The effect of microbial phytase on true and apparent ileal amino acid digestibilities in growing-finishing pigs\textsuperscript{1,2}

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ABSTRACT: Ten 56-d-old, 15-kg barrows were surgically fitted with a postvalvular T-cecum cannula at the ileo-cecal junction to evaluate the effect of microbial phytase on apparent and true ileal AA digestibility and N utilization. A semipurified cornstarch- and soybean meal-based diet was formulated to contain 3.4 Mcal of DE/kg, 17.0% CP, 0.8% Ca, and 0.6% P but had a low phytate-P concentration (0.13%; all on an as-fed basis). Chromic oxide and dysprosium chloride were used as indigestible markers. The basal diet was supplemented with 0 or 1,000 phytase units/kg of microbial phytase. Postprandial plasma urea N and \(\alpha\)-amino N concentrations, excretion of Ca, P, and N in feces and urine, and ileal AA digestibilities were determined 3 times at 4-wk intervals beginning at 70 d of age. The homoarginine (HA) method was used to determine endogenous AA flow by replacing 50% of the basal protein with guanidinated protein. Microbial phytase had no effect on apparent ileal digestibility (AID) or on true ileal digestibilities of N and most AA but did increase AID for arginine \((P = 0.006)\) and methionine \((P = 0.037)\). However, in HA diets, phytase increased the AID of CP \((P = 0.01)\) and several AA. Addition of microbial phytase had no effect on the postprandial \(\alpha\)-amino N concentrations in plasma but increased overall plasma urea N concentrations \((P = 0.035)\). Barrows fed phytase-supplemented diets had decreased P in feces \((P = 0.003)\) and greater P in urine \((P = 0.001)\) but comparable total P excretion compared with barrows fed no phytase-supplemented diets. In conclusion, the addition of phytase to a semipurified soybean meal-based diet did not affect the AID of several AA. In addition, differences between the basal and HA diets in N digestibilities indicated that that guanidination may limit the use of the HA method in determining endogenous protein losses.

Key words: amino acid, apparent ileal digestibility, homoarginine, phytase, pig, true ileal digestibility

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INTRODUCTION

Most of the P in cereal grains and products from oilseeds is in the form of phytate-P, which has low bio-

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availability for pigs and poultry (Kornegay, 2001). Phytases may also be implicated in the inhibition of trypsin, tyrosinase, and pepsin (Nair et al., 1991; Caldwell, 1992). Microbial phytases are used to hydrolyze phytate complexes in nonruminant animals, liberating P and essential minerals (Jongbloed et al., 1992; Mroz et al., 1994). Although phytase can clearly increase P availability, its effect on protein digestibility is less clear and often controversial (Mroz, 2002; Adeola and Sands, 2003). In pigs, the addition of microbial phytase improved apparent ileal protein digestibility in some studies (Officer and Batterham, 1992; Mroz et al., 1994; Li et al., 1998; Kemme et al., 1999; Liao et al., 2002; Mroz, 2002), whereas it had no effect in others (Bruce and Sundstol, 1995; Lantsch et al., 1995; Valaja et al., 1998; Traylor et al., 2001).

Phytase-mediated effects on protein utilization, if any, are of low magnitude (Kornegay, 2001), and its effect can be influenced by age (Noblet and van Milgen,
In poultry, the addition of phytases seems to improve protein availability (Cowieson et al., 2006b) by reducing the excretion of endogenous AA (Cowieson et al., 2004, 2006a). Apparent ileal digestibility (AID) does not distinguish between exogenous and endogenous protein sources. Therefore, to assess the possible effect of microbial phytase on protein utilization in pigs, the true AA digestibilities and the endogenous losses of protein should be evaluated.

The homoarginine (HA) method is used to determine true ileal AA digestibilities (Hagemeyer and Erbersdorfer, 1985; Marty et al., 1994; Nyachoti et al., 2002; Stein et al., 2007). In this technique, dietary lysine is converted to HA, which is an analog of lysine (Hagemeyer and Erbersdorfer, 1985; Moughan and Rutherford, 1990; Nyachoti et al., 2002), in a process referred to as guanidination. Because less than 0.2% of absorbed guanidinated protein will reappear in the digestive tract (Schmitz et al., 1991), HA concentration in chyme can be used to distinguish between endogenous and exogenous lysine entering the distal ileum. This assumes that guanidination does not modify AA composition and digestibilities, which has not been studied extensively in swine. The objectives of this study were to evaluate the effect of microbial phytase on true and apparent ileal AA and N digestibilities, postprandial protein absorption and utilization, and the effect of guanidination on AA composition and digestibilities at different pig ages.

**MATERIALS AND METHODS**

**Animals and Housing**

Animals were cared for according to a recommended code of practice (Agriculture and Agri-Food Canada, 1993) and the guidelines of the Canadian Council on Animal Care (1993).

