ABSTRACT: Seven ruminally cannulated Holstein steers (194 ± 16 kg) housed in metabolism crates were used in a 6 × 6 Latin square, with one additional steer, to study effects of ruminal ammonia load on methionine (Met) use. All steers received a diet based on soybean hulls (2.6 kg DM/d), ruminal infusions of 200 g/d of acetate, 200 g/d of propionate, and 50 g/d of butyrate, as well as abomasal infusion of 300 g/d of glucose to provide energy without increasing microbial protein supply, and abomasal infusions of a mixture (248 g/d) of all essential AA except Met. Treatments were arranged as a 3 × 2 factorial and included urea (0, 40, or 80 g/d) infused ruminally to supply metabolic ammonia loads and Met (2 or 5 g/d) infused abomasally. Supplementation with the greater amount of Met decreased urinary N excretion from 68.8 to 64.8 g/d and increased (P < 0.05) retained N from 22.0 to 27.5 g/d. Urea infusions linearly increased (P < 0.05) urinary N excretions, plasma urea concentrations, and urinary urea excretions, but retained N was not affected. The efficiency of deposition of supplemental Met, calculated by assuming that Met deposition is 2.0% of protein deposition (6.25 × retained N), ranged between 18 and 27% when steers received 0 or 80 g/d of urea, respectively. There were no (P ≥ 0.40) effects of treatments on serum insulin or IGF-I concentrations. In our model, increasing ammonia load did not affect whole-body protein deposition in growing steers when Met was limiting.

Key Words: Amino Acids, Ammonia, Cattle, Growth, Methionine, Utilization

Introduction

Ammonia generated from the degradation of protein and other nitrogenous compounds in the rumen is transported to the liver, where it is predominantly detoxified to urea. Some studies indicate that AA N is required for ureagenesis, and this may contribute to inefficient use of dietary AA. Urea N released by the liver exceeded the hepatic uptake of NH₃ when ammonium salts were infused into the mesenteric vein of sheep (Barej et al., 1987; Orzechowski et al., 1988) and cattle (Symonds et al., 1981; Wilton et al., 1988), suggesting that additional N sources, such as AA, are required for NH₃ detoxification (Lobley et al., 1995). Ammonia loading increased leucine oxidation in vivo (Lobley et al., 1995) and methionine (Met) deamination in vitro (Mutsvangwa et al., 1999). Furthermore, hepatocytes isolated from sheep fed diets containing excess ruminally available N demonstrated greater alanine oxidation than those from sheep fed a more typical diet (Mutsvangwa et al., 1996, 1997).

However, other studies demonstrated no obligatory need of AA N for urea synthesis. Challenging ovine hepatocytes with NH₄Cl in vitro had no effect on alanine, glutamate, leucine, or phenylalanine oxidation (Mutsvangwa et al., 1996, 1997, 1999), and NH₃ contributed both N atoms for ureagenesis in rat and sheep liver (Cooper et al., 1987; Luo et al., 1995; Brosnan et al., 1996). Furthermore, ammonia loading in sheep had no negative effects on N retention (Norton et al., 1982), nor on the hepatic uptake of total, essential, or branched-chain AA (Milano et al., 2000; Milano and Lobley, 2001), suggesting that NH₃-enhanced ureagenesis has no obligatory requirement for AA N (Lobley et al., 1996).

At this moment, the exact nature of the effect of ammonia loading on AA use has not been well assessed. Our objective was to study the effects of ruminal ammonia loading on Met use by growing cattle.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved procedures involving animals in this study.

Seven ruminally cannulated Holstein steers (194 ± 16 kg initial BW) fitted with ruminal and abomasal infusion
adaptation periods are adequate because cattle rapidly and 4 d for total fecal and urinary collections. The 2-d period lasted for 6 d, with 2 d for adaptation to treatment. Each experimental period lasted for 6 d, with 2 d for adaptation to treatment and 4 d for total fecal and urinary collections. The 2-d adaptation periods are adequate because cattle rapidly adapt to changes in nutrients supplied postruminally (Moloney et al., 1998).

