Comparison of ileal apparent, standardized and true digestibilities of amino acids in pigs fed wheat and lupine seeds

U. Hennig,⁎ W. Hackl, Antje Priepke, A. Tuchscherer, W.B. Souffranta, C.C. Metges

Abstract

The aims of the investigation were to compare calculation methods for ileal apparent digestibility (AD) of amino acids (AA) and to demonstrate the calculation of ileal true digestibility (TD) using the regression approach and additionally, to compare the TD with the standardized digestibility (SD). Eight Goettingen minipigs, average 52 kg initial BW, were fitted with ileo-rectal anastomoses and fed consecutively with assay diets in four experiments according to a Latin rectangle design. To determine AD by using the ‘direct method’, diets contained wheat, lupine, or casein as the sole sources of protein. Furthermore, the ‘difference method’ was used by applying diets with wheat plus casein or wheat plus lupine. To determine AD using the regression (‘Reg2’) method, three graded levels of lupine mixed with wheat were fed. The TD and the basal ileal endogenous losses of AA (ELbAA) were determined using the regression approach (‘Reg1’) by feeding both 5 graded levels of lupine and 5 graded levels of wheat, each added with N-free mixture at 1000 g. In each experiment, one repetition comprised 10 days for adaptation and 4 days for quantitative collection of ileal effluents. Pigs were fed twice a day and the target dietary allowance was 35 g DM kg⁻⁰·⁷⁵ BW d⁻¹ in all experiments. There were no differences in the AD of any AA in lupine or in wheat when those were determined with the direct, difference or Reg2 method. The TD values of the most indispensable AA were higher than the AD (in lupine 8 to 20%-units for lysine and methionine and in wheat 6 to 11%-units for tryptophan and lysine, respectively). These differences reflected the ELbAA derived from the intercept (a) of the Reg1. The ELbAA were higher in lupine than in wheat for arginine, histidine, leucine, methionine, phenylalanine, tryptophan, cysteine, glutamine, and serine (P<0.007 to 0.047). In conclusion, the TD of AA should be determined by Reg1 regression rather than calculation of SD, i.e. AD values corrected with means of ELbAA, because the latter cannot be assumed as constant.

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Keywords: Amino acids; Wheat; Lupine; Ileal true digestibility; Standardized digestibility; Methodology; Pigs

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1. Introduction

Ileal apparent digestibility (AD) values of amino acids (AA) depend on the determination methods used and the flux of endogenous AA at the various intake levels (Mosenthin et al., 2000; Sauer et al., 2000). It has been proposed “that the true digestibility (TD) of AA could be calculated from the measured ‘plateau apparent ileal AA digestibilities’ at the threshold levels for AA intakes” (Fan et al., 1995; Mosenthin et al., 2000; Sauer et al., 2000). This TD has recently been called standardized digestibility (SD), which is defined as AD corrected by the standard deviation (SD), which is defined as AD corrected by the standard deviation (SD) (Fan and Sauer, 1995a, 1995b; Sauer et al., 2000; Hennig et al., 2003).

The TD is related to the SD because it uses the same regression method (Reg2) because it uses the same regression method. The difference method involved the AD calculation in both a basal diet and an assay diet. The daily ileal flows and AD of AA and CP of each individual pig fed lupine or casein basal diet (Lupine+N-free mixture; Casein+N-free mixture) were applied for each individual calculation of AD in wheat assay diets. The assay diets consisted of a mixture of the basal feed ingredient and the assay feed such as wheaat (Lupine+wheat; Casein+wheat). The direct, difference and regression (Reg2) methods were already described by Fan and Sauer (1995a), Fan and Sauer (1995b), Sauer et al. (2000) and GfE (2005). However, the two regression methods used will be described here again in more detail.

