Varying Protein and Starch in the Diet of Dairy Cows. I. Effects on Ruminal Fermentation and Intestinal Supply of Nutrients

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ABSTRACT

The main objective of this experiment was to examine the effects of the percentage and source of crude protein (CP) and the amount of starch in the diet of dairy cows on ruminal fermentation, nutrient passage to the small intestine, and nutrient digestibility. For this purpose, 6 multiparous Holstein cows fistulated in the rumen and duodenum that averaged 73 d in milk were used in a 6 × 6 Latin square design with a 2 × 3 factorial arrangement of treatments. Two sources of CP [solvent-extracted soybean meal (SBM) and a mixture of SBM and a blend of animal-marine protein supplements plus ruminally protected Met (AMB)] and 3 levels of dietary protein (about 14, 16, and 18%) were combined into 6 treatments. On a dry matter (DM) basis, diets contained 25% corn silage, 20% alfalfa silage, 10% cottonseed, 26.7 to 37% corn grain, and 4 to 13.5% protein supplement. Intakes and digestibilities in the rumen and total tract of DM, organic matter, acid and neutral detergent fiber were unaffected by treatments. Increasing dietary CP from 14 to 18% decreased the intake and apparent ruminal and total tract digestion of starch, but increased the proportion of starch consumed by the cows that was apparently digested in the small intestine. At 14% CP, starch intake and total tract digestion were higher for the AMB diet than for the SBM diet, but the opposite occurred at 16% CP. Across CP sources, increasing CP in the diet from 14 to 18% increased the intakes of N and amino acids (AA), and ruminal outflows of nonammonia N, nonammonia nonmicrobial N, each individual AA except Met, total essential AA, and total AA. Across CP percentages, replacing a portion of SBM with AMB increased the intake of Met and Val and decreased the concentration of ammonia N in the rumen, but did not affect the intake of other essential AA or the intestinal supply of any essential AA and starch. The ruminal outflow of microbial N, the proportional contribution of Lys and Met to total AA delivered to the duodenum,

and milk yield were unaffected by treatments. Data suggest that the intake of N by high-producing dairy cows that consume sufficient energy and other nutrients to meet their requirements can be decreased to about 600 to 650 g daily without compromising the supply of metabolizable protein if the source and amount of dietary CP and carbohydrate are properly matched.

(Key words: ruminal fermentation, intestinal supply of nutrients, dairy cow, protein)

Abbreviation key: AMB = blend of animal-marine protein supplements plus ruminally protected Met, EAA = essential amino acids, HCP = high CP, LCP = low CP, MCP = medium CP, MN = microbial N, MP = metabolizable protein, NANMN = nonammonia nonmicrobial N, NEAA = nonessential amino acids, SBM = solvent-extracted soybean meal.

INTRODUCTION

During the last few years, awareness regarding the potential contribution of intensive dairy farming to nitrogen pollution has escalated. As a result, research and modeling efforts have been conducted to identify management and nutritional strategies for reducing the excretion of N into the off-farm environment (e.g., Kohn et al., 1997; St-Pierre and Thraen, 1999; Jonker et al., 2002). Results from these studies suggest that improving the efficiency of N use by dairy cows, which in most production schemes rarely exceeds 25 to 30%, is the most promising way to decrease N losses from dairy farms with minimal detrimental effects, if any, on their productivity and profitability. Reducing the output of N in urine provides the greatest opportunity for achieving that goal (Hvelplund and Madsen, 1996). This is because most of the N present in urine arises from inefficient N transactions in the rumen and peripheral tissues that may be amenable to manipulation (Lobley, 2002).

The supply of metabolizable protein (MP) to dairy cows mainly comprises proteins of microbial (MN) and feed origin that pass from the rumen and are absorbed as AA from the small intestine (NRC, 2001). To max-

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imize the efficiency of N use for milk production without compromising cow productivity, it is expected that first the supply of total AA in MP must be sufficient, but not excessive, for meeting maintenance and production needs of the cow. Second, a further improvement would be expected when the profile of absorbable essential AA (**EAA**) that reaches the small intestine exactly matches the demand of the cow for EAA. This additional benefit would be obtained because the efficiency with which AA are used for protein synthesis is maximized (Sloan, 1997; NRC, 2001).

These likely events are partly supported by recent studies that showed that reducing N intake while correcting for predicted shortages of limiting AA for milk production by including RUP supplements (Castillo et al., 2001; Noftsger and St-Pierre, 2003) and (or) rumen-protected AA (Kröber et al., 2000; Leonardi et al., 2003; Noftsger and St-Pierre, 2003) in the diet can reduce excretion of N in urine and sustain production of milk and milk protein. In these studies, dietary CP was reduced by replacing high-protein supplements with cereal grains, sources of nonforage fiber, or protein supplements lower in CP. In some cases, urea was added to the low CP diets to prevent limiting microbial growth because of a deficiency of RDP. Consequently, in these experiments the amount and form of N and carbohydrate available in the rumen and intestines might have differed between treatments. These factors and their interaction are major determinants of the ruminal outflow of MN and RUP (Clark et al., 1992), ruminal fermentation (Nocek and Russell, 1988), and, ultimately, the efficiency or pattern of use of absorbed nutrients for milk production (Oldham, 1984). In none of those studies, however, were the impact of treatments on ruminal fermentation and the intestinal supply of nutrients assessed. Therefore, it remains unclear whether the reported effects on N excretion and cow performance were achieved independently of alterations in ruminal metabolism and the postruminal supply of N and carbohydrate.

Recently, Bateman et al. (2001) reported that some of the most widely used models for predicting the supply of MP to dairy cows underestimated both the amount of feed CP and total CP that exited the rumen. These researchers explained their findings by proposing that the proportion of individual feed protein that is degraded in the rumen of dairy cows that consume large amounts of feed is less than that assumed by current feeding standards (Bateman et al., 2001). As follows, use of these models to examine the adequacy of diets for meeting the requirements of dairy cows for MP may contribute to overfeeding N and exacerbate its inefficient use for milk production. To solve this limitation, more data for the outflow of N fractions from the rumen

of dairy cows that consume large quantities of feed is needed.

Two experiments were conducted concurrently to test the broad hypothesis that the CP content of the diet can be decreased to improve the efficiency of N use for milk production without affecting negatively the supply of MP and the lactational performance of dairy cows if the source and amount of CP are properly matched with the source and amount of carbohydrate in the diet. The objectives of the experiment were 1) to determine the effects of the amount and source of CP and the amount of starch in the diet of dairy cows on ruminal fermentation, passage of nutrients to the small intestine, and nutrient digestibility; 2) to relate differences in conversion of feed N to milk N and productivity of cows determined in a companion experiment (Ipharraguerre and Clark, 2005b) to alterations in ruminal fermentation, intestinal supply of nutrients, and use of nutrients by dairy cows; 3) and to examine the passage of N fractions to the small intestine of dairy cows that consume large quantities of diets supplemented with proteins reported to be high in either RDP or RUP.

MATERIALS AND METHODS

Animals, Experimental Design, and Management

Six multiparous Holstein cows that averaged 73 DIM (39 to 98 d) at the onset of the experiment were surgically fitted with ruminal and duodenal cannulas according to procedures approved by the University of Illinois Laboratory Animal Care Advisory Committee. The ruminal cannulas were constructed of soft plastic (Bar Diamond, Parma, ID) and measured 10.2 cm in diameter. The duodenal cannulas were an enclosed T-shaped design made of stainless steel (Berzins Vet Laboratory Ltd., Edmonton, Alberta, Canada). They were placed proximal to the common bile and pancreatic duct, about 10 cm distal to the pylorus.

The experimental design was a 6×6 Latin square with a 2×3 factorial arrangement of treatments. Two protein supplements (source) of different ruminal degradability and 3 concentrations (percentage) of dietary protein were combined into the following treatments: 1) low CP (**LCP**), solvent-extracted soybean meal (**SBM**); 2) LCP, blend of animal-marine protein supplements (i.e., meals of blood, porcine meat and bone, menhaden fish, and hydrolyzed feather) plus ruminally protected Met (**AMB**); 3) medium CP (**MCP**), SBM; 4) MCP, AMB; 5) high CP (**HCP**), SBM; and 6) HCP, AMB. Diets were formulated to contain 14% (LCP), 16% (MCP), or 18% CP (HCP) by replacing an increasing proportion of corn with protein supplements as the CP content of the diet increased (Table 1). Consequently, the effects of the concentration of starch and protein in

Table 1. Ingredient and chemical composition of the experimental diets¹ (DM basis).