Ten crossbred (Canabrid × C15, PIC International Inc., Airdrie, Alberta, Canada) barrows of approximately 15 kg of BW were surgically fitted with silicone rubber, postvalvular, T-cecum cannulas at the ileocecal junction, according to the method of van Leeuwen et al. (1991). The barrows were anaesthetized by i.m. injections of atropine sulfate, xylazine (Rompun, Bayer Canada Inc., Toronto, Ontario, Canada), and ketamine (Rogarsetic, Pfizer Canada Inc., London, Ontario, Canada). Halothane (MTC Pharmaceuticals, Cambridge, Ontario, Canada) was used at the maximal dose of 2% to maintain anesthesia. After surgery, the barrows received a commercial 19% CP starter diet. After a recovery period of at least 12 d, the 10 barrows were randomly assigned to 1 of the 2 treatments: the basal diet with or without 1,000 phytase units (FTU)/kg (as-fed basis; Engelen et al., 1994) from microbial phytase (5,000 FTU/g; Natuphos, BASF Canada Inc., Toronto, Ontario, Canada; Table 1). Basal diets, diets with chromic oxide, and diets with guanidinated protein were offered sequentially during the first 21 d of each collection period, as described later. During these 21 d, feed intake was adjusted for each pig and collection period at 2.5 times the maintenance energy requirements (2.5 × 114 kcal of DE/kg of BW0.75), which covered between 65 and 85% of the ad libitum feed intake (NRC, 1998).

During each 21-d period, the barrows ate the same daily amount of feed, 50% of which was offered at 0800 h and the remaining 50% at 1600 h. The barrows were fed ad libitum with a commercial diet, with or without microbial phytase according to treatment, for the rest of the collection period between d 21 and 28. Commercial diets were used to avoid having the barrows consume semi-purified diets for long periods of time. The barrows were kept in stainless steel metabolism cages, and they had free access to water for the whole trial.

**Diet Preparation**

Diets were formulated to contain soybean meal as the sole source of protein. The soybean meal protein was guanidinated as described by Schmitz et al. (1991), Marty et al. (1994), and Nyachoti et al. (2002). Briefly, material calculated to contain 200 g of CP was thoroughly mixed with 1 L of a methylisoureacra (MIU) solution and adjusted to pH 10.5 by using 1-M NaOH. The mixture was incubated in a refrigerator at 4°C for 4 d. The MIU solution was prepared by reacting O-methylisourea (Pfalzer and Bayer, Connecticut, OH) with barium hydroxide (Sigma Chemical, St. Louis, MO), followed by centrifugation at 4,000 × g to remove the precipitated barium sulfate. During incubation, the pH of the mixture was monitored twice daily and adjusted accordingly, with the material being thoroughly stirred to ensure uniform conditions. At the end of incubation, the guanidination reaction was stopped by lowering the pH to the isoelectric point of the soybean meal (pH 4.5) with 1-M HCl. Samples were then centrifuged at 4,000 × g and 4°C to recover the guanidinated protein. Nonreacted MIU was removed by resuspending the precipitate after adjusting it to pH 4.5 in water and centrifuging it again. The latter was repeated 3 times, each time discarding the supernatant. The clean material was freeze-dried before it was used in diet preparations. The efficiency of conversion of lysine in soybean meal to HA was 69.5%.

Cornstarch was the main source of energy, whereas dextrose and cellulose were included to improve palatability and to increase dietary fiber, respectively. Fat was added to obtain the desired energy concentration. All diets were formulated to contain approximately 17% CP and 3.4 Mcal of DE/kg (Tables 1 and 2). Chromic oxide (0.5%) and dysprosium chloride (100 mg/kg) were used as markers for apparent and true digestibility determinations, respectively (Marty et al., 1994; Nyachoti et al., 2002). Fifty percent of the protein provided by the untreated soybean meal was replaced by guanidinated protein in the HA diets (Table 1). The composition of the treated and the untreated soybean meal is presented in Table 2.
Table 1. Composition of diets, as-fed basis

<table>
<thead>
<tr>
<th>Ingredient, %</th>
<th>Basal Control</th>
<th>Phytase Control</th>
<th>Basal HA</th>
<th>Phytase HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>45.25</td>
<td>45.24</td>
<td>47.49</td>
<td>47.60</td>
</tr>
<tr>
<td>Cellulose</td>
<td>3.61</td>
<td>3.61</td>
<td>3.84</td>
<td>3.84</td>
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<tr>
<td>Soybean meal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated, 47.2% CP</td>
<td>35.45</td>
<td>35.45</td>
<td>18.82</td>
<td>18.86</td>
</tr>
<tr>
<td>Guanidinated, 70.0% CP</td>
<td>—</td>
<td>—</td>
<td>12.69</td>
<td>12.45</td>
</tr>
<tr>
<td>Canola oil</td>
<td>2.00</td>
<td>2.00</td>
<td>2.12</td>
<td>2.13</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
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<td>1.72</td>
<td>1.83</td>
<td>1.83</td>
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<tr>
<td>Limestone</td>
<td>1.17</td>
<td>1.17</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Chronic oxide</td>
<td>—</td>
<td>—</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>Dysprosium chloride</td>
<td>—</td>
<td>—</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Salt</td>
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<td>0.20</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Dextrose</td>
<td>10.00</td>
<td>10.00</td>
<td>10.62</td>
<td>10.64</td>
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<tr>
<td>Vitamin premix</td>
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<td>0.50</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>0.10</td>
<td>0.10</td>
<td>0.11</td>
<td>0.11</td>
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<tr>
<td>Phytase</td>
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<td>0.02</td>
<td></td>
<td></td>
</tr>
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</table>