The regression method is called ‘Reg2’ because it uses the digestibility measures of a nutrient in two feeds as feed A and feed B (FA and FB). The two feeds wheat and lupine, respectively, were mixed at graded levels in three assay diets. The relations between digestibility of AA in the total assay diets and the contribution of AA from feeds FA and FB to the assay diets can be expressed according to the following equations.

\[ AD_{TF} = AD_{FA} \times CF_{FA} + AD_{FB} \times CF_{FB} \]  

It can be written as: \( AD_{FA} = \alpha \) and \( AD_{FB} = \beta \) and therefore the new equations are:

\[ AD_{TF} = \alpha \times CF_{FA} + \beta \times CF_{FB} \]  

Using the above equations, the ileal true digestibility of AA in the total diet can be calculated as:

\[ y = \left( \frac{1}{\beta} \right) - \left( \frac{\alpha}{\beta} \right) \times x \]  

From this it can be determined that AA from feed A to the ileal apparent digestibility of AA in the total assay diets. AD_{TF} is ileal apparent digestibility of AA in the feed A; CF_{FA} is ileal apparent digestibility of AA from feed A to the ileal total diet; AD_{FB} is ileal apparent digestibility of AA from feed B to the ileal total diet; \( \alpha \) is ileal apparent digestibility coefficient of FA; \( \beta \) is ileal apparent digestibility coefficient of FB.

The AD of AA (% of feed A and B can be calculated by: AD_{FA} (%) = \( \alpha \times 100 \) and AD_{FB} (%) = \( \beta \times 100 \), according to Poppe (1981) and Hackl et al. (2007).

To determine the true digestibility (TD) of AA in wheat and lupine we used a modified regression analysis technique termed ‘Reg1’, which is characterized by the fact that only one assay feed as the only protein source is blended with graded levels of protein-free components (such as starch and others). It requires the use of at least three assay diets containing graded levels of assay feed. In our investigations using quantitative digesta collections, the excretion (\( y \)) of an undigested AA at levels \( y_{1..n} \) is linearly correlated to the intake (\( x \)) of an

2. Materials and methods

2.1. Determination methods

The AD of CP and AA in single diets with wheat, lupine or casein as the only protein sources was determined by the direct method. The difference method involved the AD calculation in both a basal diet and an assay diet. The daily ileal flows and AD of AA and CP of each individual pig fed lupine or casein basal diet (Lupine+N-free mixture; Casein+N-free mixture) were applied for each individual calculation of AD in wheat assay diets. The assay diets consisted of a mixture of the basal feed ingredient and the assay feed such as wheaat (Lupine+wheat; Casein+wheat). The direct, difference and regression (Reg2) methods were already described by Fan and Sauer (1995a), Fan and Sauer (1995b), Sauer et al. (2000) and GfE (2005). However, the two regression methods used will be described here again in more detail.

The regression method is called ‘Reg2’ because it uses the digestibility measures of a nutrient in two feeds as feed A and feed B (FA and FB). The two feeds wheat and lupine, respectively, were mixed at graded levels in three assay diets. The relations between digestibility of AA in the total assay diets and the contribution of AA from feeds FA and FB to the assay diets can be expressed according to the following equations.
AA at levels $x_1$...$x_n$. The ‘Reg1’ regression is valid for both the excreted undigested AA to determine $E_{LB}$ and the digested AA to determine TD. The slopes $(b)$ of both regressions are identical. The TD was calculated from the amounts of digested AA at the terminal ileum $(y; g/d)$ and the dietary intake of AA from the assay protein $(x; g/d)$ according to Furuya et al. (1986). The calculations in our experiments were modified so the TD of AA was determined from the slope $(b)$ of the linear regression $(y=a+b \times x)$ by the equation:

$$TD(\%) = (1 - b) \times 100 \quad (6)$$

The $E_{LB}$AA were derived from the intercept $(a)$ of the regression equation of excreted undigested AA by extrapolation of the regression line to zero intakes.

### 2.2. Animal trials, housing, and feeding

The Animal Care Committee of the Ministry of Nutrition, Agriculture, Forestry, and Fishery, Schwerin, State Mecklenburg-Vorpommern, Germany (Permission No. 462a-7221/3-013/93), approved the experimental protocol.

