

## EFFECTS OF AMMONIA LOAD ON AMINO ACID UTILIZATION BY GROWING STEERS

*M. S. Awawdeh, E. C. Titgemeyer, K. C. McCuiston, and D. P. Gnad*

### Summary

Ruminally cannulated steers were used in two experiments to study effects of rumen ammonia load on methionine and leucine utilization. All steers were limit-fed a diet based on soybean hulls, received ruminal infusions of volatile fatty acids and abomasal infusions of glucose to provide energy, and received an abomasal infusion containing a mixture of all essential amino acids except methionine in Exp. 1 or leucine in Exp. 2. Treatments were arranged as  $3 \times 2$  factorials and included urea (0, 40, or 80 g/day) infused ruminally and methionine (2 or 5 g/day) infused abomasally in Exp. 1 and leucine (0, 4, or 8 g/day) infused abomasally and urea (0 or 80 g/day) infused ruminally in Exp. 2. In Exp. 1, supplementation with the greater amount of methionine improved retained nitrogen, but urea infusions did not alter nitrogen retention. In Exp. 2, leucine linearly increased retained nitrogen, and urea infusions also increased nitrogen retention. The efficiency of deposition of supplemental methionine ranged between 18 and 27%, whereas that for leucine ranged from 24 to 43%. Increasing ammonia load did not negatively impact whole-body protein deposition in growing steers when either methionine or leucine was limiting.

### Introduction

Ammonia is generated within the rumen from the degradation of protein and non-protein nitrogenous compounds, subsequently absorbed, and detoxified predominantly into

urea in the liver. Some previous studies indicate that ammonia detoxification might require additional nitrogen from non-ammonia nitrogen to support ureagenesis and that ammonia load might have metabolic costs that could decrease protein deposition by the animal. This negative effect of an ammonia load has been used to explain the inefficient utilization of nitrogen in forage-fed animals. In contrast, some studies have demonstrated little or no effect of ammonia loading on animal performance. Our objective was to study the effects of rumen ammonia loading on methionine and leucine utilization by growing cattle.

### Experimental Procedures

**Experiment 1.** Six ruminally cannulated Holstein steers (initially weighing 428 pounds) fitted with ruminal and abomasal infusion lines were used in a  $6 \times 6$  Latin square to study the effects of ammonia load on methionine utilization. Steers were housed in individual metabolism crates in a temperature-controlled room. All steers received the same basal diet at 5.7 lb/day dry matter in equal proportions at 12-hour intervals. The basal diet contained 83% soybean hulls and was formulated to provide adequate ruminally degraded protein but small amounts of amino acids to the small intestine. All steers received continuous ruminal infusions of volatile fatty acids, as well as abomasal infusions of glucose to supply additional energy without increasing microbial protein supply. All steers received continuous abomasal infusions of an amino acid mixture that supplied all essential

amino acids, except methionine, to ensure that methionine was the most limiting amino acid for nitrogen retention.

Treatments were arranged as a  $3 \times 2$  factorial and included three levels of urea (0, 40, and 80 g/day) infused continuously into the rumen to serve as ammonia loads and two levels of L-methionine (2 and 5 g/day) infused continuously into the abomasum. Each experimental period lasted for 6 days, consisting of 2 days for adaptation to treatment and 4 days for fecal and urinary collections.

**Experiment 2.** Six ruminally cannulated Holstein steers (initially weighing 417 pounds) were used to study the effects of ammonia load on leucine utilization. Experimental housing, periods, diet, treatment administration, and collections were the same as for Exp. 1 except that leucine was restricted instead of methionine. Treatments were arranged as a  $3 \times 2$  factorial, and included three levels of L-leucine (0, 4, and 8 g/day) infused abomasally and two levels of urea (0 and 80 g/day) infused ruminally.

