

Ruminally Protected Lysine or Lysine and Methionine for Lactating Dairy Cows Fed a Ration Designed to Meet Requirements for Microbial and Postruminal Protein¹

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ABSTRACT

The purpose of this study was to separate the effects of ruminally protected Lys from effects of ruminally protected Met on the performance of lactating dairy cows fed a ration calculated to be first-limiting in intestinally delivered Lys and second-limiting in intestinally delivered Met. Thirty multiparous Holstein cows were examined in a 20-wk study that started on wk 5 postpartum. Rations contained timothy silage, corn silage, barley, corn, corn gluten meal, and soybean meal. Treatments were 1) no supplemental amino acids, 2) 21 g/d of intestinally available Lys, and 3) 22 g/d of intestinally available Lys and 6 g/d of intestinally available Met. Post-experimental calculations suggested that, in contrast to the objective, the unsupplemented ration was colimiting in intestinally available His (0.96 of requirement), followed by Lys (1.00), digestible ruminally undegraded protein (1.01), Ile (1.03), Arg (1.04), Val (1.10), and Met (1.14). In this context, the virtually identical performance of cows fed the unsupplemented ration and cows fed the ration supplemented with ruminally protected Lys demonstrated that dairy cows did not respond to enhanced intestinal supplies of Lys when Lys was not calculated to be the first-limiting nutrient. In contrast, for cows fed rations supplemented with both ruminally protected Lys and ruminally protected Met, the production of both milk protein (40 g/d) and fat (40 g/d) was numerically increased to an extent that was consistent with earlier reported studies, although calculations did not indicate that performance was

limited by intestinal supplies of Lys or Met. This result, which may be disputed because of a lack of statistical significance, suggests that Met, apparently unlike Lys, may enhance the production of milk components beyond an enhancement expected because of its role as a limiting amino acid.

(**Key words:** lysine, methionine, ruminally protected)

Abbreviation key: BCS = body condition score, MR = mixed ration, RP = ruminally protected, RPL = RP Lys product, RPLM = RP Lys and RP Met product.

INTRODUCTION

Dairy cows have metabolic requirements for specific AA in addition to those necessary for intestinally available protein (1). Although intestinal delivery of any essential AA may limit animal performance, suggestions by both Clark (2) and Schwab et al. (16) that Lys and Met are the AA that are most likely to be deficient in intestinally available protein appear to have resulted in a research focus on these AA in studies that utilized ruminally protected (RP) AA over the past 20 yr. A potential deficiency of Lys is understandable because of its high metabolic requirement for milk protein synthesis (5), particularly in situations in which proteins from corn with high RUP and low Lys contents dominate the dietary protein sources. In contrast, Met deficiencies have most often been suggested to affect milk fat synthesis because Met is a methyl donor in the transmethylation reactions of lipid biosynthesis (6).

A number of published studies (10) have examined production responses of lactating dairy cows to supplementation with combinations of RP Lys and RP Met and, in general, production of milk protein (mean daily increase, 51 g/d) and milk fat (mean

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daily increase, 30 g/d) was enhanced. However, the milk protein response was more consistent (i.e., numerical increases in 12 of 12 comparisons) than was the increase in milk fat (i.e., numerical increases in 9 of 12 comparisons). These results appear to be conclusive in demonstrating that supplementation of various basal diets with various amounts of RP Lys and RP Met products (**RPLM**) does enhance production of milk protein and milk fat in dairy cows, albeit at rather moderate levels that are generally difficult to detect statistically. However, because RP Lys and RP Met were provided concomitantly in all of the studies reviewed by Robinson et al. (10), individual effects of these AA cannot be separated.

Schwab et al. (14, 15) attempted to separate the influence of supplemental Met from that of supplemental Lys in dairy cows at early, mid, and late stages of lactation. Under the circumstances of those studies, Schwab et al. (14, 15) concluded that Lys was first-limiting and Met was second-limiting in early lactation, Lys and Met were colimiting in mid-lactation, and no AA limited production in late lactation. The rations utilized consisted of corn silage, haycrop silage, corn meal, distillers dried grains with solubles, wheat middlings, and soybean meal. Results led those researchers (14, 15) to conclude that the optimal delivery of Lys to the duodenum was 15.2% of total essential AA, which was consistent with an earlier finding (14.8% of total essential AA) by Rulquin et al. (12). However, this approach to the expression of Lys requirements appears to be flawed because some essential AA could lead to deficiencies of other essential AA, even in situations in which Lys was delivered at the optimal proportion of total essential AA.

