Effects of Methionine and Lysine on Fermentation In Vitro and In Vivo, Nutrient Flow to the Intestine, and Milk Production¹

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ABSTRACT

Three experiments were conducted to investigate the feasibility of using crystalline methionine and lysine as protein supplements for lactating Holstein cows. In the first experiment, Met (DL-methionine) and Lys (L-lysine-HCl) were added to diets used in continuous culture bioreactors to estimate optimal concentrations for use in subsequent in vivo experiments. The second experiment measured ruminal fermentation and nutrient flow to the small intestine when Met and Lys were top-dressed on diets fed to nonlactating cows. The third experiment measured lactation performance when Met and Lys were added to diets fed to late-lactation cows. Providing 0.29 and 2.27% of dry matter as Met and Lys, respectively, provided the largest improvement in fermentation in vitro and these concentrations were used in subsequent experiments. When Met and Lys were top-dressed on diets fed to nonlactating cows, no changes in total tract N digestion were observed. No changes in microbial protein production or ruminal fermentation were observed. Adding Met and Lys did not change production or efficiency of production of milk or milk components by late lactation cows. These data indicate that providing supplemental Met and Lys during late lactation does not significantly improve the protein status of the cow and therefore may not improve milk production.

Key words: fermentation, methionine, lysine, nutrient flow

INTRODUCTION

Although ruminal microorganisms can survive and grow with only nonprotein N, a growth response is usually measured when they are supplied with α amino N (Maeng et al., 1976; Maeng and Baldwin, 1976). Addi-

tionally, a substantial portion of crystalline AA has been reported to escape ruminal degradation and arrive at the small intestine intact (Froidmont et al., 2002; Bateman et al., 2004). However, supplementing ruminants with crystalline AA historically has been cost prohibitive. Crystalline AA are almost universally included in commercial swine and poultry diets. The increased usage in these species, coupled with advances in technology, has greatly reduced the cost of crystalline AA. Furthermore, feeding strategies for dairy cattle have changed dramatically in the recent past (NRC, 1985, 1996, 2001) and these dietary changes may influence the ruminal response to added AA.

Protein may be included in diets above the absolute requirements of ruminant animals to provide adequate RDP for microbial fermentation and growth (NRC, 2001). If the AA requirements of ruminal microorganisms could be better defined and met, total dietary protein could be lowered without adverse effects on production. We hypothesized that crystalline AA could be fed to lactating dairy cows to stimulate ruminal fermentation and to increase flow of those AA to the small intestine. We chose to use Met and Lys in this trial because they are usually the first- and second-limiting AA in many diets (Schwab et al., 1992).

A series of experiments was conducted to investigate the use of crystalline DL-methionine and L-lysine-HCl as protein supplements for lactating dairy cows. The first experiment studied the effects of Met and Lys supplementation on ruminal fermentation in vitro. The second experiment determined the effects of Met and Lys on flow of nutrients to the small intestine of cows. The third experiment determined the effects of Met and Lys on production of milk and milk components.

MATERIALS AND METHODS

In Vitro Fermentation Experiment

Two single-effluent continuous culture bioreactors (maximum capacity, 3 L; Biovessel with microbial package, Applikon Inc., Foster City, CA) were used to investigate the effects of supplemental Met and Lys on in

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vitro fermentation. Prior to inoculation, 25 g of each treatment diet was added to 2 L of buffer (Goering and Van Soest, 1970) and allowed to hydrate for 24 h at 39°C with constant stirring. Trypticase was removed from the original buffer recipe to maximize any impact of supplemental AA in this study. Immediately before inoculation, 100 mL of reducing solution (Goering and Van Soest, 1970) was added to each bioreactor, and then each bioreactor was inoculated with 500 mL of strained ruminal fluid. The ruminal fluid used to inoculate the bioreactors was sampled and stored frozen (−20°C) for further analyses.

Inoculant was obtained from 3 ruminally cannulated, nonlactating Holstein cows maintained on bermudagrass pasture at the Louisiana State University Dairy Science Research and Teaching Farm in Baton Rouge between January and September 2002. Approximately 500 mL of ruminal fluid was collected by hand from each cow. Ruminal contents were strained through 4 layers of cheesecloth to separate fluid from large feed particles. The fluid from all 3 cows was combined. Strained ruminal fluid mixture was immediately transported to the laboratory and mixed well before inoculation. All donor cows were handled and housed under protocols approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center.

