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Improving Intestinal Amino Acid Supply of Pre- and Postpartum Dairy Cows with Rumen-Protected Methionine and Lysine*,†

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ABSTRACT

Eighty-four Holstein cows were assigned to a randomized block experiment to determine effects of supplementing pre- and postpartum diets containing high-Lys protein supplements with rumen-protected Met and Lys. Before parturition, cows received a basal diet with 1) no rumen-protected amino acids (AA), 2) 10.5 g/d of Met from rumen-protected Met, or 3) 10.2 g/d of Met and 16.0 g/d of Lys from rumen-protected Met plus Lys. After parturition, cows continued to receive AA treatments but switched to diets balanced for 16.0 or 18.5% crude protein (CP). Diets were corn-based; supplemental protein was provided by soybean products and blood meal. Cows received treatments through d 105 of lactation. Compared with basal and Met-supplemented diets, Met + Lys supplementation increased yield of energy-corrected milk, fat, and protein, and tended to increase production of 3.5% fat-corrected milk. Significant CP × AA interactions were observed only for milk protein and fat content. Supplementation of the 16% CP diet with Met and Met + Lys had no effect on milk true protein and fat content. However, Met and Met + Lys supplementation of the 18.5% CP diet increased milk protein content by 0.21 and 0.14 percentage units, respectively, and Met supplementation increased fat content by 0.26 percentage units. Results of this study indicate that early-lactation cows fed corn-based diets are responsive to increased intestinal supplies of Lys and Met and that the responses depend on dietary CP concentration, supply of metabolizable protein, and intestinal digestibility of the rumen-undegradable fraction of supplemental proteins.
(**Key words:** rumen-protected amino acids, lysine, methionine, lactating cow)

Abbreviation key: ECM = energy-corrected milk, **MP** = metabolizable protein; **RPAA** = rumen-protected AA, **RPMet** = rumen-protected Met, **RPMet+Lys** = rumen-protected Met plus Lys, **16B** = 16.0% CP basal diet, **16M** = 16.0% CP diet with 10.5 g of Met from rumen-protected Met, **16ML** = 16.0% CP diet with 10.2 g of Met and 16.0 g of Lys from rumen-protected Met and rumen-protected Met plus Lys, **18.5B** = 18.5% CP basal diet, **18.5M** = 18.5% CP diet with 10.5 g of Met from rumen-protected Met, **18.5ML** = 18.5% CP diet with 10.2 g of Met and 16.0 g of Lys from rumen-protected Met and rumen-protected Met plus Lys.

INTRODUCTION

Production responses of dairy cows to improved Lys and Met nutrition include variable increases in feed intake, milk production, and content and yield of milk protein. Literature summaries confirm that responses to postruminal Lys and Met supplementation are greater when basal levels of Lys and Met in RUP are low rather than high, when RUP supplies a greater portion of the metabolizable protein (**MP**), when cows are in early rather than mid or late lactation, and in high-producing cows rather than low producing cows (Rulquin and Vérité, 1993; NRC, 2001). There are 4 additional and noteworthy observations regarding improvements in duodenal concentrations of Lys and Met. First, content of milk protein is more sensitive than milk yield (Rulquin et al., 1993; NRC, 2001). Second, results of several experiments indicate that milk casein is affected more than the whey and NPN fractions (Donkin et al., 1989; Chow et al., 1990; Le Henaff et al., 1990; Armentano et al., 1993). Third, increases in content of milk protein are greater than what would be expected

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by increasing dietary CP (NRC, 2001). Finally, increases in milk yield to supplemental Lys and Met generally are limited to cows in early lactation when the need for absorbable AA, relative to absorbable energy, is greatest (Polan et al., 1991; Schwab et al., 1992a,b; Rulquin and Vérité, 1993).

The advantage of improving the balance of absorbable AA is the increased efficiency of use of absorbed AA for milk protein production. It has been demonstrated that improved Lys and Met nutrition reduced the amount of dietary CP needed to achieve similar yields of milk protein (Robert et al., 1989; Rulquin et al., 1990).

In most of the studies referred to above, a Latin Square with short experimental periods (generally 2 wk or less) was used as the experimental design, and in only a few experiments (Overton et al., 1996; Carson et al., 1998; Xu et al., 1998) did cows receive supplemental AA before or immediately after calving. The objectives of this study were: 1) to determine the effects of supplementing corn-based diets of prepartum and early postpartum cows with rumen-protected Met (**RPMet**) and rumen-protected Met plus Lys (**RPMet+Lys**) on early lactation performance, 2) to determine if the use of high-Lys protein supplements provided adequate intestinal supplies of Lys, and 3) to determine the effect of postpartum dietary CP on response to rumen-protected AA (**RPAA**) supplementation.

MATERIALS AND METHODS

Experimental Design and Treatments

Eighty-four multiparous cows, blocked by calving date (14 blocks), were assigned to an experiment using a randomized complete block design, 14 d before expected calving. The experiment consisted of 3 prepartum diets and 6 postpartum diets. The 3 prepartum diets were the basal diet, the basal diet supplemented with 15 g/d of Smartamine M (Adisseo, Atlanta, GA) which supplied 10.5 g of Met, or the basal diet supplemented with 6 g/d of Smartamine M plus 40 g/d of Smartamine ML (Adisseo), which together supplied 10.2 g of Met and 16.0 g of Lys (Table 1). At calving, cows continued to receive their respective RPAA treatment, in similar amounts, but were switched to either a 16.0 or 18.5% CP postcalving diet (Table 1), forming a 2×3 factorial arrangement of treatments during lactation. The 6 lactation treatments were the 16.0% CP basal diet (**16B**), 16B diet supplemented with RPMet (**16M**), the 16B supplemented with RPMet+Lys (**16ML**), the 18.5% CP basal diet (**18B**), the 18B supplemented with RPMet (**18M**), and the 18B supplemented with RPMet+Lys (**18ML**). Cows remained on their assigned diets through wk 15 of lactation. All procedures related to animal care were conducted with the approval of the University of New Hampshire Institutional Animal Care and Use Committee.

Table 1. Ingredient composition of basal diets.

	Diet		
Ingredient, % DM	Precalving	18.5% CP	16.0% CP
Corn silage[1]	31.1	22.3	22.3
Grass-legume silage, mid-mat.	16.7	12.6	12.6
Alfalfa hay, mid-mat.	7.2	9.7	9.7
Corn, coarse grind	32.0	31.8	37.1
Soybean meal, solvent-extracted	6.9	11.5	0.8
Raw soybeans, cracked	2.8	5.3	5.3
Soybean meal, expeller[2]	0.0	0.0	5.3
Blood meal, ring-dried	0.7	1.4	1.4
Fat[3]	0.7	1.4	1.4
Mineral-vitamin mix[4]	1.9	4.0	4.0

[1]Treated at ensiling with 0.5% urea.

[2]SoyPlus (West Central Co-op, Ralston, IA).

[3]Alifet (Alifet USA, Inc., Cincinnati, OH).

[4]Mineral-vitamin mix composition: 15.5% Ca, 5.6% P, 4.6% Mg, 1.5% K, 2.1% S, 0.2% Zn, 0.13% Mn, 0.04% Cu, 0.23% Fe, 0.0025% I, 0.0041% Co, 0.0013% Se, 143,424 IU/kg of vitamin A, 45,895 IU/kg of vitamin D, and 457 IU/kg of vitamin E.

