DIGESTIBILITY AND AVAILABILITY DETERMINATION OF NITROGEN AND AMINO ACIDS IN FEED FOR PIGS : 1. DIGESTIBILITY MEASUREMENT

S. Prawirodigdo
Assessment Institute for Agricultural Technology Central Java, Ungaran 50501

ABSTRACT

This paper discusses aspect of digestion estimate in amino acids and nitrogen of feed for pigs. It has been highlighted that low utilization of protein does not only being a high cost to the pig industry, but also results in an environmental hazard. In formulation of diets for pigs, it is necessary to pay attention to the profile and character of feed protein. It is well understood that before being absorbed in the gut and synthesized for replacement and improvement of body tissue, dietary protein is broken down into small molecules, the so-called amino acids. In order to estimate the amount of available protein of feed, it is urge to determine the digestibility of such feed. There are four different expressions to explain that there is a distinction between the so-called apparent, net, true, and real ileal nitrogen and amino acid digestibilities. However, the present paper only focuses on the apparent digestibility.

Keywords : feed, apparent, faecal, ileal, digestibility, amino acid, nitrogen, pigs

INTRODUCTION

In a symposium directed at improving the utilization of amino acids by the pig, some major issues relating to the use of dietary protein supplements in the pig industry were highlighted (Batterham, 1993). Since protein is a costly item in pig diets it is essential that the protein is used as efficiently as possible (Baker, 1993). Besides low utilization of protein being a high cost to the pig industry, it also results in an environmental hazard through increasing nitrogen (N) output in effluent (Jongbloed and Lenis, 1992; Lee et al., 1993; Schutte et al., 1993; Kornegay, 1994). Thus, studies should be directed towards maximizing the efficiency of protein utilization. It also follows that diets for pigs should be formulated properly and based on the amino acid requirements and their availability estimates in the feedstuffs (Prawirodigdo, 1999).
The term feed digestibility has long been used to express feed availability. It was assumed that the nutrients of ingested feed not recovered in the small intestine had been digested and absorbed, and therefore available to be utilized.

In the past Batterham et al. (1990) compared directly the utilization of ileal digestible (ID) amino acids of soybean meal (SBM) and cottonseed meal (CSM) in pigs. They highlighted that ID value reflected amino acids availability of SBM, but was not appropriate for amino acids availability estimate of CSM. Therefore, it can be emphasized that ID value is useful to express nutrient availability of feed, except for heat-processed meal protein. The present paper reviews several studies that have been conducted previously to evaluate the digestibility value of feedstuffs as a dietary protein for pigs.

MEASUREMENT TECNIQUES

There are few techniques to determine the digestibility of amino acids and N in feeds for pigs. Fuller (1991) suggested that for measuring feed digestibility and absorption, the accuracy of measurement should be taken into account. As well, the nature of the processes of the accessible methods should be considered before measuring the digestibility of feeds (Fuller, 1991).

Recently, dietary protein and amino acid digestibility in feed for pigs has been reviewed extensively by Boisen and Moughan (1996). They defined four different expressions to explain that there is a distinction between the so-called apparent, net, true, and real ileal nitrogen and amino acid digestibilities. However, in the present paper only the apparent digestibility will be discussed intensively.

Apparent Digestibility

The apparent digestibility measurement of nutrients has traditionally been conducted by determining nutrient disappearance in the small intestine or the whole alimentary tract of animals. Such a technique is simply practiced by measuring the proportion of ingested nutrient that is not recovered in the voided faeces (apparent faecal digestibility) or in the terminal ileum digesta (apparent ileal digestibility).