During each experimental period, each bioreactor was fed 20 g of its assigned treatment diet twice daily at 0800 and 1600. The fluid was constantly stirred throughout the fermentation period. Fermentation was maintained at 39°C under anaerobic conditions by bubbling CO₂ through the bioreactor fluid for 5 min to force out O₂ each time the system was opened to the atmosphere. Bioreactor fluid pH was maintained at 6.5 or higher by infusing 0.5 M Na₂HPO₄ and 0.5 M NaHCO₃ in equal volumes using peristaltic pumps controlled by automatic pH meters. Excess amounts of fluid were removed and the volume recorded daily during the stabilization period to maintain a working volume of 2 L.

One diet, which consisted of 50% (as-fed basis) commercial chopped alfalfa hay (Bert & Wetta Larned, Inc., Larned, KS) and 50% (as-fed basis) commercial concentrate pellet (ADM Animal Health and Nutrition Division, Quincy, IL) was used as the base for formulating experimental treatments. Concentrate and hay were ground to pass a 2-mm screen. The Met (DL-methionine) and Lys (L-Lysine HCl), which were mixed by hand with the basal diet, were feed grade from commercial sources (Rhodimet NP99, Rhône-Poulenc Animal Nutrition, Antony Cedex, France; L-Lys HCl, Biokyowa Inc., Cape Girardeau, MO). Three levels of supplemental Met, 0, 0.29, and 0.58% of the diet (DM basis), and 3 levels of supplemental Lys, 0, 1.14, and 2.29% of the

diet (DM basis), were added to the basal diet in a 3 × 3 factorial arrangement to formulate 9 dietary treatments. Both the concentrate pellet and alfalfa hay, along with the 9 experimental diets, were analyzed for DM by drying at 105°C for 24 h. Ash was analyzed by combustion at 450°C for 24 h, total N was measured by Kjeldahl procedure (AOAC, 1980), and ADF and ADIN were measured according to Van Soest et al. (1991).

Intermediate concentrations of supplemental Met and Lys used in this study were similar to those used by Froidmont et al. (2002) when measuring ruminal escape of free Met and Lys. They also were chosen to mimic the ideal dietary requirements of Met and Lys for lactating sows consuming a corn-soybean based diet with 90% DM (NRC, 1998). The ideal dietary requirements for lactating sows are expressed as the concentration of Met and Lys in the total diet (NRC, 1998); however, treatments in this study used those concentrations as supplemental AA. Therefore, intermediate treatments in this study would have provided approximately 7-fold excess of the Lys requirement for lactating sows (NRC, 1998). Zero added AA and 2 times the intermediate concentrations were also evaluated to determine the optimal concentrations for ruminal microorganisms. Each treatment was evaluated twice in each bioreactor. Therefore, the experiment was conducted in 2 replicates of 9 periods. Within each replicate, treatments were randomly assigned to each bioreactor. Therefore, there were 4 observations per treatment during the entire experiment. Experimental period length during the first replicate was 5 d, which included 4 d of stabilization followed by 1 d of sampling. Experimental period length during the second replicate was 6 d, which included 4 d of stabilization followed by 1 d of sampling and 1 d of microbial isolation. On the day of sampling for each replicate, 2 samples of fluid (approximately 45 mL each) were collected from the bioreactors by vacuum immediately prior to the morning feeding at 0800 h (corresponding to 0 h postfeeding). Bioreactor fluid was then sampled again at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 h postfeeding. One of the samples was subsampled (4 mL) and prepared for VFA analysis. The remainder of this sample was then acidified by adding 1 mL of 20% (vol/vol) H₃PO₄ and stored frozen (−20°C) for NH₄⁺ analysis. The 4-mL subsample of bioreactor fluid was mixed with 1 mL of 25% (wt/wt) meta-phosphoric acid containing 2 g of 2-ethylbutyric acid /L, which was used as an internal standard for VFA quantification. The mixture of bioreactor fluid and meta-phosphoric acid was then clarified by centrifugation at 30,000 × g for 25 min. Concentrations of individual VFA were measured by GLC (Bateman et al., 2002). The second sample was immediately frozen (−20°C) without any reagent addition for later analyses of purine and total

N content. Before NH_4^+ analysis, the acidified fluid sample was thawed at room temperature and clarified by centrifuging at $30,000 \times g$ for 20 min. The clarified supernatants were then decanted and analyzed for NH_4^+ using a modified phenol-hypochlorite reaction (Bateman et al., 2002).