Feeding and Management of Cows

Diets (Table 1) were fed as a TMR and were prepared by weighing each ingredient and blending in a drum-type mixer (Data Ranger; American Calan, Inc., Northwood, NH). Alfalfa hay was chopped before incorporation into the TMR using a bale chopper (model 6-90, Wic Inc., Johnson, Quebec, Canada). Cows were fed 67% of the total daily allotment at 1530 h and 33% of the daily allotment at 0530 h. Feed allotments were adjusted to achieve 5 to 10% orts. Orts were collected at 1300 h. The RPAA supplements were top dressed at time of feeding. Before initiation of the experiment, and every 4 wk thereafter, feed ingredients were analyzed for CP, NDF, ADF, ether extract, Ca, P, K, Mg, and S (Dairy One Forage Laboratory, Ithaca, NY). Forage to grain ratio was adjusted to maintain a forage NDF of 21% of DM. Amounts of corn and expeller form of soybean meal (SoyPlus, West Central Co-op, Ralston, IA) were altered to achieve a CP level of 16.0% for the 16.0% CP diets. For the 18.5% CP diets, amounts of corn and solvent soybean meal were altered to achieve a CP level of 18.5%.

Measurements, Collection, and Analysis of Samples

Feed ingredients and orts were sampled weekly, analyzed for DM (60°C under 760 mm of vacuum for 24 h), ground to pass through a 1-mm screen, and then composited across experiment by treatment. The orts were composited by treatment and analyzed for CP,

Table 2. Chemical composition of feeds used in pre- and postpartum diets.

Item, DM basis	Corn silage	Grass-legume silage	Alfalfa hay	Corn, coarse grind	Soybean meal, solvent-extracted	Soybean meal, expeller	Raw soybeans, cracked	Blood meal, ring-dried
DM, %	33.0	32.7	90.1	87.8	91.4	94.0	89.0	92.0
CP, %	9.8	16.8	17.8	9.5	52.2	48.2	40.2	95.5
ADF, %	25.3	36.3	33.6	2.7	4	11.4	20.8	—
NDF, %	43.7	54.6	44.9	8.6	12.1	30.0	27.8	—
ADFICP,[1] %	1.2	1.9	1.5	0.9	1.1	1.9	1.7	—
NDFICP,[2] %	1.6	4.9	5.6	1.8	1.6	13.4	5.8	—
Lignin, %	3.6	6.9	7.1	1.1	1.3	2.1	1.8	—
Ash, %	4.1	9.3	11.1	2.7	7.5	6.8	5.6	3.2
Ca, %	0.16	0.75	0.66	0.02	0.24	0.29	0.18	0.40
P, %	0.17	0.29	0.24	0.24	0.67	0.64	0.60	0.23
Mg, %	0.10	0.21	0.18	0.10	0.28	0.28	0.23	0.03
K, %	0.86	2.52	3.11	0.38	2.29	2.10	1.79	0.15
S, %	0.07	0.18	0.12	0.06	0.35	0.43	0.28	0.71
Fe, ppm	118	157	74	42	152	143	125	1900
Zn, ppm	12	27	24	21	47	33	24	26
Cu, ppm	5	7	8	2	14	18	8	5

[1]ADFICP = Acid detergent insoluble CP.
[2]NDFICP = Neutral detergent insoluble CP.

NDF, ADF, ether extract, Ca, P, K, Mg, and S (Dairy One Forage Laboratory, Ithaca, NY). Composited samples of feed ingredients and orts were analyzed for AA concentrations using procedures described by Putnam et al. (1997). Composited samples of blood meal and expeller soybean meal were analyzed for RUP digestibility at the University of Minnesota using the 3-step procedure of Calsamiglia and Stern (1995).

Milk weights were recorded twice daily; milk samples were taken from 2 consecutive milkings each week. Milk samples were preserved with 2-bromo-2-nitropropane-1,3 diol and analyzed for fat and true protein by Dairy One Forage Laboratory (Ithaca, NY) using infrared technology. Body condition scores were obtained weekly by 3 individuals and averaged. Cows in blocks 1 through 12 were weighed daily. Body weights for the complete duration of the study were not available for cows in blocks 13 and 14, due to mechanical failure of the scale.

Blood samples were obtained by venipuncture of the coccygeal vein at approximately 2 h after the morning feeding during wk 1, 2, 3, 4, and 8 of lactation. Blood was collected into one 10-mL evacuated tube containing no additive and one 10-mL evacuated tube containing sodium heparin and 4% sodium fluoride (Vacutainer, Becton Dickinson, Rutherford, NJ). Tubes containing the anticoagulant were placed in an ice bath until centrifuged at 3300 × *g* for 20 min at 5°C. One aliquot of plasma was removed and frozen (–20°C) for determination of glucose (Sigma kit Trinder 500, Sigma Chemical Co., St. Louis, MO) (Barham and Trinder, 1972) and NEFA (WAKO NEFA C kit, WAKO Chemicals USA, Inc., Richmond, VA) (Johnson and Peters, 1993) concentrations. An additional aliquot was deproteinized; 4 volumes were vortexed with 1 volume of 15% sulfosalicylic acid, centrifuged at 3300 × *g* for 20 min at 5°C, and the supernatant frozen (–20°C) for BHBA analysis (Gibbard and Watkins, 1968). Blood in tubes containing no additive was allowed to clot at room temperature (15 to 21°C), centrifuged (3300 × *g* for 20 min), and the serum was frozen for determination of urea concentrations (Sigma kit 640, Sigma Chemical Co.) (Crocker, 1967). All plasma and serum samples were thawed at 5°C before analysis.

Statistical Analysis and Calculations

Production data were analyzed using the MIXED procedure of SAS (SAS Institute, 1999) according to the following model:

$$Y_{ijkl} = \mu + A_i + P_j + AP_{ij} + \beta X_{ijk} + c_{kij} + T_l + AT_{il} + PT_{jl} + APT_{ijl} + E_{ijkl}$$

where Y_{ijkl} is the dependent, continuous variable, μ is the overall mean, A_i is the fixed effect of the ith level of AA (i = 1, ..., 3), P_j is the fixed effect of the jth level of protein (j = 1,2), β is the regression coefficient, X_{ijk} is the covariate measurement, c_{kij} is the random effect of the kth cow with the ijth treatment subclass (k = 1, ..., 14), T_l is the fixed effect of the lth week of experiment (l = 1,..., 15), E_{ijkl} is the residual error, and AP_{ij}, AT_{il}, PT_{jl}, and APT_{ijl} are fixed effects due to the interactions of the main effects.

In this model, the random effect of cows within treatment subclasses is used as the error term for the effect of AA and protein levels and their interactions. Residual errors, which are errors within cows across time and

Table 3. Amino acid composition of feeds used in pre- and postpartum diets.

AA, % of CP	Corn silage	Grass-legume silage	Alfalfa hay	Corn, coarse grind	Soybean meal, solvent-extracted	Soybean meal, expeller	Raw soybeans, cracked	Blood meal, ring-dried
Ala	7.96	6.43	4.10	7.68	4.02	3.92	4.38	6.95
Asp	4.80	7.26	11.46	7.47	10.57	10.25	11.32	8.77
Cys	1.53	1.55	0.28	2.21	1.15	1.12	1.12	0.02
Glu	9.69	7.02	10.11	18.63	16.59	15.73	17.51	8.07
Gly	3.27	4.05	5.06	4.00	3.85	3.78	4.18	3.92
Ser	2.86	3.10	4.89	4.74	4.41	4.40	4.80	4.59
Tyr	2.14	2.44	3.82	3.89	3.05	2.95	3.41	2.73
Arg	1.84	2.62	4.61	4.74	6.67	6.06	7.19	3.70
His	1.33	1.37	2.25	2.74	2.36	2.24	2.56	4.73
Ile	2.65	3.21	3.88	3.16	3.75	3.36	4.18	0.94
Leu	7.55	6.37	8.20	12.32	6.88	6.64	7.56	10.44
Lys	2.14	3.63	4.83	3.16	5.65	4.75	6.19	7.70
Met	1.02	0.95	0.84	1.79	0.92	0.89	1.09	1.48
Phe	2.86	3.63	5.06	4.84	4.35	4.13	4.80	5.87
Thr	2.65	3.27	4.55	3.68	3.58	3.57	3.88	4.41
Val	3.98	4.76	6.80	4.84	4.21	3.88	4.65	7.35

represent errors from repeated measurements from the experimental units (cows), were modeled using a first-order autoregressive covariance structure. Degrees of freedom were calculated using the Kenward-Roger option of the MIXED procedure (SAS Institute, 1999). A covariate term was included in the model to reduce the variance due to cow within treatment subclasses. The covariate variables were taken from the prior lactation of each cow and consisted of mature equivalents for milk production, milk component yields, or milk composition, as appropriate. The cows had not been assigned to an experiment in their previous lactation. Mature equivalent milk production was used as the covariate for DMI analysis. The covariate term was removed from the final statistical model in the analysis of BW, blood, and prepartum DMI data because analyzing the data without covariates resulted in smaller Bayesian information criteria values. Block effect was initially included in the model but was removed in the final analysis because it was found to be insignificant. Least square means were determined for AA source, protein level, and the interaction between AA source and protein level. The DIFF option in SAS was used to test treatment differences among least square means. Significant treatment responses were declared at $P \leq 0.05$ and trends for treatment responses were declared at $P > 0.05$ but $P \leq 0.15$.