Abomasal infusate for each steer was prepared by dissolving the branched-chain AA (L-valine, L-leucine, and L-isoleucine) in 1 kg of water containing 60 g of 6 M HCl. Once the branched-chain AA were dissolved, the remaining AA, except glutamate, were added to the mixture. Glutamate was dissolved separately in 500 g water containing 30 g of NaOH. After all AA were dissolved, the two solutions of AA were mixed together, 300 g of glucose was added, and water was added to bring the total weight of the daily infusate to 4 kg. The pH of the infusate was 5.5. Pyridoxine-HCl (10 mg/d), folic acid (10 mg/d), and cyanocobalamin (100 μg/d) were added to the mixture because data demonstrated that steers maintained under our experimental conditions were deficient in one or more of these vitamins (Lambert et al., 2004). Methionine was dissolved separately in water and added to the mixture according to treatment (2 or 5 g/d).

Ruminal infusates for each steer were prepared by mixing 200 g/d of acetate, 200 g/d of propionate, and 50 g/d of butyrate. Water was added to bring the final weight of the mixture to 4 kg/d. Urea was added to the mixture according to treatment (0, 40, or 80 g/d). Infusion lines of flexible polyvinylchloride tubing (2.4 mm i.d.) were placed in the rumen and abomasum through the ruminal cannula. A perforated vial was attached to the end of the ruminal infusion lines to avoid direct infusion of VFA onto the ruminal wall. Rubber flanges (8-cm diameter) were attached to the end of the abomasal infusion lines to ensure that they remained in the abomasum. Solutions were continuously infused into the rumen and abomasum using a peristaltic pump.

Representative samples of the basal diet for each period were collected daily and stored (−20°C) for later analysis. Orts, if any, on d 2 through 5 were collected, composited, and stored (−20°C) for later analysis. Feces and urine for each steer were collected from d 3 through 6 of each period and weighed to determine the total output. Urine was collected in buckets containing 300
<table>
<thead>
<tr>
<th>Item</th>
<th>No urea</th>
<th>40 g/d urea</th>
<th>80 g/d urea</th>
<th>No urea</th>
<th>40 g/d urea</th>
<th>80 g/d urea</th>
<th>SEM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Infused</td>
<td>34.4</td>
<td>53.0</td>
<td>71.5</td>
<td>34.7</td>
<td>53.3</td>
<td>71.8</td>
<td>—</td>
</tr>
<tr>
<td>Dietary</td>
<td>56.6</td>
<td>57.0</td>
<td>57.2</td>
<td>57.9</td>
<td>58.2</td>
<td>57.5</td>
<td>0.6</td>
</tr>
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<td>Total intakebc</td>
<td>91.0</td>
<td>110.0</td>
<td>128.7</td>
<td>92.6</td>
<td>111.5</td>
<td>129.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Fecal</td>
<td>18.4</td>
<td>19.7</td>
<td>18.8</td>
<td>17.8</td>
<td>20.2</td>
<td>18.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Urinarybc</td>
<td>50.1</td>
<td>70.0</td>
<td>86.2</td>
<td>46.8</td>
<td>64.7</td>
<td>82.9</td>
<td>1.4</td>
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<td>1.5</td>
<td>2.8</td>
<td>4.0</td>
<td>1.8</td>
<td>1.6</td>
<td>1.6</td>
<td>0.7</td>
</tr>
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<td>Ureabd</td>
<td>41.2</td>
<td>57.0</td>
<td>68.3</td>
<td>35.7</td>
<td>52.2</td>
<td>71.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Retainedd</td>
<td>22.5</td>
<td>20.2</td>
<td>23.5</td>
<td>28.0</td>
<td>26.6</td>
<td>27.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Diet digestibility, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>69.7</td>
<td>68.8</td>
<td>69.4</td>
<td>69.5</td>
<td>69.3</td>
<td>69.3</td>
<td>1.0</td>
</tr>
<tr>
<td>OM</td>
<td>71.9</td>
<td>71.6</td>
<td>72.6</td>
<td>72.3</td>
<td>71.1</td>
<td>72.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>

aFor n = 6.
bEffect of methionine, P < 0.05.
cLinear effect of urea, P < 0.05.
dLinear effect of urea × methionine interaction, P < 0.05.

Nitrogen balance and plasma metabolite data were analyzed statistically using the Mixed procedure of SAS (Release 8.1, SAS Inst., Inc., Cary, NC). The model contained the effects of Met, urea, Met × urea, and period. Steer was included as a random effect. Linear and quadratic effects of urea and their interactions with Met were tested using single degree of freedom contrasts. Treatment means were computed using the LSMEANS option. Ruminal NH₃ concentrations were analyzed using the Mixed procedure of SAS. The model contained the effects of time of sampling (3 or 6 h after infusions), level of urea supplementation, and urea supplementation × sampling time.