Analyzed composition

<table>
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<tr>
<th>Item</th>
<th>Basal</th>
<th>Phytase</th>
<th>Basal</th>
<th>Phytase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>17.0</td>
<td>17.0</td>
<td>18.1</td>
<td>17.9</td>
</tr>
<tr>
<td>DM, %</td>
<td>90.5</td>
<td>90.5</td>
<td>91.8</td>
<td>91.7</td>
</tr>
<tr>
<td>Phytase activity, phytase units/kg</td>
<td>&lt;100</td>
<td>1,200</td>
<td>160</td>
<td>1,340</td>
</tr>
<tr>
<td>Phytate-P, %</td>
<td>0.16</td>
<td>0.13</td>
<td>0.11</td>
<td>0.13</td>
</tr>
</tbody>
</table>

1Formulated to contain 3.4 Mcal of DE/kg, 0.8% Ca, and 0.6% P, and to have soybean meal as the sole source of protein.
2Chromic oxide (0.50%) and dysprosium chloride (0.01%) were added at the expense of cornstarch.
3Formulated on a DM basis to contain 50% of the CP provided by guanidinated protein.
4Formulated to contain 1,000 phytase units from microbial phytase/kg.
5Provided per kg of diet: vitamin A as retinyl palmitate and acetate, 8,000 IU; vitamin D3 as cholecalciferol, 1,500 IU; vitamin E as α-tocopherol, 34 IU; vitamin K as menadione, 2.2 mg; thiamine as thiamine monohydrate, 2 mg; riboflavin, 6 mg; niacin, 30 mg; pantothenic acid as Ca-pantothenate, 16 mg; folie acid, 10 mg; pyridoxine as pyridoxine hydrochloride, 4 mg; biotin, 300 μg; cyanocobalamin, 40 μg; and choline as choline chloride, 400 mg.
6Provided per kg of diet: Mn as manganous oxide, 30 mg; Zn as zinc oxide, 100 mg; Fe as ferrous sulfate, 100 mg; Cu as copper sulfate, 25 mg; I as calcium iodate, 300 μg; and Se as selenite, 300 μg.

Table 2. Analyzed protein and AA composition of the untreated soybean meal and of the guanidinated soybean meal

<table>
<thead>
<tr>
<th>Item</th>
<th>Untreated</th>
<th>Guanidinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>52.98</td>
<td>71.43</td>
</tr>
</tbody>
</table>

Indispensable

<table>
<thead>
<tr>
<th>AA</th>
<th>Untreated</th>
<th>Guanidinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>8.49</td>
<td>8.55</td>
</tr>
<tr>
<td>Cystine</td>
<td>1.29</td>
<td>1.35</td>
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<tr>
<td>Histidine</td>
<td>1.94</td>
<td>1.69</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.20</td>
<td>2.59</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.64</td>
<td>1.37</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.38</td>
<td>6.25</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.79</td>
<td>1.91</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.25</td>
<td>4.69</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.19</td>
<td>4.02</td>
</tr>
<tr>
<td>Valine</td>
<td>3.20</td>
<td>2.79</td>
</tr>
</tbody>
</table>

Dispensable

<table>
<thead>
<tr>
<th>AA</th>
<th>Untreated</th>
<th>Guanidinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>6.41</td>
<td>4.88</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>11.73</td>
<td>12.89</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>20.56</td>
<td>19.95</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.80</td>
<td>5.18</td>
</tr>
<tr>
<td>Serine</td>
<td>6.86</td>
<td>6.61</td>
</tr>
</tbody>
</table>

1DM basis.

General Conduct of Study

Blood, total feces, urine, and ileal digesta samples were collected 3 times at 4-wk intervals beginning at 66 d of age (d 1). Average BW at the beginning of each collection period was 24.0 ± 1.02, 41.7 ± 1.51, and 61.0 ± 2.83 kg, respectively. During the first week of each collection period, semipermanent catheters were nonsurgically installed in the jugular vein according to the technique of Matte (1997). The barrows were not fed in the afternoon of d 5. On d 6, after a 16-h fast, the barrows were offered a 1-kg meal, and blood samples were collected from the semipermanent catheters at 0, 30, 60, 90, 120, 150, 180, 240, 300, and 360 min after feeding. Blood samples were collected directly into Vacutainer tubes containing 100 USP of sodium heparin as an anticoagulant (Becton, Dickinson and Company, Franklin Lakes, NJ) and placed on ice until processed. Within 20 min of collection, blood samples were centrifuged at 1,800 × g for 10 min and the supernatant was collected and frozen until subsequent analysis. Total urine and feces were collected from d 10 to 13. Feces were collected twice daily after each meal and immediately stored at −20°C. Urine samples were collected after the morning and afternoon meals and stored in
refrigerated containers, to which 1 M HCl was added to keep the pH below 3. Urine samples were filtered and frozen after the 4-d collection period.

The barrows received the experimental diets supplemented with 0.5% chromium oxide from d 14 to 20. Total ileal digesta was collected on d 17 and 19 for 8 h after the morning meal. Digesta was collected in sterile polyethylene bags attached to the external part of the cannula. Bags were removed every 30 min and were immediately frozen. All of the digesta collected was thawed, pooled on a per-animal basis, and mixed. On d 21, a single meal containing HA was given at 0800 h and digesta samples were collected as indicated before.

At the end of each collection period, feces, urine, and digesta were pooled to form 1 sample of each material for each pig. At the end of the experiment, the barrows were slaughtered for postmortem examinations.

