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## ROLE OF METHIONINE AS A METHYL GROUP DONOR IN CATTLE

*C. A. Löest, E. C. Titgemeyer, and R. H. Greenwood*

### Summary

Holstein steers were used in two  $5 \times 5$  Latin square experiments to evaluate the sparing of methionine by alternative sources of methyl groups (betaine or choline). Steers were housed in metabolism crates and limit fed a diet high in rumen degradable protein. To increase energy supply, volatile fatty acids were infused into the rumens, and glucose was infused into the abomasum. An amino acid mixture, limiting in methionine, was infused abomasally to ensure that non-sulfur amino acids did not limit protein synthesis. Treatments for Exp. 1 were abomasal infusion of 1) water (control), 2) 2 g/day additional L-methionine, 3) 1.7 g/day L-cysteine, 4) 1.6 g/day betaine, and 5) 1.7 g/day L-cysteine + 1.6 g/day betaine. Treatments for Exp. 2 were abomasal infusion of 1) water (control), 2) 2 g/day additional L-methionine, 3) 8 g/day betaine, 4) 16 g/day betaine, and 5) 8 g/day choline. In both experiments, nitrogen retention increased ( $P < .05$ ) in response to methionine, demonstrating a deficiency of sulfur amino acids. Responses to cysteine, betaine and choline were small. The low response to cysteine indicates that either the response to methionine is not due to transsulfuration to cysteine, or that cysteine supply does not alter the flux of methionine through transsulfuration. The small responses to betaine and choline suggest that they do not substitute for methionine. Thus, under our experimental conditions, responses to methionine likely were due to a correction of a deficiency of methionine per se rather than of methyl group donors.

(Key Words: Methionine, Cysteine, Betaine, Choline, Steers.)

### Introduction

Methionine is an essential amino acid often identified as limiting for growing ruminants. Methionine functions as a precursor for protein synthesis, and a deficiency of this amino acid can cause inefficient use of dietary protein for lean muscle (protein) deposition. However, methionine has many other functions in the body, including methyl group donation and use for cysteine and polyamine biosynthesis.

More than half the methionine requirement of rats can be replaced by cysteine. However, recent research with cattle has indicated that cysteine does not effectively spare methionine. The lack of response to cysteine may have been due to methyl groups being limiting, such that methionine was needed as a methyl group donor. Therefore, our objective was to evaluate the sparing of methionine by alternative sources of methyl groups (betaine or choline).

### Experimental Procedures

**Experiment 1.** Five ruminally cannulated Holstein steers (343 lb initial BW) were maintained in metabolism crates to facilitate total collection of feces and urine. Steers were limit fed (5.3 lb/day, dry basis) a diet based on soybean hulls (84% soybean hulls, 7% wheat straw, 3% molasses, 5% minerals/vitamins, and 0.5% urea). This diet was formulated to contain a low amount of undegradable intake protein so that only limited amounts of dietary amino acids were available postruminally. To increase energy supply without increasing ruminal microbial growth, steers received a continuous infusion of volatile fatty acids (180 g acetate, 180 g

propionate, and 45 g butyrate/day) into the rumen and a continuous infusion of glucose (300 g/day) into the abomasum. Also, an amino acid mixture containing 150 g L-glutamate; 50 g glycine; 20 g L-valine; 30 g L-leucine; 20 g L-isoleucine; 40 g L-lysine-HCl (feed grade, 79.8%); 10 g L-histidine-HCl-H<sub>2</sub>O (74%); 20 g L-arginine; 20 g L-threonine (feed grade, 98%); 35 g L-phenylalanine; 7 g L-tryptophan (feed grade, 98%); and 2 g L-methionine per day was infused continuously into the abomasum to ensure that nonsulfur amino acids did not limit tissue protein synthesis. The abomasal infusions were made by placing flexible tubing (inside diameter: 1/16 in.) through the rumen cannula and the reticulo-omasal orifice.

A 5 × 5 Latin square design was used with periods of 7 days. This allowed for a 2-day adaptation to the abomasal infusions and 5 days for total collection of feces and urine. Treatments were abomasal supplementation with 1) water (control), 2) 2 g/day additional L-methionine, 3) 1.7 g/day L-cysteine, 4) 1.6 g/day betaine, and 5) 1.7 g/day L-cysteine + 1.6 g/day betaine. The L-cysteine and betaine were provided in amounts that were equimolar to the L-methionine supplement. Only four observations were obtained for the 1.7 g/day L-cysteine + 1.6 g/day betaine treatment because of an infusion problem in the last period.

