Short communication: Supplementing lysine and methionine in a lactation diet containing a high concentration of wet corn gluten feed did not alter milk protein yield

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ABSTRACT

Primiparous ($n = 33$) and multiparous ($n = 63$) lactating Holstein cows (186 ± 51 d in milk) were used to evaluate the effects of supplementing metabolizable amino acids using lysine in a matrix of Ca salts of fatty acids (Megamine-L, Arm & Hammer Animal Nutrition, Princeton, NJ) and the isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid (MetaSmart, Adisseo Inc., Antony, France) in diets containing $>26\%$ wet corn gluten feed (dry matter basis). Cows were blocked by production level, parity, and pregnancy status, then randomly assigned to 1 of 8 pens and allowed a 7-d adaption period before receiving dietary treatments for 28 d. Pens were assigned randomly to either of 2 diets formulated to differ by metabolizable amino acid supply. Dry matter intake and production were monitored daily and milk components analyzed 3 d/wk. Data were analyzed using mixed models with repeated measures. The original design of the study consisted of a control diet predicted to be deficient in lysine and methionine; however, after ingredient nutrients were analyzed and modeled with animal requirements at dry matter intake [26.6 ± 0.35 kg/d (mean $\pm$ SEM)] and milk production levels achieved during the study (40.1 ± 0.46 kg/d), only marginal deficiencies were predicted for the control (-8.1 g/d for lysine; -1 g/d for methionine) according to the National Research Council method, whereas the Cornell Net Carbohydrate and Protein System 5.0 and 6.1 models indicated positive balances for these amino acids (25.9 and 21.8 g/d for lysine, 14.7 and 18.9 g/d for methionine, respectively). Supplementing 30 g/d of metabolizable lysine in a Ca soap matrix and 2.4 g/d of metabolizable methionine as 2-hydroxy-4-(methylthio) butanoic acid led to positive predicted lysine and me-

thionine balances by all 3 models, and predicted metabolizable lysine-to-methionine ratios ranging from 2.9 to 3.1. No treatment effects were observed for dry matter intake, milk yield, milk component concentrations or yields, or energy-corrected milk yield. Despite the negative lysine balance and low lysine-to-methionine ratio predicted by the National Research Council model, results provided no evidence of a lysine deficiency in the control diet.

Key words: dairy cattle, by-product, amino acid

Short Communication

Formulation of diets to meet the protein requirements of dairy cattle has evolved from a focus simply on CP to estimating MP supply, which attempts to account for ruminal protein degradation, microbial yield, and ruminal bypass protein. Formulating for MP has been shown to improve the efficiency of dietary nitrogen utilization (Leonardi et al., 2003; Noftsger and St Pierre, 2003; Chen et al., 2011), while possibly improving production (Schwab, 2010). However, formulating for an adequate MP supply may still fail to meet the requirements of the cow if AA profile is not considered. This concept has been widely recognized by the US dairy industry; a survey conducted in 2010 indicated that 81% of nutritionists and veterinarians formulate diets to balance for AA (Dairy Herd Network, 2010). Enhanced knowledge of protein nutrition has been directly responsible for this progression; still, the cost of protein sources, combined with environmental stewardship, demand even more efficient use of dietary protein.

By-products from corn biofuel production are often used to provide protein and energy in lactation diets. In 2010, of the nutritionists and veterinarians who formulate rations and completed the survey, 92% used distillers grains or considered using them (Dairy Herd Network, 2010). Many other by-products of corn milling are also fed to dairy cattle, including corn germ meal, corn bran, corn meal, and corn gluten feed. As in corn grain, the predominant protein in these by-products is zein, which is known to be low in Lys (Coleman and

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Larkins, 1999). Kelzer et al. (2010) characterized the AA profile of 7 corn by-products and observed that RUP Lys was below 3.5% in all 7 feedstuffs. Therefore, it is not surprising when nutrition models predict that diets containing large concentrations of corn by-products do not supply enough Lys to high-producing cows. It is also recognized that many lactation diets do not supply adequate Met; thus, Lys and Met have often been deemed first-limiting AA in lactation diets (Schwab et al., 1976; Schwab et al., 1992; NRC, 2001).

