

Effect of Dietary Level of Rumen-Degraded Protein on Production and Nitrogen Metabolism in Lactating Dairy Cows*

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ABSTRACT

Twenty-eight (8 with ruminal cannulas) lactating Holstein cows were assigned to 4 × 4 Latin squares and fed diets with different levels of rumen-degraded protein (RDP) to study the effect of RDP on production and N metabolism. Diets contained [dry matter (DM) basis] 37% corn silage, 13% alfalfa silage, and 50% concentrate. The concentrate contained solvent and lignosulfonate-treated soybean meal and urea, and was adjusted to provide RDP at: 13.2, 12.3, 11.7, and 10.6% of DM in diets A to D, respectively. Intake of DM and yield of milk, fat-corrected milk, and fat were not affected by treatments. Dietary RDP had positive linear effects on milk true protein content and microbial non-ammonia N (NAN) flow at the omasal canal, and a quadratic effect on true protein yield, with maximal protein production at 12.3% RDP. However, dietary RDP had a positive linear effect on total N excretion, with urinary N accounting for most of the increase, and a negative linear effect on environmental N efficiency (kg of milk produced per kg of N excreted). Therefore, a compromise between profitability and environmental quality was achieved at a dietary RDP level of 11.7% of DM. Observed microbial NAN flow and RDP supply were higher and RUP flow was lower than those predicted by the NRC (2001) model. The NRC (2001) model overpredicted production responses to RUP compared with the results in this study. Replacing default NRC degradation rates for protein supplements with rates measured in vivo resulted in similar observed and predicted values, suggesting that in situ degradation rates used by the NRC are slower than apparent rates in this study.

(Key words: rumen-degraded protein, dairy cow, nitrogen metabolism, microbial protein)

Abbreviation key: FAB = fluid-associated bacteria, FP = omasal fluid phase, HMSC = high-moisture shelled corn, IADF = indigestible ADF, INDF = indigestible NDF, LP = omasal large particle phase, LSBM = lignosulfonate-treated soybean meal, MP = metabolizable protein, ¹⁵NB = ¹⁵N background, NANMN = nonammonia nonmicrobial N, NDIN = neutral-detergent insoluble N, OTD = omasal true digesta, PAB = particle-associated bacteria, PD = purine derivatives, RC = ruminally cannulated, SP = omasal small particle phase, SSBM = solvent soybean meal.

INTRODUCTION

In the United States, a typical dairy farm exports as milk and meat protein only 21 to 38% of the total N imported as feed, fertilizer, and via N fixation by legumes (Klausner, 1993). Approximately 70% of the remaining N can be lost into the environment through volatilization, denitrification, leaching, and runoff, contributing to environmental pollution (Tamminga, 1992; Van Horn et al., 1994; Hutson et al., 1998). Nitrogen use by dairy cows must be maximized by reducing N losses while maintaining or improving production of saleable product.

Microbial protein synthesized in the rumen, RUP, and endogenous protein contribute AA to the small intestine, with microbial protein accounting for the majority of the total AA flow (Clark et al., 1992). However, substantial amounts of dietary AA are deaminated by bacteria, converting dietary AA-N into ammonia-N. Most of the ammonia-N that is not incorporated into microbial protein in the rumen is eventually excreted as urea (Broderick et al., 1991). On the other hand, feeding RDP below the requirements for maximal rumen microbial growth can compromise microbial protein production, ruminal digestion, and energy and protein availability to the cow (Stokes et al., 1991; Clark et al., 1992). Therefore, determining the level of dietary RDP required for optimum N use by ruminal microbes would allow for reductions in dietary CP levels without compromising milk production, thereby increasing feed efficiency and reducing feed costs and N losses to the environment. Moreover, quantitative in vivo estimates

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of RDP requirements will be useful for the accuracy of the predictions of ration formulation models.

The objectives of this experiment were 1) to determine the level of dietary RDP required to maximize microbial protein synthesis; 2) to determine the effect of RDP level on production and digestion; and 3) to test NRC (2001) predictions of microbial and RUP flows from the rumen.

MATERIALS AND METHODS

Experimental Procedures

Twenty-eight (8 with ruminal cannulas) lactating Holstein cows, averaging 72 (SD 62) DIM and 634 (SD 47) kg of BW at the beginning of the study, were blocked by DIM into seven 4 × 4 Latin squares [2 squares of ruminally cannulated (**RC**) cows]. Cows were randomly assigned within squares to 4 dietary treatment sequences. Diets were fed as TMR containing (DM basis) 37% corn silage, 13% alfalfa silage, and 50% concentrate. Proportions of rolled high-moisture shelled corn (**HMSC**), solvent soybean meal (**SSBM**), lignosulfonate-treated soybean meal (**LSBM**; SoyPass, Ligno-Tech USA, Inc., Rothschild, WI), and urea were adjusted to provide similar levels of CP from ingredients other than urea to achieve 4 levels of RDP across diets (Table 1). Based on the DMI and composition of the diets as fed, RDP levels predicted by the NRC (2001) model were: 12.5, 10.9, 9.2, and 7.7% of DM for diets A, B, C, and D, respectively. All cows were injected with bST (500 mg of Posilac; Monsanto, St. Louis, MO) beginning on d 1 of the trial and at 14-d intervals throughout. Cows were housed in tie stalls and had free access to water throughout 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 18 d for adaptation and 10 d for sample collection. Diets were offered once daily at 1000 h, except for the RC cows during the sampling period when diets were offered twice daily at 1000 and 2200 h. Orts were collected and weights recorded once daily at 0900 h and the feeding rate was adjusted daily to yield orts of about 5 to 10% of intake. Weekly composites of corn silage, alfalfa silage, HMSC, TMR, and orts were taken from daily samples of about 0.5 kg that were stored at -20°C. Weekly samples also were taken of urea, SSBM, and LSBM and stored at room temperature. The DM was determined in weekly composites of corn silage, alfalfa silage, and HMSC by drying at 60°C for 48 h and in weekly samples of urea, 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. Jo-

seph, MI). The DM and N content of the feeds was used to adjust dietary composition weekly to obtain similar levels of CP from sources other than urea. 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. 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 (Hintz et al., 1995), and for indigestible ADF (**IADF**; ADF remaining after a 12-d in situ incubation; Huhtanen et al., 1994). Body weights were measured on 3 consecutive days at the start of the trial and at the end of each period to compute BW change. Milk yield was recorded at all daily a.m. and p.m. milkings. Milk samples were taken at 2 consecutive milkings (p.m. and a.m.) on d 19 and 26 of each period. Composite milk samples were prepared from each cow each sampling day by mixing 5-mL subsamples from consecutive a.m. and p.m. samples. Composite samples were deproteinized (Shahani and Sommer, 1951) and analyzed for MUN by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to a flow-injection analyzer (QuikChem 8000 FIA, Lachat Instruments, Milwaukee, WI). The remaining individual a.m. and p.m. samples were analyzed for fat, true protein, lactose, and SNF by infrared analysis (Ag-Source, Verona, WI).

On d 21 of each period, 2 spot urine and 2 fecal grab samples were collected from all 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 N, DM, ash, OM, NDF, ADF, and IADF as described earlier. Indigestible ADF was used as an internal marker to estimate apparent nutrient digestibility and fecal output (Cochran et al., 1986). Urine samples were immediately acidified after collection by diluting 1 volume of urine with 4 volumes of 0.072 N H₂SO₄ 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 assay (Oser, 1965) adapted to a flow-injection analyzer (Lachat QuikChem 8000), for total N (Mitsubishi N Analyzer), for allantoin using the method of Vogels and van der Grift (1970) adapted to a 96-well plate reader, for uric acid using a commercial kit (No. 1830-200, Thermo DMA, Arlington, TX), and for urea with the colorimetric method also used for MUN. Daily urine volume and excretion of urea N, total N, allantoin, uric acid, and total purine derivatives (**PD**; allantoin plus

Table 1. Composition of diets.

	Diet			
	A	B	C	D
Ingredients	(% of DM)			
Corn silage	37.1	37.1	37.1	37.1
Alfalfa silage	12.7	12.7	12.7	12.7
Rolled high-moisture shelled corn	32.4	32.1	31.9	31.7
Solvent soybean meal	16.43	10.95	5.48	—
Lignosulfonate-treated soybean meal	—	5.87	11.74	17.61
Urea	0.50	0.33	0.17	—
Sodium bicarbonate	0.45	0.45	0.45	0.45
Salt	0.25	0.25	0.25	0.25
Dicalcium phosphate	0.10	0.10	0.10	0.10
Vitamin-mineral mix ¹	0.10	0.10	0.10	0.10
Nutrient content of diets				
DM, %	50.3	50.3	50.3	50.3
CP, % of DM	18.8	18.3	17.7	17.2
RDP, ² % of DM	13.2	12.3	11.7	10.6
RUP, ³ % of DM	5.8	6.2	6.0	6.6
RDP, ⁴ % of DM	12.5	10.9	9.2	7.7
RUP, ⁴ % of DM	6.3	7.4	8.5	9.5
NE _L , ⁵ Mcal/kg of DM	1.56	1.55	1.55	1.55
NFC, ⁴ % of DM	49.2	49.9	50.6	51.4
NDF, % of DM	27.5	28.3	27.8	30
ADF, % of DM	16.0	16.4	14.6	15.4
Neutral detergent insoluble N, % of total N	4.06	7.82	12.86	19.26
Total N, % of DM	3.00	2.92	2.83	2.75
Nonurea N, % of DM	2.77	2.77	2.75	2.75
NPN, ⁶ % of total N	56.6	52.5	48.7	41.4
True protein-N, % of nonurea N	47.0	50.1	52.7	58.6
NH ₃ -N, % of total N	3.75	3.70	3.93	3.90
Free AA-N, ⁷ % of total N	12.2	12.6	13.2	13.0
Total AA-N, % of total N ⁸	64.5	67.1	68.5	72.1
NAN, % of total N	96.3	96.3	96.1	96.1

¹Provided (per kilogram 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, 6440 IU of vitamin A, 2000 IU of vitamin D, and 16 IU of vitamin E.

²Least squares means of RDP measured in vivo as: RDP, % of DM = (Total CP intake, kg/d – omasal RUP flow, kg/d) × 100/DMI, kg/d.

³Least squares means of RUP measured in vivo as: RUP, % of DM = (Total omasal CP flow, kg/d – microbial CP flow, kg/d) × 100/DMI, kg/d.

⁴Predicted by the NRC (2001) model.

⁵Computed by discounting dietary energy content based on actual intake (NRC, 2001).

⁶Proportion of total N soluble in 10% (wt/vol) TCA (Muck, 1987).

⁷Free AA-N = total free AA, mmol × (40.3 mg of N/mmol of total free AA; Broderick, 1987).

