An in vitro method for the estimation of ileal crude protein and amino acids digestibility using the dialysis tubing for pig feedstuffs

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Received 28 May 1999; received in revised form 15 December 1999; accepted 31 July 2000

Abstract

A new in vitro method using the dialysis tubing for predicting the ileal apparent digestibility (IAD) of crude protein (CP) and amino acids (AA) was proposed for pig feedstuffs. Under the laboratory condition, the procedure of the in vitro dialysis tubing method was as follows: a 1.0 g sample of feedstuff was placed in a 100 ml Erlenmeyer flask, 10 ml hydrochloric acid (0.01 M, pH = 20) pepsin solution (1.0 mg pepsin/ml HCl) was added, and the mixture was incubated for 4 h at 37°C. Then after neutralization with 0.2 M sodium hydroxide, this mixture was transferred into a prepared dialysis tubing with 40 ml phosphate buffered saline (PBS) solution (0.02 M NaHPO₄, 0.02 M Na₂HPO₄, and pH = 7.6) and 10 ml trypsin PBS solution (2.0 mg trypsin/ml PBS) was added. Then, the open side of the dialysis tubing was tied tightly with nylon threads so that the liquid could not leak out. Finally, the dialysis tubing was put into a 1000 ml conical flask which was sealed with parafilm containing 300 ml dialytic liquid (PBS solution). The flask was incubated for an additional 24 h at 37°C. During the incubation period, the digested CP or AA could infiltrate into the 300 ml PBS solution. After the two-stage incubation, the in vitro CP and AA digestibility was calculated on the basis of the original CP and AA contents of feedstuffs and the CP and AA contents of the dialyzed solution. Linear regression equations were obtained between in vitro digestibilities and IAD of CP and AA for four feedstuffs commonly used for growing pigs (0.96 < r < 0.99). The results show that it is possible to predict IAD of feedstuffs using the in vitro digestibility data for growing pigs. This method is accurate, rapid, reproducible and particularly suited for the evaluation of a large number of samples under laboratory condition. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Crude protein; Amino acids; In vitro digestibility; Pig
1. Introduction

The ileal digestibility value of crude protein (CP) and amino acids (AA) is one of the most valuable measurements for the estimation of the nutritive value of feedstuffs. Although the ileal digesta collection method (ileal apparent digestibility, IAD) (Yin et al., 1991, 1993a,b, 1994) is commonly used in digestibility experiments, the procedures are tedious, time-consuming, and require large quantities of feedstuffs. The in vitro two-stage incubation with intestinal fluid or semi-purified enzyme preparations has been widely used for evaluating the nutritive value of pig feedstuffs, but it has some disadvantages in the determination of CP and AA IAD (Furuya et al., 1979; Zhang and Nie, 1986; Liang and Zhang, 1988). However, there has been no attempt to use the dialysis tubing technique that imitates the gastrointestinal environment of growing pigs. In non-ruminant animals, such as the pig, the digestion of major nutrients starts in the stomach and is essentially completed in the small intestine. During the intestinal digestion phase, the pancreatic enzymes hydrolyze feedstuff components to the terminal stage just preceding final hydrolysis or absorption by brush-border-bound systems. In the stomach phase of digestion, hydrochloric acid and pepsin are the hydrolytic agents. In the intestinal digestion phase, trypsin is the hydrolytic agent (Lu, 1995). Furthermore, the dialysis tubing and phosphate buffered saline (PBS) solution are the ideal materials to imitate the digestion environment of the intestinal tract. Therefore, it is reasonable that CP and AA IAD of feedstuffs may be estimated by the in vitro dialysis tubing method, if the actual digestion occurring in the stomach and intestine can be simulated. The objectives of the current study were to establish an in vitro dialysis tubing method so that IAD of CP and AA can be estimated according to the regression equations between in vitro and IAD of CP and AA for pig feedstuffs.

