Extent of Methionine Limitation in Peak-, Early-, and Mid-Lactation Dairy Cows^{1,2}

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ABSTRACT

Five multiparous, ruminally and duodenally cannulated Holstein cows were assigned to 5 × 5 Latin squares at wk 2 (experiment 1), wk 11 to 13 (experiment 2), and wk 17 to 19 postpartum (experiment 3) to determine extent of Met limitation. Treatments were duodenally infused and consisted of 10 g/d of L-Lys plus 0, 3.5, 7.0, 10.5, or 16.0 g/d of DL-Met in experiments 1 and 2 and 8 g/d of L-Lys plus 0, 5, 10, 15, or 20 g/d of DL-Met in experiment 3. Calculated Lys contributions to total AA (TAA) in duodenal digesta for control treatments were 8.6, 7.5, and 9.0% for experiments 1, 2, and 3, respectively. Methionine contributions to TAA for the 5 infusion treatments were 1.9, 2.1, 2.2, 2.4, and 2.7% for experiment 1; 2.1, 2.3, 2.4, 2.5, and 2.7% for experiment 2; and 1.8, 2.0, 2.2, 2.4, and 2.5% for experiment 3, respectively. Milk protein yield increased linearly in experiments 1 and 2, indicating that Met contribution to TAA in duodenal digesta for maximal milk protein synthesis exceeded 2.7 for early-lactation cows. In experiment 2, a quadratic relationship was found between level of infused Met and milk protein content, with the response reaching a plateau when 12.2 g of Met was infused, corresponding with a Met contribution to TAA in duodenal digesta of 2.4%. In experiment 3, milk protein content increased quadratically, but milk yield declined linearly with increasing levels of infused Met; hence, milk protein yield was unaffected by treatment. The calculated plateau point of the milk protein content response curve was determined to be 12.4 g of infused Met, which corresponds to a Met contribution to TAA in

duodenal digesta of 2.3%. Experiment 3 results indicate that the required level of Met in duodenal digesta for maximizing milk protein yield is lower than that required for maximizing milk protein content.

Key words: methionine limitation, milk protein, lactating dairy cow

INTRODUCTION

Optimizing the balance of total AA (TAA) in MP is fundamental to maximizing lactation performance with minimal dietary protein. Realizing this goal should reduce urinary N output per unit of milk produced as well as spare metabolic energy for milk production or other body functions. Fine tuning of diets with respect to AA will require knowledge of the cow's requirements and the ability to formulate diets that provide a balanced quantity of absorbable AA. Presently, the literature on determining AA requirements of lactating dairy cows is extremely limited.

Research identifies Lys and Met as the first 2 limiting AA when corn-based diets are fed (Rulquin, 1987; Schwab et al., 1976, 1992a,b). Research also demonstrates that milk protein percentage is the most sensitive of the production responses to alterations in intestinal supply of Lys and Met (NRC, 2001; Rulquin et al., 1993; Rulquin and Vérité, 1993). Furthermore, the casein fraction of milk protein is most affected by alterations in Lys and Met content of duodenal digesta (Donkin et al., 1989; Robert et al., 1989; Chow et al., 1990; Le Henaff et al., 1990). The sequence of AA limitation has not been elucidated beyond determining that Lys and Met are most limiting when corn-based diets are fed.

Progress has been made toward the determination of Lys requirements of lactating dairy cows fed corn-based diets. By posttruminally infusing incremental amounts of Lys, Schwab et al. (1992b) determined that yield and content of milk protein reached a plateau when Lys contributed 7.2, 6.4, and 6.9% of TAA (mean of 3 experiments was 6.8%) in duodenal digesta for

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peak-, postpeak-, and mid-lactation cows fed diets containing 17.3, 14.4, and 14.1% CP, respectively. This first approximation of a Lys "requirement", expressed on the basis of profiles, probably underestimates the true Lys requirement; some AA undoubtedly were supplied in excess relative to need, whereas the supply of others may have become deficient as additional Lys was supplied, thereby limiting the response to additional Lys. Therefore, a more precise estimate of the Lys requirement cannot be obtained using the empirical, dose-response approach without knowledge of requirements for other AA.

The principal objective of this study was to determine, in the presence of supplemental Lys, the dose-response relationships between contributions of Met to TAA in duodenal digesta and milk protein production during peak, early, and mid lactation, and from those relationships, identify the Met requirements for milk protein synthesis.

MATERIALS AND METHODS

Experimental Design and Treatments

Three 5 × 5 Latin square experiments were conducted during peak (experiment 1), early (experiment 2), and mid (experiment 3) lactation, each using the same 5 multiparous Holstein cows. At 6 to 8 wk prepartum, cows were fitted surgically with a plastisol 10.2-cm ruminal cannula (Bar Diamond Inc., Parma, ID) and a plastisol closed-flange, T-shaped duodenal cannula (provided by J. H. Herbein, Virginia Tech, Blacksburg). Animals were sedated with intravenous xylazine (0.02 mg/kg of BW) and butorphenol (0.01 mg/kg of BW), and the cannulation surgeries were conducted with the animals standing. An inverted L-block was completed with 200 to 300 mL of 2% lidocaine hydrochloride plus epinephrine before the 20-cm vertical incision. The 20-cm vertical incision was made starting approximately 10 cm below the transverse processes, just caudal to the last rib. The pylorus was located and exteriorized. The duodenal cannula was inserted proximal to the pancreatic and bile duct, approximately 10 cm distal to the pylorus. The intestine was internalized, allowed to reassume its natural position, and the duodenal cannula was exteriorized usually in the tenth intercostal space at the level of the costochondral junction and positioned with the barrel of the cannula ascending. Cannulas were immobilized with a soft plastisol, external o-ring for 2 to 3 wk to ensure intestinal adhesion to the body wall. Ruminal cannulas were inserted immediately following the duodenal cannulation. Total surgical time was 2 to 3 h. All procedures related to animal care were conducted with the approval of the

Table 1. Ingredient composition of diets

Ingredient, % of DM	Experiment		
	1	2	3
Corn silage	20.7	21.1	22.0
Alfalfa hay	10.3	10.6	11.0
Haycrop silage	10.4	10.5	11.0
Corn, ground shelled	33.9	36.9	38.7
Soybean meal, solvent	11.9	8.9	5.8
Raw soybeans, cracked	6.0	6.0	6.0
Mineral-vitamin mix ¹	4.0	4.0	4.0
Blood meal, ring-dried	1.4	1.0	0.7
Fat ²	1.4	1.0	0.7

¹Mineral-vitamin composition (% of DM): 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, 29,510 IU/kg of vitamin A, 9,443 IU/kg of vitamin D, 94 IU/kg of vitamin E.

²Alifet (Alifet USA Inc., Cincinnati, OH).

University of New Hampshire Institutional Animal Care and Use Committee.

