Recovery of Amino Acids at the Distal Ileum for Determining Apparent and True Ileal Amino Acid Digestibilities in Growing Pigs Fed Various Heat-Processed Full-Fat Soybean Products

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ABSTRACT: Ten crossbred gilts fitted with simple T-cannulas at the distal ileum were used to determine the apparent and true ileal amino acid digestibilities in five different soybean products: extruded, jet-sploded, micronized, or roasted full-fat soybeans (FFSB) and soybean meal (SBM). The gilts with an average initial body weight of 36 kg were fed the different diets according to a replicated 5 × 5 Latin square design. Gilts were fed twice daily at 0800 and 1830 at 2.6 times maintenance energy requirement. All diets were cornstarch-based and formulated to contain 16% CP from one of the five soybean products. The recovery of endogenous lysine at the distal ileum was determined using the hornoarginine technique. This technique involved the guanidination of dietary lysine to homoarginine, to allow for a differentiation between undigested dietary lysine, represented by homoarginine, and endogenous lysine in the digestive tract of pigs consuming diets that contain guanidinated proteins. Chromic oxide and dysprosium chloride were included as indigestible markers in the normal and homoarginine diets, respectively. True digestibilities were only determined with the five gilts of one Latin square. Ileal digesta were collected for 24 h on d 8 and 10 of each 10-d experimental period. The apparent ileal protein digestibility was higher in SBM than in other soybean products (P < .05). In the heat-treated FFSB, the apparent protein digestibility varied between 69.0 and 81.6%. Recovery of endogenous lysine was affected by the diet (P < .01) and varied between 1,329 and 2,448 mg/kg of DM intake. True lysine digestibility was higher in SBM (P < .05) than in roasted or jet-sploded FFSB; extruded and micronized FFSB were intermediate. With the exclusion of extruded FFSB, protein nitrogen flows were higher when FFSB were fed than when SBM was fed (P < .05). Free amino acid nitrogen flows expressed as a percentage of total amino acid nitrogen flow were not influenced by treatment (P > .05).

Key Words: Pigs, Soybeans, Heat Treatment, Homoarginine, Endogenous Protein, Lysine

Introduction

Full-fat soybeans (FFSB) must be heat-treated before they are included in practical swine diets. This is to inactivate the antinutritional factors that are present in raw FFSB, especially the trypsin inhibitors (Grant, 1989). However, when protein-containing ingredients are overheated, the availability of amino acids, especially of lysine, can be reduced (Van der Poel et al., 1990). Of the different processes to commercially heat FFSB, extrusion, roasting, micronization, and jet-sploding are the methods most commonly used.

At present, the ileal analysis method is the preferred method for the determination of amino acid digestibilities in feedstuffs for pigs (Sauer and Ozi-mek, 1986), but in conventional digestibility studies, no difference is made between exogenous and endogenous protein. Recently, a procedure to distinguish between exogenous and endogenous lysine in the digesta was proposed (Hagemeister and Erbersdobler, 1985; Moughan and Rutherfurd, 1990). In this procedure, the lysine residues of dietary proteins are chemically converted (guanidinated) to homoarginine (HA), a synthetic analogue of lysine. If it is assumed that guanidination of dietary proteins does not affect protein digestion and absorption, and that HA is not incorporated into endogenous protein, then this approach can be used to determine directly the true

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digestibilities of HA and, thus, of dietary lysine (Moughan and Rutherfurd, 1990).

The objectives of the present study were to determine apparent protein and amino acid digestibilities in growing pigs fed heat-treated FFSB or soybean meal (SBM). The true digestibility of amino acids other than lysine were estimated from the recovery of endogenous lysine (HA method) and a previously published composition of endogenous amino acids (de Lange et al., 1990).

**Materials and Methods**

**Animals and Housing**

Each of 10 female pigs (Landrace × Yorkshire × Hampshire) with an average initial BW of 36 kg was surgically fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The design of the cannulas was modified according to de Lange et al. (1989). After surgery, the pigs were housed individually in stainless steel metabolism crates in a temperature-controlled room (19 to 21°C). During recovery from surgery, at least 10 d, the pigs were fed increasing amounts of an 18% CP starter diet. Body weights of the individual pigs were determined in the morning of the first day of each experimental period.

The present experiment was reviewed and approved by the Animal Care Committee of McGill University and the pigs were cared for under the guidelines of the Canadian Council on Animal Care. At the end of the experiment, the pigs were used for another study and were then killed to determine whether cannulation had caused intestinal abnormalities.

**Preparation of Diets**

Five different soybean products, extruded, jet-sploded, micronized, and roasted FFSB and SBM, were included in five experimental diets (Table 1). All soybean products were obtained from commercial suppliers, where they were exposed to the various heat treatments as described by Marty and Chavez (1993). They were ground in a hammer mill through a 4-mm screen before diet preparation. All diets were cornstarch-based and formulated to contain approximately 16% CP from the individual soybean products. Sugar was included in an attempt to improve palatability of the purified diets. Soybean oil was added to the SBM diet to obtain a fat level similar to that of the FFSB diets. Vitamins and minerals were supplemented to meet or exceed NRC (1988) requirements. Chromic oxide (0.5%) was included in each diet as an indigestible marker for determining nutrient digestibilities.

