The impact of lower gut nitrogen supply on nitrogen balance and urea kinetics in growing pigs fed a valine-limiting diet

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ABSTRACT: An N-balance and isotope dilution study was performed to determine the effect of lower gut N supply on N retention and CO(NH2)2 kinetics in growing pigs. Nine cecally cannulated and jugular-catheterized barrows (initial BW 22.4 ± 1.2 kg) were randomly assigned to 1 of 3 cecal N infusion treatments: saline, casein, or CO(NH2)2; the latter 2 treatments were infused at a rate of 40% of daily N intake. All pigs were fed a Val-limiting corn (Zea mays) starch and soybean (Glycine max) meal-based diet. Cecal N infusions did not affect apparent total tract digestibility of N (P > 0.05). The efficiency of using N [% of apparent ileal digestible intake; 72.9 ± 1.9, 84.9 ± 1.9, and 85.6 ± 2.3% (P = 0.01) for saline, casein, and CO(NH2)2, respectively] and Val (76.9 ± 1.9, 86.5 ± 1.9, and 86.5 ± 2.4; P = 0.02) for whole body protein and Val retention increased for casein and CO(NH2)2. Urea flux and urinary N excretion increased (P < 0.05) similarly for both N infusions, but this increase did not fully account for lower gut N disappearance. Lower gut N disappearance is in the form of NPN, which can be used for microbial AA production in the upper gut and should be considered when determining N and AA supply and requirements.

Key words: lower gut, microbial amino acids, nitrogen, urea recycling

INTRODUCTION

It is generally believed that AA disappearance in the lower gut (LG) is of little nutritional importance. However, AA transporters and AA uptake have been shown in porcine colonocytes (James and Smith, 1976; Blachier et al., 2007). Fuller and Reeds (1998) suggested that N absorbed from the LG is largely in the form of NH3, which is converted to CO(NH2)2 and excreted with urine or recycled into the gastrointestinal tract. Recycled CO(NH2)2 can be used by resident gut microbes for de novo AA synthesis, which may benefit the host (Torrallardona et al., 2003). The objective of this trial was to determine the impact of LG N supply on N balance and CO(NH2)2 kinetics in growing pigs fed a Val-limiting diet.

MATERIALS AND METHODS

The trial protocol was approved by the Animal Care Committee of the University of Guelph. Nine barrows (22.8 ± 1.34 kg initial BW) were fitted with a simple T-cannula in the cecum for infusion of saline, casein, or CO(NH2)2, solutions and implanted with catheters in the left and right external jugular veins for infusion of 15N15N-CO(NH2)2 and blood sampling (Libao-Mercado et al., 2009). Among essential AA, the largest potential contribution of microbial protein to the host’s AA supply is for Val (Torrallardona et al., 2003). Therefore, all pigs received the same Val-limiting cornstarch and soybean meal-based diet (13.7% CP) fed at 2.8 × maintenance DE requirements per day (800 kJ/kg of BW0.60). Pigs were fed in 3 equal meals per day at 0830, 1230, and 1630 h. Infusion solutions were continuously infused into the cecum at 1.4 mL/min and providing N from casein or CO(NH2)2, equivalent to 40% of dietary N intake.

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The experimental period consisted of a 5-d adaptation followed by a 4-d N balance (Möhn et al., 2000). To determine CO(NH$_2$)$_2$ kinetics during the N balance, a 4-d intravenous infusion of $^{15}$N$^{15}$N-CO(NH$_2$)$_2$ (16.1 mM) supplied 0.4 mmol/kg BW per d of the labeled CO(NH$_2$)$_2$ (Libao-Mercado, 2009). Means were separated; *P* $<$ 0.05 was considered significant and *P* $<$ 0.10 a trend.

