Varying Protein and Starch in the Diet of Dairy Cows. II. Effects on Performance and Nitrogen Utilization for Milk Production

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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 the lactational performance and use of N for milk production. Sixty multiparous Holstein cows were used in a 210-d lactational trial with a completely randomized 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 CP (means = 14.8, 16.8, and 18.7%) were combined into 6 treatments. On a dry matter (DM) basis, diets contained 25.0% corn silage, 20.0% alfalfa silage, 10.0% cottonseed, 26.7 to 37.0% corn grain, and 4.8 to 13.5% protein supplement, plus minerals and vitamins. Across the 210 d of lactation, the productive response of dairy cows to the source of supplemental CP depended on the concentration of CP in the diet. At 18.7% CP, cows fed SBM consumed more DM and produced more milk, 3.5% fat-corrected milk, fat, and true protein, but had lower efficiency of feed use and body condition score than cows fed AMB. At 16.8% CP, cows fed AMB produced more 3.5% fat-corrected milk, fat, and true protein than cows fed SBM. At 14.8% CP, cows fed SBM consumed more DM but produced less true protein and had lower feed efficiency than cows fed AMB. Across CP sources, cows fed 14.8% CP produced less fat-corrected milk and true protein than cows fed 16.8 and 18.7% CP. Across CP percentages, cows fed AMB produced more fat-corrected milk per kilogram of DM consumed than cows fed SBM. Despite these interactions, improvements in the gross efficiency of N use for milk production were achieved through reductions in the intake of N independently of the source of CP. 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 if the source of RDP and RUP are properly matched with the source and amount of carbohydrate in the diet.

(**Key words:** dairy cow, milk production, gross efficiency of nitrogen utilization, 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, ME = metabolizable energy, MP = metabolizable protein, SBM = solvent-extracted soybean meal.

INTRODUCTION

Mass nitrogen balance studies showed that on typical dairy farms only 12 to 36% of the N input is retained in salable products, whereas up to about 70% is lost mainly through volatilization and leaching into the off-farm environment (Klausner, 1993; Castillo et al., 2000; Spears et al., 2003). This inefficient use of N for productive purposes may be exacerbated by the current trend among dairy producers to feed more CP than is apparently required by lactating dairy cows (Castillo et al., 2000; Jonker et al., 2002). Overfeeding N to dairy cows may have detrimental effects not only on the productive performance but also on the efficiency of nutrient use for milk production, reproductive performance, economic returns, environment, and public perception of dairy farming (NRC, 2001; CAST, 2002). It is widely recognized, however, that advancing our knowledge about the protein requirements of today's high-producing dairy cow is a prerequisite for optimizing cow productivity and minimizing nutrient excretion into the environment (NRC, 2001; CAST, 2002).

Results from various research and modeling approaches 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 (Kohn et al., 1997; Rotz et al., 1999; St-Pierre and Thraen, 1999). Reducing the output of N in urine provides the greatest opportunity for achieving that goal (Hvelplund and Madsen, 1996). This is because most of

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the N present in urine arises from inefficient N transactions in the rumen and tissues of cows that may be amenable to manipulation (Lobley, 2002). In recent years, several researchers reported that decreasing the amount of dietary CP while correcting for predicted shortages of limiting AA for milk production by including RUP supplements (Castillo et al., 2001; Noftsker and St-Pierre, 2003) and (or) rumen-protected AA (Dinn et al., 1998; Kröber et al., 2000b; Noftsker and St-Pierre, 2003) in the diet can reduce excretion of N in urine and sustain production of milk and milk protein. However, crossover designs with short experimental periods were used in most studies (Dinn et al., 1998; Kröber et al., 2000b; Noftsker and St-Pierre, 2003) and negative controls were not included in others (Dinn et al., 1998; Noftsker and St-Pierre, 2003). In addition, in most cases the CP content of the diet was reduced by replacing high-protein supplements with sources of structural or nonstructural carbohydrates. Therefore, it is uncertain 1) whether those desirable effects are attainable during long feeding periods; 2) whether the effects of reducing dietary CP and feeding RUP supplements and (or) rumen-protected AA are additive or mutually independent; and 3) whether the effects of reducing dietary CP are related to alterations in the interrelationship between protein and carbohydrates in the digestive tract of dairy cows.

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 metabolizable protein (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 reported here were 1) to examine the effects of the amount and source of CP and the amount of starch in the diet of dairy cows on the lactational performance and use of N for milk production, and 2) to relate differences in cow productivity and conversion of feed N to milk N to alterations in ruminal fermentation, intestinal supply of nutrients, and use of nutrients by dairy cows determined in the companion experiment (Ipharreguerre et al., 2005).

MATERIALS AND METHODS

Experimental Design and Treatments

A 210-d lactation trial using a completely randomized design with a 2×3 factorial arrangement of treatments was conducted according to procedures approved by the University of Illinois Laboratory Animal Care Advisory Committee.

Two sources of protein of different ruminal degradability and 3 concentrations (percentage) of dietary CP were

combined into 6 dietary 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). The AMB supplement (ProLak; H. J. Baker & Bros., Inc., Westport, CT) partially replaced SBM in the concentrate mixtures with the aim of enhancing the content of RUP in 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).

Animals and Management

Immediately after calving, 60 multiparous Holstein cows were housed in individual tie stalls equipped with water bowls and bedded with sawdust. During the first 14 d postcalving, all cows were fed the same pretreatment diet that was prepared by mixing equal quantities (50:50, wt/wt) of diets MCP/SBM and MCP/AMB. In addition, cows were offered 2 kg of alfalfa hay daily for the first 7 d postcalving. Measurements taken during d 7 to 14 of the pretreatment period were used for covariance adjustment of response parameters. On d 15 postcalving, cows were randomly assigned to 1 of the 6 dietary treatments and fed their assigned diet until d 210 of lactation. Cows were fed diets as TMR twice daily at 1000 and 1700 h in amounts to ensure 10%orts. The TMR were adjusted weekly to reflect changes in DM content of forages and concentrate mixtures by drying the forages and concentrates overnight at 105°C. During the 210 d, cows were milked twice daily at 0300 and 1500 h and were let outside in a dry lot to exercise from 0700 to 0900 h daily. The study was conducted over 2 yr, and 30 cows were assigned to treatment each year. Two cows, one fed the LCP/SBM treatment and a second fed the MCP/SBM treatment, were removed from the experiment because of retained placenta followed by metritis and low milk production.