Eight adult Goettingen minipig barrows (Ellegard, Dalmoose, Denmark) were fitted with an end-to-end ileo-rectal anastomosis (IRA) conserving the ileo-ceco-colic valve and isolating the colon completely (Hennig et al., 1990; Laplace et al., 1994). Four consecutive experiments (E1–E4) were carried out using a Latin rectangle design according to Hennig et al. (2004) as shown in Table 1. The compositions of the experimental diets are shown in Table 2. As shown in Tables 1 and 2 in E1 the AD of AA was determined in three repetitions with 8 pigs (52±4.9 kg BW; average during the measurement periods) and for wheat calculated by the ‘direct’ (diet 1.1) and ‘difference’ method (diet 1.2). In three groups graded levels of the lupine cultivar ‘Borweta’ mixed with wheat (diets 1.2, 1.3, 1.4) were fed and the AD was determined by Reg2 regression. The E2 was performed with 8 pigs (54±5.0 kg BW; average during the measurement periods) again in three repetitions to determine the AD of AA from lupine and casein each blended with N-free mixture using the ‘direct’ method (diet 2.1, diet 2.2). In addition, diet 2.3 composed of wheat and casein was used to determine AD of wheat AA by ‘difference’ method. The two further experiments E3 and E4 were designed to determine the TD of AA of lupine and of wheat, respectively, using Reg1 regression. The E3 was performed with 6 pigs (62±6.9 kg BW; average during the measurement periods) in three repetitions. Pigs were subdivided into five groups (five graded levels of lupine with N-free mixture) in three repetitions with 5 pigs (67±7.7 kg BW mean average at the measurement periods) subdivided into five groups (five graded levels of wheat with N-free mixture blended: diets 4.1 to 4.5). Each assay diet was fed in consecutive repetition periods each lasting 14 days.

During all experiments the feed supply was restricted to a daily level of 35 g DM kg BW$^{-0.75}$ and adjusted to BW change every second week. The daily rations were divided into two equal meals mixed with water (1/2.5 wt/wt) and fed at 0700 and 1400. Following the meals and in addition to drinking water, pigs were offered twice daily 300 mL of an electrolyte solution (composition, g/L: 5.38 NaCl, 6.8 CH$_3$COONa×3 H$_2$O, 0.372 KCl, 0.584 CaCl$_2$×6 H$_2$O, 0.304 MgCl$_2$×6 H$_2$O; concentrations, g/L: 3.218 Na, 0.195 K, 0.036 Mg, 0.100 Ca, 3.651 Cl) to prevent mineral depletion due to IRA (Hennig et al., 1986). The pigs consumed solution volumes of 20 to
24 mL kg BW$^{-0.75}$ d$^{-1}$, and the daily Na supply was adjusted to 2.5-fold of requirements of intact pigs.

Pigs were housed individually in floor pens (3 m$^2$ per pig) for 1 week adaptation to the diet and during the following 7 days in metabolic cages (0.8 m$^2$ per pig), which comprised 3 days for adaptation and 4 days for collection of ileal effluents. The animal room was air conditioned, and temperature- and light-controlled (18 to 20 °C; 0600 to 1800 lighting). The IRA pigs were used because their ileal effluent can be collected quantitatively via the rectum and no marker substance is necessary to calculate digestibility. All animals were in good health, they were washed daily with warm water and soap, and the anal region was regularly treated with ointment containing zinc oxide.

2.3. Chemical composition of dietary ingredients

The analyzed crude protein (CP) and AA concentrations in feedstuffs used (wheat, lupine, casein) are shown in Table 3. To minimize the effect of endogenous AA on AD values, and based on data of Furuya and Kaji (1989) and Fan et al. (1994), the German Society of Nutrition Physiology (GfE, 2002) introduced the following threshold levels in assay diets (g kg$^{-1}$ DM): lysine 9.0, threonine 7.0, methionine 3.0, and leucine 12.0. The assay diets were supplemented with crystalline AA up to the above-mentioned levels to determine the AD. Crystalline AA are considered to be 100% digestible (Chung and Baker, 1992) and thus should not be added to the intake. The experimental design, collection, treatment and analyses of feed and ileal effluent were conducted according to the recommendation (GfE, 2005). The wheat and lupine batches were ground using a hammer mill (Mühlenbau GmbH, Dresden, Germany) to pass a 2.5 mm sieve. 

