well as linear increases ($P < 0.01$) in concentrations of MUN and blood urea. Much of the depression in production of milk and milk components with increasing dietary urea can be attributed to lower DM intake. The maximum reduction in DM intake corresponded to 2.0 Mcal less NE_L /d; this would amount to 2.8 kg/d depression in production of milk with the mean composition secreted in this trial (NRC, 2001). Actual milk yield declined a maximum of 3.3 kg/d (Table 3). Previously, we observed a quadratic response in protein yield when reducing RDP (by reducing urea and replacing SSBM with LSBM) in diets containing substantially more CP (Reynal and Broderick, 2005). However, those effects were confounded because RDP from NPN and true protein were altered together. Brito and Broderick (2007) found that replacing CP from urea with CP from 1 of 3 different oilseed meals, in diets containing 16.5% CP, increased DM intake by 2.1 to 2.8 kg/d, milk yield by 7 to 8 kg/d, fat yield by 170 to 280 g/d, and protein yield by 260 to 350 g/d. The urea diet in that study contained about 2 percentage units more RDP than the diets supplemented with true protein. Charbonneau et al. (2007) reported that replacing CP from LSBM (11% RDP in dietary DM) with that from SSBM (13% RDP) or urea (15% RDP) resulted in a linear decrease in milk protein yield, and linear increase in MUN as dietary RDP was elevated. Moharrery (2004) found that substituting CP from cottonseed meal or urea for CP from SSBM reduced yield of milk and milk N and increased MUN concentration; the effect of adding urea was more negative than adding cottonseed meal. Thus, it appears that replacing RDP from true protein sources with RDP from NPN reduces yield of milk and milk components.

The depressed production observed with increasing RDP from urea was not accompanied by any other indication of impaired N utilization and, except for the linear increase ($P < 0.01$) in urinary urea excretion, there were no alterations in total N excretion in the urine or urine plus feces, or in estimated N-balance (Table 3). Moreover, milk-N/N-intake was not affected by RDP source, averaging 32.5% across diets. Apparent N efficiency was relatively high; previously we observed apparent N efficiency to decline from 36.5% at 13.5% dietary CP to 25.4% at 19.4% dietary CP (Olmos Colmenero and Broderick, 2006). Brito and Broderick (2007) found that replacing oilseed meal CP with CP from urea in lactating cows increased the proportion of consumed N excreted in the urine and reduced apparent N efficiency from a mean of 30 to 25%. Linear

declines ($P < 0.01$) in fecal DM and N excretion were observed as urea RDP replaced that from soybean meal. However, the pattern of mean separation for both traits was similar to that for DM intake, suggesting these effects were related. The NRC (2001) estimated that metabolic fecal CP is excreted at a rate of 30 g/kg of DM intake; thus, the 1.3 kg/d lower DM intake on the highest urea diet would result in an estimated 39 g/d reduction in fecal CP, or about 6 g/d of N excretion versus the zero-urea diet. This accounts for only part of the observed difference in fecal N excretion. Increased apparent NDF digestion may be attributed to replacement of SSBM, which contained 5% NDF, with LSBM, which contained 23% NDF. The linear increase in apparent N digestion with increasing dietary urea was surprising because little net change was anticipated. Urea (with true digestibility approaching 100%) and LSBM (with a total-tract digestibility of CP similar to SSBM; Reynal and Broderick, 2005) replaced SSBM, which was reported to have a true digestibility of 94% (Shingfield et al., 2003).

