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Addition of Ruminal Escape Methionine and Lysine to Meat and Bone Meal¹

M. J. Klemesrud, T. J. Klopfenstein², and A. J. Lewis

Department of Animal Science, University of Nebraska, Lincoln 68583-0908

ABSTRACT: A growth study was conducted to determine the effects of adding ruminal escape methionine and lysine to meat and bone meal (MBM). A basal diet of 44% sorghum silage, 44% corncobs, and 12% supplement (DM basis) was individually fed to 60 crossbred steers (234 ± 14 kg). Supplements contained either urea, MBM, MBM plus protected methionine (MBM + M), or MBM plus protected methionine and lysine. Protein sources were fed to supply 30, 40, 50, and 60% of the supplemental CP, with urea supplying the remainder. Protein efficiency, calculated as gain above the urea control vs natural protein intake using the slope ratio technique, was used to evaluate the protein sources. The most efficiently used protein source was MBM + M, which was greater than MBM alone ($P < .10$). Meat and

bone meal plus protected methionine and lysine had a protein efficiency similar to MBM + M ($P > .30$), indicating that lysine was not limiting. True protein digestibility of MBM in the gastrointestinal tract of lambs was determined to be 86.1%. In situ analysis performed by 12-h ruminal incubation of MBM determined the escape CP to be 53.0% of CP. Amino acid analysis was conducted to compare supplies to requirements for live animal gain. The urea control failed to meet the metabolizable protein requirement. Feeding MBM to provide additional metabolizable protein failed to provide an adequate amount of the essential amino acid methionine, which was first-limiting. These data indicate that protein efficiency of MBM can be enhanced by the addition of ruminal escape methionine.

Key Words: Beef Cattle, Meat and Bone Meal, Protein, Amino Acids, Methionine, Lysine

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Introduction

Meat and bone meal (**MBM**) is a rendered animal by-product often used as a source of escape protein for ruminants. However, using MBM as an escape protein source has had variable results (Stock et al., 1981). This may be a result of heat damage due to rendering (Leibholz, 1979; Batterham and Darnell, 1986; Knabe et al., 1989) and/or the type of tissues rendered into MBM (Eastoe and Long, 1960; Atkinson and Carpenter, 1970).

Commercial MBM may contain between 50 and 65% collagen (Eastoe and Long, 1960), which is devoid of tryptophan and low in methionine (Atkinson and Carpenter, 1970). Adding tryptophan to MBM did not improve efficiency of protein utilization (Gibb et al., 1992b) suggesting that tryptophan was not the first-limiting amino acid in MBM. Efficiency of protein

utilization in steers was moderately correlated to metabolizable methionine content in MBM and poultry by-product meal supplements (Klemesrud et al., 1997), implicating methionine as the first-limiting amino acid in MBM.

This research was conducted to determine whether methionine or lysine was the first-limiting amino acid in MBM. A secondary objective was to estimate the metabolizable amino acid supply to steers fed MBM diets relative to predicted amino acid requirements.

Materials and Methods

Growth Trial. A calf growth trial was conducted using 60 steer calves (234 ± 14 kg) individually fed diets of 44% sorghum silage (7.2% CP, 68% TDN), 44% corncobs (2.3% CP, 48% TDN), and 12% supplement (DM basis, Table 1). Treatments consisted of 1) urea (control); 2) MBM; 3) MBM plus rumen protected methionine (**MBM + M**) to supply methionine at the rate of 7.5g/kg of supplement; 4) MBM plus rumen protected methionine and lysine (**MBM + ML**) to supply methionine and lysine each at the rate of 7.5g/kg of supplement (Smartamine M[®])

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²To whom correspondence should be addressed.

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Table 1. Supplement composition for the steer growth trial^a

Ingredient	Treatment			
	Urea	MBM ^b	MBM + M ^b	MBM + ML ^b
Meat and bone meal	—	87.54	87.54	87.54
Soyhulls	72.73	2.27	1.2	—
Urea	15.08	5.23	5.23	5.23
Smartamine M ^c	—	—	1.07	.76
Smartamine ML ^d	—	—	—	1.51
Dicalcium phosphate	7.23	—	—	—
Salt	2.50	2.50	2.50	2.50
Ammonium sulfate	1.67	1.67	1.67	1.67
Trace minerals ^e	.42	.42	.42	.42
Vitamins ^f	.25	.25	.25	.25
Selenium ^g	.12	.12	.12	.12

^aValues are expressed as percentage of DM; supplement was fed at 12% of DMI.

