Evaluation of the Apparent Ileal Digestibility (AID) of Protein and Amino Acids in Nursery Diets by \textit{In vitro} and \textit{In vivo} Methods

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\textbf{ABSTRACT} : The objective was to evaluate \textit{in vitro} prediction of ileal digestibility of protein and amino acids (AA) for current nursery pig diets (n = 10) by using pepsin and pancreatin incubations. To compare \textit{in vivo} ileal digestibility, forty nursery pigs (4 pigs per diet) with an initial BW of 12.2 ± 2.7 kg were surgically equipped with T-cannula in the distal ileum. In all cases, the values of \textit{in vitro} digestibility were higher than those of \textit{in vivo} digestibility (p<0.05). With regard to the relationships of essential and non essential AA (CP), the $r^2$ value was 0.76. With regard to AA, high relationships were observed in Ile, Thr, and Gly (0.85, 0.83, and 0.89, respectively). Also, there was a lower relationship for Arg, Met, Ala, Asp, Glu, Pro, Ser, and Tyr with $r^2$ values of 0.56, 0.54, 0.40, 0.54, 0.45, 0.24, 0.49, and 0.35, respectively between \textit{in vitro} and \textit{in vivo} digestibility. The EAA relationship ($r^2 = 0.71$) was generally higher than that of NEAA ($r^2 = 0.50$) numerically. In conclusion, there were strong linear relationships between \textit{in vivo} and \textit{in vitro} ileal digestibility (CP, Ile, Thr, and Gly). \textit{In vitro} prediction of ileal digestibility (CP, Ile, Thr, and Gly) seems to have significant potential for practical application. (\textbf{Key Words} : \textit{In vivo}, \textit{In vitro}, Ileal Digestibility, Nursery Pigs)

\textbf{INTRODUCTION}

Ileal and total tract digestibility should be measured by \textit{in vivo} trial. The ileal digestibility of amino acid and nitrogen is one of the most valuable measurements for the evaluation of nursery pig diets. However, \textit{in vivo} methods to evaluate of digestibility require complicated surgery and are time-consuming and costly. In 1991, a model for feed evaluation based on \textit{in vitro} digestible dry matter and protein was developed by Boisen (1991). Since \textit{in vitro} digestibility was not influenced by endogenous losses, Boisen and Fernandez (1995) reported that values of \textit{in vitro} digestibility for protein were higher than those of apparent ileal digestibility and equation for prediction of apparent ileal digestibility of protein and amino acid for pigs diets. Recently, Huang et al. (2000) demonstrated that dialysis tubing and phosphate buffered saline (PBS) solution might be ideal materials for imitating the digestion environment of the intestinal tract. There were significant linear relationships (0.96<r-values<0.99) between \textit{in vivo} and \textit{in vitro} digestibility for amino acids. Most \textit{in vitro} researches have predicted the equations for relationship and reported

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In each period, the 7 d was a period of adaptation to the experimental diets and ileal digesta were collected on d 8 and 9 (12 h/d) for each pig. The ileal digesta were collected between 0600 and 1800 for 2 days by attaching a transparent 100-ml latex collection bag to the cannulas. During the 12 h collection period, digesta were collected every 30 min and immediately frozen at -20°C. The samples were then freeze-dried and finely grounded prior to analysis for chromium (Kimura and Miller, 1957).

### In vitro method

**Step 1**: About 0.5 g of feed was weighed within an accuracy of ±0.1 mg into 100-ml conical flasks with a blank was included in each series. Each diet has 20 replicates because 0.5 g feed is not enough for analysis. A small magnetic rod and 25 ml of phosphate buffer (0.1 M, pH 6.0) were added to each flask, the sample and buffer then were mixed carefully by gentle magnetic stirring. 10 ml a 0.2 M HCl was added to the slurry, and pH was then adjusted to pH 2 using a 1 M HCl or a 1 M NaOH solution. 1 ml of a freshly prepared pepsin solution containing 10 mg pepsin (porcine, 2000 FIP U/g, Merck art no. 7190) was then added to the mixture. In order to prevent bacteria growth, 0.1 ml of a chloramphenicol solution (0.5 g chloramphenicol (ICN no. 190321) per 100 ml ethanol) was also added to the mixture. The flasks were then closed with a rubber stopper and the flasks were incubated in a heating chamber at 40°C for 75 minutes with constant magnetic stirring.