Guanidinated soybean protein was prepared from each of the five soybean products as described by Schmitz et al. (1991). The extent of guanidination was calculated from the levels of lysine and HA in the guanidinated soybean products as determined by HPLC. The average conversion rate of lysine to HA

| Table 1. Composition and analysis of air-dry experimental diets\(^a\) |
|-----------------|--------|--------|--------|--------|--------|--------|
| **Item**        | **Ex** | **Js** | **Mi** | **Ro** | **SBM** | **CPF** |
| Composition     |        |        |        |        |        |        |
| Cornstarch, %   | 41.07  | 41.47  | 42.17  | 39.47  | 43.07  | 73.37  |
| Soybean product, % | 43.30  | 42.90  | 42.10  | 45.00  | 33.60  |        |
| Sugar, %        | 10.00  | 10.00  | 10.00  | 10.00  | 10.00  | 10.00  |
| Vegetable oil, % |        |        |        |        |        |        |
| Alphacel, %\(^b\) | 2.50   | 2.50   | 2.60   | 2.40   | 3.60   | 4.60   |
| Vitamin-mineral premix, %\(^c\) | 2.50   | 2.50   | 2.50   | 2.50   | 2.50   | 2.50   |
| Vitamin premix, %\(^d\) | .13    | .13    | .13    | .13    | .13    | .13    |
| Cr\(_2\)O\(_3\), % | .50    | .50    | .50    | .50    | .50    | .50    |
| Dry matter, %   | 92.2   | 92.3   | 92.1   | 91.9   | 91.4   | 92.8   |
| Crude protein, % | 16.8   | 16.9   | 16.8   | 16.8   | 16.9   |        |
| Lysine, %       | .78    | .82    | .76    | .81    | .78    |        |
| TIU/mg of CP\(^e\) | 12.9   | 14.9   | 10.4   | 11.8   | 9.5    |        |

\(^a\)Ex, extruded full-fat soybeans (FFSB); Js, jet-sploded FFSB; Mi, micronized FFSB; Ro, roasted FFSB; SBM, soybean meal; CPF, crude protein-free.

\(^b\)Alpha-cellulose, ICN Biomedicals St-Laurent, Quebec, Canada.

\(^c\)Supplied the following per kilogram of complete feed: vitamin A, 10,250 IU; vitamin D\(_3\), 1,025 IU; vitamin E, 25 IU; P, 2.25 g; Ca, 5.75 g; NaCl, 3 g; Mg, 225 mg; Fe, 175 mg; Zn, 100 mg; Mn, 42.5 mg; Cu, 15 mg; I, 450 \(\mu\)g; Se, 300 \(\mu\)g; Co, 100 \(\mu\)g.

\(^d\)Supplied the following per kilogram of complete feed: choline chloride, 300 mg; niacin, 10 mg; d-pantothenic acid, 8 mg; riboflavin, 2.5 mg; thiamin, 1 mg; vitamin B\(_6\), 1 mg; folic acid, 300 \(\mu\)g; d-biotin, 50 \(\mu\)g; vitamin B\(_12\), 10 \(\mu\)g.

\(^e\)Trypsin inhibitor units per milligram of CP determined in the individual soybean products.
was 78% among the different batches with similar variabilities within and among soybean products. To prepare the experimental diets containing the guanidinated proteins, 50% of the untreated soybean protein (Table 1) was replaced with the respective guanidinated soybean proteins. In the HA diets, chromic oxide was replaced with dysprosium chloride (Sigma Chemical, St. Louis, MO), which was added at a level of 100 ppm. This replacement was done to have an indigestible marker, which was unique for the HA meals.

General Conduct of Study

After recovery from surgery, the pigs were fed one of the five experimental diets according to a replicated 5 × 5 Latin square design. Feed intake was adjusted for individual pigs at the start of each experimental period to 2.6 times maintenance energy requirement (ARC, 1981) and feed was provided twice daily in two equal meals at 0800 and 1830. The diets were given as a wet mash, but water was available at all times through low-pressure drinking nipples. Feed refusals and spillages were recorded and considered in the calculation of DM intake.

Each experimental period consisted of 10 d. After a 7-d adaptation period, ileal digesta were collected continuously for 24 h on d 8 and 10 as described by de Lange (1989). Digesta were collected through soft plastic tubing (Pellicule, 5-cm width, Baxter Corporation, Montreal, Canada). One end of the tubing was attached to the ileal cannula and the other end was clamped shut and kept immersed in a container filled with ice water. Digesta were removed frequently (at least once every hour) from the immersed tubing and immediately frozen.

True digestibility using the homoarginine method was determined in five pigs representing one of the two Latin squares. A single meal containing the guanidinated protein was given at the start of the second 24-h ileal digesta collection period on d 10. The meal preceding (1830, d 9) and following (1830, d 10) the meal containing guanidinated protein was given as a N-free diet (Table 1). The aim of administering the N-free diets before and after the homoarginine protein was to determine the clearance and the flow of amino acids at the distal ileum from meals other than the HA meal. Therefore, it was not possible to calculate the endogenous flows of amino acids other than lysine in pigs fed homoarginine.