Because fermentation periods were conducted over a broad time period, samples of inoculums were retained and subjected to the same chemical analysis as samples that were collected from the bioreactors. No differences in pH, VFA concentrations or amounts, or NH_4^+ concentrations were observed in these samples (data not shown). However, this does not rule out the possibility that experimental period impacted our results. Due to limitations within our laboratory, we were unable to control for this variation.

A modified isolation procedure adapted from Steinhour et al. (1982) and Zinn and Owens (1986) was used to isolate bacteria. Briefly, the entire bioreactor contents were blended in a commercial blender at low speed for 5 min. The blended fluid was strained through 4 layers of cheesecloth, and solids were discarded. Strained fluid was centrifuged at $500 \times g$ for 5 min to remove the remaining feed particles and protozoa. The supernatant was decanted, and the pellet was discarded. The supernatant was recentrifuged at $20,000 \times g$ for 20 min. The supernatant from this second centrifugation step was decanted and discarded. The pellet was resuspended in 0.9% saline solution and recentrifuged at $20,000 \times g$ for 20 min. Again, the supernatant was decanted and discarded. This step was repeated once before harvesting the bacterial pellet, which was stored frozen (-20°C) until later analysis.

Nonacidified fluid samples from each time point were thawed at room temperature and combined. Composite samples were centrifuged at $20,000 \times g$ for 25 min. Effluent pellets and isolated bacteria were dried at 55°C overnight, and dry weight was recorded. Oven-dried effluent pellets and bacteria were ground by hand. Subsamples of effluent pellets and bacteria were then dried at 105°C and analyzed for their contents of ash and purines (Zinn and Owens, 1986). Contents of N in effluent and bacterial pellets were analyzed using an N auto-analyzer (Quickchem 8000, Lachat Instruments, Loveland, CO).

Data for NH_4^+ , total and individual VFA, and bacterial N synthesis were analyzed as a replicated 3×3 factorial arrangement of treatments by ANOVA using a fixed effects model. The statistical model used was $Y_{ijk} = \mu + M_i + L_j + H_k + M_iL_j + M_iH_k + L_jH_k + M_iL_jH_k + \varepsilon_{ijk}$, where Y_{ijk} is the observed response, μ is the overall mean, M_i is the effect of Met level i , L_j is the effect of Lys level j , H_k is the effect of time of sampling k , and ε_{ijk} is residual error that was used to test all treatment

effects. When interactions of Met and Lys were observed, Fisher's least significant differences were used to separate means. All data are presented as least squares means. All calculations were completed using SAS (SAS Inst. Inc., Cary, NC). Significance was declared at $P < 0.05$.

Ruminal Degradability and Nutrient Flow Experiment

Cannulas were surgically fitted into rumens and proximal duodenums of 4 nonlactating Holstein cows (average BW = 642 kg) by the School of Veterinary Medicine at Louisiana State University under protocols approved by the LSU Agricultural Center's Institutional Animal Care and Use Committee. After recovering, these cows were then used in a replicated switch-back designed experiment to measure ruminal degradability of Met and Lys. Duodenal cannulas were a simple T gutter type design made from soft plastic material (B. Hess, Univ. Wyoming). Ruminant cannulas were from a commercial source (Bar-Diamond, Inc., Parma, ID).

Cows were fed a single diet once daily at 0800 h at a restricted intake or the same diet top dressed with 190 g of Lys and 20 g of Met. Diets were fed at restricted intake to ensure complete consumption by the cows. Levels of AA used in this experiment were chosen to provide 0.29% supplemental Met and 2.27% supplemental Lys in the diet DM offered. The basal diet used in this experiment consisted of (DM basis) 45% corn silage, 20% cottonseed hulls, 15% ground shelled corn, 15% soybean meal (48% CP), and 5% vitamin and mineral premix. The basal diet was 42.83% DM and contained 91.0% OM, 33.03% ADF, 72.40% NDF, and 2.74% N on a DM basis.

Period length for this experiment was 7 d. During each period, cows were allowed to adapt to diets for the first 5 d and then data were collected. Gelatin capsules containing 10 g of Cr_2O_3 were placed in the rumen once daily beginning on d 4 of each period to be used as an external marker of digesta flow. Starting on d 6 of each period, samples of duodenal contents (250 mL), ruminal contents (100 mL of liquid plus approximately 400 g of solids), and feces were collected starting at 0800 h and every 6 h thereafter. On d 7 of each period, sample collection times were advanced 3 h. Therefore, over the course of the period, a sample was collected every 3 h over the entire 24-h period. Six observations for each treatment are represented in this data set.