**Analytical Procedures**

Feces and digesta were freeze-dried and ground to pass through a 1-mm screen. Urinary and fecal Ca, P, and N were determined according to the method described by Barnett (1994). Dry matter was determined by freeze-drying and corrected by oven drying to a constant weight at 100°C. Freeze-dried samples were analyzed for Ca by atomic absorption spectrophotometry and P by colorimetric methods using standard procedures (AOAC, 1990) after ashing the sample at 100°C. Freeze-dried samples were analyzed for Ca by atomic absorption spectrophotometry and P by colorimetric methods using standard procedures (AOAC, 1990) after ashing the sample at 100°C. The HA concentration was determined (Marty et al., 1994) by using dysprosium chloride as the indigestible marker.

The endogenous flow of lysine at the distal ileum (EndoLysflow) was estimated as follows:

**Flows and Digestibility Calculations of AA and N**

All units of the following formulas are expressed in milligrams per gram of DM unless otherwise indicated.

**Table 3. The effect of adding microbial phytase to a semipurified, cornstarch- and soybean meal-based diet on excretion in feces and urine and on the efficiency of retention of Ca, P, and N in growing-finishing pigs**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Phytase</th>
<th>SEM</th>
<th>P-value</th>
<th>Control</th>
<th>Phytase</th>
<th>SEM</th>
<th>P-value</th>
<th>Control</th>
<th>Phytase</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine, g/d</td>
<td>0.14</td>
<td>0.16</td>
<td>0.05</td>
<td>NS</td>
<td>0.47</td>
<td>1.01</td>
<td>0.11</td>
<td>0.001</td>
<td>13.3</td>
<td>16.4</td>
<td>1.31</td>
<td>0.064</td>
</tr>
<tr>
<td>Feces, g/d</td>
<td>5.48</td>
<td>5.48</td>
<td>0.44</td>
<td>NS</td>
<td>4.13</td>
<td>3.37</td>
<td>0.24</td>
<td>0.003</td>
<td>2.97</td>
<td>2.68</td>
<td>0.25</td>
<td>0.068</td>
</tr>
<tr>
<td>Retained, g/d</td>
<td>5.93</td>
<td>5.92</td>
<td>0.52</td>
<td>NS</td>
<td>4.60</td>
<td>4.38</td>
<td>0.27</td>
<td>NS</td>
<td>23.0</td>
<td>20.3</td>
<td>1.57</td>
<td>NS</td>
</tr>
<tr>
<td>Efficiency, %</td>
<td>51.9</td>
<td>51.0</td>
<td>4.08</td>
<td>NS</td>
<td>48.0</td>
<td>49.3</td>
<td>3.04</td>
<td>NS</td>
<td>59.6</td>
<td>51.7</td>
<td>3.69</td>
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<td>P</td>
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<td>Ca</td>
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<td>0.44</td>
<td>NS</td>
<td>4.13</td>
<td>3.37</td>
<td>0.24</td>
<td>0.003</td>
<td>2.97</td>
<td>2.68</td>
<td>0.25</td>
<td>0.068</td>
</tr>
<tr>
<td>Retained, g/d</td>
<td>5.93</td>
<td>5.92</td>
<td>0.52</td>
<td>NS</td>
<td>4.60</td>
<td>4.38</td>
<td>0.27</td>
<td>NS</td>
<td>23.0</td>
<td>20.3</td>
<td>1.57</td>
<td>NS</td>
</tr>
<tr>
<td>Efficiency, %</td>
<td>51.9</td>
<td>51.0</td>
<td>4.08</td>
<td>NS</td>
<td>48.0</td>
<td>49.3</td>
<td>3.04</td>
<td>NS</td>
<td>59.6</td>
<td>51.7</td>
<td>3.69</td>
<td>0.072</td>
</tr>
</tbody>
</table>

1All of the interactions with age were nonsignificant (NS) at P > 0.10.
2Average values for the 3 sampling periods.
3Efficiency = (retention/intake) × 100.
Table 4. The effect of adding microbial phytase on apparent ileal digestibilities of N in growing-finishing pigs fed a semipurified, cornstarch- and soybean meal-based diet containing 50% guanidinated or 100% basal untreated protein.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Phytase</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>76.49</td>
<td>78.4</td>
<td>0.91</td>
<td>NS</td>
</tr>
<tr>
<td>Guanidinated</td>
<td>73.04</td>
<td>76.64</td>
<td>0.96</td>
<td>0.010</td>
</tr>
<tr>
<td>Overall</td>
<td>74.77</td>
<td>75.22</td>
<td>0.70</td>
<td>0.005</td>
</tr>
<tr>
<td>Basal Guanidinated Overall</td>
<td>77.45</td>
<td>74.84</td>
<td>0.66</td>
<td>0.007</td>
</tr>
</tbody>
</table>

1All of the interactions with age were nonsignificant (NS) at P > 0.10.

### Statistical Analysis

The barrows were the experimental units. Data were subjected to ANOVA by using the GLM procedure (SAS Inst. Inc., Cary, NC). Apparent and true ileal digestibilities of AA and balances of N, Ca, and P were subjected to ANOVA as repeated measures by using polynomial contrasts to evaluate the effects of the treatment during the growing-finishing period. Results of the postprandial evolution of plasma α-amino N and urea N concentrations were also analyzed with repeated measures analysis by using polynomial contrasts for each sampling period.