Item	LCP		MCP		HCP	
	SBM	AMB	SBM	AMB	SBM	AMB
Ingredient						
Alfalfa silage, %	20.00	20.00	20.00	20.00	20.00	20.00
Corn silage, %	25.00	25.00	25.00	25.00	25.00	25.00
Cottonseed (w/lin), %	10.00	10.00	10.00	10.00	10.00	10.00
Ground shelled corn, %	36.42	37.01	30.80	34.01	26.65	30.67
Soybean meal (49% CP), %	4.75	2.33	9.13	4.48	13.50	6.62
Animal-marine protein blend, ² %	...	1.73	...	3.32	...	4.90
Soyhulls, %	...	...	1.50	...	1.50	...
Inert fat, ³ %	...	0.36	...	...	...	...
Sodium bicarbonate, %	1.00	1.00	1.00	1.00	1.00	1.00
Sodium chloride, %	0.25	0.25	0.25	0.25	0.25	0.25
Mineral and vitamin mix, ⁴ %	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate, %	0.62	0.52	0.54	0.32	0.45	0.12
Limestone, %	1.08	0.93	1.07	0.95	1.10	0.94
Magnesium oxide, %	0.17	0.18	0.15	0.17	0.14	0.17
Sodium sulfate, %	0.46	0.44	0.31	0.25	0.16	0.08
Chemical composition						
DM, %	65.5	65.3	65.4	65.4	65.7	65.7
OM, %	93.4	93.5	93.3	93.6	93.4	93.5
CP, %	13.9	13.9	16.1	16.0	17.9	18.3
RUP, ⁵ %	4.8	5.2	5.6	6.4	6.4	7.6
RUP, ⁵ % of CP	34.5	37.4	34.8	40.0	35.8	41.5
RDP, ⁵ %	9.1	8.7	10.5	9.6	11.5	10.7
NDF, %	31.2	30.3	30.9	30.8	30.6	30.9
ADF, %	19.5	19.6	20.4	19.7	20.5	19.9
Starch, %	32.8	33.0	29.6	30.5	27.0	29.4
NE _L , Mcal/kg of DM ⁵	1.53	1.51	1.53	1.52	1.54	1.53

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = solvent-extracted soybean meal.

²ProLak (H. J. Baker & Bros., Inc., Westport, CT)

³Megalac (Church & Dwight, Co., Inc., Princeton, NJ)

⁴Contained 5.0% Mg, 7.5% K, 10.0% S, 3.0% Zn, 3.0% Mn, 2.0% Fe, 0.5% Cu, 0.025% I, 0.015% Se, 0.004% Co, 2200 IU of vitamin A/g, 660 IU of vitamin D₃/g, and 8 IU of vitamin E/g.

⁵Calculated using tabulated values from NRC (2001) and assuming an intake of 4 times maintenance. It was also assumed that 70% of the CP in ProLak is RUP.

the diet were confounded. The AMB supplement (ProLak; H. J. Baker & Bros., Inc., Westport, CT) partially replaced SBM in the concentrate mixtures with the aim of enhancing the RUP content of the diets (Table 1). Our ultimate goal was to improve quantitatively and qualitatively the supply of MP at each concentration of dietary CP through increased input of digestible RUP with an AA composition balanced to complement microbial protein. The remaining dietary ingredients were supplied from the same source and in the amounts needed to meet the NRC (2001) nutrient requirements (Table 1). Each cow was randomly assigned to 1 of 6 treatment sequences in which a treatment never followed the same treatment for all sequences. Experimental periods lasted 14 d; the first 9 d of each period were used to adapt the cows to treatments and the remaining 5 d were used to collect data.

Cows were fed diets as TMR for ad libitum intake to ensure 10% orts. The TMR were offered twice daily at 0600 and 1700 h and were adjusted weekly to reflect

changes in DM of forages and concentrate mixtures by drying each component overnight in an oven at 105°C. Cows were housed in individual stanchions equipped with water bowls and bedded with rubber mats and straw and were milked twice daily at 0600 and 1800 h. With the exception of the last 3 d of each period when samples were being collected, cows were allowed to exercise in a dry lot from 0800 to 0900 h.

Sampling, Measurements, and Analyses

Dry matter intake and orts were measured and recorded daily. Samples of individual feed ingredients, TMR, and orts were collected for the last 5 d of each period. Samples of individual feedstuffs and TMR were dried at 55°C in an oven for 72 h, ground through a 2-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA), composited on an equal weight basis by period, and ground through a 1-mm screen. Samples of orts were dried at 55°C in an oven for 72 h, ground

through a 2-mm screen, composited by the amount and dry weight of the daily orts for each cow, and ground through a 1-mm screen. Samples of feeds, TMR, and orts were analyzed for DM, OM, Kjeldahl N (AOAC, 1990), ADF (Van Soest et al., 1991), NDF with heat-stable α -amylase (Thermamyl 120L; Novo Nordisk Biochem, Franklinton, NC) and sodium sulfite (Van Soest et al., 1991), and starch (Kartchner and Theurer, 1981). Samples of feeds and TMR were prepared for AA determination, except Met, by acid hydrolysis with 6 N HCl (McCarthy et al., 1989). For Met determination, samples were first oxidized with performic acid, lyophilized after dilution with water to remove excess performic acid (Overton et al., 1995), and hydrolyzed with 6 N HCl as outlined by Moore (1963). Individual AA were then separated by ion exchange chromatography on a Beckman 126AA amino acid analyzer equipped with a Beckman 12-cm column (Beckman Instruments Inc., Palo Alto, CA).

Samples of ruminal fluid and duodenal digesta were collected every 3 h during the last 3 d of each period. The sampling time was adjusted ahead 1 h daily so that a sample was obtained for each 1-h interval of the day (24 total samples). Ruminal fluid samples were taken from multiple sites in the rumen and pH of ruminal fluid was measured immediately by glass electrode. After measurement of pH, a subsample of 50 mL was acidified to pH < 2 with 50% H₂SO₄ (vol/vol), centrifuged at 27,000 $\times g$ for 10 min at 4°C, and the supernatant was frozen at -20°C for later analyses. Following removal of the cap of the duodenal cannula, accumulated digesta was discarded, and when the flow appeared normal, 500 mL of duodenal contents were collected. Samples were pooled by cow and stored frozen in 20-L buckets at -20°C until analyses.

Ruminal fluid samples taken at 0, 1, 2, 3, 5, 7, 9, and 10 h after the morning feeding, and at 0, 1, 2, 3, 5, 7, 9, 11, and 12 h after the afternoon feeding (17 total samples) were thawed, centrifuged at 27,000 $\times g$ for 20 min at 4°C, and an aliquot of 4 mL was diluted with 25% metaphosphoric acid (4:1 ratio). These subsamples were assayed with a gas chromatograph (model 5890 Series II; Hewlett-Packard, Avondale, PA) equipped with a 1.8-m glass column packed with 10% SP 1200/1% H₃PO₄ on 80/100 chromosorb W AW (Supelco Inc., 1975) to determine the concentration of VFA. Nitrogen was the carrier gas, and the temperature of the injection port and column was 175 and 125°C, respectively. Ruminal ammonia N was determined according to the procedures outlined by Chaney and Marbach (1962) as modified by Cotta and Russell (1982).

Duodenal samples were thawed and homogenized for 5 min using a propeller-type mixer set at high speed. During continuous stirring, a representative subsample

(1000 mL) of digesta was collected by vacuum. Samples then were poured into shallow pans, lyophilized, ground through a 1-mm screen, and analyzed for DM, OM, Kjeldahl N, ADF, NDF, starch, and AA as described above. The duodenal digesta concentration of NH₃N was determined by steam distillation with MgO (Bremner and Keeney, 1965), and purines, used as a bacterial marker, were measured by the method of Zinn and Owens (1986).

Ruminal bacteria were isolated from samples (1000 mL) of whole ruminal contents obtained from the reticulum near the reticulo-omasal orifice at 6 separate post-feeding times (0, 2, 4, 6, 8, and 10 h) during the last 3 d of each period. Ruminal contents were blended in a Waring blender (Waring Products Division, New Hartford, CT) for 1 min at low speed, strained through 6 layers of cheesecloth, and the effluents were used to prepare a bacteria-rich sample by differential centrifugation (Overton et al., 1995). Bacterial samples were pooled by cow within period and frozen at -20°C. These samples were lyophilized and analyzed for DM, OM, Kjeldahl N, AA, and purines by the methods described above.

During the last 6 d of each period, fecal grab samples were collected twice daily at 12-h intervals. The sampling time was adjusted ahead 2 h daily so that a sample was obtained for each 2-h interval of the day (12 total samples). Samples were composited on an equal wet weight basis, dried at 55°C, ground through a 1-mm screen, and assayed for DM, OM, Kjeldahl N, ADF, NDF, and starch as described above.

Chromic oxide was used as an indigestible marker to assess the passage of digesta to the duodenum and fecal excretion by the cows. Gelatin capsules that contained 10 g of Cr₂O₃ powder were administered via the ruminal cannula at 0800 and 2000 h during the last 10 d of each period. Concentration of Cr in duodenal and fecal samples was quantified by atomic absorption spectroscopy (air plus acetylene flame; Perkin-Elmer, Norwalk, CT) after preparation of samples by the procedure of Williams et al. (1962).

Passage of MN and microbial OM to the duodenum was calculated from the passage of DM and from the proportion of N or OM of bacterial origin, respectively. These proportions were estimated by dividing the N to purine ratio or the OM to purine ratio of isolated bacteria by the N to purine ratio or the OM to purine ratio of duodenal digesta. Passage of nonammonia nonmicrobial N (NANMN) to the duodenum was calculated by subtracting passage of MN from passage of total NAN. Apparent digestibility of OM in the rumen was corrected for the passage of microbial OM to the duodenum to establish the amount of OM truly digested in the rumen.

Milk weights were recorded at each milking throughout the last 7 d of each period. Milk samples were taken at each milking during the last 7 d of each period, preserved with 2-bromo-2-nitropropane-1,3-diol, and stored at 4°C. Samples were sent to Dairy One Cooperative Inc. (Ithaca, NY) for analyses of fat, CP, true protein, total solids, and urea N by infrared procedures (AOAC, 1990; Foss 4000; Foss North America, Eden Prairie, MN).