Cows were assigned to experiment 1 at similar stages of lactation (10 to 15 d postpartum) with a range in parturition dates of 21 d; the same cows were assigned to treatments in experiment 2 (11 to 13 wk postpartum) and experiment 3 (17 to 19 wk postpartum) on the same calendar date. Each period of the Latin square lasted 8 d. Cows were allowed 3 d to adapt to the new infusate, and milk weights and samples were collected on d 4 to 8 of each period. The 5 treatments were infused into the duodenum via the duodenal cannula and consisted of 10 g/d of L-Lys plus 0, 3.5, 7.0, 10.5, or 16.0 g/d of DL-Met in experiments 1 and 2 and 8 g/d of L-Lys plus 0, 5, 10, 15 and 20 g/d of DL-Met in experiment 3. Amino acids were dissolved daily in 4 L of tap water. Solutions were infused continuously by using two 4-channel peristaltic pumps (Haake Buchler Instruments Inc., Saddlebrook, NJ).

Feeding and Management of Cows

Diets were formulated to meet or slightly exceed all nutrient requirements for lactating dairy cows (NRC, 2001) and fed as a TMR (Table 1). Cows started receiving the experimental diets 10 d before each experiment. Diets were prepared by weighing each ingredient and blending in a drum-type mixer (Data Ranger, American Calan Inc., Northwood, NH). The alfalfa hay was chopped before incorporation into the TMR by using a bale chopper (model 6-90, Wic Inc., Johnson, Quebec, Canada). Feed offered was adjusted daily to achieve 5 to 10% orts. Cows were fed 67% of the total daily allotment at 1600 h and the remaining 33% at 0500 h. Orts were collected at 1400 h. Feed ingredients were sampled for chemical analysis before initiation of each experiment. Forage to grain ratio was adjusted to achieve

21% forage NDF in the diet. Amounts of corn and soybean meal were adjusted to achieve dietary CP concentrations of 18.5, 17.3, and 16.0% of DM for experiments 1, 2, and 3, respectively.

Measurements, Collection and Analyses of Samples

Samples of orts were obtained daily during d 4 to 8 of each period, composited by cow, and frozen (-20°C) immediately. After each period was completed, orts were thawed at room temperature (21°C), dried at 60°C for 18 h in a convection oven, ground to pass through a 1-mm screen, and composited by experiment according to the amount of orts for each cow in each period before analysis. Samples of feed ingredients were collected weekly, dried at 60°C under 760 mm of vacuum for 24 h, ground to pass through a 1-mm screen, and composited across experiment. Composited feed ingredients and orts were analyzed for CP, NDF, ADF, ether extract, Ca, P, K, Mg, and S (NEDHIC, Ithaca, NY).

Milk weights were recorded and samples collected at each milking during d 4 to 8 of each period. Milk samples were composited according to production for d 4 to 6 and d 7 to 8, preserved with 2-bromo-2-nitropropane-1,3 diol, and analyzed for fat and true protein content (Dairy One, Ithaca, NY). Dry matter intake was recorded for d 4 to 8 of each period to correspond with milk sampling.

Chromium oxide was used as the unabsorbable digesta flow marker for intestinal flow measurements. Five grams of chromium oxide was wrapped in filter paper (P8, Fisher Inc., Pittsburgh, PA) and placed in the ruminal mat at 0400, 1000, 1600, and 2200 h via the ruminal cannula. Administration began 10 d before duodenal sampling in period 1 of each experiment and continued daily throughout each experiment.

Duodenal digesta samples (approximately 500 mL) were collected every 3 h on d 1 to 3 of each period; the initial sample was frozen (-20°C) immediately and subsequent samples were added to the frozen composite. On each successive day, sampling advanced 1 h; thus, samples were taken at 1-h intervals for a 24-h period. Digesta collected during the first 30 s after removing the cannula plug (generally a minimum of 200 mL) was discarded. At the end of each 3-d collection period, duodenal digesta was allowed to thaw at room temperature (21°C) and homogenized using a 3.8-L commercial blender (Waring Products Division, Hartford, CT). Duplicate samples of duodenal digesta were analyzed for DM (60°C for 18 h in a convection oven) and NH_3N . Samples collected for NH_3N were strained through 59- μm Dacron mesh and frozen for analysis. A portion of duodenal digesta was lyophilized and ground through a 0.4-mm screen. Lyophilized samples were

ashed at 500°C for 16 h in a muffle furnace for measurement of OM. Chromium content of duodenal samples was determined by using atomic absorption spectrophotometry (model Smith-Hieftje 12, Thermo Jarrell Ash, Franklin, MA) according to the procedure of Williams et al. (1962). Concentrations of ADF and N were determined according to AOAC methods 973.18 and 976.06 (AOAC, 1990) respectively, and NDF content was determined using the procedure of Van Soest et al. (1991). Nonstructural carbohydrate content of feed and duodenal samples was determined by using the procedure of Smith (1981) modified to use ferricyanide as the colorimetric indicator. Concentrations of AA were determined by using the procedure outlined by Cunningham et al. (1993).

Ruminal fluid (approximately 200 mL) was obtained on d 7 of each period at 2, 4, 6, and 10 h after the a.m. feeding. Samples were obtained from 4 different locations in the rumen by using manual vacuum applied through a 1.27-cm polyvinyl chloride pipe to minimize disturbance of the ruminal mat. Ruminal fluid was strained immediately through 59- μm Dacron mesh, and 40 mL of strained fluid was added to 2.4 mL of 6 N HCl and frozen for NH_3N analysis. Strained ruminal fluid collected for VFA analysis (1 mL/sample) was composited by cow across the 4 time points; the initial sample was frozen immediately and subsequent samples were added to the frozen composite. The samples were thawed and analyzed via a Hewlett-Packard gas chromatograph (model 5880, Hewlett-Packard Co., Boise, ID) following the procedure of Erwin et al. (1961), modified to use cyclohexanone as an internal standard. The cyclohexanone was added to samples acidified with 25% metaphosphoric acid before injection. Ruminal fluid and duodenal digesta were analyzed for NH_3N using a gas-ammonium electrode (Orion model 407A meter with 95-12 electrode, Orion Research Inc., Boston, MA).

Blood samples were obtained by venipuncture of the coccygeal vein at approximately 2 h after the a.m. feeding on d 7 and 8 of each period. 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 $3,300 \times g$ for 20 min at 4°C . One aliquot of plasma was removed and frozen (-20°C) for glucose and NEFA analyses. A second aliquot was deproteinized; 4 volumes were vortexed with 1 volume of 15% sulfosalicylic acid, centrifuged at $3,300 \times g$ for 20 min at 4°C , and the supernatant frozen for BHBA analysis. Blood in tubes containing no additive was allowed to clot at room temperature (15 to 21°C), centrifuged ($3,300 \times g$ for 20 min), and the serum frozen. Plasma samples were

thawed at 5°C and analyzed for glucose (Sigma kit Trinder 500, Sigma Chemical Co., St. Louis, MO) and NEFA concentrations (Wako NEFA C kit, Wako Chemicals USA Inc., Richmond, VA). Deproteinized plasma and serum samples were thawed at 5°C and analyzed for concentrations of BHBA (Gibbard and Watkins, 1968) and urea (Sigma kit 640, Sigma Chemical Co.).