Duodenal samples were collected by opening the cannula and allowing contents to flow freely to clear the cannula of sedimentary material. After approximately 100 mL of contents had flowed from the cannula, 250-mL samples were collected, composited within each cow

and period observational unit, and stored frozen (-20°C) until analysis. Samples of ruminal fluid were collected by placing a 100-mL bottle into the rumen and into the water layer of the ruminal contents, allowing it to fill, and then removing it from the rumen. Gross contamination of solids that were inadvertently collected were then removed by hand and a 40-mL sample of the fluid was retained and immediately frozen (-20°C). Samples of ruminal solids were collected by hand from the ruminal mat directly in front of the cannula. Samples were composited within each cow-period observation and stored frozen until analysis. The ruminal fluid remaining after removal of the 40-mL subsample was combined with the solids that were collected when freezing so that samples of ruminal contents contained both solids and liquid. Fecal grab samples were collected from immediately inside the rectum and composited within each cow-period observation. At the completion of each period, samples were thawed and further prepared. Samples of duodenal contents were mixed by hand and a subsample (45 mL) was refrozen. Bacteria were isolated from the samples of ruminal contents using procedures previously outlined then stored frozen (-20°C). Fecal and duodenal samples were dried to a constant weight at 40°C in a forced air oven. Samples of ruminal fluid were composited within each cow-period observation and pH measured. A 50-mL subsample was then prepared for VFA and NH_4^+ analyses as previously described. Content of Cr in samples of duodenal and fecal contents were determined by atomic absorption spectroscopy (Williams et al., 1962) and used to estimate nutrient flows, which were then used to calculate digestibility.

All data from this experiment were reduced to period means and analyzed with a mixed model using SAS. The statistical model used was: $Y_{ijk} = \mu + T_i + P_j + C_k + \varepsilon_{ijk}$, where Y_{ijk} is the observed response, μ is the overall mean, T_i is the fixed effect of treatment i , P_j is the random effect of period j , C_k is the random effect of cow k , and ε_{ijk} is residual error. Significance was declared when $P < 0.05$ and trends when $P \geq 0.05$ but < 0.1 .

Lactation Experiment

Sixteen Holstein cows in late lactation (mean DIM = 223) were paired by their current milk production (average 20.4 kg/d), parity, and DIM, and then randomly assigned to either the control or treatment diet (Table 1). Both diets were offered as TMR twice daily at 0700 and 1500 h using electronic feeding gates (American Calan, Northwood, NH). Cows were adapted to the control diet for 8 d before data collection. The AA in the treatment group were mixed with the TMR. Cows were milked twice daily at 0600 and 1530 h. All cows were

Table 1. Composition of the control and treatment diets used in the lactation experiment.

Ingredient	Control	Treatment
	% of DM	
Corn silage	48.59	47.35
Protein concentrate	15.39	15.00
Alfalfa hay	12.41	12.09
Cottonseed	11.07	10.78
Corn	10.82	10.54
Sodium bicarbonate	0.74	0.72
Limestone	0.49	0.48
Trace mineral salt ¹	0.49	0.48
L-Lys-HCl	0	2.27
DL-Met	0	0.29

¹Contains 99% DM, 60.60% Cl, 39.34% Na, 70 mg/kg Co, 400 mg/kg Cu, 70 mg/kg I, 1,750 mg/kg Fe, 2,800 mg/kg Mn, 0 mg/kg Se, and 3,500 mg/kg Zn.

handled and housed under protocols approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center.

The entire experiment was a 36-d trial that included 8-d of adaptation (pre-experimental period) followed by a 28-d experimental period. During the experimental period, as-fed intake and milk production were recorded daily and milk was sampled at each milking and sent to the Louisiana DHIA lab (Baton Rouge, LA) for analyses of percentage of milk fat and protein, and SCC. The TMR of the control and treatment groups were sampled daily at each feeding and feed ingredients were sampled weekly to analyze for total N, ADIN, ash, DM (AOAC, 1980), and ADF (Van Soest et al., 1991). Body weight for each cow was recorded prior to (d -2) and at the end (d 28) of the experiment.