2. Materials and methods

2.1. Feedstuffs, dialysis tubing and digestion enzymes

Four feedstuffs, fish meal (Peru), fish meal (China), rapeseed meal and cottonseed meal were sampled, and all samples were ground in the laboratory mill to pass through a 1 mm screen. The chemical composition is shown in Table 1. The dialysis tubing (D-9402) was purchased from Sigma (St Louis, USA), its diameter was 49 mm. The dialysis tubing was cut into pieces of 20 cm. Firstly glycerin was removed by washing the tubing in running water for 3–4 h, then sulfur compounds were removed by treating the tubing with a 0.3% (w/v) solution of sodium sulfide at 80°C for 1 min. Subsequently, the tubing was washed with hot water (60°C) for 2 min, followed by acidification with a 0.2% (v/v) solution of sulfuric acid, and finally the tubing was rinsed with hot water to remove the residual acid. This tubing will retain most of the molecular compounds of molecular weight 12,000 Da or greater in the dialyzed liquid. Finally, the dialysis tubing was soaked in distilled water at 4°C before usage. The pepsin (EC 3.4.23.1, P-7000, 1:10,000) and trypsin (EC 3.4.21.4, T-4799, 1:250) were also supplied by Sigma.
2.2. In vitro procedures

Considering that the conditions of the first stage incubation (pepsin incubation) are relatively simple and have been studied previously (Furuya et al., 1979; Zhang and Nie, 1986; Liang and Zhang, 1988), it was concluded that it was especially important to determine the conditions of the second stage incubation individually. Thus, if an experimental condition was determined, it would not be changed in the following experiments. The suitable incubation conditions depend on the digestibility of the experimental feedstuff. Experimental conditions of the two-stage in vitro digestibility were determined in experiment 1 using the Peru fish meal as experimental feedstuff, and the standard procedures were developed on the basis of these results.

2.2.1. The standard conditions of pepsin incubation

The initial standard conditions of pepsin incubation were as follows: a 1.0 g sample was placed in a 100 ml Erlenmeyer flask, 10 ml hydrochloric acid (0.01 M, pH = 2.0) pepsin solution (1.0 mg pepsin/ml HCl) was added, and the mixture was incubated for 4 h at 37°C.

2.2.2. In vitro and in vivo CP and AA digestibility

Experiment 1. The effect of incubation period, volume of dialytic liquid (PBS), trypsin concentration and pH of trypsin solution in the second stage on CP in vitro digestibility were investigated. Firstly, the incubation period was determined under standard