Ruminal Metabolism Trial

Concentrations of ruminal metabolites observed in this study are in Table 4. There was a linear increase ($P < 0.01$) in ammonia with increasing RDP from NPN, reflecting the rapid rate of urea hydrolysis in the rumen. Total free AA concentration was the only trait in the study to show a quadratic effect ($P = 0.05$), suggesting that this was possibly a random observation. As expected, isobutyrate, isovalerate, and total branched-chain VFA (isobutyrate plus isovalerate), which are formed from catabolism of branched-chain AA (Allison, 1970), were altered ($P < 0.01$) as RDP from true protein was deleted from the diet; however, a pattern of linear decline was not observed. Concentrations of ruminal metabolites are the net result of formation and utilization; although AA formation would be expected to decline as RDP from SSBM was removed from the diet, uptake of peptides and AA for microbial growth probably was greatest on the zero-urea diet (because of greater microbial protein yield). There are several reports of decreased ruminal concentrations of branched-chain VFA (Broderick, 1986; Sannes et al., 2002) and total AA (Reynal and Broderick, 2005; Brito and Broderick, 2007) when RDP from true protein is removed from the diet.

Digesta flows at the omasal canal and ruminal digestion coefficients are in Table 5. There was an effect of diet on the amount of apparent DM ($P = 0.03$) and OM ($P = 0.06$) leaving the rumen; however, there were no differences when apparent DM and OM digestibility was expressed as a proportion of intake. Correcting OM

Table 3. Effect of dietary RDP source on production, excretion, and apparent digestion¹

Item	Total ² RDP (RDP from urea), % of DM				SE	<i>P</i> > <i>F</i> ³		
	10.6 (0)	10.5 (1.2)	10.4 (2.4)	10.5 (3.7)		RDP source	Linear	Quadratic
DM intake, kg/d	23.6 ^a	23.2 ^a	23.0 ^{ab}	22.3 ^b	0.4	0.02	<0.01	0.75
BW change, kg/d	0.57	0.53	0.34	0.34	0.10	0.18	0.04	0.84
Milk yield, kg/d	39.3 ^a	38.6 ^a	38.5 ^a	36.0 ^b	0.9	<0.01	<0.01	0.20
Milk:DM intake	1.68	1.67	1.69	1.63	0.03	0.19	0.19	0.18
3.5% FCM, kg/d	36.4 ^a	36.0 ^{ab}	34.6 ^{bc}	33.7 ^c	0.9	0.01	<0.01	0.78
FCM:DM intake	1.54	1.55	1.51	1.51	0.04	0.51	0.21	0.85
Milk fat, %	3.05	3.17	2.92	3.05	0.09	0.15	0.50	0.89
Milk fat, kg/d	1.20	1.19	1.10	1.11	0.04	0.07	0.02	0.70
Milk true protein, %	3.22	3.18	3.18	3.18	0.04	0.85	0.51	0.63
Milk true protein, kg/d	1.27 ^a	1.22 ^b	1.21 ^{bc}	1.17 ^c	0.03	<0.01	<0.01	0.59
Lactose, %	5.00	4.96	4.96	4.93	0.03	0.20	0.04	0.93
Lactose, kg/d	1.98 ^a	1.92 ^a	1.90 ^{ab}	1.81 ^b	0.05	0.01	<0.01	0.79
SNF, %	9.09	9.02	9.03	9.00	0.05	0.39	0.13	0.52
SNF, kg/d	3.59 ^a	3.48 ^a	3.45 ^{ab}	3.32 ^b	0.08	0.01	<0.01	0.90
MUN, mg/dL	6.77 ^b	7.45 ^b	8.13 ^b	9.08 ^a	0.31	<0.01	<0.01	0.65
BUN, mg/dL	8.87 ^c	9.89 ^c	11.39 ^b	12.78 ^a	0.45	<0.01	<0.01	0.80
Milk-N: N intake, %	33.2	32.1	32.4	32.1	0.7	0.38	0.19	0.47
Excretion ⁴								
Urine volume, L/d	20.1	19.2	18.0	19.0	0.8	0.13	0.12	0.11
Urinary urea-N, g/d	136 ^c	138 ^{bc}	152 ^a	150 ^{ab}	6	0.01	<0.01	0.41
Total urinary-N, g/d	191	192	195	203	6	0.37	0.10	0.54
Fecal DM, kg/d	6.99 ^a	6.69 ^a	6.61 ^{ab}	6.17 ^b	0.24	0.03	<0.01	0.76
Fecal N, g/d	179 ^a	170 ^a	167 ^a	151 ^b	7	0.01	<0.01	0.59
Total N excretion, g/d	370	361	363	355	11	0.67	0.26	0.98
Total N excretion, % of intake	65.3	64.6	65.5	65.8	1.4	0.94	0.69	0.73
Apparent N-balance, ⁵ g/d	35	43	36	38	9	0.91	0.97	0.71
Apparent total-tract digestibility ⁶								
OM, %	69.8	70.1	70.3	71.3	0.8	0.51	0.15	0.66
NDF, %	47.2 ^b	49.5 ^{ab}	52.3 ^a	52.0 ^a	1.3	0.02	<0.01	0.28
ADF, %	48.5	49.7	52.0	49.5	1.3	0.25	0.37	0.12
N, %	66.8 ^b	69.7 ^{ab}	70.0 ^{ab}	72.0 ^a	1.3	0.04	<0.01	0.69