Sampling, Analyses, and Calculations

From calving until d 210, feed offered was recorded daily. Orts were weighed daily and were scored on a 4-

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, %	63.3	63.5	63.3	63.7	63.4	63.3
CP, %	14.8	14.9	16.6	17.0	18.5	19.0
Soluble CP, % of CP	36.4	36.2	36.0	35.6	36.1	34.9
RUP, ⁵ %	5.0	5.4	5.8	6.5	6.6	7.7
RUP, ⁵ % of CP	33.8	36.2	34.9	38.2	35.7	40.5
RDP, ⁵ %	9.8	9.5	10.8	10.5	11.9	11.3
NDF, %	28.0	28.2	28.9	28.3	29.2	28.6
ADF, %	18.6	18.7	19.3	18.9	19.7	19.1
NFC, ⁵ %	45.3	45.0	42.6	43.5	40.8	41.6
Starch, ⁶ %	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
Ca, %	1.02	1.01	0.99	1.03	1.01	1.04
P, %	0.53	0.52	0.52	0.52	0.52	0.52
Mg, %	0.32	0.32	0.31	0.32	0.32	0.32
K, %	1.12	1.07	1.21	1.13	1.30	1.17

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 ProLak contains 70% of the CP as RUP and 1.50 Mcal of NE_L per kg of DM.

⁶Data from Ipharraguerre et al. (2005) for the same diets.

point scale, where 1 = dry and 4 = wet. On d 7 and 14 postcalving and, afterward, during the last day of each 4-wk period, samples of orts were collected and DM content was determined by drying samples from individual cows at 105°C for 24 h. For each cow, these determinations were used to assign a percentage of DM to each bunk score. Dry matter intake was calculated from the amount of feed offered and its DM content, the amount of orts, and the DM percentage that was assigned to each bunk score for each cow. Samples of forages, concentrates, and TMR were collected once a week and divided into 2 representative subsamples. One subsample of feed was used to determine DM as described for orts and the other subsample was stored frozen at -20°C for later analyses. At the end of each 4-wk period, feed subsamples from the

weekly collections were thawed, combined on an equal weight basis, and sent to the Dairy One, Inc., Forage Testing Laboratory (Ithaca, NY) where they were analyzed for Kjeldahl N (AOAC, 1990), ADF (Van Soest et al., 1991), NDF with heat-stable α -amylase and sodium sulfite (Van Soest et al., 1991), and Ca, P, Mg, and K by inductively coupled plasma spectrometry.

Milk production was recorded automatically at each milking throughout the experiment. Milk samples were collected at each milking on d 11 to 13 and weekly from 2 consecutive milkings for the duration of the experiment. Individual milk samples were preserved with 2-bromo-2-nitropropane-1,3-diol and stored at 4°C until shipment to Dairy One Cooperative, Inc., Milk Check Laboratory (Ithaca, NY) for analyses of fat, CP, true protein, lactose,

total solids, MUN, and somatic cells by infrared procedures (AOAC, 1990; Foss 4000; Foss North America, Eden Prairie, MN). Daily milk composition was estimated as an average of the a.m. and p.m. milk samples corrected by the proportion of daily production at that milking.

Over the course of the study, BW were recorded weekly at 0700 h starting on d 1 postcalving. Measurement of BW was conducted after the morning milking and before the morning feeding. Body condition scores were determined by the same 2 individuals on d 7 postcalving and thereafter every 28 d until the end of the trial using a 5.00-point scale in quarter-point increments, where 1 = thin and 5 = fat (Wildman et al., 1982).

The intake of N was estimated using the calculated DMI and the determined N content of the TMR. Secretion of N in milk (g/d) was calculated by multiplying daily milk production by the percentage of milk CP divided by 6.38. The concentration of MUN and BW were used to estimate excretion of N (g/d) in urine according to the equation developed by Kauffman and St-Pierre (2001). The difference between N intake and N output in milk plus urine was assumed to represent excretion of N in feces.

Statistical Analyses

Before analysis, data for DMI, milk production, and milk composition were reduced to weekly means for each cow. These data were analyzed from d 15 to 112 and from d 15 to 210 to test whether animal responses to treatments were altered by stage of lactation. Likewise, data from low-producing ($n = 29$) and high-producing ($n = 29$) cows were stratified based on milk yield ($< \text{or} \geq 40$ kg/d) and analyzed from d 15 to 112 to assess whether animal responses to treatments were dependent on cow productivity. In both cases, data were subjected to covariance adjustment using data from d 8 to 14 of the pretreatment period and analyzed as repeated measures in time using the MIXED procedure of SAS (Littell et al., 1996). The mixed model contained the effects of year, covariate, treatment (percentage, source, and percentage $\times$ source of CP), cow within year and treatment, time (week of lactation), and all possible interactions among year, treatment, and time. Year and cow within year and treatment were treated as random factors. If the variance estimate for the random effect of year was zero or close to zero, then year was dropped from the model. For each model tested, the smallest value for the Akaike's information criterion was used to identify the most appropriate covariance structure (Littell et al., 1996).

The maximum loss of BW and BCS were estimated by subtracting the lowest BW and BCS for each cow during the experimental period from the BW (d 14) and

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

Item	Silages		Cottonseed
	Alfalfa	Corn	
DM, %	55.7	41.6	91.3
NDF, %	47.6	41.0	43.8
ADF, %	37.3	25.3	33.1
NFC, %	21.1	43.2	5.1
CP, %	20.4	7.8	29.8
Soluble CP, % of CP	51.4	57.0	26.9
Ca, %	1.36	0.25	0.17
P, %	0.36	0.29	0.92
Mg, %	0.25	0.16	0.45
K, %	2.48	1.10	1.16

BCS (d 7) measured during the pretreatment period, respectively. These data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Institute, 2000). In this case, the mixed model contained the effects of year (random), treatment (percentage, source, and percentage $\times$ source of CP; fixed), cow within year and treatment (random), and interactions of year by treatment. The analysis was conducted according to criteria described above.