The Univariate Procedure of SAS (SAS Institute, 1999) was used to determine outlier cows for DM intake during the prepartum period. An observation that was greater than 2.5 standard deviations (SD = 2.97) from the mean (mean = 14.66) for the last 7 d of gestation was considered an outlier. The results of the outlier analysis indicated that 2 cows were outliers due to extremely low (5.7 kg/d) and extremely high (25.1 kg/d) DMI; therefore these cows were removed from the final statistical analysis.

RESULTS

The average time required to complete a block was 6.4 wk (range: 3 to 11 wk). The length of the experiment from the time the first cow was assigned to treatment until the last cow completed the experiment was 535 d. The RPAA supplements were readily consumed by the cows with no cows refusing to eat the supplements. Four cows were removed from the experiment; 3 due to the development of pendulous udders which hindered milking of the cows, and 1 because of an immobilizing calving injury. Information collected on these cows was not included in the statistical analysis of the data.

Chemical Composition of Ingredients and Diets

The chemical and AA compositions of feed ingredients are shown in Tables 2 and 3. The measured RUP digestibility coefficients for expeller soybean meal and blood meal were 92.8 and 60.7%, respectively.

The chemical compositions of the consumed diets are shown in Table 4. The chemical composition of the consumed diet was calculated by measuring the concentrations of chemical components in each ingredient and in orts, and subtracting the amount of each chemical component in orts from the total amount of each chemical component offered. Based upon average milk production, milk composition, and DMI for the 15-wk postpartum experimental period (Table 5), the RDP, RUP, and metabolizable protein (**MP**) balances as predicted by NRC (2001) were –183, –205, and –165 g/d, respectively, for cows fed 16B; and 352, 11, and 9 g/d, respec-

Table 4. NRC (2001) evaluation of the basal diets.[1]

Chemical component	Precalving diet[2]	Lactation diet[3]	
		18.5% CP	16.0% CP
NDF, % of DM	30.9	26.6	27.4
Forage NDF, % of DM	26.5	21.0	21.0
RDP, % of DM	10.3	11.5	9.3
RUP, % of DM	5.3	7.1	6.7
CP, % of DM	15.6	18.5	16.0
RDP balance, g/d	0	352	–183
RUP balance, g/d	826	11	–205
MP balance,[4] g/d	628	9	–165
NE_L balance, Mcal/d	12.5	–2.5	–2.5
Lys, % of MP			
Basal	6.6	6.3	6.1
With rumen-protected Met	6.5	6.2	6.1
With rumen-protected Met + Lys	7.5	6.7	6.5
Met, % of MP			
Basal	1.8	1.6	1.6
With rumen-protected Met	2.4	1.9	1.9
With rumen-protected Met + Lys	2.4	1.9	1.9
MP-Lys, g/d			
Basal	101	172	157
With rumen-protected Met	101	172	157
With rumen-protected Met + Lys	117	185	170
MP-Met, g/d			
Basal	27	44	42
With rumen-protected Met	37	52	50
With rumen-protected Met + Lys	37	52	50

[1]Diets were evaluated using nutrient profiles listed in Tables 2 and 3. For nutrients and feed components not listed in Tables 2 and 3, default values were used, with the exception of RUP digestibility of blood meal. Analysis of the blood meal using the 3-step procedure of Calsamiglia and Stern (1995) yielded an RUP digestibility value of 60.7% (lower than the NRC default value of 80%).

[2]Animal inputs: Holstein cow, 50 mo of age, 635 kg of BW, 270 d pregnant, 15.2 kg/d of DMI, and default environmental conditions.

[3]Animal inputs: Holstein cow, 52 mo of age, 630 kg of BW, 45 DIM, 44.0 kg/d of milk, 3.7% milk fat, 2.9% milk true protein, 0 d pregnant, 23.6 kg/d of DMI, and default environmental conditions.

[4]MP = Metabolizable protein.

tively, for cows fed 18.5B (Table 4). Corn sources (silage and grain) supplied 36 and 31% of dietary RUP for the 16 and 18.5% CP diets, respectively; the higher Lys feeds, soybean meal, raw soybeans, expeller soybean meal, and blood meal supplied 53 and 58% of dietary RUP for the 16 and 18.5% CP diets, respectively.

According to NRC (2001), the predicted percentages of Lys and Met in MP were 6.6 and 1.8; 6.1 and 1.6; and 6.3 and 1.6% for the precalving basal diet and the 16B and 18.5B diets, respectively (Table 4). These predicted concentrations of Lys and Met in MP are less than the concentrations of 7.2 and 2.4% suggested to be required for maximum yield and content of milk protein (NRC, 2001). Due to the Lys:Met ratios being well in excess of the optimum of 3.0 (NRC, 2001), diets appeared to be more limiting in Met than Lys.

DMI and Lactation Responses

There was no effect of treatment ($P > 0.15$) on prepartum DMI (Table 5). Cows receiving RPMet tended to ($P \leq 0.15$) consume less DM than cows receiving no RPAA or RPMet+Lys (22.9 vs. 23.7 and 24.3 kg/d, respectively, Table 5). Supplementing the basal diets with RPMet numerically reduced ($P > 0.15$) milk yield, whereas supplementing with RPMet+Lys numerically increased milk yield; the net result was that cows fed RPMet+Lys produced more ($P \leq 0.05$) milk than cows fed RPMet (44.9 vs. 41.8 kg/d). Improving intestinal supply of Lys and Met through feeding RPMet+Lys compared with no RPAA or RPMet supplementation increased ($P \leq 0.05$) yields of energy corrected milk (**ECM**; 45.9 vs. 43.6 and 43.0 kg/d), true protein (1306 vs. 1221 and 1218 g/d), and fat (1632 vs. 1550 and 1543 g/d; Table 5). There was a trend ($P \leq 0.15$) for cows fed RPMet+Lys to have higher yields of 3.5% FCM than cows fed the basal diets or the basal diets supplemented with RPMet (45.9 vs. 43.8 and 43.1 kg/d).

There was no effect ($P > 0.15$) of dietary CP on yield of DMI, milk, ECM, FCM, and fat. Increases in yield of milk true protein in response to dietary CP content were dependent on week postpartum (CP × week interaction, $P \leq 0.05$; Figure 1). Cows fed the 16% CP diets produced more milk protein in the weeks immediately

Table 5. Intake and milk production responses during the first 105 d of lactation of multiparous Holstein cows fed rumen-protected Met or rumen-protected Met plus Lys at 2 levels of dietary CP.