⁸Proportion of total N detected as 18 protein AA in 6 N HCl hydrolysates of each diet.

uric acid) were estimated from urinary creatinine concentration and BW assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Microbial NAN flow from the rumen was estimated based on the excretion of PD using the equation of Vagnoni et al. (1997).

Digesta flow from the rumen was quantified using the omasal sampling technique developed by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2000). Indigestible NDF (**INDF**; Huhtanen et al., 1994), CoEDTA (Uden et al., 1980), and YbCl₃ (modified from Siddons et al., 1985) were used as digesta flow markers. The YbCl₃ was prepared by adding 230 g of Yb₂O₃ to 200 mL of distilled water plus 320 mL of concentrated

HCl, heating and stirring until the solution cleared, then diluting to 5 L with distilled water. The external microbial marker ¹⁵N was used to measure microbial N flows from the rumen. Before starting the infusion of markers, a sample of whole ruminal contents was taken from each RC cow to determine the ¹⁵N background (¹⁵NB). The ruminal ¹⁵NB samples were stored at –20°C until freeze-dried and then ground sequentially through a 1-mm screen (Wiley mill) and a 0.5-mm screen (Udy mill; Udy Corporation, Fort Collins, CO) for later analysis. Cobalt-EDTA, YbCl₃, and 10% atom excess ¹⁵NH₄SO₄ were dissolved in distilled water and infused into the rumen at a constant rate of 2.6 g of Co, 2.2 g of Yb, and 182 mg of ¹⁵N/d in 3.12 L of

solution. Markers were continuously infused for 158 h from d 20 at 0900 h to d 26 at 2300 h using 2 syringe pumps (model 33; Harvard Apparatus, Inc., Holliston, MA). Using the omasal sampling technique, spot samples were collected from the omasal canal 6 times daily at 1-h intervals on d 23 to 26. Sampling was at 1000, 1100, 1200, 1300, 1400, 1500 h (d 23 and 25), and at 1600, 1700, 1800, 1900, 2000, and 2100 h (d 24 and 26), such that the samples taken represented two 12-h feeding cycles over 2 d each, the first feeding cycle corresponding to sampling days 23 and 24, and the second feeding cycle corresponding to sampling days 25 and 26. On each sampling day, 285-mL omasal spot samples were taken at the first 5 sampling times and a 785-mL spot sample was taken at the last sampling time from each cow. The 285-mL omasal spot samples were divided into subsamples of 85 and 200 mL, and the 785-mL spot sample was divided into subsamples of 85, 200, and 500 mL. The 85-mL subsamples were held in an ice-bath as they were collected, and pooled over the 6 sampling times to yield one 510-mL composite from each cow on each sampling day. The 200-mL subsamples were pooled and stored at -20°C as they were collected over the 12 sampling times to yield two 2.4-L omasal composites, one corresponding to sampling d 23 and 24 and another corresponding to d 25 and 26, from each cow in each period. The 500-mL subsample from each cow was processed immediately after collection to isolate protozoa; these data are reported in a companion paper (Reynal et al., 2005).

The 2.4-L pooled omasal composites were thawed at room temperature and separated into 3 digesta phases as follows. Samples were squeezed through 1 layer of cheesecloth, and the retained solids were defined as the omasal large particle phase (**LP**). The filtrate was centrifuged at $1000 \times g$ (5°C , 5 min), and the supernatant was carefully decanted from the pellet. The supernatant was defined as the omasal fluid phase (**FP**) and the pellet was defined as the omasal small particle phase (**SP**). The separated phases were frozen, freeze-dried, and then ground through a 1-mm screen (Wiley mill) for later analysis. Concentrations of Co, Yb, and INDF (determined in LP and SP) and concentrations of Co and Yb (determined in FP) were used to recombine DM from freeze-dried FP, SP, and LP in the correct proportions to reconstitute the omasal true digesta (**OTD**) flowing out of the rumen based on the triple-marker method of France and Siddons (1986). Concentrations of Co, Yb, and INDF were distinctly higher than the other markers in, respectively, FP, SP and LP, allowing for the successful application of the triple-marker approach. Aliquots of SP and LP phases were mixed in the correct proportions based on the markers to yield a 2-g sample that was ground through a 0.5-

mm screen in a Udy Cyclone Sample mill (Udy Corp.) and defined as small plus large particle phase (**SP+LP**).

At the end of each sampling day, the 510-mL omasal composites were squeezed through 1 layer of cheesecloth; solids retained on the cheesecloth were washed with 400 mL of 0.85% (wt/vol) NaCl solution and squeezed again; filtrates (equivalent to the FP) were pooled and held on ice until processed for isolation of fluid-associated bacteria (**FAB**). The solids retained on the cheesecloth (equivalent to LP) were transferred to a 500-mL bottle, to which 350 mL of cold (5°C) solution containing 0.85% (wt/vol) NaCl and 0.1% (vol/vol) Tween-80 solution were added. Bottle contents were mixed thoroughly and held on ice until processed for isolation of particle-associated bacteria (**PAB**). Filtrates for FAB isolation were centrifuged ($1000 \times g$, 5°C , 5 min) and the pellets were added to the bottles to be used for PAB isolation. Supernatants were carefully decanted and centrifuged at higher speed ($11,300 \times g$, 5°C , 30 min); these supernatants were decanted and discarded. Pellets were resuspended in 100 mL of McDougall's buffer and recentrifuged ($11,300 \times g$, 5°C , 30 min). The resulting FAB pellets were stored at -20°C until freeze-dried and ground with a mortar and pestle for later analysis. Contents of the PAB bottles were blended for 20 s in a one-speed Waring blender (Waring Products Division, New Hartford, CT), transferred back to the bottles and stored at 5°C . After 24 h at 5°C , contents were squeezed through 2 layers of cheesecloth and the filtrates were centrifuged ($1000 \times g$, 5°C , 5 min); supernatants were carefully decanted and pellets discarded. Supernatants were centrifuged at higher speed ($11,300 \times g$, 5°C , 30 min), decanted, and discarded, and the pellets resuspended in 100 mL of McDougall's buffer and recentrifuged ($11,300 \times g$, 5°C , 30 min). The resulting PAB pellets were stored at -20°C until freeze-dried and ground with a mortar and pestle. Two separate FAB and PAB composites were prepared for each cow in each period by mixing equal DM from d 23 and 24 and from d 25 and 26. Thus, each bacterial composite represented one 12-h feeding cycle.

Indigestible NDF was determined in LP, SP, and TMR samples (but not FP; Ahvenjärvi et al., 2000) as follows. Samples (1 g for LP and TMR; 4 g for SP) were weighed into duplicate 5- \times 10-cm bags made of 6- μm pore-size Dacron mesh (part no. 03-6/5, SEFAR America Inc., Depew, NY) incubated in the rumens of 2 cows for 12 d, rinsed with water, and then subjected to conventional NDF analysis (Hintz et al., 1995). For Co and Yb analysis, 1-g samples of SP and LP and 0.5-g samples of FP were ashed at 500°C for 16 h and then solubilized in 15 mL of concentrated HCl. A solution of 0.6% (wt/vol) LiOH was added to a final mass of 100 g. Aliquots (0.5 mL) of the infused marker solutions were

diluted with 15 mL of concentrated HCl and 84.5 mL of LiOH solution before analysis. Concentrations of Co and Yb were analyzed by direct current plasma emission spectroscopy (Combs and Satter, 1992; SpectraSpan V; Fison Instruments, Valencia, CA).

Whole ruminal contents (100 to 200 mL) were obtained from all RC cows at 0, 1, 2, 4, 6, 8, 12, 18, and 24 h after the morning feeding of d 21 of each period, strained through 2 layers of cheesecloth, and pH measured immediately. Ten-milliliter subsamples of strained ruminal fluid were preserved by addition of 0.2 mL of 50% (vol/vol) H₂SO₄, and stored at -20°C. Just before analysis, samples were thawed, centrifuged (15,000 × g, 4°C, 15 min), and analyzed for ammonia and total free AA (Reynal and Broderick, 2003) using flow-injection analysis (Lachat QuikChem 8000 FIA), and for VFA (Brotz and Schaeffer, 1987). Blood samples were taken from the coccygeal artery or vein of each cow 4 h after feeding on d 20 of each period. Blood was heparinized and held at 4°C for 8 h; 1.25 mL of 25% (wt/vol) TCA was added to a 5-mL subsample. After being held for 30 min at 25°C, samples were centrifuged (15,000 × g, 4°C, 15 min) and the supernatant stored at -20°C until analyzed for blood urea N by the colorimetric assay used for MUN and urine.

The OTD samples and weekly composites of TMR and feed ingredients were analyzed for total N, DM, ash, OM, NDF, and ADF as described earlier, and for neutral-detergent insoluble N (NDIN; AOAC, 1980). Weekly composites of TMR were also analyzed for IADF as described earlier. Extracts were prepared from weekly composites of silages and TMR and from OTD samples in distilled water (Muck, 1987) as follows: 10 mL of pH 2.2 Na-citrate buffer was added to 0.5 g of sample; after mixing, samples were held at 39°C for 30 min, centrifuged (15,000 × g, 4°C, 15 min) and supernatants stored at -20°C for later analysis. These extracts were analyzed for ammonia and total free AA as described above for strained ruminal fluid. Extracts from TMR and silages were also analyzed for NPN (Muck, 1987) using a combustion assay (Mitsubishi TN-05 Nitrogen Analyzer; Mitsubishi Chemical Corp., Tokyo).