**Results and Discussion**

Ruminal NH₃ concentrations averaged 4.5, 21.5, and 19.7 mM for steers receiving 0, 40, and 80 g/d of urea, respectively (data not shown). The increases (P < 0.05) in ruminal NH₃ concentrations and plasma urea (see Table 4) in response to urea infusions indicate that urea infusions increased ammonia absorption and that ruminal ammonia loading was achieved. In vitro (Slyter et al., 1979) and in vivo (Satter and Slyter, 1974) studies showed that microbial growth was maximized when ruminal NH₃ concentrations were 1.6 and 3.5 mM, respectively. This, in addition to unchanged DM and OM digestibilities in response to urea infusion (Table 3), indicates that rumen NH₃ concentrations in our study were adequate to support microbial requirements and that treatments did not affect ruminal fermentation.

There were no Met × urea interactions for diet digestibilities or N retention data (Table 3). Nitrogen intake was increased (P < 0.05) in response to Met and urea infusions as a result of the additional N infused. Fecal N excretion was not altered by treatments. The higher level of Met supplementation increased (P < 0.05) N mL of 6 M HCl to prevent NH₃ loss. Representative samples of feces (10%) and urine (1%) were saved, composited by period, and stored (−20°C) for later analysis.

Samples of the diet, orts, and feces were analyzed for DM (105°C in forced-air oven for 24 h) and OM (weight loss on ashing at 450°C for 8 h) to calculate digestibilities. Composite samples of the diet, orts, wet feces, and urine were analyzed for N using a Leco FP 2000 nitrogen analyzer (Leco Corp., St. Joseph, MI) to calculate N retention.

Jugular blood samples were collected 4 h after the morning feeding on the last day of each period. Blood was collected into vacuum tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, immediately chilled on ice, and centrifuged for 20 min at 1,000 × g to obtain plasma. Blood also was collected into vacuum tubes without additives, allowed to clot for 30 min at room temperature, and then centrifuged for 20 min at 1,000 × g to obtain serum. Samples were stored (−20°C) for later analysis of plasma glucose, urea, and AA and of serum insulin and IGF-I.

After completion of the study, steers were maintained on their treatments for one more day, and ruminal fluid samples were collected at 3 and 6 h after morning feeding and stored (−20°C) for later analysis of NH₃.

Plasma glucose concentrations were measured using methods of Gochman and Schmitz (1972). Plasma and urinary urea were measured using the method of Marsh et al. (1965), and urinary and ruminal fluid NH₃ concentrations by the method of Broderick and Kang (1980). Plasma AA were measured by gas chromatography using a commercial kit (EZ:faast; Phenomenex, Torrance, CA). Insulin was measured using an insulin RIA kit (DSL-1600; Diagnostic Systems Laboratories, Webster, TX), and IGF-I was measured using an active IGF-I coated-tube IRMA kit (DSL-5600; Diagnostic Systems Laboratories).
Table 4. Effects of methionine supplementation and ammonia load on blood hormone and metabolite concentrations in steers

<table>
<thead>
<tr>
<th>Item</th>
<th>2 g/d of l-methionine</th>
<th>5 g/d of l-methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No urea</td>
<td>40 g/d urea</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>1.02</td>
<td>1.16</td>
</tr>
<tr>
<td>IGF-1</td>
<td>386</td>
<td>410</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>4.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.60</td>
<td>5.41</td>
</tr>
<tr>
<td>Methionine</td>
<td>7.7</td>
<td>7.2</td>
</tr>
<tr>
<td>Valine</td>
<td>162</td>
<td>146</td>
</tr>
<tr>
<td>Leucine</td>
<td>94</td>
<td>85</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>70</td>
<td>62</td>
</tr>
<tr>
<td>Threonine</td>
<td>54</td>
<td>43</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Lysine</td>
<td>106</td>
<td>107</td>
</tr>
<tr>
<td>Histidine</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>Tryptophan</td>
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<td>26</td>
</tr>
<tr>
<td>Glutamate</td>
<td>123</td>
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<tr>
<td>Glutamine</td>
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<td>191</td>
</tr>
<tr>
<td>Alanine</td>
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<td>121</td>
</tr>
<tr>
<td>Glycine</td>
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<td>359</td>
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<tr>
<td>Serine</td>
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<td>74</td>
</tr>
<tr>
<td>Proline</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>Aspartate</td>
<td>5.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Asparagine</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Ornithine</td>
<td>68</td>
<td>66</td>
</tr>
</tbody>
</table>

For n = 6.

Linear effect of urea, P < 0.05.