Statistical Analyses

Data were analyzed as a 6×6 Latin square using the MIXED procedure of SAS (SAS Institute, 2000), with cow treated as a random variable and according to the following model:

$$Y_{ijkl} = \mu + C_i + P_j + L_k + S_l + LS_{kl} + \varepsilon_{ijkl}$$

where μ = overall mean; C_i = effect of cow i ($i = 1, 2, 3, 4, 5, 6$); P_j = effect of period j ($j = 1, 2, 3, 4, 5, 6$); L_k = effect of the k percentage of dietary CP ($k = 1, 2, 3$); S_l = effect of the l source of supplemental CP ($l = 1, 2$); LS_{kl} = effect of the interaction between the k percentage and l source of CP; and ε_{ijkl} = residual error.

Least square means were separated into significant main effects by the PDIF option of SAS (SAS Institute, 2000). Ruminal VFA, NH_3N , and pH data were analyzed as repeated measures in time using the MIXED procedure of SAS (Littell et al., 1996). The smallest value for the Akaike's information criterion was used to select the most appropriate covariance structure (Littell et al., 1996). The model was similar to the model described previously except for the addition of the effect of hour and the interaction of treatment and hour. Because the treatment by hour interaction was not significant ($P > 0.25$) for the ruminal parameters measured, treatment effects were compared across treatment sampling times using the procedure described above.

Differences among treatments were considered significant when $P < 0.05$, whereas when $P > 0.05$ but < 0.10 , differences were considered to indicate a trend toward a significant effect.

RESULTS AND DISCUSSION

Chemical Composition of Diets and Feed Ingredients

The percentage of CP in the diet ranged from 13.9 to 18.3 with minimal variation between protein sources within each percentage of dietary CP (Table 1). The AMB supplement provided 9.3, 15.6, and 20.1% of the dietary CP for the LCP, MCP, and HCP diets, respec-

tively. Estimation of the RUP content of the diets as described in Table 1, indicated that the percentage of dietary DM supplied as RUP for the AMB diets was higher than that of the SBM diets at all concentrations of CP (0.4, 0.8, and 1.2 percentage units). For the AMB diets, the estimated proportion of dietary CP supplied as RUP increased by 2.6 and 1.5 percentage units when the CP content increased from 13.9 to 16.0 and 18.3, respectively. These increases were 0.3 and 1.0 percentage units for the SBM diets. As intended, the starch content declined as the percentage of CP increased and this effect was less pronounced for the AMB diets. The concentration of DM, OM, ADF, NDF, and energy (i.e., NE_L) remained constant across percentages and sources of CP.

Compared with SBM, the AMB product contained more CP per unit of DM with higher mean concentrations of His, Leu, Lys, Met, Thr, Phe, and Val (Table 2). The chemical composition of the protein supplements and other feed ingredients was within the range reported elsewhere (Beede et al., 1994; Degussa Corp., 2001; NRC, 2001).

Intakes and Digestibilities of DM and OM

The intakes of DM and OM were not affected by the percentage and source of dietary CP, although they tended to be influenced by the interaction between percentage and source of CP (Table 3). For the MCP diets, cows fed SBM tended to consume more DM (+1.4 kg/d) and OM (+1.2 kg/d) than those fed ABM, but the opposite was observed for the LCP diets (−1.6 and −1.5 kg/d, respectively). In the companion experiment (Ipharraguerre and Clark, 2005b), differences in DMI during the first 210 d of lactation were also small and not significant across percentages and sources of dietary CP. However, a significant ($P < 0.002$) interaction for DMI was detected because the HCP diet containing SBM resulted in greater DMI (+2.5 kg/d) than the diet containing AMB. These results are in general agreement with results showing that the replacement of SBM with RUP supplements of marine and (or) animal origin can depress, but rarely increase, DMI (Santos et al., 1998; Ipharraguerre and Clark, 2005a), and that variability in this response is in part dictated by the percentage of dietary CP (Ipharraguerre, 2004; Ipharraguerre and Clark, 2005a). Likewise, results agree with previous data indicating that decreasing the CP content of the diet of lactating dairy cows generally has minor effects on DMI unless dietary CP is reduced to extremely low percentages ($\leq 12\%$; Allen, 2000; Ipharraguerre, 2004).

The amount and proportion of DM apparently digested in the total tract were unaffected by the percent-

Table 2. Chemical composition of feed ingredients (DM basis).

Item	Silages		Energy and protein supplements ¹			
	Alfalfa	Corn	Corn grain	Cottonseed	SBM	AMB
OM, %	91.0	95.9	98.9	96.5	93.1	87.2
NDF, %	47.0	38.8	ND ²	48.4	8.8	ND
ADF, %	38.5	24.9	ND	40.1	5.1	ND
Starch, %	0.8	28.1	64.8	0.3	ND	ND
CP, %	20.1	7.9	8.4	23.1	52.0	75.0
EAA, ³ % of CP						
Arg	3.08	2.09	ND	ND	7.35	6.37
His	1.36	1.67	ND	ND	2.73	3.54
Ile	4.10	3.42	ND	ND	5.12	3.43
Leu	6.45	8.78	ND	ND	7.85	9.06
Lys	3.93	2.38	ND	ND	7.06	7.23
Met	1.70	2.08	ND	ND	1.29	1.57
Phe	4.20	3.62	ND	ND	5.25	5.37
Thr	3.86	2.99	ND	ND	4.40	4.68
Val	5.22	4.54	ND	ND	5.35	7.32

¹AMB = Animal-marine protein blend, SBM = solvent-extracted soybean meal.²ND = Not determined.³EAA = Essential amino acids.

age and source of dietary CP (Table 3). Similarly, the amount and proportion of OM apparently digested in the rumen and total tract and the amount of OM that passed to and was digested in the small intestine were not different among treatments. Several researchers also reported that varying the CP content of SBM-based

diets within a range wider (11.3 to 19.9%) than the one used in the experiment (13.9 to 18.3%) had no effects on the extent and site of OM digestion (Klusmeyer et al., 1990; Christensen et al., 1993; Cunningham et al., 1996). Likewise, in a number of trials those parameters were not altered by the replacement of SBM with RUP

Table 3. Least square means for intakes and digestibilities of DM and OM in various segments of the gastrointestinal tract of lactating dairy cows fed diets containing different percentages and sources of CP.¹

Item	LCP		MCP		HCP		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% × source
DM										
Intake, kg/d	22.2	23.8	24.4	23.0	22.9	22.9	0.8	NS ³	NS	0.08
ADTT ⁴										
kg/d	14.7	15.6	16.3	15.4	15.4	15.1	0.7	NS	NS	NS
%	66.0	65.4	66.9	67.2	67.7	65.9	1.4	NS	NS	NS
OM										
Intake, kg/d	20.8	22.3	22.7	21.5	21.4	21.3	0.7	NS	NS	0.09
Apparently digested in the rumen										
kg/d	6.2	6.0	7.2	7.4	5.1	4.6	1.3	NS	NS	NS
% of Intake	29.7	26.8	32.0	34.6	24.3	21.1	6.1	NS	NS	NS
Truly digested in the rumen										
kg/d	8.1	8.2	10.2	10.3	8.2	8.1	1.5	0.10	NS	NS
% of Intake	39.0	37.0	45.1	48.2	38.1	32.8	6.5	NS	NS	NS
Passage to duodenum, kg/d	14.6	16.0	15.5	14.0	16.2	16.4	1.3	NS	NS	NS
Apparently digested postruminally										
kg/d	7.9	8.1	8.2	8.2	10.4	9.0	1.2	NS	NS	NS
% of Intake	37.8	36.8	36.1	37.5	47.2	43.1	5.2	0.07	NS	NS
% of Passage to duodenum	52.5	52.2	52.8	52.4	60.7	56.3	3.6	0.06	NS	NS
ADTT										
kg/d	14.0	15.0	15.4	14.7	14.8	14.3	0.6	NS	NS	NS
%	67.4	66.9	68.1	68.5	69.3	67.1	1.3	NS	NS	NS

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP.³Nonsignificant (*P* > 0.10).⁴ADTT = Apparently digested in the total gastrointestinal tract.