^bMeat and bone meal (MBM), meat and bone meal plus methionine (MBM + M), and meat and bone meal plus methionine and lysine (MBM + ML) were mixed with urea supplement to supply 30, 40, 50, or 60% of supplemental protein.

^c70% methionine.

^d15% methionine and 50% lysine.

^e10% Mg, 6% Zn, 4.5% Fe, 2% Mn, .5% Cu, .3% I, .05% Co.

^f30,000 IU of vitamin A, 6,000 IU of vitamin D, and 7.5 IU of vitamin E per gram of premix.

^gPremix contained .06% Se.

and Smartamine ML[®]; Rhône-Poulenc Animal Nutrition, Atlanta, GA). These products contain methionine and methionine plus lysine encapsulated in a pH-sensitive coating (poly-2-vinyl-pyridine-co-styrene) that is stable at a ruminal pH of 5.4 yet loses its integrity when it enters the abomasum (Polan et al., 1991).

Protein sources were fed to supply 30, 40, 50, or 60% of the supplemental CP, with urea supplying the remainder. Therefore, regardless of the assigned level, all steers consumed a diet containing 10.7% CP (DM basis). Steers were assigned randomly to treatment and level of treatment protein, with 12 steers receiving the urea supplement and 16 steers receiving each of the other three supplements, or four steers per level. Steers were individually fed, at an equal percentage of body weight, once daily with Calan electronic gates (American Calan, Northwood, NH). This percentage was adjusted as needed to minimize orts (taken weekly) while maintaining intake near ad libitum. Weight data were collected before feeding every 28 d, and intakes were recalculated based on current weights. Weights were taken on three consecutive days at the beginning, d 56, and end of the 84-d trial. Least significant differences (SAS, 1985) were used to separate treatment means.

Efficiency of protein utilization was determined for each treatment using the slope-ratio technique (Klopfenstein et al., 1985) with the urea-supplemented steers as the control. Protein efficiency was calculated as the units of gain obtained greater than the urea

Table 2. Basal diet for the digestion trial with lambs.

Ingredient	%, DM
Ensiled corncobs	72.70
Alfalfa pellets	15.00
Ground corn	10.00
Urea	1.48
Dicalcium phosphate	.26
Salt	.30
Ammonium sulfate	.17
Trace minerals	.04 ^a
Vitamins	.03 ^b
Selenium	.02 ^c

^aContains 10% Mg, 6% Zn, 4.5% Fe, 2% Mn, .5% Cu, and .3% I.

^b15,000 IU vitamin A, 3,000 IU vitamin D, and 3.75 IU vitamin E per gram of premix.

^cPremix contains .06% Se.

control steers per unit of natural protein consumed greater than the control diet. Slopes (protein efficiency), determined using the NLIN procedure of SAS (1985), were compared using a two-tailed *t*-test (Steel and Torrie, 1980).

In Situ Study. Approximately 4 g of MBM was placed in each of four Dacron[®] bags (10 × 20 cm; 50- μ pore size; Du Pont, Wilmington, DE). Each bag was sealed by wrapping the top around a #8 rubber stopper and secured with a #18 rubber band. The bag was then folded over the rubber band and a second rubber band was added. Sample bags were placed in a polyester bag 36 × 42 cm made of mesh material and closed with a nylon zipper. To facilitate hydration, bags were soaked in 39°C water for 20 min before ruminal incubation. Bags were then placed in the liquid phase of the ruminal ventral sac of a cannulated crossbred steer (534 kg) maintained on a grass hay diet (Wilkerson et al., 1995).

Following 12 h of ruminal incubation (Wilkerson et al., 1993), bags were removed from the rumen and washed by hand until rinse water was clear. Total N (AOAC, 1975) was determined before and after ruminal incubation to estimate the amount of ruminal escape protein. Additionally, MBM was analyzed for ash content (AOAC, 1975) as an estimate of the amount of bone.

Residue remaining after ruminal incubation was composited and analyzed for amino acid composition to estimate intestinal flow of amino acids. Residue was hydrolyzed in 6 N HCl, and amino acid content of hydrolyzates was determined by ion-exchange chromatography (AOAC, 1975). Separate samples were oxidized with performic acid for analysis of cystine and methionine (AOAC, 1975). A separate analysis for tryptophan was also conducted using the procedure of Lewis et al. (1976) modified for manual analysis. All analyses were conducted in duplicate.