Samples of OTD, FAB, PAB, and TMR were hydrolyzed without pretreatment in 6 N HCl containing 0.5 mM norleucine and 1 g/L of phenol (Gehrke et al., 1985; Nagel and Broderick, 1992) or after oxidation with performic acid (for Met and Cys determination; Elkin and Griffith, 1985). Hydrolysates were filtered (Whatman No. 1 filter paper; Whatman International Ltd., Maidstone, UK) and diluted with distilled water to a final volume of 50 mL. A 2-mL aliquot was evaporated to moistness using a concentrator (SpeedVac Concentrator SVC-200H, Savant Instruments, Inc., Farmingdale, NY), redissolved in 1 mL of distilled water,

re-evaporated, dissolved in 2 mL of pH 2.2 sodium citrate buffer (Na-S, Beckman Instruments, Inc., Palo Alto, CA), and analyzed for individual AA using norleucine as internal standard by ion-exchange chromatography with ninhydrin detection (Beckman 6300 Amino Acid Analyzer, Beckman Instruments, Inc., Palo Alto, CA). The OTD, FAB, PAB, and TMR samples also were analyzed for Trp using an HPLC method adapted from Delhaye and Landry (1986) and Landry and Delhaye (1992). An amount of sample containing approximately 8 mg of N was weighed into a glass tube and 4.2 g of barium hydroxide, 2 mL of 6.25 mM 5-methyltryptophan, and 6 mL of distilled water were added. After aerating the solution with N₂ for 2 min, tubes were tightly capped and placed in a heating-block at 125°C for 16 h. After cooling, tubes were held at 25°C for 1 h and then placed in an ice bath for 30 min. Samples were adjusted to pH 3 with 6 N HCl, then centrifuged (3000 × g, 4°C, 10 min) and filtered (0.2 μm). Samples (15 μL) were injected into an HPLC system (Shimadzu Class-VP, version 5.03, Shimadzu Scientific Instruments, Inc., Columbia, MD), which consisted of an SIL-10ADvp autosampler, 2 LC-10ADvp pumps, an SCL-10Avp system controller, and an SPD-M10Avp photodiode array detector. The analytical column was a 250 × 4.6-mm reverse phase C18 column (Inertsil ODS-3; MetaChem Technologies Inc., Torrance, CA) protected by a guard column containing the same material as in the main column. Elution solvents were: A) methanol (300 mL) added to 700 mL of 70 mM-sodium acetate and 0.7 mL of triethylamine with pH adjusted to 4.5 using acetic acid; and B) 100% methanol. All solvents were filtered through a 0.45-μm filter and degassed by ultrasonication under vacuum. Samples were eluted starting with solvent A for 14 min at 0.5 mL/min, followed by a 2-min linear gradient from 0 to 100% solvent B and with flow increasing from 0.5 to 1 mL/min. Solvent B was maintained at 1 mL/min for 4 min to complete the chromatogram. The column was returned to starting conditions using a 2-min linear gradient from 0 to 100% solvent A; after 4 min at 1 mL/min, flow was decreased to 0.5 mL/min over 2 min and solvent A maintained at that rate for 4 min before injecting the next sample. Separation was performed at 45°C and the effluent was monitored at 280 nm. Tryptophan and 5-methyltryptophan (the internal standard) eluted at 10 and 16 min, respectively. Recovery of Trp was estimated by carrying ovalbumin (A5503; Sigma-Aldrich, St. Louis, MO) through the assay. Based on its AA sequence (Stein et al., 1991), pure ovalbumin is 16.32% N and 1.43% Trp; N content of the ovalbumin used was 14.22% by combustion assay (Leco Instruments, Inc.), indicating a purity of the ovalbumin used of 87%. Mean recovery of Trp from ovalbumin, after correction for

purity, was 99.5% (SD 1.8). Mean recoveries of Trp added as ovalbumin to bacterial, omasal, and TMR samples were 93.8 (SD 6.7), 105.5 (SD 2.0), and 99.0% (SD 2.4), respectively.

Total purines were determined in samples of isolated PAB and FAB using the HPLC method of Balcells et al. (1992) as modified by Makkar and Becker (1999). The N:purine ratios of FAB and PAB were used for estimating microbial protein flow to the small intestine from the urinary excretion of PD. Samples of ^{15}N B, FAB, PAB, FP, and SP+LP (100 μg of N) for ^{15}N determination were weighed into $5 \times 9\text{-mm}$ tin capsules and, after addition of 50 μL of 72-mM K_2CO_3 solution, were placed in a 60°C oven for 24 h to volatilize ammonia. Samples were analyzed for NAN and ^{15}N enrichment of NAN by isotope ratio mass spectrometry (Stable Isotope Facility, Department of Agronomy and Range Science, University of California-Davis, Davis, CA).

Calculations

Nonammonia N content of OTD was calculated by difference between total N and ammonia N. Total NAN entering the omasal canal was attributed to 3 fractions: PAB-NAN, FAB-NAN, and nonammonia nonmicrobial N (NANMN). The NANMN fraction was assumed to comprise dietary and endogenous NAN. Based on the similar background of ^{15}N in microbes and digesta observed by Ahvenjärvi et al. (2002), the ^{15}N B used for computing ^{15}N -enrichment in both bacterial and digesta fractions was defined as ^{15}N content of ruminal contents immediately before infusion. The mean ^{15}N B was 0.3677 (SD 0.0002) atom percent N over all 4 periods. The atom percent ^{15}N above this background (enrichment) was calculated for digesta and bacterial samples for each cow in each period as follows:

$$^{15}\text{N}\text{-enrichment} = ^{15}\text{N}\text{-atom}\% - ^{15}\text{N}\text{B}$$

Assuming that isolated FAB and PAB were representative of the bacterial biomass flowing with the FP and the SP+LP phases, respectively, the FAB-NAN, PAB-NAN, and total-NAN flows into the omasal canal were calculated using the ^{15}N microbial marker as follows:

$$\begin{aligned} \text{FAB-NAN flow} &= \text{FP flow} \times \text{NAN in FP} \\ &\times (\text{FP } ^{15}\text{N enrichment} / \text{FAB } ^{15}\text{N enrichment}) \\ \text{PAB-NAN flow} &= \\ &(\text{SP flow} \times \text{NAN in SP} + \text{LP flow} \times \text{NAN in LP}) \\ &\times (\text{SP+LP } ^{15}\text{N enrichment} / \text{PAB } ^{15}\text{N enrichment}) \end{aligned}$$

Microbial NAN flow = FAB-NAN flow + PAB-NAN flow,

where flows are in grams per day and NAN contents in grams per gram of DM.

Microbial flows to the small intestine were also estimated for each cow in each period from the urinary excretion of PD using the regression equation of Vagnoni et al. (1997) and the average N:purine ratio of FAB and PAB measured in the present study.

Apparent and true digestion of nutrients in the rumen were calculated as follows (using DM as an example):

$$\text{DM apparently digested in the rumen} =$$

$$\text{DM intake} - \text{omasal DM flow}$$

$$\text{DM truly digested in the rumen} = \text{DM intake}$$

$$- (\text{omasal DM flow} - \text{microbial DM flow})$$

where DM digested, intake, and flow are in kilograms per day.

Flow NANMN at the omasal canal was calculated as follows:

$$\text{NANMN flow} = \text{total NAN flow} - \text{microbial NAN flow.}$$

Assuming that all nonmicrobial N was of dietary origin, flow of RUP at the omasal canal and daily supply of RDP to the rumen were calculated as follows:

$$\text{RUP flow} = \text{total CP flow} - \text{microbial CP flow}$$

$$\text{RDP supply} = \text{Total CP intake} - \text{RUP flow}$$

where flows, supply, and intake are in grams per day.

Statistical Analyses

Data were analyzed using Proc Mixed in SAS (SAS Institute, 1999–2000). For variables measured only in the RC cows (except for rumen pH, ammonia, total free AA, and VFA), the 2 sets of samples taken from each cow in each period were considered repeated measures and, therefore, correlated on time, and were analyzed together using the following model:

$$Y_{ijklm} = \mu + S_i + P_j + Ck_{(i)} + T_l + ST_{il} + Wm_{(k)} + \varepsilon_{ijklm},$$

where Y_{ijklm} = dependent variable, μ = overall mean, S_i = effect of square i , P_j = effect of period j , $Ck_{(i)}$ = effect of cow k (within square i), T_l = effect of treatment l , ST_{il} = interaction between square i and treatment l , $Wm_{(k)}$ = effect of sample duplication m , and ε_{ijklm} = residual error. The spatial covariance structure CS was used for estimating covariances and the subject of the

repeated measurements was defined as period \times treatment \times cow(square). All terms were considered fixed except for $W_{m(k)}$, $Ck_{(i)}$, and ε_{ijklm} , which were considered random. The following model was fitted to data from all variables measured in all cows:

$$Y_{ijkl} = \mu + S_i + P_j + Ck_{(i)} + T_l + ST_{il} + \varepsilon_{ijkl},$$

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = effect of square i , P_j = effect of period j , $Ck_{(i)}$ = effect of cow k (within square i), T_l = effect of treatment l , ST_{il} = interaction between square i and treatment l , and ε_{ijkl} = residual error. All terms were considered fixed, except for $Ck_{(i)}$ and ε_{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 VFA):

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

where Y_{ijklm} = dependent variable, μ = overall mean, S_i = effect of square i , P_j = effect of period j , $Ck_{(i)}$ = effect of cow k (within square i), T_l = effect of treatment l , ST_{il} = interaction between square i and treatment l , ε_{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 covariances and the subject of the repeated measurements were defined as cow(square \times period \times treatment). All terms were considered fixed, except for $Ck_{(i)}$, ε_{ijkl} , and ω_{ijklm} , which were considered random. For all models used, the interaction term ST_{il} was removed from the models when $P > 0.25$ and differences between least squares means were reported only if the F -test for treatment was significant at $P < 0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P < 0.10$. Because dietary RDP levels among treatments were not equally spaced, linear, quadratic, and cubic effects of RDP level were tested by partitioning the degrees of freedom for diet into single degree of freedom variables corresponding to linear, quadratic and cubic effects. Cubic effects were not statistically significant for any of the variables measured, and, therefore, are not reported.

Dietary RDP levels giving maximal responses were determined by taking the first derivative of quadratic equations for which the squared terms were significant ($P < 0.05$). Quadratic maxima for treatment effects on the variables measured in this trial will be reported using the in vivo estimates of dietary RDP concentration in the Results and Discussion sections.

Table 2. Amino acid composition of diets.

	Diet			
	A	B	C	D
	(% of total AA)			
Essential AA				
Arg	5.26	5.16	5.15	5.05
His	2.26	2.23	2.25	2.22
Ile	4.21	4.16	4.19	4.27
Leu	9.49	9.42	9.57	9.52
Lys	4.83	4.70	4.53	4.38
Met	1.34	1.25	1.10	1.12
Phe	4.67	4.66	4.70	4.78
Thr	4.39	4.52	4.62	4.61
Trp	1.21	1.29	1.26	1.24
Val	4.82	4.76	4.79	4.85
Nonessential AA				
Ala	7.55	7.60	7.48	7.42
Asp	11.1	11.2	11.1	11.3
Cys	1.23	1.24	1.21	1.17
Glu	18.6	18.7	18.9	19.1
Gly	4.62	4.63	4.65	4.66
Pro	6.20	6.37	6.27	6.16
Ser	4.93	4.90	4.92	4.96
Tyr	3.21	3.19	3.25	3.22

RESULTS

Diet Composition

Overall, the alfalfa silage fed in this trial averaged 49.5% NDF, 36.1% ADF, and 21.8% CP. Corn silage contained (DM basis) 43.9% NDF, 23.3% ADF, 8.1% CP, and the rolled HMSC contained 10.4% NDF, 2.4% ADF, and 8.6% CP. The SSBM and LSBM fed in the experiment were each from single batches and contained, respectively, 52.4 and 49.7% CP, 0.33 and 32.4% of total N as NDIN, and 8.8 and 28.3% NDF. Diets were formulated to be isoenergetic and provide equal levels of nonurea N, but with decreasing levels of RDP and increasing RUP, by varying the proportions of urea, SSBM, and LSBM and maintaining constant proportions of forage and HMSC in the diets (Table 1). Measured nonurea N content varied only slightly and ranged from 2.75 to 2.77% of dietary DM. The RDP and RUP contents of the diets, computed from the observed DMI and omasal flows of NAN fractions, ranged from 13.2 to 10.6% RDP and from 5.79 to 6.61% RUP in dietary DM. However, the values predicted by the NRC (2001) model, based on observed DMI and diet composition, ranged from 12.5 to 7.7% RDP and from 6.3 to 9.5% RUP in dietary DM. The energy content of the diets predicted by the NRC (2001) model, using observed DMI of each diet, ranged from 1.55 to 1.56 Mcal/kg of DM (Table 1). The AA compositions determined for the TMR fed in this trial are in Table 2.

