Nonprotein Nitrogen Is Absorbed from the Large Intestine and Increases Nitrogen Balance in Growing Pigs Fed a Valine-Limiting Diet\textsuperscript{1–3}

Daniel A. Columbus,\textsuperscript{4} Hélène Lapierre,\textsuperscript{5} John K. Htoo,\textsuperscript{6} and Cornelis F. M. de Lange\textsuperscript{4*}

\textsuperscript{4}Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada; \textsuperscript{5}Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada; and \textsuperscript{6}Evonik Industries AG, Hanau, Germany

Abstract

Nitrogen absorption from the large intestine, largely as ammonia and possibly as amino acids (AAs), is generally thought to be of little nutritional value to nonruminant animals and humans. Ammonia-nitrogen absorbed from the large intestine, however, may be recycled into the small intestine as urea and incorporated into microbial AAs, which may then be used by the host. A cecal infusion study was performed to determine the form in which nitrogen is absorbed from the large intestine and the impact of large intestine nitrogen supply on nitrogen balance in growing pigs. Eighteen cecally cannulated barrows (initial body weight: 22.4 ± 1.2 kg) were used to determine the effect of supplying nitrogen into the large intestine from either casein or urea on whole-body nitrogen retention and urea kinetics. Treatments were cecal infusions of saline (control), casein, or urea with nitrogen infused at a rate of 40% of nitrogen intake. In a subsample of 9 pigs, \textsuperscript{15}N\textsubscript{15}N-urea was infused via i.v. during the nitrogen-balance period to determine urea kinetics. All pigs were fed a valine-limiting cornstarch-soybean meal–based diet. More than 80% of infused nitrogen was apparently absorbed. Urea flux and urinary nitrogen excretion increased (\(P < 0.01\)) and did not differ (\(P > 0.05\)) between nitrogen sources. Absorption of nitrogen from the large intestine appears to be in the form of nonprotein nitrogen, which appears to be returned to the small intestine via urea and used there for microbial AA production and should therefore be considered when determining nitrogen and AA supply and requirements. J. Nutr. 144: 614–620, 2014.

Introduction

It is generally thought that amino acid (AA)\textsuperscript{7} and nitrogen disappearance or absorption from the large intestine is of little nutritional value to nonruminant animals and humans. This is based on the results of a number of studies conducted using pigs and reviewed by Fuller and Reeds (1) that show little or no benefit of infusing either individual AAs or protein-bound AAs into the large intestine. For example, the infusion of lysine into the colon of pigs receiving a lysine-deficient diet did not improve whole-body nitrogen balance (2). Likewise, Darragh et al. (3) found no improvement in whole-body nitrogen balance of pigs fed a diet limiting in sulfur AAs when methionine was infused into the colon.

The results of these studies, however, are in contrast to evidence of the presence of AA transporters and AA uptake in colonocytes (4–6). Moreover, Gargallo and Zimmerman (7) showed an improvement in whole-body nitrogen balance in pigs fed low-nitrogen diets and infused with casein into the terminal ileum. In addition, Heine et al. (8) showed that when labeled yeast was infused into the colon of infants, the majority of the label that was absorbed (i.e., not excreted in feces) was retained in body protein and not excreted in urine. The results of these studies suggest that AAs are absorbed in the large intestine. However, these studies were performed in newborn animals or in isolated cells and therefore do not necessarily indicate what would occur in vivo and in animals with mature digestive systems.

Another possible fate of nitrogen absorbed from the large intestine is recycling into the upper gut for microbial AA synthesis. It has been suggested that, because of the susceptibility of undigested protein to microbial fermentation in the large intestine, nitrogen absorption from the large intestine is largely in the form of ammonia (1). Hepatic detoxification of absorbed ammonia contributes to urea flux, which is largely excreted in the urine in nonruminants (9). However, it has been demonstrated in...
Both nonruminant and ruminant animals that a proportion of urea flux is recycled into the gastrointestinal tract (10–12). In pigs, the majority of recycled urea enters via the small intestine (12), where resident microflora can use the nitrogen from hydrolyzed urea-nitrogen for de novo AA synthesis. Pigs are capable of absorbing and utilizing microbially produced AAs (13) and, therefore, this pathway may be an important method of nitrogen salvage during dietary AA or nitrogen deficiency.