All data were analyzed as repeated measurements using a mixed model using SAS (Littell et al., 1998). The statistical model used was: $Y_{ijk} = \mu + T_i + D_j + T_i D_j + C_k + \varepsilon_{ijk}$, where Y_{ijk} is the observed response, μ is the overall mean, T_i is the fixed effect of treatment i , D_j is the repeated effect of day of study j , C_k is the random effect of cow k nested within treatment i , and ε_{ijk} is residual error. The cow k nested within treatment i was used as the subject for the repeated effect of day j , and the repeated effect was assumed to have an autoregressive type 1 covariance structure. Significance was declared at $P < 0.05$ and trends when $P \geq 0.05$ but < 0.1 .

RESULTS AND DISCUSSION

In Vitro Fermentation Experiment

Chemical analyses of the 9 treatments are presented in Table 2. As expected, supplemental AA increased diet N content but did not change ADIN content. Diets were similar in contents of DM, ADF, and ash.

Table 2. Chemical analysis of experimental treatments used in bioreactors

Item	0% Met			0.26% Met			0.52% Met		
	0% Lys	1.03% Lys	2.06% Lys	0% Lys	1.03% Lys	2.06% Lys	0% Lys	1.03% Lys	2.06% Lys
DM, %	89.89	90.83	90.33	90.49	90.17	90.51	90.88	91.08	90.24
	(% of DM)								
N	3.10	3.27	3.52	3.19	3.30	3.38	3.22	3.35	3.38
CP	19.40	20.45	22.00	19.95	20.60	21.15	20.10	20.95	21.10
ADIN	0.34	0.33	0.35	0.42	0.33	0.29	0.33	0.38	0.38
ADF	19.82	17.71	17.53	16.85	17.25	17.07	18.19	17.09	16.35
Ash	8.69	8.86	8.62	8.78	8.65	8.49	8.83	8.73	8.49

Concentrations of total VFA and proportions of individual VFA are presented in Table 3. No effects of time of sampling ($P > 0.1$) on concentrations of total VFA or proportions of individual VFA were observed; therefore, only main effects are presented. Concentrations of VFA were altered by the interaction of Lys and Met supplementation ($P < 0.01$) and the addition of Lys ($P < 0.05$) or Met ($P < 0.01$). The highest concentration of total VFA was observed with 1.03% Lys and 0.52% Met supplementation and the lowest concentration of total VFA was observed with no AA supplementation.

The concentration of acetate was altered ($P < 0.01$) by both the interaction of Lys and Met as well as Lys supplementation, but was not affected by Met supplementation alone. The concentration of propionate was influenced by the interaction of Lys and Met ($P < 0.05$) and the main effects of Lys and Met ($P < 0.01$) supplementation. These changes resulted in alterations in the ratio of acetate to propionate. The ratio of acetate to propionate was numerically lowest when 1.03% Lys and 0.52% Met were added to the diet. This corresponds to the diet with the greatest concentrations of total VFA. The ratio of acetate to propionate decreased as the amount of Met supplementation increased ($P < 0.01$).

There were no effects of Lys and Met or their interaction on the concentration of isobutyrate. The concentration of butyrate was affected by the interaction of Lys and Met ($P < 0.05$) and increased with Lys supplementation ($P < 0.01$). The concentration of isovalerate was not affected by Lys ($P > 0.05$) but was influenced by both the interaction of Lys and Met ($P < 0.01$) and decreased ($P < 0.01$) with Met supplementation. The concentration of valerate was decreased ($P < 0.01$) by the interaction of Lys and Met and decreased ($P < 0.01$) as the amount of Lys or Met increased.

Concentration of NH_4^+ increased as both the amount of Lys and Met ($P < 0.01$) supplementation increased. There was an interaction ($P < 0.01$) of Lys and Met for NH_4^+ concentration from the continuous culture system. Supplementing 2.06% Lys and 0.26% Met resulted in the numerically highest concentration of NH_4^+ among treatments studied in this experiment. This is probably due to microbial deamination of the supplemental AA and the subsequent use of the carbon in those AA as energy substrates by the ruminal bacteria. There were no effects of Lys, Met, or their interaction in the proportion of total N that was microbial N (Table 4). Similarly, there were no effects of Lys, Met, or their

Table 3. Concentrations of VFA and ammonia from bioreactors supplied with differing combinations of Lys and Met