Chemical Analysis

After the conclusion of the experiment, digesta were pooled per pig and collection day. The pooled samples were freeze-dried, ground through a 1-mm mesh screen (Tecator Cyclotec Sample Mill, Fisher Scientific, Montreal, Canada), and thoroughly mixed before analysis. Analyses for N and DM content were carried out according to AOAC (1990) procedures. Trypsin inhibitor units (TIU) were determined in all soybean products according to della Gatta et al. (1988). Digesta and feed samples were hydrolyzed for 24 h with 6 N HCl at 110°C for the determination of amino acid levels including HA, using the Pico-Tag system from Waters Chromatography Division, Millipore Corporation (Milford, MA) (Sarwar et al., 1988). Methionine, cystine/cysteine, and tryptophan, because they were partly destroyed under acid hydrolysis, were not determined.

The Waters Pico-Tag system was also used to determine free amino acids in digesta employing the Waters 30-cm Pico-Tag column for free amino acids (Sarwar and Botting, 1990). For determination of chromium and dysprosium, diets and digesta samples were wet-digested with concentrated nitric and perchloric acids (Reagent Grade, Caledon Laboratories, Georgetown, Ont., Canada), and the two minerals were determined by flame atomic absorption spectrophotometry (Perkin Elmer, Model 2380, Norwalk, CT) (Emerson, 1975). All analyses were performed in duplicate and all calculations were conducted on an absolute DM basis, which was determined by drying to a constant weight in a vacuum oven at 100°C.

Calculations and Statistical Analysis

If not otherwise specified all units in the following formulas are in milligrams/kilogram of DM and the flows at the terminal ileum of total amino acids, free amino acids, and endogenous amino acids are relative to the ingestion of 1 kg of feed DM. Apparent ileal amino acid and protein digestibilities were calculated with pigs fed non-guanidinated soybean diets using the indigestible markers:

\[
AD_{AA} = 100 - \left( 100 \times \frac{[AA]_{\text{digesta}} \times [Cr]_{\text{diet}}}{[AA]_{\text{diet}} \times [Cr]_{\text{digesta}}} \right)
\]

\[
= \frac{[AA]_{\text{diet}} - [AA]_{\text{flow}}}{[AA]_{\text{diet}}} \times 100
\]

where \(AD_{AA}\) are the apparent digestibilities of individual amino acids in percentage, \([AA]_{\text{digesta}}\) is the
amino acid concentration in the digesta, \([AA]_{\text{diet}}\) is the amino acid concentration in the diet, \([Cr]_{\text{diet}}\) is the chromic oxide concentration in the diet, and \([Cr]_{\text{digesta}}\) is the chromic oxide concentration in the ileal digesta. AAflow is the flow of an amino acid at the ileal level as determined by Equation [2] using the corresponding individual amino acid concentrations and chromic oxide as the indigestible marker.

Observations made on pigs fed the guanidinated proteins were used to determine true lysine digestibilities only. True lysine digestibilities were calculated from HA digestibilities as discussed by Moughan and Rutherfurd (1990).

\[
AA_{\text{flow}} = [AA]_{\text{digesta}} \times \frac{[IDM]_{\text{diet}}}{[IDM]_{\text{digesta}}} \quad [2]
\]

\[
TD_{\text{Lys}} = \frac{[HA]_{\text{diet}} - HA_{\text{flow}}}{[HA]_{\text{diet}}} \times 100 \quad [3]
\]

where AAflow is the flow of an amino acid at the distal ileum and [IDM]diet and [IDM]digesta are the concentration of the indigestible marker in diet and digesta, respectively. TD_{Lys} is the true lysine digestibility expressed in percentage and [HA]diet is the dietary homoarginine concentration. The flow of HA (HAflow) was determined according to Equation [2] using dysprosium chloride as the indigestible marker.

All amino acid flows and the true digestibilities of amino acids, other than lysine, were derived from observations on d 8 in those pigs that received guanidinated soybean diets on d 10 (Equations [4] and [5]).

\[
\text{EndoLys}_{\text{flow}} = \text{TotLys}_{\text{flow}} - \left( [Lys]_{\text{diet}} - TD_{\text{Lys}} \times \frac{[Lys]_{\text{diet}}}{100} \right) \quad [4]
\]

where EndoLysflow is the endogenous flow of lysine at the distal ileum, TotLysflow is the total flow of lysine at the distal ileum as determined by acid hydrolysis, [Lys]diet is the lysine concentration in the diet, and TD_{Lys} is the true lysine digestibility determined in Equation [3]. Exogenous flow of lysine at the distal ileum was calculated by difference between the flows of total lysine and endogenous lysine.