Table 3. Effect of dietary RDP on production and ruminal metabolism.

Item	Diet (RDP, %)				SE	$P > F^1$		
	A (13.2)	B (12.3)	C (11.7)	D (10.6)		RDP	Linear	Quadratic
Production (data from all 28 cows)								
DMI, kg/d	25.1	25.7	25.7	25.4	0.6	0.46	0.42	0.17
Milk yield, kg/d	42.3	42.8	42.4	41.5	0.9	0.43	0.33	0.26
3.5% FCM, kg/d	38.4	40.0	40.2	39.4	1.3	0.36	0.33	0.13
Milk fat, %	3.21	3.36	3.28	3.40	0.15	0.51	0.26	0.82
Milk fat, kg/d	1.23	1.31	1.33	1.32	0.07	0.39	0.16	0.31
Milk true protein, %	3.14 ^a	3.14 ^a	3.07 ^b	3.04 ^b	0.05	<0.01	<0.01	0.56
Milk true protein, kg/d	1.30 ^a	1.34 ^a	1.30 ^a	1.23 ^b	0.03	<0.05	<0.05	<0.05
SNF, %	8.78	8.86	8.78	8.77	0.08	0.55	0.59	0.37
SNF, kg/d	3.66	3.78	3.74	3.56	0.11	0.11	0.32	<0.05
MUN, ² mg/dL	15.9 ^a	15.6 ^a	13.6 ^b	12.8 ^b	0.5	<0.01	<0.01	0.55
Blood urea N, ² mg/dL	13.8 ^a	14.0 ^a	11.8 ^b	12.4 ^b	0.4	<0.01	<0.01	0.66
BW change, kg/d	0.51	0.58	0.43	0.52	0.13	0.88	0.85	0.97
Ruminal metabolism (data from ruminally cannulated cows only)								
pH	6.28	6.21	6.18	6.26	0.05	0.27	0.67	<0.10
NH ₃ -N, mg/dL	12.33 ^a	11.76 ^a	8.68 ^b	5.71 ^c	0.77	<0.01	<0.01	<0.05
Total free AA, mM	4.89 ^a	4.51 ^{ab}	3.79 ^{bc}	3.38 ^c	0.27	<0.01	<0.01	0.95
Total VFA, mM	91.3	81.0	85.9	92.0	3.9	0.11	0.63	<0.05
Acetate (A), mM	55.0	50.9	52.6	56.1	2.2	0.27	0.57	<0.10
Propionate (P), mM	21.7 ^a	15.5 ^b	18.6 ^{ab}	19.7 ^a	1.5	<0.05	0.66	<0.05
A:P ratio	2.56	3.49	2.93	3.11	0.26	0.12	0.35	0.16
Butyrate, mM	10.0 ^b	10.4 ^b	9.7 ^b	11.5 ^a	0.4	<0.01	<0.05	<0.10
Isobutyrate, mM	1.25	1.09	1.21	1.13	0.06	<0.10	0.27	0.44
Valerate, mM	1.76	1.63	2.02	1.88	0.25	0.11	0.16	0.93
Isovalerate, mM	1.60	1.67	1.80	1.65	0.09	0.34	0.49	0.17

^{a,b,c}Least squares means within the same row without a common superscript differ ($P < 0.05$).

¹Probability of a significant effect of RDP or of a linear or quadratic effect of RDP level in the diet.

²MUN and blood urea N determined using a colorimetric assay (Broderick and Clayton, 1997).

Production and Ruminal Metabolism

The RDP content of the diet had no effect on DMI and yields of milk, FCM and fat, and milk fat content (Table 3). Decreasing dietary RDP from 13.2 to 10.6% of DM resulted in linear decreases in milk true protein content, MUN, and blood urea-N ($P < 0.01$). Milk urea-N and blood urea-N also decreased linearly with decreasing dietary CP content ($P < 0.01$). There was a quadratic effect of RDP level on milk true protein yield ($P < 0.05$), with quadratic maxima at 12.3% RDP. Dietary RDP had no effect on ruminal pH and concentrations of acetate, isobutyrate, valerate, and isovalerate. However, decreasing RDP from 13.2 to 10.6% resulted in significant linear decreases in ruminal concentration of ammonia and total free AA ($P < 0.01$), and a linear increase in ruminal butyrate concentration ($P < 0.05$; Table 3).

Omasal Flows and Ruminal Digestibilities

Surprisingly, dietary RDP had no effect on DMI and OM intake when all the cows were included in the statistical analysis, but had significant quadratic effects (P

< 0.05; Table 4) when only the RC cows were considered. Cows fed diet D had the lowest DMI and the quadratic maxima corresponded to a dietary RDP concentration of 12.2%. Level of RDP had a significant quadratic effect (Table 4) on the amount of OM apparently ($P < 0.05$) and truly ($P < 0.05$) digested in the rumen, with quadratic maxima at 11.9 and 12.2% RDP. Amount of OM apparently digested in the rumen also was affected by RDP level, with diet C being higher than diets A and D ($P < 0.05$) but not significantly different from diet B (Table 4). Intake, omasal flow, amount digested in the rumen, and apparent ruminal digestibility of NDF were not affected by dietary RDP concentration. Although ADF intake and amount apparently digested in the rumen both decreased linearly with decreasing dietary RDP ($P < 0.01$), the percentage of ADF apparently digested in the rumen was not affected by diet (Table 4).

The quadratic effect of dietary RDP on DMI of RC cows, together with the decrease in dietary CP of diets A to D from 18.8 to 17.2% of DM (Table 1), resulted in CP intakes, flows at the omasal canal, and true digestibilities in the rumen that were significantly lower for cows fed diet D ($P < 0.01$; Table 5). As intended when formulating the diets, the supply of RDP decreased lin-

Table 4. Effect of dietary RDP on intake, flow at omasal canal and ruminal digestibility of DM, OM, NDF, and ADF.¹

Item	Diet (RDP, %)				SE	<i>P</i> > <i>F</i> ²		
	A (13.2)	B (12.3)	C (11.7)	D (10.6)		RDP	Linear	Quadratic
DM								
Intake, kg/d	23.6 ^a	23.7 ^a	24.4 ^a	22.4 ^b	1.0	<0.05	0.15	<0.05
Flow at omasal canal, kg/d	15.7	15.3	15.5	14.6	0.7	<0.10	<0.05	0.48
OM								
Intake, kg/d	22.4 ^a	22.4 ^a	23.1 ^a	21.2 ^b	0.9	<0.01	0.11	<0.05
Flow at omasal canal, kg/d	12.8 ^a	12.5 ^a	12.6 ^a	11.7 ^b	0.5	<0.05	<0.05	0.39
Apparently digested in the rumen kg/d	9.51 ^b	9.97 ^{ab}	10.52 ^a	9.44 ^b	0.52	<0.05	0.86	<0.05
% of OM intake	42.7	44.5	45.5	44.6	1.0	0.14	0.12	0.14
Truly digested in the rumen kg/d	14.5 ^{ab}	14.6 ^a	15.2 ^a	13.7 ^b	0.6	<0.05	0.23	<0.05
% of OM intake	65.2	64.9	65.8	65.0	1.0	0.81	0.93	0.72
NDF								
Intake, kg/d	6.48	6.51	6.60	6.69	0.31	0.53	0.19	0.77
Flow at omasal canal, kg/d	3.17	3.33	3.35	3.37	0.15	0.69	0.28	0.59
Apparently digested in the rumen kg/d	3.29	3.18	3.24	3.32	0.24	0.80	0.79	0.37
% of NDF intake	50.3	48.5	49.3	49.6	2.3	0.85	0.87	0.47
ADF								
Intake, kg/d	3.61 ^a	3.53 ^a	3.32 ^b	3.22 ^b	0.17	<0.01	<0.01	0.96
Flow at omasal canal, kg/d	1.71	1.68	1.76	1.63	0.09	0.42	0.58	0.40
Apparently digested in the rumen kg/d	1.88 ^a	1.85 ^a	1.55 ^b	1.59 ^b	0.14	<0.01	<0.01	0.69
% of ADF intake	51.6	52.0	46.8	49.3	2.4	<0.10	<0.10	0.51

^{a,b}Least squares means within the same row without a common superscript differ ($P < 0.05$).

¹Data from ruminally cannulated cows only.

²Probability of a significant effect of RDP or of a linear or quadratic effect of RDP level in the diet.

early from 3076 to 2403 g/d ($P < 0.01$) and from 13.2 to 10.6% of DMI ($P < 0.01$) for diets A to D, respectively (Table 5). Although the flow of RUP at the omasal canal was not significantly different among diets (mean 1442 g/d), when expressed as a percentage of DMI, RUP tended to increase linearly from 5.79 to 6.61% ($P < 0.10$) for diets A to D. Similarly, the flow of NANMN, as a percentage of NAN flow, increased linearly from 30.4 to 37.8% for diets A to D ($P < 0.01$). The linear decrease in RDP supply paralleled linear decreases in total N, ammonia-N, free AA-N, and NAN flows at the omasal canal ($P < 0.01$). Conversely, the flow of NDIN (g/d) increased linearly from diets A to D ($P < 0.01$), reflecting the contribution from LSBM (NRC, 2001). The NAN flows of FAB, PAB, and total microbial NAN decreased linearly from 199 to 167 g/d ($P < 0.05$), from 271 to 217 g/d ($P < 0.01$), and from 470 to 384 g/d ($P < 0.01$), respectively, as dietary RDP decreased from 13.2 to 10.6% of DM (Table 5). The slope of the regression of microbial NAN flow on dietary RDP levels was 32.6 ($P < 0.01$). When expressed as a proportion of total NAN, microbial NAN flow decreased linearly ($P < 0.01$) from 69.9 to 62.1% for diets A to D, respectively. Proportions of FAB-NAN and PAB-NAN in total microbial NAN were not affected by RDP level and averaged 43.4 and

56.6%, respectively (Table 5). Decreasing RDP also resulted in a linear decrease in microbial efficiency from 32.3 to 28.0 g of microbial NAN per kilogram of OM truly digested in the rumen ($P < 0.01$; Table 5) with a slope of 1.65 ($P < 0.01$).