Item	0% Met			0.26% Met			0.52% Met			SEM
	0% Lys	1.03% Lys	2.06% Lys	0% Lys	1.03% Lys	2.06% Lys	0% Lys	1.03% Lys	2.06% Lys	
Total VFA, mM ^{1,2,3}	269 ^c	274 ^c	339 ^b	316 ^b	322 ^b	334 ^b	334 ^b	372 ^a	320 ^b	10.7
Acetate, mol/100 mol ^{1,3}	60.7 ^{ab}	59.7 ^c	61.0 ^a	61.0 ^a	59.6 ^c	59.8 ^{bc}	60.0 ^{bc}	60.0 ^{bc}	60.4 ^{abc}	0.33
Propionate, mol/100 mol ^{1,2,3}	16.9 ^c	17.4 ^{bc}	15.9 ^d	18.0 ^{ab}	17.3 ^c	17.0 ^c	18.0 ^{ab}	18.1 ^a	17.0 ^c	0.20
Isobutyrate, mol/100 mol	1.6	1.5	1.4	1.5	1.5	1.6	1.5	1.4	1.4	0.07
Butyrate, mol/100 mol ^{1,3}	14.6 ^{ef}	15.4 ^{cd}	16.4 ^a	14.2 ^f	15.7 ^{bcd}	15.8 ^{bc}	15.0 ^{de}	15.2 ^{cd}	16.1 ^{ab}	0.23
Isovalerate, mol/100 mol ^{2,3}	3.2 ^a	3.2 ^a	2.7 ^c	2.8 ^c	3.0 ^{ab}	3.1 ^a	2.8 ^{bc}	2.7 ^c	2.6 ^c	0.08
Valerate, mol/100 mol ^{1,2,3}	3.0 ^a	2.8 ^{bc}	2.5 ^e	2.6 ^{de}	2.9 ^{ab}	2.7 ^{cde}	2.7 ^{cd}	2.6 ^{de}	2.6 ^{de}	0.05
Acetate:propionate ^{1,2,3}	3.6 ^b	3.4 ^{cde}	3.9 ^a	3.4 ^{de}	3.5 ^{bcd}	3.5 ^{bcd}	3.4 ^e	3.3 ^e	3.6 ^{bc}	0.05
NH_4^+ , mg/dL ^{1,2,3}	6.1 ^e	6.6 ^d	7.4 ^{bc}	6.0 ^e	7.3 ^c	7.9 ^a	6.8 ^d	7.8 ^{ab}	7.0 ^{cd}	0.14

^{a-f}Means in the same row with different superscripts differ ($P < 0.05$).

¹Main effect of Lys ($P < 0.05$).

²Main effect of Met ($P < 0.05$).

³Interaction of Met and Lys ($P < 0.05$).

Table 4. Chemical composition of bacterial and effluent pellet from bioreactors supplied with different combinations of Lys and Met

Item	0% Met			0.26% Met			0.52% Met			SEM
	0%	1.03%	2.06%	0%	1.03%	2.06%	0%	1.03%	2.06%	
	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	
Bacteria										
DM, %	8.21	6.59	10.20	9.20	9.12	10.84	8.58	8.04	9.57	1.46
Ash, % of DM	10.48	7.63	12.83	13.97	9.85	13.15	10.47	10.34	13.22	2.00
N, % of DM	10.20	10.40	9.90	9.90	10.30	9.50	10.20	10.50	10.00	0.32
N, % of total N	42.16	42.90	24.14	23.28	35.13	28.05	23.40	39.66	30.58	8.27
Effluent										
DM, %	13.77	13.65	14.08	13.54	13.55	14.61	14.06	13.41	13.35	0.73
Ash, % of DM	21.79	21.68	20.74	21.36	20.59	22.05	20.34	23.07	21.65	1.25
N, % of DM	4.27	4.27	4.49	4.40	4.42	4.80	4.55	4.42	4.14	0.16

interaction on the composition of the ruminal bacteria or fermenter effluent.

Ruminal Degradability and Nutrient Flow Experiment

Addition of the top-dressed AA supplement resulted in numerical increases to DMI and OM intake (Table 5). Apparent digestibility of DM and OM in the rumen and total tract were not affected by supplemental AA. Supplemental AA increased ($P < 0.01$) N intake in this experiment (Table 6). There was a numeric increase in apparent digestibility of N and amount of N digested in the rumen when AA were top dressed. This is probably further evidence that the supplemental AA were being deaminated by the ruminal microorganisms and their carbon skeletons used as an energy source. There was no effect of supplemental AA on apparent total tract N digestibility (Table 6). Supplemental AA did not affect the amount or proportion of duodenal DM flow that was bacterial or feed in origin (data not shown). Supplemental AA did not affect the amount or proportions of microbial or feed N that flowed to the small intestine.