It was hypothesized that nitrogen absorption from the large intestine is largely in the form of ammonia, which may contribute to the AA supply of the pig through urea recycling to the small intestine and de novo microbial AA synthesis. The objectives of this study were to determine the form in which nitrogen is absorbed from the large intestine and to determine the effect of supplying nitrogen into the large intestine from either nonprotein nitrogen (NPN; urea) or intact protein (casein) on whole-body nitrogen balance and urea kinetics in pigs fed a valine-limiting diet. Diets were formulated to be limiting in valine because the potential contribution of microbial protein to the host’s requirements for indispensable AAs appears largest for valine (14,15).

Materials and Methods

The experimental protocol was reviewed and approved by the Animal Care Committee of the University of Guelph. Pigs were cared for according to the guidelines of the Canadian Council on Animal Care (16).

Animals, diets, and experimental design. To determine the effect of nitrogen absorption from the large intestine on whole-body nitrogen balance and urea kinetics, 2 groups of pigs were used. For the main nitrogen-balance study, a total of 9 Yorkshire barrows with an initial body weight (BW) of 22.0 ± 1.78 kg were obtained from the Arkell Swine Research Station at the University of Guelph. For the isotope tracer study, which also included nitrogen-balance observations, 9 barrows with an initial BW of 22.8 ± 1.34 kg were obtained from the same source. During recovery from surgery to fit pigs with cecal cannulas and jugular catheters, the pigs were individually housed in smooth-sided floor pens in a temperature-controlled room at 21°C with free access to water. During the adaptation and collection periods, pigs were individually housed in smooth-sided metabolic crates (17) and only received water with meals to avoid excessive water spillage and contamination of urine. All pigs received the same cornstarch-soybean meal-based, valine- (14,15). The experimental diet was formulated based on published nutrient contents of ingredients according to the guidelines of the Canadian Council on Animal Care.

The diet was formulated based on the expected response in whole-body protein deposition, and therefore whole-body nitrogen balance, of increased nitrogen absorption from the large intestine and based on estimates of incorporation of NPN into microbial valine, microbial valine absorption, and whole-body AA content in pigs. Valine has been shown among indispensable AAs to have the highest amount of incorporation of 15N from NPN sources in ileal digesta and ileal microbes (14) and plasma-free AAs (15). Valine also exhibits the highest ratio of 15N in plasma AAs to 15N in microbial AAs (15), indicating a high rate of absorption of microbially produced valine. These findings indicate that microbial production of valine and absorption of microbial valine are the highest among indispensable AAs and would therefore have the largest impact on whole-body nitrogen balance among indispensable AAs based on the AA profile of body-protein gain (22).

Prior to surgery, pigs were fed a standard corn and soybean meal-based grower pig diet. After surgery, pigs were fed increasing amounts of the experimental diet until they had returned to presurgical intake amounts. Thereafter, pigs were fed to 2.8 times maintenance digestible energy requirements per day (800 kJ/kg of BW0.60) according to Birkett and de Lange (23) and based on the estimated mid-period BW. It was confirmed that body protein deposition in the pigs was limited by valine, rather than energy, intake by estimating the expected body protein deposition rate based on dietary valine intake and based on the effect of metabolic energy intake on body protein deposition reported by Möhn et al. (17). The estimated protein deposition was below the maximum protein deposition for this group of pigs (17). Pigs were given their daily ration as a wet mash (water-feed of 3:1) in 3 equal meals per day at 0830, 1230, and 1630 h.

Surgery and infusions. Two weeks prior to the start of infusions, pigs in both the main nitrogen-balance study and isotope tracer study were fitted with a simple T-cannula in the cecum for infusion of saline, casein, or urea using the method for ileal cannulation as previously described (24,25) using a smaller cannula barrel (7.5-cm height × 2-cm external diameter) and with minor modifications. The cecum was visualized and incised between the cecal taenia. An additional plastic retaining ring (6.5-cm diameter) was placed at the base of the cannula and inserted into the cecum to provide additional support for the cannula. The cannula barrel was exteriorized through the body wall caudal to the ribs and as dorsal as possible. Pigs in the isotope tracer study were also implanted with catheters in the left and right external jugular veins to allow for infusion of 15N15N-urea and blood sampling (26).