Data for energy balance and N use were reduced to weekly means for each cow and analyzed from d 15 to 210 using the aforementioned mixed model and repeated measures approach, but without covariance adjustment. In addition, data for N use were also analyzed from d 42 to 49, d 112 to 119, and d 196 to 203 to evaluate the influence of the stage of lactation on the impact of treatments on the gross conversion of feed N into milk N, as described for the maximum loss of BW and BCS.

Least square means were separated into significant main effects by the PDIF option of SAS (SAS Institute, 2000). Differences among treatments were considered to be 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 averaged 14.8, 16.8, and 18.7 for the LCP, MCP, and HCP diets, respectively (Table 1). As indicated earlier, diets were formulated to contain 0.8 percentage units less CP than these amounts. Probably, this discrepancy occurred because cottonseed had on average 22.5% more CP (Table 2) than the sample of cottonseed analyzed for formulating the diets (i.e., 29.8 vs. 23.1%). As intended, however, the difference between concentrations of dietary CP (i.e., LCP vs. MCP and MCP vs. HCP) was on average 2 percentage units with minimal variation between sources of supplemental CP.

Table 3. Least square means for performance of 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
DMI, kg/d										
d 15 to 112	24.7	23.2	24.6	25.6	26.3	24.2	0.56	0.04	0.04	0.01
d 15 to 210	24.5	23.9	24.7	25.1	26.0	23.5	0.48	NS ³	NS	0.002
DMI, % of BW										
d 15 to 112	3.92	3.65	3.81	4.00	4.09	3.70	0.09	NS	0.02	0.002
d 15 to 210	3.85	3.65	3.75	3.87	4.05	3.54	0.07	NS	0.001	0.0001
Milk, kg/d										
d 15 to 112	39.4 ^b	40.0 ^b	41.6 ^a	43.1 ^a	43.3 ^a	42.8 ^a	1.05	0.005	NS	NS
d 15 to 210	36.3	37.3	37.3	38.7	39.4	37.8	1.31	NS	NS	NS
3.5% FCM, ⁴ kg/d										
d 15 to 112	39.3	38.1	38.9	42.2	42.4	40.8	0.89	0.04	NS	0.06
d 15 to 210	35.8	35.8	35.2	38.1	39.0	36.8	0.81	0.03	NS	0.005
FCM/DMI										
d 15 to 112	1.60	1.67	1.62	1.66	1.66	1.72	0.04	NS	0.08	NS
d 15 to 210	1.46	1.52	1.46	1.52	1.52	1.58	0.04	0.07	0.04	NS

^{a,b}Means within a row with different superscripts 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 = solvent-extracted soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% $\times$ source = interaction between percentage and source of CP. The 2-way interactions and 3-way interaction between factors and week of lactation were not significant ($P > 0.10$).

³Nonsignificant ($P > 0.10$).

⁴3.5% FCM = $0.4324(\text{kg of milk}) + 16.216(\text{kg of fat})$.

At all concentrations of dietary CP, the replacement of SBM with AMB increased the RUP content of the diet (Table 1) either expressed as percentage of dietary DM (+0.4, 0.7, and 1.1 percentage units) or as proportion of dietary CP (+2.4, 3.3, and 4.8 percentage units). Conversely, the content of RDP decreased 0.3 to 0.6 percentage units when ABM replaced SBM in the diets. The content of ADF, NDF, NE_L, and macrominerals was similar among diets.

Lactational Performance

The effect of the interaction between percentage and source of CP on DMI was significant during both d 15 to 112 and d 15 to 210 (Table 3). It is noteworthy that this effect was more pronounced during the first 112 d of lactation (Figure 1). At LCP in the diet, cows fed SBM consumed more DM (expressed as kilograms/day and proportion of BW) than those fed AMB, but this effect was significant only during d 15 to 112 (+1.5 kg/d and 0.27 percentage units). When dietary CP increased from LCP to MCP, DMI was numerically higher for cows fed AMB than for those fed SBM. In contrast, with HCP diets SBM resulted in higher DMI than AMB during d 15 to 112 (+2.1 kg/d and 0.39 percentage units) and d 15 to 210 (+2.5 kg/d and 0.51 percentage units). Grant and Haddad (1998) observed that replacing SBM with a mixture of feather and blood meal to supply 4% of the

dietary DM depressed the intake of DM (−3.5 kg/d) by dairy cows when the CP content of the diet increased from 17.5 to 19.5%. In other experiments with lactating cows, the factorial effects of the percentage (from 16.2 to 19.9) and source (SBM and various RUP supplements) of dietary CP on DMI were not significant (Christensen et al., 1993; Cunningham et al., 1996). Nonetheless, results reported here agree with those from a meta-analytic review of 93 studies (Ipharraguerre, 2004) showing that the amount of DM consumed by dairy cows was numerically (−0.5%, $P > 0.05$) or significantly (−1.4%, $P < 0.05$) decreased by the replacement of RDP sources with RUP supplements in diets that contained either more than 18% or less than 15.9% CP, respectively; this effect, however, was positive (+0.2%) but not significant ($P > 0.05$) when dietary CP ranged from 16 to 17.9%.