^{a-c}Least squares means within the same row with different superscripts differ ($P < 0.05$) when effect of RDP source differs ($P < 0.05$).¹Data from all 28 cows.²Predicted from the NRC (2001) model.³Probability of a significant effect of RDP source or of a linear or quadratic effect of dietary urea content.⁴Urinary excretion estimated using creatinine as a volume marker and fecal excretion estimated using indigestible ADF as an internal marker.⁵Apparent nitrogen balance computed assuming milk true protein contained 6.38% N.⁶Apparent digestibilities estimated from spot fecal sampling, using indigestible ADF as an internal marker.**Table 4.** Effect of dietary RDP source on concentrations of ruminal metabolites¹

Item	Total ² RDP (RDP from urea), % of DM				SE	<i>P</i> > <i>F</i> ³		
	10.6 (0)	10.5 (1.2)	10.4 (2.4)	10.5 (3.7)		RDP source	Linear	Quadratic
pH	6.51	6.52	6.51	6.59	0.05	0.25	0.12	0.30
Ammonia-N, mg/dL	8.2 ^b	9.3 ^{ab}	10.3 ^a	10.7 ^a	0.73	0.03	<0.01	0.52
Total free AA, mM	16.8	19.5	19.4	18.0	1.7	0.21	0.52	0.05
Acetate, mM	83.0	78.8	86.5	86.1	3.6	0.23	0.20	0.61
Propionate, mM	29.3	30.6	33.4	31.6	1.6	0.32	0.20	0.28
Butyrate, mM	17.3	16.6	17.5	17.3	1.1	0.87	0.75	0.77
Isobutyrate, mM	1.87	1.72	1.90	1.73	0.09	0.08	0.42	0.81
Isovalerate, mM	2.71 ^{ab}	2.29 ^c	2.97 ^a	2.60 ^b	0.16	<0.01	0.41	0.96
Total branched-chain VFA, mM	4.58 ^{ab}	4.01 ^c	4.87 ^a	4.33 ^{bc}	0.23	<0.01	0.81	0.88
Valerate, mM	2.75	2.89	3.30	2.78	0.28	0.35	0.72	0.16
Total VFA, mM	137	133	146	142	5.8	0.28	0.21	0.97

^{a-c}Least squares means within the same row with different superscripts differ ($P < 0.05$) when effect of RDP source differs ($P < 0.05$).¹Data from the 8 ruminally cannulated cows.²Predicted from the NRC (2001) model.³Probability of a significant effect of RDP source or of a linear or quadratic effect of dietary urea content.