### RESULTS

The barrows remained healthy and consumed their meal allowances throughout the experiment. Postmortem examinations conducted at the end of the experiment revealed no intestinal adhesions or other abnormalities.

### Ca, P, and N Balances

The effects of the addition of microbial phytase on Ca, P, and N excretion or retention were similar among collection periods; therefore, only pooled values are presented (Table 3). The addition of microbial phytase had no effect on the amount of Ca excreted in feces or urine. However, microbial phytase reduced P excretion in feces (P = 0.003) and increased it in urine (P = 0.001), with the total amount of P excreted or retained being unaffected. Phytase tended to decrease the amount of N excreted in feces (P = 0.068) but tended to increase it in urine (P = 0.064). It also tended to increase the total amount of N excreted (P = 0.073) without affecting the amount of N retained in the body. Overall, microbial phytase tended to reduce the efficiency of N retention (P = 0.072).

### AID and TID

Barrows receiving the diet containing 50% of guanidinated protein had lower AID of N than did those fed the basal diet (P = 0.007; Table 4). The addition of

Table 5. Effect of adding microbial phytase on apparent ileal digestibilities (AID) of individual AA in growing-finishing pigs fed a semipurified, cornstarch- and soybean meal-based diet

<table>
<thead>
<tr>
<th>AA</th>
<th>Control</th>
<th>Phytase</th>
<th>SEM</th>
<th>Overall phytase effect</th>
<th>Age × phytase interaction^2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indispensable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>86.8</td>
<td>90.2</td>
<td>0.94</td>
<td>0.006</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cystine</td>
<td>78.7</td>
<td>82.8</td>
<td>1.14</td>
<td>0.073</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Histidine</td>
<td>80.8</td>
<td>81.8</td>
<td>1.05</td>
<td>NS</td>
<td>0.079</td>
<td>NS</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>87.9</td>
<td>87.4</td>
<td>1.67</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Leucine</td>
<td>86.6</td>
<td>86.7</td>
<td>1.31</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lysine</td>
<td>87.8</td>
<td>88.2</td>
<td>1.24</td>
<td>NS</td>
<td>ns</td>
<td>NS</td>
</tr>
<tr>
<td>Methionine</td>
<td>81.2</td>
<td>84.5</td>
<td>1.17</td>
<td>0.037</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>86.3</td>
<td>86.7</td>
<td>1.14</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Threonine</td>
<td>73.4</td>
<td>74.5</td>
<td>1.57</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Valine</td>
<td>80.3</td>
<td>80.0</td>
<td>1.66</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Dispensable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>83.0</td>
<td>81.7</td>
<td>1.31</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>75.4</td>
<td>76.6</td>
<td>1.18</td>
<td>0.061</td>
<td>NS</td>
<td>0.049</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>84.2</td>
<td>86.7</td>
<td>1.13</td>
<td>NS</td>
<td>0.084</td>
<td>0.036</td>
</tr>
<tr>
<td>Glycine</td>
<td>70.5</td>
<td>70.1</td>
<td>2.85</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Serine</td>
<td>79.7</td>
<td>80.7</td>
<td>1.11</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Average AID for the 3 sampling periods; NS = nonsignificant effect at P > 0.10.
2For age × phytase interactions, tendencies (P < 0.1) were always characterized by similar AID at 75 and 131 d of age and by a greater AID for pigs fed with phytase diets at 103 d of age.
Table 6. Effect of an addition of microbial phytase on apparent ileal digestibilities (AID) of individual AA in growing-finishing pigs fed a semipurified, cornstarch- and soybean meal-based diet with half of the protein guanidinated.

<table>
<thead>
<tr>
<th>AA</th>
<th>Control</th>
<th>Phytase</th>
<th>SEM</th>
<th>Overall phytase effect</th>
<th>Age × phytase interaction²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>Linear</td>
<td>Quadratic</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Indispensable</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>85.3</td>
<td>88.8</td>
<td>0.76</td>
<td>0.005</td>
<td>0.052</td>
</tr>
<tr>
<td>Cystine</td>
<td>74.3</td>
<td>78.2</td>
<td>1.15</td>
<td>0.011</td>
<td>0.062</td>
</tr>
<tr>
<td>Histidine</td>
<td>78.9</td>
<td>82.8</td>
<td>1.24</td>
<td>0.046</td>
<td>NS</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>81.4</td>
<td>82.8</td>
<td>1.14</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Leucine</td>
<td>82.1</td>
<td>83.7</td>
<td>1.01</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lysine</td>
<td>78.8</td>
<td>81.0</td>
<td>2.27</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Methionine</td>
<td>80.5</td>
<td>82.4</td>
<td>0.87</td>
<td>0.073</td>
<td>0.026</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>82.6</td>
<td>84.5</td>
<td>0.84</td>
<td>0.063</td>
<td>0.062</td>
</tr>
<tr>
<td>Threonine</td>
<td>69.7</td>
<td>72.4</td>
<td>1.31</td>
<td>0.090</td>
<td>0.074</td>
</tr>
<tr>
<td>Valine</td>
<td>74.9</td>
<td>76.5</td>
<td>1.35</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Dispensable</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>72.3</td>
<td>75.8</td>
<td>1.59</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>74.8</td>
<td>77.9</td>
<td>0.87</td>
<td>0.077</td>
<td>0.073</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>81.4</td>
<td>85.8</td>
<td>0.86</td>
<td>0.007</td>
<td>0.080</td>
</tr>
<tr>
<td>Glycine</td>
<td>67.4</td>
<td>71.7</td>
<td>2.00</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Serine</td>
<td>77.8</td>
<td>80.1</td>
<td>0.92</td>
<td>0.051</td>
<td>NS</td>
</tr>
<tr>
<td>Homoarginine</td>
<td>98.8</td>
<td>97.3</td>
<td>0.33</td>
<td>0.025</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Average AID for the 3 sampling periods; NS = nonsignificant effect at P > 0.10.
2 For age × phytase interactions, significant differences or tendencies (P < 0.10) were always characterized by similar AID at 75 d of age followed by an increase at 103 d of age and by a slight (linear) or a more important reduction (quadratic) effect at 131 d of age in pigs fed with phytase diets.