Table 4. Least square means for intakes and digestibilities of ADF, NDF, and starch in various segments of the gastrointestinal tract of lactating dairy cows fed diets containing different percentages and sources of CP.¹

Item	LCP		MCP		HCP		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% × source
ADF										
Intake, kg/d	4.1	4.5	4.7	4.2	4.4	4.4	0.2	NS ³	NS	0.03
Apparently digested in the rumen										
kg/d	1.8	1.8	2.1	2.1	1.8	1.9	0.3	NS	NS	NS
% of Intake	42.9	40.3	45.8	49.6	41.2	41.3	5.3	NS	NS	NS
Passage to duodenum, kg/d	2.3	2.6	2.6	2.1	2.6	2.5	0.2	NS	NS	NS
ADTT ⁴										
kg/d	1.7	1.5	1.8	1.6	1.8	1.5	0.2	NS	NS	NS
%	35.6	37.2	38.9	37.9	40.8	34.3	2.7	NS	NS	NS
NDF										
Intake, kg/d	6.6	7.0	7.1	6.7	6.6	6.7	0.3	NS	NS	NS
Apparently digested in the rumen										
kg/d	2.8	2.6	2.8	3.3	2.1	2.7	0.4	NS	NS	NS
% of Intake	42.3	37.2	40.1	46.4	34.3	39.6	5.9	NS	NS	NS
Passage to duodenum, kg/d	3.8	4.3	4.3	3.6	4.3	4.0	0.3	NS	NS	NS
ADTT										
kg/d	2.8	2.9	3.0	2.9	2.8	2.7	0.2	NS	NS	NS
%	42.2	41.7	41.8	44.0	43.4	39.9	2.5	NS	NS	NS
Starch										
Intake, kg/d	7.7	8.4	7.5	6.7	6.7	6.8	0.2	0.0001	NS	0.002
Apparently digested in the rumen										
kg/d	3.2 ^a	3.6 ^a	3.0 ^a	2.5 ^a	1.5 ^b	1.6 ^b	0.6	0.006	NS	NS
% of Intake	41.9 ^a	42.3 ^a	39.7 ^a	37.8 ^a	24.3 ^b	25.8 ^b	8.3	0.05	NS	NS
Passage to duodenum, kg/d	4.4	4.9	4.5	4.1	5.2	5.1	0.5	NS	NS	NS
Apparently digested postruminally										
kg/d	4.1	5.1	4.5	3.7	5.0	5.6	0.6	0.08	NS	NS
% of Intake	53.2 ^b	61.7 ^b	59.3 ^b	53.4 ^b	76.2 ^a	83.5 ^a	8.2	0.006	NS	NS
% of Passage to duodenum	91.1	91.5	91.9	90.6	93.2	92.6	1.6	NS	NS	NS
ADTT										
kg/d	7.3	7.9	7.1	6.3	6.3	6.4	0.2	0.0001	NS	0.003
%	95.1	94.6	94.7	94.7	94.6	93.8	0.9	NS	NS	NS

^{a,b}Means within a row with different superscript differ significantly for the main effect of CP percentage. Pairwise comparisons were calculated using the appropriate standard error.

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP.

³Nonsignificant (*P* > 0.10).

⁴ADTT = Apparently digested in the total gastrointestinal tract.

supplements of animal, marine, and (or) plant origin (Waltz et al., 1989; Christensen et al., 1993; Cunningham et al., 1994). Compared with the LCP and HCP diets, however, the amount of OM truly digested in the rumen tended to be greater for the MCP diet. Because true digestibility of OM did not change significantly, this trend might have been caused by the slightly greater intake of OM (~0.7 kg/d) by cows fed the MCP diet. A positive relationship between the intake and true digestibility of OM in the rumen of dairy cows has been shown previously (Clark et al., 1992). Compared with the LCP and MCP diets, cows fed HCP diets tended to have higher postruminal digestibility of OM (expressed as percentage of intake and passage to duodenum). As discussed later, this trend might have resulted from a proportionally higher contribution of digestible starch and protein to the ruminal outflow of OM for cows fed HCP diets. Improvements in the intestinal

digestibility of OM were reported when urea was used to increase the CP content of diets supplemented with fish meal (Cameron et al., 1991), but such a response was not observed when the percentage of dietary CP of SBM-based diets was increased through addition of true protein supplements (Klusmeyer et al., 1990; Christensen et al., 1993).

Intakes and Digestibilities of Carbohydrates

Intakes, apparent ruminal and total tract digestibilities (amount and percentage), and flows to duodenum of ADF and NDF were not altered by treatments (Table 4). Although the amount (Cunningham et al., 1996) or source (Reynal and Broderick, 2003) of dietary CP influenced fiber digestibility in some studies, most frequently manipulation of these factors in the diet of lactating cows did not impact the extent and site of

fiber digestion (Stern et al., 1983; Klusmeyer et al., 1990; Stokes et al., 1991; Christensen et al., 1993; Chan et al., 1997).

When the concentration of dietary CP increased from LCP to HCP, the intake of starch decreased on average from 8.0 to 6.7 kg/d. However, cows fed AMB consumed 0.7 kg/d more starch than those fed SBM when diets contained LCP, whereas cows fed SBM consumed 0.8 kg/d more starch than those fed AMB when diets contained MCP (Table 4). These differences arose from variations in both DMI (Table 3) and chemical composition of experimental diets (Table 1).

The percentage of dietary CP, but not the source or interaction of these 2 factors, influenced the digestion of starch in the rumen and intestines (Table 4). As CP increased in the diet from LCP to HCP, the amount and percentage of starch apparently digested in the rumen decreased on average 1.8 kg/d (53%) and 17 percentage units (40.5%), respectively. This difference in ruminal starch digestibility compensated for the differences in starch intake, resulting in similar ruminal outflows of starch among treatments. However, as the dietary CP content increased, a slightly ($P < 0.08$) larger amount (mean = 0.7 kg/d), but a significantly larger proportion of the starch consumed by the cows (mean = 22.4 percentage units or 39%) was digested in the intestines. Consequently, the percentage of starch apparently digested in the total tract was unaffected by treatments, but the quantity digested paralleled intake. Voelker and Allen (2003) also observed that reducing starch intake from 8.2 to 6.4 kg/d by replacing corn grain with beet pulp to supply 12% of dietary DM decreased the amount and proportion of starch apparently digested in the rumen by 1.9 kg/d and 16 percentage units (38.1%), respectively. In turn, the percentage of starch intake that was digested in the intestines increased 16.9 percentage units (37%), whereas its total tract digestibility remained unaltered by treatments. Other researchers reported similar relationships between the intake and the ruminal and postruminal digestion of starch in dairy cows (Cameron et al., 1991; Oba and Allen, 2003). Likewise, ruminal and postruminal digestibilities of starch were similar to values previously reported for diets that contained equivalent concentrations of starch (Cameron et al., 1991; Lynch et al., 1991; Reynolds et al., 1997; Voelker and Allen, 2003), in which corn grain and corn silage were the predominant source of starch. To our knowledge, few studies with dairy cows have evaluated the factorial effects of the percentage and source of CP on starch digestibility. Increasing dietary CP through additions of urea (Cameron et al., 1991) or a mix of RUP supplements plus urea (Christensen et al., 1993) did not affect the extent, percentage, or site of starch digestion.

The composition of the prevalent microbial population, or its pattern of metabolism, is at least one factor that might explain why there were differences in digestibility and site of starch digestion. As the concentration of CP in the diet increased and that of starch decreased, it is likely that nonstructural carbohydrate-fermenting bacteria declined whereas protein-, peptide-, and AA-fermenting bacteria proliferated, comprising a significant proportion of the microbial population inhabiting the rumen. As a result, proportionally more digestible starch escaped ruminal fermentation and reached the duodenum. This hypothesis is supported in part by recent data from experiments in which the effects of feeding dairy cows diets with a variable nonstructural carbohydrate content on proteolytic bacteria were evaluated (Reilly et al., 2002). Populations of *Prevotella* and total Eubacteria were not affected by changes in the diet, but the numbers of *Butyrivibrio* increased, and the numbers of *Eubacterium* spp. decreased when carbohydrate was supplemented. Additionally, evidence is mounting that bacterial species that ferment peptides and AA as energy sources (e.g., *Peptostreptococcus anaerobius*, *Clostridium sticklandii*, *Clostridium aminophilum* spp. nov., *Eubacterium pyruvovarans* spp. nov.) are commonly present in the rumen under numerous conditions (Attwood et al., 1998; Morrison, 2000). Most of these protein-, peptide-, and AA-degrading bacteria either do not use or poorly ferment carbohydrate, in particular starch, and predominantly produce long- and branched-chain organic acids such as butyrate, isobutyrate, valerate, isovalerate, and caproate as well as NH_3N (Hungate, 1966; Attwood et al., 1998). As is discussed in the next section, changes in ruminal pH, molar proportions of VFA, and concentration of NH_3N (Table 5) in response to higher inputs of dietary CP corresponded to such a pattern of fermentation, supporting further the above hypothesis.

Interestingly, the amount of starch digested in the intestines was positively correlated with the passage of NAN to duodenum:

$$Y = 0.008X \text{ (SE 0.02, } P < 0.0001) \\ - 0.108 \text{ (SE 1.09, } P < 0.92); (n = 30; R^2 = 0.41)$$

where Y = apparent postruminal digestion of starch (kg/d) and X = intestinal supply of NAN (g/d). The proportion of starch consumed by the cows that was digested in the intestines also was related, although only slightly ($R^2 = 0.26$), to the ruminal outflow of NAN. Because cows were not cannulated in the ileum, it is not possible to establish if these relationships indicate that increased intestinal supply of NAN (protein) improved the digestion of starch (i.e., quantity) in the small intestines and subsequently the supply of glucose

Table 5. Least square means for ruminal fermentation parameters of lactating dairy cows fed diets containing different percentages and sources of CP.¹

Item	LCP		MCP		HCP		SEM	Effect ² ($P > F$)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% $\times$ source
pH	5.87 ^b	5.93 ^b	5.89 ^b	5.92 ^b	5.91 ^a	6.04 ^a	0.04	0.01	0.002	NS ³
Total VFA, mM	122.6 ^b	123.1 ^b	129.1 ^a	124.6 ^a	128.0 ^a	126.1 ^a	2.04	0.05	NS	NS
Acetate, mol/100 mol	63.6	65.2	64.0	64.9	63.9	64.3	0.64	NS	0.006	NS
Propionate, mol/100 mol	22.8	21.7	21.6	21.6	21.0	21.1	0.65	NS	NS	NS
Isobutyrate, mol/100 mol	0.7 ^c	0.7 ^c	0.8 ^b	0.8 ^b	0.9 ^a	0.9 ^a	0.03	0.0001	NS	NS
Butyrate, mol/100 mol	10.1 ^b	10.0 ^b	10.7 ^{ab}	10.2 ^{ab}	11.0 ^a	10.4 ^a	0.28	0.06	0.09	NS
Isovalerate, mol/100 mol	1.3 ^b	1.3 ^b	1.4 ^b	1.4 ^b	1.5 ^a	1.6 ^a	0.04	0.02	NS	NS
Valerate, mol/100 mol	1.6 ^b	1.2 ^b	1.5 ^b	1.3 ^b	1.7 ^a	1.5 ^a	0.15	0.06	0.008	NS
NH ₃ N, mg/dL	11.9 ^c	10.3 ^c	17.2 ^b	15.1 ^b	20.2 ^a	18.8 ^a	1.16	0.0001	0.02	NS

^{a,b,c}Means within a row with different superscript differ significantly for the main effect of CP percentage. Pairwise comparisons were calculated using the appropriate standard error.