Table 5. Effect of dietary RDP source on intake, flow at omasal canal, and ruminal digestibility of DM, OM, NDF, and ADF¹

Item	Total ² RDP (RDP from urea), % of DM				SE	<i>P</i> > <i>F</i> ³		
	10.6 (0)	10.5 (1.2)	10.4 (2.4)	10.5 (3.7)		RDP source	Linear	Quadratic
DM								
Intake, kg/d	23.7	22.0	23.9	22.5	1.1	0.26	0.64	0.94
Flow at omasal canal, kg/d	15.3 ^a	13.9 ^b	14.8 ^{ab}	13.9 ^b	0.6	0.03	0.38	0.68
Apparently digested in the rumen % of DM intake	34.9	36.0	37.9	38.3	2.7	0.67	0.59	0.84
OM								
Intake, kg/d	22.9	20.6	22.3	21.0	0.9	0.10	0.22	0.59
Flow at omasal canal, kg/d	12.4	11.4	12.1	11.3	0.5	0.06	0.13	0.92
Apparently digested in the rumen kg/d	6.79	6.37	6.80	6.57	0.61	0.87	0.93	0.88
% of OM intake	32.9	34.3	33.6	34.2	2.5	0.92	0.70	0.92
Truly digested in the rumen kg/d	15.3	13.1	15.2	14.2	0.9	0.13	0.56	0.61
% of OM intake	66.1	64.7	67.1	66.9	1.2	0.33	0.28	0.73
NDF								
Intake, kg/d	6.14	5.82	6.51	5.96	0.27	0.14	0.52	0.52
Flow at omasal canal, kg/d	3.59 ^a	3.04 ^b	3.50 ^a	3.48 ^a	0.20	0.02	0.14	0.09
Apparently digested in the rumen kg/d	2.35	2.82	3.10	2.48	0.26	0.08	0.01	0.01
% of NDF intake	39.2 ^b	46.9 ^a	45.4 ^a	40.7 ^b	2.2	<0.01	<0.01	<0.01
ADF								
Intake, kg/d	3.52	3.30	3.67	3.25	0.16	0.07	0.29	0.20
Flow at omasal canal, kg/d	2.02 ^a	1.63 ^b	1.91 ^a	1.76 ^{ab}	0.10	0.04	0.36	0.33
Apparently digested in the rumen kg/d	1.42	1.66	1.76	1.48	0.14	0.10	0.02	0.02
% of ADF intake	41.6	49.3	45.7	44.6	2.5	0.15	0.07	0.08

^{a,b}Least squares means within the same row with different superscripts differ ($P < 0.05$) when effect of RDP source differs ($P < 0.05$).

¹Data from the 8 ruminally cannulated cows.

²Predicted from the NRC (2001) model.

³Probability of a significant effect of RDP source or of a linear or quadratic effect of dietary urea content.

flow at the omasum for microbial OM allowed estimation of OMTDR; this also was not altered by diet in the present trial. Ruminal NDF flow was lower on the diet with 1.2% dietary urea RDP than on the other 3 diets. Linear and quadratic effects ($P \leq 0.01$) also were detected for the amount of NDF and the proportion of consumed NDF that were digested in the rumen. Although there was no effect of diet on NDF intake by the cannulated cows used for omasal sampling, dietary NDF content increased with increasing inclusion of LSBM (Table 1). As discussed earlier, greater total-tract NDF digestibility with increasing dietary LSBM was likely related to the higher level of NDF in that protein source. This is partly NDF-bound CP, which contributes to N fraction B3 of the Cornell Model, much of which would be expected to escape the rumen but still be digestible in the intestine (Fox et al., 2004). The quadratic response of NDF flow and digestion may have occurred because an increasing proportion of dietary NDF came from LSBM, and may not represent an effect of urea supply or ruminal ammonia on fiber digestion. Alteration of ADF digestion was not as pronounced, although there was an effect of diet on ADF flow ($P = 0.04$) and linear and quadratic effects ($P =$

0.02) on amount apparently digested. However, there were no significant effects when ruminal digestion was expressed as a proportion of ADF intake. Moreover, estimated total-tract ADF digestion was not altered (Table 3).