Quadratic effect of urea, P < 0.05.

Effect of methionine, P < 0.05.

Quadratic effect of urea × methionine interaction, P < 0.05.

retention from 22.0 to 27.5 g/d. The observed increase in retained N was a result of the decreased (P < 0.05) urinary N excretion from 68.8 to 64.8 g/d with Met supplementation. Similar responses to Met supplementation were observed by Campbell et al. (1996, 1997). Supplementation with the higher level of Met decreased (P < 0.05) urinary urea N from 55.5 to 53.1 g/d and urinary NH₃-N from 2.7 to 1.7 g/d.

Although urea infusions linearly increased (P < 0.05) urinary N excretions from 48.5 to 67.3 and 84.5 g/d for steers infused with 40 and 80 g/d urea, respectively, retained N was not affected (P = 0.2) by urea treatments. Of the additional N infused as urea, 73 and 97% were excreted as urea N when 2 and 5 g/d Met was supplemented, respectively (Table 3). Urea infusions linearly increased (P < 0.05) urinary urea N (Table 3) from 38.9 to 54.5 and 70.2 g/d for the steers infused with 40 and 80 g/d of urea, respectively. These increases in urinary urea N in response to ammonia loading closely reflect the increase in total urinary N. Of the additional increases in total urinary N when 40 and 80 g/d of urea was infused, 83 and 87% were excreted as urea N, respectively.

To assess the efficiency of AA use, it is important that the input levels being tested are below animal requirements. Our levels of Met supplementation (2 and 5 g/d) were selected to be below the requirements of growing steers under our experimental conditions to ensure we were working in a range that provided a linear response to N retention with Met supplementation. Our diet (2.6 kg DM/d) provided 2.6 g/d of Met (Campbell et al., 1997), so even with the higher level of supplemental Met (5 g/d) the total Met supply (7.6 g/d) was below the requirements of steers maintained under similar conditions (Campbell et al., 1997). Although plasma Met concentrations demonstrated a significant increase in response to the higher level of Met supplementation (Table 4), the observed increases were relatively small in magnitude and, in comparison to the greater increases in plasma Met observed by Campbell et al. (1997) in response to Met supplementation, suggest that 5 g/d of supplemental Met was below the steers’ requirement.

Assuming that retained N was deposited completely as tissue protein (retained N × 6.25) and that the protein of tissue gain contains 2.0% Met (Ainslie et al., 1993),
the calculated efficiencies of use of supplemental Met
(i.e., between 2 and 5 g/d of supplemental Met) were 23,
27, and 18% for steers receiving 0, 40, or 80 g/d of urea,
respectively. Thus, our average efficiency of use of supple-
mental Met (23%) was similar to those reported by
Campbell et al. (1996, 1997) and Titgemeyer and
Merchen (1990), higher than those of 12 to 14% reported
by Froidmont et al. (2000) and Lambert et al. (2002),
but much lower than the 65% efficiency value predicted
by Ainslie et al. (1993), which was adopted by the NRC
estimated that Met requirements were 3.0% of metabo-
lizable protein requirements; thus, if body protein con-
tains 2.0% Met, the efficiency of Met use was approxi-
mately two-thirds as great for Met as for metabolizable
protein as a whole.

The efficiencies of use of the basal Met supply (4.6 g/
d; calculated as 2.6 g/d provided from the diet [Campbell
et al., 1997] plus 2 g/d provided to all steers via abomasal
infusions) were much greater than those for the supple-
mental methionine. Nitrogen retention averaged 22.1 g/
d for steers receiving the lower level of infused methio-
nine, and this would correspond to 2.8 g/d of Met being
deposited by the steers at an efficiency of approximately
60%. Consideration of a maintenance requirement would
increase this calculated efficiency. Although it seems that
lesser supplies of Met are used more efficiently
than greater ones, the efficiency of use of the basal Met
supplies is likely overestimated due to the overestima-
tion of protein deposition by N retention.

Our estimates of Met use do not consider the role of
Met as a precursor for the synthesis of cysteine. In our
experimental model, we have observed either no change
in N retention in response to supplemental cysteine
(Campbell et al., 1997; Lœst et al., 2002) or only rela-
tively small increases (Lambert et al., 2004). Thus, it
does not seem advantageous to consider the role of Met
as a precursor for cysteine synthesis; however, if cysteine
production is considered as an important end product of
Met, then the efficiencies of Met use could be nearly
twice those calculated from our data.