In the main nitrogen-balance study, 3 pigs were assigned to each infusion treatment per period according to the protocol for a crossover design with 3 treatments and 3 consecutive periods (27). In the isotope tracer study, with only 1 experimental period, 3 pigs were assigned at random to each infusion treatment. Pigs were infused intracecally with a solution of saline (0.9% NaCl) or saline with sodium-caseinate (Dana Foods) or urea (Fisher Scientific). Solutions were infused at 1.4 mL/min using a peristaltic pump (Watson-Marlow 323S, 4-roller pump head; Watson-Marlow Pumps Group), and the concentration of the infusion solution was adjusted for each period to provide nitrogen from casein or urea equivalent to 40% of dietary nitrogen intake.

For determination of urea kinetics in the isotope tracer study, a 24-h infusion of saline solution (0.9% NaCl; Baxter Corp) was followed by a 4-d continuous i.v. infusion of a saline solution containing 16.1 mmol/L of 15N-urea (99% moles percent excess; ACP Chemicals). Solutions were infused during the 4-d collection period at a rate of 0.3 mL/min using a peristaltic pump (Masterflex L/S, model #7523-60, 8-roller pump head; Cole-Palmer Instruments) to provide mmol · kg BW–1 · d–1 of the labeled urea (26).

Sample collection, processing, and analysis. Each experimental period consisted of a 5-d adaptation period and 2 consecutive 2-d collection periods. In the main nitrogen-balance study, a 2-d rest period occurred between experimental periods. Feeces from each 2-d collection period were pooled per pig and stored at −20°C until further processing. Samples of feces were prepared for analysis by freeze drying (Virtis Model 100; SP Scientific) and were then ground to a fine powder using a mortar and pestle. Urine was collected quantitatively by funneling urine into the cecum for infusion of 15N15N-urea and blood sampling (26).

Dry matter (DM) and organic matter (OM) content of feces was determined as previously described (28). Titanium dioxide was determined according to Myers et al. (29). The AA content of the experimental diet was determined by Evonik Industries according to Llamas and Fontaine (30) and the Commission Directive (31,32). Total nitrogen content was determined in the experimental diet, feces, and

Large intestinal nitrogen supply and balance
Calculations. Apparent absorption of nitrogen from the large intestine was calculated based on mass balance (ileal nitrogen flow + cecal nitrogen infusion – fecal nitrogen flow), with ileal flow based on ileal digestibility values determined previously with identical diet and treatments (35). The percentage of nitrogen absorbed was determined relative to total nitrogen supply (ileal nitrogen flow + cecal nitrogen infusion), and a proportion of infused nitrogen via the increase in nitrogen absorption was determined for casein or urea infusion relative to the saline treatment (i.e., subtracting basal nitrogen absorption). Body protein deposition was estimated based on whole-body nitrogen balance and multiplying nitrogen retention by 6.25. The amount of nitrogen absorbed from the large intestine that could be accounted for by the increase in urinary urea-nitrogen or urinary total nitrogen excretion was determined as the increase in large intestinal nitrogen absorption with nitrogen infusion relative to the saline divided by the increase in urinary urea-nitrogen or urinary total nitrogen excretion with nitrogen infusion relative to the saline.

The efficiency of utilizing ileal-digestible nitrogen or valine intake for body protein deposition was estimated as nitrogen or valine retained in whole-body protein as a percentage of digestible nitrogen or valine intake. The concentration of valine in body protein deposition was assumed to be 4.7% (22). The efficiency of utilizing total nitrogen supply for whole-body protein deposition was estimated as for digestible nitrogen intake with the addition of cecal nitrogen infusion. The marginal efficiency of utilizing nitrogen absorbed from the large intestine for whole-body protein deposition was estimated as the increase in nitrogen retention with infusion of nitrogen relative to the saline treatment divided by the increase in large intestinal nitrogen disappearance with infusion of nitrogen relative to the saline treatment. Ileal digestibility of nitrogen and valine was determined in a previous study using the same diet and treatments; that study showed that the infusion of casein or urea into the large intestine did not influence ileal digestibility of AAAs and nitrogen (35).