There were substantial effects of replacing soybean meal RDP with urea RDP on ruminal N metabolism and omasal flow of N fractions of dietary and microbial origin (Table 6). A linear decrease ($P < 0.01$) in total NAN flow at the omasum with increasing dietary urea was observed; this may be partly attributed to the linear decline ($P = 0.04$) in total N intake in the cannulated cows. Moreover, there were significant effects of diet ($P < 0.01$) and linear reductions ($P < 0.01$) in omasal flow of every one of the 17 AA measured when soybean meal RDP was replaced with that from urea (Table 7). The magnitude of these depressions, from zero-urea to the highest urea diet, was about 22%, a somewhat greater effect than the 16% decline in total NAN flow (Table 6). A reduction of this magnitude would not have occurred if microbial growth were not affected by source of RDP. Measured RDP supply, expressed as proportions of either dietary CP or DM, was not completely constant across diets as expected,

Table 6. Effect of dietary RDP source on intake and flow of N fractions at the omasal canal¹

Item	Total ² RDP (RDP from urea), % of DM					<i>P</i> > <i>F</i> ³		
	10.6 (0)	10.5 (1.2)	10.4 (2.4)	10.5 (3.7)	SE	RDP source	Linear	Quadratic
Dietary N intake, g/d	570	551	561	534	16	0.08	0.04	0.63
Omasal flows								
Total NAN, g/d	604 ^a	562 ^a	573 ^a	509 ^b	27	<0.01	<0.01	0.41
N truly digested in the rumen, g/d	439	362	425	423	25	0.12	0.24	0.20
RDP supply								
kg/d	2.75	2.26	2.66	2.64	0.16	0.12	0.81	0.23
% of diet CP	69.2 ^{ab}	64.8 ^b	68.1 ^b	72.5 ^a	1.8	0.02	0.05	0.01
% of DM intake	11.2 ^{ab}	10.4 ^b	11.0 ^b	11.7 ^a	0.3	0.02	0.04	0.02
RUP flow								
kg/d	1.19 ^a	1.22 ^a	1.24 ^a	1.01 ^b	0.07	0.01	0.02	0.01
% of diet CP	30.8 ^{ab}	35.2 ^a	31.9 ^a	27.5 ^b	1.8	0.02	0.03	0.01
% of DM intake	5.0 ^{ab}	5.6 ^a	5.1 ^a	4.4 ^b	0.29	0.02	0.03	0.01
NMNAN, ⁴ % of total NAN	31.1	34.7	34.9	31.6	1.5	0.12	0.98	0.02
Microbial NAN flows								
FAB-NAN ⁴								
g/d	183	164	166	154	13	0.41	0.13	0.82
% of microbial-NAN	41.7	44.8	46.0	45.2	3.9	0.85	0.48	0.56
PAB-NAN ⁴								
g/d	260 ^a	199 ^b	207 ^b	189 ^b	20	0.05	0.02	0.26
% of microbial-NAN	58.3	55.2	54.0	54.8	3.9	0.85	0.48	0.56
Total microbial NAN								
g/d	440 ^a	363 ^b	374 ^b	342 ^b	19	<0.01	<0.01	0.22
% of total NAN	68.9	65.3	65.1	68.4	1.5	0.12	0.98	0.02
Microbial efficiency, g of NAN/kg of OMTDR ⁴	29.3	28.6	24.4	24.5	2.4	0.23	0.05	0.75

^{a,b}Least squares means within the same row with different superscripts differ ($P < 0.05$) when effect of RDP source differs ($P < 0.05$).

¹Data from 8 ruminally cannulated cows.

²Predicted from the NRC (2001) model.

³Probability of a significant effect of RDP source or of a linear or quadratic effect of dietary urea content.