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% $\times$ source = interaction between percentage and source of CP.

³Nonsignificant ($P > 0.10$).

to the cows. However, if those positive correlations arose from enhanced cecal fermentation of starch rather than digestion in the small intestines, a decline in the intestinal or total tract digestibility of N would have occurred because of synthesis of microbial protein that could not be digested and absorbed from that segment of the digestive tract (Ørskov, 1992). As will be discussed later, this was not the case when the percentage of CP increased in the diet. A stimulatory effect of protein on the intestinal disappearance of starch or nonstructural carbohydrate has been observed in experiments with steers (Richards et al., 2002) and dairy cows (Abramson et al., 2002) infused abomasally with starch and casein. The relevance of this interaction between protein and carbohydrate resides in the beneficial effects that improved postruminal digestion of starch could have in the efficiency of use of energy (Huntington, 1997) and N (Oldham, 1984) for milk production. Therefore, results suggest that generalizations about the effects of the percentage and source of CP on performance of cows and (or) efficiency of nutrient use for milk production without considering the protein-carbohydrate interaction may be misleading.

Ruminal Fermentation

The interaction between the percentage and source of dietary CP for the ruminal fermentation parameters examined in this experiment was not significant (Table 5). These 2 factors, however, independently altered ruminal metabolism. Mean ruminal pH increased from 5.90 to 5.98 as the percentage of dietary CP increased from LCP to HCP, but differences were not significant between LCP and MCP. This response might be explained by the progressive decrease in the amount of

starch digested in the rumen that resulted from the increase of CP in the diet (Table 4). Alternatively, the increase in ruminal NH₃N concentration that was associated with the increasing input of dietary CP might have contributed to buffer the acidity of the ruminal fluid. In previous experiments, varying the CP content of the diet of dairy cows did not affect ruminal pH (Klusmeyer et al., 1990; Christensen et al., 1993; Cunningham et al., 1996). The concentration of VFA in the ruminal fluid was higher for HCP and MCP than for LCP, which is in contrast with changes in ruminal pH. However, the ruminal concentration of VFA is the net result of production and absorption (Owens and Goetsch, 1988) and the rate of absorption increases as ruminal pH decreases (Merchen, 1988). Therefore, it is possible that differences detected for the concentration of VFA arose from a faster rate of removal of VFA from the rumen of cows fed LCP. More importantly, these differences were small and probably were not biologically relevant.

The molar proportions of acetate and propionate were not affected by the percentage of dietary CP. In contrast, the molar proportions of butyrate ($P < 0.06$), isobutyrate, valerate ($P < 0.06$), and isovalerate were increased by the HCP diet. Except for butyrate, these VFA originate from oxidative deamination and decarboxylation of branched-chain AA (Allison, 1978; Andries et al., 1987) and this along with the increase in ruminal NH₃N concentration, suggest that additional true protein was degraded in the rumen as the input of CP increased from LCP to HCP. In other experiments with dairy cows, increasing the CP content of the diet consistently enhanced the concentration of NH₃N in the rumen but not the molar proportions of branched-chain VFA (Klusmeyer et al., 1990; Christensen et al.,

Table 6. Least square means for intake and digestibility of N in various segments of the gastrointestinal tract of lactating dairy cows fed diets containing different percentages and sources of CP.¹

Item	LCP		MCP		HCP		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% × source
N intake, g/d	494 ^c	526 ^c	634 ^b	589 ^b	662 ^a	674 ^a	22.6	0.0001	NS ³	NS
Passage to duodenum										
Total N, g/d	510	611	580	544	638	653	48.8	0.10	NS	NS
Total N, % of N intake	103.7 ^b	110.4 ^b	91.3 ^a	92.1 ^a	96.1 ^{ab}	98.8 ^{ab}	6.7	0.04	NS	NS
NAN, g/d	488 ^b	555 ^b	554 ^{ab}	518 ^{ab}	608 ^a	626 ^a	41.0	0.03	NS	NS
NAN, % of N intake	99.1 ^b	106.7 ^b	87.1 ^a	88.5 ^a	91.4 ^{ab}	93.9 ^{ab}	6.4	0.04	NS	NS
NANMN, ⁴ g/d	220 ^b	227 ^b	276 ^b	252 ^b	341 ^a	388 ^a	38.3	0.003	NS	NS
NANMN, % of NAN	45.4 ^b	40.1 ^b	49.1 ^b	48.7 ^b	56.4 ^a	61.5 ^a	5.0	0.003	NS	NS
NANMN, % of N intake	45.8	43.6	43.2	43.5	51.4	58.2	6.0	0.10	NS	NS
Microbial N, g/d	267	292	278	266	267	251	23.7	NS	NS	NS
Microbial N, % of NAN	54.6 ^a	59.9 ^a	50.9 ^a	51.3 ^a	43.6 ^b	38.4 ^b	5.0	0.002	NS	NS
Microbial N, g/kg of OMAD ⁵	34.9	46.3	42.5	38.6	48.4	41.2	8.7	NS	NS	NS
Microbial N, g/kg of OMTD ⁶	26.8	37.4	28.5	27.5	32.9	26.8	5.4	NS	NS	NS
Microbial N, g/kg of OMADTT ⁷	19.2	20.0	18.0	18.3	18.0	16.4	1.6	NS	NS	NS
N digested postruminally										
g/d	340	328	384	344	416	438	45.5	0.09	NS	NS
% of N intake	68.8	61.4	60.2	58.8	64.3	65.8	6.7	NS	NS	NS
% of N passage to duodenum	66.3	61.5	65.4	64.0	68.0	67.8	2.8	NS	NS	NS
N ADTT ⁸										
g/d	324 ^c	328 ^c	438 ^b	401 ^b	471 ^a	468 ^a	22.1	0.0001	NS	NS
%	65.2 ^b	62.0 ^b	68.9 ^a	67.9 ^a	71.4 ^a	69.5 ^a	1.8	0.003	0.08	NS

^{a,b,c}Means within a row with different superscript differ significantly for the main effect of CP percentage. Pairwise comparisons were calculated using the appropriate standard error.

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP.

³Nonsignificant (*P* > 0.10).

⁴NANMN = Nonammonia nonmicrobial N.

⁵OMAD = Organic matter apparently digested in the rumen.

⁶OMTD = Organic matter truly digested in the rumen.

⁷OMADTT = Organic matter apparently digested in the total gastrointestinal tract.

⁸ADTT = Apparently digested in the total gastrointestinal tract.

1993; Cunningham et al., 1996). However, feeding increasing amounts of CP to sheep decreased the intake and amount of starch digested in the rumen and increased both the ruminal concentration of NH₃N and molar proportion of valerate and isovalerate (Ludden et al., 2002).

Compared with AMB, feeding SBM decreased ruminal pH and the molar proportion of acetate but increased the molar percentages of butyrate (*P* < 0.09) and valerate and the concentration of NH₃N in the ruminal fluid (Table 5). Because the intake and ruminal fermentation of OM and carbohydrates were not affected by the source of CP, these differences were likely caused by a more rapid rate of degradation of OM and protein in SBM than in AMB. Replacing SBM with RUP supplements of diverse origin, including meals of blood, fish, feather, meat, corn gluten, and soybeans, led to similar shifts in ruminal fermentation in some studies (Waltz et al., 1989; Christensen et al., 1993; Mansfield and Stern, 1994) but not in others (Cunningham et al., 1996; O'Mara et al., 1998; Reynal and Broderick, 2003). In all these studies, however, feeding RUP supplements

consistently decreased the concentration of NH₃N in the ruminal fluid compared with SBM.

Intake and Ruminal Outflow of Nitrogen

The effects of the source of CP and the interaction between the percentage and source of CP were not significant for N intake, ruminal outflow of N fractions, and N digestibility in the intestines and total tract (Table 6). In a summary of studies, Santos et al. (1998) indicated that replacing SBM with RUP supplements in 25 treatment comparisons did not increase the intake of N. In contrast to results reported here, a meta-analytic review of studies designed to examine the impact of RUP supplements on the outflow of N fractions from the rumen of dairy cows (Ipharraguerre and Clark, 2005b) showed that the intestinal supply of NAN and NANMN was significantly (*P* < 0.05) increased (mean = 6.2 and 22.9%, respectively), whereas that of MN was significantly (*P* < 0.05) decreased (mean = -6.7%) when SBM was replaced with a variety of RUP sources in 34 treatment comparisons. In those 34 comparisons, cows

consumed on average 18.7 kg of DM daily. The detrimental effect of replacing degradable protein sources with RUP supplements on the ruminal outflow of MN has been associated with a shortage of energy, AA, peptides, or NH_3N in the rumen (Hoover and Stokes, 1991; Clark et al., 1992). However, in some studies in which DMI was as high as in this experiment (~ 23 kg/d), the replacement of SBM with blood meal, fish meal, extruded SBM, or corn gluten meal did not affect the ruminal outflow of NAN, NANMN (Klusmeyer et al., 1991; Reynal et al., 2003), and MN (Reynal et al., 2003).