Species ‘Borweta’ from breeding station Bornhof, Mecklenburg-Vorpommern, donated from IG Pflanzenzucht GmbH, Dr. Kendelbacher, München, Germany.
2.4. Sampling and analysis of intestinal effluents and feeds

The ileal effluents were quantitatively collected in containers with a solution of methanol and formaldehyde (99.5/0.5 vol/vol; ≈10 mL to 100 g effluent) to prevent microbial activity. Total amounts were sampled twice a day, pooled and frozen at −18 °C. At the end of the collection period, the total effluent was weighed, homogenized and samples were taken for freeze-drying in order to avoid N losses. The weights of samples were recorded before and after lyophilisation. Dried digesta samples as well as feed samples were ground through a 1 mm mesh screen. The contents of DM and N were determined using standard procedures (AOAC, 1995). Three hydrolysates of two samples of each diet and dried effluents of all individual animals were prepared. Cysteine and methionine were determined as cysteic acid and methionine sulfone after oxidation with performic acid (16 h at 0 °C) followed by hydrolysis with 6 N HCl (22 h at 110 °C). An alkaline hydrolysis was used to determine tryptophan (4 N NaOH, 26 h at 110 °C). All other AA were determined following hydrolysis with redistilled 6 N HCl (22 h at 110 °C). The AA contents in the dried effluents and diets were determined quantitatively with liquid ion exchange chromatography and post-column ninhydrin derivatization (“Biochrom 20”, Pharmacia LKB Biochrom Ltd., Cambridge, England).

2.5. Calculations and statistical analysis

To calculate the LSM and SE using Reg2 the AD of AA for each individual pig was calculated separately. For that reason, pigs A, B, C, D, G and H received two diets and pigs E and F received three diets with varied graded portions of lupine and wheat. In E3 and E4, the amounts of AA excreted in ileal effluents were expressed as g kg⁻¹ DMI. From these values, the amounts of digestible CP and AA (g kg⁻¹ DM) and the digestibility (%) were calculated.

Due to constant environmental conditions during the experiments (see housing and feeding), the results of consecutive periods in E1 and E2 were comparable, and in addition, the results of E3 and E4 were comparable also. Therefore, no time-sequence analyses were needed between E1 + E2 and E3 + E4. Body weight-dependent changes in AD of CP and AA could be excluded (Laplace et al., 1994).

All statistical analyses were done with the SAS System for Windows, release 9.1.3 (SAS/STAT Institute Inc., 2004). The effect of different determination methods of AD was analyzed by one-way ANOVA using the GLM procedure where the fixed effect was the method (direct, difference, regression). Each feedstuff (lupine, wheat) was evaluated separately. Additionally, the LS means and their standard errors (SE of LSM) were determined and tested pairwise. The TD was investigated by linear regression analyses using the REG procedure for both lupine and wheat. The intercepts and slopes of the two linear regression functions were estimated and tested (equal or not) for each feedstuff using the GLM procedure, where the class variable feed was fixed, and the nutrient intake and the interaction feed×nutrient intake were variables.

Table 3
Analyzed chemical composition of wheat, lupine, and casein (g kg⁻¹ DM)

<table>
<thead>
<tr>
<th>Items</th>
<th>Wheat</th>
<th>Lupine</th>
<th>Casein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter</td>
<td>983</td>
<td>964</td>
<td>979</td>
</tr>
<tr>
<td>Crude protein</td>
<td>159</td>
<td>371</td>
<td>956</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>26</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>23</td>
<td>156</td>
<td>0</td>
</tr>
<tr>
<td>Sugar</td>
<td>29</td>
<td>73</td>
<td>n.d.</td>
</tr>
<tr>
<td>Starch</td>
<td>591</td>
<td>7</td>
<td>n.d.</td>
</tr>
<tr>
<td>Amino acids:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>6.0</td>
<td>36.4</td>
<td>34.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>4.1</td>
<td>10.7</td>
<td>33.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.4</td>
<td>15.0</td>
<td>51.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>9.7</td>
<td>25.1</td>
<td>82.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.3</td>
<td>17.4</td>
<td>72.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.0</td>
<td>1.9</td>
<td>25.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>7.1</td>
<td>14.0</td>
<td>49.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.4</td>
<td>12.1</td>
<td>41.7</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.6</td>
<td>2.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Valine</td>
<td>7.0</td>
<td>15.6</td>
<td>59.9</td>
</tr>
<tr>
<td>Cysteine</td>
<td>3.0</td>
<td>4.6</td>
<td>4.2</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.2</td>
<td>12.7</td>
<td>28.5</td>
</tr>
<tr>
<td>Asparagine</td>
<td>7.5</td>
<td>34.9</td>
<td>65.3</td>
</tr>
<tr>
<td>Glutamine</td>
<td>31.0</td>
<td>69.7</td>
<td>159.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.0</td>
<td>15.0</td>
<td>19.0</td>
</tr>
<tr>
<td>Proline</td>
<td>16.9</td>
<td>16.2</td>
<td>107.5</td>
</tr>
<tr>
<td>Serine</td>
<td>6.6</td>
<td>16.3</td>
<td>50.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.7</td>
<td>10.4</td>
<td>54.8</td>
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</table>