⁴NMNAN = nonmicrobial NAN; FAB- and PAB-NAN = fluid- and particle-associated bacterial NAN; OMTDR = OM truly digested in the rumen.

but was subject to linear ($P \leq 0.05$) and quadratic ($P \leq 0.02$) effects of urea level. However, measured RDP ranged only from 10.4 to 11.7% of DM intake, with a mean of 11.0%. This mean approached the formulated value of 10.5%, computed from the NRC (2001) model, indicating that our objective of similar total RDP supply from different sources was achieved overall. Effects on RUP supply were of comparable magnitude and, of course, in the opposite direction. Flow of RUP, when expressed as amount/day or as proportion of total dietary CP and DM, was altered by diet ($P \leq 0.02$) and displayed linear ($P \leq 0.03$) and quadratic ($P = 0.01$) trends. Mean observed RUP was 5.0%, rather than the formulated 4.5% of dietary DM and, because it was not constant across diets, suggested that the NRC (2001) model overestimated RUP contents of diets fed in this trial.

Omasal NAN flows contributed by FAB, PAB, and total ruminal microbes (FAB plus PAB), quantified using ¹⁵N enrichment, are reported in Table 6. A linear decrease ($P = 0.02$) in PAB NAN was detected as urea RDP increased. More importantly, linear declines were observed for amount ($P < 0.01$) and proportionate flow ($P = 0.02$) of total microbial NAN and for microbial

growth efficiency (g of NAN/kg of OMTDR; $P = 0.05$). The reduction in total microbial NAN flow from zero to highest dietary urea was 22% and appeared to account for all of the depression in AA flow observed in this trial (Table 7). This is clear evidence that replacing RDP from true protein with that from NPN depressed microbial protein formation in vivo in the lactating dairy cow. Earlier, we observed a linear reduction in FAB NAN flow when dietary RDP supply decreased from 3,076 to 2,403 g/d; however, that decrease was accomplished by removing both SSBM and urea from the diet. Several in vitro studies have shown that supplying RDP as free AA and peptides improves microbial growth and efficiency (Maeng and Baldwin, 1976; Cotta and Russell, 1982; Argyle and Baldwin, 1989; Hristov and Broderick, 1994; Atasoglu et al., 1999; Carro and Miller, 1999). Chikunya et al. (1996) observed that microbial yield in sheep, estimated in vivo from urinary excretion of purine derivatives, increased more with casein than urea supplementation and was 10% greater on low-energy hay but 80% greater on a higher energy beet pulp diets. Kozloski et al. (2000) found that replacing soybean meal with isonitrogenous amounts of urea depressed in vivo microbial protein

Table 7. Effect of RDP source on omasal flow of individual and total AA¹

Item	Total ² RDP (RDP from urea), % of DM				SE	<i>P</i> > <i>F</i> ³		
	10.6 (0)	10.5 (1.2)	10.4 (2.4)	10.5 (3.7)		RDP source	Linear	Quadratic
AA flows, g/d								
Essential								
Arg	153 ^a	138 ^b	134 ^b	119 ^c	6	<0.01	<0.01	0.93
His	61.1 ^a	55.0 ^b	55.2 ^b	47.4 ^c	2.5	<0.01	<0.01	0.52
Ile	155 ^a	141 ^{ab}	133 ^b	113 ^c	8	<0.01	<0.01	0.63
Leu	279 ^a	250 ^b	253 ^b	222 ^c	11	<0.01	<0.01	0.73
Lys	136 ^a	126 ^a	124 ^a	106 ^b	7	<0.01	<0.01	0.47
Met	92.9 ^a	82.4 ^{bc}	85.2 ^{ab}	74.7 ^c	4.2	<0.01	<0.01	0.87
Phe	175 ^a	162 ^a	164 ^a	144 ^b	8	<0.01	<0.01	0.47
Thr	175 ^a	152 ^b	153 ^b	134 ^c	7	<0.01	<0.01	0.89
Val	168 ^a	153 ^{ab}	145 ^b	122 ^c	8	<0.01	<0.01	0.47
Nonessential								
Ala	225 ^a	197 ^b	201 ^b	179 ^c	8	<0.01	<0.01	0.82
Asp	393 ^a	349 ^b	345 ^b	304 ^c	17	<0.01	<0.01	0.95
Cys	132 ^a	118 ^{ab}	115 ^{bc}	102 ^c	8	0.02	<0.01	0.94
Glu	554 ^a	503 ^b	507 ^b	442 ^c	23	<0.01	<0.01	0.55
Gly	178 ^a	156 ^b	158 ^b	137 ^c	7	<0.01	<0.01	0.95
Pro	160 ^a	143 ^b	149 ^{ab}	129 ^c	7	<0.01	<0.01	0.57
Ser	172 ^a	152 ^b	153 ^b	136 ^c	8	<0.01	<0.01	0.91
Tyr	144 ^a	131 ^b	131 ^b	116 ^c	6	<0.01	<0.01	0.58
Total essential AA	1,395 ^a	1,259 ^b	1,245 ^b	1,082 ^c	58	<0.01	<0.01	0.62
Total nonessential AA	1,956 ^a	1,750 ^b	1,758 ^b	1,544 ^c	79	<0.01	<0.01	0.80
Total AA	3,350 ^a	3,009 ^b	3,001 ^b	2,623 ^c	137	<0.01	<0.01	0.71
Total AA-N, g/d	434 ^a	389 ^b	388 ^b	339 ^c	19	<0.01	<0.01	0.72