Statistical Analysis and Calculations

Repeated measurements (i.e., milk production, milk composition, ruminal ammonia) were reduced to period means for each cow before analysis. Data were analyzed as a 5 × 5 Latin square design according to the following model:

$$Y_{ijk} = \mu + M_i + P_j + C(S)_{ik} + E_{ijk}$$

where Y_{ijk} = the dependent variable; μ = overall mean; M_i = the fixed effect of the i th level of methionine, $i = 1, \dots, 5$; P_j = the random effect of the j th period, $j = 1, \dots, 5$; $C(S)_{ik}$ = the random effect of the k th cow within the i th level of methionine, $k = 1, \dots, 5$; and E_{ijk} = the random residual $\sim N(0, \sigma_e^2)$.

The MIXED procedure of SAS (release 8.2; SAS Institute, 1999) was used to solve the model. Degrees of freedom were calculated using the Satterthwaite option of the MIXED procedure (SAS Institute, 1999). Fixed effects included treatment, and the random effects were cow and period. Because levels for the infused Met were not equally spaced, appropriate orthogonal coefficients were generated using PROC IML of SAS. Linear, quadratic, and cubic contrasts were fitted within each Met level. Least squares means were determined for Met level. Significant treatment effects were noted at $P \leq 0.05$ and trends for treatment effects were noted at $P > 0.05$ and ≤ 0.15 . There were no missing observations. Each stage of lactation was analyzed individually.

With the mixed model that was used (*observed Y = predicted Y + residual*), *predicted Y* accounts for all the effects in the model (e.g., cow, period). Thus, a corrected (also called adjusted) observation for cow i infused the j th level of Met is equal to the least squares means for the corresponding mean plus the residual error for the observation. This in effect corrects the observation for all random and fixed effects of the mixed model as the multidimensional space was collapsed into a plane consisting of the response variable (Y) on the first axis and the levels of Met on the second axis. A similar procedure has been used before in the context of a meta-analysis using mixed model methodology (St-Pierre, 2001).

The corrected observations, noted as Y_{adj} , were then modeled for each Met level using 2 alternative continu-

ous nonsmooth models. Model A was the traditional threshold and plateau model:

$$Y_{adjij} = B_0 + B_1 L_i + E_{ij} \text{ if } L_i < L^*$$

and

$$Y_{adjij} = B_0 + B_1 L^* + E_{ij} \text{ if } L_i \geq L^*$$

where Y_{adj} = the adjusted dependent variable; B_0 = the intercept; B_1 = the slope of the straight line (regression) linking Y to L at levels under the threshold L^* ; L_i = the level of Met fed; and L^* = the threshold level of Met at which cows cease to respond (plateau).

Model B was also a threshold model, but it does not assume a plateau past the threshold level but only a change of slope as follows:

$$Y_{ij} = B_0 + B_1 L_i + E_{ij} \text{ if } L_i < L^*$$

and

$$Y_{ij} = B_0 + B_1 L^* + B_2 (L_i - L^*) \text{ if } L_i \geq L^*.$$

Model B is an expansion of model A and requires the estimation of one additional parameter. The significance of this parameter was tested using the reduction in error mean square. In both models B_0 , B_1 , and L^* are parameters to be estimated. The NLIN procedure (SAS Institute, 1999) was used to estimate parameters, and results were deemed significant at the 5% probability level.

RESULTS

Chemical Composition of Diets

The ingredient and chemical compositions of the diets are shown in Tables 1 and 2. Chemical composition of the consumed diet was calculated from chemical components measured in each ingredient and subtracting the amount of corresponding components inorts from the total amounts offered. Concentrations of RDP and RUP in the diets were calculated by using the NRC (2001) model and the model default values for all N fractions and digestion rates of the ingredients. Based upon average milk production and DMI in each of the 3 infusion studies (Table 2), the RDP, RUP, and MP requirements, respectively, in experiment 1 were 2,112, 1,484, and 2,458 g/d; experiment 2: 2,511, 1,359, and 2,596; and experiment 3: 2,526, 1,337, and 2,527 g/d. The RDP, RUP, and MP balances, respectively, in experiment 1 were 336, -61, and -50 g/d; experiment 2: 266, 343, and 280 g/d; and experiment 3: 100, 179, and 145 g/d. Corn

Table 2. National Research Council (2001) evaluation of the basal diets¹ fed in peak (experiment 1), early (experiment 2), and mid (experiment 3) lactation using actual DM intakes

Chemical component	Experiment ²		
	1	2	3
NDF, % of DM	27.0	26.8	26.7
Forage NDF, % of DM	21.0	20.9	21.0
RDP, % of DM	11.9	10.9	10.3
RUP, % of DM	6.9	6.7	5.9
CP, % of DM	18.8	17.6	16.2
RDP requirement, g/d	2,112	2,511	2,526
RUP requirement, g/d	1,484	1,359	1,337
MP requirement, g/d	2,458	2,596	2,577
RDP balance, g/d	336	266	100
RUP balance, g/d	-61	343	179
MP balance, g/d	-50	280	145
NE _L balance, g/d	-5.1	3.7	3.5
MP AA, ³ % of MP			
Arg	4.7	4.6	4.6
His	2.2	2.2	2.2
Ile	4.5	4.4	4.5
Leu	8.9	9.0	9.1
Lys	6.4	6.2	6.3
Met	1.6	1.6	1.7
Phe	4.9	4.9	4.9
Thr	4.7	4.7	4.7
Val	5.4	5.4	5.4
MP-Lys, ³ g/d	153	179	171
MP-Met, ³ g/d	39	47	46
MP flow, ³ g/d	2,408	2,877	2,722

¹Diets were evaluated using nutrient profiles listed in Tables 3 and 4. For nutrients not listed in Tables 3 and 4, default values were used with the exception of RUP digestibility of blood meal. Analysis of blood meal indicated that the RUP digestibility of blood meal fed in this study was 60.7% (M. Stern, University of Minnesota, personal communication).

²Model inputs for evaluation of diets were: peak lactation diet (experiment 1); lactating Holstein cow, 38 mo of age, 632 kg of BW, 28 DIM, 40.6 kg/d milk, 4.11% milk fat, 2.84% milk true protein, 0 d pregnant, 20.6 kg/d DMI, second lactation, default environmental conditions; early lactation diet (experiment 2) lactating Holstein cow, 40 mo of age, 614 kg of BW, 98 DIM, 38.2 kg/d milk, 3.73% milk fat, 3.00% milk true protein, 0 d pregnant, 25.4 kg/d DMI, second lactation, default environmental conditions; and mid lactation diet (experiment 3) lactating Holstein cow, 42 mo of age, 632 kg of BW, 140 DIM, 35.1 kg/d milk, 4.25% milk fat, 3.22% milk true protein, 30 d pregnant, 25.5 kg/d DMI, second lactation, default environmental conditions.

³Excludes AA contributed by infusates.

sources (silage and grain) supplied 31, 37, and 43% of dietary RUP in experiments 1, 2, and 3, respectively; soybean meal, raw soybeans, and blood meal supplied 59, 52, and 43%. The chemical and AA compositions of feed ingredients are shown in Tables 3, 4, and 5.