It is well known that decreases in ruminal retention time associated with increases in DMI can decrease the extent of protein degradation in the rumen (NRC, 1985; Satter, 1986). The magnitude of this effect, however, would be expected to be relatively small even for large increases in DMI (Satter, 1986). Loerch et al. (1983) reported that the *in situ* disappearance of N for various sources of supplemental CP declined as the ruminal pH approached 6.0 and this effect was more pronounced for SBM than for sources of RUP (e.g., blood meal, corn gluten meal). In this experiment (Table 5), as in those in which DMI averaged about 23 kg/d (Klusmeyer et al., 1991; Reynal et al., 2003), the mean ruminal pH for the SBM-based diets was 6.0 or less. Therefore, it would seem reasonable to suggest that high DMI and relatively low ruminal pH might have contributed to reduce the ruminal degradability of proteins in SBM to a larger extent than for those in AMB, resulting in more passage of NANMN to the duodenum from these sources of CP. At the same time, high DMI coupled with extensive fermentation of OM in the rumen of cows fed AMB might have resulted in an adequate supply of nutrients in the rumen, preventing in turn a depression of the synthesis and the efficiency of synthesis of microbial protein. Additionally, the proportion of dietary CP supplied from AMB, which ranged from 9 to 20%, might have been insufficient for improving quantitatively the ruminal escape of NANMN and (or) limiting microbial growth. In part, this suggestion is supported by results from a summary of studies (Ipharraguerre, 2004; Ipharraguerre and Clark, 2005a) indicating that the mean outflow of NANMN from the rumen of dairy cows fed diets that contained RUP supplements was significantly increased when RUP provided more than 25% of the dietary CP.

The intake of N increased from 510 to 668 g/d as CP increased in the diet from LCP to HCP (Table 6). The passage to the small intestine of total N and NAN (expressed as g/d and as percentage of N intake) followed the same trend as N intake, but differences between MCP and HCP were not significant. For the LCP diets, the ruminal outflow of N and NAN was on average 7 and 2.9% greater than N intake, suggesting that the

supply of RDP from this diet might have been slightly marginal. However, the passage of MN to the duodenum and the efficiency of microbial growth were not affected by the percentage of dietary CP. Consequently, it is possible that more endogenous N was recycled into the rumen of cows fed the LCP diet to sustain microbial growth and fermentation. This observation is supported by studies with steers (Marini and Van Amburgh, 2003) and dairy cows (Al-Dehneh et al., 1997) in which it was estimated that the amount and proportion of endogenous urea N used by ruminal microbes increased when the availability of N in the rumen, but not of energy, became increasingly deficient.

The escape of NANMN from the rumen increased as the concentration of CP increased in the diet, but differences between LCP and MCP were not significant. As a result, proportionally less MN and more NANMN contributed to the intestinal supply of NAN as dietary CP increased from LCP to HCP. These results agree with previous research indicating that the ruminal outflow of NAN increased, whereas the passage to duodenum of MN and the efficiency of microbial protein synthesis remained unaffected when the percentage of dietary CP varied from 11.3 to 15.0 (Klusmeyer et al., 1990), from 15.8 to 20.3 (Reynal et al., 2003), or from 13.1 to 23.1 (Stern et al., 1983).

The amount of N apparently digested in the intestines tended to increase in response to increments in the input of dietary CP (Table 6). This response, however, can be attributed to increases in N intake because the post-ruminal digestibility of N expressed as percentage of N intake or as percentage of N passage to duodenum was similar among treatments. Likewise, the amount of N apparently digested in the total tract reflected changes in N intake, being lowest for LCP, intermediate for MCP, and highest for HCP. Compared with the LCP diet, feeding the MCP and HCP diets increased the percentage of N intake apparently digested in the total tract. Because the intestinal digestibility did not differ among treatments, it is likely that this effect corresponded to 1) a dilution of metabolic fecal N losses and 2) increased protein degradation and losses of NH_3N from the rumen of cows fed the MCP and HCP diets. Additionally, the suggested larger incorporation of endogenous N into microbial protein synthesized in the rumen of cows fed the LCP diet might have contributed to explain these differences. Similar outcomes have been reported in other experiments in which the intake of N was enhanced by increasing the percentage of dietary CP (Klusmeyer et al., 1990; Cunningham et al., 1996).

Intake and Ruminal Outflow of AA

As shown in Table 7, the interaction between the percentage and source of CP was significant for the

Table 7. Least square means for intakes of AA (g/d) by lactating dairy cows fed diets containing different percentages and sources of CP.¹

AA								Effect ² (<i>P</i> > <i>F</i>)		
	LCP		MCP		HCP		SEM	CP%	Source	CP% × source
	SBM	AMB	SBM	AMB	SBM	AMB				
Essential										
Arg	166 ^c	167 ^c	218 ^b	224 ^b	245 ^a	238 ^a	7.2	0.0001	NS ³	NS
His	71 ^c	74 ^c	91 ^b	92 ^b	101 ^a	104 ^a	3.2	0.0001	NS	NS
Ile	127	125	164	170	181	166	5.3	0.0001	NS	0.08
Leu	269 ^c	284 ^c	322 ^b	324 ^b	361 ^a	354 ^a	11.3	0.0001	NS	NS
Lys	129	128	177	187	215	199	6.0	0.0001	NS	0.05
Met	55 ^b	60 ^b	54 ^b	59 ^b	63 ^a	71 ^a	2.2	0.0001	0.001	NS
Phe	147 ^c	152 ^c	186 ^b	191 ^b	211 ^a	202 ^a	6.3	0.0001	NS	NS
Thr	115	117	142	145	169	155	4.9	0.0001	NS	0.09
Val	160	171	201	204	193	231	7.0	0.0001	0.002	0.02
Total	1240 ^c	1278 ^c	1555 ^b	1597 ^b	1740 ^a	1721 ^a	53.3	0.0001	NS	NS
Nonessential										
Ala	183	195	214	283	236	243	7.9	0.0001	0.0001	0.0002
Asp	298	294	389	402	455	403	12.7	0.0001	NS	0.02
Glu	480	473	616	636	712	621	20.0	0.0001	0.06	0.008
Gly	132 ^c	146 ^c	168 ^b	170 ^b	190 ^a	208 ^a	6.2	0.0001	0.01	NS
Pro	207 ^c	220 ^c	245 ^b	246 ^b	274 ^a	269 ^a	8.7	0.0001	NS	NS
Ser	126	131	157	160	206	179	5.6	0.0001	NS	0.005
Tyr	79 ^c	80 ^c	88 ^b	88 ^b	100 ^a	101 ^a	3.1	0.0001	NS	NS
Total	1506	1540	1877	1985	2173	2025	64.1	0.0001	NS	0.07
Total AA	2746 ^c	2818 ^c	3432 ^b	3582 ^b	3914 ^a	3746 ^a	117.4	0.0001	NS	NS

^{a,b,c}Means within a row with different superscript differ significantly for the main effect of CP percentage. Pairwise comparisons were calculated using the appropriate standard error.

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% $\times$ source = interaction between percentage and source of CP.

³Nonsignificant ($P > 0.10$).

intake of Ile ($P < 0.08$), Lys, Thr ($P < 0.09$), Val, Ala, Asp, Glu, Ser, and total nonessential AA (NEAA). With the exception of Val and Ala, the HCP/SBM diet resulted in higher intakes of those AA than the HCP/AMB diet, but differences between sources of CP at lower concentrations of dietary CP were not significant. In contrast, the intakes of Val and Ala were higher for AMB than for SBM for HCP and MCP diets, respectively. Across percentages of CP, replacing a portion of SBM with AMB increased the intake of Met (mean = 6 g/d), Val (mean = 17 g/d), and Ala (mean = 29 g/d), but decreased that of Glu (mean = -26 g/d). Across sources of CP, increasing the CP of the diet increased the intake of all individual AA, EAA, NEAA, and total AA, but differences between LCP and MCP were not significant for the intake of Met. Because the source of supplemental CP and corn grain were the only ingredients that varied in the diets, these findings can be attributed to variations in DMI (Table 3) and differences in the AA composition of SBM, AMB (Table 2), and corn grain.