<table>
<thead>
<tr>
<th>Composition</th>
<th>Fish meal (Peru)</th>
<th>Fish meal (China)</th>
<th>Rapeseed meal</th>
<th>Cottonseed meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (DM)</td>
<td>885.9</td>
<td>857.7</td>
<td>874.7</td>
<td>874.6</td>
</tr>
<tr>
<td>Organic matter (OM)</td>
<td>726.4</td>
<td>678.8</td>
<td>803.5</td>
<td>784.1</td>
</tr>
<tr>
<td>Crude protein (CP)</td>
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<td>530.5</td>
<td>389.6</td>
<td>381.0</td>
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<tr>
<td>Aspartic acid (Asp)</td>
<td>39.5</td>
<td>22.9</td>
<td>29.4</td>
<td>32.2</td>
</tr>
<tr>
<td>Glutamic acid (Glu)</td>
<td>69.4</td>
<td>58.0</td>
<td>68.6</td>
<td>69.7</td>
</tr>
<tr>
<td>Serine (Ser)</td>
<td>24.3</td>
<td>41.7</td>
<td>19.1</td>
<td>16.7</td>
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<td>Glycine (Gly)</td>
<td>76.4</td>
<td>40.4</td>
<td>19.6</td>
<td>15.3</td>
</tr>
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<td>Histidine (His)</td>
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<td>9.5</td>
<td>11.9</td>
<td>9.8</td>
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<td>Arginine (Arg)</td>
<td>50.1</td>
<td>48.4</td>
<td>26.0</td>
<td>40.7</td>
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<tr>
<td>Threonine (Thr)</td>
<td>25.5</td>
<td>26.5</td>
<td>19.0</td>
<td>13.1</td>
</tr>
<tr>
<td>Alanine (Ala)</td>
<td>51.4</td>
<td>28.9</td>
<td>21.2</td>
<td>21.0</td>
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<tr>
<td>Proline (Pro)</td>
<td>56.2</td>
<td>41.6</td>
<td>28.1</td>
<td>15.0</td>
</tr>
<tr>
<td>Tyrosine (Tyr)</td>
<td>15.2</td>
<td>26.0</td>
<td>12.1</td>
<td>8.7</td>
</tr>
<tr>
<td>Valine (Val)</td>
<td>26.5</td>
<td>30.6</td>
<td>19.6</td>
<td>14.1</td>
</tr>
<tr>
<td>Methionine (Met)</td>
<td>14.6</td>
<td>8.2</td>
<td>3.6</td>
<td>3.0</td>
</tr>
<tr>
<td>Isoleucine (Ile)</td>
<td>20.9</td>
<td>21.9</td>
<td>15.3</td>
<td>9.8</td>
</tr>
<tr>
<td>Leucine (Leu)</td>
<td>38.9</td>
<td>44.7</td>
<td>29.0</td>
<td>20.4</td>
</tr>
<tr>
<td>Phenylalanine (Phe)</td>
<td>23.7</td>
<td>23.5</td>
<td>15.8</td>
<td>17.6</td>
</tr>
<tr>
<td>Lysine (Lys)</td>
<td>36.2</td>
<td>19.0</td>
<td>18.0</td>
<td>12.9</td>
</tr>
<tr>
<td>Total amino acids (ΣAA)</td>
<td>587.8</td>
<td>491.8</td>
<td>356.3</td>
<td>320.0</td>
</tr>
</tbody>
</table>
conditions of pepsin incubation, volume of dialysis liquid 200 ml, trypsin concentration
0.5 mg/ml, and pH of trypsin solution 7.60, the selected incubation periods were 4, 8, 12, 16, 20, 24 and 28 h with three replicates for each incubation period. The most suitable
incubation period was determined when the in vitro CP digestibility of fishmeal (Peru)
reached a maximum, and this condition was not changed when the other incubation
conditions continued to be determined. According to the above principles, volume of the
dialysis liquid, trypsin concentration and pH of PBS were determined one-by-one. The
selected volumes of dialysis liquids were 100, 200, 300, 400 and 500 ml; trypsin
concentrations were 0.1, 0.3, 0.5, 1, 2 and 5 mg/ml; pH values of PBS were 7.00, 7.60 and
8.00, respectively. The in vitro digestibility of CP and AA (I) was calculated as follows:

\[ I = \frac{D}{S} \]

where \( D \) is the weight of CP or AA in the dialytic liquid and \( S \) the weight of CP or AA in
the feed sample.

Experiment 2. The in vitro digestibility of CP and AA was performed following the
determined procedures described above for four pig feedstuffs: Peru fish meal, China fish
meal, rapeseed meal and cottonseed meal.

Experiment 3. CP and AA IAD values of the same four feedstuffs were determined.
Four ileo-rectal anastomosed Yorkshire × Chinese Black barrows, with an average initial
weight of 50 kg, were used to measure IAD of CP and AA for four protein rich feedstuffs
in a 4×4 Latin square design. The detailed in vivo procedure was described by Yin et al.
(1993a).

2.3. Analytical procedures

Samples were analyzed for dry matter (DM), organic matter (OM) and CP using the
technique outlined by the Association of Official Analytical Chemists (1980). AA
compositions of samples (i.e. feedstuffs and ileal digesta) were determined by
derivatization with phenylisothiocyanate (PITC) and detection by fluorescence using a
high performance liquid chromatograph (Pico-Tag, Waters, USA), after hydrolysis in 6 N
HCl solution in sealed vacuum tubes at 110°C for 24 h. The procedure for the analysis of
AA composition in the dialyzed liquid was as follows: 1000 μl dialyzed liquid was
transferred into a Waters™ 4 mm × 4 cm glass tube and freeze-dried, then protected and
acidified according to the method described by Hoogerheide and Campbell (1992). Its
AA composition was measured using a high performance liquid chromatograph (Pico-
Tag, Waters, USA) after derivatization with PITC.