Digestion Study. Sixteen crossbred wether lambs (33.1 ± 3.5 kg) housed in individual metabolism crates were fed a basal diet (Table 2) containing

Table 3. Daily gain and feed efficiency of calves fed urea, meat and bone meal (MBM), meat and bone meal plus methionine (MBM + M), or meat and bone meal plus methionine and lysine (MBM + ML)

Item ^a	Treatment				SEM
	Urea	MBM	MBM + M	MBM + ML	
Daily DMI, % of BW	2.10	2.10	2.10	2.10	—
Daily gain, kg	.19 ^b	.32 ^c	.41 ^d	.38 ^{cd}	.05
Gain:feed	.038 ^b	.062 ^c	.078 ^d	.074 ^{cd}	.003

^aExpressed as the average of levels fed.

^{b,c,d}Means within a row with different superscripts differ ($P < .05$).

ensiled corncobs and alfalfa pellets. The basal diet was fed to lambs at 2.0% of BW (DM basis) throughout the trial. This maintenance diet was balanced to provide a minimum of 10% CP, 52% TDN, .42% Ca, and .18% P. Urea was included in the basal diet to ensure that rumen NH_3 was not limiting digestion and provided 44% of the basal dietary CP.

The trial consisted of a 14-d adaptation period and a 7-d fecal collection period. Lambs were assigned randomly to receive either MBM, soybean meal (SBM), or an unsupplemented control. Supplemental protein sources were fed at 3.75% of the basal diet DMI as units of additional CP. Therefore, the supplemental DMI in addition to the basal diet was dependent on the CP content of the protein source. All diets containing treatment proteins were isonitrogenous and contained 13.75% CP, and the unsupplemented control diet contained 10% CP. Treatment protein sources were individually weighed and hand-mixed into the basal diet at time of feeding.

Lambs were weighed before the trial to enable feeding diets on an equal percentage of BW. Lambs were fitted with fecal collection bags to allow for total fecal collection. Feces was collected daily and weighed, and a 10% subsample was taken. Subsamples were composited by lamb for the 7-d collection period. Feed, feces, and orts were oven-dried (60°C) and analyzed for DM and CP (AOAC, 1975). True protein digestibility was calculated by difference from urea-supplemented sheep as outlined by Blasi et al. (1991). Results were analyzed as a completely randomized design using the GLM procedure of SAS (1985). Least significant differences (SAS, 1985) were used to separate treatment means.

Results and Discussion

Growth Trial. Gain and feed efficiency of steers were increased ($P < .05$) when MBM, MBM + M, or MBM + ML treatments were fed compared with urea (Table 3). The increase in gain presumably was due to additional metabolizable protein supplied by these sources. The increase in feed efficiency was due to the increase in gain, because daily feed intake as a

percentage of body weight was equal for all treatments. The greatest and most efficient gains were obtained when MBM + M was fed compared with MBM alone ($P < .05$).

Maximal gain was .47 kg/d (.28 kg/d above the urea control; Figure 1). The MBM + M and MBM + ML had greater efficiency of protein utilization ($P < .10$) than MBM. Because the proportion of MBM fed in the three treatments was equal, any differences in protein efficiency were due to additional rumen escape methionine or lysine. The MBM + M and MBM + ML had similar ($P > .30$) protein efficiencies, indicating that MBM provided adequate amounts of metabolizable lysine. The improvement in protein efficiency for MBM + M and MBM + ML over MBM was likely due

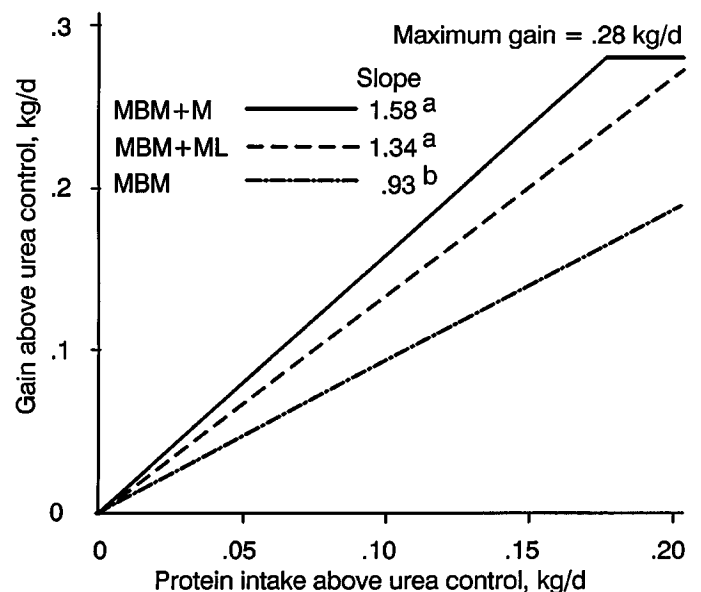


Figure 1. Regression of daily protein intake above urea control against daily gain above urea control. Resulting values (slopes) represent the protein efficiencies. Standard errors of the slopes are .16, .19, and .15 for meat and bone meal (MBM), meat and bone meal plus methionine (MBM + M), and meat and bone meal plus methionine and lysine (MBM + ML). Values with different superscripts differ ($P < .10$).