Flows of individual AA at the omasal canal are presented in Table 6. Except for Arg, Met, and Trp, individual AA flows were not affected by diet. The flow of Arg was lowest for diet A, whereas Met flow decreased linearly from diets A to D ($P < 0.05$). Dietary RDP had a quadratic effect on Trp flow ($P < 0.05$), with quadratic maxima at 12.3% RDP.

Excretion and Total Tract Digestibility of Nutrients

Decreasing dietary RDP from 13.2 to 10.6% resulted in significant linear decreases in estimated urine volume (from 22.3 to 17.9 l/d), urinary excretion of N (from 295 to 239 g/d), urea-N (from 240 to 163 g/d), allantoin (from 418 to 327 mmol/d), and PD (from 435 to 352 mmol/d) for diets A to D, respectively (Table 7). Microbial NAN flow to the small intestine, computed from the urinary excretion of PD, decreased linearly from 396 to 299 g/d for diets A to D ($P < 0.01$). There was a trend ($P < 0.10$) for a linear decline in fecal N output

Table 5. Effect of dietary RDP on intake and flow at omasal canal of N fractions.¹

Item	Diet (RDP, %)				SE	$P > F^2$		
	A (13.2)	B (12.3)	C (11.7)	D (10.6)		RDP	Linear	Quadratic
N intake, g/d	715 ^a	701 ^a	690 ^a	619 ^b	26	<0.01	<0.01	<0.10
CP								
Intake, g/d	4468 ^a	4377 ^a	4310 ^a	3872 ^b	156	<0.01	<0.01	<0.10
Flow at omasal canal, kg/d	4.31 ^a	4.20 ^a	4.12 ^a	3.87 ^b	0.15	<0.01	<0.01	0.49
Truly digested in the rumen								
kg/d	3.08 ^a	2.92 ^a	2.84 ^a	2.40 ^b	0.15	<0.01	<0.01	0.14
% of CP intake	68.8 ^a	66.3 ^a	65.7 ^a	61.5 ^b	1.6	<0.01	<0.01	0.54
RDP supply								
g/d	3076 ^a	2918 ^a	2839 ^a	2403 ^b	143	<0.01	<0.01	0.14
% of DMI	13.2 ^a	12.3 ^{ab}	11.7 ^b	10.6 ^c	0.4	<0.01	<0.01	0.68
RUP flow								
g/d	1370	1459	1470	1469	77	0.61	0.39	0.58
% of DMI	5.79	6.18	6.04	6.61	0.28	0.12	<0.10	0.66
Flow at omasal canal								
Total N, g/d	689 ^a	672 ^a	660 ^a	619 ^b	24	<0.01	<0.01	0.49
NH ₃ -N, g/d	9.29 ^a	7.33 ^b	6.71 ^b	5.17 ^c	0.56	<0.01	<0.01	0.74
NAN, g/d	684 ^a	664 ^a	653 ^{ab}	615 ^b	26	<0.05	<0.01	0.55
Free AA-N, g/d	36.5 ^a	33.8 ^a	32.5 ^a	26.9 ^b	2.6	<0.01	<0.01	0.42
NDIN, g/d	20.9 ^d	30.9 ^c	37.7 ^b	45.4 ^a	2.2	<0.01	<0.01	0.49
Total AA-N, g/d	420	454	442	437	19	0.37	0.59	0.22
NANMN, ³ g/d	209	226	229	233	13	0.36	0.24	0.62
NANMN, ³ % of NAN	30.4 ^c	34.2 ^b	34.9 ^{ab}	37.8 ^a	1.3	<0.01	<0.01	0.85
FAB-NAN ³								
g/d	199 ^a	189 ^a	191 ^a	167 ^b	10	<0.05	<0.05	0.45
% of microbial-NAN	42.1	43.3	44.9	43.5	1.9	0.51	0.33	0.34
PAB-NAN ³								
g/d	271 ^a	249 ^b	233 ^{bc}	217 ^c	14	<0.01	<0.01	0.80
% of microbial-NAN	57.9	56.7	55.1	56.5	1.9	0.51	0.33	0.34
Microbial NAN								
g/d	470 ^a	438 ^b	425 ^b	384 ^c	18	<0.01	<0.01	0.71
% of total NAN	69.6 ^a	65.8 ^b	65.1 ^{bc}	62.1 ^c	1.3	<0.01	<0.01	0.85
Microbial efficiency								
g of NAN/kg of OMTDR ³	32.3 ^a	30.1 ^b	28.1 ^c	28.0 ^c	0.8	<0.01	<0.01	0.28

^{a,b,c,d}Least squares means within the same row without a common superscript differ ($P < 0.05$).

¹Data from ruminally cannulated cows only.

²Probability of a significant effect of RDP or of a linear or quadratic effect of RDP level in the diet.

³NANMN = Nonammonia nonmicrobial N; FAB- and PAB-NAN = fluid- and particle-associated bacteria-NAN, respectively; OMTDR = OM truly digested in the rumen.

and total N excretion decreased linearly ($P < 0.01$) from 517 to 437 g/d for diets A to D. Fecal excretion of DM and OM decreased linearly with decreasing dietary RDP ($P < 0.01$). Total tract apparent digestibilities of DM, OM and NDF increased by 5.7, 5.4, and 12.2 percentage units, respectively ($P < 0.01$), when dietary RDP decreased from 13.2 to 10.6% of the DM (Table 7). Total tract apparent digestibility of CP and N use coefficient (expressed as N secreted in milk as a percentage of total N intake) were not affected by diet and averaged, respectively, 69 and 30% across diets (Table 7). However, environmental N efficiency increased linearly from 84.5 to 99.8 kg of milk/kg of N excreted when dietary RDP decreased from 13.2 to 10.6% (Table 7).

DISCUSSION

Experimental Design

Production responses to substitution of dietary RUP for RDP observed in dairy cows have been inconsistent.

In a literature review covering 12 yr, Santos et al. (1998) reported that when SSBM was replaced by high RUP sources in 127 treatment comparisons, milk yield was significantly higher in only 17%, milk protein percentage was decreased in 22%, and microbial protein synthesis was significantly decreased in 37% and numerically decreased in 93% of the comparisons. Santos et al. (1998) suggested that, if dietary RUP is increased at the expense of RDP, protein supply to the rumen could become limiting for microbial growth. This is the reason why the adequacy of RDP and RUP in dairy cow rations should be considered independently. In most experiments studying the effects of ruminal protein degradability on production of dairy cows, it has not been possible to determine whether a production response is an effect of the flow of RUP to the intestines or the supply of RDP to the rumen microorganisms. Kalscheur et al. (1999) fed 52 cows in early, mid and late lactation diets containing (% of DM) from 5.5 to 7.3% RUP and

Table 6. Effect of dietary RDP on omasal flow of individual and total AA.¹

Item	Diet (RDP, %)				SE	$P > F^2$		
	A (13.2)	B (12.3)	C (11.7)	D (10.6)		RDP	Linear	Quadratic
Essential AA	(AA flows, g/d)							
Arg	136 ^b	163 ^a	157 ^a	160 ^a	8	<0.05	<0.10	0.12
His	52.1	59.1	57.6	58.7	3.0	0.11	0.11	0.23
Ile	150	166	160	159	7	0.31	0.45	0.24
Leu	283	300	293	289	12	0.59	0.81	0.32
Lys	153	165	159	161	9	0.60	0.57	0.49
Met	53.4	52.7	52.4	50.4	2.4	0.23	<0.05	0.81
Phe	137	151	146	146	7	0.30	0.40	0.24
Thr	162	173	167	163	8	0.49	0.89	0.28
Trp	46.4 ^{ab}	48.6 ^a	48.2 ^a	43.9 ^b	2.0	<0.05	0.14	<0.05
Val	150	167	163	160	8	0.29	0.46	0.16
Nonessential AA								
Ala	248	251	244	228	10	0.14	<0.10	0.36
Asp	395	421	412	406	17	0.54	0.77	0.25
Cys	69.2	74.9	71.2	70.9	4.3	0.68	0.90	0.41
Glu	550	592	587	589	25	0.34	0.24	0.30
Gly	174	182	178	173	8	0.59	0.75	0.30
Pro	155	167	163	161	7	0.46	0.60	0.28
Ser	164	174	170	169	7	0.62	0.76	0.34
Tyr	147	158	153	149	6	0.36	0.99	0.23
Total AA	3228	3466	3381	3337	152	0.37	0.59	0.22

^{a,b}Least squares means within a row without a common superscript differ ($P < 0.05$).

¹Data from ruminally cannulated cows only.

²Probability of a significant effect of RDP or of a linear or quadratic effect of RDP level in the diet.

Table 7. Effect of dietary RDP on urinary N and purine derivative excretion, estimated microbial N synthesis, fecal excretion and apparent total tract digestibilities.¹

Item	Diet (RDP, %)				SE	$P > F^2$		
	A (13.2)	B (12.3)	C (11.7)	D (10.6)		RDP	Linear	Quadratic
N intake, g/d	715 ^a	701 ^a	690 ^a	619 ^b	26	<0.01	<0.01	<0.10
Urinary excretion								
Total volume, l/d	22.3 ^a	19.9 ^{ab}	19.7 ^{ab}	17.9 ^b	1.1	<0.05	<0.01	0.65
Total N, g/d	295 ^a	293 ^a	237 ^b	239 ^b	13	<0.01	<0.01	0.58
Urea-N, g/d	240 ^a	216 ^{ab}	195 ^{bc}	163 ^c	12	<0.01	<0.01	0.69
Allantoin, mmol/d	423 ^a	368 ^b	356 ^b	338 ^b	17	<0.01	<0.01	0.14
Uric acid, mmol/d	12.2	10.3	10.5	12.9	1.5	0.46	0.64	0.13
Total PD excretion, mmol/d	435 ^a	378 ^b	368 ^b	352 ^b	17	<0.01	<0.01	0.11
Microbial NAN flow, ³ g/d	396 ^a	329 ^b	317 ^b	299 ^b	20	<0.01	<0.01	0.11
Fecal excretion								
DM, kg/d	7.93 ^a	7.41 ^{ab}	7.02 ^b	6.70 ^b	0.34	<0.05	<0.01	0.74
OM, kg/d	7.27 ^a	6.76 ^{ab}	6.39 ^b	6.12 ^b	0.32	<0.05	<0.01	0.65
N, g/d	222	220	219	197	10	0.18	<0.10	0.28
Total N excretion								
g/d	517 ^a	514 ^a	456 ^b	437 ^b	18	<0.01	<0.01	0.82
% of N intake	73.4 ^a	72.1 ^{ab}	66.7 ^{bc}	66.2 ^c	2.3	<0.05	<0.01	0.79
Total tract apparent digestibility, %								
DM	66.4 ^b	69.8 ^a	71.2 ^a	72.1 ^a	1.2	<0.01	<0.01	0.27
OM	67.6 ^b	70.9 ^a	72.3 ^a	73.0 ^a	1.2	<0.01	<0.01	0.25
NDF	40.2 ^c	46.4 ^b	49.7 ^{ab}	53.1 ^a	2.1	<0.01	<0.01	0.46
CP	68.5	69.4	68.2	70.2	1.3	0.67	0.49	0.66
N efficiency								
Milk N, % of N intake	29.6	29.5	30.4	30.4	0.9	0.65	0.37	0.68
Kg of milk:kg of N excreted	84.5 ^b	87.2 ^b	94.3 ^a	99.8 ^a	3.9	<0.01	<0.01	0.56

^{a,b,c}Least squares means within a row without a common superscript differ ($P < 0.05$).