	Treatments[1]							
	18.5% CP			16.0% CP				
Item	Basal	M	M+L	Basal	M	M+L	SE	Effects[2]
Prepartum DMI,[3] kg/d	15.1	13.9	15.1	15.0	14.5	15.3	0.7[4]	
Postpartum DMI, kg/d	23.9	22.6	24.2	23.5	23.1	24.3	0.5[5]	m vs. basal,m+l
Milk, kg/d	43.4	40.9	45.8	42.9	42.7	44.0	1.3[5]	M vs. M+L
3.5% FCM, kg/d	44.0	42.8	46.5	43.6	43.4	45.2	1.1[5]	basal, m vs. m+l
ECM,[6] kg/d	43.7	42.7	46.5	43.5	43.3	45.2	1.1[5]	Basal, M vs. M+L
Milk component yield								
True protein, g/d	1213	1211	1328	1229	1225	1284	38[5]	Basal, M vs. M+L; CP × WK
Fat, g/d	1555	1548	1651	1544	1538	1614	35[5]	Basal, M vs. M+L
Milk composition								
True protein, %	2.80^{x}	3.01^{z}	2.94^{yz}	2.90^{y}	2.91^{y}	2.93^{yz}	0.04[5]	Basal vs. M, M+L; AA × CP; CP × WK
Fat, %	3.62^{y}	3.88^{z}	3.65^{y}	3.69^{y}	3.63^{y}	3.74^{yz}	0.06[5]	AA × CP; CP × WK; AA × CP × WK
ECM/DMI[6]	1.85	1.94	1.97	1.89	1.92	1.91	0.03[5]	basal vs. m, m+l
Milk N/N intake,[7] g	0.29^{a}	0.31^{b}	0.32^{b}	0.35^{c}	0.35^{c}	0.35^{c}	0.01[5]	Basal vs. M, M+L; CP; aa × cp

^{a,b,c}Within a row, means with uncommon superscripts differ at $P \leq 0.15$.

^{x,y,z}Within a row, means with uncommon superscripts differ at $P \leq 0.05$.

[1]Treatments were basal, 15 g/d of a rumen-protected Met product which supplied 10.5 g of Met (M), and 6 g/d of rumen-protected Met product plus 40 g/d of a rumen-protected Met plus Lys product which together supplied 10.2 g of Met and 16.0 g of Lys (M+L).

[2]Only treatment effects with $P \leq 0.15$ reported: M vs. M+L, $P \leq 0.05$; Basal, M vs. M+L, $P \leq 0.05$; basal, m vs. m+l, $P \leq 0.15$; basal vs m, m+l, $P \leq 0.15$; CP = crude protein effect, $P \leq 0.05$; AA × CP = amino acid × crude protein interaction, $P \leq 0.05$; aa × cp = amino acid × crude protein interaction, $P \leq 0.15$; CP × WK = dietary crude protein by week interaction, $P \leq 0.05$; and AA × CP × WK = amino acid × dietary crude protein × week interaction, $P \leq 0.05$.

[3]Mean represents DMI of all cows for 7 d before parturition.

[4]Standard error of the least squares mean, n = 26.

[5]Standard error of the least squares mean, n = 13.

[6]ECM = Energy-corrected milk; 3.5% fat, 3.2% protein.

[7]Milk N yield (kg) per kilogram of N intake.

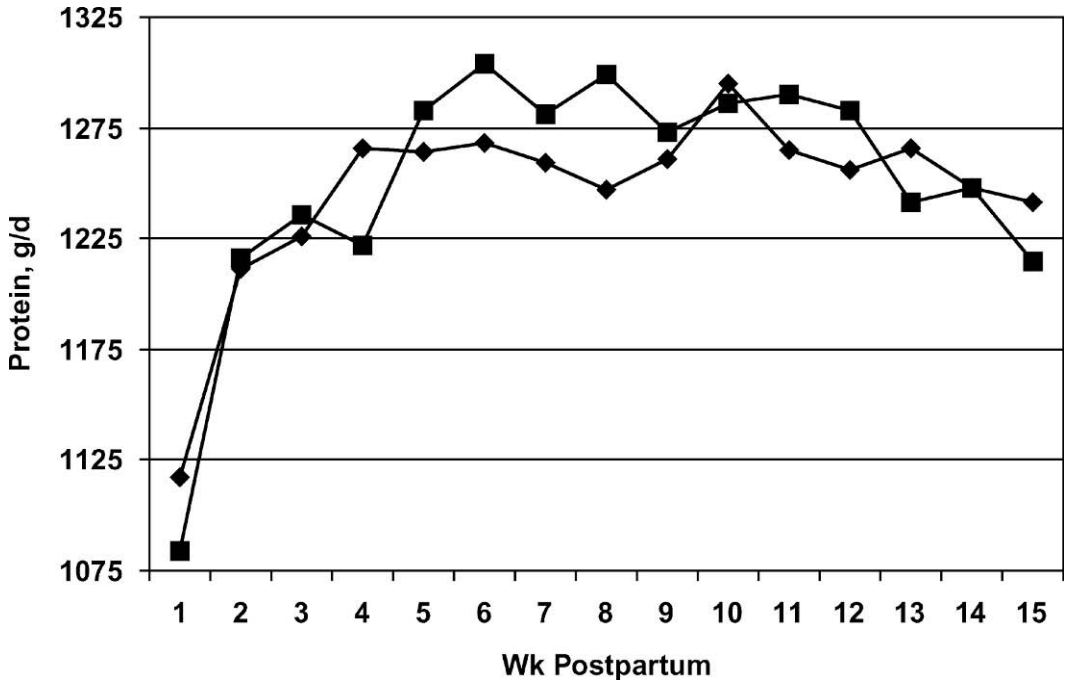

Figure 1. Response to dietary CP content across time: Production of milk true protein. 16.0% CP diet (◆), 18.5% CP diet (■). Week × dietary CP content, ($P \leq 0.05$). Pooled SEM = 31.0.

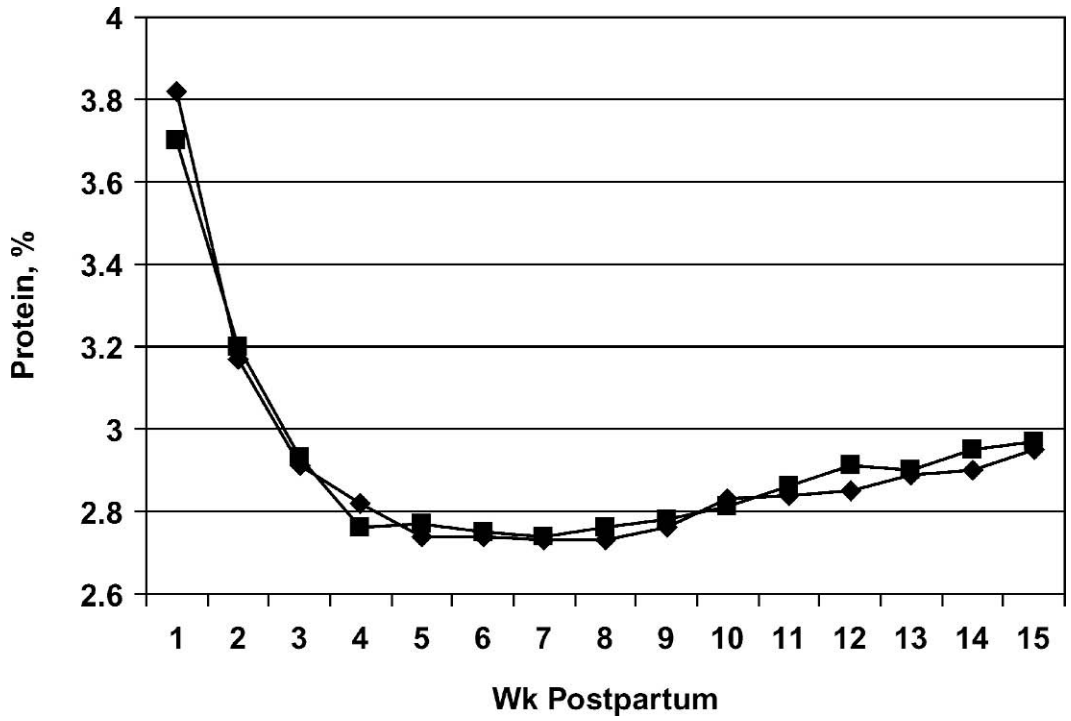

Figure 2. Response to dietary CP across time: Milk true protein content. 16.0% CP diet (◆), 18.5% CP diet (■). Week × dietary CP content, $P \leq 0.05$. Pooled SEM = 0.04.

after calving and as cows entered midlactation, whereas cows fed the 18.5% CP diets produced more milk protein when cows were at peak production.