The objective of this study was to supplement RP Lys, either alone or in combination with RP Met, to a ration balanced to be first-limiting in intestinally available Lys and second-limiting in intestinally available Met. In addition, the ration was designed to meet but not exceed N requirements for ruminal microbial growth and to exceed requirements for intestinally digestible protein (i.e., other essential AA) to allow a production response to postruminal supplementation of Lys alone or Lys and Met together when these AA truly limited performance.

MATERIALS AND METHODS

Cows and Design

Thirty multiparous Holstein cows were used in this study. To be eligible for assignment, cows had to be in good health and had to have a mean production of at

least 30 kg/d of milk during d 15 to 28 postpartum inclusive. Cows were blocked into groups of 3 based on milk production, milk composition, BW, and body condition score (**BCS**) and randomly assigned to treatment for the entire 20-wk experimental period.

All cows were housed in tie stalls bedded with softwood shavings on rubber mats. Access to water was ad libitum, including the daily exercise period in a drylot between 0730 and 0830 h.

Management Prior to Assignment

Cows were maintained on grass pasture from the time that they completed their previous lactation until they were brought into the research unit approximately 3 wk prepartum. At that time, all cows received 2.5 kg/d of a concentrate based on barley that contained 14.9% CP (DM basis). In addition, all cows received 1 kg/d of corn silage DM, 1 kg/d of timothy silage DM, and ad libitum access to a poor quality timothy hay (5.72% CP, DM basis) of which they consumed a mean of 6.7 kg/d of DM.

At calving, all cows were changed abruptly to a TMR, which was also the mixed ration (**MR**) fed during the subsequent experimental period (described subsequently).

Rations

Cows were assigned to the study on the first Wednesday following d 27 postpartum and continued to receive the MR that they had been receiving since parturition. This MR was composed of a mixed concentrate (Tables 1 and 2), silage composed largely of timothy grass, and corn silage (Table 3). This MR was fed for ad libitum intake during the entire 20-wk measurement period; 67% was allocated at 1630 h, and the balance was allocated at 0600 h. Orts were weighed between 1330 and 1400 h. In addition to the MR, all cows received 0.5 kg/d of a high molasses concentrate (Table 1) at 0700 h. Cows assigned to the MR supplemented with RP Lys received an experimental matrix embedded RP Lys product (**RPL**; Ajinomoto Co. Inc., Tokyo, Japan) blended with 0.5 kg of the high molasses concentrate. Cows assigned to the MR supplemented with RP Lys and RP Met received the same experimental RPL in addition to an experimental matrix-embedded RPLM (Ajinomoto Co. Inc.). Neither product contained free AA; the portion of the products that was not Lys-HCl or Lys-HCl and Met was the fat-based matrix. Both RP AA products were fed in relation to DMI to mimic its feeding in a TMR. However, because of the potential for degradation if these products remained in

TABLE 1. Ingredient composition of concentrates.

Composition	Main concentrate ¹	High molasses concentrate ²
	— (kg/tonne as mixed) —	
Barley, ground	470	440
Corn, ground	272	272
Soybean meal, 49% CP	135	135
Corn gluten meal	46	46
Dicalcium phosphate	7.5	7.5
Dynamate ^{® 3}	3.8	3.8
Trace-mineralized salt ⁴	4.5	4.5
Iodized salt ⁵	6.0	6.0
Limestone	18.8	18.8
Magnesium oxide	1.5	1.5
Copper sulfate	0.038	0.038
Se-Mar 200 ^{® 6}	3.0	3.0
Vitamin premix ⁷	1.7	1.7
Liquid molasses	30	60

¹Mixed with the silages to create the TMR (preexperimental period) and mixed ration (experimental period).