2.4. Statistical analyses

The relationships between the in vitro digestibilities and IAD, and the analyses of
variance to test the effect of incubation period, volume of dialytic liquid, trypsin
concentration and pH of trypsin solution in the second stage on the in vitro digestibility
were performed by the CORR and ANOVA procedures of the Statistical Analysis
3. Results

3.1. Effect of in vitro conditions on the in vitro digestibility

The effect of incubation period on CP in vitro digestibility is shown in Fig. 1. When trypsin incubation period was increased from 4 to 24 h, CP in vitro digestibility increased significantly ($P < 0.05$) for fish meal (Peru), whereas incubation period had no significant effect on the CP in vitro digestibility from 24 to 28 h. It was concluded that the trypsin incubation period should be 24 h.

The effect of volume of PBS on CP in vitro digestibility is represented in Fig. 2. When the volume of PBS was increased from 100 to 300 ml, CP in vitro digestibility increased significantly ($P < 0.05$). There was no significant effect on CP in vitro digestibility in the range 300–500 ml dialytic liquid. In view of the small benefit in the digestibility by increasing the dialysis liquid volume from 300 to 500 ml, the 300 ml volume was chosen as a practical volume. Thus, the suitable volume of dialysis liquid should be 300 ml or so if thinking of simplification of operation procedure.

When trypsin concentration was increased from 0.1 to 2 mg/ml, CP in vitro digestibility also increased significantly ($P < 0.01$), see Fig. 3. There was little difference between 2 and 5 mg/ml trypsin concentration. Therefore, the effect of pH of trypsin solution of 2 mg/ml was selected as a suitable concentration.

Finally, the effect of pH of trypsin solution on CP in vitro digestibility was examined (see Fig. 4). From the three experimental trypsin solution pH values, the pH of 7.60 reached the highest CP in vitro digestibility. These results indicated that pH of 7.60 is suitable for the second stage of the standard in vitro procedure.

![Fig. 1. Effect of trypsin incubation time on CP in vitro digestibility. Standard conditions of pepsin incubation are as in Experiment 1.](image-url)
3.2. CP and AA in vitro digestibilities of four feedstuffs

The CP and AA in vitro digestibilities of Peru fish meal, China fish meal, rapeseed meal and cottonseed meal, measured using the standard in vitro digestion procedures, are given in Table 2. The results show that there were significant differences \((P < 0.05\) or \(P < 0.01\)) in CP and AA in vitro digestibility among the four feedstuffs. Their in vitro digestibility decreased significantly in the following order: Peru fish meal > rapeseed meal > cottonseed meal > China fish meal.

![Graph](image1)

Fig. 2. Effect of dialysis liquid volume on CP in vitro digestibility. Standard conditions of pepsin incubation are as in Experiment 1.

![Graph](image2)

Fig. 3. Effect of trypsin concentration on CP in vitro digestibility. Standard conditions of pepsin incubation are as in Experiment 1.
3.3. IAD of CP and AA for four pig feedstuffs

The IAD of CP and AA for four feedstuffs showed similar tendencies as the CP and AA in vitro digestibilities (see Table 3). There were significant differences ($P < 0.05$ or $P < 0.01$) among the four feedstuffs, their IAD of CP and AA differed significantly between feedstuffs.

Table 2
In vitro digestibility of CP and amino acids in four pig feedstuffs (means all followed by their standard deviations; means within a row with different superscripts are significantly different ($P < 0.05$))