There were no effects of supplemental AA on ruminal VFA concentrations or proportions observed in this experiment (Table 7). Similarly, there was no effect of supplemental AA on ruminal pH. Unlike in the in vitro fermentation experiments, supplemental AA did not result in increased NH_4^+ concentrations in ruminal fluid. However, ruminal NH_4^+ concentrations in this experiment were low (average 2.9 mg/dL) and may be an indication that ruminal N availability was limiting in the experiment. Diets in this experiment were formulated to be adequate in N before addition of the AA (NRC, 2001). The basal diet contained 2.74% N (data not shown), which was approximately 0.5% more N than formulated levels. It is possible that because the cows in this experiment were limit fed, the absolute

amount of N provided was limiting for optimal ruminal fermentation, and the supplemental AA were used as a source of RDP instead of as the specific AA provided.

Table 5. Nutrient intakes and passage of DM, OM, NDF, and ADF from the rumen and through the gastrointestinal tract of cows fed diets top dressed with DL-Met and L-Lys-HCl

	Basal diet	Top-dressed diet	SEM
DM			
Intake, kg/d	9.8	10.1	0.43
Apparently digested in the rumen			
kg/d	4.7	4.5	0.54
%	48.0	44.6	4.9
Truly digested in the rumen			
kg/d	5.57	5.31	0.49
%	56.9	52.6	5.05
Apparently digested in the total tract			
kg/d	6.2	6.5	0.39
%	62.8	64.1	2.5
OM			
Intake, kg/d	8.9	9.2	0.38
Apparently digested in the rumen			
kg/d	4.8	4.8	0.51
%	53.9	52.8	4.8
Truly digested in the rumen			
kg/d	5.23	5.23	0.49
%	58.8	56.9	9.14
Apparently digested in the total tract			
kg/d	5.9	6.2	0.36
%	66.0	69.5	2.3
ADF			
Apparently digested in the rumen			
kg/d	1.3	1.3	0.20
%	41.5	39.7	7.2
Apparently digested in the total tract			
kg/d	1.3	1.4	0.12
%	38.5	42.6	3.3
NDF			
Apparently digested in the rumen			
kg/d	4.4	4.3	0.35
%	61.8	59.0	5.7
Apparently digested in the total tract			
kg/d	4.4	4.7	0.26
%	62.5	64.9	2.4

Table 6. Nitrogen intakes and passage of nitrogen from feed and bacteria from the rumen and through the gastrointestinal tract of cows fed diets top dressed with Met and Lys

	Basal diet	Top-dressed diet	SEM
N intake, g/d ¹	267.2	309.4	11.7
Apparently digested in the rumen			
g/d	72.7	106.8	20.6
%	25.4	34.8	7.5
Apparently digested in the total tract			
g/d	181.3	215.0	14.7
%	67.0	70.2	3.4
Apparently digested postruminally			
g/d	112.2	108.2	24.7
% of intake	41.6	35.4	8.7
% of duodenal flow	53.8	53.0	6.8
Flow to the small intestine			
Microbial N, g/d	59.0	65.7	7.5
% of intake	29.6	33.6	4.49
Nonmicrobial N, g/d	140.6	135.4	22.8
% of intake	70.4	66.4	4.49

¹Effect of treatment ($P < 0.05$).

Lactation Experiment

Diets for the control and treatment groups were formulated to slightly exceed the requirements for ME and MP of a 545-kg, 30-mo-old cow at 280 DIM producing 28 kg of 3.7% fat milk/d (NRC, 2001) and consuming 21 kg of DM/d prior to the addition of the AA. The average DMI for each cow of the treatment group in this study was 13.18 kg/d. Therefore, daily supplementation of Met was 38.22 g/cow per d and L-Lys-HCl was 299.19 g/cow per d. Chemical analyses of the control and treatment diets are presented in Table 8.