Microbial phytase also increased the AID of N when barrows were fed diets containing guanidinated protein (P = 0.01), but not when they were fed untreated soybean meal. Although phytase increased the AID of N for the entire experimental period (P = 0.005), this effect was mainly associated with HA and not with the basal diets.

For individual AA in the basal diet (Table 5), addition of microbial phytase increased overall AID of arginine (P = 0.006) and methionine (P = 0.037) and tended to increase cystine (P = 0.073). Concerning the effect of time, the addition of phytase tended to increase AID of aspartic acid (P = 0.061), glutamic acid (P = 0.084), and histidine (P = 0.079) but only at 103 d of age.

In the HA diets, the addition of microbial phytase increased overall AID of arginine (P = 0.005), cystine (P = 0.011), histidine (P = 0.046), glutamic acid (P = 0.007), and HA (P = 0.025; Table 6). Phytase supplementation also tended to increase AID of methionine (P = 0.073), phenylalanine (P = 0.063), threonine (P = 0.090), aspartic acid (P = 0.077), and serine (P = 0.051). As the barrows became older, the addition of microbial phytase increased AID of methionine (P = 0.026) and it also tended to increase those of arginine (P = 0.052), cystine (P = 0.062), phenylalanine (P = 0.062), threonine (P = 0.074), aspartic acid (P = 0.073), and glutamic acid (P = 0.080) until 103 d of age. The addition of microbial phytase had no effect on true ileal AA digestibilities (Table 7). However, phytase increased the TID values for lysine in older, but not in young, barrows (P = 0.046).

**Postprandial Protein Absorption and Utilization**

The addition of microbial phytase had no effect on plasma α-amino N concentration at any age studied (Figure 1a, 1b, and 1c). By contrast, plasma urea N concentration was greater in barrows receiving phytase at 70 (P = 0.010) and 127 d of age (P = 0.035; Figure 1d, 1e, and 1f), but not at 99 d of age. However, urea N concentration increased faster with time in 99-d-old barrows fed phytase-supplemented diets (P = 0.023).

**DISCUSSION**

Cereal grains and legumes that are commonly used as feed ingredients all have similar phytate-P concentrations of approximately 0.25% of DM (Maenz, 2000). However, the semipurified soybean meal diets used in the present experiment contained only 0.13% phytate-P compared with normal commercial diets, which can contain more than 0.22%. Therefore, in this experiment, the effect of microbial phytase on reducing phytate-P concentration may not have been as pronounced as it would have been in commercial diets.

**Balances of Ca, P, and N**

It is generally accepted that Ca and P retention, ash deposition, and bone mineralization are increased by the addition of microbial phytase (Cromwell et al., 1993; Mroz et al., 1994; Kornegay, 2001). In the present exper-
Table 7. Effect of addition of microbial phytase on true ileal digestibilities (TID) of individual AA in growing-finishing pigs fed a semipurified, cornstarch- and soybean meal-based diet with half of the proteins guanidinated

<table>
<thead>
<tr>
<th>AA</th>
<th>Control</th>
<th>Phytase</th>
<th>SEM</th>
<th>Overall phytase effect</th>
<th>Age × phytase interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall</td>
<td>Linear</td>
</tr>
<tr>
<td>Indispensable</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Arginine</td>
<td>94.1</td>
<td>96.6</td>
<td>1.36</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cystine</td>
<td>115.2</td>
<td>120.4</td>
<td>6.96</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Histidine</td>
<td>90.6</td>
<td>92.9</td>
<td>1.61</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>100.9</td>
<td>100.9</td>
<td>3.18</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Methionine</td>
<td>95.8</td>
<td>95.6</td>
<td>2.43</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Leucine</td>
<td>95.3</td>
<td>95.1</td>
<td>1.86</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lysine</td>
<td>95.3</td>
<td>96.0</td>
<td>0.38</td>
<td>NS 0.046</td>
<td>0.090</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>95.7</td>
<td>95.7</td>
<td>1.96</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Threonine</td>
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<td>111.8</td>
<td>6.79</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Valine</td>
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<td>104.3</td>
<td>4.82</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Dispensable</td>
<td></td>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Alanine</td>
<td>94.1</td>
<td>94.9</td>
<td>2.99</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>86.8</td>
<td>85.7</td>
<td>1.94</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>91.0</td>
<td>93.1</td>
<td>1.40</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Glycine</td>
<td>102.7</td>
<td>104.8</td>
<td>5.68</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Serine</td>
<td>97.5</td>
<td>98.0</td>
<td>2.86</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1Average TID for the 3 sampling periods; NS = nonsignificant effect at P > 0.10.
2The age × phytase interaction was characterized by an equal TID at 75 d of age followed by a reduction of TID at 103 and 131 d of age in pigs receiving the control diet.

iment, the addition of microbial phytase to diets reduced the amount of P in feces but increased the urinary P losses. These observations indicate that microbial phytase has been effective in increasing P digestibility even in low-phytate-P experimental diets. However, because the diets already met the P requirements, the surplus P absorbed was simply eliminated via the urine. Therefore, the addition of microbial phytase had no effect on total P excreted and retained, and consequently is irrelevant in terms of P balance when dietary P already fulfills the animal requirements.