<table>
<thead>
<tr>
<th>Item</th>
<th>Fish meal (Peru)</th>
<th>Fish meal (China)</th>
<th>Rapeseed meal</th>
<th>Cottonseed meal</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>0.580$\pm$0.0084</td>
<td>0.476$\pm$0.0081</td>
<td>0.555$\pm$0.0089</td>
<td>0.520$\pm$0.0044</td>
<td>0.0044</td>
</tr>
<tr>
<td>Asp</td>
<td>0.616$\pm$0.0013</td>
<td>0.476$\pm$0.0022</td>
<td>0.585$\pm$0.0038</td>
<td>0.540$\pm$0.0046</td>
<td>0.0019</td>
</tr>
<tr>
<td>Glu</td>
<td>0.621$\pm$0.0027</td>
<td>0.476$\pm$0.0023</td>
<td>0.595$\pm$0.0013</td>
<td>0.527$\pm$0.0042</td>
<td>0.0016</td>
</tr>
<tr>
<td>Ser</td>
<td>0.577$\pm$0.0029</td>
<td>0.464$\pm$0.0011</td>
<td>0.553$\pm$0.0018</td>
<td>0.513$\pm$0.0037</td>
<td>0.0015</td>
</tr>
<tr>
<td>Gly</td>
<td>0.581$\pm$0.0004</td>
<td>0.476$\pm$0.0020</td>
<td>0.546$\pm$0.0033</td>
<td>0.516$\pm$0.0027</td>
<td>0.0014</td>
</tr>
<tr>
<td>His</td>
<td>0.585$\pm$0.0004</td>
<td>0.472$\pm$0.0031</td>
<td>0.530$\pm$0.0030</td>
<td>0.513$\pm$0.0034</td>
<td>0.0016</td>
</tr>
<tr>
<td>Arg</td>
<td>0.590$\pm$0.0033</td>
<td>0.473$\pm$0.0023</td>
<td>0.594$\pm$0.0035</td>
<td>0.520$\pm$0.0034</td>
<td>0.0018</td>
</tr>
<tr>
<td>Thr</td>
<td>0.581$\pm$0.0024</td>
<td>0.489$\pm$0.0012</td>
<td>0.532$\pm$0.0013</td>
<td>0.509$\pm$0.0036</td>
<td>0.0013</td>
</tr>
<tr>
<td>Ala</td>
<td>0.578$\pm$0.0030</td>
<td>0.469$\pm$0.0025</td>
<td>0.521$\pm$0.0022</td>
<td>0.494$\pm$0.0033</td>
<td>0.0016</td>
</tr>
<tr>
<td>Pro</td>
<td>0.572$\pm$0.0028</td>
<td>0.472$\pm$0.0023</td>
<td>0.567$\pm$0.0017</td>
<td>0.512$\pm$0.0014</td>
<td>0.0012</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.625$\pm$0.0024</td>
<td>0.476$\pm$0.0030</td>
<td>0.556$\pm$0.0003</td>
<td>0.517$\pm$0.0021</td>
<td>0.0013</td>
</tr>
<tr>
<td>Val</td>
<td>0.636$\pm$0.0027</td>
<td>0.482$\pm$0.0021</td>
<td>0.574$\pm$0.0040</td>
<td>0.538$\pm$0.0024</td>
<td>0.0017</td>
</tr>
<tr>
<td>Met</td>
<td>0.590$\pm$0.0014</td>
<td>0.478$\pm$0.0033</td>
<td>0.544$\pm$0.0024</td>
<td>0.537$\pm$0.0045</td>
<td>0.0018</td>
</tr>
<tr>
<td>lle</td>
<td>0.606$\pm$0.0010</td>
<td>0.480$\pm$0.0021</td>
<td>0.550$\pm$0.0034</td>
<td>0.525$\pm$0.0044</td>
<td>0.0017</td>
</tr>
<tr>
<td>Leu</td>
<td>0.591$\pm$0.0030</td>
<td>0.473$\pm$0.0022</td>
<td>0.541$\pm$0.0008</td>
<td>0.514$\pm$0.0028</td>
<td>0.0014</td>
</tr>
<tr>
<td>Phe</td>
<td>0.580$\pm$0.0022</td>
<td>0.463$\pm$0.0014</td>
<td>0.545$\pm$0.0011</td>
<td>0.511$\pm$0.0036</td>
<td>0.0013</td>
</tr>
<tr>
<td>Lys</td>
<td>0.593$\pm$0.0028</td>
<td>0.477$\pm$0.0015</td>
<td>0.561$\pm$0.0022</td>
<td>0.537$\pm$0.0065</td>
<td>0.0022</td>
</tr>
<tr>
<td>Total AA</td>
<td>0.595$\pm$0.0025</td>
<td>0.475$\pm$0.0020</td>
<td>0.556$\pm$0.0012</td>
<td>0.520$\pm$0.0026</td>
<td>0.0012</td>
</tr>
</tbody>
</table>
Table 3
Ideal apparent digestibility of CP and AA for four feedstuffs (means all followed by their standard deviations; means within a row with different superscripts are significantly different ($P < 0.05$))