The objective of this study was to evaluate the effects of supplementing commercial rumen escape AA products in a diet that the NRC (2001) model predicted to be deficient in Lys and Met supply. The products used to provide the additional AA contained Lys embedded within Ca salts of FA (Megamine-L, Arm & Hammer Animal Nutrition, Princeton, NJ) and the Met precursor isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid (**HMBi**; MetaSmart, Adisseo Inc., Antony, France). We hypothesized that providing additional essential AA with these products would increase milk protein production in lactating dairy cattle.

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University. Primiparous ($n = 33$) and multiparous ($n = 63$) Holstein cows [186 ± 51 DIM (mean $\pm$ SD)] from the Kansas State University Dairy Cattle Teaching and Research Facility were used in this production study. Cows were blocked by milk production, parity, and pregnancy status. Cows within each block were then randomly assigned to 1 of 8 identical pens with 12 freestalls in each pen. Cows were moved into pens on May 3, 2010, and the study began on May 10, 2010, allowing for a 1-wk adaptation period. During the adaption period, all pens received a common diet.

The study consisted of a single 28-d treatment period. During this period, pens (4/diet) were offered 1 of 2 diets that were formulated to differ in metabolizable AA supply (Table 1). Treatment diets were randomly assigned to pen. Cows were fed once daily at 110% of the expected intake. The amounts of feed delivered and refused were recorded on d 19, 20, 21, 26, 27, and 28 (3 times in each of the final 2 wk). Total mixed ration samples from both treatments were analyzed for DM on those days. Samples of all dietary ingredients were collected on d 19, 21, 26, and 28 and composited for wet chemistry analysis by Dairy One Forage Laboratory (Ithaca, NY).

Cows were milked 3 times daily in a milking parlor and milk yields were recorded at each milking. Milk samples were collected from every milking on each Monday, Wednesday, and Friday throughout the experiment. Samples were analyzed for concentrations of fat, true protein, lactose (B-2000 Infrared Analyzer;

Bentley Instruments, Chaska, MN), and urea nitrogen (MUN spectrophotometer, Bentley Instruments; Heart of America DHIA, Manhattan, KS). Energy-corrected milk yield was calculated as $0.327 \times \text{milk yield} + 12.86 \times \text{fat yield} + 7.65 \times \text{protein yield}$ (Dairy Record Management Systems, 2010).

The chemical composition of the feed ingredients, intake data, production data, and animal descriptors were all entered into 3 computer programs or models: Spartan Dairy Ration Evaluator 3.0 (Michigan State University, East Lansing, MI), which is based on the NRC (2001) model with some additional improvements developed at Michigan State University (East Lansing, MI); CPM-Dairy 3.0.10 from Cornell University (Ithaca, NY), University of Pennsylvania (Philadelphia, PA), and Miner Institute (Chazy, NY), which uses the Cornell Net Carbohydrate and Protein System (**CNCPS**) 5.0 (Fox et al., 2004); and Nutritional Dynamic System v3 (RUM&M, Emilia, Italy), which uses the CNCPS 6.1 model. Models were used to determine the predicted supply of AA against model-specific requirements. For all 3 programs, a feed library was created so the nutrient profile of each dietary ingredient matched laboratory analyses. Megamine-L was assumed to be 16% digestible rumen bypass Lys on a DM basis, and HMBi was assumed to be 17% digestible rumen bypass Met on a DM basis.

A total of 95 of the 96 cows completed the study; 1 cow was removed from the study due to mastitis. Intake data for each pen was divided by the number of cows present in that pen on that day. All data were analyzed using the mixed procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). All models included the fixed effects of day, treatment, and day $\times$ treatment interaction and the random effect of pen. Repeated measures over time were modeled with a first-order autoregressive covariance structure; the subject was pen for intake data and cow within pen for milk production and component data. Denominator degrees of freedom were estimated by the Kenward-Roger method. Significance was declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

