Estimating fermentative amino acid losses in the upper gut of pigs

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ABSTRACT

Fermentative catabolism of dietary and endogenous amino acids (AA) in the upper gut of pigs (FAAC) can result in significant loss of AA available for protein synthesis and body maintenance functions. A continuous infusion trial was performed using isotope tracers to determine ammonia flux in the upper gut, whole body urea flux, urea recycling (urea flux – urinary urea excretion), and FAAC (ammonia flux in the upper gut – urea recycling) in ileal-cannulated growing pigs fed a control diet (C, 19.3% CP), the control diet with added fibre (F, 12% pectin added at expense of cornstarch), or a diet low in protein (LP, 13.6% CP). 15N-ammonium chloride and 13C-urea were infused intragastrically and intravenously, respectively, for a period of 4 days. In samples obtained on days 3 and 4 of infusion, 15N-enrichments in blood urea (6.21 ± 1.65, 8.93 ± 2.01, and 9.78 ± 1.40 atoms percent excess (APE) for C, F and LP, respectively) were higher than those in ileal ammonia (0.44 ± 0.12, 0.37 ± 0.14, and 0.71 ± 0.10 APE). This suggests a rapid absorption of ammonia prior to the distal ileum and lack of uniformity for enrichment in the digesta ammonia pool. Simple isotope dilution calculations are, therefore, inadequate for calculating FAAC and ammonia flux in the upper gut of pigs. A two compartment (ileal ammonia and plasma urea) model was developed to determine possible value ranges for FAAC in the upper gut (0.0 to 13.3 ± 1.42, 15.5 ± 1.65, and 10.7 ± 1.20 mmol N/kg/d for the three treatments), but this model also has limitations. Quantifying FAAC in the upper gut of pigs offers a number of challenges, but warrants further investigation.

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1. Introduction

Fermentative catabolism of dietary and endogenous amino acids (AA) in the upper gut of pigs (FAAC) can result in significant loss of AA available for protein synthesis and body maintenance functions. On the other hand, ammonia from FAAC and urea that is secreted into the upper gut of pigs, i.e. recycled urea, can be used for de novo synthesis of AA that may be available to the host (Libao-Mercado et al., 2009). To date, no estimates of FAAC are available for pigs. The objective of this trial was to explore approaches to estimate FAAC losses in the upper gut of growing pigs. An additional objective was to determine the impact of dietary fibre and protein content on FAAC.

2. Materials and methods

Six barrows with an initial BW of 19.9 kg (SD 0.99) were fitted with a simple T-cannula at the distal ileum, a gastric catheter, and two jugular catheters (de Lange et al., 1989). During two consecutive experimental periods and based on a cross-over design, pigs were fed a cornstarch–soybean meal based control diet (C, 19.3% CP), a high-fibre diet (F, control diet with 12% pectin added at the expense of cornstarch), or a low-protein diet (LP, 13.6% CP, cornstarch replacing soybean meal) for a total of 12 observations. Pigs were allowed to adapt to their assigned diets for 7 days prior to infusion of isotopes. 15N-ammonium chloride and 13C-urea were infused intragastrically

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and intravenously at a rate of 0.5 mL/min to provide 0.8 and 0.2 mmol/kg/d, respectively, for a period of 4 days.

Samples of blood, urine and ileal digesta were collected on days 0, 3, and 4 of the infusion period. Day 0 samples were used to determine background enrichment values while samples from days 3 and 4 were used for plateau enrichment values. Ileal digesta and blood plasma samples were obtained and processed according to Libao-Mercado et al. (2009). Urine was collected into vessels containing sulphuric acid to maintain pH below 2.