**RESULTS**

Pigs, except for 1, fully recovered from surgery and had achieved presurgical dietary intake levels within 3 d. Body weight and N intake were consistent across treatments and the amount of N infused did not differ between the 2 N infusions (Table 1; *P* $>$ 0.10). Nitrogen infusion into the cecum did not affect (*P* $>$ 0.05) fecal N excretion (Table 1). Urinary N and CO(NH$_2$)$_2$ excretion were increased (*P* $<$ 0.05) by the infusion of casein and CO(NH$_2$)$_2$ compared to saline-infused pigs. Protein deposition in N-infused pigs tended to be higher (*P* $<$ 0.10) compared to saline-infused pigs. Lower gut N infusion increased (*P* $<$ 0.05) the use of apparent ileal digestible N intake for protein deposition but decreased (*P* $<$ 0.05) the use of total N supply. The calculated efficiency of using apparent ileal digestible Val for Val retention in body protein was increased (*P* $<$ 0.05) by infusing N in the LG, regardless of N source. The use of absorbed N from the LG for N retention averaged 28% and was not different between CO(NH$_2$)$_2$ and casein infusions (*P* $>$ 0.10). Urea flux was increased (*P* $<$ 0.05) in N-infused pigs compared to saline-infused pigs (Table 2). The rate of CO(NH$_2$)$_2$ recycled to the gut was increased (*P* $<$ 0.05) by N infusions and was largest for the CO(NH$_2$)$_2$ infusion.

**Table 1.** Body weight, N intake and infused, and N balance in pigs fed a Val-limiting diet and infused with saline, casein, or CO(NH$_2$)$_2$ into the lower gut (LG)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>Casein</th>
<th>Urea [CO(NH$_2$)$_2$]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>29.7 ± 0.8</td>
<td>30.6 ± 0.8</td>
<td>30.9 ± 1.0</td>
<td>0.599</td>
</tr>
<tr>
<td>N intake, g/d</td>
<td>24.6 ± 0.6</td>
<td>25.2 ± 0.6</td>
<td>24.7 ± 0.7</td>
<td>0.809</td>
</tr>
<tr>
<td>N infused, g/d</td>
<td>0.00$^a$</td>
<td>10.47 ± 0.24$^b$</td>
<td>9.63 ± 0.29$^b$</td>
<td>$&lt;$0.001</td>
</tr>
<tr>
<td>Fecal N excretion, g/d</td>
<td>4.72 ± 0.36</td>
<td>6.30 ± 0.36</td>
<td>5.62 ± 0.44</td>
<td>0.068</td>
</tr>
<tr>
<td>LG N disappearance$^c$, g/d</td>
<td>–1.13 ± 0.34$^a$</td>
<td>8.34 ± 0.34$^a$</td>
<td>8.28 ± 0.42$^a$</td>
<td>$&lt;$0.001</td>
</tr>
<tr>
<td>Urinary excretion, g N/d</td>
<td>3.48 ± 1.10$^a$</td>
<td>10.06 ± 1.10$^a$</td>
<td>10.08 ± 1.34$^a$</td>
<td>0.014</td>
</tr>
<tr>
<td>N</td>
<td>4.58 ± 0.47$^a$</td>
<td>11.50 ± 0.47$^a$</td>
<td>11.20 ± 0.57$^b$</td>
<td>$&lt;$0.001</td>
</tr>
<tr>
<td>Protein deposition$^d$, g/d</td>
<td>94.8 ± 3.9</td>
<td>111.4 ± 3.9</td>
<td>109.6 ± 4.8</td>
<td>0.075</td>
</tr>
<tr>
<td>$K_{\text{valuation}}$, % of AID$^e$ intake</td>
<td>73.7 ± 1.9$^a$</td>
<td>85.8 ± 1.9$^a$</td>
<td>86.6 ± 2.4$^a$</td>
<td>0.010</td>
</tr>
<tr>
<td>$K_{\text{valuation}}$, % of total N supply</td>
<td>81.6 ± 1.9$^a$</td>
<td>61.6 ± 1.9$^b$</td>
<td>58.8 ± 2.3$^b$</td>
<td>$&lt;$0.001</td>
</tr>
<tr>
<td>$K_{\text{valuation}}$, % of LG disappearance</td>
<td>–</td>
<td>29.0 ± 8.4</td>
<td>25.8 ± 10.3</td>
<td>0.824</td>
</tr>
</tbody>
</table>

$^a$bWithin a row, means without a common superscript differ (*P* $<$ 0.05).