The addition of microbial phytase did not affect Ca absorption or excretion in this study, as reported in the literature (Lantzsch et al., 1995; Jongbloed et al., 1996; Traylor et al., 2001). In this study, the Ca:P ratio was adequate (NRC, 1998) and phytate-P concentrations were low; therefore, the formation of insoluble phytate complexes should also have been low (Wise, 1983; Gifford, and Clydesdale, 1990; Kornegay, 2001). Nevertheless, the effect on Ca of adding microbial phytase was always less marked than that on P, particularly with higher concentrations of dietary Ca.

Nitrogen excretion in feces or urine and N retention were only slightly affected by the addition of microbial phytase. Similar results have been observed in the literature (Ketaren et al., 1993; Mroz et al., 1994; Näsi et al., 1995; Gagné et al., 2002) although other data have shown some effects of phytase on N excretion (Pallauf et al., 1994a,b; Schone et al., 1995).

AID

The AID of N and individual AA are similar to those observed in the literature (Sauer and Ozimek, 1986; de Lange et al., 1990; Marty et al., 1994). Although Officer and Batterham (1992) observed an improved AID of N with phytase, others found no effect (Näsi and Helander, 1994; Pallauf et al., 1994b). Although not statistically significant, the 2.5% increase in AID of N observed in this experiment with the addition of microbial phytase to basal diets may indicate that the enzyme increased dietary AA digestibility, as reported in the literature (Mroz, 2002). However, these results seem to support the hypothesis that phytase-mediated responses on protein digestibility, if any, are low.

Microbial phytase only increased AID of arginine and methionine in the basal diets, which is also consistent with the report by Mroz et al. (1994). However, these increases were small and they may not be able to contribute to any increase in protein retention. The increase in blood urea N concentrations observed in pigs receiving phytase and the tendency to have decreased retention efficiencies support this contention.

In diets in which 50% of the soybean meal protein was guanidinated, microbial phytase was more effective in improving the AID of N than with diets containing untreated protein. Furthermore, the addition of microbial phytase in HA diets increased the AID of several AA, whereas it seemed to have no effect in basal diets. These results indicate that the guanidination reaction may affect protein characteristics and endogenous losses, and thus AA digestibility.

The HA Technique

Nyachoti et al. (2002) indicated that guanidination did not interfere with digestion, which supports the
use of the HA method for determining true ileal AA digestibilities in pigs fed commercial diets. However, other studies showed that guanidination might have detrimental effects on protein digestibility. For example, feeding guanidinated casein for a long period of time reduced feed intake and growth in chickens (Aoyagi and Baker, 1994; Bryden et al., 1996) and rats (Moughan and Rutherfurd, 1990). One reason for the reduced feed intake may have been the palatability of the treated protein (Leterme and Thewis, 1995). However, in the present study, no effect of guanidination was observed on feed intake, probably because pigs were fed guanidinated soybean meal for short periods of time and were subjected to restricted feeding, which caused them to eat all their meal quickly. However, the AID of N in diets containing guanidinated soybean meal protein was reduced by 4.5%. Drescher (1995) observed a similar reduction of digestibilities in miniature pigs fed guanidinated casein. In the present experiment, AID were reduced for alanine, lysine, and isoleucine in pigs receiving the guanidinated soybean meal protein.  

Siriwan et al. (1994) suggested that uniform guanidination of lysine in a protein source is required if HA is used as a marker for determining endogenous recoveries of AA. The efficiency of lysine conversion to HA in the current study was 69.5%, which is within the range of values reported in the literature (73 to 78%, Marty et al., 1994; 68.8%, Siriwan et al., 1994; 80.1 to 64 to 83% Caine et al., 1997; 94.1%, Nyachoti et al., 1997; 72.3 to 84.6%, Nyachoti et al., 2002). Roos et al. (1994) used guanidinated casein with 90 and 95% of lysine converted to HA to determine the TID of N. Despite these different efficiencies of guanidination, these authors did not detect any effect of guanidination on the TID of AA, and they concluded that guanidination was probably uniform over the protein in all cases. They suggested, however, that this reaction may preferentially affect terminally positioned peptides from other protein sources. The AID of lysine was reduced by more than 10% after guanidination. This may be explained by the fact that guanidination occurred mainly in the terminal position of peptides and that the nontransformed lysine may have been less accessible. During guanidination, multiple washings are performed to remove the excess methylisourea, which also removes soluble proteins and