Lactation and DMI Responses

Experiment 1 began 2 wk postpartum and continued for 40 d for each cow. Milk fat content and yield of milk fat, FCM, and ECM were not affected by level of infused Met (Table 6). Yield and content of milk true protein increased linearly with increasing levels of infused Met. Milk yield tended to increase quadratically ($P \leq 0.15$)

with increasing levels of infused Met, with milk yield being greatest when either 0 or 16.0 g/d of Met was infused (40.6 and 40.9 kg/d, respectively) and lowest when 3.5 or 10.5 g/d of Met was infused (39.0 and 39.3 kg/d, respectively). Conversion of DMI to ECM tended to decline linearly ($P \leq 0.15$) with increasing levels of infused Met, reflecting a trend for a linear increase ($P \leq 0.15$) in DMI and no effect of treatment on yield of ECM.

Experiment 2 began 11 to 13 wk postpartum and also continued for 40 d. Dry matter intake tended to increase linearly ($P \leq 0.15$) with increasing amounts of infused Met (Table 7). However, milk yield was not affected by treatment (Table 7). Increasing the amounts of infused Met resulted in linear increases in yields of true protein, fat, ECM, and 3.5% FCM. These responses can be partially attributed to linear increases in fat and protein secretions in milk in response to increased Met supply. Milk true protein content responses to increasing levels of infused Met were also quadratic in nature, with the increases in milk protein content diminishing with increasing amounts of infused Met. The calculated plateau point of the response curve was determined to be 12.2 g of infused Met. Conversion of DMI to ECM, and dietary plus infused N to milk N were not affected by infusion of Met (Table 7).

In experiment 3, DMI and milk fat content were not affected by level of infused Met. Milk true protein content increased linearly with increasing amounts of infused Met (Table 8). However, true protein yield was not affected by treatment because of a linear decline in milk yield with increasing levels of infused Met. Increases in milk protein content diminished with each increment of infused Met; thus, responses in content of milk protein were also quadratic ($P \leq 0.15$). The calculated plateau point of the response curve was determined to be 12.4 g of infused Met. Yields of FCM, ECM, and milk fat tended to decline linearly ($P \leq 0.15$) with increasing amounts of infused Met. Conversions of DMI to ECM and dietary plus infused N to milk N were not affected by treatment.

Blood Metabolites

Plasma NEFA concentrations tended to decline linearly ($P \leq 0.15$) in experiment 1 with increasing levels of infused Met, indicating either a reduction in fat mobilization or an increase in uptake of NEFA by liver or peripheral tissue (Table 9). In contrast, plasma glucose and BHBA concentrations and serum urea concentrations were not affected by treatment. In experiment 2, plasma glucose concentrations responded quadratically to increasing levels of infused Met. Plasma glucose lev-

Table 3. Chemical composition of forages fed during peak (experiment 1), early (experiment 2), and mid (experiment 3) lactation

Item, DM basis	Corn silage			Haycrop silage			Alfalfa hay		
	1	2	3	1	2	3	1	2	3
DM, %	38.6	32.1	34.4	39.5	33.5	31.7	89.4	89.8	90.4
CP, %	9.8	10.6	11.0	17.7	17.1	20.7	17.0	18.9	15.8
ADF, %	28.0	29.2	26.9	32.4	32.5	37.0	36.3	32.0	37.3
NDF, %	50.0	50.0	45.3	53.8	55.0	50.7	49.0	43.6	49.1
ADFICP, ¹ %	0.8	0.8	0.8	1.1	1.1	1.1	1.2	1.2	1.2
NDFICP, ² %	1.8	1.8	1.8	4.9	4.9	4.9	5.9	5.9	5.9
Lignin, %	3.4	3.4	3.4	6.3	6.3	6.3	8.2	8.2	8.2
Ash, %	3.8	3.8	3.8	9.9	9.9	9.9	8.9	8.9	8.9
Ca, %	0.16	0.16	0.16	0.75	0.75	0.75	0.66	0.66	0.66
P, %	0.17	0.17	0.17	0.29	0.29	0.29	0.24	0.24	0.24
Mg, %	0.10	0.10	0.10	0.21	0.21	0.21	0.18	0.18	0.18
K, %	0.86	0.86	0.86	2.52	2.52	2.52	3.11	3.11	3.11
S, %	0.07	0.07	0.07	0.18	0.18	0.18	0.12	0.12	0.12
Fe, mg/kg	118	118	118	157	157	157	74	74	74
Zn, mg/kg	12	12	12	27	27	27	24	24	24
Cu, mg/kg	5	5	5	7	7	7	8	8	8

¹ADF insoluble CP.²NDF insoluble CP.

els peaked at 70.3 mg/dL with 3.5 g/d of infused Met and then declined with each additional increment of infused Met. Plasma BHBA levels increased linearly with increasing amounts of infused Met (Table 9). Serum urea concentrations tended to respond quadratically ($P \leq 0.15$) to increasing amounts of infused Met. Serum urea concentrations increased from 14.9 mg/dL with 0 g/d of infused Met to 16.0 mg/dL with 10.5 g/d of infused Met and decreased to 13.4 mg/dL with 16.0 g/d of infused Met. The increase in serum urea concentrations with 10.5 g/d of infused Met may be the result of increased DMI. There was no effect of treatment on plasma NEFA concentrations.

In experiment 3, plasma glucose and BHBA concentrations and serum urea concentrations were not affected by amount of infused Met (Table 9). Plasma NEFA concentrations declined quadratically; the lowest concentration occurred when 10 g/d of Met was infused into the duodenum.

Ruminal and Duodenal Measurements

There was no effect of infused Met on ruminal or duodenal measurements. Hence, treatment means were combined and are presented in Table 10. Average ruminal pH was 6.12, 6.30, and 6.41 in experiments 1,

Table 4. Chemical composition of concentrates fed during peak-, early-, and mid-lactation experiments

Item, DM basis	Corn, coarse grind	Soybean meal, solvent-extracted	Raw soybeans, cracked	Blood meal, ring dried
DM, %	87.8	91.4	89.0	92.0
CP, %	9.5	52.2	40.2	95.5
ADF, %	2.7	4	20.8	—
NDF, %	8.6	12.1	27.8	—
ADFICP, ¹ %	0.2	0.7	1.0	1.3
NDFICP, ² %	1.9	2.1	4.9	31.8
Lignin, %	1.0	0.6	1.8	2.3
Ash, %	2.1	7.6	5.6	3.2
Ca, %	0.02	0.24	0.18	0.40
P, %	0.24	0.67	0.60	0.23
Mg, %	0.10	0.28	0.23	0.03
K, %	0.38	2.29	1.79	0.15
S, %	0.06	0.35	0.28	0.71
Fe, mg/kg	42	152	125	1,900
Zn, mg/kg	21	47	24	26
Cu, mg/kg	2	14	8	5

¹ADF insoluble CP.²NDF insoluble CP.