## Results and Discussion

**Experiment 1.** There were no methionine  $\times$  urea interactions for diet digestibilities or nitrogen-retention data (Table 1). Nitrogen intake was increased in response to both methionine and urea infusions as a result of the additional nitrogen infused. Fecal nitrogen excretions were not altered by treatments. The higher level of methionine supplementation increased nitrogen retention from 22.0 to 27.5 g/day. The observed increase in retained nitrogen was a result of the decreased urinary nitrogen excretions from 68.8 to 64.8 g/day because of methionine supplementation. Although urea infusions linearly increased urinary nitrogen excretions, from 48.5 to 67.3 and 84.5 g/day for steers infused with 40 and 80 g/day urea, respectively, retained nitrogen

was not affected by the ammonia load provided by the urea supplementation.

If we assume that retained nitrogen was deposited completely as tissue protein (retained nitrogen  $\times$  6.25) and that the protein of tissue gain contains 2.0% methionine, the calculated efficiencies of methionine utilization were 23, 27, and 18% for steers receiving 0, 40 and 80 g/day urea, respectively. Thus, our average efficiency of utilization of supplemental methionine (23%) was similar to previous observations from our laboratory, but much less than the 65% efficiency value utilized by the current Beef NRC publication.

**Experiment 2.** There were no leucine  $\times$  urea interactions for diet digestibilities or nitrogen retention data (Table 2). Diet digestibilities of dry matter were linearly increased in response to leucine supplementation, which matches the observed decrease in fecal nitrogen excretions in response to leucine supplementation (Table 2). Changes in fecal output are not typically observed in response to changes in supply of a limiting amino acid, so we have no explanation for these small, but significant, changes in fecal output. Digestibilities of dry matter were not affected by urea infusion.

Nitrogen retention linearly increased with leucine supplementation, from 21.4 to 24.5 and 26.9 g/day for 4 and 8 g/day leucine, respectively. The increase in retained nitrogen in response to leucine supplementation was a result of decreases in both urinary and fecal nitrogen excretions. Leucine supplementation linearly decreased urinary nitrogen excretions, from 65.3 to 63.2 and 62.2 g/day for 4 and 8 g/day leucine, respectively, and linearly decreased fecal nitrogen excretions, from 22.1 to 21.2 and 19.9 g/day for 4 and 8 g/day leucine, respectively. The increase in retained nitrogen in response to supplementation of leucine in our study was an expected result. The ob-

served linear responses to leucine suggest that steer requirements for supplemental leucine are clearly more than 4 g/day and probably close to 8 g/day under our experimental conditions.

Nitrogen intake was increased with urea infusions as a result of the additional nitrogen infused. Retained nitrogen increased from 22.4 to 26.2 g/day when 80 g/day urea was infused. Fecal nitrogen excretions were not affected by urea infusions. Urea infusions increased total urinary nitrogen excretion from 47.1 to 80.0 g/day (Table 2).

The increase in retained nitrogen with urea infusions is in contrast to our initial hypothesis that an ammonia load might decrease nitrogen retention by increasing catabolism of the limiting amino acid (leucine). The reasons for the increased nitrogen retention with urea infusion are unknown, but it is possible that the observed increase in retained nitrogen in response to ammonia loading in our study was a result of decreasing the rate of leucine transamination (catabolism) by altering the substrate available for this reaction.

If we assume that retained nitrogen was deposited completely as tissue protein (retained nitrogen  $\times$  6.25) and that the protein of tissue gain contains 6.7% leucine, the calculated efficiency of leucine utilization between 0 and 4 g/day of leucine supplementation was 24 and 43% for steers receiving 0 and 80 g/day urea, respectively. The seemingly greater efficiency of leucine utilization in the presence of the urea infusion might be explained by ammonia loading decreasing the degradation of leucine, the limiting amino acid in our study, which resulted in increases in retained nitrogen and utilization efficiency.

Most typical diets fed to growing cattle, particularly diets containing significant amounts of corn protein, would not be ex-

pected to be limiting in leucine supply. Thus, there is not a great opportunity to directly apply the benefits of improving leucine utilization to a production setting.