Values are least square means ± SE; n = 3 for saline and casein and n = 2 for CO(NH$_2$)$_2$.

Calculated as ileal N flow + N infusion – fecal N flow. Ileal N flow was based on ileal digestibility values determined previously (Columbus et al., 2012).

Calculated as (N intake + N infusion – fecal N excretion – urine N excretion) × 6.25.

Calculated as (N retention)/[apparent ileal digestible (AID) N intake – N losses with skin and hair] according to NRC (2012).

AID = apparent ileal digestible.

Calculated as (N retention)/[AID N intake + LG N disappearance – N losses with skin and hair] according to NRC (2012).

Calculated as (change in N retention vs. saline)/(change in LG N disappearance vs. saline).

Calculated as (protein deposition × Val % body protein)/(AID Val intake – Val losses with skin and hair) according to NRC (2012).

Table 2. Urea enrichment [moles percent excess (MPE)] and CO(NH$_2$)$_2$ kinetics (mmol N/d) in pigs fed a Val-limiting diet and infused with saline, casein, or CO(NH$_2$)$_2$ into the lower gut (LG)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>Casein</th>
<th>Urea [CO(NH$_2$)$_2$]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{15}$N$^{15}$N-CO(NH$_2$)$_2$ enrichment</td>
<td>6.29 ± 1.33</td>
<td>1.99 ± 1.33</td>
<td>1.67 ± 1.63</td>
<td>0.114</td>
</tr>
<tr>
<td>Urea flux$^f$</td>
<td>360 ± 77$^b$</td>
<td>1,039 ± 77$^b$</td>
<td>1,220 ± 94$^b$</td>
<td>0.002</td>
</tr>
<tr>
<td>Urea recycling$^g$</td>
<td>112 ± 64$^a$</td>
<td>321 ± 64$^a$</td>
<td>500 ± 78$^b$</td>
<td>0.030</td>
</tr>
<tr>
<td>LG N disappearance$^h$, % of CO(NH$_2$)$_2$ flux increase</td>
<td>–</td>
<td>101.3 ± 6.9</td>
<td>78.9 ± 8.4</td>
<td>0.131</td>
</tr>
</tbody>
</table>

$^a$bWithin a row, means without a common superscript differ (*P* $<$ 0.05).

Values are least square means ± SE; n = 3 for saline and casein and n = 2 for CO(NH$_2$)$_2$.

Calculated as infusion rate × [(MPE infusate/MPE urine) – 1].

Calculated as CO(NH$_2$)$_2$ flux – urinary CO(NH$_2$)$_2$ excretion.

Calculated as (change in LG N disappearance vs. saline)/(change in CO(NH$_2$)$_2$ flux vs. saline).
DISCUSSION

In this trial the majority of the N infused into the cecum was absorbed as NH$_3$, as indicated by the increase in CO(NH$_2$)$_2$ flux and urinary N excretion in both the casein- and CO(NH$_2$)$_2$–infused pigs. The lack of a protein deposition response to casein infusion above that observed with CO(NH$_2$)$_2$ is further evidence for the lack of absorption of intact AA from the LG.

Between 20 to 50% of CO(NH$_2$)$_2$ flux is recycled into the gastrointestinal tract of the pig (Mosenthin et al., 1992) and may be used by the resident gut microbes for de novo production of AA that can be absorbed and use by the host (Torrallardona et al., 2003). Recycling of CO(NH$_2$)$_2$ may therefore be an important N salvage mechanism during periods of AA imbalance or limited N intake (Fuller and Reeds, 1998). In the current study, the LG N supply improved the use of dietary N and Val. This provides evidence for the nutritional importance of NPN absorption from the LG.

In summary, LG N disappearance is in the form of NPN, which can be used for microbial AA production in the upper gut. Lower gut N metabolism should be considered when determining N and AA supply and requirements.

LITERATURE CITED


Columbus, D., M. F. Fuller, J. K. Htoo, and C. F. M. de Lange. 2012. Lower gut nitrogen supply has no effect on apparent ileal digestibility of nitrogen or amino acids in growing pigs. Page 60 in Proc. 12th Int. Symp. Dig. Physiol. Pigs. Keystone, CO.