**Experiment 2.** Five ruminally cannulated Holstein steers (348 lb initial BW) were used in a design similar to Exp. 1 and were housed in similar conditions. They were limit-fed 5.5 lb/day, dry basis.

Treatments were abomasal supplementation with 1) water (control), 2) 2 g/day additional L-methionine, 3) 8 g/day betaine, 4) 16 g/day betaine, and 5) 8 g/day choline. The betaine was provided in amounts that were 5 and 10 times the amount in Exp. 1 in order to test the efficiency of betaine as a methyl group donor. Choline was infused to examine its role as an alternative methyl group donor.

## Results and Discussion

**Experiment 1.** Increases in retained nitrogen were due to decreases in urinary nitrogen excretion (Table 1). Nitrogen retention increased in response to supplementation with 2 g/day methionine ( $P < .05$ ), but responses to equimolar amounts of cysteine and betaine alone or in combination were less dramatic. The response to methionine verifies the sulfur amino acid-deficient conditions intentionally created by our model, whereas the low response to cysteine supplementation may indicate that the response to additional methionine supplementation is not due to conversion of methionine to cysteine. However, both cysteine and betaine tended ( $P < .16$ ) to increase nitrogen retention relative to the control treatment. Because of the insignificant response to methyl groups (betaine), the sparing of methionine by methyl donors could not be demonstrated. However, replacement of methionine by betaine appeared to be relatively inefficient (the response to betaine was 20% as large as that to methionine). Therefore, we hypothesized that more betaine may be required to yield responses similar to that observed for methionine.

**Experiment 2.** As in Exp. 1, observed increases in retained nitrogen resulted from decreases in urinary nitrogen excretion (Table 2). Also, large increases in retained nitrogen occurred for steers supplemented with 2 g/day methionine ( $P < .05$ ). However, betaine infused at levels that were 5 and 10 times higher than that supplied in Exp. 1 only tended ( $P < .22$ ) to increase nitrogen retention. Nitrogen balance responses were only 23% (for 8 g/day betaine) and 20% (for 16 g/day betaine) as large as that observed for methionine. These responses were similar to that for the lower level of betaine in Exp. 1. Choline also failed to improve nitrogen balance.

Under our experimental conditions, responses to methionine likely were due to a correction of a deficiency of methionine per se rather than its role as a methyl group donor.

**Table 1. Nitrogen Balance of Steers Supplemented with Methionine, Cysteine, and/or Betaine**

Item	Treatments <sup>a</sup>					SEM
	Control	MET-2	CYS-1.7	BET-1.6	BET-1.6 + CYS-1.7	
No. observations	5	5	5	5	4	
Nitrogen	----- g of N/day -----					
Intake	101.1	101.4	101.5	101.5	101.6	.12
Fecal	22.4	22.9	24.0	23.7	24.1	.54
Urinary	59.0 <sup>b</sup>	51.9 <sup>c</sup>	56.6 <sup>b</sup>	56.8 <sup>b</sup>	56.1 <sup>b</sup>	1.01
Retained	19.7 <sup>c</sup>	26.6 <sup>b</sup>	21.0 <sup>c</sup>	21.0 <sup>c</sup>	21.4 <sup>c</sup>	.64

<sup>a</sup>MET-2 = 2 g/day methionine, CYS-1.7 = 1.7 g/day cysteine, and BET-1.6 = 1.6 g/day betaine.

<sup>b,c</sup>Means not bearing common letter differ (P<.05).

**Table 2. Nitrogen Balance of Steers Supplemented with Methionine, Betaine, or Choline**

Item	Treatments <sup>a</sup>					SEM
	Control	MET-2	BET-8	BET-16	CHO-8	
No. observations	5	5	5	5	5	
Nitrogen	----- g of N/day -----					
Intake	97.3 <sup>f</sup>	97.5 <sup>e</sup>	98.3 <sup>c</sup>	99.3 <sup>b</sup>	98.0 <sup>d</sup>	.06
Fecal	22.8	22.0	23.8	23.1	22.9	.64
Urinary	54.5 <sup>b</sup>	46.2 <sup>c</sup>	52.4 <sup>b</sup>	54.3 <sup>b</sup>	54.8 <sup>b</sup>	1.03
Retained	20.0 <sup>c</sup>	29.3 <sup>b</sup>	22.2 <sup>c</sup>	21.9 <sup>c</sup>	20.4 <sup>c</sup>	1.02

<sup>a</sup>MET-2 = 2 g/day methionine, BET-8 = 8 g/day betaine, BET-16 = 16 g/day betaine, and CHO-8 = 8 g/day choline.

<sup>b,c,d,e,f</sup>Means not bearing common letter differ (P<.05).