**Step 2**: After incubation, 10 ml of a phosphate buffer (0.2 M, pH 6.8) plus 5 ml of a 0.6 M NaOH solution were added. The slurry was adjusted to a pH of 6.8 with a 1 M HCl or a 1 M NaOH solution, then mixed with 1 ml freshly prepared pancreatin solution containing 50 mg pancreatin (porcine, grade IV, Sigma no p 1750). After closing with a rubber stopper, the sample was incubated under constant magnetic stirring in a heating chamber at 40°C for three hours and thirty minutes.

A minimum of 0.5 g Celite (545, Tecator) was added to glass filter crucibles and rinsed. Then, samples were dried at 100°C for at least 4 h and crucibles were weighed after cooling in a dessicator. The undigested residues were then collected in a filtration unit (Fibertee System M, Tecator, Sweden) by using dried and pre-weighted glass filter crucibles (d: 3 cm; pore size: 40-90 pm) containing about 0.5 g celite (545, Tecator) as a filter aid. All material was then transferred with 1% sulphosalicylic acid to the crucible. After consecutive washings with 2×10 ml of ethanol and acetone, respectively, the crucible was suctioned (with the water pump) to be as dry as possible. The undigested residues were then dried at 100°C overnight. The crucible was placed in an ashing oven and the content was ashed at 525°C for about 4 hours. After ashing, the crucibles were cooled in a dessicator and subsequently weighed.

### Table 1. Analysed contents of ME, CP, and amino acids of 10 nursery pig diets

<table>
<thead>
<tr>
<th>Items</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>Mean</th>
<th>Min.</th>
<th>Max.</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys (%)</td>
<td>1.43</td>
<td>1.45</td>
<td>1.50</td>
<td>1.43</td>
<td>1.38</td>
<td>1.39</td>
<td>1.43</td>
<td>1.51</td>
<td>1.55</td>
<td>1.45</td>
<td>1.38</td>
<td>1.55</td>
<td>1.55</td>
<td>0.05</td>
</tr>
<tr>
<td>Met (%)</td>
<td>0.54</td>
<td>0.55</td>
<td>0.56</td>
<td>0.54</td>
<td>0.51</td>
<td>0.56</td>
<td>0.50</td>
<td>0.50</td>
<td>0.49</td>
<td>0.54</td>
<td>0.53</td>
<td>0.49</td>
<td>0.56</td>
<td>0.03</td>
</tr>
<tr>
<td>Thr (%)</td>
<td>0.94</td>
<td>0.93</td>
<td>0.92</td>
<td>0.94</td>
<td>0.89</td>
<td>0.92</td>
<td>0.94</td>
<td>0.95</td>
<td>0.94</td>
<td>0.94</td>
<td>0.89</td>
<td>0.99</td>
<td>0.99</td>
<td>0.03</td>
</tr>
<tr>
<td>Trp (%)</td>
<td>0.24</td>
<td>0.24</td>
<td>0.25</td>
<td>0.24</td>
<td>0.28</td>
<td>0.28</td>
<td>0.28</td>
<td>0.27</td>
<td>0.31</td>
<td>0.26</td>
<td>0.24</td>
<td>0.31</td>
<td>0.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Val (%)</td>
<td>1.10</td>
<td>1.11</td>
<td>1.18</td>
<td>1.09</td>
<td>1.08</td>
<td>1.11</td>
<td>1.20</td>
<td>1.10</td>
<td>1.17</td>
<td>1.12</td>
<td>1.08</td>
<td>1.20</td>
<td>1.20</td>
<td>0.04</td>
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<tr>
<td>Ile (%)</td>
<td>0.92</td>
<td>0.94</td>
<td>0.93</td>
<td>0.97</td>
<td>0.99</td>
<td>0.96</td>
<td>0.94</td>
<td>0.91</td>
<td>0.95</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1 Each mean represents 40 observations for in vivo and in vitro, respectively.
2 Standard error. * p<0.05.
undigested residue, correction was made for dry matter in the blank.

Undigested materials together with the celite were wrapped into a piece of nitrogen-free paper, and undigested nitrogen was measured by using the Kjeldahl method in a semi-automatic Kjellfoss apparatus (Foss Electric, Denmark). The in vitro digestibility of protein was calculated from the difference between nitrogen found in the sample and the undigested residue after correction for nitrogen in the blank.