²Fed with the ruminally protected AA products at 0600 h.

³Pitman Moore, Inc. (Mundelein, IL); guaranteed analysis: 25% S, 18% K, and 11% Mg.

⁴Guaranteed analysis: 37.6% Na, 37.5 ppm of Co, 75 ppm of I, 1875 ppm of Fe, 3000 ppm of Mn, 5625 ppm of Zn, 400 ppm of Cu, and 10 ppm of Se.

⁵Guaranteed analysis: 96.5% NaCl, 4000 ppm of Zn, 1600 ppm of Fe, 1200 ppm of Mn, 330 ppm of Cu, 70 ppm of I, and 40 ppm of Co.

⁶Central Soya Ltd. (Woodstock, ON, Canada); guaranteed analysis: 200 ppm of Se and 11,000 IU/kg of vitamin E.

⁷Guaranteed analysis: 2,600,000 IU/kg of vitamin A and 92,000 IU/kg of vitamin D.

contact with silages for prolonged periods, and because of a desire to provide specific amounts of AA daily, the RP products were fed once daily with a highly palatable carrier. Actual feeding concentrations of the AA products, as well as their AA composition, are presented in Table 4.

Measurements and Analytical Methods

Cows were milked between 1530 and 1630 h and between 0630 and 0730 h daily. Milk production was recorded twice daily for all cows, and milk samples were collected from Sunday p.m. and Monday a.m. milkings beginning at parturition. Individual milk samples were analyzed for fat, protein, and lactose by methods previously described (11), and were arithmetically pooled in proportion to production at each milking.

Silages and the MR were sampled on Thursday, Saturday, and Tuesday of each week and were composited weekly. Batch lots of concentrate ingredients were sampled as received and analyzed individually. The mixed concentrate was prepared in 252 batches, and the batches were composited and analyzed as 13

monthly samples. Orts were sampled weekly and composited within cow over 4-wk periods to create five samples per cow.

All analytical methods were completed as described by Robinson and Burgess (8).

Calculations

Composited orts samples within cow representing five periods of 4 wk each during the 20-wk measurement period were assayed for chemical composition. This analysis was applied to all orts during that period within cow to calculate actual consumption of dietary analytical components. The 0.5 kg of high molasses concentrate fed to all cows at 0700 h was completely consumed on virtually all occasions by all cows.

Energy balance of the cows was determined individually by cow within period. Milk energy was calculated with the equation of Tyrrell and Reid (18) that utilized milk protein, fat, and lactose; the energy required for BW change was calculated as 5.12 Mcal/kg of gain or 4.92 Mcal/kg of BW loss (7); and energy of maintenance was calculated as $BW^{0.75} \times 0.08$ (7). Body condition was scored by two experienced scorers using the system of Edmonson et al. (3).

Results were evaluated utilizing The Atlantic Protein System (an unpublished software program available from the authors upon request). This program estimates requirements for RDP based on the production of bacterial CP, which is estimated from measured DMI, the measured proportion of NDF in the DM, and the measured intake of buffer-soluble CP. Requirements for digestible RUP were calculated based on the difference between the estimated intestinal requirement for CP and the estimated delivery of digestible bacterial CP. Requirements for digestible AA were calculated based on their assumed proportions in milk protein and an assumed fixed transfer coefficient for each AA absorbed from the intestine. Intestinal delivery of RDP and digestible RUP for each feedstuff was calculated from the measured proportions of soluble CP and indigestible CP and the estimated rates of ruminal passage and digestion of the measured potentially degradable CP. Intestinal delivery of AA was calculated from the estimated digestible RUP delivery from each dietary feedstuff and its assumed AA proportions based on prior analysis of other similar feedstuffs.