¹Data obtained from all 28 cows.

²Probability of a significant effect of RDP or of a linear or quadratic effect of RDP level in the diet.

³Estimated from urinary PD excretion of all experimental cows using equations from Vagnoni et al. (1997).

from 10.3 to 7.9% RDP. Although milk production of early lactation cows increased linearly as dietary RUP increased, the proportions of 7 protein and energy supplements were changed across diets, making it difficult to determine which dietary factor or factors affected milk production in this study. In the present trial, diets were formulated to provide RUP from the same protein sources, altering RDP supply from both urea and soybean meal, and maintaining similar energy contents across diets. This allowed the study of the effects of dietary RDP level without confounding effects of dietary RDP and RUP source and energy concentration and source. Although dietary CP contents were not constant across diets, differences in dietary CP originated only from addition of urea, whereas nonurea N contents were similar across diets. Nevertheless, ammonia from ruminal degradation of dietary urea may have affected animal performance not only through an effect on microbial growth and fermentation but also through an effect on physiological function related to excess ammonia (e.g., ammonia detoxification by the liver), possibly confounding the effects of dietary RDP and CP.

Effect of RDP Level on Microbial Protein Synthesis

The requirements of rumen microbes for ammonia and preformed AA for protein synthesis depend on bacterial species (Ling and Armstead, 1995; Jones et al., 1998) and dietary characteristics (Cruz Soto et al., 1994; Chikunya et al., 1996). Conflicting results have been reported concerning the optimal ruminal ammonia-N concentrations required to achieve maximal microbial synthesis both in vivo and in vitro. In the early work of Satter and Slyter (1974), ammonia-N concentrations greater than 5 mg/dL had no effect on microbial protein production in continuous culture fermentors. Similarly, Russell and Strobel (1987) reported that protein synthesis of mixed ruminal bacteria grown in vitro declined when ammonia-N concentration in the media was less than 5 mg/dL. An ammonia-N concentration of 1.4 mg/dL was required by rumen bacteria species grown as pure cultures to achieve 95% of maximal growth rate (Schaefer et al., 1980). However, most of these results were obtained with bacterial suspensions grown in media without substrates for attachment.

When Kang-Meznarich and Broderick (1980) added incremental amounts of urea to a basal diet of corn and cottonseed hulls fed to 2 nonlactating cows, microbial protein synthesis, estimated using diaminopimelic acid as microbial marker, was maximized at a ruminal ammonia-N concentration of 8.5 mg/dL. When sheep were fed diets supplemented with increasing amounts of urea, microbial N flow at the duodenum was maximized at ruminal ammonia-N concentrations of 13.3 mg/dL

(Hume et al., 1970) and increased linearly when rumen ammonia-N increased from 7.9 to 15.9 mg/dL (Allen and Miller, 1976). Odle and Schaefer (1987) incubated barley grain in situ in steers fed barley-based diets and reported that the minimum ruminal ammonia-N concentration required to maximize the fractional rate of barley degradation was 12.5 mg/dL, whereas 6.1 mg/dL of ammonia-N was required to maximize the degradation rate of corn in steers fed corn-based diets. Moreover, the maximum degradation rate for barley was greater than that for corn (0.036 vs. 0.024/h, respectively; $P < 0.01$). The authors concluded that the optimal ammonia concentration required to maximize ruminal digestion rates is influenced by the chemical or structural characteristics of the degradable substrate.

Erdman et al. (1986) infused increasing amounts of urea into the rumen of cows fed a 7.4% CP diet consisting of 50:50 ground corn:cottonseed hulls to study the effect of ruminal ammonia-N concentration on in situ DM digestibility of different feedstuffs. Maximum DM digestion occurred at a ruminal ammonia-N concentration of 25 mg/dL for ground corn and SSBM, at 17 mg/dL for cottonseed meal and corn gluten meal, but at less than 5 mg/dL for alfalfa hay. Erdman et al. (1986) concluded that the minimum ruminal ammonia concentration required for maximizing digestion and microbial yield increased with increasing fermentability of the feed. Balcells et al. (1993) studied the effects of urea supplementation of alkali-treated barley straw and, similar to Erdman et al. (1986), found that a minimum ruminal ammonia-N concentration of 5 mg/dL was necessary to avoid reductions in intake and in situ fermentation rate of slowly fermented alkali-treated barley straw. However, a concentration of 11 mg/dL was needed to maximize microbial protein flow from the rumen (estimated from urinary excretion of PD). Odle and Schaefer (1987) suggested that the higher estimates of optimal ruminal ammonia concentration obtained in vivo, compared with in vitro, were due to differences in nutrient concentrations between the microenvironments within the microbial consortium colonizing on the surface of feed particles and in the surrounding environment.

In the present study, decreasing dietary CP from 18.8 to 17.2 and RDP from 13.2 to 10.6% resulted in a linear decrease in ruminal ammonia-N from 12.3 to 5.7 mg/dL. Although ruminal ammonia-N concentrations of cows fed diets A, B and C were above 5 mg/dL (Satter and Slyter, 1974) during the entire feeding cycle (except for diet C at 8 h after feeding; Figure 1), microbial NAN flows at the omasal canal were significantly higher for cows fed diet A compared with those fed diets B and C (Table 5). Cows fed diet D had ruminal ammonia concentrations below 5 mg/dL for 16 h/d (Figure 1) and

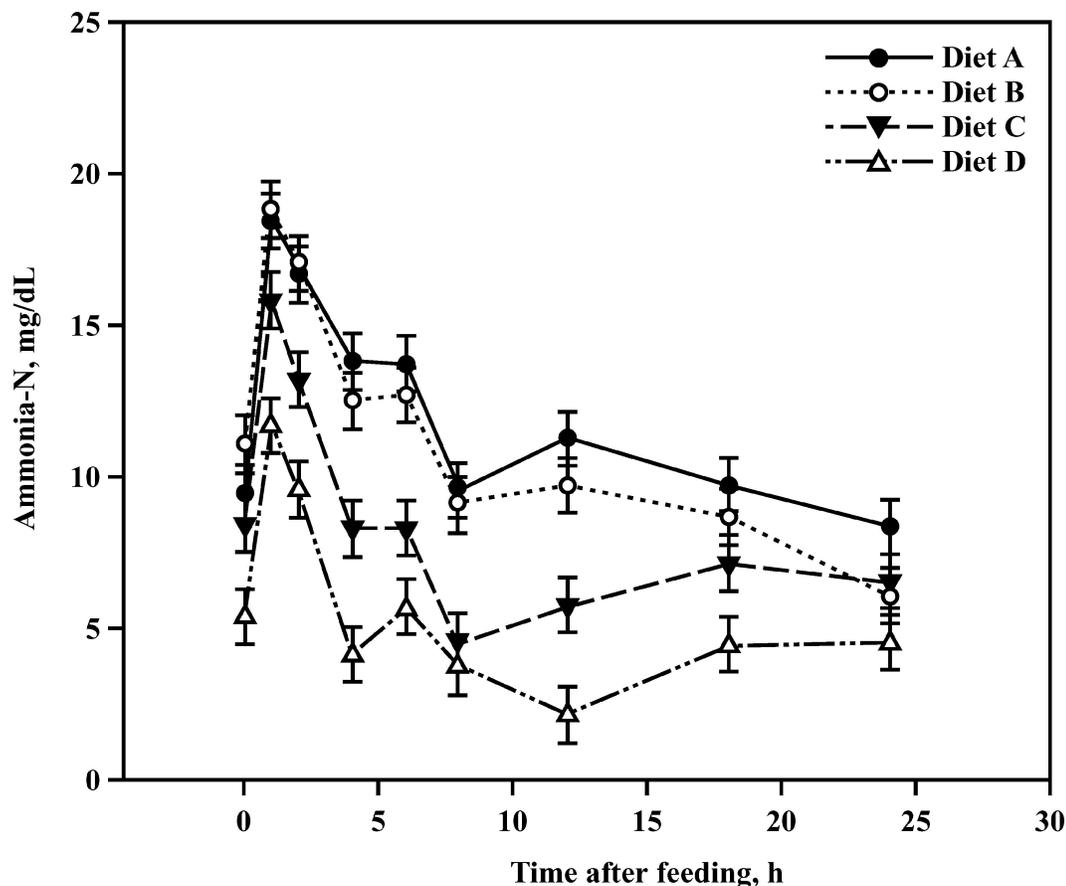


Figure 1. Rumen ammonia concentrations (means \pm SE) after feeding.

had the lowest microbial NAN flows. These results suggest that ammonia-N concentrations above 11.8 mg/dL (the least squares mean for diet B) might be needed to maximize microbial protein synthesis, which is similar to the minimum requirement of 11 mg/dL reported by Balcells et al. (1993) in vivo. However, evidence from other in vivo (Chikunya et al., 1996) and in vitro (Argyle and Baldwin, 1989; Carro and Miller, 1999) studies showed that growth rates and yields of ruminal microorganisms are stimulated by AA and peptides. In the present study, increasing dietary RDP resulted in linear increases of free AA concentrations in the rumen ($P < 0.01$). Moreover, Chen et al. (1987) observed that replacing half of the SSBM fed to lactating dairy cows with extruded SBM resulted in a 26% decrease of peptide-N flow from the rumen. Although ruminal peptide concentrations were not measured in our study, it might be speculated that peptide availability increased with increasing dietary protein degradability. Therefore, AA and peptides might have stimulated microbial protein synthesis. If ammonia requirement for maximal microbial growth is 5 mg/dL and preformed AA were limiting

microbial yield, then urea added to diets A and B would have exceeded ammonia requirements, and would have been lost from the rumen and contributed to the higher urinary N excretion without improving microbial yield. On the other hand, if the ammonia requirement were higher than 11.8 mg/dL, then maximum microbial protein yields would be achieved only at the expense of higher ruminal ammonia-N and greater urinary losses. As availability of N increased in relation to the amount of OM fermented, efficiency of microbial protein synthesis increased linearly from 28.0 to 32.3 g of NAN per kg of OM truly digested in the rumen for cows fed diets D to A, suggesting that the limiting factor for microbial growth was not digestible OM but rather availability of RDP. However, it is not possible to determine which RDP source or sources were necessary to stimulate microbial growth.