The partial replacement of SBM with AMB in the diet did not affect the intestinal supply of total EAA, total NEAA, and total AA of NANMN origin (Table 8). However, feeding AMB increased the amount of His

and Pro ($P < 0.07$) that passed to the duodenum in the NANMN fraction. The ruminal outflow of individual AA, total EAA, total NEAA, and total AA of microbial origin (Table 9) and NAN origin (Table 10) were unaffected by the source of CP. In a summary of 41 treatment comparisons (Ipharraguerre, 2004) it was shown that increasing the RUP content of the diet of dairy cows that consumed on average 19.5 kg/d of DM enhanced the amount of Lys, Met, EAA, and total AA that reached the small intestine. The magnitude and significance of these responses, however, usually depended on the RUP supplement and the source of CP in the control diet. For instance, when mean responses were computed using only 25 comparisons in which SBM was the CP supplement in the control diet (Ipharraguerre and Clark, 2005a), the improvement in the ruminal outflow of Lys and Met caused by the feeding of RUP supplements in the experimental diet was small (3.4 and 2.5%, respectively) and not significant ($P > 0.05$). Rulquin and Vérité (1993) indicated that most of the variation in the profile of EAA that exit the rumen is accounted for by the individual EAA content of the RUP and the proportion of RUP that is consumed and delivered to the lower gastrointestinal tract. Even though the percentage of many EAA was higher in AMB

Table 8. Least square means for passage of nonammonia nonmicrobial AA (g/d) to the duodenum of lactating dairy cows fed diets containing different percentages and sources of CP.¹

AA	LCP		MCP		HCP		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% × source
Essential										
Arg	60 ^b	71 ^b	73 ^b	71 ^b	85 ^a	110 ^a	12.5	0.02	NS ³	NS
His	33 ^b	41 ^b	39 ^b	44 ^b	45 ^a	63 ^a	5.7	0.01	0.03	NS
Ile	53 ^b	57 ^b	65 ^b	56 ^b	78 ^a	90 ^a	12.9	0.05	NS	NS
Leu	139 ^b	161 ^b	160 ^b	164 ^b	178 ^a	234 ^a	24.9	0.05	NS	NS
Lys	65 ^b	71 ^b	79 ^b	77 ^b	124 ^a	122 ^a	22.6	0.03	NS	NS
Met	36 ^b	32 ^b	30 ^b	32 ^b	44 ^a	50 ^a	7.5	0.07	NS	NS
Phe	61 ^b	75 ^b	76 ^b	75 ^b	87 ^a	115 ^a	13.3	0.03	NS	NS
Thr	60 ^b	66 ^b	72 ^b	69 ^b	87 ^a	97 ^a	14.2	0.07	NS	NS
Val	63 ^b	76 ^b	78 ^b	81 ^b	91 ^a	127 ^a	15.4	0.03	NS	NS
Total	571 ^b	653 ^b	641 ^b	673 ^b	819 ^a	1012 ^a	121.5	0.02	NS	NS
Nonessential										
Ala	71 ^b	77 ^b	85 ^b	81 ^b	101 ^a	139 ^a	19.8	0.05	NS	NS
Asp	98 ^b	102 ^b	120 ^b	110 ^b	152 ^a	175 ^a	28.2	0.05	NS	NS
Glu	190 ^b	205 ^b	224 ^b	207 ^b	259 ^a	314 ^a	38.3	0.04	NS	NS
Gly	65 ^b	81 ^b	86 ^b	76 ^b	100 ^a	132 ^a	18.4	0.04	NS	NS
Pro	84 ^b	98 ^b	97 ^b	103 ^b	107 ^a	148 ^a	14.3	0.03	0.07	NS
Ser	65 ^b	74 ^b	75 ^b	79 ^b	87 ^a	112 ^a	14.0	0.07	NS	NS
Tyr	22 ^b	18 ^b	27 ^b	21 ^b	35 ^a	50 ^a	10.0	0.04	NS	NS
Total	596 ^b	655 ^b	714 ^b	676 ^b	841 ^a	1069 ^a	139.1	0.04	NS	NS
Total AA	1167 ^b	1311 ^b	1323 ^b	1352 ^b	1662 ^a	2082 ^a	261.8	0.004	NS	NS

^{a,b}Means within a row with different superscript differ significantly for the main effect of CP percentage. Pairwise comparisons were calculated using the appropriate standard error.

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP.

³Nonsignificant (*P* > 0.10).

than in SBM (Table 2), the CP supplement supplied only 9 to 20% of total N intake and NANMN contributed on average only 50% of the NAN that passed to the duodenum when diets that contained SBM and AMB were fed (Table 6). Therefore, it appears that the small proportion of feed CP reaching the small intestine and the lack of significant differences in degradability obtained between SBM- and AMB-containing diets probably prevented finding significant differences between these sources of CP for their ability to supply AA to the small intestine. As previously suggested, the large amount of feed consumed by the cows combined with a ruminal pH of about 6 and relatively small proportions but recommended amounts of dietary CP supplied from AMB might have contributed to equalize the ruminal escape of protein from these diets that contained SBM and AMB.

Increasing CP in the diet enhanced or tended to enhance the passage to the duodenum of all AA of NANMN origin; however, differences were not significant between LCP and MCP (Table 8). Because the ruminal outflow of microbial AA was not affected by the percentage of dietary CP (Table 9), the amount of all AA that reached the small intestine in the NAN fraction, except for Met, also increased or tended to

increase only when the HCP diets were fed (Table 10). Apparently, changes in the intestinal supply of AA in response to additional dietary CP paralleled the described differences in AA intake. These results agree with data reviewed in other reports (Clark et al., 1992; Ipharraguerre, 2004), which indicate that a positive and high correlation exists between N intake and flow of NAN, NANMN, and total AA to the duodenum.

Research suggests that Lys and Met are the 2 EAA that most frequently limit the performance of dairy cows fed a broad variety of diets (Sloan, 1997; NRC, 2001). To maximize milk protein output, it has been suggested that Lys and Met must provide about 7.0 and 2.5%, respectively, of the total AA that pass to the small intestine of cows fed corn-based diets (NRC, 2001). Neither the percentage nor the source of CP significantly altered the proportion of Lys (Figure 1) and Met (Figure 2) in the intestinal supply of AA. In relation to numerical trends, when CP increased in the diet, the percentage of Lys in total AA reaching the duodenum increased, whereas that of Met decreased, probably denoting a decline in the relative contribution of corn protein to the ruminal outflow of NANMN. This trend was less evident when AMB replaced a portion of SBM in the diet. Because the passage to duodenum

Table 9. Least square means for passage of microbial AA (g/d) to the duodenum of lactating dairy cows fed diets containing different percentages and sources of CP.¹

AA	LCP		MCP		HCP		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% × source
Essential										
Arg	58	67	65	62	69	61	6.2	NS ³	NS	NS
His	22	25	24	24	26	24	2.3	NS	NS	NS
Ile	67	77	76	74	78	72	6.8	NS	NS	NS
Leu	101	115	113	110	120	107	10.0	NS	NS	NS
Lys	85	99	99	96	103	96	9.8	NS	NS	NS
Met	27	28	29	29	25	22	3.4	NS	NS	NS
Phe	66	72	71	70	75	68	6.5	NS	NS	NS
Thr	66	79	74	72	75	76	7.8	NS	NS	NS
Val	77	88	85	84	88	81	7.7	NS	NS	NS
Total	568	651	637	620	659	607	58.2	NS	NS	NS
Nonessential										
Ala	108	125	120	120	121	113	10.4	NS	NS	NS
Asp	161	191	187	180	189	185	17.8	NS	NS	NS
Glu	178	207	200	193	208	186	17.9	NS	NS	NS
Gly	82	95	92	91	92	87	7.9	NS	NS	NS
Pro	49	56	54	51	58	49	4.6	NS	NS	NS
Ser	67	86	78	77	82	83	7.9	NS	NS	NS
Tyr	64	73	71	69	73	72	6.3	NS	NS	NS
Total	710	834	801	781	823	770	71.1	NS	NS	NS
Total AA	1278	1485	1438	1401	1482	1377	128.9	NS	NS	NS

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP.

³Nonsignificant (*P* > 0.10).

of MN and NANMN were not affected by the source of supplemental CP, it is likely that the profile of EAA in AMB, which was balanced to supply adequate amounts of limiting AA for milk protein synthesis (Beede et al., 1994), ameliorated fluctuations in the composition of total AA reaching the small intestine. The HCP/SBM and MCP/AMB diets resulted in percentages of Lys and Met in duodenal digesta that most closely approached the aforementioned targets. However, none of the diets resulted in an ideal profile of Lys and Met in total AA delivered to the duodenum. Based on estimations by the NRC (2001), it is recommended that, under practical situations, the contents of Lys and Met in MP should be 6.6 and 2.2%, respectively, to achieve maximum milk protein production (Schwab et al., 2003). Because the proportion of individual ingredients in the AMB product has not been disclosed, it is not possible to estimate the proportion of EAA in MP that was supplied to the cows by the experimental diets.

Production of Milk and Milk Composition

Yields of milk and 3.5% FCM were not affected significantly by treatments (Table 11). Likewise, neither the percentage nor the source of CP altered significantly mean milk production during the first 210 d of lactation

(range = 36.3 to 39.4 kg/d) in the companion study (Ipharraguerre and Clark, 2005b). In a number of experiments, it was observed that decreasing the CP content of the diet of high-producing cows from 18 and 19 to 16 and 17% did not depress DMI or milk production (Cunningham et al., 1996; Wu and Satter, 2000; Broderick, 2003). However, reducing dietary CP to within a range of 13 to 15% depressed the intake of DM and the output of milk (Wu and Satter, 2000; Frank and Swensson, 2002; Broderick, 2003). Collectively, these results support the view that lower inputs of dietary CP exert negative effects on milk production partly by decreasing DMI (Wohlt et al., 1978; Clark and Davis, 1980; Oldham, 1984), which did not occur in this experiment. Additionally, the amount of milk produced by cows in this study (mean = 30.1 kg/d) appeared to be insufficient for detecting improvements in milk yield in response to increased supply of AA to the duodenum. Data from several experiments showed that the replacement of SBM with RUP supplements of marine, animal, and (or) plant origin generally failed to improve significantly milk yield in dairy cows that produced on average up to about 30 kg/d (Santos et al., 1998; Ipharraguerre and Clark, 2005a).