Table 5. Amino acid composition of feeds

AA, % of CP	Corn silage	Haycrop 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

Table 6. Dry matter intake and milk production responses of peak-lactation cows (experiment 1) receiving 10 g of Lys and 0, 3.5, 7.0, 10.5, or 16.0 g of Met by duodenal infusion

Item	Infusates, g/d of Met					SE ¹	Treatment effect, <i>P</i> =	
	0	3.5	7.0	10.5	16.0		Linear	Quadratic
DMI, kg/d	20.6	20.6	20.9	21.7	22.0	0.7	0.056	0.830
Milk, kg/d	40.6	39.0	40.1	39.3	40.8	2.5	0.588	0.094
3.5% FCM, kg/d	44.5	42.6	44.0	43.1	44.6	2.0	0.725	0.238
ECM, ² kg/d	43.5	41.8	43.4	42.4	44.3	1.9	0.417	0.207
Milk true protein, g/d	1,144	1,108	1,182	1,133	1,227	44.3	0.034	0.233
Milk fat, g/d	1,656	1,587	1,639	1,608	1,656	74.3	0.846	0.448
Milk true protein, %	2.84	2.87	2.97	2.94	3.02	0.12	0.001	0.704
Milk fat, %	4.11	4.10	4.12	4.23	4.08	0.26	0.940	0.470
ECM/DMI	2.13	2.04	2.08	1.96	2.02	0.11	0.104	0.331
Milk N/N intake ³	0.32	0.31	0.32	0.30	0.32	0.014	0.773	0.205

¹Standard error of the mean, *n* = 5.²3.5% fat and 3.2% protein = (0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein).³Kilograms of milk N per kg of N intake (dietary + infused).**Table 7.** Dry matter intake and milk production responses of early-lactation cows (experiment 2) receiving 10 g of Lys and 0, 3.5, 7.0, 10.5, or 16.0 g of Met by duodenal infusion

Item	Infusates, g/d of Met					SE ¹	Treatment effect, <i>P</i> =	
	0	3.5	7.0	10.5	16.0		Linear	Quadratic
DMI, kg/d	25.4	25.1	25.8	25.9	25.9	1.1	0.101	0.834
Milk, kg/d	38.2	38.2	38.4	38.2	38.1	2.3	0.948	0.657
3.5% FCM, kg/d	39.5	40.1	40.0	40.4	42.0	1.8	0.052	0.506
ECM, ² kg/d	39.5	40.3	40.3	40.6	42.0	1.6	0.033	0.666
Milk true protein, g/d	1,137	1,162	1,183	1,181	1,195	39.9	0.013	0.341
Milk fat, g/d	1,413	1,452	1,438	1,471	1,570	71.2	0.030	0.406
Milk true protein, %	3.00	3.07	3.09	3.13	3.15	0.12	<0.001	0.048 ³
Milk fat, %	3.73	3.86	3.78	3.91	4.15	0.25	0.017	0.391
ECM/DMI	1.56	1.61	1.57	1.57	1.62	0.06	0.390	0.585
Milk N/N intake ⁴	0.28	0.28	0.28	0.28	0.28	0.011	0.566	0.618

¹Standard error of the mean, *n* = 5.²3.5% fat and 3.2% protein = (0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein).³Curve reached a plateau when 12.2 g/d of Met was infused.⁴Kilograms of milk N per kg of N intake (dietary + infused).

Table 8. Dry matter intake and milk production responses of mid-lactation cows (experiment 3) receiving 8 g of Lys and 0, 5, 10, 15, or 20 g of Met by duodenal infusion

Item	Infusates, g/d of Met					SE ¹	Treatment effect, <i>P</i> =	
	0	5	10	15	20		Linear	Quadratic
DMI, kg/d	25.5	26.0	24.9	25.4	25.9	1.1	0.802	0.384
Milk, kg/d	35.1	35.1	33.8	34.5	34.0	2.7	0.037	0.470
3.5% FCM, kg/d	38.9	38.6	37.3	37.5	37.6	1.6	0.066	0.199
ECM, ² kg/d	38.9	38.8	37.5	38.0	37.9	1.7	0.121	0.307
Milk true protein, g/d	1,118	1,137	1,105	1,137	1,120	58.3	0.943	0.817
Milk fat, g/d	1,457	1,441	1,393	1,390	1,409	36.9	0.124	0.185
Milk true protein, %	3.22	3.26	3.30	3.32	3.32	0.10	<0.001	0.043 ³
Milk fat, %	4.25	4.18	4.22	4.09	4.22	0.27	0.518	0.236
ECM/DMI	1.52	1.49	1.51	1.50	1.47	0.06	0.214	0.776
Milk N/N intake ⁴	0.29	0.29	0.29	0.30	0.29	0.014	0.988	0.262

¹Standard error of the mean, *n* = 5.²3.5% fat and 3.2% protein = (0.327 × kg milk) + (12.95 × kg fat) + (7.2 × kg protein).³Curve reached a plateau when 12.4 g of Met was infused.⁴Kilograms of milk N per kilogram of dietary plus infused N.

2, and 3 respectively, indicating that diets contained adequate amounts of effective fiber; the desirable ruminal acetate:propionate ratios of 2.6:1, 2.6:1, and 2.4:1 in experiments 1, 2, and 3, respectively, and high concentrations of milk fat (Tables 6, 7, and 8) corroborate these findings. Despite the acetate:propionate ratio being lowest in experiment 3, milk fat content was greater in this experiment than in experiments 1 and 2 (4.19

vs. 4.13 and 3.89%, respectively). Ruminal ammonia concentrations were above 5.0 mg/dL in all experiments, indicating that microbial protein synthesis and fiber digestion were probably not limited by rumen ammonia levels. Duodenal pH was 3.15, 2.87, and 3.15 in experiments 1, 2, and 3, indicating there was very little, if any, contamination of samples by bile and pancreatic secretions.

Table 9. Blood metabolite concentrations of peak-, early-, and mid-lactation cows receiving 10 g (experiments 1 and 2) or 8 g (experiment 3) of Lys and 0, 3.5, 7.0, 10.5, or 16.0 g of Met (experiments 1 and 2) and 0, 5, 10, 15, or 20 g of Met (experiment 3) by duodenal infusion

Plasma metabolite	Experiment					SE ¹	Treatment effect, <i>P</i> =	
							Linear	Quadratic
Experiment 1 (peak lactation): infusates, g/d								
	10 g of Lys							
	0 g of Met	3.5 g of Met	7.0 g of Met	10.5 g of Met	16.0 g of Met			
Glucose, mg/dL	66.8	67.6	65.2	70.6	72.3	3.2	0.175	0.562
NEFA, μ eq/L	331.1	279.1	265.7	204.9	252.3	73.3	0.098	0.199
BHBA, mg/dL	4.8	4.3	4.7	4.7	5.2	0.6	0.395	0.387
Serum urea, mg/dL	18.0	15.1	17.8	15.3	16.3	1.0	0.270	0.371
Experiment 2 (early lactation): infusates, g/d								
	10 g of Lys							
	0 g of Met	3.5 g of Met	7.0 g of Met	10.5 g of Met	16.0 g of Met			
Glucose, mg/dL	55.0	70.3	65.3	64.6	64.2	4.0	0.275	0.052
NEFA, μ eq/L	117.7	86.7	63.3	76.0	89.4	24.2	0.426	0.157
BHBA, mg/dL	3.6	3.9	5.0	5.2	5.2	0.4	0.004	0.161
Serum urea, mg/dL	14.9	15.5	14.3	16.0	13.4	1.0	0.173	0.150
Experiment 3 (mid lactation): infusates, g/d								
	8 g of Lys							
	0 g of Met	5 g of Met	10 g of Met	15 g of Met	20 g of Met			
Glucose, mg/dL	70.2	66.4	71.9	67.6	67.0	3.1	0.521	0.774
NEFA, μ eq/L	102.2	99.6	74.2	80.5	143.0	16.3	0.113	0.008
BHBA, mg/dL	4.0	5.1	3.6	5.0	5.0	0.6	0.243	0.862
Serum urea, mg/dL	13.5	13.3	11.9	13.4	12.1	0.8	0.197	0.849

¹Standard error of the mean, *n* = 5.