<table>
<thead>
<tr>
<th>Item</th>
<th>Fish meal (Peru)</th>
<th>Fish meal (China)</th>
<th>Rapeseed meal</th>
<th>Cottonseed meal</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>0.927±0.0068</td>
<td>0.811±0.0113</td>
<td>0.893±0.0043</td>
<td>0.856±0.0108</td>
<td>0.0051</td>
</tr>
<tr>
<td>Asp</td>
<td>0.927±0.0048</td>
<td>0.816±0.0074</td>
<td>0.905±0.0109</td>
<td>0.854±0.0041</td>
<td>0.0042</td>
</tr>
<tr>
<td>Glu</td>
<td>0.926±0.0066</td>
<td>0.811±0.0040</td>
<td>0.917±0.0019</td>
<td>0.850±0.0027</td>
<td>0.0024</td>
</tr>
<tr>
<td>Ser</td>
<td>0.936±0.0066</td>
<td>0.813±0.0054</td>
<td>0.878±0.0129</td>
<td>0.849±0.0033</td>
<td>0.0046</td>
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<tr>
<td>Gly</td>
<td>0.930±0.0129</td>
<td>0.810±0.0082</td>
<td>0.887±0.0165</td>
<td>0.851±0.0027</td>
<td>0.0065</td>
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<td>His</td>
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<td>0.809±0.0056</td>
<td>0.913±0.0017</td>
<td>0.849±0.0010</td>
<td>0.0024</td>
</tr>
<tr>
<td>Arg</td>
<td>0.933±0.0026</td>
<td>0.812±0.0046</td>
<td>0.893±0.0041</td>
<td>0.849±0.0013</td>
<td>0.0020</td>
</tr>
<tr>
<td>Thr</td>
<td>0.923±0.0059</td>
<td>0.813±0.0068</td>
<td>0.893±0.0133</td>
<td>0.854±0.0056</td>
<td>0.0049</td>
</tr>
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<td>0.887±0.0051</td>
<td>0.847±0.0058</td>
<td>0.0023</td>
</tr>
<tr>
<td>Pro</td>
<td>0.927±0.0052</td>
<td>0.814±0.0078</td>
<td>0.893±0.0123</td>
<td>0.850±0.0008</td>
<td>0.0045</td>
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<tr>
<td>Tyr</td>
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<td>0.887±0.0066</td>
<td>0.827±0.0062</td>
<td>0.0042</td>
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<td>0.0025</td>
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<tr>
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<td>0.897±0.0013</td>
<td>0.850±0.0007</td>
<td>0.0017</td>
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<tr>
<td>Ile</td>
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<td>0.892±0.0057</td>
<td>0.853±0.0068</td>
<td>0.0041</td>
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<tr>
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<td>0.890±0.0123</td>
<td>0.852±0.0044</td>
<td>0.0040</td>
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<tr>
<td>Phe</td>
<td>0.929±0.0016</td>
<td>0.809±0.0034</td>
<td>0.887±0.0140</td>
<td>0.852±0.0034</td>
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<td>Lys</td>
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<td>0.814±0.0080</td>
<td>0.909±0.0153</td>
<td>0.851±0.0038</td>
<td>0.0053</td>
</tr>
<tr>
<td>Total AA</td>
<td>0.928±0.0010</td>
<td>0.811±0.0014</td>
<td>0.898±0.0047</td>
<td>0.855±0.0015</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