True digestibilities of individual amino acids other than lysine were calculated as follows:

\[
TD_{AA} = \frac{[AA]_{\text{diet}} - (AA_{\text{flow}} - \text{EndoAA}_{\text{flow}})}{[AA]_{\text{diet}}} \times 100 \quad [5]
\]

where TD_{AA} is the true digestibility of an amino acid other than lysine expressed in percentage, [AA]_{\text{diet}} is the concentration of an amino acid other than lysine in the diet, AAflow is the flow of an amino acid other than lysine at the distal ileum, and EndoAAflow is the endogenous flow of an amino acid other than lysine at the distal ileum. Flows of endogenous amino acids other than lysine were calculated based on the observed endogenous lysine flows and the composition of endogenous amino acids, relative to lysine, as reported by de Lange et al. (1990).

To calculate flows of total amino acid N and of free amino acid N at the distal ileum, total and free amino acid concentrations were expressed on their N basis using the corresponding molecular weights for transformation. Nitrogen from peptide and protein sources was estimated as the difference between hydrolyzed amino acid N and free amino acid N. Peptide plus protein N is hereafter referred to as protein N. Flows of free amino acid N and protein N were estimated using Equation [2] with the corresponding variables and chromic oxide (percentage) as the indigestible marker.

The results were subjected to analyses of variance using the GLM procedures of SAS (1985). The statistical design was a replicated 5 x 5 Latin square and the model included the effects of period (df = 4), Latin square (df = 1), animal nested within square (df = 8), and diet (df = 4). The effect of collection day in pigs fed normal diets on d 8 and 10 was not significant (P > .05); therefore, it was not included in the model and data from these pigs were pooled across days for statistical analysis. Animals fed HA diets on d 10 contributed data from d 8 only, with the exception of true lysine digestibilities and lysine flow, which were calculated on d 10 and for which the statistical model included the effects of period (df = 4), animal (df = 4), and diet (df = 4). The Scheffe multiple-range test was used to determine differences between diets when a significant F-value (P < .05) was obtained (Steel and Torrie, 1980).

**Results and Discussion**

The pigs remained healthy, readily consumed the experimental diets, and had normal body weight gains (Table 2). The average body weight over the five experimental periods was between 50 and 55 kg with daily feed intakes of approximately 1.5 kg of DM (Table 2). As determined by postmortem investigations, cannulation did not result in intestinal abnormalities in the 10 female pigs from which observations were obtained.

The apparent ileal digestibilities of CP and individual amino acids in SBM (Table 3) were within the range of previously published values (Sauer and Ozimek, 1986; de Lange et al., 1990) with the exception of serine and tyrosine, which were lower in the present experiment. Thus, our data support earlier reports that suggest that the range of heat treatment normally found among commercially available SBM
Table 2. Dry matter intake, average body weight, and body weight gain of pigs fed the experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Ex</th>
<th>Js</th>
<th>Mi</th>
<th>Ro</th>
<th>SBM</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM intake, g/d</td>
<td>1,463</td>
<td>1,481</td>
<td>1,508</td>
<td>1,534</td>
<td>1,506</td>
<td>32.166</td>
<td>.59</td>
</tr>
<tr>
<td>DM intake, g/(d·MBwt)(^b)</td>
<td>72.9</td>
<td>74.7</td>
<td>77.4</td>
<td>76.6</td>
<td>76.3</td>
<td>2.084</td>
<td>.62</td>
</tr>
<tr>
<td>Avg BW, kg</td>
<td>54.7</td>
<td>54</td>
<td>52.9</td>
<td>54.9</td>
<td>53.9</td>
<td>968</td>
<td>.57</td>
</tr>
<tr>
<td>Weight gain, g/d</td>
<td>640</td>
<td>610</td>
<td>575</td>
<td>565</td>
<td>640</td>
<td>26.961</td>
<td>.17</td>
</tr>
</tbody>
</table>

\(^a\)Ex, extruded full-fat soybeans (FFSB); Js, jet-sploded FFSB; Mi, micronized FFSB; Ro, roasted FFSB; SBM, soybean meal; SEM, pooled standard error of the mean.

\(^b\)MBwt, metabolic body weight in kilogram.

has little effect on nutritive value when fed to swine (Chang et al., 1987). Ileal apparent digestibilities of CP were higher \((P < .05)\) in SBM than in any of the heat-treated FFSB (Table 3). Among the heat-treated FFSB, CP digestibility was higher in extruded FFSB \((P < .05)\) than in FFSB that were either jet-sploded or roasted. Apparent digestibilities of individual amino acids in the different soybean products followed a trend similar to those observed for CP, although differences for some amino acids were not significant (Table 3). Among heat-treated FFSB apparent lysine digestibilities for extruded FFSB were between 6.1 and 9% higher \((P < .05)\) than those found in other heat-treated FFSB. The present observation of lower ileal N and amino acid digestibilities in extruded FFSB vs SBM is in accordance with earlier findings (Rudolph et al., 1983). Similarly, CP digestibilities of extruded FFSB have been reported as superior to those of FFSB exposed to other heat treatments (Marty and Chavez, 1993).