Statistical Analysis

Ten cows were assigned to each treatment at approximately 4 wk postpartum. One cow was lost to the study prior to completion of the 20-wk measurement period because of lameness caused by physical

injury. However, this cow completed 12 wk of the measurement period, and her data were included in the analysis. Data were reduced to least squares means by cow and subjected to ANOVA using PROC GLM of SAS (13). All data were adjusted for the appropriate pretreatment parameter recorded during wk 2 through 4 postpartum (e.g., milk fat percentage during the preliminary period was used as the covariable for milk fat percentage during the experimental period) using covariable analysis (14). Treatment differences were determined using the PDIF option of SAS (13). Significance was considered to exist when $P < 0.05$, and tendencies were considered to exist when $0.05 \leq P \leq 0.10$.

RESULTS

Dietary Ingredients

The chemical compositions of the concentrate ingredients listed in Table 2 are typical of these materials (7), except possibly barley grain. However, this

material originated in the Atlantic region of Canada where CP concentrations are typically lower and NDF concentrations are typically higher than those in barley obtained from other parts of North America.

The timothy silage (Table 3) was only of moderate quality based on relatively high fiber concentrations, low CP, and moderate ADIN. However, the timothy silage was well ensiled as determined by the low pH and the lack of visible mold or spoilage. The chemical composition of the corn silage was normal.

Supplemental AA Intake

The mean value for supplemented RPL was 131 or 70 g/d of Lys-HCl for cows supplemented with RPL alone (Table 4). The estimated intestinal availability of 30% (information supplied by the manufacturer) indicated that approximately 21 g/d of intestinally available Lys were supplied. The mean value for supplemental RPL was 65 g/d or 35 g/d of Lys for cows fed rations supplemented with both Lys and Met. These cows were also fed 114 g/d of RPLM. Thus, the

TABLE 2. Chemical composition of some concentrate ingredients and the main concentrate.

	Ground barley	Cracked corn	Corn gluten meal	Soybean meal	Concentrate ¹
DM, ² %	87.71	86.91	92.42	89.07	86.85
	(% of DM at 105°C)				
OM	97.08	98.28	94.64	92.61	93.83
NDF	20.8	11.6	3.6	9.1	14.9
ADF	7.4	2.9	3.8	7.0	5.9
Fat	ND ³	ND	ND	ND	2.27
NE _L ⁴	1.90	1.80	2.00	2.00	1.79
CP					
Total	10.63	9.57	65.25	51.09	18.87
Buffer insoluble	8.87	8.56	62.13	46.50	15.75
ND ⁵ Insoluble	1.31	1.19	1.00	0.94	1.31
AD ⁶ Insoluble	0.56	0.31	2.06	0.69	0.63
Ca	0.05	0.02	0.08	0.38	0.85
P	0.41	0.32	0.64	0.82	0.59
K	0.51	0.44	0.40	2.55	0.85
Mg	0.16	0.12	0.12	0.33	0.27
S	0.14	0.11	1.00	0.46	0.29
Na	<0.01	<0.01	<0.01	<0.01	0.40
	(ppm of DM at 105°C)				
Zn	30.1	32.0	49.2	63.8	51.7
Fe	57	43	154	324	177
Mn	20.0	7.3	17.7	53.4	40.4
Cu	3.50	2.77	15.06	19.94	11.16
Se	ND	ND	ND	ND	0.385

¹Ingredient composition specified in Table 1.

²At 105°C.

³Not determined.

⁴Estimated from NRC (7) tables.

⁵Neutral detergent.

⁶Acid detergent.

total estimated intestinally available supply was 22 g/d of Lys and 6 g/d of Met for cows fed RPLM.

Feed Intake

All cows were provided with 0.5 kg of the high molasses concentrate (Table 1) at 0700 h in a small tub that was placed in the manger. The concentrate was the carrier for the RPL and RPLM, and the mixture of concentrate and the RP AA product was consumed rapidly by all cows on virtually all occasions. Thus, to calculate DMI, it was assumed that the intake of the high molasses concentrate was complete.

TABLE 3. Chemical composition of the silages, TMR, and mixed ration (MR).