Table 4
Linear regression analysis between IAD (as dependent variable $Y$) and in vitro digestibility (as independent variable $X$) of CP and AA$^a$

<table>
<thead>
<tr>
<th>Item</th>
<th>Equations</th>
<th>RSD</th>
<th>$r$</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>$Y = 0.330 + 1.013X$</td>
<td>0.0006</td>
<td>0.999</td>
<td>**</td>
</tr>
<tr>
<td>Asp</td>
<td>$Y = 0.425 + 0.814X$</td>
<td>0.0085</td>
<td>0.990</td>
<td>**</td>
</tr>
<tr>
<td>Glu</td>
<td>$Y = 0.412 + 0.837X$</td>
<td>0.0067</td>
<td>0.995</td>
<td>**</td>
</tr>
<tr>
<td>Ser</td>
<td>$Y = 0.373 + 0.936X$</td>
<td>0.0184</td>
<td>0.971</td>
<td>*</td>
</tr>
<tr>
<td>Gly</td>
<td>$Y = 0.262 + 1.147X$</td>
<td>0.0028</td>
<td>0.999</td>
<td>**</td>
</tr>
<tr>
<td>His</td>
<td>$Y = 0.314 + 1.058X$</td>
<td>0.0271</td>
<td>0.972</td>
<td>*</td>
</tr>
<tr>
<td>Arg</td>
<td>$Y = 0.411 + 0.848X$</td>
<td>0.0218</td>
<td>0.941</td>
<td>*</td>
</tr>
<tr>
<td>Thr</td>
<td>$Y = 0.263 + 1.153X$</td>
<td>0.0168</td>
<td>0.957</td>
<td>*</td>
</tr>
<tr>
<td>Ala</td>
<td>$Y = 0.315 + 1.074X$</td>
<td>0.0121</td>
<td>0.981</td>
<td>*</td>
</tr>
<tr>
<td>Pro</td>
<td>$Y = 0.338 + 1.005X$</td>
<td>0.0148</td>
<td>0.970</td>
<td>*</td>
</tr>
<tr>
<td>Tyr</td>
<td>$Y = 0.233 + 1.186X$</td>
<td>0.0135</td>
<td>0.978</td>
<td>*</td>
</tr>
<tr>
<td>Val</td>
<td>$Y = 0.398 + 0.848X$</td>
<td>0.0058</td>
<td>0.961</td>
<td>*</td>
</tr>
<tr>
<td>Met</td>
<td>$Y = 0.292 + 1.087X$</td>
<td>0.0200</td>
<td>0.998</td>
<td>*</td>
</tr>
<tr>
<td>Ile</td>
<td>$Y = 0.370 + 0.930X$</td>
<td>0.0104</td>
<td>0.982</td>
<td>*</td>
</tr>
<tr>
<td>Leu</td>
<td>$Y = 0.321 + 1.038X$</td>
<td>0.0060</td>
<td>0.985</td>
<td>*</td>
</tr>
<tr>
<td>Phe</td>
<td>$Y = 0.329 + 1.030X$</td>
<td>0.0044</td>
<td>0.996</td>
<td>*</td>
</tr>
<tr>
<td>Lys</td>
<td>$Y = 0.318 + 1.030X$</td>
<td>0.0166</td>
<td>0.976</td>
<td>*</td>
</tr>
<tr>
<td>Total AA</td>
<td>$Y = 0.347 + 0.978X$</td>
<td>0.0061</td>
<td>0.994</td>
<td>**</td>
</tr>
</tbody>
</table>

$^a$ Statistical significance: $^* P < 0.05$; $^{**} P < 0.01$. 
3.4. The relationships between IAD and in vitro digestibility of CP and AA

Table 4 shows the relationships between IAD and in vitro digestibility of CP and AA as linear regression equations. Linear regression was performed with \( Y \) (IAD) as the dependent variable, and \( X \) (in vitro digestibility) as the independent variable. The results show that there were significant linear relationships between IAD and in vitro digestibility for Ser, His, Arg, Thr, Ala, Pro, Tyr, Val, Ile, Leu and Lys \( (P < 0.05) \), and for CP, Asp, Glu, Gly, Met, Phe and total AA \( (P < 0.01) \). The values of \( r \) were between 0.96 and 0.99. The values of RSD were between 0.06 and 2.71, in fact the RSD values of the linear regressions were very small for the prediction of IAD for CP and AA from the in vitro digestibility. Thus, it was concluded that it was possible to assess IAD using the in vitro digestibility for CP and AA.