In determining apparent ileal amino acid digestibilities, no differentiation is made between endogenous and exogenous amino acids at the distal ileum. As suggested by Souffrant (1991), a substantial part of amino acids or proteins at the distal ileum can be of endogenous origin, mainly in the form of enzymes, mucins, amides, amines, and mucosa cells. Traditionally, endogenous amino acid excretion has been determined by feeding N-free diets. However, this latter approach yields very low values and there is evidence that the presence of dietary peptides in the gut lumen enhances the endogenous excretion of amino acids (Butts et al., 1993). A relatively new technique to differentiate between exogenous and endogenous lysine is the homoarginine technique (Hagemeister and Erbersdobler, 1985; Moughan and Rutherfurd, 1990). Homoarginine is a synthetic analogue of lysine, which is not found in animal body protein and can thus be used to distinguish between

### Table 3. Apparent ileal dry matter, protein, and amino acid digestibilities (%) in pigs fed the experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Ex</th>
<th>Js</th>
<th>Mi</th>
<th>Ro</th>
<th>SBM</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>76.1(^b)</td>
<td>73.8(^b)</td>
<td>74.4(^bc)</td>
<td>71.8(^c)</td>
<td>77.2(^b)</td>
<td>1.761</td>
<td>.01</td>
</tr>
<tr>
<td>Crude protein</td>
<td>75.6(^c)</td>
<td>69.0(^d)</td>
<td>71.7(^cd)</td>
<td>69.5(^d)</td>
<td>81.6(^b)</td>
<td>1.051</td>
<td>.01</td>
</tr>
<tr>
<td>Amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>81.0(^c)</td>
<td>78.5(^c)</td>
<td>78.9(^c)</td>
<td>80.0(^c)</td>
<td>89.2(^b)</td>
<td>1.71</td>
<td>.01</td>
</tr>
<tr>
<td>Histidine</td>
<td>81.3(^bc)</td>
<td>76.6(^c)</td>
<td>76.9(^c)</td>
<td>75.9(^c)</td>
<td>86.4(^b)</td>
<td>1.62</td>
<td>.01</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>77.8(^c)</td>
<td>70.9(^d)</td>
<td>75.4(^cd)</td>
<td>70.1(^d)</td>
<td>85.1(^b)</td>
<td>1.13</td>
<td>.01</td>
</tr>
<tr>
<td>Leucine</td>
<td>79.6(^c)</td>
<td>72.2(^d)</td>
<td>76.7(^cd)</td>
<td>71.9(^d)</td>
<td>85.7(^b)</td>
<td>1.28</td>
<td>.01</td>
</tr>
<tr>
<td>Lysine</td>
<td>77.4(^b)</td>
<td>68.4(^c)</td>
<td>69.8(^c)</td>
<td>71.3(^c)</td>
<td>81.1(^b)</td>
<td>2.06</td>
<td>.01</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>78.3(^b)</td>
<td>72.4(^c)</td>
<td>74.8(^c)</td>
<td>71.8(^c)</td>
<td>84.3(^b)</td>
<td>1.47</td>
<td>.01</td>
</tr>
<tr>
<td>Threonine</td>
<td>68.9(^bc)</td>
<td>62.8(^d)</td>
<td>67.6(^bc)</td>
<td>61.8(^c)</td>
<td>74.3(^b)</td>
<td>2.41</td>
<td>.01</td>
</tr>
<tr>
<td>Valine</td>
<td>75.4(^c)</td>
<td>66.0(^d)</td>
<td>74.0(^c)</td>
<td>66.8(^d)</td>
<td>82.9(^b)</td>
<td>1.27</td>
<td>.01</td>
</tr>
<tr>
<td>Dispensable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>70.6(^c)</td>
<td>63.4(^d)</td>
<td>68.6(^cd)</td>
<td>65.1(^cd)</td>
<td>78.8(^b)</td>
<td>1.51</td>
<td>.01</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>80.9(^b)</td>
<td>75.9(^bc)</td>
<td>77.8(^b)</td>
<td>69.2(^c)</td>
<td>82.0(^b)</td>
<td>1.79</td>
<td>.01</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>81.6(^bc)</td>
<td>79.3(^cd)</td>
<td>79.1(^cd)</td>
<td>75.1(^d)</td>
<td>86.9(^b)</td>
<td>1.27</td>
<td>.01</td>
</tr>
<tr>
<td>Glycine</td>
<td>69.3(^bc)</td>
<td>60.3(^d)</td>
<td>66.8(^bc)</td>
<td>62.6(^c)</td>
<td>74.3(^b)</td>
<td>2.07</td>
<td>.01</td>
</tr>
<tr>
<td>Proline</td>
<td>82.5</td>
<td>76.5</td>
<td>78.5</td>
<td>80.2</td>
<td>84.2</td>
<td>2.71</td>
<td>.29</td>
</tr>
<tr>
<td>Serine</td>
<td>63.2(^b)</td>
<td>49.6(^d)</td>
<td>57.0(^bc)</td>
<td>66.5(^b)</td>
<td>68.6(^b)</td>
<td>2.74</td>
<td>.01</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>62.9</td>
<td>50.4</td>
<td>55.9</td>
<td>58.4</td>
<td>70.0</td>
<td>5.56</td>
<td>.16</td>
</tr>
</tbody>
</table>

\(^a\)Ex, extruded full-fat soybeans (FFSB); Js, jet-sploded FFSB; Mi, micronized FFSB; Ro, roasted FFSB; SBM, soybean meal; SEM, pooled standard error of the mean.