Urea flux was determined according to the equation of Matthews and Downey (36) for simple isotope dilution. Urea recycling was calculated as the difference between urea flux and urinary urea excretion (12). The amount of nitrogen absorbed from the large intestine that could be accounted for by the increase in urea flux was determined as the increase in large intestinal nitrogen disappearance with the infusion of nitrogen relative to the saline treatment divided by the increase in urea flux with the infusion of nitrogen relative to the saline treatment.

Statistical analysis. All data were analyzed using the mixed model procedure (PROC MIXED) of the SAS statistical program (SAS 9.1; SAS Institute). The effect of previous treatment was not significant (P > 0.05) and therefore nitrogen-balance data from both the main nitrogen-balance study and the isotope tracer study were analyzed together. For analyses of nitrogen-balance data, pig (n = 18, minus excluded pig) was considered a random effect; treatment (n = 3), period (n = 3), and block (n = 2; main nitrogen-balance study and isotope tracer study) were considered fixed effects. For the nitrogen-balance observations obtained in the tracer study, pig and period effects were not estimated. For the urea kinetics data from the isotope tracer study, only treatment was considered as a fixed effect. Differences between least square means were determined using Tukey’s test and were considered significantly different at P ≤ 0.05.

Results

All pigs fully recovered from surgery and had achieved presurgical feed intake amounts within 3 d of surgery. During the main nitrogen-balance study, some of the cannulas failed and 3 pigs were removed from the study, 1 at the start of each period, resulting in a total of 6 missing observations. One pig in the isotope tracer study developed diarrhea and was removed from the study, resulting in 1 additional missing observation for nitrogen balance and urea kinetics observations. Based on determined nutrient contents, the experimental diet was prepared accurately (Supplemental Table 1). The amount of nitrogen infused was as anticipated and did not differ between the casein and urea infusion (P > 0.05). Body weight as well as intake of DM and nitrogen were consistent across treatments (Table 1; P > 0.05). The mid-period mean BW for pigs used in the main nitrogen-balance study in periods 1, 2, and 3 was 29.9 ± 0.7 kg, 37.2 ± 0.8 kg, and 44.4 ± 0.8 kg, respectively, whereas it was 30.4 ± 0.7 kg for pigs used in the isotope tracer study.

Apparent fecal digestibility of DM and OM was not different across treatments (Table 1; P > 0.05), but there was an increase (P ≤ 0.05) in the fecal excretion of DM and OM with the infusion of casein compared with saline infusion. The infusion of nitrogen, regardless of the source, resulted in a decrease in the apparent fecal digestibility of nitrogen with subsequent increase in fecal excretion of nitrogen compared with the saline treatment (P < 0.001), with casein-infused pigs having the lowest digestibility and highest excretion among all treatments. The disappearance of DM and OM from the large intestine was increased with the infusion of casein (P < 0.01), but not urea (P > 0.05), when compared with saline-infused pigs. Absorption of nitrogen from the large intestine was higher in both the casein- and urea-infused pigs compared with pigs on the saline treatment (P < 0.001) and did not differ between the casein and urea infusions (P > 0.05). Urinary excretion of urea (P ≤ 0.05) and nitrogen (P < 0.001) was increased with the infusion of casein and urea into the large intestine, and this increase did not differ between the casein and urea infusions (P > 0.05). Neither the increase in urea or nitrogen excretion in urine completely accounted for the increment of nitrogen absorbed from the large intestine with cecal infusions of nitrogen.

The infusion of both casein and urea into the large intestine resulted in an increase (Table 1; P < 0.01) in calculated body protein deposition (nitrogen retention × 6.25) compared with saline-infused pigs. The efficiency of ileal-digestible nitrogen utilization for body protein deposition was improved (P < 0.001) in pigs receiving either casein or urea. The efficiency of using total nitrogen, including large intestinal nitrogen supply, for body protein deposition was decreased (P < 0.001) in both nitrogen infusion treatments compared with the saline treatment. The mean utilization of absorbed nitrogen from the large intestine was 18% and did not differ between casein and urea (P > 0.05). Infusion of nitrogen into the large intestine resulted in an increase in the utilization of ileal-digestible valine intake for body protein deposition (P < 0.01). Infusion of casein and urea yielded similar efficiencies of using nitrogen or valine intake for body protein deposition (P > 0.05).