In formulating experimental diets, the strategy was to feed a control diet that would be deficient in Lys and Met. As part of that strategy, the goal was to maintain large concentrations of corn by-products within diets. Therefore, the control diet (Table 1) contained 26.7% wet corn gluten feed on a DM basis. Wet corn gluten feed has been reported to contain as little as 6.5% RUP (% of CP; Kononoff et al., 2007), so a diet containing such a great quantity of wet corn gluten feed could exacerbate any MP deficiency. The treatment diet was similar to the control, with the primary differences being replacement of 190 g/cow of the Ca salts of FA

Table 1. Ingredient and nutrient compositions of diets fed to lactating Holstein cows

Item (% of DM)	Dietary treatment	
	Control	AA supplemented
Ingredient		
Corn silage	23.4	23.4
WCGF ¹	26.7	26.6
Alfalfa hay	18.5	18.7
Cottonseed	5.4	5.3
Ground corn grain	14.2	14.2
Ground milo	2.7	2.7
Expeller soybean meal ²	4.9	4.9
Fish meal, Menhaden	0.3	0.3
Ca salts of long-chain FA ³	0.7	—
Ca salts of FA-Lys matrix ⁴	—	0.7
HMBi ⁵	—	0.04
Micronutrient premix ⁶	3.3	3.3
Nutrient		
DM (% as fed)	55.6	55.6
CP	17.8	17.9
NDF	33.3	32.9
Crude fat	4.7	4.5
Starch	24.0	23.5
NFC ⁷	37.6	37.9
Ash	6.9	6.8
NE _L ⁸ (Mcal/kg)	1.68	1.67
Ca	0.83	0.79
P	0.54	0.53
Mg	0.35	0.33
K	1.41	1.42
Na	0.42	0.44
Net energy allowable milk ⁸ (kg/d)	47.4	47.1
Metabolizable protein-allowable milk ⁸ (kg/d)	45.6	45.9

¹WCGF = wet corn gluten feed, Sweet Bran (Cargill Inc., Blair, NE).

²Soybest (Grain States Soya Inc., West Point, NE).

³Megalac-R (Arm & Hammer Animal Nutrition, Princeton, NJ).

⁴Calcium salts of FA plus lysine monohydrochloride, Megamine-L (Arm & Hammer Animal Nutrition).

⁵Isopropyl ester of 2-hydroxy-4-methylthio butanoic acid, MetaSmart (Adisseo Inc., Antony, France).

⁶The premix consisted of 41.6% limestone, 32.5% sodium bicarbonate, 6.50% Diamond V XP (Diamond V, Cedar Rapids, IA), 5.40% trace mineral salt, 5.40% magnesium oxide, 5.20% vitamin E premix, 1.66% 4-plex, 0.93% Se premix, 0.36% vitamin A premix, 0.16% vitamin D premix, 0.05% ethylenediamine dihydroiodide, and 0.21% Rumensin 80 (Elanco Animal Health, Greenfield, IN).

⁷Calculated as DM – (CP + NDF + ether extract + ash).

⁸Estimated according to NRC (2001) at actual intake.

(Megalac-R, Arm & Hammer Animal Nutrition) with a source of Ca salts of FA that was embedded with Lys (Megamine-L), and addition of 14 g/cow of HMBi (MetaSmart). As expected, the predicted supplies of metabolizable Lys and Met were slightly elevated with AA supplementation (Table 2), whereas other nutrient concentrations remained similar across treatments (Table 1).

Model predictions for percent of MP and Lys-to-Met ratio are listed in Table 2. The NRC model predicted an inadequate ratio of Lys to Met (2.92), and these AA as a percentage of MP were less than NRC (2001) guidelines for maintenance and milk protein production (7.2 and 2.4% of MP for Lys and Met, respectively). Model predictions obtained through the NRC (2001) indicated negative Lys (–8.1 g) and Met (–1 g) bal-

ances for the control cows; however, CNCPS 5.0 and 6.1 predicted positive balances for these AA (25.9 and 21.8 g for Lys, 14.7 and 18.9 g for Met, respectively). Amino acid supplementation using the commercial products led to a predicted positive Lys and Met balance by all 3 models.