Table 4. Crude protein, escape protein^a, and amino acid content^b of meat and bone meal (MBM)

Item	MBM
Crude protein, %	39.5
Ash, %	41.7
Escape CP, %	53.0
Arginine	10.3
Cystine	1.1
Histidine	1.9
Isoleucine	3.5
Leucine	7.1
Lysine	5.9
Methionine	1.7
Phenylalanine	4.0
Threonine	3.9
Valine	5.3
Tryptophan	.3

^aExpressed as a percentage of CP.

^bExpressed as a ratio to CP remaining after 12-h incubation in situ.

to additional metabolizable methionine.

In Situ and Digestion Study. Meat and bone meal used for these trials contained 39.5% crude protein and 41.7% ash (DM basis, Table 4). After 12 h of ruminal incubation, escape protein value of MBM averaged 53.0% (± 2.5). This was similar to escape protein values of 53.6 and 51.0% previously reported for MBM with similar CP and ash content (Gibb et al., 1992b; Klemesrud et al., 1997).

Amino acid profiles before and after in situ ruminal incubation have been reported to be similar for SBM, blood meal, feather meal, and corn gluten meal (Weakley et al., 1984; Goedecken et al., 1990). However, differences have also been reported between amino acid profiles before and after ruminal incubation for MBM (Klemesrud et al., 1997). For this reason, amino acid profile after in situ ruminal incubation was determined (Table 4) and used to predict the amino acid composition of MBM escape protein flowing to the small intestine.

There were no differences ($P > .20$) in apparent diet CP or true protein digestibility among SBM and MBM (Table 5). Numerically, SBM had a higher true protein digestibility (91.1%) than MBM (86.1%). Loerch et al. (1983) found true protein digestibility of MBM, calculated from duodenal and ileal flow, to be 86.4%, which was similar to the value we obtained. Overheating during processing, which has been blamed for reduced protein digestibility, does not seem to affect this product. Results indicate that MBM was highly digestible with minimal damage from processing or contamination.

Growth trial treatments were compared at an equal level of supplemental natural protein intake, 177 g, which corresponds to the break point at which maximum gain was achieved for the most efficiently used protein source, MBM + M (Figure 1). The

Table 5. Crude protein digestibility of test proteins by lambs

Item	Apparent diet CP digestibility, %	True supplemental CP digestibility, % ^a
Unsupplemented control	67.3 ^b	—
Soybean meal	73.3 ^c	91.1
Meat and bone meal	72.0 ^c	86.1
SEM	.9	3.9

^aCalculated by difference from apparent CP digestibility of urea control. No significant difference between diets ($P > .20$).

^{b,c}Means within a column with different superscripts differ ($P < .01$).

amounts of metabolizable protein and metabolizable amino acids supplied by each treatment (Table 6) were calculated using the equations of Goedecken et al. (1990), which account for digestible escape protein from the supplement, digestible escape protein from the basal diet, and microbial protein production. Ruminal microbial amino acid absorption from the small intestine was based on the conversion of 10.4% of the TDN to digestible microbial CP (Burroughs et al., 1974). Amino acid content of microbial protein was estimated by Goedecken et al. (1990) by feeding a diet containing only nonprotein N and analyzing whole ruminal contents. Escape protein and amino acid composition for basal diet ingredients were reported by Gibb et al. (1992a). Calculations of amino acids supplied were based on a daily DMI of 2.10% of BW for calves weighing 248 kg (average midtrial BW).

The total metabolizable protein and amino acids supplied were compared with the requirements estimated by Wilkerson et al. (1993), where the metabolizable protein requirement was $3.8 \times \text{BW}^{.75}$ (grams/day, where BW is expressed as kilograms) for maintenance and 305 g/kg of live weight gain. The amount of metabolizable protein supplied by each treatment at 177 g of supplemental natural protein intake was in excess of the metabolizable protein requirement, with the exception of the urea control, which was deficient in metabolizable protein (Table 6). Metabolizable protein, which was equal between MBM treatments, failed to account for differences in protein efficiency between these treatments.