Isotopic steady state was confirmed by comparing urea enrichment in either plasma or urine from day 3 and day 4 of the labeled urea infusion, which was not different between days.
TABLE 1  Nutrient intake and nitrogen and valine utilization in pigs fed a valine-limiting diet and infused with saline, casein, or urea into the cecum1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline (n = 9)</th>
<th>Casein (n = 10)</th>
<th>Urea (n = 10)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-period BW, kg</td>
<td>37.1 ± 0.8</td>
<td>37.3 ± 0.8</td>
<td>37.4 ± 0.8</td>
<td>0.63</td>
</tr>
<tr>
<td>DM intake, kg/d</td>
<td>1.18 ± 0.02</td>
<td>1.18 ± 0.02</td>
<td>1.16 ± 0.02</td>
<td>0.54</td>
</tr>
<tr>
<td>Dietary nitrogen intake, g/d</td>
<td>30.8 ± 0.7</td>
<td>30.6 ± 0.7</td>
<td>30.8 ± 0.7</td>
<td>0.46</td>
</tr>
<tr>
<td>N infused, g/d</td>
<td>0.00b</td>
<td>11.5 ± 0.3a</td>
<td>11.1 ± 0.3a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N infused, % of N intake</td>
<td>0.00b</td>
<td>38.5 ± 1.3a</td>
<td>36.6 ± 1.3a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apparent fecal digestibility, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>90.6 ± 0.8</td>
<td>88.6 ± 0.6</td>
<td>90.0 ± 0.7</td>
<td>0.12</td>
</tr>
<tr>
<td>OM</td>
<td>92.0 ± 0.7</td>
<td>90.2 ± 0.7</td>
<td>91.4 ± 0.6</td>
<td>0.12</td>
</tr>
<tr>
<td>N</td>
<td>86.2 ± 1.1a</td>
<td>79.8 ± 1.0a</td>
<td>83.4 ± 1.0b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fecal excretion, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>104 ± 7.3b</td>
<td>128 ± 6.9a</td>
<td>112 ± 6.9b</td>
<td>0.05</td>
</tr>
<tr>
<td>OM</td>
<td>90.9 ± 7.3b</td>
<td>114 ± 6.6a</td>
<td>101 ± 6.5b</td>
<td>0.06</td>
</tr>
<tr>
<td>N</td>
<td>4.11 ± 0.29c</td>
<td>6.07 ± 0.26a</td>
<td>5.04 ± 0.26c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Large intestinal disappearance or absorption2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, g/d</td>
<td>91.6 ± 8.5b</td>
<td>138 ± 7.8a</td>
<td>103 ± 7.5b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OM, g/d</td>
<td>90.8 ± 8.1b</td>
<td>134 ± 7.2a</td>
<td>101 ± 7.2b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>N, g/d</td>
<td>0.95 ± 0.43a</td>
<td>10.6 ± 0.43a</td>
<td>11.5 ± 0.43c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N, % of N supply</td>
<td>−4.00 ± 5.47b</td>
<td>63.0 ± 4.91b</td>
<td>68.7 ± 4.90b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N, % of N infused</td>
<td>—</td>
<td>91.3 ± 2.9b</td>
<td>103 ± 2.9b</td>
<td>0.026</td>
</tr>
<tr>
<td>Urinary excretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea-N, g/d</td>
<td>4.65 ± 0.79b</td>
<td>10.1 ± 0.68a</td>
<td>11.0 ± 0.70a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N, g/d</td>
<td>3.73 ± 0.57b</td>
<td>14.2 ± 0.52a</td>
<td>15.3 ± 0.53a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urea-N, % large intestinal N absorption</td>
<td>—</td>
<td>54.3 ± 6.1</td>
<td>61.7 ± 6.2</td>
<td>0.36</td>
</tr>
<tr>
<td>N, % large intestinal N absorption</td>
<td></td>
<td>68.3 ± 5.0</td>
<td>73.3 ± 5.1</td>
<td>0.45</td>
</tr>
<tr>
<td>Body protein deposition, g/d</td>
<td>114 ± 3.4b</td>
<td>128 ± 3.1a</td>
<td>130 ± 3.1a</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Knu, % of N intake</td>
<td>71.0 ± 1.7b</td>
<td>80.7 ± 1.5a</td>
<td>80.3 ± 1.5b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Knu, % total N supply</td>
<td>72.4 ± 1.4a</td>
<td>56.3 ± 1.2a</td>
<td>57.9 ± 1.2a</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Knu, % of large intestinal absorption</td>
<td>—</td>
<td>18.7 ± 5.5</td>
<td>16.5 ± 5.5</td>
<td>0.74</td>
</tr>
<tr>
<td>Kvaline, % of valine intake</td>
<td>90.1 ± 2.2b</td>
<td>99.7 ± 2.0a</td>
<td>103 ± 2.0e</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1 Values are least square means ± SEs. Means within a row without a common letter differ, P ≤ 0.05. BW, body weight; DM, dry matter; Knu, efficiency of utilizing ileal-digestible N; Kvaline, efficiency of utilizing ileal-digestible valine; N, nitrogen; OM, organic matter.
2 Main effect of treatment.
3 Calculated as: ileal flow + cecal infusion − fecal flow. Ileal flow based on ileal digestibility values determined previously (35).
4 Calculated as: (lower gut N absorption)/ileal N flow + N infused.
5 Calculated as: (lower gut N absorption for casein or urea treatment − lower gut N absorption for saline treatment)/N infused.
6 Calculated as: (increase in urinary urea-N or N excretion relative to saline treatment)/increase in lower gut N absorption relative to saline treatment.
7 Calculated as: (N intake + N infusion − fecal N excretion − urine N excretion) × 6.25.
8 Calculated as: (N retained in body protein)/ileal-digestible N intake. Ileal-digestible N intake determined previously (35).
9 Calculated as: (N retained in body protein)/ileal-digestible N intake + large intestinal N absorption. Ileal-digestible N intake determined previously (35).
10 Calculated as: (increase in N retention relative to saline treatment)/increase in large intestinal N relative to saline treatment.
11 Calculated as: (protein deposition × valine % of body protein)/ileal-digestible valine intake. Ileal-digestible N intake determined previously (35). Valine content of body protein assumed to be 4.70% (22).