Intake and production means for both groups are shown in Table 3. Mean DMI was 26.6 kg/d and mean milk yield was 40.1 kg/d, with means of 3.10% fat and 3.06% protein. No treatment effects were observed for any of the parameters measured. Milk protein yield, the variable of greatest interest, was numerically lower for the AA-supplemented diet compared with control (1.21 vs. 1.23 ± 0.02 kg/d; *P* = 0.54).

Results did not support the hypothesis that increasing the Lys and Met supply would increase production

Table 2. Predicted metabolizable Lys and Met supplies and predicted metabolizable Lys-to-Met ratio by 3 different models

Item ¹	Control			AA supplemented		
	Lys	Met	Ratio ²	Lys	Met	Ratio ²
g/d						
NRC (2001)	191	65	2.92	206	67	3.07
CNCPS 5.0	181	64	2.84	197	66	2.99
CNCPS 6.1	165	62	2.66	194	67	2.89
Metabolizable protein (%)						
NRC (2001)	6.3	2.2		6.8	2.2	
CNCPS 5.0	6.1	2.1		6.6	2.2	
CNCPS 6.1	5.3	2.0		6.0	2.1	

¹CNCPS = Cornell Net Carbohydrate and Protein System.²Lysine-to-methionine calculated based on grams of predicted AA supply.

of cows fed a corn by-product-based diet. Several possible explanations exist for the lack of response. One possibility is that the products used to provide supplemental Met and Lys did not increase the metabolizable supply of these AA. Exposing this Lys source to TMR ingredients can cause up to 20% of Lys to be released from the protective matrix before being consumed by the cow (Ji et al., 2012). However, past research has indicated that the Lys source used in this study provides 70% protection from ruminal degradation at 3 h and 47% protection at 12 h, suggesting effective postruminal delivery of Lys (Block et al., 2010; Zuccarello et al., 2011). For the HMBi, a calibration curve established by modeling the area under the curve response to increasing doses of Met indicates that approximately 50% of HMBi is absorbed through the rumen wall, and, thus, escapes ruminal degradation (Graulet et al., 2005). The efficacy of HMBi is further supported by production responses showing that HMBi supplementation increases

milk protein when production appears to be limited by metabolizable Met supply (St-Pierre and Sylvester, 2005; Phipps et al., 2008; Chen et al., 2011). Although estimates of ruminal protection for these products may be imprecise, it is likely that at least some additional Lys and Met were absorbed.

A more likely reason why Lys and Met supplementation did not increase production is that another nutrient, or possibly mammary synthetic capacity, was first-limiting in this scenario. Our study narrowly focused on Lys and Met because substantial research has supported the focus on a Lys and Met deficiency in diets similar to those fed in our study (Nichols et al., 1998; Wickersham et al., 2004; Vyas and Erdman, 2009; Patton, 2010), and infusion of Lys or Met has increased production in several dairy nutrition studies (Schwab et al., 1976; Pisulewski et al., 1996; Varvikko et al., 1999). Histidine has sometimes been identified as a limiting AA in dairy cattle diets, but the controlled research

Table 3. Effects of supplementing Lys embedded within Ca salts of FA¹ and HMBi² on performance of lactating Holstein cows

Item	Treatment		SEM	P-value
	Control	AA supplemented		
DMI (kg/d)	26.4	26.7	0.49	0.65
Yield (kg/d)				
Milk	40.1	40.1	0.65	0.98
Milk fat	1.22	1.25	0.03	0.59
Milk protein	1.23	1.21	0.02	0.54
Milk lactose	1.95	1.96	0.04	0.96
ECM ³	38.2	38.5	0.69	0.81
Milk fat (%)	3.07	3.12	0.06	0.59
Milk protein (%)	3.08	3.04	0.04	0.51
Milk lactose (%)	4.88	4.88	0.03	0.97
MUN (mg/dL)	13.9	14.3	0.24	0.25

¹Megamine-L (Arm & Hammer Animal Nutrition, Princeton, NJ).²HMBi = 2-hydroxy-4-(methylthio) butanoic acid; the methionine precursor isopropyl ester of 2-hydroxy-4-methylthio butanoic acid (MetaSmart, Adisseo Inc., Antony, France).³ECM = (0.327 × milk yield) + (12.86 × fat yield) + (7.65 × protein yield); per Dairy Record Management Systems (2010).