	Silage		TMR and MR ²
	Timothy ¹	Corn	
pH	4.27	3.72	4.93
DM, %			
105°C	35.87	29.17	45.49
Toluene	37.09	31.06	46.59
	————— (% of DM at 105°C) —————		
OM	91.02	94.81	92.94
NDF	59.1	51.3	35.5
ADF	38.7	30.7	21.7
Fat	ND ³	ND	2.68
NE _L ⁴	1.20	1.65	1.58
CP			
Total	12.94	8.50	14.00
Ammonia ⁵	1.00	0.69	0.50
Buffer Insoluble	6.75	4.88	11.38
ND ⁶ Insoluble	3.19	1.19	2.13
AD ⁷ Insoluble	1.13	0.63	0.69
Ca	0.79	0.31	0.86
P	0.36	0.28	0.46
K	3.23	1.24	1.76
Mg	0.23	0.20	0.28
S	0.19	0.13	0.26
Na	<0.01	<0.01	0.29
	————— (ppm of DM at 105°C) —————		
Zn	28.0	44.7	43.7
Fe	592	1716	506
Mn	34.3	45.7	46.2
Cu	6.05	6.61	11.88
Se	ND	ND	0.366

¹Estimated from botanical composition at ensiling to be 69.7% timothy, 16.4% clover, 8.7% alfalfa, and 5.2% weeds (DM basis).

²Calculated to be 21.9% corn silage, 30.2% timothy silage, and 48.0% mixed concentrate (DM basis). The TMR was fed during the preexperimental period, and the MR was fed during the experimental period.

³Not determined.

⁴Estimated from NRC (7) tables.

⁵As CP equivalent.

⁶Neutral detergent.

⁷Acid detergent.

Intakes of DM and its components were influenced by treatment (Table 4). However, cows fed rations supplemented with RPLM consumed a ration that was higher in NDF and lower in CP than did cows fed the unsupplemented ration, although all cows were offered the same MR. However, the numerical extent of these differences was small and unlikely to be of biological significance.

Milk Production

Production of milk and milk components was not influenced by treatment (Table 5). However, consistent with results of many other studies in which RP Met has been utilized (10), a small (i.e., 40 g/d) numerical increase occurred in the production of both milk fat and protein in cows fed the MR supplemented with RPLM.

Body and Energy Status

Cows on all treatments were gaining weight as determined by either BW or BCS (Table 5), although neither was influenced by treatment.

The output of energy in milk for maintenance and for changes in BW was not influenced by treatment (Table 6). The calculated dietary energy density also did not differ among treatments and was virtually identical to the values estimated from NRC (7) tables.

DISCUSSION

In general, the performance of the dairy cows utilized in this study was consistent with expectations based on their production in previous lactations as well as performance of multiparous cows from this herd in previous and subsequent years. Cows on all treatments peaked at about 40 kg/d of milk during wk 5 to 6 of lactation and declined over the 20-wk measurement period to about 30 kg/d of milk. The DMI averaged about 21 kg/d at the initiation of the measurement period (wk 5 of lactation); DMI increased consistently to peak at about 24 kg/d at wk 11 to 12 of lactation followed by a slow decline to about 22.5 kg/d at the termination of the measurement period at 24 wk postpartum.

The bulk of the following discussion evaluates these rations using equations of The Atlantic Protein System to determine which, if any, AA in these rations actually limited performance. The relevance of this section is dependent on these unpublished equations and the estimated AA proportions in the protein from each dietary feedstuff that entered the small intestine. Clearly, these equations may be incorrect.

TABLE 4. Supplementation of ruminally protected (RP) AA products for each treatment as well as feed intake as influenced by supplementation of a RP Lys product (RPL) or a RP Lys and RP Met product (RPLM).

	Treatment				Contrast		
	Control	RPL	RPLM	SEM	Control vs.		RPL vs. RPLM
					RPL	RPLM	
P							
Supplementation levels, g/d							
RPL ¹	0	131.4	65.2
RPLM ²	0	0	113.7
Feed intake ³							
DM							
kg/d	23.26	23.04	23.31	0.43	0.74	0.94	0.69
% of BW	3.59	3.54	3.61	0.07	0.61	0.89	0.53
OM							
kg/d	21.64	21.43	21.67	0.39	0.73	0.97	0.70
% of DMI	93.01	93.01	93.02	0.01	0.85	0.70	0.56
NDF							
kg/d	7.89	7.79	7.98	0.13	0.64	0.70	0.38
% of BW	1.22	1.19	1.23	0.02	0.40	0.78	0.26
% of DMI	33.8	34.0	34.4	0.2	0.50	0.03	0.10
CP							
kg/d	3.23	3.21	3.27	0.07	0.83	0.73	0.57
% of DMI	13.98	13.97	13.90	0.02	0.65	0.04	<0.10