(data not shown; P > 0.05). It has also been shown previously that the infusion protocol in this study results in an isotopic steady state within 2 d of the start of infusion (26). Urea flux increased (Table 2; P ≤ 0.05) with the infusion of nitrogen into the large intestine, and this increase was not different between casein and urea (P > 0.05). When calculated using urinary enrichment, the amount of urea recycled increased (P ≤ 0.05) when infusing urea into the cecum, but did not change (P > 0.05) when infusing casein, compared with saline infusion. The increase in urea flux accounted for nearly all of the nitrogen absorbed in the large intestine of pigs infused with casein and urea. Urea recycling estimated with urine enrichment increased (P ≤ 0.05) with the urea infusion. The proportion of urea flux that was recycled to the gut was not affected (P > 0.05) by cecal nitrogen infusions.

**Discussion**

Because of evidence showing little or no benefit of infusing either individual AAAs or protein-bound AAAs into the large intestine (1), it is generally thought that AA and nitrogen absorption from the large intestine is of little nutritional value to nonruminant animals and humans. This is in contrast, however, to evidence of the presence AA transporters and AA uptake in colonocytes (4–6), which would suggest that AAAs can be absorbed from the large intestine and used by the animal. The objectives of this study were to determine the form in which nitrogen is absorbed from the large intestine and to determine the impact of a large intestinal nitrogen supply, as either protein or NPN, on urea kinetics and nitrogen balance in growing pigs fed a diet limiting in an indispensable AA. To achieve these objectives, 2 infusion
and nitrogen-balance studies were performed using a continuous intracecal infusion of saline, casein, or urea in pigs fed a diet first-limiting in valine. In addition, in the second study, a continuous i.v. infusion of isotopic-labeled urea was performed to evaluate urea kinetics.