showing this was conducted with diets containing large proportions of grass silages, barley, oats, and canola, instead of corn sources (Schwab et al., 2005). Corn grain contains almost no Trp (Coleman and Larkins, 1999), so diets with large amounts of corn silage and corn by-products, such as the diets in the current study, could possibly be deficient in Trp. NRC (2001) also suggests that Phe and Ile are frequently limiting, behind Lys and Met, in corn-based diets. Energy intake could have also been limiting, but model predictions indicated a positive energy balance.

Hormonal signaling (Burgos et al., 2010) and energy status (Proud, 2007) of the mammary cells will also influence protein synthesis. It is possible that the fermentability of the carbohydrates in these diets triggered an insulin response (Grant et al., 1990), which led to greater efficiency of milk protein production, compensating for any marginal AA deficiency. We hypothesize this based on the low milk fat concentration observed ($\approx 3.1\%$) and on past results demonstrating that similar diets can induce milk fat depression if particle size is not carefully maintained (Bradford and Mullins, 2012). Given the low dietary fat concentrations ($<5\%$) in these diets, the milk fat depression was likely related to carbohydrate fermentability. In such a situation, a large proportion of the dietary NFC is fermented to produce propionate, which is a secretagogue of insulin (Harmon, 1992). An abundance of research has been published linking elevated insulin concentration to improved milk protein production (McGuire et al., 1995; Mackle et al., 1999; Molento et al., 2002). Blood samples were not collected in our study, so this hypothesis cannot be evaluated. We also recognize that the effects of AA supply on mammary intracellular signals promoting translation can be independent of insulin (Appuhamy et al., 2011), but this was observed in a media that was considerably deficient in AA supply (5% of normal AA supply). If AA were supplied at levels near the requirements, such as in our study, the response to elevated insulin and essential AA could be less independent; therefore, milk protein production responses would be smaller.

Focusing on AA as a proportion of MP was misleading in this study. The NRC model estimated an MP balance of 250 g/d for the control diet, which allowed for a relatively high level of milk production to be supported with lower levels of AA as a percent of MP. For example, if MP had been supplied at exactly the required amount, 6.8% Lys may be necessary to meet a cow's requirement; but if MP is oversupplied by 10%, then 6.2% Lys would provide the same amount of Lys and similarly meet the requirement. Not surprisingly, a meta-analysis examining 281 published diets indicated that neither Met nor Lys supply, expressed as a propor-

tion of MP, was correlated with milk protein content (Patton et al., 2003).

It is also possible that the NRC model prediction, which was used to suggest a Lys limitation in the control diet, was imprecise. It would be unrealistic to expect perfect model predictions in these diets because most of the diets within the databases used to create these models did not contain high inclusion rates of nonforage fiber sources, and nonforage fiber sources alter the kinetics of digestion and passage rate (Firkins, 1997). Another concern is that models do not account for associative effects within diets, which is likely to be a major factor when substantial amounts of NFC are replaced by nonforage NDF (Beckman and Weiss, 2005).

Even if passage rate was estimated correctly, the Lys limitation could be related to an inaccurate prediction of metabolizable Lys supply. Schingoethe et al. (2009) pointed out that recoverable Lys content of distillers grains currently on the market is actually greater than the NRC (2001) suggests (3.15 vs. 2.24% of CP), which is likely due to improvements in the dry corn-milling process that minimizes heat damage. It is probable that similar progress has been made with wet corn milling, although surveys have not been conducted to validate this; regardless, database values should be used cautiously.

Feeding supplemental Lys embedded in a matrix of Ca salts of FA combined with an HMBi source did not affect performance and intake in diets containing large proportions of wet corn gluten feed. This is likely because the diet fed to control cows was either not deficient in Lys and Met, or the supplemental AA products used did not provide effective levels of ruminal escape AA. More information is needed for field nutritionists to effectively incorporate AA balancing when formulating diets with high levels of wet corn gluten feed.

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