Supplementing the basal diets of lactating cows with RPMet or RPMet+Lys increased ($P \leq 0.05$) percentage of true protein in milk (2.96 and 2.94 vs. 2.85) (Table 5). However, responses were not consistent across dietary CP level (CP × AA interaction, $P \leq 0.05$). Supplementing 18.5B with RPMet and RPMet+Lys increased ($P \leq 0.05$) milk true protein content 0.21 and 0.14 percentage units, whereas supplementing 16.0B with RPMet and RPMet+Lys had no effect. Similarly, RPAA supplementation of the basal diets had an inconsistent effect on milk fat content (CP × AA interaction, $P \leq 0.05$). Adding RPMet to 18.5B increased ($P \leq 0.05$) milk fat content by 0.26 percentage units, but did not affect milk fat content when added to 16B.

The effect of dietary CP content on milk true protein and fat concentrations was dependent on stage of lactation (CP × wk interaction, $P \leq 0.05$). Compared with cows fed the 18.5% CP diets, cows fed the 16.0% CP diets produced milk with a higher protein content immediately following calving and a similar or lower protein content thereafter (Figure 2). Feeding the 16% CP diets increased milk fat content in the immediate postpartum period and lowered milk fat content during wk 3 and 4 of lactation (Figure 3). Feeding the 18.5% CP diets increased milk fat content as cows entered midlactation (Figure 3).

Effect of treatment on milk fat content was dependent on stage of lactation (AA × CP × wk interaction, $P \leq 0.05$). Cows fed 18.5B produced milk with the lowest milk fat content immediately following parturition and at peak production. Cows fed 18.5M produced milk with the highest fat content immediately following calving and as cows approached peak production, whereas cows fed 16M produced milk with the lowest fat content as cows approached peak and midlactation (Figure 4).

Supplementing the basal diets with RPMet and RPMet+Lys tended to increase ($P \leq 0.15$) the efficiency of conversion of DMI to ECM (1.93 and 1.94 vs. 1.87 kg of ECM/kg of DMI; Table 5). Similarly, efficiency of conversion of feed N to milk N increased ($P \leq 0.05$) with RPMet and RPMet+Lys supplementation (0.33 and 0.34 vs. 0.32 kg of milk N/kg of feed N). However, responses tended to be inconsistent across dietary CP levels (AA × CP interaction, $P \leq 0.15$), with efficiency of conversion of feed N to milk N tending to improve when RPMet and RPMet+Lys were added to the 18.5% CP diet, but not the 16% CP diet. Reducing dietary CP from 18.5 to 16.0% increased ($P \leq 0.05$) efficiency of conversion of consumed N to milk N (0.31 to 0.35).

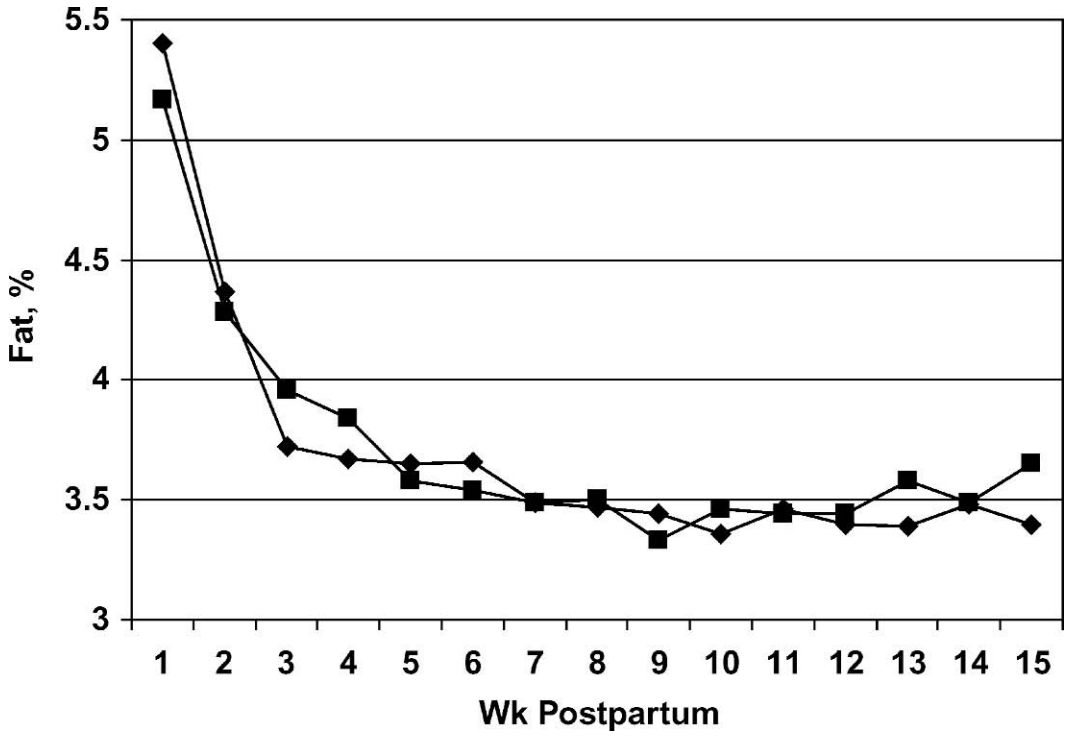

Figure 3. Response to dietary CP across time: Milk fat content. 16.0% CP diet (◆), 18.5% CP diet (■). Week × dietary CP content, $P \leq 0.05$. Pooled SEM = 0.09.

There were no effects ($P > 0.15$) of treatment on BW or BW changes (Table 6). Cows receiving 18.5ML tended to have lower BCS ($P \leq 0.15$) at wk 1 postpartum compared with cows receiving 18B, whereas 16ML cows tended to have higher BCS than 16B cows (AA × CP interaction, $P \leq 0.15$). There was no effect ($P > 0.15$) of treatment on body condition at wk 15 postpartum.

Blood Metabolite Concentrations

There were no effects of treatment ($P > 0.15$) on postpartum plasma NEFA and BHBA concentrations (Table 7). Cows fed RPMet+Lys tended to have lower plasma glucose concentrations ($P \leq 0.15$) than cows fed only the basal diet. Feeding RPMet reduced serum urea concentrations ($P \leq 0.05$) compared with feeding no RPAA or feeding RPMet+Lys. Cows fed the 16.0% CP diets had lower serum urea concentrations ($P \leq 0.05$) than cows fed the 18.5% CP diets (Table 7).

The effect of RPAA supplementation on plasma glucose concentrations was dependent upon week postpartum (AA × week interaction, $P \leq 0.05$; Figure 5). Between wk 1 and 4 postpartum, plasma glucose concentrations of cows receiving RPMet+Lys declined at a more rapid rate than cows receiving either the basal diet or RPMet. However, between wk 4 and 7 postpartum, plasma glucose concentrations of RPMet+Lys supplemented cows increased, whereas plasma glucose concentrations of cows receiving no RPAA or RPMet remained essentially unchanged.

The effect of dietary CP and RPAA supplementation on plasma glucose concentration was not consistent across time (AA × CP × week interaction, $P \leq 0.05$). Immediately following parturition, glucose concentrations decreased for cows receiving 16M, 16ML, 18.5B, and 18.5ML, and increased for cows receiving 18.5M (Figure 6). As cows approached peak production, plasma glucose concentrations decreased for cows receiving the 16M, 18.5B, and 18.5M treatments and increased for cows receiving 16ML and 18.5ML. The large drop in glucose concentrations following parturition for cows fed RPMet+Lys may be attributed at least in part to the higher yield of ECM (Table 7).