Microbial NAN flows at the omasal canal ranged from 384 to 470 g/d and were within the range of 334 treatment means summarized by the NRC (2001) from published literature since 1985. More recently, Oba and Allen (2003) reported similar microbial N flows at the

Table 8. Regression coefficients and quadratic maxima for variables with linear and quadratic effects of RDP level.

Item	Intercept	SE	Coefficient	SE	Linear coefficient	Quadratic SE	Quadratic maxima, %	
							In vivo ¹	NRC ¹
DMI, kg/d (cannulated cows only)	-72.8	39.1	15.7*	6.6	-0.64*	0.28	12.2	10.7
Milk true protein, %	2.98**	0.19	0.044**	0.012				
Milk true protein yield, kg/d	-3.48	2.21	0.78*	0.37	-0.032*	0.016	12.3	10.9
Milk urea-N, mg/dL	2.37	2.97	1.30**	0.24				
Blood urea-N, mg/dL	4.51	2.65	0.69**	0.21				
Ruminal NH ₃ , mM	-16.4**	2.6	1.93**	0.20				
Ruminal total free AA, mM	-4.48*	1.48	0.61*	0.12				
Ruminal butyrate, mM	15.4**	2.5	-0.51*	0.20				
OMADR, ³ kg/d	-58.6	26.0	11.6*	4.4	-0.49*	0.19	11.9	10.1
OMTDR, ⁴ kg/d	-53.1	29.2	11.1*	4.9	-0.46*	0.21	12.2	10.4
Microbial NAN flow, g/d								
Omasal ⁵	12.1	97.7	32.6**	7.9				
Purine derivatives ⁶	-185	115	36.3**	9.0				
Microbial efficiency, g of N/kg of OMTDR ⁴	8.65	5.69	1.65**	0.46				
Omasal Met flow, g of AA/d	34.3**	8.3	1.33*	0.65				
Omasal Trp flow, g of AA/d	-249	120	6.67*	2.79	-0.27*	0.12	12.3	10.9
Urinary N excretion, g/d	-49.6	77.9	25.1**	6.3				
Total N excretion, g/d	36.7	98.1	34.0**	7.8				
N efficiency, kg of milk:kg of N excreted	155**	19	-6.20**	1.39				
Total tract digestibility, %								
DM	99.0**	7.5	-2.16**	0.61				
OM	99.0**	7.4	-2.08*	0.60				
NDF	110**	13	-4.97**	1.1				

¹Dietary RDP measured in vivo at maximum response = $-\text{linear coefficient}/(2 \times \text{quadratic coefficient})$.

²Dietary RDP predicted by NRC (2001) at maximum response = $-\text{linear coefficient}/(2 \times \text{quadratic coefficient})$.

³Organic matter apparently digested in the rumen.

⁴Organic matter truly digested in the rumen.

⁵Microbial NAN flow measured using the omasal sampling technique.

⁶Microbial NAN flow estimated from the urinary excretion of purine derivatives.

* $P < 0.05$; ** $P < 0.01$.

duodenum of 347 and 484 g/d in cows consuming 19.6 and 22.5 kg of DM per day of low and high starch diets. Microbial NAN flow to the small intestine, estimated from the urinary excretion of PD, decreased linearly from 396 to 299 g/d for diets A to D, respectively (Table 7). Compared with microbial flows measured at the omasal canal, microbial NAN flows estimated using PD were 74 to 109 g/d lower across treatments. However, when regressed against observed RDP levels, estimated and measured microbial NAN flows had nearly parallel slopes (35.4 and 36.3 g/d, respectively; Table 8). Therefore, the use of purine derivatives yielded estimates of differences among treatments in microbial NAN flow from the rumen that were similar to those measured at the omasal canal.

Effect of RDP Level on Digestion and Production

Surprisingly, dietary RDP had a quadratic effect on ruminal propionate concentration ($P < 0.05$). Earlier, replacing SSBM with heat-treated SBM either depressed (Windschitl and Stern, 1988) or did not change

(Broderick, 1986; Schingoethe et al., 1988) ruminal propionate concentrations in lactating cows fed corn-based diets. In the present trial however, cows supplemented with either SSBM plus urea (diet A) or LSBM (diet D) had similar ruminal propionate concentrations, while cows fed a combination of these 3 CP sources (diet B) had the lowest propionate concentrations. On the other hand, ruminal butyrate concentrations increased linearly with decreasing RDP ($P < 0.05$). When SSBM was replaced with expeller SBM in corn-based diets fed to lactating dairy cows, ruminal butyrate concentration either increased (McCormick et al., 2001), or decreased (Broderick, 1986). These findings are difficult to explain.

Dietary RDP level had no effect on DMI or yields of milk, FCM, milk fat, and SNF, but had a linear effect on milk true protein content and a quadratic effect on milk true protein yield, with quadratic maximum at 12.3% (10.9% NRC-predicted) RDP. In their 12-yr literature review on the effects of RUP on dairy cow performance, Santos et al. (1998) reported that, when SSBM was replaced by SBM treated to increase ruminal es-

cape, milk protein content was decreased in 8 of the 29 comparisons. When SSBM was replaced with expeller SBM in corn-based diets fed to cows producing 33 to 35 kg of milk per day, milk protein content decreased from 2.98 to 2.84% ($P < 0.05$; Broderick, 1986) and from 3.02 to 2.92% ($P < 0.05$; Schingoethe et al., 1988). Both Broderick (1986) and Schingoethe et al. (1988) speculated that heat treatment reduced the availability of Lys from expeller SBM, resulting in lower milk protein contents. Although dietary Lys content decreased from 4.83 to 4.38% of total AA from diets A to D, flows of Lys at the omasal canal were not affected and averaged 160 g/d. However, although dietary Met content decreased from 1.34 to 1.12% of total AA from diets A to D, omasal flow of Met decreased linearly (slope = 1.33; $P < 0.05$; Table 8) with decreasing dietary RDP. Moreover, dietary RDP had a quadratic effect on Trp flow at the omasal canal ($P < 0.05$) with the highest flow corresponding to 12.3% RDP. Furthermore, omasal flow of Arg was lowest ($P < 0.05$) on diet A. Therefore, the quadratic effect of RDP level on milk true protein yield may have resulted partly from the quadratic effect on Trp flow, the linear effect on Met flow, and the numerically highest flows of total AA corresponding to this RDP level. Similar to our findings, Windschitl and Stern (1988) reported that replacing SSBM with LSBM significantly decreased the amount of Met absorbed in the small intestine of lactating cows by 26% and significantly increased Arg absorption by 18%. Mainly due to analytical difficulties, literature data on digestion of Trp are lacking, preventing comparison of our results with other findings. On the other hand, differences among treatments in milk true protein yield may have resulted, at least in part, from numerical differences in total milk secretion. The Lys:Met ratios in omasal digesta flows were 2.9, 3.1, 3.0, and 3.2 for diets A, B, C, and D, respectively. These ratios are similar to the estimated optimum ratios of 2.9 (Rulquin et al., 1993) and 3.0 (NRC, 2001) required for maximum milk production.

Total tract digestibility of NDF increased linearly with decreasing dietary CP and RDP ($P < 0.01$), with digestibility for cows fed diet D being 12.9 percentage units higher than for diet A. Because digestibility of NDF in the rumen was not affected by treatment (mean of 49.4% of NDF intake), differences in total tract digestibility must have been due to differences in intestinal digestibility. However, digestibility estimates of NDF probably were biased by the high NDIN content of LSBM (32.4% of total N). Higher NDF content of LSBM compared with SSBM (28.2 vs. 8.8% in the present trial and 29.7 vs. 9.8% reported by NRC, 2001) is an artifact of the fiber analysis. Therefore, NDF intakes and, to a lesser extent, omasal flows were overestimated

in cows fed LSBM. Because the RUP fraction of LSBM is very digestible in the small intestine (Mansfield and Stern, 1994; NRC, 2001; Prestlokken and Rise, 2003), it contributes relatively little to the NDF detected in the feces. Approximately 5% of the DM in diet D was NDF from LSBM, whereas only 1.4% of the DM in diet A was NDF from SSBM. Assuming that the nonNDIN fiber content of LSBM is the same as SSBM, the overestimation of NDF intake from diet D was 0.9 kg/d. After subtracting this amount from the total NDF intake of cows fed diet D (6.7 kg - 0.9 kg = 5.8 kg), total tract digestibility (using the NDF excretion observed on diet D of 2.93 kg/d) was 49.4% instead of 53.1% (Tables 4 and 7). Therefore, ruminal and the total tract digestibilities of NDF were confounded by the presence of intestinally digestible NDIN.

Total tract digestibilities estimated for NDF were lower than corresponding ruminal digestibilities in cows fed diets A and B. This discrepancy was due, at least in part, to the use of additional cows at generally greater intakes when total tract digestibilities were estimated. Although ruminal digestibilities were measured using the 8 RC cows eating an average of 23.5 kg of DM per day, total tract digestibilities were estimated using data from all 28 cows in the experiment with average DMI of 25.5 kg. When only data from RC cows were considered, total tract NDF digestibilities for diets A and B were 3.6 and 1.3 percentage units higher than those measured on all cows (data not shown). Moreover, use of different markers for estimating ruminal (Co, Yb, and INDF) and total tract (IADF) digestibilities probably also contributed to these discrepancies. Indigestible NDF was estimated using nylon bags with pore size of 6 μm , whereas Ankom bags (Ankom Technology, Macedon, NY) with an average pore size of 25 μm were used for indigestible ADF determination. Huhtanen et al. (1994) suggested that the lower fecal recovery of dietary INDF estimated in situ using bags with a pore size of 41 μm , compared with 6- μm pore size bags, was due to a greater relative loss of particles from fecal than feed samples. In the present experiment, the use of 25- μm pore size bags for determination of indigestible ADF could have resulted in loss of that internal marker and, hence, overestimation of fecal excretion and underestimation of the total tract digestibility of nutrients, compared with ruminal digestibilities estimated using 6- μm pore bags. Therefore, comparison between rumen and total tract digestibility in the present study should be done with caution.