For the in vivo measure, the chromium concentration was determined via UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) and the ileal apparent digestibility was calculated via indirect method. N content was determined by using a Kjeltac 2300 Analyzer (Foss Tecator AB, Hoeganaes, Sweden). Amino acids (excluding tryptophan) were analyzed by dansylation (Beckman Instruments Inc., Fullerton, CA) and HPLC after acid hydrolysis for 24 h in 6 M HCl. Sulfur-containing amino acids were analyzed after overnight cold performic acid oxidation and subsequent hydrolysis.

Statistical analyses

Pig means served as the experimental unit. The means of the treatments were also compared by using Duncan’s multiple range test (Duncan, 1955) with an alpha level of p<0.05. Variability in the data was expressed as the SEM. All 40 pigs were fed the experimental diets at the 2 different times (20 pigs per month). However, there was no significant difference about time factor. The relationship between in vitro digestibility and in vivo digestibility measured in nursery pigs was determined by regression analyses using a general linear model (GLM) in a standard SAS package (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

In vitro and in vivo Digestibility of CP and AA

The in vitro digestibility of CP and AA of 10 nursery diets are presented in Table 3. In all cases, the values of in vitro digestibility were higher than those of in vivo digestibility (p<0.05). Boisen and Fernandez (1995) compared the in vitro ileal digestibility of protein of 17 feedstuffs with the in vivo digestibility by using the in vitro enzyme digestion method, and reported that ileal protein digestibility of the in vitro was higher than those of in vivo. Endogenous losses of protein at the ileal level might have a great influence on in vivo digestibility (Boisen and Eggum, 1991). Thus the reduced values of in vivo digestibility, compared with values of in vitro digestibility, can be explained by the presence in the feces of endogenous loss. Boisen and Fernandez (1995) reported that the in vivo digestibility of CP and AA could be predicted from in vitro digestibility, since in vitro digestibility is not influenced by endogenous losses.

The relationships between in vitro and in vivo digestibility of CP and AA

The statistical relationships between in vitro and in vivo digestibility of CP and AA as linear regression equations are shown in Table 4. With regard to relationships concerning CP, the r² value was 0.76. This relationship was stronger than those of EAA and NEAA numerically. With regard to AA, Ile, Thr, and Gly had strong relationships (0.85, 0.83, and 0.89, respectively). Also, several equations had relatively weak relationships (Arg, Met, Ala, Asp, Glu, Pro, Ser, and Tyr were 0.56, 0.54, 0.40, 0.54, 0.45, 0.24, 0.49, and 0.35, respectively) between in vitro and in vivo digestibility. The EAA relationship (r² = 0.71) was generally stronger than that of NEAA (r² = 0.50) numerically. In vitro digestibility techniques using enzymes and length of incubations that mimic in vivo digestion can be used to predict the AID of protein and AA among feedstuffs and compound feeds in swine. And these techniques are valid, less expensive and with reasonable accuracy (Boisen and Fernández, 1997; Noblet and Jaguelin-Peyraud, 2007). The accuracy of the equation of the in vitro to predict the AA or CP availability may be affected by many factors, such as the feedstuffs characteristics, the enzymatic method
used during the fermentation step (Regmi et al., 2008), and the endogenous losses of the animals. Boisen and Fernandez (1995) found that the relationship between predicted and determined apparent ileal digestibility of protein in the diets was substantially lower than that found in single feedstuffs ($r^2 = 0.57$ vs. $r^2 = 0.92$). Similarly, Huang et al. (2000) demonstrated that the statistical equations between in vivo and in vitro digestibility of CP and AA for single protein stuffs (fish meals, rapeseed meal, and cottonseed meal) had higher linear relationships ($r^2 = 0.96$ to 0.99). Jezierny et al. (2010) demonstrated that there were strong linear relationships ($p<0.05$) between in vivo and in vitro ileal digestibility ($CP$, $Ile$, $Thr$, and $Gly$). In conclusion, there were strong linear relationships between in vivo and in vitro ileal digestibility ($CP$, $Ile$, $Thr$, and $Gly$). In vitro prediction of ileal digestibility ($CP$, $Ile$, $Thr$, and $Gly$) seems to have significant potential for practical application.

REFERENCES