Table 10. Ruminal pH and ammonia and VFA concentrations, and duodenal pH and ammonia concentrations in peak (experiment 1), early (experiment 2) and mid (experiment 3) lactation cows

Item	Experiment		
	1	2	3
Ruminal			
pH	6.12	6.30	6.41
Ammonia, mg/dL	12.6	7.9	6.7
VFA, mM			
Acetate (A)	73.9	71.4	66.4
Propionate (P)	28.5	27.2	28.1
A:P ratio	2.6	2.6	2.4
Isobutyrate	2.2	2.2	2.0
Butyrate	14.0	13.3	12.7
Isovalerate	2.1	2.0	2.1
Valerate	1.6	1.8	1.8
Duodenal			
pH	3.15	2.87	3.15
Ammonia, mg/dL	6.5	6.6	5.5

Intake and Passage of AA to the Duodenum

Intake and passage of nutrients to the duodenum and calculated apparent digestibility coefficients were not affected by treatment; therefore, only experiment means are presented (Tables 11, 12, and 13). True digestibility coefficients and passage of N fractions to the small intestine are not presented because ruminal bacteria were not isolated for analysis (Table 11). Means by experiment for AA intake and passage to the duodenum are given in Table 12. With the exception of ADF apparent digestibility and Leu flow to the duodenum, flows to the duodenum of OM and most other analyzed constituents (Tables 11 and 12) were greater and apparent digestibilities were lower (Table 11) in experiments 2 and 3 compared with experiment 1. Both

Table 11. Experimental means for intake, apparent digestibility, and passage of OM, nitrogen, and fiber fractions to the duodenum of peak (experiment 1), early (experiment 2) and mid (experiment 3) lactation cows

Item	Experiment		
	1	2	3
OM			
Intake, kg/d	19.5	24.1	23.8
Flow to duodenum, kg/d	12.3	14.4	13.0
Apparent ruminal digestibility, %	36.9	40.2	45.4
N			
Intake, g/d	634	729	663
Flow to duodenum, g/d	529	630	958
NDF			
Intake, kg/d	5.5	6.6	6.8
Flow to duodenum, kg/d	3.2	3.8	3.4
Apparent ruminal digestibility, %	41.8	42.4	50.0
ADF			
Intake, kg/d	3.3	3.9	3.9
Flow to duodenum, kg/d	1.6	2.3	1.8
Apparent ruminal digestibility, %	51.5	41.0	53.8

observations can be attributed in large part to the greater intake of OM by cows in experiments 2 and 3 compared with experiment 1 (24.1 and 23.8 vs. 19.5 kg/d). When cows consume increasing amounts of OM, the percentage of OM intake that is both apparently and truly digested in the rumen decreases (Clark et al., 1992).

Measured contributions of Lys and Met to TAA in duodenal digesta were 8.1 and 1.9%, respectively, for the basal diet in experiment 1, 7.2 and 2.1% in experiment 2, and 8.8 and 1.8% in experiment 3 (Table 13). In comparison, NRC (2001) predicted contributions of MP-Lys and MP-Met to MP in duodenal digesta for the basal diet to be 6.4 and 1.6% in experiment 1, 6.2 and 1.6% in experiment 2, and 6.3 and 1.7% in experiment 3. By using measurements of AA flow to the duodenum and assumed intestinal availabilities of 100% for infused Lys and Met and 80% for microbial and RUP AA, the additional 10 g of infused Lys in experiments 1 and 2 (equivalent to 12.5 g from microbial and RUP sources) increased the Lys content of duodenal digesta to 8.6 and 7.5% of TAA, respectively; in experiment 3 the 8 g/d of infused Lys increased Lys content of duodenal digesta to 9.0% of TAA. These values for duodenal Lys exceeded the concentrations observed to be necessary for maximal synthesis of milk protein (Schwab et al., 1992b). The relative proportions of Met in duodenal digesta as a result of infusates were 1.9, 2.1, 2.2, 2.4, and 2.7% of TAA in experiment 1; 2.1, 2.3, 2.4, 2.5, and 2.7% of TAA for experiment 2; and 1.8, 2.0, 2.2, 2.4, and 2.5% of TAA for experiment 3.

DISCUSSION

Determining Met Requirements

To determine the optimum profile of absorbable AA in duodenal digesta using the empirical dose-response approach, requirements for all essential AA (EAA) must be determined. As part of the initial step, the primary objective of our experiments was to obtain estimates of the required level of Met in TAA of duodenal digesta for maximum synthesis of milk protein. A basal level of Lys was infused along with the graded levels of Met to meet Lys requirements as determined by Schwab et al. (1992b) and to ensure that Met was first limiting. Similar to the approach of Schwab et al. (1992b), no attempt was made to determine differences in intestinal availability of individual AA among sources of MP. Reviews of studies in which Met, Lys, or both were either infused postruminally or fed in rumen-stable form indicated that milk protein content and yield are more sensitive than milk yield to short-term alterations in Met and Lys content of duodenal digesta (NRC, 2001; Rulquin et al., 1993; Rulquin and Vérité,

Table 12. Experimental means for intake and flow of AA to the duodenum of peak- (experiment 1), early- (experiment 2), and mid- (experiment 3) lactation cows

AA	AA intake in each experiment, g/d			AA flows to duodenum in each experiment, ¹ g/d		
	1	2	3	1	2	3
Essential						
Arg	194	210	178	121	168	180
His	91	97	85	54	65	75
Ile	125	137	124	138	166	149
Leu	312	346	315	321	313	279
Lys	186	197	169	171	228	288
Met	46	50	46	43	69	61
Phe	170	183	162	140	175	183
Thr	136	144	136	121	163	153
Val	178	193	179	133	209	177
Total	1,436	1,556	1,392	1,250	1,514	1,549
Nonessential						
Ala	195	222	231	125	304	254
Asp	332	360	329	109	169	193
Cys	50	57	55	27	53	58
Glu	514	569	508	233	414	539
Gly	143	158	149	141	254	226
Pro	203	238	225	201	257	238
Ser	163	176	156	116	165	177
Tyr	120	130	115	96	113	113
Total	1,720	1,910	1,768	1,042	1,704	1,798
Total AA	3,156	3,466	3,160	2,292	3,218	3,346

¹Flow of AA to the duodenum do not include contributions of infusates.

1993) and were, therefore, chosen as our response criteria.