In the present study, the majority of the cecally infused nitrogen was absorbed, regardless of the source of nitrogen being infused. Although this could be interpreted as an indication that the large intestine is capable of absorption of both intact AAs and NPN, it is more likely that nitrogen was absorbed as NPN with both infusion treatments. Evidence for this conclusion is provided by the fact that the increase in urinary nitrogen and urea excretion, urea flux, and protein deposition was the same for the casein- and urea-infused pigs. In addition, the increase in urea flux observed with both treatments accounted for nearly all of the nitrogen absorbed from the large intestine.

Increasing the supply of nitrogen via the large intestine resulted in an increase in protein deposition and in the estimated efficiency of utilizing ileal-digestible nitrogen and valine for whole-body protein deposition. In general, infusing nitrogen or AAs directly into the large intestine (1,3) or providing supplemental NPN in swine diets (37–40) has been shown to have little effect on whole-body nitrogen retention in pigs. Previous studies have demonstrated very little effect of large intestinal protein or AA infusion on fecal digestibility but a significant increase in urinary nitrogen excretion, indicating that although nitrogen is absorbed from the large intestine, it is not used by the pig for body protein synthesis. In contrast, the results of the current study show that nitrogen absorption from the large intestine can contribute to body protein synthesis. This is in agreement with Gargallo and Zimmerman (7) who demonstrated an increase in nitrogen retention in pigs infused with casein at the terminal ileum. In the review by Fuller and Reeds (1), although nitrogen balance was not significantly improved, there was almost always a numerically positive response in nitrogen balance to nitrogen infusion into the large intestine, indicating that with proper experimental methods—including an AA-limiting experimental diet—and precision the utilization of large intestinal nitrogen may have been significant.

The lack of consistent results in studies examining the utilization of nitrogen absorbed from the large intestine for supporting growth and nitrogen retention in pigs is likely due to the low efficiency of utilization and diet composition. The results of the current study indicate that nitrogen absorbed from the large intestine is not used as efficiently for body protein deposition (e.g., 20% in the current study) as nitrogen that is absorbed from the small intestine, largely in the form of AAs (e.g., ~75% (18)).

The ability of nonruminants to correct an indispensable AA deficiency by using NPN appears limited and is likely dependent on the AA that is deficient in the diet. As stated previously, based on estimates of the incorporation of NPN into microbial AAs and the microbial valine (15) and body protein valine content (22), microbial valine has the largest potential impact on body protein deposition. Because pigs are incapable of endogenous production of indispensable AAs such as valine, it can be assumed that the observed improvement in nitrogen balance in the current study, in which pigs were fed a valine-limiting diet, is the result of intestinal microbial valine production. This same degree of improvement may not be achieved with other indispensable AAs, such as lysine, where the rate of microbial production and supply to the host may not be sufficient (13–15) to overcome a dietary deficiency.

It is important to note that the increase in urinary urea and nitrogen excretion observed with both nitrogen infusions relative to the saline treatment did not fully account for the amount of nitrogen absorbed from the large intestine, with the difference between absorption and excretion contributing to nitrogen retention. This demonstrates that nitrogen absorbed from the large intestine, regardless of the source and form, can contribute to whole-body protein deposition. The ability to

### TABLE 2  Nitrogen utilization, urea enrichment, and urea kinetics, based on either urine or plasma enrichment, in pigs fed a valine-limiting diet and infused with saline, casein, or urea into the cecum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline (n = 3)</th>
<th>Casein (n = 3)</th>
<th>Urea (n = 2)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma urea-N, mg/d</td>
<td>33.9 ± 5.0</td>
<td>79.3 ± 5.0</td>
<td>100 ± 6.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>¹¹⁵N¹⁵N urea, MPE</td>
<td>6.29 ± 1.33</td>
<td>1.99 ± 1.33</td>
<td>1.67 ± 1.63</td>
<td>0.11</td>
</tr>
<tr>
<td>Urea-N flux, mg/(kg-d)</td>
<td>170 ± 37.8</td>
<td>476 ± 37.8</td>
<td>553 ± 46.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urea-N recycling, mg/(kg-d)</td>
<td>51.8 ± 28.6</td>
<td>147 ± 28.6</td>
<td>226 ± 35.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Urea recycling, % of urea flux</td>
<td>27.7 ± 6.8</td>
<td>30.6 ± 6.8</td>
<td>42.4 ± 8.4</td>
<td>0.44</td>
</tr>
<tr>
<td>Lower gut N absorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea flux increase (urine), %</td>
<td>—</td>
<td>101 ± 6.9</td>
<td>78.9 ± 8.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Urea flux increase (plasma), %</td>
<td>—</td>
<td>94.6 ± 12.2</td>
<td>96.7 ± 15.0</td>
<td>0.92</td>
</tr>
</tbody>
</table>