Across CP sources, feeding the LCP diet decreased DMI by 1.1 and 1.3 kg/d from d 15 to 112 compared with the MCP and HCP diets, respectively. Similar decreases in DMI were reported when the CP content of the diet fed to moderate- (Weigel et al., 1997) or high-producing (Broderick, 2003) cows was decreased from about 18 to about 15%. However, this negative response to similar decreases in the input of dietary CP has not always been observed (Kalscheur et al., 1999; Wu and Satter, 2000). A lack of major changes in DMI of high-producing cows fed diets in which the concentration of CP was decreased from 19 or 18 to about 16% appears to be a more consis-

Figure 1. Least square means for DMI (A; SEM = 0.48), production of 3.5% FCM (B; SEM = 0.81), and ratio FCM/DMI (C; SEM = 0.04) of cows fed diets containing different percentages and sources of CP. The 3-way interaction (CP percentage $\times$ CP source $\times$ week postpartum) was not significant ($P > 0.10$) for all variables.

tent outcome (Christensen et al., 1993; Cunningham et al., 1996; Komaragiri and Erdman, 1997; Leonardi et al., 2003). In the companion study (Ipharraguerre et al., 2005), the percentage of dietary CP did not alter significantly the ruminal fermentation of organic matter or the amount of microbial N that passed to the small intestine, suggesting that reduced DMI for the LCP diet was probably independent of a shortage of available N in the rumen that impaired nutrient digestibility. Even though the molar proportion of propionate was unaffected by the percentage of dietary CP, the amount of starch consumed and fermented in the rumen decreased by 16 and 54%, respectively, as the concentration of CP increased from LCP to HCP (Ipharraguerre et al., 2005). Therefore, it seems reasonable to speculate that more propionate was produced and absorbed from the rumen of cows fed the LCP diet compared with cows fed higher dietary CP. Increased supply of propionate to the liver might have provided a signal for hypophagia, leading in turn to reduced DMI (Allen, 2000; Oba and Allen, 2003). Another potential explanation is that as CP increased in the diet the concentration of ammonia N in blood might have increased because of an increase in the ruminal production of ammonia N (Ipharraguerre et al., 2005), resulting in a decline of the glucose-mediated release of insulin by the pancreas (Fernandez et al., 1990) and, consequently, in reduced hunger (Allen, 2000).

Across CP percentages, DMI expressed as kilograms per day and percentage of BW was higher for cows fed SBM than for cows fed AMB during d 15 to 112 (+0.9 kg/d and 0.16 percentage units) and during d 15 to 210 when DMI was expressed as percentage of BW (+0.19 percentage units; Table 3). In contrast, replacing SBM with the same AMB product (i.e., ProLak) in the diet of dry (Huyler et al., 1999; Underwood, 2003) or lactating (Santos et al., 1999; Underwood, 2003) cows did not affect DMI. It should be noted, however, that the numerical trend reported by Underwood (2003) for DMI of lactating cows (−1.0 kg/d) paralleled the significant differences reported here and that the use of the AMB product has depressed feed intake on a number of farms involved in a large field study (Ferguson et al., 2000). Additionally, research summarized elsewhere (Santos et al., 1998; Ipharraguerre and Clark, 2005) indicated that the replacement of SBM with RUP supplements of marine and (or) animal origin has generally depressed DMI. The AMB product contained meals of menhaden fish and hydrolyzed feather (see previous section for details) and the negative impact of these RUP supplements on DMI has been partly attributed to poor palatability of the diet (Mäntysaari et al., 1989; Grant and Haddad, 1998; Abu-Ghazaleh et al., 2001). Considering that in the companion experiment (Ipharraguerre et al., 2005) the source of supplemental CP had minor effects on rumen fermenta-

tion and intestinal supply of nutrients, it is probable that in this experiment reduced palatability might have contributed to decreased DMI of cows fed the AMB diets.

Although the interaction between percentage and source of CP was not significant for milk production (Table 3), the MCP/AMB and HCP/SBM diets resulted in the highest numerical yields of milk during both d 15 to 112 and d 15 to 210. As shown in Table 3 and Figure 1, this interaction was significant for the yield of 3.5% FCM. During both d 15 to 112 and d 15 to 210, differences between sources of CP at LCP in the diet were negligible and not significant; however, AMB resulted in a higher yield of FCM than SBM at MCP (+3.3 and 2.9 kg/d, respectively), whereas the opposite occurred when dietary CP increased to HCP (−1.6 and 2.2 kg/d, respectively). Compared with the MCP and HCP diets, the LCP diet decreased the production of milk during early lactation and the yield of FCM during both d 15 to 112 and d 15 to 210 (Table 3). In contrast, no significant differences for these variables were observed between MCP and HCP. Likewise, the source of CP did not affect the output of milk or FCM. Collectively, these findings indicate that in this experiment the marginal returns in milk and FCM output to the source of supplemental CP depended on the amount of CP in the diet and that this dependency was particularly marked during the first 112 d in lactation. When the concentration of dietary CP averaged 16.8% (i.e., MCP), partially replacing SBM with AMB increased yields of milk and FCM. Conversely, the benefits of adding AMB to SBM-based diets were nil when dietary CP averaged 14.8 (i.e., LCP) or 18.7% (i.e., HCP). Although these significant interactions have not been observed in previous experiments (Christensen et al., 1993; Cunningham et al., 1996; Weigel et al., 1997), they are in agreement with results reported in a summary of 244 treatment comparisons (Ipharraguerre, 2004), indicating that the replacement of RDP sources with RUP supplements significantly ($P < 0.05$) increased milk yield (2.1%) when diets contained from 16 to 17.9% CP, whereas below ($\leq 15.9\%$) and above ($\geq 18\%$) that range the magnitude of this positive effect was less important (1 and 0.6%, respectively) and not significant ($P > 0.05$). Similar interactions between the amount and source of dietary CP might have been partly involved in negating an improvement in milk production by dairy cows fed the AMB product in 2 experiments (Santos et al., 1999; Underwood, 2003) and in 16 of 35 herds involved in a field study (Ferguson et al., 2000). As discussed later, alterations in the supply of MP, metabolizable energy (ME), or the interaction of these 2 factors might be at least 3 reasons that could help explain these responses.

During d 15 to 210, the FCM/DMI ratio tended to be higher for cows fed HCP than for cows fed MCP and

Table 4. Least square means for BW and BCS of 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
BW, kg										
d 15 to 112	636 ^b	638 ^b	644 ^a	653 ^a	652 ^a	653 ^a	6.7	0.05	NS ³	NS
d 15 to 210	645	671	664	636	649	667	20.1	NS	NS	NS
Maximum loss ⁴	-57.4 ^b	-72.6 ^b	-38.3 ^a	-22.4 ^a	-34.9 ^a	-44.9 ^a	12.79	0.02	NS	NS
BCS ⁵										
d 15 to 112	2.68	2.65	2.73	2.69	2.61	2.77	0.10	NS	NS	NS
d 15 to 210	2.81	2.79	2.89	2.81	2.66	2.93	0.10	NS	NS	0.03
Maximum loss ⁴	-0.46	-0.37	-0.29	-0.29	-0.41	-0.32	0.09	NS	NS	NS

^{a,b}Means within a row with different superscripts 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 = solvent-extracted soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% $\times$ source = interaction between percentage and source of CP. The 2-way interactions and 3-way interaction between factors and week of lactation were not significant ($P > 0.10$).