¹Ruminally protected Lys product (Ajinomoto Co., Inc., Tokyo, Japan). Analysis by the supplier: 53.6% Lys as Lys-HCl with an estimated intestinal availability of 30% of total Lys.

²Ruminally protected Lys and Met product (Ajinomoto Co., Inc.). Analysis by the supplier: 34.2% Lys as Lys-HCl and 17.3% DL-Met with an estimated intestinal availability of 30% for each AA.

³Exclusive of the RPL and RPLM.

Nevertheless, in the absence of a published and accepted AA evaluation model, this approach allows the performance results to be evaluated in terms of the treatments. Data have been presented as completely as possible so that other models can be used to evaluate the data. Other models may lead to different conclusions, which is consistent with the state of knowledge in this area of animal biology.

Evaluation of the Rations

The objective of the study required that the unsupplemented ration provide sufficient RDP to meet but not substantially exceed microbial N requirements as well as to provide approximately 110% of the calculated requirements for intestinally digestible RUP. Thus, performance would be limited by the desired intestinally available AA balance of Lys as the first-limiting AA and Met as the second-limiting AA with all other essential AA provided at levels at least 10% in excess of calculated requirements. This combination, if achieved, would allow the response to RP Lys to be measured when intestinally digestible supplies of Lys were actually calculated to limit performance (i.e., RPL treatment). In addition, the response to RP

Met could be measured when the Lys limitation had been alleviated by supplementation with RP Lys, leaving Met as the first-limiting AA (i.e., RPLM treatment).

Clearly, this objective was optimistic, and, not surprisingly, was only partially achieved. Indeed, examination of the protein and AA status of the rations actually fed (Tables 7 and 8) revealed a somewhat different composition of the unsupplemented ration than was the objective. The objective to meet but not substantially to exceed microbial N requirements was probably achieved because delivery of RDP was calculated to be 1.07 of requirements (Table 7). However, the objective to provide intestinally available protein to at least 1.10 of calculated requirements was not achieved because delivery of digestible RUP was calculated to be only 1.01 of requirements. The secondary objective, to achieve an intestinally available AA profile in which Lys was first-limiting and Met was second-limiting, was also not achieved. Although Lys was indeed more limiting than Met in the unsupplemented ration (i.e., 1.00 vs. 1.14 of calculated requirements), His (0.96 of calculated requirement) was more limiting than Lys, and Ile (1.03), Arg (1.04), and Val (1.10) were more limiting than Met.

TABLE 5. Milk production, milk composition, BW, BW change, body condition score (BCS),¹ and BCS change as influenced by supplementation of a ruminally protected (RP) Lys product (RPL) or a RP Lys and RP Met product (RPLM).

	Treatment			SEM	Contrast		
	Control	RPL	RPLM		Control vs.		RPL vs. RPLM
					RPL	RPLM	
P							
Production, kg/d							
Milk	33.85	33.53	33.92	0.74	0.79	0.95	0.75
Fat	1.27	1.26	1.31	0.04	0.88	0.53	0.45
Protein	1.07	1.07	1.11	0.03	0.90	0.51	0.47
Lactose	1.59	1.58	1.60	0.04	0.85	0.86	0.72
Milk composition, %							
Fat	3.79	3.80	3.85	0.12	0.97	0.75	0.79
Protein	3.21	3.21	3.26	0.04	0.99	0.39	0.40
Lactose	4.70	4.71	4.72	0.03	0.81	0.72	0.90
BW							
Mean, kg	649	652	648	3	0.62	0.82	0.47
Change, kg/d	0.35	0.38	0.30	0.04	0.67	0.45	0.24
BCS							
Mean, units	3.21	3.21	3.17	0.04	0.98	0.50	0.48
Change, units/wk	0.015	0.016	0.013	0.004	0.89	0.74	0.65

¹Scored on a five-point scale where 1 = emaciated and 5 = obese (3).