The infusion of 40 g/d of urea, but not 80 g/d, decreased
(quadratic; \( P < 0.05 \)) plasma glucose concentrations (Ta-
ble 4). The reasons for the decreased plasma glucose
concentrations with the infusion of 40 g/d of urea are
unknown, but the magnitude of change was small. Me-
thionine supplementation did not affect plasma urea or
glucose concentrations. Serum insulin and IGF-I were
not affected by any treatment (Table 4).

Methionine supplementation increased \( (P < 0.05) \)
plasma concentrations of Met and decreased \( (P < 0.05) \)
concentrations of valine, leucine, serine, ornithine, and
tyrosine (Table 4). The same trends were observed for
valine, leucine, and serine in growing steers infused with
L-Met (Titgemeyer and Merchen, 1990; Campbell et al.,
1996, 1997) and similar trends were observed for tyro-
sine in growing steers infused with D-Met (Campbell et
al., 1996). The decrease in plasma valine, leucine, and
tyrosine concentrations might be a result of increased
uptake and use of these AA for protein synthesis as
Met, the first-limiting AA, became available in greater
amounts. Serine is used in cystathionine synthesis dur-
ing transsulfuration, and this may have been the reason
for the observed decrease in plasma concentrations of
serine in response to Met supplementation.

Plasma concentrations of glutamate and alanine were
linearly \( (P < 0.05) \) decreased in response to urea infusions
(Table 4). The decrease in plasma glutamate could be
due to the amidation of glutamate to glutamine as a
secondary means of hepatic NH\(_3\) detoxification. Numeri-
cal increases in plasma glutamine concentrations in re-
sponse to urea infusion were also observed, which sup-
ports this hypothesis. Trends for decreased plasma gluta-
matase and increased glutamine in response to ammonia
loading were similarly observed in growing steers when
His was the AA most limiting for animal performance
(McCuistion et al., 2004). The decrease in plasma alanine
in response to urea infusion may reflect increased ala-
nine oxidation, as was observed in hepatocytes isolated
from sheep fed high-urea diets (Mutsvanga et al.,
1996). Moreover, alanine may be used as a source of
aspartate N for detoxification of ammonia load.

We studied the effects of ammonia load under condi-
tions where Met supply was limiting. To achieve that,
the diet was formulated to provide deficient amounts of
AA, and all essential AA, except Met, were supple-
mented. If protein (AA) supply were not limiting, nega-
tive effects of ammonia loading on AA use might not
lead to any changes in performance because an excess
supply of AA could allow for optimal performance even
in the face of decreased efficiency of use. Ammonia
loading had no negative effects on N retention in sheep
(Norton et al., 1982) or cattle (Slyter et al., 1979; Moorby
and Theobald, 1999), but those studies were not conducted
under conditions where AA supply limited performance.
Our data suggest that catabolism of Met is not impacted
by increases in ureagenesis in response to an ammo-
nia load.

Ammonia loading did not have negative effects on N
retention or on the efficiency of use of supplemented Met
by growing steers. McCuistion et al. (2004) similarly
observed that N retention was not affected by ammonia
loading in growing steers when histidine supply limited
animal performance. Our results suggest that the addi-
tional urea synthesis to support NH\(_3\) detoxification does
not require an obligatory input of AA N, at least not
from Met, as was suggested by the data of Mutsvanga
et al. (1999), where Met deamination was increased with
ammonia loading in vitro. Our results also contrast with
the data of Lobley et al. (1995), where ammonia loading
increased leucine oxidation in sheep.

The efficiency of supplemental Met use was lower than
the 65% efficiency value predicted by Ainslie et al. (1993)
and used for estimating AA requirements by the NRC
(1996). The NRC (1996) assumes the same use efficiency
value for all AA, and the efficiency is based only on the
equivalent BW of the animal. The overestimation by the
NRC (1996) for efficiency of use of supplemental Met
has been noted previously (Titgemeyer and Merchen, 1990; Campbell et al., 1996, 1997; Froidmont et al., 2000; Lambert, 2001). Recently, we have observed an efficiency of use for supplemental histidine greater than that for Met (65%; McCuistion et al., 2004), suggesting that there are differences among AA in how efficiently they are used by cattle.

Implications

Under our experimental conditions, ruminal ammonia loading did not lead to metabolic costs with regard to methionine use for growth. Although ammonia loading did not negatively affect whole-body protein deposition when methionine was limiting, excessive amounts of dietary protein may have environmental and economical costs that are generally considered unacceptable.

Literature Cited