\(^b,c,d\)Means in the same row with different superscripts differ.
Table 4. Total, endogenous, and exogenous lysine flows at the terminal ileum and true ileal amino acid digestibilities of pigs fed different soybean products

<table>
<thead>
<tr>
<th>Item</th>
<th>Ex</th>
<th>Js</th>
<th>Mi</th>
<th>Ro</th>
<th>SBM</th>
<th>SEM</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Lysine flows at the ileum, mg/kg DM intake</td>
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<tr>
<td>Total flow</td>
<td>1,876bcd</td>
<td>2,448b</td>
<td>1,988bcd</td>
<td>2,164bc</td>
<td>1,527d</td>
<td>187.83</td>
<td>.01</td>
</tr>
<tr>
<td>Endogenous flow</td>
<td>1,485bcd</td>
<td>2,448b</td>
<td>1,988bcd</td>
<td>2,164bc</td>
<td>1,329d</td>
<td>180.77</td>
<td>.01</td>
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<tr>
<td>Exogenous flow</td>
<td>385bc</td>
<td>431bc</td>
<td>330bc</td>
<td>598b</td>
<td>198c</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>True amino acid digestibilities</td>
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<tr>
<td>Indispensable amino acids, %</td>
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<tr>
<td>Arginine</td>
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<td>94.9</td>
<td>93.9</td>
<td>91.7</td>
<td>96.2</td>
<td>2.93</td>
<td>.85</td>
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<tr>
<td>Histidine</td>
<td>92.4</td>
<td>91.9</td>
<td>90.5</td>
<td>87.8</td>
<td>94.2</td>
<td>1.52</td>
<td>.08</td>
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<tr>
<td>Isoleucine</td>
<td>94.8</td>
<td>95.9</td>
<td>95.7</td>
<td>96.2</td>
<td>97.3</td>
<td>2.11</td>
<td>.94</td>
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<tr>
<td>Leucine</td>
<td>93.8</td>
<td>92.9</td>
<td>94.0</td>
<td>93.9</td>
<td>95.9</td>
<td>1.92</td>
<td>.83</td>
</tr>
<tr>
<td>Lysine</td>
<td>95.5bc</td>
<td>95.3c</td>
<td>96.0bc</td>
<td>94.1c</td>
<td>97.7b</td>
<td>.73</td>
<td>.05</td>
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<tr>
<td>Phenylalanine</td>
<td>94.5</td>
<td>96.5</td>
<td>95.8</td>
<td>94.5</td>
<td>97.0</td>
<td>1.39</td>
<td>.59</td>
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<tr>
<td>Threonine</td>
<td>105.7</td>
<td>110.9</td>
<td>109.6</td>
<td>107.9</td>
<td>101.7</td>
<td>4.32</td>
<td>.55</td>
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<tr>
<td>Valine</td>
<td>97.4</td>
<td>101.1</td>
<td>100.6</td>
<td>100.2</td>
<td>98.8</td>
<td>2.72</td>
<td>.88</td>
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<tr>
<td>Dispensable amino acids, %</td>
<td></td>
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<td></td>
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<tr>
<td>Alanine</td>
<td>95.7</td>
<td>98.4</td>
<td>98.3</td>
<td>98.9</td>
<td>98.7</td>
<td>3.29</td>
<td>.95</td>
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<tr>
<td>Aspartic acid</td>
<td>98.7</td>
<td>101.9</td>
<td>97.5</td>
<td>98.3</td>
<td>96.7</td>
<td>2.78</td>
<td>.73</td>
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<tr>
<td>Glutamic acid</td>
<td>94.2</td>
<td>93.5</td>
<td>92.2</td>
<td>90.0</td>
<td>95.5</td>
<td>1.43</td>
<td>.14</td>
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<tr>
<td>Glycine</td>
<td>104.7</td>
<td>114.8</td>
<td>109.4</td>
<td>105.2</td>
<td>101.7</td>
<td>3.85</td>
<td>.24</td>
</tr>
<tr>
<td>Proline</td>
<td>99.7</td>
<td>104.9</td>
<td>96.3</td>
<td>99.4</td>
<td>98.7</td>
<td>3.45</td>
<td>.51</td>
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<tr>
<td>Serine</td>
<td>93.8</td>
<td>94.8</td>
<td>89.2</td>
<td>92.1</td>
<td>90.8</td>
<td>2.85</td>
<td>.68</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>93.7</td>
<td>104.1</td>
<td>98.9</td>
<td>100.8</td>
<td>99.6</td>
<td>6.60</td>
<td>.41</td>
</tr>
</tbody>
</table>

endogenous lysine in intestinal digesta and lysine from undigested dietary protein (Moughan and Rutherfurd, 1990). For this method to be valid it is assumed that 1) the lysine in the test protein is homogeneously guanidinated, 2) HA is released during digestion from the protein and absorbed to the same extent as lysine, 3) the hydrolysis of HA to lysine and urea in the digestive tract, catalyzed by arginase, is negligible, 4) the presence per se of HA in the gut or plasma does not affect endogenous protein losses, and 5) absorbed HA is not resecreted into the gut lumen. These assumptions have been extensively discussed in previous papers (Schmitz, 1988; Moughan and Rutherfurd, 1990) and found to be valid. Thus, by applying this methodology to pigs fed different soybean products, the endogenous flow and true digestibility of lysine could be calculated.