³Nonsignificant ($P > 0.10$).

⁴Lowest BW and BCS recorded during the experimental period subtracted from BW and BCS on d 14 and d 7 of the pretreatment period, respectively.

⁵BCS on a 5.00-point scale in quarter-point increments where 1 = thin and 5 = fat.

LCP (Table 3; Figure 1). This trend, however, likely arose from the combination of intermediate FCM yield with numerical lowest DMI for the HCP/AMB diet. In addition, maximum losses of BW and BCS were significantly and numerically largest, respectively, for cows fed the LCP diet (Table 4), suggesting that increased mobilization of body reserves along with reduced DMI and FCM output prevented a decline in the efficiency of feed use by cows fed LCP. Therefore, it seems that the marginal benefits in efficiency of nutrient use for milk production in response to increases in dietary CP reached a plateau at MCP. Previous research with high-producing cows also showed that reducing the input of dietary CP from $\geq 18\%$ to within the 16 to 17% range sustained (Cunningham et al., 1996) or improved (Broderick, 2003) feed efficiency without negatively affecting productivity of cows. The partial replacement of SBM with AMB improved the FCM/DMI ratio (2.5 to 4.4%) at all percentages of dietary CP, and this effect was significant during d 15 to 210 (Table 3; Figure 1). Underwood (2003) reported that replacing SBM with AMB to supply 4.75% of the DM of a diet fed to dairy cows from parturition to 63 d postpartum, increased the yield of 4% FCM per kilogram of DM consumed by about 11% during d 15 to 63 in lactation. Underwood highlighted, however, that this response was confounded with a larger contribution of labile energy reserves to milk synthesis in cows fed AMB. Based on the evolution of BW, BCS, and FCM/DMI across the 210 d of lactation (Figures 1 and 2), different use of body reserves for cows fed either SBM or AMB did not appear to account for the improvement

in feed efficiency elicited by AMB in this experiment. Additionally, digestibility of nutrients in the rumen and total digestive tract was not affected by the source of supplemental CP (Ipharraguerre et al., 2005). Consequently, it seems reasonable to speculate that replacing SBM with AMB might have ameliorated imbalances in the profile of essential AA in MP (Ipharraguerre et al., 2005), which contributed to improve the conversion of absorbed nutrients into milk by reducing the energetic cost associated with the required removal of AA supplied in excess. However, changes in the supply of energy-yielding substrates resulting from different patterns of ruminal fermentation cannot be ruled out because of the lower ruminal pH and molar proportion of acetate but higher molar percentages of butyrate and valerate found in the rumen of cows fed SBM compared with cows fed AMB (Ipharraguerre et al., 2005).

An interaction between the percentage and source of CP was detected for the yield of fat, CP ($P < 0.09$), and total solids during d 15 to 112 and for the same components as well as true protein during d 15 to 210 (Table 5). Compared with AMB, feeding SBM increased significantly or numerically the yield of these milk components at HCP in the diet, but the opposite happened when dietary CP was decreased to MCP. Because concentrations in milk of fat, CP, true protein, and total solids were not affected by treatments (Table 5), it appears that the nonsignificant response in milk production was sufficient to account for this interaction. The percentage of lactose in milk tended to be altered by the 2-way interaction and by the concentration of dietary CP dur-

Figure 2. Least square means for BW (A; SEM = 20.1) and BCS (B; SEM = 0.10) of cows fed diets containing different percentages and sources of CP. The 3-way interaction (CP percentage $\times$ CP source $\times$ week postpartum) was not significant ($P > 0.10$) for all variables.

Table 5. Least square means for concentration and production of milk components from 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% × source
Fat, %										
d 15 to 112	3.48	3.20	3.14	3.35	3.38	3.26	0.13	NS ³	NS	NS
d 15 to 210	3.53	3.30	3.20	3.37	3.45	3.31	0.14	NS	NS	NS
Fat, kg/d										
d 15 to 112	1.37	1.26	1.30	1.45	1.47	1.38	0.04	NS	NS	0.05
d 15 to 210	1.25	1.20	1.18	1.31	1.36	1.25	0.04	NS	NS	0.04
CP, %										
d 15 to 112	3.00	3.05	3.03	3.08	3.05	3.02	0.06	NS	NS	NS
d 15 to 210	3.09	3.18	3.15	3.18	3.13	3.09	0.06	NS	NS	NS
CP, kg/d										
d 15 to 112	1.18	1.22	1.25	1.33	1.33	1.28	0.03	0.002	NS	0.09
d 15 to 210	1.11	1.18	1.17	1.23	1.23	1.18	0.03	0.07	NS	0.03
True protein, %										
d 15 to 112	2.81	2.85	2.86	2.88	2.84	2.85	0.06	NS	NS	NS
d 15 to 210	2.90	2.98	2.97	2.98	2.92	2.91	0.06	NS	NS	NS
True protein, kg/d										
d 15 to 112	1.10 ^b	1.14 ^b	1.18 ^a	1.24 ^a	1.25 ^a	1.20 ^a	0.03	0.001	NS	NS
d 15 to 210	1.03	1.10	1.10	1.15	1.15	1.10	0.03	0.04	NS	0.03
MUN, mg/dL										
d 15 to 112	9.02 ^c	8.20 ^c	11.06 ^b	11.03 ^b	14.70 ^a	13.58 ^a	0.47	0.0001	NS	NS
d 15 to 210	9.30 ^c	8.48 ^c	11.48 ^b	11.56 ^b	14.97 ^a	14.00 ^a	0.43	0.0001	NS	NS
Lactose, %										
d 15 to 112	5.04	4.94	4.93	4.98	4.94	4.86	0.04	0.06	NS	0.06
d 15 to 210	4.96	4.90	4.87	4.93	4.89	4.83	0.04	NS	NS	NS
SCC, log10										
d 15 to 112	1.94	1.93	1.90	1.94	1.91	2.10	0.15	NS	NS	NS
d 15 to 210	2.02	2.08	2.04	1.97	2.04	2.20	0.11	NS	NS	NS
Total solids, %										
d 15 to 112	12.19	11.95	11.82	12.19	12.07	11.94	0.21	NS	NS	NS
d 15 to 210	12.26	12.13	11.94	12.25	12.18	12.03	0.22	NS	NS	NS
Total solids, kg/d										
d 15 to 112	4.84	4.81	4.89	5.26	5.22	5.07	0.11	0.009	NS	0.05
d 15 to 210	4.38	4.49	4.45	4.78	4.81	4.55	0.12	0.09	NS	0.03