Least squares means for lactation performances of cows fed diets with or without AA supplementation are presented in Table 9. No effects of AA were observed for any production measure. These results are in agreement with Harrison et al. (2000) who reported no

Table 7. Concentrations of VFA and ammonia, and pH in rumen fluid of cows fed diets top dressed with DL-methionine and L-lysine-HCl

	Basal diet	Top-dressed diet	SEM
Total VFA, mM	148.12	143.00	12.79
Acetate, mol/100 mol	67.1	67.7	0.57
Propionate, mol/100 mol	17.2	17.5	0.80
Isobutyrate, mol/100 mol	0.99	0.94	0.04
Butyrate, mol/100 mol	11.9	11.0	0.78
Isovalerate, mol/100 mol	1.4	1.4	0.13
Valerate, mol/100 mol	0.92	0.84	0.08
Hexanoate, mol/100 mol	0.47	0.41	0.04
Acetate:propionate	3.90	3.93	0.20
NH ₄ ⁺ , mg/dL	3.55	4.03	0.69
pH	6.46	6.45	0.06

Table 8. Least squares means for chemical analysis of the experimental diets used in the lactation experiment

Item	Control	Treatment
DM, %	44.30	44.69
OM, %	40.40	40.78
	% of DM	
N	2.14	2.49
ADIN	0.39	0.38
ADF	32.84	30.30
Ash	8.81	8.74

significant differences of DMI or components of milk from cows fed diets with or without supplemental L-Lys and rumen protected-Met. These data also agree with Koudele et al. (1999) who reported no effect of Lys supplementation on milk production or milk components from high-producing cows fed diets supplemented with 50 g of Lys.

Least squares means for BW of the control and treatment groups were numerically increased after the experiment, as expected (Table 9). Although there was no AA treatment effect observed on BW gain between the control and treatment groups in this study, the numerically greater weight gain found in the treatment group (21.42 vs. 9.2 kg) suggests that AA supplementation may have favored energy partition to body tissue rather than production of milk and milk contents for cows in

Table 9. Least squares means for lactation performances and BW gains of cows fed diets with or without supplemental DL-methionine and L-lysine-HCl

Parameter	Control	Treatment	SEM
DMI, kg/d	14.66	13.18	0.73
OM intake, kg/d	13.29	11.97	0.63
Milk yield, kg/d	18.41	19.05	0.61
4% FCM ¹ , kg/d	18.31	19.26	0.63
ECM ² , kg/d	19.23	20.20	0.67
4% FCM efficiency ³	1.33	1.41	0.10
ECM efficiency ⁴	1.40	1.48	0.10
Milk efficiency ⁵	1.27	1.33	0.09
BW gain ⁶ , kg	9.20	21.42	5.95
Milk fat, %	3.96	4.05	0.05
kg/d	0.73	0.78	0.03
Milk protein, %	3.01	3.02	0.03
kg/d	0.55	0.57	0.02
SCS ⁷	3.54	3.69	0.12

¹4% FCM = $0.4 \times \text{milk yield} + 15 \times \text{fat yield}$.

²ECM = $0.3246 \times \text{milk yield} + 12.86 \times \text{fat yield} + 7.04 \times \text{protein yield}$.

³4% FCM efficiency = $\text{kg of 4\% FCM production} \div \text{kg of DMI}$.

⁴ECM efficiency = $\text{kg of ECM production} \div \text{kg of DMI}$.

⁵Efficiency = $\text{kg of milk production} \div \text{kg of DMI}$.

⁶Total gain or loss of BW over the 28-d experimental period.

⁷SCS = $3 + [\text{Ln}(\text{SCC} + 100) \div 0.693147]$ (National Mastitis Council, 1996).

late lactation (Samuelson et al., 2001). The AA treatment had no effect on productions and percentages of milk fat and protein of cows in this experiment.

Although there were no differences in percentages of milk fat and milk protein between the control and treatment groups, the treatment group had numerically greater percentages of milk fat and milk protein. The milk protein percentage of the treatment group exhibited a tendency for increasing during the experimental period and resulted in a numerically higher milk protein percentage than the control group. The SCS for both groups were not affected by AA supplementation.

CONCLUSIONS

Addition of supplemental AA changed fermentation *in vitro*. However, changes observed *in vitro* were small and may not have been large enough to impact observed fermentation responses *in vivo*. When AA were supplemented *in vivo*, there was a small increase in N digestion in the rumen but no changes in fermentation, microbial protein production, or postruminal flow of N fractions. This increase in N digestion was probably a result of increased microbial deamination of the supplemental AA and did not result in improved milk production by cows in late lactation.

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