^{a-c}Least squares means within the same row with different superscripts differ ($P < 0.05$) when effect of RDP source differs ($P < 0.05$).

¹Data from the 8 ruminally cannulated cows.

²Predicted from the NRC (2001) model.

³Probability of a significant effect of RDP source or of a linear or quadratic effect of dietary urea content.

synthesis in steers, despite positive effects of urea addition on ruminal cellulose digestion. Additional *in vivo* studies have confirmed the effects of RDP from true protein (Hume, 1970; Olmos Colmenero and Broderick, 2006; Brito et al., 2007b). However, Ahvenjärvi et al. (1999) reported that replacing urea CP with that from rapeseed meal or rapeseed cake did not affect microbial protein flow from the rumen of dairy cows fed diets based on barley and grass silage.

Across diets, FAB and PAB NAN accounted for, respectively, 44 and 56% of total microbial NAN flow from the rumen (Table 6). We observed proportions of total microbial NAN flow of 45% from FAB and 55% from PAB in one earlier experiment (Brilo et al., 2007b) but somewhat greater contributions from PAB in other trials (Reynal and Broderick, 2005; Brito et al., 2006, 2007a). Although about 75% of microbial OM in whole ruminal contents has been found to be associated with particulate matter (Craig et al., 1987), Hristov and Broderick (1996) found similar amounts of total microbial NAN left the rumen as FAB and PAB because of 3 times more rapid outflow of the fluid phase than particulate phase. Olmos Colmenero and Broderick (2006) observed about equal contribution from FAB and PAB to total microbial NAN flow.

Linear Responses to Increasing Urea RDP

Coefficients from regression on proportion of RDP from urea (as % of dietary DM) for several traits describing production and omasal flow are reported in Table 8. Only linear regressions are reported. All intercepts were different from zero ($P < 0.01$). Slopes for the 6 production traits indicated substantial depression ($P \leq 0.02$) in performance as urea RDP was added at the expense of soybean meal RDP. Per unit replacement of RDP from true protein with that from urea, intake declined 0.3 kg/d, milk and FCM yield 0.7 to 0.8 kg/d, and fat and protein yield 28 and 24 g/d, and MUN increased about 0.5 mg/dL. Decreases in flow of essential AA, nonessential AA, total AA, and microbial NAN from the rumen were even more dramatic, with slopes for these traits all being highly significant ($P < 0.01$). The linear decline of microbial efficiency, although not as statistically significant ($P = 0.05$), reflected the degree to which microbial growth was impaired as the supply of RDP from true protein was reduced.