^{a,b,c}Means within a row with different superscripts 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 = solvent-extracted soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP. The 2-way interactions and 3-way interaction between factors and week of lactation were not significant ($P > 0.10$).

³Nonsignificant ($P > 0.10$).

ing d 15 to 112, but not during d 15 to 210. Additionally, somatic cell count (log10) was unaffected by experimental diets. Similar to results reported here, the replacement of SBM with a mixture of feather and blood meals at 2 concentrations of dietary CP resulted in a significant interaction for the yield of milk, leading to similar trends in the daily outputs of milk fat and protein (Grant and Haddad, 1998). This response, however, has not been observed in a number of studies in which the factorial effects of the amount and source CP on cow performance were investigated (Christensen et al., 1993; Cunningham et al., 1996; Weigel et al., 1997).

Compared with HCP, decreasing dietary CP to LCP, but not to MCP, depressed the production of CP, true protein, and total solids during both d 15 to 112 and d

15 to 210 (Table 5). These findings support data from experiments with high-producing cows that showed negligible improvements in the output of milk components in response to increased dietary CP that exceed 16.4 to 17.4% CP (Cunningham et al., 1996; Wu and Satter, 2000; Broderick, 2003). During d 15 to 210, the secretion of urea in milk (MUN) on average increased 2.53 mg/dL when dietary CP was increased from LCP to MCP and 3.03 mg/dL when dietary CP was increased from MCP to HCP. This is consistent with increases in the concentration of ammonia N in ruminal fluid that were observed at higher concentrations of dietary CP in the companion experiment (Ipharraguerre et al., 2005) as well as with results obtained in studies in which dietary CP was increased from 15.1 to 18.4% (Broderick, 2003)

Table 6. Least square means for estimated energy intake, energy requirements, and energy balance of cows fed diets containing different percentages and sources of CP.¹

NE _L	LCP		MCP		HCP		SEM	Effect ² (<i>P</i> > <i>F</i>)		
	SBM	AMB	SBM	AMB	SBM	AMB		CP%	Source	CP% × source
	(Mcal/d)									
Intake ³	36.9	35.4	38.0	38.5	40.4	36.2	1.01	NS ⁴	NS	NS
Requirements										
Maintenance ⁵	10.2	10.5	10.4	10.2	10.3	10.5	0.24	NS	NS	NS
Milk ⁶	24.5	24.8	25.1	26.0	27.3	25.0	1.03	NS	NS	0.08
Total ⁷	34.7	35.3	35.6	36.1	37.6	35.5	1.18	NS	NS	NS
Balance ⁸	2.3	0.2	2.5	2.4	2.9	0.7	1.16	NS	NS	NS

¹Data are least square means for d 15 to 210 of lactation, AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = solvent-extracted soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP. The 2-way interactions and 3-way interaction between factors and week of lactation were not significant (*P* > 0.10).

³Intake = (DMI, kg/d) × (NE_L/kg of DMI) where NE_L was calculated as described in Table 1.

⁴Nonsignificant (*P* > 0.10).

⁵Maintenance = 0.080 Mcal/kg BW^{0.75} (NRC, 2001).

⁶Milk = (0.0929 × Fat% + 0.0547 × CP% + 0.192) × (milk yield, kg/d) (NRC, 2001).

⁷Total = Maintenance + milk.

⁸Balance = Intake – total requirements.

or from 16.1 to 18.9% (Leonardi et al., 2003). Across percentages of dietary CP, the impact of the source of CP on the content and yield of milk components was not significant. Likewise, other experiments designed to evaluate the effects of replacing SBM with the AMB product did not find changes in milk composition (Santos et al., 1999; Underwood, 2003); although in one of these reports (Underwood, 2003) feeding AMB increased milk fat output during early lactation, whereas in the other trial (Santos et al., 1999) milk protein production was increased during midlactation. In both studies, these positive responses were associated with numerical increases in milk yield, which also occurred in this experiment when AMB replaced SBM at LCP and MCP. With the exception of fish meal, the replacement of SBM with RUP supplements of plant and (or) animal origin has frequently failed to improve the secretion of milk components, particularly of protein (Santos et al., 1998; Ipharraguerre and Clark, 2005).

An explanation for the described interactions might be that at HCP in the diet, the supply of MP exceeded the requirements of the cows and, consequently, SBM resulted in higher yields of FCM and milk components than AMB by increasing the intake of ME. Estimation of energy balance across the 210 d of lactation based on criteria outlined by the NRC (2001) and estimated dietary concentrations of NE_L (Table 1) showed that feeding SBM at HCP in the diet elicited a nonsignificant increase in NE_L intake (+4.2 Mcal/d) that could have accounted for 1.8 times the estimated increase in NE_L

output in milk (+2.3 Mcal/d) when compared with the HCP/AMB diet (Table 6). In the companion study (Ipharraguerre et al., 2005), feeding the HCP diets maximized the intestinal supply of nonammonia N and nonammonia nonmicrobial N independently of the source of supplemental CP, which supports further the suggestion that the supply of MP might not have been a limiting factor for the production of milk and milk components when HCP diets were fed. When SBM was partially replaced with AMB in the MCP diet, the estimated intake of NE_L (Table 6), and the ruminal digestibilities of OM and carbohydrates as well as the postruminal supply of N fractions remained unaffected (Ipharraguerre et al., 2005). Therefore, differences in the outputs of FCM and milk components between SBM and AMB at MCP in the diet might have been related to an attenuation of the imbalance in the profile of EAA in total AA delivered to the small intestine of cows fed the MCP/AMB diet. When cows were fed the LCP diets, however, the quantity of MP supplied to the cows, rather than its AA profile, and (or) the intake of ME might have been less than the requirements of the cows, explaining the lack of effect of the source of supplemental CP on the production of FCM and milk components.