Apparent faecal digestibility. The traditional method to estimate nutrient digestibility in feed is the apparent faecal digestibility (AFD) method. Apparent faecal digestibility of nitrogen and amino acids can be calculated by deduction of the amount of ingested nitrogen or amino acids with the quantity of excreted nitrogen or amino acids in the faeces. An example of the formula for calculating apparent faecal amino acid digestibility is given in the following equation:

\[ \text{AFDL} (\%) = \frac{\text{LI} - \text{LF}}{\text{LI}} \times 100 \]

where AFLD is apparent faecal digestibility of lysine, LI is lysine intake and LF is lysine in faeces.

The apparent faecal digestibility method for estimating amino acid digestibility in feed was developed by Kuiken and Lyman (1948) who used the AFD analysis for estimating the availability of foods in rats. In the study of Kuiken and Lyman (1948), AFD of N and amino acids was expected to be similar to the availability of both nutrients in the examined foods, on the assumption that the nutrients that disappeared after passage through the alimentary canal were digested and absorbed in an available form for utilization by the rats. Without measurement of the carcass or growth rate of the rats, Kuiken and Lyman (1948) claimed that AFD of some foods, except for cottonseed flour, have a close relation with their availability. In the past the AFD analysis method was also employed to measure faecal digestibility of nutrients in feed in other monogastric animals. For example Sauer and Ozimek (1986) noted that the AFD analysis method has been used extensively in pigs by Eggum (1989).

However, it is now recognised that the AFD method is an inaccurate technique for assessment of N and amino acid availability of feed, since there is interference from the microbial activity in the hindgut causing either overestimation or underestimation on the amino acid and N availability.

Apparent ileal digestibility. As a replacement for the AFD, the apparent ileal digestibility (AID) analysis method for determining
N and or amino acid digestibility of feeds was developed. The technique to measure apparent ileal digestibility of N and amino acids is similar to the apparent faecal digestibility, except that the calculated AID value is based on the intake and the amount of N and each amino acid passing at the terminus of the small intestine rather than at the hindgut. The AID analysis method has been accepted and practiced world-wide, and the AID value has become the most important estimate of the nutritive value of feeds for pigs.

There are four techniques to obtain ileal digesta samples for determining the AID of feed in pigs. The first technique involves collecting the total ileal digesta using a fitted cannula. This method requires the surgical preparation of animals to fit a cannula to allow digesta collection from the distal ileum (Prawirodigdo et al., 1998). In addition, in order to conduct the total collection of ileal digesta from canulated pig, either a plastic tube or a balloon is commonly attached to the cannula to allow the ileal digesta to be withdrawn into a digesta container. Low (1980) noted several disadvantages of gut cannulation. When a simple cannula is employed, the proper time to collect ileal digesta samples and the extent to which the ileal digesta are typical of the digesta flowing past the cannula can be uncertain. There is also the possibility that if sedimentation of digesta occurs in or around the cannula, the collection of the sample may not be entirely representative of luminal digesta. When re-entrant cannulas are used, other problems arise including the requirement for more complex surgery and more laborious collection procedures; the surgery may result in a reduction in, and disturbance to intestinal activity (particularly for diets with large particle size) and hypertrophy near the cannula due to total separation of the nervous system and the animals usually only survive for a short time. Furthermore, digestibility studies in pigs fitted with re-entrant

Table 1. The Use of different Slaughter Techniques for Estimating Aapparent Ileal Digestibility of Amino Aacids in Cottonseed Mmeal (CSM) and Ssoybean Meal (SBM) Diets