The percentage and yield of fat, CP, true protein, and total solids in milk were not significantly altered by the

Table 10. Least square means for passage of total AA (g/d) to the duodenum of lactating dairy cows fed diets containing different percentages and sources of CP.¹

AA	LCP		MCP		HCP		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% × source
Essential										
Arg	118 ^b	129 ^b	136 ^b	128 ^b	155 ^a	166 ^a	15.2	0.02	NS ³	NS
His	55 ^b	60 ^b	63 ^b	64 ^b	71 ^a	84 ^a	6.8	0.008	NS	NS
Ile	120 ^b	127 ^b	141 ^b	124 ^b	157 ^a	157 ^a	14.6	0.03	NS	NS
Leu	240 ^b	251 ^b	272 ^b	258 ^b	299 ^a	338 ^a	29.0	0.02	NS	NS
Lys	150 ^b	163 ^b	178 ^b	164 ^b	228 ^a	212 ^a	26.4	0.02	NS	NS
Met	63	58	60	58	69	67	8.0	NS	NS	NS
Phe	127 ^b	135 ^b	146 ^b	137 ^b	163 ^a	179 ^a	15.2	0.01	NS	NS
Thr	126 ^b	137 ^b	147 ^b	133 ^b	163 ^a	169 ^a	15.6	0.04	NS	NS
Val	140 ^b	153 ^b	163 ^b	156 ^b	180 ^a	203 ^a	15.0	0.02	NS	NS
Total	1140 ^b	1213 ^b	1280 ^b	1225 ^b	1488 ^a	1573 ^a	143.3	0.02	NS	NS
Nonessential										
Ala	180 ^b	190 ^b	205 ^b	191 ^b	223 ^a	248 ^a	18.7	0.03	NS	NS
Asp	259 ^b	278 ^b	307 ^b	274 ^b	343 ^a	354 ^a	32.2	0.02	NS	NS
Glu	368 ^b	379 ^b	423 ^b	379 ^b	469 ^a	493 ^a	44.1	0.03	NS	NS
Gly	148 ^b	173 ^b	178 ^b	165 ^b	193 ^a	210 ^a	20.9	0.06	NS	NS
Pro	133 ^b	140 ^b	150 ^b	145 ^b	166 ^a	191 ^a	16.0	0.01	NS	NS
Ser	133 ^b	142 ^b	153 ^b	147 ^b	171 ^a	193 ^a	16.3	0.01	NS	NS
Tyr	85 ^b	89 ^b	99 ^b	87 ^b	108 ^a	112 ^a	10.6	0.04	NS	NS
Total	1305 ^b	1391 ^b	1515 ^b	1389 ^b	1676 ^a	1801 ^a	160.0	0.03	NS	NS
Total AA	2445 ^b	2605 ^b	2761 ^b	2615 ^b	3163 ^a	3367 ^a	305.9	0.02	NS	NS

^{a,b}Means within a row with different superscript differ significantly for the main effect of CP percentage. Pairwise comparisons were calculated using the appropriate standard error.

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP.

³Nonsignificant (*P* > 0.10).

Figure 1. Least square means for the proportion of Lys in total AA in duodenal digesta of dairy cows fed diets containing high, medium, and low percentages (HCP, MCP, LCP, respectively) and 2 sources [soybean meal (SBM) or animal-marine blend (AMB)] of CP. The effects of the interaction (*P* < 0.43), percentage (*P* < 0.21), and source of CP (*P* < 0.37) were not significant (SEM = 0.34).

Figure 2. Least square means for the proportion of Met in total AA in duodenal digesta of dairy cows fed diets containing high, medium, and low percentages (HCP, MCP, LCP, respectively) and 2 sources [soybean meal (SBM) or animal-marine blend (AMB)] of CP. The effects of the interaction (*P* < 0.77), percentage (*P* < 0.15), and source of CP (*P* < 0.58) were not significant (SEM = 0.20).

Table 11. Least square means for production and composition of milk from lactating dairy cows fed diets containing different percentages and sources of CP.¹

Item	LCP		MCP		HCP		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% × source
Milk, kg/d	28.6	30.5	31.0	30.3	30.2	30.1	0.8	NS ³	NS	NS
3.5% FCM, ⁴ kg/d	27.2	29.5	29.7	29.2	29.6	28.9	0.9	NS	NS	NS
Fat										
%	3.21	3.31	3.24	3.30	3.43	3.27	0.17	NS	NS	NS
kg/d	0.91	1.01	1.00	0.99	1.02	0.98	0.05	NS	NS	NS
CP										
%	3.18	3.27	3.21	3.23	3.26	3.18	0.10	NS	NS	0.10
kg/d	0.91	0.99	0.99	0.97	0.98	0.96	0.04	NS	NS	NS
True protein										
%	2.97	3.07	3.00	3.02	3.06	2.98	0.10	NS	NS	0.09
kg/d	0.86	0.91	0.91	0.92	0.90	0.88	0.04	NS	NS	NS
Total solids										
%	12.05	12.18	12.05	12.14	12.17	12.08	0.29	NS	NS	NS
kg/d	3.46	3.70	3.73	3.66	3.65	3.63	0.12	NS	NS	NS
MUN, mg/dL	9.53	7.98	11.52	11.17	13.51	14.25	0.93	0.0001	NS	0.05

¹AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP.

³Nonsignificant (*P* > 0.10).

⁴3.5% FCM = 0.4324(kg of milk) + 16.216(kg of fat).

percentage and source of dietary CP (Table 11). At LCP in the diet, cows fed AMB tended to produce milk with a higher concentration of CP and true protein than those fed SBM. These trends were possibly caused by the slightly higher intake of nutrients by cows fed the LCP/AMB diet compared with those fed the LCP/SBM diet. In the companion study (Ipharraguerre and Clark, 2005b), the percentage and source of dietary CP did not influence milk composition; however, the yields of CP and true protein increased when dietary CP increased from LCP to HCP. In the literature, changes in the concentration and production of milk components in response to higher inputs of dietary CP within the range used in this experiment have been variable and, frequently, more strongly related to other dietary factors (NRC, 2001; Ipharraguerre, 2004). For instance, Cunningham et al. (1996) reported that increasing dietary CP from 16.4 to 18.4% did not affect the percentage and yield of protein and fat in milk. However, Broderick (2003) observed that increasing the dietary CP from 15.1 to 18.4% increased the percentage and yield of milk protein but did not affect the concentration of fat in milk. In contrast, Frank and Swensson (2002) found that increasing the amount of dietary CP from 13.5 to 17.0% decreased the percentage of protein in milk but did not influence the production of protein or the percentage and yield of fat. In relation to the source of CP, Santos and coworkers (1999) found that replacing SBM with the AMB product to supply 5% of the dietary DM increased the output of protein without affecting the

concentration of protein and fat in milk from dairy cows that produced on average 37.2 kg/d of milk. When mean milk production was about 32 kg/d per cow, however, the replacement of SBM with RUP supplements significantly decreased milk CP percentage but did not affect the yield of milk CP or the percentage and yield of milk fat (Ipharraguerre and Clark, 2005a).

When the LCP/AMB diet was fed, the secretion of urea in milk (i.e., MUN) decreased compared with the LCP/SBM diet, but differences among sources of CP were not significant at higher percentages of dietary CP (Table 11). Moreover, mean MUN increased from 8.8 to 13.8 mg/dL as the amount of CP increased in the diet from LCP to HCP. This response is consistent with increases in the concentration of NH₃N in ruminal fluid of cows fed incremental amounts of dietary CP, suggesting that the amount of NH₃N absorbed from the rumen into the blood, converted to urea in the liver, and released back into blood increased as additional supplemental CP replaced corn in the diets. Increasing the CP content of the diet of dairy cows has consistently increased the concentration of MUN (Frank and Swensson, 2002; Leonardi et al., 2003; Broderick, 2003; Ipharraguerre and Clark, 2005b).

CONCLUSIONS

In this experiment, the percentage of dietary CP, but not the source of supplemental CP, profoundly influenced the intake, ruminal fermentation, intestinal sup-

ply, and digestion of N and starch. Increasing the CP content of the diet from about 14 to 18% increased the intake, apparent ruminal degradation, and intestinal supply of AA. In contrast, the intake, ruminal fermentation, and total tract digestion of starch decreased as CP increased in the diet. The proportion of starch consumed by the cows that was apparently digested in the small intestine increased in response to higher inputs of dietary CP. Replacing a portion of SBM with AMB increased the intake of Met and Val and decreased the concentration of NH_3N in the rumen, but did not affect the intake of other EAA or the intestinal supply of any EAA and starch. Neither the percentage nor the source of CP improved the proportion of Lys and Met in total AA that reached the absorption sites in the small intestine.

These results underscore the central role that the interaction between protein and carbohydrate in various segments of the digestive tract plays in modulating the responses to changes in the input of dietary CP. In this context, it appears that the intake of N by high-producing dairy cows that consume sufficient energy and other nutrients to meet their requirements can be decreased to about 600 to 650 g daily without compromising the supply of MP if the source and amount of dietary CP and carbohydrate are properly matched. Data also suggest that the supply of RUP to the small intestines of dairy cows that consume large quantities of corn-SBM-based diets might be underestimated by current feeding standards.

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