In a literature review of 127 treatment comparisons, Santos et al. (1998) indicated that the productive response of dairy cows to RUP could be influenced by the amount of milk produced. Stratifying and analyzing data according to milk yield (< or ≥40 kg/d) during d 15 to 112 showed that high-producing cows produced more

Table 7. Least square means for performance of high and low producing cows¹ fed diets containing different percentages and sources of CP.²

Item	LCP		MCP		HCP		SEM	Effect ³ (<i>P</i> > <i>F</i>)			
	SBM	AMB	SBM	AMB	SBM	AMB		Group	Group × CP%	Group × Source	Group × CP% × source
Number of cows											
High producing cows	4	3	4	5	8	5					
Low producing cows	5	7	5	5	2	5					
DMI, kg/d											
High producing cows	24.9	22.8	25.5	26.2	26.6	24.8	1.2	NS ⁴	NS	NS	NS
Low producing cows	24.7	23.5	24.0	25.1	25.3	23.6					
Milk, kg/d											
High producing cows	42.3	41.5	45.8	44.7	44.7	45.4	1.8	0.0001	NS	NS	NS
Low producing cows	37.6	39.7	38.6	40.8	38.6	39.4					
3.5% FCM, ⁵ kg/d											
High producing cows	40.5	39.5	41.7	43.1	44.0	42.7	1.8	0.002	NS	NS	NS
Low producing cows	38.0	37.1	37.3	40.5	38.9	38.8					
FCM/DMI											
High producing cows	1.68	1.81	1.69	1.67	1.69	1.76	0.08	0.02	NS	NS	NS
Low producing cows	1.55	1.61	1.57	1.61	1.53	1.65					
BW, kg											
High producing cows	632	603	631	649	651	652	13.3	0.08	0.08	NS	NS
Low producing cows	643	652	653	664	651	651					
Maximum BW loss, ⁶ kg											
High producing cows	-63.0	-75.3	-73.8	-76.4	-58.2	-52.5	20.6	NS	NS	NS	NS
Low producing cows	-65.0	-71.1	-48.7	-44.3	-67.5	-69.5					

¹High producing cows ≥ 40 kg of milk/d (mean = 45.6), low producing cows < 40 kg of milk/d (mean = 37.9).

²Data from d 15 to 112 of lactation, AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = solvent-extracted soybean meal.

³Group = high vs. low producing cows, CP% = percentage of CP, Source = source of CP.

⁴NS = Nonsignificant (*P* > 0.10).

⁵3.5% FCM = 0.4324(kg of milk) + 16.216(kg of fat).

⁶Lowest BW and BCS recorded during the experimental period subtracted from BW and BCS on d 14 and d 7 of the pretreatment period, respectively.

milk (+4.9 kg/d) and FCM (+3.5 kg/d), but consumed similar amounts of feed (+0.8 kg/d), than lower producing cows, and that this response was largely mediated by an increase in the efficiency of nutrient use for milk synthesis (i.e., FCM/DMI; Table 7). Although maximum losses of BW were not significantly different between groups, high-producing cows tended to have lower BW than the other cows. These findings are consistent with data indicating that cows of superior genetic potential are capable of supporting greater lactogenic drive by using nutrients more efficiently rather than increasing their consumption (Veerkamp et al., 1995). With the exception of BW, none of the described responses was modulated by the interaction between level of production and the percentage and (or) source of CP. Likewise, the fat and urea content of milk and the yield of milk fat were not affected by the interaction between these factors (Table 8). Westwood et al. (2000) also found that the yield of milk and DMI of cows of low and high genetic merit were not affected differently by the RUP content of the diet (7.7 and 5.0% of the dietary DM). Conversely, the percentage of true protein in milk of high-producing cows increased when they were fed AMB compared with

SBM at LCP and MCP in the diet. This response, however, was not observed for the HCP diets or for the low-producing group. Additionally, reducing dietary CP to LCP limited the output of true protein but only by the group of high-producing cows. These results suggest that high-producing cows largely accounted for the effects of the percentage and source of dietary CP on milk protein output discussed earlier in this report. Nonetheless, the number of cows used in this study limited the power with which interactions between groups and dietary factors could be detected.

Nitrogen Use

Across the 210 d of lactation, the intake of N tended to parallel the significant interaction found for DMI (Table 9). That is, SBM resulted in higher N intake than AMB at HCP in the diet, but this trend was larger for AMB at MCP and not evident at LCP. The percentage of CP, but not the source, profoundly influenced the intake and use of N. Increasing dietary CP from LCP to HCP increased the amount of N consumed (+184 g/d) and estimated to be excreted in urine (+93 g/d) and feces

Table 8. Least square means for concentration and production of milk components of high and low producing cows¹ fed diets containing different percentages and sources of CP.²

Item	LCP		MCP		HCP		SEM	Effect ³ (<i>P</i> > <i>F</i>)			
	SBM	AMB	SBM	AMB	SBM	AMB		Group	Group × CP%	Group × Source	Group × CP% × source
Number of cows											
High producing cows	4	3	4	5	8	5					
Low producing cows	5	7	5	5	2	5					
Fat, %											
High producing cows	3.27	3.26	2.95	3.17	3.35	3.12	0.25	NS ⁴	NS	NS	NS
Low producing cows	3.65	3.19	3.29	3.30	3.46	3.48					
Fat, kg/d											
High producing cows	1.39	1.35	1.34	1.46	1.51	1.41	0.09	NS	NS	NS	NS
Low producing cows	1.36	1.23	1.27	1.38	1.36	1.35					
True protein, %											
High producing cows	2.67	2.80	2.71	2.87	2.85	2.82	0.07	0.0002	0.04	0.04	0.02
Low producing cows	2.95	2.88	2.98	2.88	2.79	2.88					
True protein, kg/d											
High producing cows	1.13	1.17	1.24	1.28	1.27	1.28	0.06	0.0001	0.04	NS	NS
Low producing cows	1.09	1.14	1.14	1.18	1.07	1.14					
MUN, mg/dL											
High producing cows	9.23	9.38	10.75	11.24	15.11	13.73	0.96	NS	NS	NS	NS
Low producing cows	8.93	7.82	11.39	10.92	13.44	13.57					

¹High producing cows ≥ 40 kg of milk/d (mean = 45.6), low producing cows < 40 kg of milk/d (mean = 37.9).