1 Values are least square means ± SEs. Means within a row without a common letter differ, P ≤ 0.05. MPE, moles percent excess; N, nitrogen.
2 Main effect of treatment.
3 Calculated as: (tracer infusion rate) × (MPE infusate/MPE sample − 1) according to Matthews and Downey (36).
4 Calculated as: urea flux − urinary urea excretion.
5 Calculated as: (N absorption from the large intestine for casein or urea treatment − lower gut N absorption for saline treatment)/(urea flux on casein for casein treatment − urea flux for saline treatment).
retain nitrogen absorbed from the large intestine has been demonstrated previously in infant and adult humans (8,41,42). In these studies, AA absorption and retention was determined by the difference between the amount of isotope infused, as either urea or protein, and the amount recovered in both urine and feces. Heine et al. (8) interpreted the results as demonstrating the ability of the large intestine to absorb AAs, however, because AA enrichment in the whole body or plasma was not determined, this conclusion was not supported by direct observation. The absorption of NPN from the large intestine was confirmed by Moran and Jackson (41,42) with more than half of the labeled urea dose being retained. This is in agreement with Patterson et al. (43) and Stein et al. (44) who found that >50% of orally administered ammonium chloride was retained in the form of AAs, including, but to a lesser extent, indispensable AAs (43). These studies and the results reported herein provide evidence that NPN supply, including nitrogen absorption from the large intestine, can be used and retained by nonruminant animals and humans.

It has been shown that nitrogen retention is overestimated when determined by nitrogen balance (17,45,46). This difference can be attributed to overestimation of intake and underestimation of excretion from incomplete collection of nitrogen in feces and urine (e.g., gaseous loss of nitrogen as ammonia from urine and feces). Therefore, some care should be taken with the interpretation of absolute responses and the improvement in nitrogen and valve retention and utilization efficiency reported in the current study, which should be confirmed via serial slaughter and growth performance studies. However, urea kinetics, including treatment effects on urinary urea excretion, observed in the current study support urea recycling into the gastrointestinal tract and the use of recycled urea-nitrogen for microbial synthesis of indispensable AAs.

Both casein- and urea-infused pigs showed the same degree of increase in urea flux. In addition, the increase in urea flux on both treatments completely accounted for the additional nitrogen absorbed from the large intestine. The complete conversion of absorbed nitrogen from the large intestine into urea observed in the current study is in agreement with previous findings in isolated sheep hepatocytes (47). Combined with urea balance and similar increments in nitrogen retention between the 2 nitrogen sources, these results lend confidence to the conclusion that nitrogen absorbed from the large intestine is absorbed as NPN, regardless of the source of nitrogen, and that this nitrogen can be used for protein synthesis by the host. Because of between-animal variability and the small number of pigs per treatment, despite the fact that urea recycling was almost tripled by cecal nitrogen infusions, significant differences in urea recycling were only observed in urea-infused pigs when urinary enrichment and excretion values were used. However, the increment in urea recycling was sufficient to supply approximately twice the nitrogen needed to cover the increment in nitrogen deposition assumed to have been provided by increased incorporation of nitrogen into microbial protein in the small intestine.

In summary, cecally infused nitrogen appears to be absorbed in the form of NPN, which improves whole-body nitrogen balance in pigs fed a valine-limiting diet. Nitrogen absorbed from the large intestine is likely salvaged via urea recycling and microbial AA synthesis in the small intestine. Large intestinal nitrogen metabolism should be considered when determining nitrogen and AA supply and requirements. Further studies on the metabolism of nitrogen in the large intestine and dietary supplementation with NPN in nonruminant animals and humans fed diets limiting in indispensable AAs or total nitrogen are warranted.

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Literature Cited

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