Revised Treatment Expectations and Responses

Evaluation of the protein and AA status of the unsupplemented ration based on the actual performance of the cows indicated that higher performance of cows fed this ration was probably colimited by

supplies of intestinally digestible protein and His. Only after these deficiencies had been overcome would there be an expectation that supplemental RP Lys would enhance performance. Thus, in this context, the virtually identical performance of cows fed the unsupplemented ration and cows fed the ration supplemented with RP Lys was consistent with expect-

TABLE 6. Energy balance as influenced by supplementation of a ruminally protected (RP) Lys product (RPL) or a RP Lys and RP Met product (RPLM).

	Treatment			SEM	Contrast		
	Control	RPL	RPLM		Control vs.		RPL vs. RPLM
					RPL	RPLM	
P							
Estimated ¹ energy input, Mcal/d	36.8	36.6	37.0
Energy output, Mcal/d							
Milk	23.99	23.88	24.61	0.58	0.91	0.51	0.46
Maintenance	10.28	10.31	10.27	0.04	0.65	0.79	0.48
BW Change	1.89	2.02	1.65	0.20	0.67	0.46	0.25
Total	36.16	35.81	37.15	0.74	0.76	0.40	0.26
Dietary energy density, Mcal/kg of DM							
Estimated ¹	1.58	1.58	1.58
Calculated ²	1.58	1.56	1.59	0.02	0.50	0.71	0.30

¹Estimated from NE_L values for dietary ingredients listed in Tables 2 and 3.

²Calculated from energy output in this table and DMI listed in Table 4 by individual cows.

tations based on evaluation of the rations. However, according to the suggestions of Schwab et al. (15), cows would have been expected to respond to RP Lys with enhanced performance because Lys constituted only an estimated 13.4% of total essential AA (assuming Phe made up 11.5% of total essential AA) delivered to the small intestine in cows fed the unsupplemented ration. This value (13.4%) contrasts with the optimal value of 15.2% suggested by Schwab et al. (15) and the estimated value of 15.2% achieved when RP Lys was supplemented. The similar performance of cows fed the unsupplemented ration and cows fed the ration supplemented with RP Lys in our study suggests that the appropriate criterion for Lys adequacy in the ration is its calculated quantitative intestinal delivery in relationship to its calculated intestinal requirements, rather than its intestinal delivery relative to other essential AA.

Supplementation of the ration with RP Lys alone would not have been expected to make cows responsive to supplementary RP Met because calculations (Tables 7 and 8) suggest that performance would still be colimited by supplies of intestinally available protein as well as His, Ile, Arg, and Val. The lack of

TABLE 7. Calculated protein balance by treatment.¹

	Treatment		
	Control	Lys	Lys and Met
Cow characteristics			
BW, kg	649	652	648
BW Change, kg/d	0.35	0.38	0.30
BCS, ² units	3.21	3.21	3.17
Milk production, kg/d	33.85	33.53	33.92
Milk fat, %	3.79	3.80	3.85
Milk protein, %	3.21	3.22	3.26
Days pregnant	0	0	0
Lactation number	3	3	3
Protein balance			
RDP			
Required, g/d	1926	1883	1919
Consumed, g/d	2060	2051	2067
Consumed/required	1.07	1.09	1.08
Digestible RUP			
Required, g/d	1287	1303	1324
Consumed, g/d	1296	1288	1311
Consumed/required	1.01	0.99	0.99
Total CP			
Required, g/d	3384	3357	3416
Consumed, g/d	3326	3310	3348
Consumed/required	0.98	0.99	0.98

¹According to equations developed as part of The Atlantic Protein System (version 5.0), which is an unpublished software package available by request from the senior author.

²Body condition score measured on a five-point scale where 1 = emaciated and 5 = obese (3).