**General Discussion.** The utilization efficiency of methionine and leucine was less than the 65% efficiency value utilized by the current Beef NRC to predict the requirements of growing cattle for amino acids. The NRC assumes the same utilization efficiency value for all amino acids, and the efficiency is based only on the equivalent body weight of the animal. Recently, our lab has observed an efficiency of utilization for supplemental histidine (65%) greater than that for methionine and leucine. The efficiency of histidine utilization was close to the value utilized by the NRC, suggesting that there are differences among amino acids in how efficiently they are used by cattle.

In light of our results, the utilization efficiency for amino acids should be considered separately for each amino acid when calculating the amino acid requirements of growing steers. It is clear that amino acids can have different efficiency values. Moreover, our data suggest that, at least for leucine, the efficiency may depend upon the nutritional status of the animal. For example, leucine requirements might be less for cattle fed diets containing a higher concentration of crude protein. However, formulating diets for cattle on the basis of individual amino acids may be difficult at the present time because of the lack of information for each amino acid.

In both experiments, we studied the effects of ammonia load under conditions in which amino acid supply was limiting. To achieve that, the diet was formulated to provide deficient amounts of amino acids, and all essential amino acids, except the amino acid under study, were supplemented. Ammonia loading did not have negative effects on nitrogen re-

tention or on the utilization of supplemented methionine or leucine by growing steers. Rather, increasing ruminal ammonia in excess of the concentrations recommended to optimize ruminal fermentation improved whole-animal protein deposition (nitrogen retention) in Exp. 2. Although increasing the ruminal ammonia load beyond that needed to optimize ruminal fermentation led to improvements in

whole-animal protein deposition when leucine supply limited animal performance, environmental and economical costs may not justify the use of ammonia loading as a means of improving cattle performance.

This research was supported by NRI Competitive Grants Program/CSREES/USDA, Award No. 2003-35206-12837.

**Table 1. Effects of Methionine Supplementation and Ammonia Load on Nitrogen Balance in Growing Steers (Exp. 1)**

Item	2 g/day L-methionine			5 g/day L-methionine			SEM
	No urea	40 g/day urea	80 g/day urea	No urea	40 g/day urea	80 g/day urea	
Nitrogen, g/day							
Total intake <sup>a,b</sup>	91.0	110.0	128.7	92.6	111.5	129.3	0.6
Fecal	18.4	19.7	18.8	17.8	20.2	18.5	1.1
Urinary <sup>a,b</sup>	50.1	70.0	86.2	46.8	64.7	82.9	1.4
Retained <sup>a</sup>	22.5	20.2	23.5	28.0	26.6	27.9	1.7
Dry matter digestibility, %	69.7	68.8	69.4	69.5	69.3	69.3	1.0

<sup>a</sup>Effect of methionine (P<0.05).

<sup>b</sup>Linear effect of urea (P<0.05).

**Table 2. Effects of Leucine Supplementation and Ammonia Load on Nitrogen Balance in Growing Steers (Exp. 2)**

Item	No Urea			80 g/day Urea			SEM
	No leucine	4 g/day leucine	8 g/day leucine	No leucine	4 g/day leucine	8 g/day leucine	
Nitrogen, g/day							
Total intake <sup>b</sup>	90.4	90.5	91.0	127.1	127.1	127.0	0.5
Fecal <sup>a</sup>	22.7	20.7	19.9	21.4	21.4	19.9	1.2
Urinary <sup>a,b</sup>	47.6	47.3	46.4	83.0	79.0	77.9	1.3
Retained <sup>a,b</sup>	20.1	22.4	24.7	22.6	26.7	29.2	2.0
Dry matter digestibility, % <sup>a</sup>	72.9	75.5	75.3	72.5	74.9	75.7	1.2

<sup>a</sup>Linear effect of leucine (P<0.05).

<sup>b</sup>Effect of urea (P<0.05).