Table: Amino acids, continued:

<table>
<thead>
<tr>
<th>Items</th>
<th>Wheat</th>
<th>Lupine</th>
<th>Casein</th>
</tr>
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<tbody>
<tr>
<td>Alanine</td>
<td>5.2</td>
<td>12.7</td>
<td>28.5</td>
</tr>
<tr>
<td>Glutamine</td>
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<tr>
<td>Serine</td>
<td>6.6</td>
<td>16.3</td>
<td>50.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.7</td>
<td>10.4</td>
<td>54.8</td>
</tr>
</tbody>
</table>


3. Results

3.1. Ileal apparent digestibility (AD) of amino acids determined with direct, difference and Reg2 methods

The analyzed concentrations of CP (15 to 20%), lysine, threonine, methionine and leucine in all diets of E1 and E2 were in the range recommended for assay diets (GfE, 2002, 2005). The chemical composition of all diets is calculable by means of Tables 2 and 3. Our analyzed CP and AA concentrations of the complete diets (not presented here) were very close to the calculated ones. The apparent AA digestibilities were determined on the
threshold intake levels. The adult miniature pigs remained healthy and consumed mostly completely their targeted daily rations.

As shown in Table 4, there were no significant differences in AD of CP and any AA in lupine obtained with the two determination methods (all \( P > 0.05 \)). The AD of indispensable AA in lupine ranged between 62% for methionine and 95% for arginine, whereas for dispensable AA it ranged between 76% for alanine and 92% for tyrosine.

In casein the AD of indispensable AA ranged from 91% for isoleucine up to 98% for phenylalanine and of dispensable AA from 83% for cysteine up to 93% for asparagine.

As demonstrated in Table 5 in wheat, there were no significant differences in AD of CP and any AA obtained with three methods (all \( P > 0.05 \)). In addition, there were no differences in AD of CP and any AA in wheat determined by the difference method in the event that both lupine and casein were used as basal diets. The mean AD of dispensable AA in wheat ranged from 81% for lysine and threonine up to 90% for phenylalanine, whereas for dispensable AA it ranged between 79% for asparagine and 94% for glutamine.

Table 4

<table>
<thead>
<tr>
<th>Protein source</th>
<th>Lupine</th>
<th>Casein</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Direct</td>
<td>Reg2</td>
</tr>
<tr>
<td>Crude protein(^{b})</td>
<td>83.5</td>
<td>82.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>94.9</td>
<td>95.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>87.9</td>
<td>88.5</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>82.7</td>
<td>87.2</td>
</tr>
<tr>
<td>Leucine</td>
<td>85.9</td>
<td>85.9</td>
</tr>
<tr>
<td>Lysine</td>
<td>85.5</td>
<td>83.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>83.4</td>
<td>63.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>85.5</td>
<td>85.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>79.5</td>
<td>79.4</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>82.6</td>
<td>84.5</td>
</tr>
<tr>
<td>Valine</td>
<td>79.1</td>
<td>79.9</td>
</tr>
<tr>
<td>Cysteine</td>
<td>84.6</td>
<td>83.0</td>
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<tr>
<td>Alanine</td>
<td>75.8</td>
<td>74.9</td>
</tr>
<tr>
<td>Asparagine(^{c})</td>
<td>85.6</td>
<td>85.6</td>
</tr>
<tr>
<td>Glutamine(^{d})</td>
<td>91.6</td>
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<tr>
<td>Glycine</td>
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<tr>
<td>Serine</td>
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<td>86.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>92.2</td>
<td>93.4</td>
</tr>
</tbody>
</table>

Number of animals investigated were each \( n=8 \) (lupine, direct method), \( n=8 \) (lupine, Reg2 regression method), and \( n=7 \) (casein, direct method).