Incidences of health disorders were minimal. One cow fed the 18.5ML diet was treated for displaced abomasum. Nine cows were treated for off-feed problems related to subclinical or clinical ketosis; 1 fed 16B, 2 fed 16M, 1 fed 16ML, 2 fed 18.5B, 2 fed 18.5M, and 1 fed 18.5ML.

DISCUSSION

Lactation Responses to RPMet and RPMet+Lys

The first 2 objectives of this study were to determine milk production responses of early-lactation dairy cows

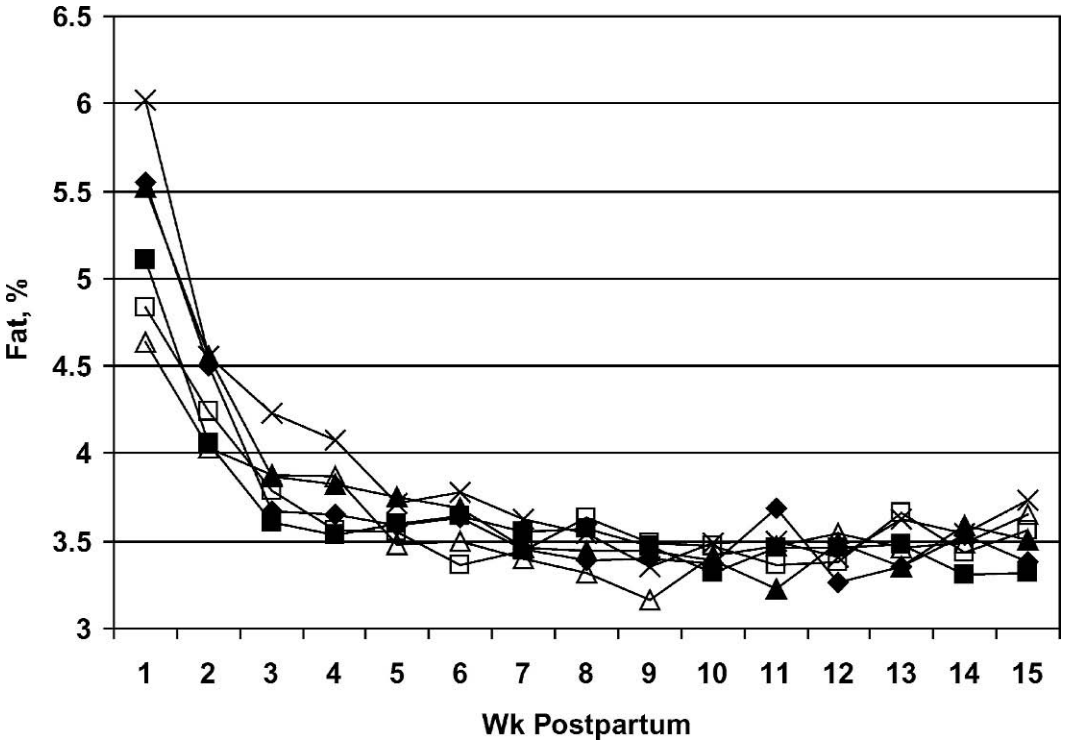

Figure 4. Response to dietary CP and amino acid supplementation across time: Milk fat content. 16% CP diet, no rumen-stable AA supplementation (◆), 16% CP diet plus rumen-protected Met product that supplied 10.5 g of Met (■), 16% CP diet plus rumen-protected Met plus Lys product that supplied 10.2 g of Met and 16.0 g of Lys (▲), 18.5% CP diet, no rumen-stable AA supplementation (△), 18.5% CP diet plus rumen-protected Met product that supplied 10.5 g Met (×), 18.5% CP diet plus rumen-protected Met plus Lys product that supplied 10.2 g of Met and 16.0 g of Lys (□). Week × AA × dietary CP supplementation effect, $P \leq 0.05$. Pooled SEM = 0.16.

to RPMet and RPMet+Lys supplementation when supplementation commenced in late gestation and cows were fed corn-based diets containing high-Lys protein supplements. Compared with cows receiving no RPAA, cows receiving RPMet+Lys produced more ECM (45.9 vs. 43.6 kg/d), true protein (1306 vs. 1221 g/d), and fat

Table 6. Body weight and body condition score responses during the first 105 d of lactation of multiparous Holstein cows fed rumen-protected Met or rumen-protected Met plus Lys at 2 levels of dietary CP.

	Treatments[1]							
	18.5% CP			16.0% CP				
Item	Basal	M	M+L	Basal	M	M+L	SE[2]	Effects[3]
BW, kg								
Wk 1	633	636	642	623	611	651	16	
Wk 15	618	619	629	612	601	626	17	
Change (wk 15 – wk 1)	–15	–16	–13	–11	–10	–25	9	
BCS								
Wk 1	3.6[c]	3.5[bc]	3.4[a]	3.3[a]	3.3[a]	3.6[c]	0.1	aa × cp
Wk 15	2.8	2.6	2.5	2.6	2.7	2.8	0.2	
Change (wk 15 – wk 1)	–0.7	–0.9	–0.9	–0.6	–0.6	–0.7	0.2	

[a,b,c]Within a row, means with uncommon superscripts differ at $P \leq 0.15$.

[1]Treatments were basal, 15 g/d of a rumen-protected Met product which supplied 10.5 g of Met (M), and 6 g/d of rumen-protected Met product plus 40 g/d of a rumen-protected Met plus Lys product which together supplied 10.2 g of Met and 16.0 g of Lys (M+L).

[2]Standard error of the least squares mean; n = 11 for BW; n = 13 for BCS.

[3]Only treatment effects with $P \leq 0.15$ reported; aa × cp = amino acid by crude protein interaction, $P \leq 0.15$.

Table 7. Blood metabolite concentrations of early-lactation multiparous Holstein cows fed rumen-protected Met or rumen-protected Met plus Lys at 2 levels of dietary CP.

	Treatments[1]							
	18.5% CP			16.0% CP				
Item	Basal	M	M+L	Basal	M	M+L	SE[2]	Effects[3]
Plasma metabolites								
Glucose, mg/dL	79.7	79.4	74.7	81.0	78.3	73.8	3.1	basal vs. m+l; AA × WK; AA × CP × WK
NEFA, μmol/L	377	447	431	399	374	461	37	
BHBA, mg/dL	5.14	4.83	5.75	4.48	4.27	4.68	0.67	
Serum urea, mg/dL	19.4	17.5	17.9	13.3	12.4	13.1	0.7	Basal, M+L vs. M; CP

[1]Treatments were basal, 15 g/d of a rumen-protected Met product which supplied 10.5 g of Met (M), and 6 g/d of rumen-protected Met product plus 40 g/d of a rumen-protected Met plus Lys product which together supplied 10.2 g of Met and 16.0 g of Lys (M+L).

[2]Standard error of the least squares mean, n = 13.

[3]Only treatment effects with $P \leq 0.15$ reported: basal vs. m+l, $P \leq 0.15$; Basal, M+L vs. M, $P \leq 0.05$; CP = crude protein effect, $P \leq 0.05$; AA × WK = amino acid by week interaction, $P \leq 0.05$; AA × CP × WK = amino acid by dietary crude protein by week interaction, $P \leq 0.05$.

(1632 vs. 1550 g/d, Table 5), and tended to produce more 3.5% FCM (45.9 vs. 43.8 kg/d). These production responses to RPMet+Lys were larger than reported previously by others (Armentano et al., 1993; Rulquin and Vérité, 1993) and support the hypothesis that the greatest responses to improved Lys and Met nutrition occur during the earliest stages of lactation when the need for absorbed AA, relative to absorbed energy, is the highest.