There was a significant difference ($P < .01$) between the flow of endogenous lysine when the different soybean products were fed. The determined endogenous lysine flows were higher ($P < .05$) with the feeding of FFSB than with that of SBM and varied between 110 to 180% of the flows observed in SBM-fed pigs (Table 4). Exogenous lysine flows increased even more dramatically with the feeding of FFSB, attaining flows of 190 to 300% of those observed with SBM. Endogenous lysine flow represented 78 and 87% of the total lysine flow at the distal ileum (Table 4) and therefore was mainly responsible for the observed low apparent ileal digestibilities of lysine in FFSB-fed pigs. The increase in lysine flow was accompanied by increases in total amino acid nitrogen flow at the distal ileum in FFSB-fed pigs (Table 5).

Table 5. Flow of free amino acid and peptide nitrogen at the terminal ileum of pigs fed different soybean products

<table>
<thead>
<tr>
<th>Item</th>
<th>Ex</th>
<th>Js</th>
<th>Mi</th>
<th>Ro</th>
<th>SBM</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free amino acid N flow, mg/kg of DM intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>as % of total N flow</td>
<td>629bcd</td>
<td>802b</td>
<td>625bcd</td>
<td>745b</td>
<td>396c</td>
<td>63.85</td>
<td>.01</td>
</tr>
<tr>
<td>as % of total AA-N flow</td>
<td>12.7</td>
<td>8.8</td>
<td>7.6</td>
<td>8.6</td>
<td>7.2</td>
<td>0.81</td>
<td>.42</td>
</tr>
<tr>
<td>Peptide N flow, mg/kg of DM intake</td>
<td>4,460bcd</td>
<td>5,819b</td>
<td>5,422bc</td>
<td>5,662b</td>
<td>3,593d</td>
<td>246.82</td>
<td>.01</td>
</tr>
</tbody>
</table>

---

*Ex, extruded full-fat soybeans (FFSB); Js, jet-sploded FFSB; Mi, micronized FFSB; Ro, roasted FFSB; SBM, soybean meal; SEM, pooled standard error of the mean.

Means in the same row with different superscripts differ.
The endogenous lysine flow for the SBM-fed pigs was 1,329 mg/kg of DM intake, which was somewhat higher than those estimated in a previous experiment using the \(^{15}\)N-isotope dilution technique (de Lange et al., 1990); approximately 1,022 and 1,223 mg/kg of DM intake in pigs fed a SBM-cornstarch diet and a canola meal-cornstarch diet, respectively. However, our estimates of endogenous lysine flows at the distal ileum, as well as those reported by de Lange et al. (1990), were much higher than those observed after feeding enzymatically hydrolyzed casein (448 mg/kg of DMI) or a protein-free diet (252 mg/kg of DMI; Butts et al., 1993). The differences are likely due to the protein sources used in the various studies (current study; de Lange et al., 1990; Butts et al., 1993), as well as the fact that Butts et al. (1993) used pigs with an average starting weight of only 13 kg, which was much smaller than the size of pigs used in the current study or in that of de Lange et al. (1990).

Souffrant (1991) compared endogenous N losses in the ileal chyme as observed by various research groups that evaluated a wide variety of protein feeds. He concluded that the amounts of endogenous N recovered at the distal ileum of pigs is variable and affected by many factors, including dietary nutrient levels, protein quality, fiber levels, animal age, and antinutritional factors. Levels of trypsin inhibitors were very similar in the various soybean products (Table 1) and represented approximately 10% of levels found in raw soybeans, which is reported to indicate adequate processing (Agunbiade et al., 1992). Thus, trypsin inhibitors are unlikely to be the cause for the variation in endogenous N secretion. It is well accepted that dietary factors such as increased dry matter intake (Wilson and Leibholz, 1981) or higher dietary fiber levels (Sauer et al., 1977; Low, 1985) will lead to increases in endogenous protein losses. However, dry matter intake as well as dietary fiber content were similar among the different soybean diets in the present study. Studies with the growing rat (Moughan and Rutherfurd, 1990; Butts et al., 1991) and pig (de Lange et al., 1990; Butts et al., 1993) have shown that endogenous amino acid losses from the distal ileum are considerably higher under peptide or protein alimentation than under protein-free feeding. In addition, it has been suggested (Schneeman, 1982) that dietary peptides and protein fragments may provide a preferred substrate for digestive enzymes, leading to a lower level of enzyme breakdown and reabsorption after the ingestion of protein or peptides. Furthermore, Schneeman (1982) and Temler et al. (1983) found that in the rat and other mammalian species, dietary peptides or proteins are much more potent stimulators of pancreatic secretion than are free amino acids. In the current experiment, the feeding of various FFSB tended to increase the quantity of dietary undigested protein present in the intestinal chyme over that found with feeding SBM. This in turn seemed to increase endogenous amino acid concentrations at the distal ileum.