\(^{a}\) \( P \)-values indicates effect of main factor ‘method’ used \( F \)-test.
\(^{b}\) Nitrogen \( \times 6.25 \).
\(^{c}\) Aspartate + asparagine.
\(^{d}\) Glutamate + glutamine.

3.2. Ileal true digestibility (TD) and ileal losses of amino acids determined by Reg1 and with casein diet

As shown in Table 6, the TD of indispensable AA in lupine ranged from 83% for methionine to 97% for arginine, whereas for dispensable AA it ranged between 85% for alanine and 95% for glutamine. Compared with the corresponding AD in Table 4 the TD values of methionine, threonine, tryptophan, and valine were 20, 11, 9, and 12%-units higher, respectively, and the difference between AD and TD for CP and most other indispensable AA in lupine was 8%-units.

The TD of indispensable AA in wheat ranged from 89% for methionine to 95% for histidine, whereas for dispensable AA it ranged from 89% for alanine and asparagine to 99% for proline. Compared with the corresponding AD in Table 5 the TD values of lysine, methionine, threonine, tryptophan, and valine were 11,
The ileal TD of CP in lupine and wheat was equal \((P = 0.180)\). This implicates an equivalent ileal TD of all indispensable and dispensable AA too, except for arginine \((P = 0.003)\), glutamine \((P = 0.030)\), and proline \((P = 0.001)\).

In contrast, as shown in Table 6, the ileal losses (g kg\(^{-1}\) DMI) of various AA as arginine, histidine, leucine, methionine, phenylalanine, tryptophan, cysteine, glutamine, and serine were significantly different in lupine and wheat \((P = 0.008\) to 0.047). The difference for ileal loss of lysine was of borderline significance \((P = 0.059)\). As a result, the ileal loss of CP and most of AA determined in wheat was lower than in lupine.

Determined by the casein diet, the assumed ileal EL\(_b\) of CP was higher than in the specific determination in lupine. There were larger ileal losses of isoleucine, valine, glutamine, proline, and serine in casein compared with the wheat diet.

3.3. Calculation of ileal standardized digestibility (SD)

In Table 7, the results of conversion AD to SD of CP and AA in lupine and wheat are presented. The SD were calculated using the equation of Furuya and Kaji (1989) considering the contents of CP and AA of lupine and wheat (g kg\(^{-1}\) DMI) of the two selected diets (diet 2.1 and diet 1.2). The SD were calculated using (a) our own corresponding lupine and wheat-specific EL\(_b\) and (b) our own casein-specific assumed EL\(_b\) and (c) means of EL\(_b\) given by (GfE, 2005).

In lupine, the SD of indispensable AA ranged from 80\% for methionine to 97\% for arginine, whereas for dispensable AA it ranged between 82\% for alanine and 95\% for glutamine. The SD of indispensable AA in wheat ranged from 88\% for lysine to 93\% for histidine, whereas for dispensable AA it ranged between 88\% for alanine and 98\% for proline. In both feeds there were no important
differences (borderline >5%-units) between the corresponding values for SD and TD of AA by using our own feed-specific ELbAA (intercepts of Reg1).

When using the ileal losses of the casein diet, the calculated SD of AA in lupine was larger for isoleucine, valine, and serine compared with calculated SD values determined by using lupine-specific ELbAA. In contrast, the SD values for methionine and cysteine were lower. The calculation of SD by using the ELbAA given by (GfE, 2005) resulted in similar values for CP and most of indispensable AA in lupine. However, larger values were calculated for methionine, threonine, tryptophan and tyrosine compared with values calculated by using own lupine-specific ELbAA.

Using the ileal losses determined with casein, the calculated SD of AA in wheat resulted in larger values for isoleucine, methionine, valine, glutamine, and serine when compared with values using wheat-specific ELbAA. The calculation of SD of AA by using the ELbAA given by (GfE, 2005) resulted in larger values for lysine, threonine, tryptophan, valine, and tyrosine.