<table>
<thead>
<tr>
<th>Digestibility (%)</th>
<th>CSM diet</th>
<th>SBM diet</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂ stunned</td>
<td>Halothane anaesthetized</td>
<td>CO₂ stunned</td>
</tr>
<tr>
<td><strong>Indispensable amino acids:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>71.1</td>
<td>81.3</td>
<td>70.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>48.5</td>
<td>66.5</td>
<td>55.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>37.0</td>
<td>60.2</td>
<td>60.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>36.8</td>
<td>59.9</td>
<td>59.4</td>
</tr>
<tr>
<td>Lysine</td>
<td>27.5</td>
<td>55.4</td>
<td>59.7</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>60.6</td>
<td>74.5</td>
<td>62.5</td>
</tr>
<tr>
<td>Threonine</td>
<td>30.4</td>
<td>50.8</td>
<td>50.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>34.8</td>
<td>56.1</td>
<td>56.2</td>
</tr>
<tr>
<td>Valine</td>
<td>42.8</td>
<td>62.6</td>
<td>52.2</td>
</tr>
<tr>
<td><strong>Dispensable amino acids:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>32.5</td>
<td>56.4</td>
<td>53.3</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>52.0</td>
<td>66.2</td>
<td>59.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>66.4</td>
<td>78.4</td>
<td>63.9</td>
</tr>
<tr>
<td>Glycine</td>
<td>24.0</td>
<td>52.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Proline</td>
<td>-9.9</td>
<td>16.5</td>
<td>50.9</td>
</tr>
<tr>
<td>Serine</td>
<td>47.4</td>
<td>61.3</td>
<td>63.7</td>
</tr>
</tbody>
</table>

* Adapted from Prawirodigdo et al. (1998) CO₂, Carbon dioxide; D, Diet; M, Method; *
P<0.05; **, P<0.01; ***, P<0.001.
cannulas are often hampered by blockage problems. The extent to which blockage occurs may be associated with the level of crude fibre in the diet, the particle size of the ground feed used and the amount and viscosity of the digesta passing through the cannula (Sauer and Ozimek, 1986; Fuller et al., 1994). When the cannula is implanted in the terminal ileum, there are few discrepancies in the estimate of apparent absorption of amino acids between the simple and the re-entrant cannulas (Low, 1980; Köhler et al., 1990a).

The second technique is the partial sampling of ileal digesta via ileal cannulation. The partial sampling technique has been practiced worldwide and is considered to be an alternative to the total collection technique for digestion studies in pigs. However, this technique requires the addition of an inert marker in the diet.

The third method is the mobile nylon bag technique which was developed initially by Sauer et al. (1983) and used by other researchers (Cherian et al., 1988; Leibholz, 1991). This technique involves surgery for cannulation to allow the insertion of nylon bags into the stomach or small intestine.

The last technique involves the slaughter of the animal in contrast to surgical implantation of cannulas. The pioneers for the application of the slaughter technique in digestibility studies were Badawy et al. (1957). They compared the slaughter and the anaesthesia techniques to obtain ileal digesta in sheep, and suggested that the slaughter technique induced an underestimate of the ileal digestibility of N in feed due to intestinal cells sloughing. The anaesthesia technique was then adopted by Moughan and Smith (1987) for determining apparent ileal digestibility of N in feed in pigs. Moughan and Smith (1987) obtained good agreement between the ileal digestibilities of amino acids measured with intact (anaesthetised) and cannulated pigs. Consequently, the anaesthesia technique has been used intensively to determine the digestibility of protein and amino acids in feed for rats (Butts et al., 1992a, b; Donkoh and Moughan, 1994; Donkoh et al., 1994b) and pigs (Kies et al., 1986; Moughan et al., 1991a, 1992; Donkoh et al., 1994a), but, the carbon dioxide (CO2) gas was used to stun the rats instead of using halothane anaesthesia.

In earlier study, Kies et al. (1986) demonstrated that the sampling site over 140 cm of small intestine did not result in any significant difference in apparent digestibility of casein N. However, a problem was encountered when the same technique of Kies et al. (1986) was employed in the study of Donkoh et al. (1994a). Donkoh et al. (1994a) highlighted that the terminal 20 cm of the ileum was empty when the ileal sample was obtained from pigs given meat and bone meal and halothane gas anaesthetised. It seems that collection of ileal digesta in pigs from the 140 cm length of the terminal ileum provides the true representative of ileal digesta sample for digestibility studies (Donkoh et al., 1994a).