Figure 1. Effect of microbial phytase supplementation (control - - - or phytase ––––) on postprandial plasma α-amino N and urea N evolution in growing-finishing pigs fed a semipurified, cornstarch- and soybean meal-based diet at 71, 99, and 127 d of age. Average BW at each collection period was 26.0 ± 1.00, 44.3 ± 1.46 and 65.89 ± 2.81 kg, respectively. Microbial phytase effect on α-amino N, P > 0.10. Microbial phytase effect on urea N concentrations, P = 0.010, 0.023, and 0.035 at 71, 99, and 127 d of age, respectively.
carbohydrates (Imbeah et al., 1996). In the present study, 25% of the initial material disappeared during guanidination, which is comparable to the results obtained in other laboratories (Caine et al., 1998). The reduction of AID for alanine, isoleucine, and other AA may also be associated with these losses of material during guanidination. Another possible explanation for the reduced AID of guanidinated proteins is the modification of the remaining proteins (i.e., some proteins may have become soluble at the high pH reached during guanidination). Furthermore, proteins do not necessarily precipitate back to the same conformation that existed before solubilization (de Rham and Jost, 1979). Consequently, more soluble proteins are washed out and the remainder may have more complex structures with more resistant links. Because microbial phytase acts specifically on phytates, the improved AID of N and AA observed with the addition of phytases on guanidinated protein may indicate less resistance to hydrolysis and perhaps enhanced digestive enzyme efficiency. If this is true, it is also possible that phytase may have reduced the amount of secreted enzymes and the total endogenous N appearing at the end of the ileum, as observed in poultry (Cowieson et al., 2004, 2006a). Nevertheless, results from this study indicate that guanidination may modify the nature of protein and the AA composition, and may also change the capacity of digestive enzymes to hydrolyze the guanidinated protein, and thus the availability of its AA and peptides for absorption. Therefore, the HA method may not be sufficiently precise to estimate total endogenous lysine losses, and, consequently, the true ileal digestibility of dietary proteins. Results obtained with this technique should therefore be interpreted with caution.

**TID**

The TID observed in the present experiment were 95.3 and 96.0% for the control and phytase-supplemented diets, respectively, which are close to the 97.7% reported by Marty et al. (1994) with the HA technique or the 96.6% observed by de Lange et al. (1990) with the \(^{15}\)N-isotope dilution technique. Standard errors are similar to those observed in the literature.

There was no effect of microbial phytase on the TID of AA with the exception of lysine, which had an interaction with time. In fact, the TID of lysine was constant over the 3 collection periods in pigs receiving microbial phytase, with a slight reduction over time in pigs fed control diets. The lack of a microbial phytase effect on the TID of other AA indicates that the differences observed in AID mainly came from differences in endogenous secretions. It should be noted, however, that results obtained with the HA technique must be interpreted with caution. Furthermore, SE for TID were generally greater than those for AID; therefore, this experimental design is less sensitive in detecting treatment effects. It is difficult to explain the greater variation observed in TID, but it may be due to the use of dysprosium chloride. This indigestible marker is added to diets in crystalline form at a very low concentration. It is possible that under these conditions, dysprosium chloride is partially responsible for the greater SE. The use of a single meal containing HA to estimate N and AA digestibilities may also have contributed to the greater SE.

**Plasma Urea N and \(\alpha\)-Amino N Concentrations, and Age**

Plasma concentration of \(\alpha\)-amino N after the meal gives an indication of the amounts of AA that are absorbed and available for metabolism. In a previous experiment with commercial diets (Gagné et al., 2002), an increase in plasma \(\alpha\)-amino N was observed in 90-d-old pigs for the first 3 h after the meal. This increase was greater in younger pigs fed phytase-supplemented diets. Using dietary phytase at 500 FTU/kg, Johnston et al. (2004) obtained similar results. In the present study, however, plasma \(\alpha\)-amino N concentration was not affected by the meal or the addition of microbial phytase. This lack of a meal effect on plasma \(\alpha\)-amino N concentration can be related to the high cornstarch concentration in the diet, which provides rapidly available energy for metabolism. However, although phytic acid may delay postprandial glucose absorption (Pallauf and Rimbach, 1997), this effect was probably minimal because phytate-P concentration in experimental diets was also small. Therefore, the rapid absorption of energy probably accelerated AA utilization, leading to the lack of a meal effect on plasma \(\alpha\)-amino N concentration.

Urea N concentration in plasma is an indicator of the rate of protein deamination. The greater plasma urea N concentrations observed in pigs fed phytase-supplemented diets may indicate that phytase increased AA digestibility. However, in this experiment, the extra AA were not utilized by the pig and were deaminated, thus explaining the greater urea N concentrations in plasma and a tendency to lower N retention.

In conclusion, results from this study support previous studies showing that the addition of microbial phytase decreases fecal P losses and increases urinary P losses when P requirements are met. Total excreted P remained unchanged. However, several results from this study indicate that the addition of microbial phytase probably increased AA digestion. The fact that phytase supplementation increased the AID of guanidinated proteins, but not the AID in untreated soybean meal protein, may also indicate that the effect of phytase on AA digestibilities differs between protein sources. Nonetheless, the experimental design used in this experiment may not have been sensitive enough to detect a microbial phytase effect on the AID of AA, particularly when phytate-P concentration in diets was low. However, this effect, if any, was small. Furthermore, protein guanidination modified the AA composition and reduced AA AID, indicating that this technique
may not always be appropriate to evaluate endogenous protein losses and TID.

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