4. Discussion

Two regression analyses techniques are available as mathematical tools to determine both the AD and the TD of nutrients. To our knowledge, we are the first to present comparisons between apparent and true digestibility in lupine and wheat derived from these two types of linear regressions in vivo. Furthermore, these are the first comparisons between experimentally determined TD and calculated SD (SD as a proxy for TD) based on AD and separately determined ELbAA.

4.1. Apparent digestibility of amino acids determined with direct, difference and Reg2 methods

Our results agree with results using peas (Fan and Sauer, 1995b) where the digestibility values were not different when they were determined with direct, difference and regression methods. A comparison with literature data showed that our lupine cultivar ‘Borweta’ compared with
L. angustifolius (Fernandez and Batterham, 1995) had similar AD of CP, valine and cysteine. However, the AD of histidine, isoleucine, leucine, lysine, phenylalanine, threonine, and tyrosine were remarkably higher, and that of methionine was lower. This could be a specific result for our lupine cultivar which contained 6% more CP, 1.6% smaller CF, and higher AA concentrations. With both methods (direct; Reg2) we obtained similarly low apparent methionine digestibility values.

In the case of wheat, our results are different from the experiment with barley reported by Fan and Sauer (1995a). In barley the AA digestibility determined by direct and difference methods differed whereas the AD of AA for our wheat sample were equal when these were determined with direct, difference and Reg2 methods, probably due to the higher CP and AA concentrations in our wheat.

To calculate the AD of wheat AA by means of the difference method we used diets 1.2, 1.3 and 1.4. Values from the diet 1.2 with 113 g lupine and 756 g wheat kg\(^{-1}\) DM were presented here only because the results obtained with the other two diets (with lower contribution levels of wheat: 472 and 189 g kg\(^{-1}\)) showed very high standard deviations in agreement with findings of Fan and Sauer (1995a). This was also reported for peas by Fan and Sauer (1995b).

The presented AD of AA for casein obtained with minipigs were equal with reported values obtained with Landrace pigs (Hennig et al., 2006) and with similar casein diets (Kies et al., 1986; Darcy-Vrillon et al., 1991; Moughan et al., 1996; Officer et al., 1998; Traylor et al., 2001). Nevertheless, all these experiments showed that the AD of casein AA cannot be assumed to be 100%. The ileal effluent probably consists of both endogenous AA and small amounts of undigested AA (or peptides).

The EL\(_b\) of AA is the main variation factor in determination of AD. According to Stein et al. (1999), the ileal AD obtained in growing pigs are not always representative of digestibilities in adults (gestating or lactating sows). On the other hand, the ileal SD of AA were equal in growing pigs and lactating sows but in gestating sows, the SD was higher than in the both other. This difference may be due to differences in daily feed intake rather than to the physiological status of the animals (Stein et al., 2001). Additional, to minimize the variation of AD and to preserve comparability in our experiments we designed an equal DMI per kg metabolic BW.

4.2. True digestibility and ileal losses of amino acids

The TD of CP and of all AA in lupine and wheat were in the same range and on the same levels with the few exceptions being arginine, glutamine, and proline (Table 6).

The TD of AA in lupine was remarkably higher than the corresponding AD presented in Table 4 especially for methionine (20%-units) but also for valine, threonine, tryptophan, lysine, leucine, and histidine (12 to 8%-units). The EL\(_b\)AA might explain these differences. The TD of indispensable AA in wheat was in the range of 89% for methionine up to 95% for histidine. The TD of isoleucine, valine, threonine, and lysine in wheat were 9 to 11%-units higher than the corresponding AD presented in Table 5. The EL\(_b\)AA might explain these differences again. Thus, it was a logical deduction to calculate the SD of AA in both feeds from their apparent AD by using the feed-specific EL\(_b\)AA as obtained by Reg1. The EL\(_b\) AA determined in lupine and wheat cannot be assumed equal as shown for seven indispensable and three dispensable AA (Table 6). The ileal loss of 195 mg methionine kg\(^{-1}\) DMI in the lupine diet is high and differed from ileal loss of 111 mg kg\(^{-1}\) DMI determined in the wheat diet (P=0.036) or 111 mg given by GfE (2005). In contrast, the ileal loss of 590 mg threonine, 140 mg tryptophan, 530 mg valine, or 290 mg tyrosine kg\(^{-1}\) DMI reported by GfE (2005) are too high and led to enlarged SD values.