The larger than expected response in milk yield to the increased intestinal supplies of Lys and Met in this study may be attributed to the fact that the cows received supplemental AA before calving. In 3 other trials in which RPMet or RPMet+Lys were added to lactating dairy diets beginning before calving and continuing through early lactation, milk yield was increased by an average of 1.2 kg/d (Overton et al., 1996; Carson et al., 1998; Xu et al., 1998). In comparison, initiating RPMet,

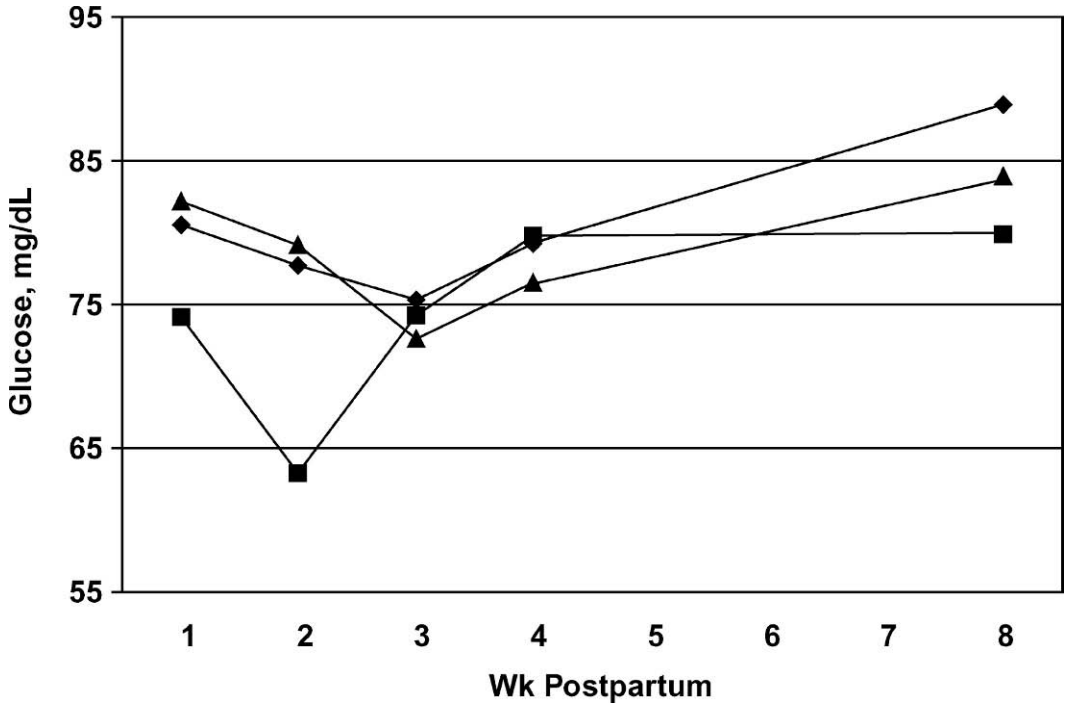

Figure 5. Response to amino acid supplementation across time: Plasma glucose concentration. Basal diet, no rumen-stable AA supplementation (◆), basal diet plus rumen-protected Met product that supplied 10.5 g Met (▲) and basal diet plus rumen-protected Met plus Lys product that supplied 10.2 g of Met and 16.0 g of Lys (■). Week × AA supplementation effect, $P \leq 0.05$. Pooled SEM = 3.4.

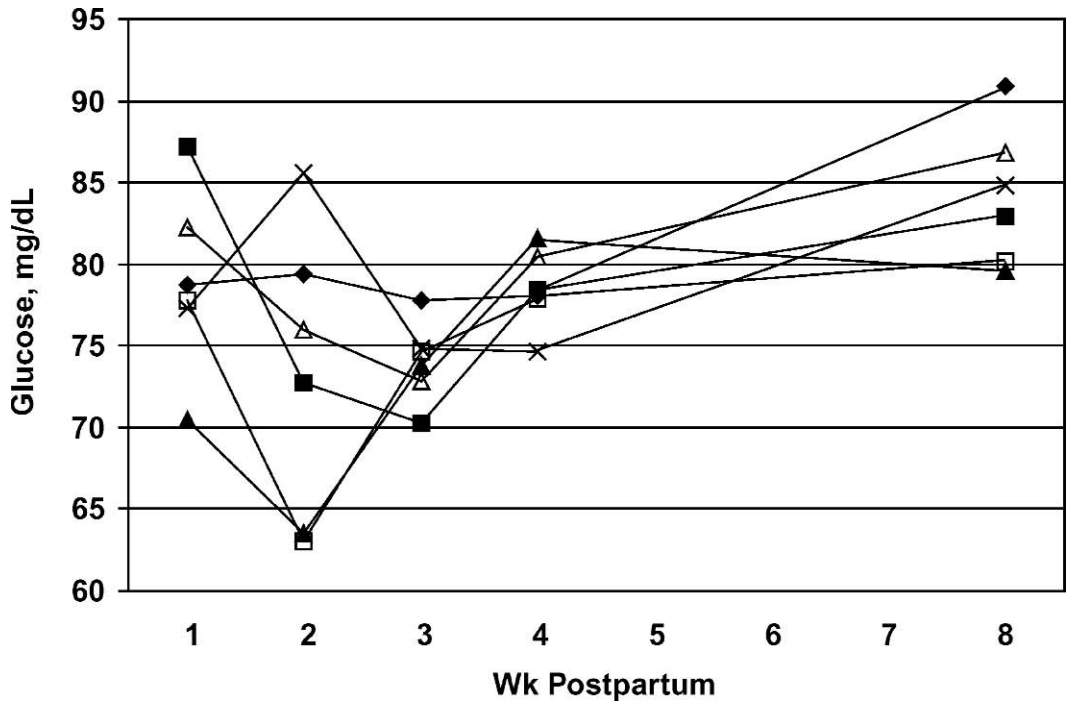

Figure 6. Response to dietary CP and amino acid supplementation across time: Plasma glucose concentration. 16% CP diet, no rumen-stable AA supplementation (◆), 16% CP diet plus rumen-protected Met product that supplied 10.5 g Met (■), 16% CP diet plus rumen-protected Met plus Lys product that supplied 10.2 g of Met and 16.0 g of Lys (▲), 18.5% CP diet, no rumen-stable AA supplementation (△), 18.5% CP diet plus rumen-protected Met product that supplied 10.5 g Met (×), 18.5% CP diet plus rumen-protected Met plus Lys product that supplied 10.2 g of Met and 16.0 g of Lys (□). Week × AA × dietary CP supplementation effect, $P \leq 0.05$. Pooled SEM = 4.0.

RPLys, or RPMet+Lys supplementation after parturition (wk 1 to 20 postpartum) increased milk yield an average of only 0.1 kg/d (Armentano et al., 1993; Wu et al., 1997; Bertrand et al., 1998; Robinson et al., 1998; Samuelson et al., 2001; Moore et al., 2003; Noftsger and St. Pierre, 2003). However, increases in milk protein yield in response to RPAA supplementation appeared to be less affected by the stage of lactation in which supplementation was initiated. In the above experiments, starting supplementation before calving increased milk protein yield an average of 82 g/d (an increase of 7.1%), whereas initiating AA supplementation after calving increased milk protein yield an average of 35 g/d (an increase of 3.1%).