Metabolizable amino acids supplied by the three MBM treatments were equal, with the exceptions of metabolizable methionine and lysine. The increase in protein efficiency due to inclusion of additional methionine indicates that methionine was the first-limiting amino acid. Other essential amino acids would then be fed in excess of their requirement. The amount of metabolizable methionine supplied by MBM + M at the breakpoint for maximal gain was 15.4 g/d. This amount is likely in excess of the requirement. At an equal level of protein intake, MBM

Table 6. Estimated metabolizable protein and amino acids supplied by diet^a

Item	Treatment				
	Urea	MBM ^b	MBM + M ^b	MBM + ML ^b	Wilkerson ^c
Protein	312	400	404	402	381
Arginine	26.4	34.0	33.8	33.6	—
Cystine	8.5	9.2	9.2	9.1	10.7
Histidine	5.6	6.9	6.8	6.8	6.1
Isoleucine	19.5	21.9	21.8	21.7	21.3
Leucine	31.2	36.3	36.1	35.9	26.3
Lysine	27.9	32.1	32.0	35.8	30.5
Methionine	10.1	11.4	15.4	15.3	11.4
Phenylalanine	18.9	21.7	21.6	21.5	14.9
Threonine	22.8	25.7	25.6	25.5	19.8
Valine	21.9	25.8	25.6	25.5	21.7
Tryptophan	3.6	3.6	3.6	3.6	3.8
TSAA ^d	18.6	20.6	24.6	24.4	22.1

^aExpressed as grams/day of metabolizable protein and amino acids flowing to the small intestine at an equal level of supplemental natural protein intake (177 g/d). Based on a 248-kg animal (average midtrial body weight) consuming 2.1% of body weight.

^bMeat and bone meal (MBM), meat and bone meal plus methionine (MBM + M), and meat and bone meal plus methionine and lysine (MBM + ML).

^cWilkerson et al. (1993) requirements based on 248-kg animal gaining .47 kg/d (.28 kg/d above the urea control).

^dTotal sulfur amino acids (methionine + cystine).

supplied only 11.4 g/d, suggesting that the requirement for metabolizable methionine lies between 11.4 and 15.4 g/d for 248 kg steers gaining .47 kg/d. Extending the protein efficiency slope for MBM to the point at which it intersects maximal gain and calculating the amount of metabolizable methionine supplied at that level of protein intake would predict the requirement for metabolizable methionine to be 12.4 g/d.

At 177 g of supplemental natural protein intake, the difference in methionine required for maximal gain and that supplied by MBM is 1.0 g, and the difference in gain is 115 g. Based on protein and amino acid composition of live weight gain, 1.0 g of metabolizable methionine is adequate for 102 g of gain (Burroughs et al., 1974; Wilkerson et al., 1993). Grams of methionine supplied by MBM was equal to the requirement estimated by Wilkerson et al. (1993). However, this is less than our predicted requirement for methionine of 12.4 g/d (Table 7). As a percentage

of metabolizable protein, our predicted requirement for methionine was similar to that of Wilkerson et al. (1993). Our predicted requirement for methionine plus cystine was also similar to that of Wilkerson et al. (1993) for total sulfur amino acids. Even though there remains a requirement for methionine (Reis et al., 1973), cystine may meet over 50% of the sulfur amino acid requirement (Ahmed and Bergen, 1983). Difference between the proposed methionine requirement and that of Wilkerson et al. (1993) may be due to the contribution of cystine in meeting the total sulfur amino acid requirement.

Although methionine is the first-limiting amino acid in meat and bone meal, amino acid requirements would suggest tryptophan to be second-limiting (Table 6). Even though the requirement for tryptophan is 1.0% of MP, MBM protein contains only .3%. Gibb et al. (1992b) concluded that tryptophan was not the first-limiting amino acid in MBM. After the methionine requirement is met, tryptophan may become next-limiting.

Implications

Adding meat and bone meal to diets of growing beef steers increased the metabolizable protein supplied, thereby increasing protein efficiency. Meat and bone meal protein contained adequate amounts of lysine, whereas methionine was the first-limiting amino acid for growing steers. Addition of rumen-escape digestible methionine to meat and bone meal increased daily gain, feed efficiency, and efficiency of protein utilization in steer calves gaining .32 kg/d.

Table 7. Metabolizable sulfur amino acids

Item	Observed, g/d	Required g/d ^a
Methionine	12.4 (3.1) ^b	11.4 (3.0)
Cystine	9.2 (2.3)	10.7 (2.8)
TSAA ^c	21.6 (5.4)	22.1 (5.8)

^aBased on Wilkerson et al., 1993.

^bValues in parentheses expressed as a percentage of metabolizable protein.

^cTotal sulfur amino acids (methionine + cystine).

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