4. Discussion

The main aims of in vitro digestibility experiments are to decrease the experimental cost and the experimental animal number. The complicated surgery is avoided and the time of assessment is reduced. The activities of digestive enzymes are influenced by many factors (i.e. types of diets, environmental temperature, and nutritional levels). For example, the trypsin activity in the intestinal fluids was 77.2 international units/ml for pigs which were fed a low protein and high energy diets, but 59.4 international units/ml when pigs were fed a high protein and low energy diet (Lu, 1995). Generally speaking, the in vitro digestion experiment was carried out by simulating the environment of stomach and intestinal tract as close as possible under strictly controlled conditions. The results of the in vitro digestibility are therefore relatively stable.

Many in vitro incubation methods were used to measure the in vitro digestibility of nutritional components and to predict the in vivo digestibility. Furuya et al. (1979) successfully established a two-stage in vitro method using pepsin and intestinal fluids for growing pigs, Zhang and Nie (1986) and Liang and Zhang (1988) successfully determined the metabolizable energy (ME) and digestible energy (DE) of some feedstuffs for poultry and pigs following the method of Furuya et al. (1979). Because the whole in vitro digestion procedure was performed in sealed containers, the products could not be removed, so there were obvious differences from the true in vivo digestion and absorption in the animal. Additionally, it was very difficult to maintain the digestive enzyme constant for each in vitro experiment. The amount of intestinal fluid had to be adapted for each in vitro digestion experiment. Thus, the in vitro digestibility by the method of Furuya et al. (1979) was subject to great variation between different in vitro digestion experiments.

Sauer et al. (1989) compared the energy and CP digestibility of some feedstuffs using the mobile nylon bag techniques, and compared the determined results between the in vivo nylon bag method and traditional feces collecting method. Because the passage time of mobile nylon bags in the intestinal tracts was difficult to control, experimental results from Sauer et al. (1989) also showed a low repeatability.

Recently, Boisen and Fernandez (1994) and Jaguelin et al. (1994) reported a new two-stage in vitro digestion procedure. The samples were incubated with pepsin in the first
stage and then incubated with trypsin in the second stage. Finally, the undigested CP was precipitated with 20% sulfonyl-salicylic acid. The CP in vitro digestibility was calculated on the basis of the original CP content and residual CP content of the precipitated feedstuffs after filtration. Although, the coefficients of variation for the CP in vitro digestibility were low (CV = 3.8%) and the instability of enzyme activity in the intestinal fluids was overcome, the entire incubation procedure still operated in tightly sealed containers and the enzyme reaction products could still not be removed.

The use of the present method for the estimation of IAD has many advantages. This method requires small amounts of sample and uses no special apparatus; the time required is very short, the determination of the CP and AA in vitro digestibility is usually completed within 2 days. The standard derivation of the CP and AA in vitro digestibility is very small for three replicates (see Table 2). The reaction products can also be easily transferred into the dialytic liquid (PBS).

From the present data it could be concluded the in vitro conditions in the second incubation stage were as follows: the incubation time of trypsin was 24 h, the volume of dialysis liquid was 300 ml, the concentration of trypsin was 2 mg/ml, and pH of dialysis liquid was 7.60. According to the above procedures, the satisfactory results for in vitro digestibility of CP and AA could be obtained, also it is possible to predict IAD of CP and AA according to the in vitro digestibility measured using the dialysis tubing technique.

Acknowledgements

The authors sincerely wish to thank the Chinese Academy of Sciences (CAS) which supported this study.

References


Lu, F.Z., 1995. The variant factors of proteinase activity in the intestinal fluids for piglet and the relation to in...