All isotopic enrichment analysis was performed in the laboratory of Metabolic Solutions, Inc. (Nashua, NH, USA). Ammonia from digesta was released by fluid aeration in a sealed tube containing a trapping well of 2% sulphuric acid after adjusting the pH to greater than 12 by adding 5 M NaOH. The 15N-enrichment of ammonia in ileal digesta was analyzed using a Europa 20/20 ANCA IRMS. The plasma and urine samples were treated with urease in a sealed tube to liberate CO2 from urea. The liberated CO2 was analyzed using a Europa 20/20 ABCA IRMS to obtain 13C-enrichment. The 15N-enrichment of urea in blood plasma and urine was determined on an Agilent 5973 GC/MS instrument using chemical ionization detection with methane gas and monitoring ions 293, 294 and 295, respectively (Nelson and Ruo, 1988). Values for 13C-enrichment were analyzed using a Europa 20/20 ANCA IRMS. The 15N-enrichment of ammonia in ileal digesta was analyzed using a Europa 20/20 ANCA IRMS. The plasma urea concentration was determined in the Animal Health Laboratory of Metabolic Solutions, Inc. (Nashua, NH, USA). Laboratory at the Ontario Veterinary College (Hitachi 911; Guelph, ON, Canada).

Isotope dilution equations were used to calculate whole body urea flux and upper gut ammonia flux values from isotope infusion rates and either 13C-enrichment in urinary urea or 15N-enrichment in ileal digesta ammonia (Matthews and Downey, 1984). Urea recycling was calculated as the difference between urea flux and urinary urea excretion (Mosenthin et al., 1992). The rate of FAAC was calculated as the difference between upper gut ammonia flux and urea recycling.

Total and isotopic fluxes were calculated based on a two compartment model (Fig. 1), where $F_{xy}$ refers to the movement of N or C from pool x to pool y; pools x or y represent plasma urea (p), urinary urea (u), ileal ammonia (i), microbial AA (m), or colonic ammonia (c); $I_{x}$ is infusion of urea or ammonium chloride; $c_{x}$ is carbon enrichment of pool x, and $n_{x}$ is nitrogen enrichment of pool x:

$$F_{ip} (\text{mmol N/d}) = I_{UREA} \times 2 \quad (1)$$

$$F_{ip} (\text{mmol } ^{13}\text{C/d}) = c_{ip} F_{ip} / 2 \quad (2)$$

$$F_{pu} (\text{mmol N/d}) = \text{urine vol.} \times \text{urea-N concentration} \quad (3)$$

$$F_{pu} (\text{mmol } ^{15}\text{N/d}) = n_{pu} F_{pu} / 2 \quad (4)$$

$$F_{pi} (\text{mmol } ^{13}\text{C/d}) = (2) - (4) \quad (6)$$

$$F_{pi} (\text{mmol } ^{15}\text{N/d}) = (6) \times 2n_{p} / c_{p} \quad (7)$$

$$F_{pi} (\text{mmol N/d}) = (7) / n_{p} \quad (8)$$

$$F_{ip} (\text{mmol } ^{15}\text{N/d}) = (5) + (7) \quad (9)$$

$$F_{li} (\text{mmol N/d}) = I_{NH3} \quad (10)$$

$$F_{ip} (\text{mmol } ^{15}\text{N/d}) = (9) - (10) \quad (11)$$

$$F_{ic} (\text{mmol N/d}) = \text{ileal ammonia flow} \quad (12)$$

$$F_{ic} (\text{mmol } ^{15}\text{N/d}) = n_{i} \times \text{ileal ammonia flow} \quad (13)$$

$$F_{im} (\text{mmol } ^{15}\text{N/d}) = (8) - (11) - (13) \quad (14)$$

$F_{ip}$ (11) assumes that all $^{15}$N infused is absorbed and, therefore, that the difference between $^{15}$N absorption and infusion is reabsorption of label. Rearranging the above equations, and assuming that all N flows must be greater than or equal to zero, yields value ranges for N flows out of the ileal pool due to microbial protein production ($F_{im}$; mmol N/d) and entry of N from FAAC ($F_{di}$; mmol N/d).

All data were analyzed as repeated measures using the mixed model procedure (PROC MIXED) of the SAS statistical program (SAS 9.1, SAS Institute, Inc.). Treatment effects were tested using the Tukey test.