²Data are least square means for d 15 to 112 of lactation, AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = solvent-extracted soybean meal.

³Group = high vs. low producing cows, CP% = percentage of CP, Source = source of CP.

⁴NS = Nonsignificant (*P* > 0.10).

(+78 g/d) without altering significantly the amount secreted in milk (+13 g/d). Differences for the estimated excretion of fecal N between MCP and HCP, however, were not significant. The gross efficiency of conversion of feed N into milk N decreased on average from 32 to 25% as the concentration of CP in the diet increased

Table 9. Least square means for N utilization by 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	570	560	660	689	776	721	26.8	0.0001	NS ³	0.08
N output, g/d										
Milk ⁴	171	184	186	190	195	184	6.8	NS	NS	NS
Urine ⁵	156 ^c	148 ^c	197 ^b	195 ^b	250 ^a	241 ^a	12.3	0.0001	NS	NS
Feces ⁶	244 ^b	229 ^b	277 ^a	304 ^a	331 ^a	296 ^a	22.0	0.0001	NS	NS
N output, % N intake										
Milk	30 ^a	33 ^a	29 ^b	28 ^b	26 ^c	25 ^c	1.3	0.0001	NS	0.09
Urine	27 ^b	27 ^b	30 ^b	28 ^b	32 ^a	33 ^a	1.8	0.004	NS	NS
Feces	43	40	41	44	42	41	2.0	NS	NS	NS

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

¹Data are least square means for d 15 to 210 of lactation, AMB = Animal-marine protein blend, HCP = high CP diet, LCP = low CP diet, MCP = medium CP diet, SBM = solvent-extracted soybean meal.

²CP% = Percentage of CP, Source = source of CP, CP% × source = interaction between percentage and source of CP. The 2-way interactions and 3-way interaction between factors and week of lactation were not significant (*P* > 0.10).

³Nonsignificant (*P* > 0.10).

⁴Calculated as milk CP (g/d)/6.38.

⁵Estimated as 0.026 × BW (kg) × MUN (mg/dL) (Kauffman and St-Pierre, 2001).

⁶Estimated as N intake – (Milk N + Urine N) and assuming no net deposition or mobilization of tissue N.

from LCP to HCP. Within the same range of dietary CP, the proportion of N intake that was estimated to be excreted in urine increased from 27 to 33%, whereas the percentage estimated to be excreted in feces remained constant among treatments and averaged 42. Similar results were obtained when data for N use were analyzed for d 42 to 49, d 112 to 119, and d 196 to 203 (data not shown). In agreement with these findings, using data from 19 N balance studies that involved low- to moderate-producing cows, Castillo et al. (2000) estimated that increasing N intake from 400 to 750 g/d markedly enhanced the partition of N to urine ($0.67 \times \text{N intake}$; $R^2 = 0.63$) and marginally improved its secretion in milk ($0.14 \times \text{N intake}$; $R^2 = 0.14$). These authors speculated that N partitioning in low- to moderate-producing cows, particularly the output of N in urine, could be different from that in cows of high genetic merit. Data from this experiment and from other studies (Leonardi et al., 2003; Noftsgger and St-Pierre, 2003) with high-producing cows that consumed from 21.7 to 23.6 kg of DM daily and produced from 41.0 to 45.2 kg/d of milk suggest that when N intake exceeds about 600 g/d (~16.5% CP on DM basis), there are negligible increases in milk N output. In some studies (Wohlt et al., 1991; Wright et al., 1998; Castillo et al., 2001), but not in others (Kröber et al., 2000a; Knowlton et al., 2001; Leonardi et al., 2003), supplementing diets for lactating cows with RUP and (or) rumen-protected AA decreased urinary N excretion. Conversely, such strategies have frequently failed to improve the gross conversion of feed N into milk (Wohlt et al., 1991; Kröber et al., 2000a; Castillo et al., 2001; Knowlton et al., 2001; Leonardi et al., 2003), which is consistent with our results.

CONCLUSIONS

In this experiment, the productive response of dairy cows to the source of supplemental CP depended on the concentration of CP in the diet. When the percentage of dietary CP averaged 18.7, supplementing diets with only SBM maximized DMI and the production of milk and milk components. As a result, the marginal returns in the output of milk and milk protein in response to replacing SBM with RUP plus rumen-protected Met (AMB) were nil, suggesting that the supply of nutrients other than MP, most likely ME, limited cow performance. When the percentage of dietary CP averaged 16.8, however, the partial replacement of SBM with RUP plus rumen-protected Met was required to sustain maximum yields of milk and milk components. This suggests that in this case the supply of MP and its AA composition may have limited the productive response of dairy cows. Supplementing diets with different sources of CP and rumen-protected Met was not effective for maintaining

cow productivity when the input of dietary CP was decreased on average to 14.8%, which indicates that the quantity of MP supplied to the cows, rather than its profile of AA, was insufficient for meeting their requirements. Despite these interactions, improvements in the gross efficiency of N use for milk production were achieved through reductions in the intake of N independently of the source of CP.

These results 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 if the source of RDP and RUP are properly matched with the source and amount of carbohydrate in the diet. This strategy would sustain optimal cow productivity throughout the entire lactation and improve the gross conversion of feed N to milk N.

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