In addition, van Barneveld et al. (1994) found a sufficient amount of ileal digesta sample when the samples were obtained using dissection of 150 cm of the terminal ileum, and suggested that the ileal digestibility of amino acids in feed determined using the slaughter technique was higher (P<0.05) than that using the cannulation technique.

Later on, Prawirodigdo et al. (1998) examined the use of (CO2) gas stunning and halothane gas inhalation techniques for determining AFD and AID of cottonseed and soybean meals (CSM and SBM) in pigs. They documented that the CO2 stunning technique consistently provided lower AID amino acids (Table 1) and nitrogen (Table 2) estimates than the halothane anaesthesia technique. In contrast to the AID amino acids and nitrogen data the AID organic matter of CSM or SBM diets were not affected by method of sampling (See Table 2). Moreover, Prawirodigdo et al. (1998) explained that the CO2 stunning technique may have caused sloughing of intestinal mucosa (Badawy et al., 1957) during the slaughter process post-CO2 stunning. Intestinal mucosal cells contributing significant amounts of protein would increase the nitrogen content of ileal digesta, thus reducing the AID aminoacids and nitrogen digestibilities (Prawirodigdo et al., 1998).

The Use of Markers in the Digestibility Studies

Apparent faecal digestibility of nutrients can be measured using the total collection method or exploitation of the partial sample collection method using indigestible markers. The total collection

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method requires data on total feed intake and the total amount of voided faeces during the measurement period. For this purpose, there is a need to house the experimental animals individually in metabolism cages, each equipped with a feeder, drinker and tray to collect faeces. The total collection AFD analysis method, however, is labor intensive and time consuming.

In contrast to the total faecal collection method, the advantage of using indigestible markers in the AFD method is that there is no need to measure either the total amount of ingested nutrients or the total voided faeces throughout the measurement period (Vogtmann et al., 1975). It is, however, necessary to take some samples of feed and faeces for determination of nutrient profiles and marker concentration in both samples.

In practice, indigestible markers are mixed with the diet to be examined. There have been several markers used as indicators in digestion studies but their exact purpose has varied. In the study of Kuiken and Lyman (1948) and Lawrence et al. (1994) ferric oxide (Fe₂O₃) was included in the examined diets for AFD analysis using the total faecal collection method, to visually ensure that the excreted faeces represented the residue of the examined diet. Total collection of faeces commenced on the first day that the change of the colour of faeces occurred. Ferric oxide is reddish-brown to yellowish-orange in colour (Kotb and Luckey, 1972), but this depends upon the size and shape of the particles and the amount of combined water (The Merck Index, 1989). There are several disadvantages with the use of Fe₂O₃ as an indigestible marker. Ferric oxide may upset the stomach and causes vomiting or even diarrhoea. In addition, Kotb and Luckey (1972) found in the literature that Fe₂O₃ is an unreliable indicator.

On the other hand, indicators can be included in the diets to predict the disappearance of nutrient from the feed in the digestive tract. The indicator method essentially involves measuring the change in concentration of the marker being used between concentration of indicator included in the diet and that recovered in the ileal digesta or excreta. Normally, concentration of marker in the ileal digesta or excreta is higher than in the diet. Assuming that a part of diet is digested and the nutrients are absorbed while the marker is intact, then the concentration of the marker in the ileal digesta or excreta will increase proportionally. In the indicator method analysis, markers can be natural constituents of the feed or added to it. The basic formula to estimate digestibility profile of feeds using the indicator analysis method is well known. Digestibility of nutrients in feed can be calculated using the following formula:

\[
\text{Digestibility of nutrient} = 100 - \left( \frac{\% \text{ marker in feed}}{\% \text{ marker in faeces}} \right) \times \left( \frac{\% \text{ nutrient in faeces}}{\% \text{ nutrient in feed}} \right) \times 100
\]

Thus, if a feed contains 220 g crude protein (CP)/kg and 0.2 % of marker, while the ileal digesta or excreta contains 150 g/kg and 0.8 % (for CP and marker concentration respectively), then the CP digestibility will be: 100 - \left( \frac{0.2}{0.8} \right) \times \left( \frac{150}{220} \right) \times 100 = 83 \%.