True digestibility of CP in the rumen decreased linearly as urea decreased and LSBM replaced SSBM from diets A to D, reflecting the greater ruminal escape of LSBM. However, total tract CP digestibility was not affected by protein supplement. Replacement of SSBM

with LSBM did not affect ruminal or total tract digestibility of CP in lactating dairy cows (Mansfield and Stern, 1994). Moreover, intestinal digestibilities for SSBM and LSBM measured using the mobile nylon bag technique were not different (Prestlokken and Rise, 2003). The NRC (2001) assigns an intestinal digestibility of 93% to RUP in both SSBM and nonenzymatically browned SBM. Because the main differences among diets fed in our experiment originated from the replacement of SSBM with LSBM, similar total tract digestibilities of CP among treatments suggested that RUP from both SBM supplements had similar intestinal digestibilities.

Quadratic effects of RDP on DMI of RC cows and similar OM digestibilities across diets resulted in quadratic responses in the amount of OM apparently and truly digested in the rumen (kg/d) to dietary RDP, with quadratic maxima at RDP concentrations of 11.9 and 12.2%, respectively. However, total tract digestibility of OM decreased linearly with increasing levels of RDP ($P < 0.01$; Table 7). Contrary to our results, Windschitl and Stern (1988) reported that lignosulfonate treatment of SBM resulted in lower apparent total tract digestibility of OM compared with SSBM; lower OM digestibility was attributed to the lower CP digestibility. In a later trial, however, replacement of SSBM with lignosulfonate-treated SBM did not affect ruminal and total tract digestion of nutrients (Mansfield and Stern, 1994). In our study, the linear effect of replacing SSBM with LSBM on total tract OM digestion cannot be explained by differences in CP digestibility. Despite the interference of NDIN with the NDF analysis described above, lower OM digestion in the total tract may have originated from a decrease in NDF digestion in the total tract with increasing RDP.

Nitrogen Use and Excretion

Decreasing dietary RDP from 13.2 to 11.7% and CP from 18.8 to 17.7% of DM for diets A to C, respectively, resulted in reduced excretion of urinary N (from 295 to 237 g/d), total N (from 517 to 456 g/d), and total N as percentage of N intake (from 73.4 to 66.7%) without affecting yields of milk and fat-corrected milk. Although cows fed diets containing 12.3% RDP had higher milk true protein content and yield than those fed diets containing 11.7% RDP, the 6.3-g/d increase in milk protein-N secretion (40 g/d as milk true protein) was at the expense of a 58-g/d increase in total N excretion, which depressed environmental N efficiency from 94.3 to 87.2 kg milk/kg N excretion. Moreover, of the 58-g/d increase in N excretion, 56 g/d was excreted in the urine. Castillo et al. (2001) studied the effect of supplementing grass silage diets with isoenergetic concentrates containing

2 levels of protein concentration at 3 levels of protein degradability on N use and milk production of dairy cows. Diets were formulated using SSBM and formaldehyde-treated SBM in different proportions of the DM. Similar to our findings, milk yield (mean 23.8 kg/d), milk composition, and fecal N excretion were not affected by protein concentration or degradability. However, increasing protein level and degradability increased urinary N excretion by 74 and 57 g of N per day, respectively. Therefore, the main route for output of dietary N in excess of requirements was via the urine. Urea is the main form of urinary N (Broderick, 2003) that, once excreted, can be rapidly degraded to ammonia, lost through volatilization, and contribute to environmental pollution (Van Horn et al., 1994). However, the recommended level of dietary RDP will depend on the criteria used to define optimum N use because dietary CP levels required to maximize milk protein production may not match those required to optimize environmental N efficiency. If optimum N efficiency were defined as the best compromise between the need for profitability (maximum income from milk production over feed costs) and the need for preservation of the environment (minimum N loss), the recommended level of RDP from the present trial would be 11.7% of DM (equivalent to 9.2% dietary RDP when predicted using the NRC model), corresponding to a dietary CP content of 17.7% of DM.

Although the lowest dietary RDP and CP concentrations (on diet D) reduced total N excretion a further 19 g/d and improved environmental N efficiency by 5.5 kg of milk production per kg of N excreted, urinary N excretion did not change and milk true protein yield decreased by 70 g/d (compared with diet C). Therefore, this small additional reduction in N excretion was associated with a substantial loss in milk protein production.

Increasing evidence shows that milk yield and N use efficiency of high-producing dairy cows can be maximized at dietary CP contents substantially lower than 17.7% (diet C). When Olmos Colmenero and Broderick (2003) fed lactating dairy cows diets ranging from 13.5 to 19.4% CP, maximum yields of milk (38.3 kg/d), FCM (36.7 kg/d), and milk protein (1.18 kg/d) were obtained when cows were fed a 16.5% CP diet. Milk N secreted as percentage of N intake was 35, 34, 32, 31, and 29% for diets containing 13.5, 15.0, 16.5, 17.9, and 19.4% CP, respectively. Broderick (2003) fed lactating dairy cows diets formulated from alfalfa and corn silages, high-moisture corn, and SSBM and containing 15.1, 16.7, and 18.4% CP. Yields of milk, FCM, milk fat and protein were lower on the 15.1% CP diet than on the other 2 diets. However, cows fed diets containing 16.7% CP had similar yields of milk, milk fat and protein,

Table 9. Comparisons between observed and NRC-predicted values for milk protein production and N flows to the small intestine.¹

Item	Diet			
	A	B	C	D
RDP concentration ²	13.2 (12.5)	12.3 (10.9)	11.7 (9.2)	10.6 (7.7)
Microbial MP flow, g/d				
Observed	1906	1893	1876	1790
Predicted by NRC (default rates)	1285	1290	1220	937
Predicted by NRC (in vivo rates)	1285	1292	1328	1248
RDP supply, g/d				
Observed	3076	2918	2839	2403
Predicted by NRC (default rates)	2955	2573	2243	1722
Predicted by NRC (in vivo rates)	3272	3027	2845	2409
RUP flow, g/d				
Observed	1370	1459	1470	1469
Predicted by NRC (default rates)	1427	1715	2067	2137
Predicted by NRC (in vivo rates)	1110	1301	1465	1448
MP flow, g/d				
Observed ³	2979	2958	2931	2797
Predicted by NRC (default rates)	2624	2897	3155	2938
Predicted by NRC (in vivo rates)	2330	2514	2703	2608
Milk protein yield, kg/d				
Observed	1.23	1.24	1.23	1.15
Predicted by NRC (default rates)	1.20	1.27	1.30	1.20
Predicted by NRC (in vivo rates)	1.00	1.13	1.24	1.22

¹Predictions are from the NRC model using actual BW, DMI and diet composition and either default degradation rates or rates measured in vivo (Reynal and Broderick, 2003) for solvent soybean meal and liginosulfonate-treated soybean meal. All data are from ruminally cannulated cows only.

²RDP concentrations, as % of DM, measured in vivo (and predicted by NRC, 2001).

³Observed MP flows were estimated from the observed microbial and nonammonia nonmicrobial N (NANMN) flows assuming that 80% of the N from microbial origin flowing at the omasal canal was protein-N with an intestinal digestibility of 80% (NRC, 2001), and assigning RUP digestibilities from the NRC to the measured flow of NANMN.

lower urinary urea-N excretion, and higher N efficiency than cows fed 18.4% CP diets.

NRC-Predicted vs. Observed N Digestion

Flows of microbial and total metabolizable protein (MP), RUP, and RDP supply, were predicted using the NRC (2001) model from diet composition and the least squares mean DMI for the RC cows. These values were compared with the corresponding least squares means determined from omasal flows (Table 9). Degradation rates used in the NRC model for SSBM and LSBM were either the default NRC values (0.075 and 0.017/h, respectively) or the in vivo estimates reported earlier (Reynal and Broderick, 2003) for SSBM and expeller SBM (0.179 and 0.077/h, respectively), assuming similar degradation rates for LSBM and expeller SBM (based on in situ data, the NRC assigns degradation rates for LSBM and expeller SBM that are 23 and 32%, respectively, of that for SSBM). When these in vivo degradation rates were used in the NRC (2001) model, the predicted RUP and total MP flows and RDP supply were, on average, more similar to in vivo observations than when using the NRC default rates (Table 9). How-

ever, the NRC model appeared to underestimate microbial NAN flows compared with our results when using either default or in vivo degradation rates (Table 9). The NRC (2001) model predicts dietary RDP and RUP using in situ-estimated degradation rates of individual feedstuffs. However, the in situ technique has theoretical limitations that call its accuracy into question (Broderick, 1994). Based on limited in vivo information, this technique appears to underestimate degradation rates of protein supplements. Degradation rates for SSBM, expeller SBM, blood meal, and corn gluten meal measured in vivo by Reynal and Broderick (2003) were 2.4, 3.2, 2.2, and 1.1 times more rapid than in situ estimates reported by NRC (2001). Similarly, degradation rates for SSBM and corn gluten meal measured in vivo by Garrett et al. (1987) were 2.4 and 2.3 times more rapid than NRC rates.

CONCLUSIONS

Decreasing dietary RDP from 13.2 to 11.7% and CP from 18.8 to 17.7% of DM did not affect production of milk or FCM but reduced estimated excretion of the environmentally labile urinary urea-N by 20%, and in-

creased estimated environmental N efficiency by 10 kg of milk per kg of N excreted. Although maximum milk true protein yield corresponded to an RDP concentration of 12.3%, the production response (40 g of milk true protein per cow per day) to increasing RDP from 11.7 to 12.3% occurred at the expense of a significant increase in estimated urinary N excretion (from 237 to 293 g/d) and a significant decrease in estimated environmental N efficiency (from 94.3 to 87.2 kg of milk per kg of N excreted). Therefore, the recommended level of dietary RDP will depend on the criteria used to define optimum N use. If optimal RDP were considered to be the point at which a satisfactory compromise is achieved between profitability (maximum income from milk production over feed costs) and environmental quality, then the recommended dietary RDP level in the present study would have been 11.7% of DM. When predicted using the NRC (2001) model, the optimal RDP concentration corresponded to 9.2% of DM.

Microbial protein yield increased linearly with dietary RDP but was not affected by the availability of digestible OM in the rumen. Although microbial NAN flow increased by 32.6 g/d for every percentage unit increase in dietary RDP, estimated total N excretion increased by 34 g/d and estimated environmental N efficiency was depressed by 6.2 kg of milk per kg of N excreted. Therefore, optimal N efficiency (best compromise between profitability and environmental quality) was not attained at maximum microbial protein yield but rather at a balance point between dietary protein degradation and microbial protein synthesis.

The NRC (2001) model overpredicted production responses to replacing SSBM with LSBM, and appeared to overpredict escape of dietary protein and underpredict microbial yield. Replacing default degradation rates for protein supplements in the NRC (2001) model with protein degradation rates previously measured in vivo resulted in NRC predictions that more closely matched observed values, suggesting that the in situ degradation rates used by the NRC (2001) were underestimated.

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