This experiment revealed no significant interactions of dietary CP and AA supplementation for milk and milk component yields. However, milk and milk component yield responses to RPMet+Lys were numerically greater with the 18.5% CP diet than with the 16.0% CP diet (Table 5). Greater responses to RPLys+Met supplementation of 18.5B could have occurred if feeding this diet resulted in Lys, Met, or both being more deficient than feeding 16.0B. However, this did not appear to be the case. The 2 basal diets were evaluated with the NRC (2001) model and predicted flows of MP-Lys and MP-Met were 172 and 44 g/d for 18.5B and 157 and 42 g/d for 16.0B when DMI was held constant at 23.6 kg/d (Table 4). The higher predicted flows of MP-Lys and MP-Met for 18.5B resulted primarily because of higher predicted flows of MP (2745 vs. 2582 g/d). The predicted concentrations of Lys and Met in MP for the 2 basal diets were similar (6.3 and 1.6% for 18.5B; 6.1 and 1.6% for 16.0B) (Table 4). These results are supported by observations in a companion experiment where the 2 basal diets were fed to early-lactation, primiparous Holstein cows that were fitted with ruminal and duodenal cannulas (Putnam et al., 1997). Measured concentrations of Lys and Met in total AA of duodenal digesta were 6.8 and 2.0% for both diets. Measured flows of total AA for 18.5B and 16.0B were 3420 and 3042 g/d, respectively. Intakes of DM for 18.5B and 16.0B were 18.3 and 18.1 kg/d, respectively.

The increase in milk protein yield with RPMet+Lys supplementation in this study is consistent with other results (Donkin et al., 1989; Chapoutot et al., 1992; Robinson et al., 1992; Armentano et al., 1993; Robinson et al., 1993). However, in contrast to the previous observations, the increased yield of milk protein in the current experiment occurred mainly because of an increase

in milk production, rather than an increase in milk protein content.

Somewhat surprising was the fact that feeding RPMet alone did not increase yield of milk protein and increased content of milk protein only when added to 18.5B (Table 5). It is concluded in NRC (2001) that the required concentrations of Lys and Met in MP for maximum content and yield of milk protein are 7.1 to 7.2 and 2.4%, respectively, when the NRC (2001) model is used to predict concentrations of AA in MP. If these required concentrations are correct, then the optimum Lys:Met ratio in MP is 3.0:1. However, the evaluation of 18.5B and 16.0B with NRC (2001) yielded Lys:Met ratios in MP of 3.9:1 (6.3:1.6) and 3.8:1 (6.1:1.6), respectively (Table 4). In the companion study, Putnam et al. (1997) observed Lys:Met ratios in duodenal digesta of 3.5:1 for both of the basal diets. Predicted duodenal supplies of Lys and Met using a factorial model (CPM-Dairy, version 3.0; New Bolton Center, University of Pennsylvania, Kennett Square, PA) resulted in Lys:Met ratios of 3.4:1 and 3.3:1, respectively, for 18.5B and 16.0B. In all cases, it is concluded that Met was more limiting than Lys and that the cows should have responded to RPMet supplementation.

One factor that may have contributed to cows not responding with increased yield of milk protein to RPMet supplementation is that the digestibility of Lys in the RUP fraction of blood meal may have been less than the measured digestibility coefficient of 60.7% for RUP. Of concern is that the blood meal was exposed to excessive heat during processing. This possibility is supported by the observations that the measured RUP digestibility (60.7% of CP) and Lys content (7.70% of CP) of the ring-dried blood meal (Table 3) are both less than the NRC (2001) default values of 80 and 8.98%, respectively. It is well documented that Lys is the most vulnerable of the essential amino acids to heat damage (Schwab, 1995). For example, increasing the amount of heat applied to cottonseed meal (Broderick and Craig, 1980; Craig and Broderick, 1981), soybean meal (Parsons et al., 1992), and whole soybeans (Faldet et al., 1992) has been shown to decrease Lys concentration and the availability of the remaining Lys. There was no attempt in this experiment to measure Lys digestibility in the blood meal. However, it is of interest to note that even if Lys digestibility in the RUP fraction of the blood meal was decreased to 30%, calculated flows of MP-Lys would be decreased by 6 g for 18.5B and 5 g for 16.0B, lowering the Lys:Met ratios in MP from 3.9:1 and 3.7:1 for the 2 basal diets to 3.8:1 and 3.6:1, respectively.

CP-Sparing Effect of RPAA

The third objective of this study was to assess the dietary CP-sparing effect of RPMet and RPMet+Lys. This was difficult to assess as CP only affected efficiency of conversion of dietary N to milk N and the only significant CP × AA effects were observed for milk fat and true protein content. However, cows receiving 16ML numerically consumed more DM (24.3 vs. 23.9 kg/d), produced more ECM (45.2 vs. 43.7 kg/d), and converted dietary N to milk N with a higher gross efficiency (35 vs. 29%) than cows receiving 18.5B (Table 5). These results suggest that 16ML was similar, if not superior, in nutritive value to 18.5B.

Accurate assessment of the CP-sparing effects of RPMet and RPMet+Lys requires a number of diets with varying levels of CP, and more specifically, different levels of RUP rather than RDP. Thus, the treatments in this study did not lend themselves to effectively determining the CP-sparing effect of RPAA, as response to RPMet and RPMet+Lys was examined at only 2 dietary CP levels, and CP levels were increased from 16 to 18.5% CP by increasing the RDP fraction rather than the RUP fraction.

Effect of RPAA on Blood Energy Metabolites

There was no effect of RPAA supplementation on clinical ketosis and other postcalving metabolic disorders. This is consistent with the lack of an observed effect of RPAA supplementation on postpartum plasma NEFA and BHBA. Effect of RPAA supplementation on plasma glucose was dependent on week postpartum, with cows supplemented with RPMet+Lys having lower plasma glucose concentrations during wk 1 and 2 postpartum than cows fed the other diets. The lower plasma glucose concentrations may be reflective of the fact that the cows supplemented with RPMet+Lys produced more ECM; across the 2-wk period, the cows produced 2.8 kg/d more ECM than the other cows (42.2 vs. 39.4 kg) while consuming only 0.9 kg/d more DM (17.7 vs. 16.8 kg). For the last 13 wk of the 15-wk treatment period, the cows fed RPMet+Lys produced 2.5 kg/d more ECM than the other cows (46.4 vs. 43.9 kg) and consumed 1.0 kg/d more DM (25.3 vs. 24.3 kg). In previous research, plasma NEFA, glucose, and BHBA were not affected when lactating dairy cows were fed RPMet+Lys (Chow et al., 1990; Chapoutot et al., 1992; Xu et al., 1998).

In contrast, postruminal infusion of a basal amount of Lys and incremental amounts of Met resulted in a linear increase in plasma BHBA of cows entering the second 100 d of lactation (Socha, 1994), but had no effect on plasma BHBA of cows assigned to the treatments before 50 DIM (Pisulewski et al., 1996; Socha, 1994) or after 150 DIM (Socha, 1994). In these same infusion studies, increasing the intestinal supply of Met linearly reduced plasma NEFA concentrations when the cows

were assigned to the studies before 50 DIM (Pisulewski et al., 1996; Socha, 1994) but not when they began receiving treatments as they approached 100 DIM or after 150 DIM (Socha, 1994). One potential reason why blood concentrations of energy metabolites such as NEFA, BHBA, and glucose are not affected by improved Lys and Met nutrition in production studies (Chow et al., 1990; Chapoutot et al., 1992; Xu et al., 1998) is that the effect may be transitory. In the production studies, the experimental periods were 21 d or longer, with blood samples usually being collected several weeks after initiation of treatments. This is in contrast to the infusion experiments where the length of experimental periods was 10 to 14 d and all blood samples were taken less than 2 wk after initiation of treatments (Socha, 1994; Pisulewski et al., 1996).

CONCLUSIONS

Supplementing the basal diets of early lactation cows with RPMet+Lys increased yield of ECM, milk true protein, and milk fat and tended to decrease concentrations of plasma glucose. It was difficult to assess the CP-sparing effect of RPMet+Lys due to the lack of a dietary CP or CP × AA effect on production of milk and milk components. However, cows receiving 16ML produced numerically more milk, FCM, ECM, protein, and fat than cows receiving 18.5B. Dairy cows in early lactation are sensitive to changes in intestinal AA balance, and their lactation performance may be enhanced considerably by optimizing Lys and Met nutrition. The lack of a response to RPMet illustrates the importance of characterizing the protein fractions of protein sources.

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