![Fig. 1. Two compartment model for N flow. $F_{ip}$ is the movement of N from pool x to pool y, $I_{NH3}$ and $I_{UREA}$ are the infusions of $^{15}$N-ammonium chloride and $^{13}$C-urea, and mAA, dAA, and cAA are microbial, dietary, and endogenous AA.](image-url)
3. Results and discussion

Out of 12 potential observations, 5 observations were missing due to loss of catheters or cannulas (n=2), poor appetite (n=1) or unrealistic values for urea excretion (n=2). The average BW at the end of the first infusion period was 24.7 kg (SD 1.44) and at the end of the second period was 30.6 kg (SD 1.82).

Surprisingly, the $^{13}$C-enrichment values in plasma urea were much lower than those in urinary urea (Table 1). This may be the result of non-urea CO$_2$ interference in the $^{13}$C-urea enrichment analysis. To maintain consistency, urinary enrichment values for both $^{13}$N and $^{13}$C were therefore, used to estimate those in plasma urea in all calculations (Tables 2 and 3).

When calculated based on isotope dilution, the inclusion of 12% pectin in the diet had no effect on urea or ammonia flux values or FAAC (Table 2; P>0.10). Lowering the dietary CP content resulted in a reduction in urea flux and urea recycling (Table 2; P<0.0001). There was no effect of dietary CP content on ammonia flux or FAAC (Table 2; P>0.10). Based on the two compartment model, estimated N flow to FAAC, microbial protein, and ammonia absorption was not affected by dietary fibre content (Table 3; P>0.10). The LP diet resulted in an increase in the minimum possible amount of N incorporation into microbial protein (P<0.0001) and a decrease in ammonia absorption (Table 3; P<0.0001). Neither increasing the dietary fibre content nor decreasing dietary CP content resulted in significant differences in estimates for FAAC (Table 3; P>0.10).

Initially, simple isotope dilution calculations were used to determine ammonia flux in the upper gut, however, this method resulted in values for ammonia flux (Table 2) that are considerably higher than total N intake. This high level of ammonia production cannot be accounted for by calculated urea flux and observed values for urinary urea excretion and ileal ammonia flow. These unrealistic values for ammonia flux are most likely a result of low $^{15}$N-enrichment values in ileal digesta ammonia, which were lower than those in plasma urea. This suggests a rapid absorption of ammonia prior to the distal ileum and lack of uniformity for enrichment in the digesta ammonia pool. Simple isotope dilution calculations are, therefore, inadequate for calculating FAAC and ammonia flux in the upper gut of pigs.

In the alternative two compartment model (Fig. 1) rapid and complete absorption of the infused ammonia is assumed, and the contribution of $^{15}$N-urea to ileal ammonia flux is calculated based on observations from $^{13}$C-urea kinetics. If this assumption is correct, isotopic fluxes through the ileal ammonia and plasma urea pool can be used to generate minimum and maximum values for FAAC and microbial protein production (Table 3). This model, however, does not allow for direct calculation of FAAC. Alternative means should be explored to better quantify FAAC.

4. Conclusions

Use of simple isotope dilution equations is inadequate for determining ammonia flux and fermentative amino acid catabolism (FAAC) in the upper gut of pigs, largely due to the non-homogenous nature of the digesta ammonia pool. An alternative two compartment model presented in this paper allows for calculation of a range of values for movement of N through the ileal ammonia pool. However, due to limitations in this model and experimental observations, absolute values

**Table 1**

| Isotopic enrichment (atoms percent excess, APE) of urea in plasma and urine and ammonia in ileal digesta as well as urinary urea excretion and ileal ammonia flow in growing pigs after a four day continuous infusion of $^{13}$C-urea and $^{15}$N-ammonia.* |
|------------------|------------------|------------------|
|                  | C (n=2)          | F (n=2)          | LP (n=3)         |
| Plasma (APE, %)  |                  |                  |                  |
| Urea-N           | $^{15}$N $^{13}$C | $^{15}$N $^{13}$C | $^{15}$N $^{13}$C |
| Urea             | $^{15}$N $^{13}$C | $^{15}$N $^{13}$C | $^{15}$N $^{13}$C |
| Urine (APE, %)   |                  |                  |                  |
| Urea-N           | $^{15}$N $^{13}$C | $^{15}$N $^{13}$C | $^{15}$N $^{13}$C |
| Urea             | $^{15}$N $^{13}$C | $^{15}$N $^{13}$C | $^{15}$N $^{13}$C |
| Ileal Digesta (APE, %) |                  |                  |                  |
| NH$_3$           | $^{15}$N         | $^{15}$N         | $^{15}$N         |
| Flow (mmol N/kg/d) |                 |                  |                  |
| Urinary Urea     |                 |                  |                  |
| Ileal Ammonia    |                 |                  |                  |