In experiment 1, the lack of a significant quadratic effect or a trend for a quadratic response in content or yield of milk protein, and the linear increase in content (Figure 1) and yield of milk true protein (Table 6) suggest that the Met requirement for maximal synthesis of milk protein was not met when 16 g/d of Met was infused into the duodenum. Assuming intestinal availabilities of 100% for infused Met and Lys and 80% for microbial and RUP AA, this amount of Met is equivalent to an additional 20 g/d of Met from microbial and RUP sources. The addition of this amount of Met to the Met

flowing to the small intestine from the rumen (75 g/d; Table 12) increases the contribution of Met from 1.9 to 2.7% of TAA. Thus, we concluded that the required contribution of Met to TAA in duodenal digesta from microbial and RUP sources for maximum synthesis of milk protein in this experiment exceeded 2.7%.

A linear relationship was also found between incremental levels of infused Met and content and yield of milk true protein in experiment 2 (Table 7). In addition, a quadratic relationship was found between level of infused Met and milk protein content, indicating diminishing responses in protein content of milk with additional Met (Figure 2). It was determined that milk true

Table 13. Experimental means for profiles of essential AA (EAA) in duodenal digesta of peak- (experiment 1), early- (experiment 2), and mid- (experiment 3) lactation cows

AA ¹	Experiment, measured AA, % of EAA			Experiment, measured AA, % of total AA		
	1	2	3	1	2	3
Arg	9.7	11.1	11.6	5.3	5.2	5.4
His	4.3	4.3	4.8	2.4	2.0	2.2
Ile	11.0	11.0	9.6	6.0	5.2	4.5
Leu	25.7	20.7	18.0	14.0	9.7	8.3
Lys	13.7	15.1	18.6	7.5	7.1	8.6
Met	3.4	4.6	3.9	1.9	2.1	1.8
Phe	11.2	11.6	11.8	6.1	5.4	5.5
Thr	9.7	10.8	9.9	5.3	5.1	4.6
Val	10.6	13.8	11.4	5.8	6.5	5.3

¹Amino acids in duodenal digesta do not include contributions of infusates.

Figure 1. Effect of infusing Met on true protein content of milk during peak lactation. There were significant linear effects of amount of Met on true protein content of milk ($P = 0.001$). A simple linear polynomial was used [$Y = 2.8487 + 0.0107x$; $R^2 = 5.6$, $SE = 0.25$, $n = 25$] where x is the amount of infused Met and Y is the milk true protein content.

protein content reached a plateau when 12.2 g/d of Met was infused. From measured flows of AA to the duodenum and making the same assumptions regarding intestinal availabilities of AA as in experiment 1, the required contribution of Met to TAA passing to the

duodenum for maximum synthesis of milk true protein was calculated to be 2.4%. Pisulewski et al. (1996) also obtained linear increases in content and yield of milk protein when incremental amounts of DL-Met (0, 6, 12, 18, and 24 g/d) and a basal amount of supplemental L-

Figure 2. Effect of infusing Met on true protein content of milk during early lactation. There was linear relationship between amounts of infused Met and true protein content of milk ($P \leq 0.001$). The threshold and plateau model was significant for the corrected data but there was no further significant improvement from fitting the threshold and hinge model [$Y = 3.0134 + 0.0115x$ for the linear part of the model and $Y = 3.0134 + 0.0115 \times 12.1807$ for the plateau ($SE = 1.34$ for x value of breakpoint); $R^2 = 53.6$; $SE = 0.03$; $n = 25$]. In this model, x is the amount of infused Met and Y is milk true protein content.

Figure 3. Effect of infusing Met on true protein content of milk during mid lactation. There was a linear relationship between amounts of infused Met and true protein content of milk ($P \leq 0.001$). The threshold and plateau model was significant for the corrected data but there was no further significant improvement from fitting the threshold and hinge model [$Y = 3.223 + 0.0078x$ for the linear part of the model and $Y = 3.223 + 0.0078 \times 12.4359$ for the plateau ($SE = 1.84$ for x value of breakpoint); $R^2 = 55.1$; $SE = 0.03$; $n = 25$]. In this model, x is the amount of infused Met and Y is milk true protein content.

Lys (10 g/d) were infused into the duodenum of early-lactation cows (4 to 16 wk postpartum). The cows were fed a 17.6% CP diet of 64% corn silage, 5% dehydrated lucerne, 5% each of corn and wheat, 4% barley, 8% formaldehyde-treated soybean, 6% soybean meal, and 0.5% urea. Using measurements of AA flow to the duodenum and assumed intestinal digestibilities of 100% for infused AA and 80% for digesta AA, calculated concentrations of Lys and Met in TAA were 7.3 and 1.5, 1.7, 1.9, 2.2, and 2.4%, respectively. Results from the experiment Pisulewski et al. (1996) and experiments 1 and 2 in this study indicate that when Lys presumably is adequate (7.0% or more of TAA), Met must contribute 2.4% or more of TAA in duodenal digesta to maximize synthesis of milk protein in early-lactation cows.

In experiment 3, a positive linear relationship was found between incremental levels of infused Met and milk protein content. In addition, the magnitude of the milk protein content response diminished with additional Met, resulting in a quadratic response to additional Met (Table 8, Figure 3). The plateau point of the milk protein content response curve was determined to be 12.4 g/d of infused Met, which corresponds to a Met concentration in duodenal digesta of 2.3% of TAA. However, because of the linear decrease in milk yield, yield of milk protein was not affected by infused Met (Table 8). The greatest yield of milk protein with the lowest amount of infused Met occurred when 5 g/d of Met was infused; this corresponds to a Met concentration in duo-

denal digesta of 2.2% of TAA. These results indicate that the required level of Met in duodenal digesta is greater for maximizing milk protein content than for maximizing milk protein yield. The lower Met requirement for mid-lactation cows, expressed as a percentage of TAA, may have been the result of cows consuming RUP well in excess of their requirements. Intake of RUP in excess of requirements would increase passage of all AA to the small intestine; thus, a qualitative deficiency of Met could be overcome.

Lysine contents of duodenal digesta in experiments 1, 2, and 3 were greater than predicted by NRC (2001; 7.5, 7.1, and 8.6% of TAA, respectively). One potential explanation for the high Lys content of duodenal digesta is that infused Lys contaminated duodenal samples. However, contamination of duodenal samples should have been minimal because digesta collected during the first 30 s after removing the cannula plug were discarded. A second potential explanation for the high Lys content of duodenal digesta is disproportionate sampling, because particles of blood meal were observed in duodenal samples. However, duodenal samples would have to contain a substantial portion of the consumed blood meal, as the Lys content of blood meal was 7.70% of CP (Table 5), whereas Lys contents in duodenal digesta were 7.5, 7.1, and 8.6%, respectively, of TAA in experiments 1, 2, and 3 (Table 13).

The Lys content of blood meal fed in this study was lower than default values for blood meal in the NRC

(2001; 7.70 vs. 8.98% of CP), indicating excess heating during processing. This was confirmed by the low measured intestinal digestibility for blood meal fed in this study (Socha et al., 2005) compared with the NRC (2001) default value (60.7 vs. 80% of CP). Thus, even though the Lys content of duodenal digesta was high, the intestinal availability of Lys appeared to be low.