TABLE 8. Calculated intestinally digestible AA balance¹ by treatment.²

	Treatment		
	Control	Lys	Lys and Met
Lys			
Required, g/d	141	141	143
Delivered, g/d	140	138 (159) ³	140 (162)
Delivered/required	1.00	0.98 (1.13)	0.98 (1.13)
Met			
Required, g/d	45	45	45
Delivered, g/d	51	50	51 (57)
Delivered/required	1.14	1.13	1.13 (1.27)
Thr			
Required, g/d	82	82	83
Delivered, g/d	109	107	109
Delivered/required	1.32	1.30	1.32
Leu			
Required, g/d	161	160	162
Delivered, g/d	230	228	232
Delivered/required	1.43	1.42	1.43
Ile			
Required, g/d	106	106	107
Delivered, g/d	109	107	109
Delivered/required	1.03	1.01	1.02
Val			
Required, g/d	115	115	116
Delivered, g/d	127	125	127
Delivered/required	1.10	1.09	1.10
His			
Required, g/d	52	52	52
Delivered, g/d	50	49	50
Delivered/required	0.96	0.95	0.69
Arg			
Required, g/d	102	102	102
Delivered, g/d	106	105	107
Delivered/required	1.04	1.03	1.04

¹Using the actual performance of the cows recorded on each treatment as specified in Table 7.

²According to equations developed as part of The Atlantic Protein System (version 5.0), which is an unpublished software package available by request from the senior author.

³Values in parentheses include the calculated concentrations of Lys, or Met, or both provided from the ruminally protected AA products.

statistical effect on the performance of cows fed rations supplemented with RP Met was consistent with expectations based on this evaluation of the rations. But can this hypothesis, that performance was limited by AA such as His and Ile, possibly be correct? Indeed, performance enhancements caused by post-ruminal supplementation of AA other than Lys and His have not been widely reported. However, Huhtanen et al. (4) and Vanhatalo et al. (19) recently reported performance improvements caused by abomasal infusion of His, and Robinson et al. (9) reported performance improvements caused by abomasal infusion of Ile. In all three of these cases, the diets were based on grass silage, as they were in the present study. These findings demonstrate that

AA other than Lys and Met, such as His and Ile, may limit the performance of lactating dairy cows fed diets based on grass.

Nevertheless, it is intriguing that the production of milk fat and milk protein was numerically higher (40 g/d) for cows fed rations supplemented with RPLM than for cows fed unsupplemented rations or that for cows fed the ration supplemented with RPL alone because the mean response to RP Lys and RP Met combinations in the 12 studies discussed earlier (10) was quantitatively similar at 51 g/d of milk protein and 30 g/d of milk fat. Thus, despite the lack of statistical support, the improved performance of cows fed rations supplemented with RPLM was consistent with the results of previous studies. Nevertheless, the performance of the cows in this study fed rations supplemented with RPLM was not consistent with the actual evaluation of the protein and AA status of the ration in this study, which clearly showed that performance was limited by intestinal delivery of protein as well as four essential AA before Met would become limiting. Therefore, the apparent, albeit not statistically significant, milk component response to RPLM may indicate that Met can impact milk component synthesis even under conditions when Met is not the AA that is calculated to limit enhanced animal performance. Alternatively, our calculations may simply be underestimating the true requirements for intestinally available Met.

CONCLUSIONS

The actual nutritional status of the dairy cows in this study, relative to the intestinal supplies of protein and AA that were designed to limit performance, differed from the objective. This situation, probably not unique to this study, emphasizes the need to discuss the results of studies that are designed to evaluate protein and AA nutrition of dairy cows based on postexperiment dietary evaluation rather than on the situation assumed from preexperimental calculations.

In this context, results demonstrated that dairy cows failed to respond productively to enhanced intestinal supplies of Lys from RPL in a situation in which Lys was not calculated to be the first-limiting nutrient. In contrast, cows fed rations supplemented with RPLM showed a numerical increase in production of both milk protein and fat to an extent consistent with results of earlier studies, although calculations did not indicate that performance was limited

by intestinal supplies of Lys or Met. Although not statistically significant, this result suggests that Met, apparently unlike Lys, may enhance production of milk components beyond its role as a limiting AA.

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