* Pigs were fed one of three diets: Control (C, 19.3% CP), High-Fibre (F, 12% pectin), Low-Protein (LP, 13.6% CP).

**Table 2**

Simple isotope dilution calculations for urea flux and recycling, ileal ammonia flux, and fermentative amino acid catabolism (FAAC, F$_{fa}$) in growing pigs after a four day continuous infusion of $^{13}$C-urea and $^{15}$N-ammonia (mmol N/kg/d).^a

<table>
<thead>
<tr>
<th></th>
<th>C (n=2)</th>
<th>F (n=2)</th>
<th>LP (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea flux</td>
<td>23.1±1.18$^{2a}$</td>
<td>23.0±2.41$^{4a}$</td>
<td>10.9±1.18$^{4b}$</td>
</tr>
<tr>
<td>Urea recycling</td>
<td>8.53±1.06$^{a}$</td>
<td>9.34±2.18$^{ab}$</td>
<td>4.58±1.06$^{b}$</td>
</tr>
<tr>
<td>Ileal ammonia flux</td>
<td>143±27.0</td>
<td>210±32.3</td>
<td>127±22.8</td>
</tr>
<tr>
<td>FAAC (F$_{fa}$)</td>
<td>115±29.4</td>
<td>180±35.2</td>
<td>103±24.9</td>
</tr>
</tbody>
</table>

^aPigs were fed one of three diets: Control (C, 19.3% CP), High-Fibre (F, 12% pectin), Low-Protein (LP, 13.6% CP).

**Table 3**

Range of total N flow values (mmol N/kg/d) for key ileal N fluxes based on isotopic flows through a two compartment model in growing pigs after a four day continuous infusion of $^{13}$C-urea and $^{15}$N-ammonia.1

<table>
<thead>
<tr>
<th></th>
<th>C (n=2)</th>
<th>F (n=2)</th>
<th>LP (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentative AA catabolism (F$_{fa}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>13.3±1.42$^{2}$</td>
<td>15.5±1.65</td>
<td>10.7±1.20</td>
</tr>
<tr>
<td>N incorporation into microbial protein (F$_{mi}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>0.69±0.16$^{a}$</td>
<td>1.26±0.33$^{ab}$</td>
<td>1.17±0.16$^{b}$</td>
</tr>
<tr>
<td>Maximum</td>
<td>2.09±0.34</td>
<td>3.60±0.45</td>
<td>3.49±0.29</td>
</tr>
<tr>
<td>Ammonia absorption (F$_{am}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>8.10±0.96$^{a}$</td>
<td>8.34±1.96$^{ab}$</td>
<td>4.08±0.96$^{b}$</td>
</tr>
<tr>
<td>Maximum</td>
<td>22.8±1.19$^{a}$</td>
<td>22.6±2.44$^{a}$</td>
<td>10.5±1.19$^{b}$</td>
</tr>
</tbody>
</table>

1Pigs were fed one of three diets: Control (C, 19.3% CP), High-Fibre (F, 12% pectin), Low-Protein (LP, 13.6% CP).

2Values are least square means± standard error.

3Calculates as (15N$^{14}$N×1)+(15N$^{15}$N×2).

4Values are least square means± standard error.

5Means in the same row with different superscripts are significantly different (P<0.05).
for FAAC could not be determined. It is recommended that future studies attempt to better quantify FAAC as it may have significant impacts on the amino acid economy in non-ruminant animals and humans.

Conflict of interest

All authors acknowledge that there are no conflicts of interest concerning the information that is provided in the paper.

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