This latter argumentation, however, did not hold for pigs fed extruded FFSB, because when this diet was fed the exogenous lysine flows, which were of similar magnitude to those of other pigs fed FFSB, seemed to be in contrast to endogenous lysine flows, which were higher in pigs fed jet-sploded FFSB (Table 4). Phillips (1989) and Camire et al. (1990) suggested that the extrusion process, in contrast to the other heat treatments, causes the soybean cells to rupture and the cell structure to undergo extensive transformations. Extrusion processing also seems to influence the fiber fraction. Lindas et al. (1988) found that the soluble fiber content was higher in extruded than in raw or boiled beans. Van der Poel et al. (1990) pointed out that such changes in the cell structure would make nutrients such as amino acids more accessible to enzymatic attack, which should result in increases in apparent, as well as true, digestibilities.

It should be pointed out that the endogenous lysine losses observed at the distal ileum represent only the balance between secretion and reabsorption. Therefore, the increased flow of endogenous lysine could also be the result of interference from undigested dietary protein with digestion and reabsorption of endogenous amino acids. The reabsorption rate for the N secreted endogenously up to the terminal ileum is very high and has been reported to exceed 70% after feeding SBM-based diets (Souffrant, 1991). This recycling of endogenous N is likely to be affected by poorly digestible exogenous proteins.

The flows of amino acid N at the distal ileum (Table 5) were higher (P < .05) when the FFSB were fed. These increases in flows when FFSB rather than SBM diets were fed were the result of large increases (P < .01) in both the free amino acid N and protein N flows. Souffrant (1991) reported that 44% of the endogenous N in the chyme at the terminal ileum was in the form of protein and peptides, 27% in the form of free amino acid, and 27% in the form of non-amino-acid N. Endogenous non-amino-acid N at the distal ileum consists mainly of amides, amino sugars, and/or urea (Souffrant, 1991). Other findings (Moughan and Schuttert, 1991; Butts et al., 1992) with both the rat and pig have shown that free amino acids and peptides of low molecular weight (M_r < 10,000 Da) from endogenous sources make up approximately 11 to 21% of the total N in ileal digesta. In the present experiment, free amino acid N flow was between 7.2 and 9.2% of the total N flow and was not influenced (P > .05) by feeding the different soybean products. This indicates that most of the free amino acids found at the distal ileum in the current study are of endogenous origin. This is turn would provide an additional argument that endogenous protein largely
contributes to the flow of protein at the distal ileum. It is unlikely that free amino acids and peptides are derived from the dietary protein. This would also suggest that the endogenous lysine flow would be representative of the total endogenous N flow.

The present experiment demonstrates that the differences in apparent lysine digestibility observed by feeding various soybean products are largely determined by endogenous lysine losses rather than by true lysine digestibilities (Table 4). However, some small but significant differences in true lysine digestibility were observed. Apparent digestibility of lysine represented 84 and 81% of the true lysine digestibility in pigs fed SBM or extruded FFSB respectively, but only between 71 and 75% in pigs fed jet-sploded, micronized, or roasted FFSB. True lysine digestibility of SBM was 97.7% in the current experiment, which was very close to the 96.6% real digestibility reported by de Lange et al. (1990) for SBM determined with the 15N-isotope dilution technique.

True digestibilities of amino acids other than lysine were calculated (Table 4) based on the observed endogenous lysine flows and a previously published endogenous amino acid composition of de Lange et al. (1990). The resulting true digestibilities for SBM were very similar to the real amino acid digestibilities determined with the 15N-isotope dilution technique (de Lange et al., 1990). True digestibilities of amino acids other than lysine reached or exceeded 100% for an increasing number of amino acids, particularly for those FFSB, which had low apparent digestibilities. Therefore, feeding dietary proteins of lower quality not only increases endogenous amino acid losses in absolute terms, but it also seems to change the amino acid composition of endogenous protein recovered at the distal ileum. This response could be due to changes in the relative contribution of the various endogenous protein sources, which would support previous data that suggested that the composition of the feed affected the amino acid composition of pancreatic juice (Souffrant, 1991).

Implications

Apparent ileal digestibilities of crude protein were higher in pigs fed soybean meal than in those fed any of the heat-treated full-fat soybeans (FFSB). In addition, the digestibility coefficients for crude protein and lysine were higher in extruded FFSB than in any other heat-treated FFSB. The data suggest that the differences in apparent amino acid digestibilities among soybean products were primarily a result of variations in endogenous amino acid losses rather than differences in true digestibilities. These variations in endogenous amino acid losses seemed to have been caused by different levels of dietary undigested proteins (and amino acids) in the intestinal tract and changes to the physical structure of the soybean products as a result of the different heat processes.

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