Benefits of Improved Balance of AA in Duodenal Digesta

The most obvious benefit of improved AA balance in duodenal digesta is increased synthesis of milk protein. For experiments 1 and 2, milk protein yield and content increased as Met content of duodenal digesta increased. Increased milk protein content has been a common observation in studies in which incremental amounts of Lys and a basal level of Met were infused into the duodenum (Schwab et al., 1992b) or when incremental amounts of Met and a basal amount of Lys were infused into the duodenum (Pisulewski et al., 1996). The increase in milk protein content appears to occur primarily in the casein fraction of milk protein (Donkin et al., 1989; Robert et al., 1989; Chow et al., 1990; Le Henaff et al., 1990).

Increasing the contribution of Met to TAA in duodenal digesta tended to increase DMI in experiments 1 and 2 and had no effect on DMI in experiment 3. Pisulewski et al. (1996) observed no effect on DMI as incremental amounts of Met were infused into the duodenum of early-lactation cows. Dry matter intake increased when incremental amounts of Lys were infused into the duodenum of peak and postpeak early-lactation cows, but not mid- and late-lactation cows (Schwab et al., 1992b). The trend for increased DMI in experiments 1 and 2 using early-lactation cows and the 2 infusion studies conducted by Schwab et al. (1992b) support the hypothesis that one of the benefits of improved balance of absorbable AA is slightly increased DMI in early lactation.

The increase in DMI may account partly for the reduction in plasma NEFA, reducing the animal's need to rely on mobilized fat to meet energy needs. Pisulewski et al. (1996) observed a linear decrease in plasma NEFA concentrations with graded levels of infused Met. Plasma NEFA concentrations of cows (9 to 21 wk postpartum) were reduced when they were given 23 g/d of L-Lys and 7 g/d of DL-Met in rumen-stable form (Chapoutot et al., 1992). For experiment 1, NEFA concentrations of plasma tended to decline linearly when cows (2 to 8 wk postpartum) were infused postruminally with increasing amounts of Met (Table 9). In experiment 3, plasma NEFA concentrations declined quadratically ($P \leq 0.05$); the lowest concentration occurred when 10 g/d

of Met was infused into the duodenum. The lack of a consistent reduction in plasma NEFA concentrations with increasing amounts of infused Met in experiment 3 may be reflective of cows being in positive energy balance (NEFA concentrations were approximately one-third of the concentrations in experiment 1; Table 9), and hence, less mobilization of body fat.

Improved liver function as a result of a more favorable balance of AA in duodenal digesta may partly account for the reduction in plasma NEFA concentrations. Durand et al. (1992) observed that rate of triglyceride export from the liver increased when 20 g/d of L-Lys and 10 g/d of DL-Met were infused into the mesenteric vein of 2 early-lactation dairy cows fed a conventional corn-based diet. Cows were fitted surgically with chronic catheters implanted in the hepatic, portal, and mesenteric veins and mesenteric artery. Hepatic balance of very low density lipoproteins was negative (-4 g/h) before infusion of Lys and Met, positive ($+8$ g/h) when Lys and Met were infused, and then became negative again (-16 g/h) after AA infusions were terminated (Durand et al., 1992). Whether Lys, Met, or both facilitated hepatic secretion of triglyceride-rich lipoproteins is not known. Methionine may be the limiting factor for the hepatic synthesis of apo B₁₀₀, very low density lipoprotein (Bauchart et al., 1992), and phospholipids (Vance and Vance, 1990).

The preliminary study by Durand et al. (1992) suggests that optimizing Lys and Met nutrition would reduce hepatic lipid infiltration in the early-lactation dairy cow. Grummer (1993) noted that strategies to increase the rate of triglyceride export by the ruminant liver may be effective management of blood NEFA levels and may aid in prevention of fatty liver and ketosis. It should be noted that the required contribution of Met to TAA in duodenal digesta for maximum synthesis of milk protein appeared to be greater (≥ 2.7 vs. 2.4 and 2.2% of TAA, respectively) for cows in experiment 1 than for those in experiments 2 and 3. Similarly, of the 3 estimates for Lys requirements (7.2, 6.4, and 6.9% of TAA; Schwab et al., 1992b), the greatest estimate was obtained when cows were at peak lactation (4 to 8 wk postpartum; Schwab et al., 1992b). These observations may be a result of a better intestinal balance of the other AA for cows in the earliest stages of lactation, or they may reflect greater Lys and Met requirements due to more energy being derived from fat in the weeks immediately following calving.

Possible Negative Implications of Excess Methionine

The linear decrease in milk yield in response to incremental levels of infused Met in experiment 3 was unex-

pected. This result can be attributed to several things. First, it cannot be ruled out as a type I error; 1 in 20 of the responses declared significant at the $P = 0.05$ are not significant. A second possible explanation is an over-supply of Met. Rulquin et al. (1993) observed similar results when they summarized the literature investigating lactational responses to supplemental Lys and Met. In the summary, additional Met either did not affect or decreased content and yield of milk protein when predicted concentrations of Lys in duodenal digesta were below 6.5% of protein truly digested in the small intestine and resulting Met levels were above 2.3% of protein truly digested in the small intestine. The results of Rulquin et al. (1993) are reinforced by results of a previous study conducted at the University of New Hampshire. Socha et al. (2005) observed no responses in milk production, milk protein content, or milk protein production to rumen-stable Met (**RS-Met**) when dietary CP was 16% of DM, but milk protein content increased and milk yield decreased in response to RS-Met supplementation with the 18.5% CP diet. This was not necessarily surprising as Lys was found to be a colimiting AA with the diets fed. The decrease in milk production with RS-Met may be the result of decreased blood flow to the mammary gland. Guinard and Rulquin (1995) noted that mammary blood flow of lactating dairy cows decreased from 5.28 to 4.55 L/min when the amount of Met infused into the duodenum increased from 0 to 16 g/d. These researchers did not observe a reduction in milk yield, although milk yield was lower in the study of Guinard and Rulquin (1995) than in the study of Socha et al. (2005; mean milk yield, 23.9 vs. 44.4 kg/d). More research is needed to determine the effect of Met on mammary blood flow.

Oversupplementing Met has been shown to decrease weight gains of swine and poultry (Edmonds and Baker, 1987a,b; Edmonds et al., 1987) and may be due to production of protein-bound methanethiol-cysteine mixed disulfides and possibly to a direct action of methanethiol on enzyme-bound metals (Benevenga and Steele, 1984). However, Met was oversupplemented to a much greater extent in the swine and poultry studies than in our study.

CONCLUSIONS

Results from experiments 1 and 2 indicate that Met must contribute more than 2.7 and 2.4%, respectively, of total TAA in duodenal digesta for maximum synthesis of milk protein in peak (2 to 8 wk postpartum) and postpeak early-lactation (11 to 19 wk postpartum) cows. These results are consistent with the observation of Pisulewski et al. (1996) that Met must exceed 2.4% of TAA in duodenal digesta when postpeak early-lactation

Holstein cows (4 to 16 wk postpartum) are fed corn-based diets. In experiment 3, the level of Met in duodenal digesta was greater for maximizing content of milk protein than for maximizing yield of milk protein. The greater requirement for Met in the earlier stages of lactation may reflect a greater need for Met because of its role in lipid metabolism. Clearly, more research is needed to determine Met requirements and to elucidate the next limiting AA and their requirements to ensure that they are not limiting the response to infused Met.

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