Furthermore, the ileal losses for most AA obtained when pigs are fed a 17% casein diet are in a similar range when determined by Reg1 as compared to those values given by GfE (2005). However, with the casein diet again several AA such as isoleucine, methionine, valine, glutamine and serine are relatively high in the ileal effluent, which will cause enlarged SD values.

4.3. Standardized digestibility

As we have assumed, the SD levels are very close to the TD levels if calculated with our feed-specific EL\(_b\)AA (Reg1). In contrast, SD values will be over- and underestimated, respectively, if the ileal losses of several AA are too large or too small (originating from casein diet or tables with means). Our present results show that the AD levels consequently differ from the SD level but the latter are mostly quite similar to the experimentally determined TD as expected. Therefore, the SD could be a valid proxy for TD. We thus support the conclusion of Fan et al. (1995) that the TD of AA should be calculated from the ileal AD AA digestibilities on the proposed threshold levels for AA intakes. This ‘TD’ has been recently termed ‘standardized digestibility’, which means AD of AA corrected by basal endogenous losses of AA. But there are some pitfalls in these calculations when we used both the losses of AA in feeding casein
diet and ‘constant’ means for EL₆AA according to GfE (2005) and Jansman et al. (2002) as shown in Table 7.

In lupine, after transforming AD to SD of AA by using lupine-specific EL₆AA, the SD agrees well with TD. On the other hand, the SD values of isoleucine, valine, and serine are too large if calculated by using ileal losses after feeding casein. Using the values of GfE (2005), the SD of methionine is underestimated and the SD of threonine, tryptophan, and tyrosine are overestimated compared with SD based on our lupine-specific EL₆AA.

After transforming AD to SD of AA in wheat by using wheat-specific EL₆AA, the numeric differences between corresponding values for SD and TD of AA were minimized against zero. However, the SD values of isoleucine, methionine, valine, glutamine, and serine are too large (several values > 100) if calculated by using ileal losses after feeding casein. These results support our assumption that a complete absorption of all AA (or peptides) from casein does not occur.

Interestingly, if the mean ileal losses of 390 mg lysine, 590 mg threonine, 140 mg tryptophan, 530 mg valine and 290 mg tyrosine kg⁻¹ DMI originating from GfE (2005) were used, the calculated SD were too large again compared with our own values. It can be assumed that the above-mentioned EL₆AA values of GfE were too large in order to estimate SD of those AA in wheat.

A weakness of the EL₆AA is that they are highly variable because of the possible different experimental approaches for their determination (see Jansman et al., 2002). Secondly, in agreement with our findings when using the RegI, the EL₆AA for most of the indispensable AA depend on the feedstuff used (Pedersen et al., 2002).

Thirdly, according to Butts et al. (1993), Hess and Seve (1999) and Moter and Stein (2004), the EL₆AA of numerous AA change with different feeding levels. At a low feeding level, the EL₆AA (g kg⁻¹ DMI) were higher than at medium or high feeding level, which further weakens the validity of using a ‘constant’ EL₆AA. However, in pigs larger than 70 kg BW the EL₆AA were constant regardless of the feeding level. A fourth problem is that the patterns of EL₆AA could be affected by the AA pattern of microbial protein as shown by Hennig et al. (1998). With protein-free feeding, 50% of the collected protein at the terminal ileum and 74, 68, 60, and 59% of the ileal lysine, methionine, threonine, and tryptophan, respectively, were of bacterial origin. Finally, Dilger et al. (2004) showed a high variability for EL₆AA among animals within one group. The results of Hess and Seve (1999) and Dilger et al. (2004) strongly indicate that an assessment of the EL₆AA must be performed for each pig to derive SD from the AD data.

5. Conclusion

In order to avoid the above-mentioned pitfalls with the use of means of EL₆AA, we conclude that the direct determination of TD by using RegI seems to be a useful alternative to replace the calculation of SD using doubtful ‘constants’.

References