We have attributed the decline in production of milk and milk components to the reduced ruminal microbial protein formation from RDP contributed by urea versus true protein. However, Ahvenjärvi et al. (1999)

Table 8. Regression on dietary RDP from urea of some production and omasal flow traits

Trait	Intercept	SE	$P > F^1$	Slope (/unit urea-RDP ²)	SE	$P > F^1$	$P > F^1$	
							Linear	Quadratic
Production								
DM intake, kg/d	26.7	1.0	<0.01	-0.32	0.10	<0.01	<0.01	0.75
Milk, kg/d	36.0	2.1	<0.01	-0.81	0.22	<0.01	<0.01	0.20
FCM, kg/d	40.5	2.1	<0.01	-0.72	0.25	<0.01	<0.01	0.79
Fat, kg/d	1.53	0.09	<0.01	-0.028	0.012	0.02	0.02	0.70
Protein, kg/d	1.25	0.06	<0.01	-0.024	0.007	<0.01	<0.01	0.86
MUN, mg/dL	13.9	1.0	<0.01	0.49	0.11	<0.01	<0.01	0.53
Omasal flow								
Essential AA, g/d	1,491	75	<0.01	-77	14	<0.01	<0.01	0.68
Nonessential AA, g/d	2,004	104	<0.01	-99	19	<0.01	<0.01	0.85
Total AA, g/d	3,492	178	<0.01	-177	33	<0.01	<0.01	0.77
Microbial NAN, g/d	463	24	<0.01	-21.9	6.1	<0.01	<0.01	0.21
Microbial efficiency, g of NAN/kg of OMTDR ³	28.6	2.6	<0.01	-1.51	0.70	0.05	0.05	0.67

¹Probability of intercepts or slopes being different from zero and of significant linear or quadratic effects of dietary urea content.

²Slopes are in units of change of production or flow trait/unit of urea-RDP as percentage of dietary DM.

³OMTDR = OM truly digested in the rumen.

observed that replacement of urea CP with that from rapeseed increased yield of milk, energy-corrected milk, and protein via increased supply of RUP without any change in microbial protein outflow from the rumen. Ipharraguerre and Clark (2005) summarized the literature showing that substituting RUP from heat-treated soybean meal for RDP from SSBM increased milk yield, but adding urea to soybean meal RUP depressed milk secretion. Thus, the confounding effects of changing RUP supply should not be ignored in the present study.

The nature of the basal diet fed in the current trial may have accounted for the additional NPN from urea being ineffective. All diets contained 55% of DM from silages. The corn silage and alfalfa silage fed in this trial contained, respectively, 47 and 55% of total CP as NPN; silage NPN accounted for approximately four-fifths of total NPN in the zero-urea diet. These NPN proportions are typical for both silages; most of the NPN in silages will be present as free AA and small peptides (Broderick, 1995). The zero-urea diet already contained 20% of total CP equivalent in the form of NPN without any urea and the proportion of total CP present as NPN increased to nearly 44% at the highest level of urea addition (Table 1). Charmley and Veira (1990) found, in sheep fed only alfalfa silage, that reducing NPN in the alfalfa from 65 to 38% of CP increased NAN flow at the duodenum; 70% of this increase was accounted for via greater flow of microbial NAN. On our basal diets with 25% NDF, already containing 20% NPN, the impairment of animal performance observed when substituting urea for true protein RDP likely occurred because providing only ammonia-N did not satisfy microbial requirements for AA and peptides and, hence, depressed microbial protein formation in the rumen.

CONCLUSIONS

Stepwise replacement of RDP from soybean with RDP from urea in a diet formulated mainly from corn and alfalfa silages and high-moisture corn and containing 16.1% CP and 10.5% total RDP resulted in linear depression of DM intake, yield of milk, 3.5% FCM, fat, protein, lactose and SNF, and weight gain, and linear increases in excretion of urinary urea-N and fecal N, and concentration of MUN, BUN, and ruminal ammonia-N. Increasing the proportion of RDP from urea resulted in linear decrease in omasal flow of dietary NAN and in microbial NAN flow and growth efficiency. These changes were paralleled by large linear reductions in omasal flows of essential AA, nonessential AA, and total AA. Overall, these results indicate that replacing RDP from true protein with that from NPN reduces yield of milk and milk components by depressing microbial protein formation in the rumen.

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