Maynard et al. (1979) highlighted five criteria for an ideal indicator for determining nutrient

<table>
<thead>
<tr>
<th>Digestibility (%)</th>
<th>Ileal</th>
<th>Faecal</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂ stunned</td>
<td>Halothane anaesthetized</td>
<td>CO₂ stunned</td>
</tr>
<tr>
<td>N</td>
<td>CSM</td>
<td>51.0</td>
<td>64.5</td>
</tr>
<tr>
<td></td>
<td>SBM</td>
<td>54.9</td>
<td>70.5</td>
</tr>
<tr>
<td>OM</td>
<td>CSM</td>
<td>69.7</td>
<td>71.8</td>
</tr>
<tr>
<td></td>
<td>SBM</td>
<td>72.0</td>
<td>77.2</td>
</tr>
</tbody>
</table>

| 1 Adapted from Prawirodigdo et al. (1998); CO₂, Carbon dioxide; M, Method; S, Site of collection; *, P<0.05; **, P<0.01; *** P<0.001. |  |

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Table 2. Influence of Slaughter Method and Site of Digesta Collection on Apparent Nitrogen (N) and Organic Matter (OM) Digestibility in Cottonseed Meal (CSM) and Soybean Meal (SBM) Diets

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digestibility in feeds. (1) The indicators should be indigestible and unabsorbable, (2) the presence of added marker in the feed should not induce digestion, (3) the passage along the alimentary tract should be at a uniform rate, (4) measurement should be sensitive and easy determined chemically and (5) a substance that is naturally presents in the feed is preferable.

Commonly the reference substances to trace the fate of ingested nutrients of feed or diet are inert markers. These markers include chromium oxide (Fenton and Fenton, 1979, Jagger et al., 1992; Saha and Gilbreath, 1993), titanium dioxide (Kotb and Luckey, 1972; Jagger et al., 1992), and acid-insoluble ash (van Keulen and Young, 1977; Vogtmann et al., 1975; McCarthy et al., 1974, Moughan et al., 1991b, Prawirodirdgo, 1999).

Chromic oxide (Cr₂O₃), is the most common external marker used in digestibility determinations in livestock. Kotb and Luckey (1972) concluded that Cr₂O₃ was not toxic and almost completely recovered in the faecal digesta of animals and humans, and was suggested to be acceptable as an inert marker in digestibility studies (Bakker and Jongbloed, 1994; van Leeuwen et al., 1996). However, there have been several controversial reports on the use of Cr₂O₃ as an inert marker in digestibility studies (Steele and Clapperton, 1982; Jagger et al., 1992). Likewise, Saha and Gilbreath (1991a) reported that 93.3% of dietary chromium used as an inert marker was recovered in the faeces of mature pigs. Saha and Gilbreath (1991b) suspected that the variations in the mineral contents of diet and faeces may have influenced recovery of chromium from the samples. Therefore, Saha and Gilbreath (1993) modified a Cr₂O₃ indicator ratio technique to accurately estimate nutrient digestibility in sows. Unfortunately, since Cr₂O₃ is a heavy metal, the use of this reference substance may no longer be tolerated in routine experiments because of concerns over environmental pollution (van Leeuwen et al., 1996). Consequently, acid insoluble ash (AIA) has been proposed as an alternative to Cr₂O₃ (McCarthy et al., 1974; Moughan et al. 1991b). McCarthy et al. (1974) concluded the results that the 4 M-HCl insoluble ash method was superior to Cr₂O₃ as an inert markers for determining the digestibility of diets in pigs. Rowan et al. (1991) found similar apparent faecal digestibility coefficients for dry matter in the diet when AIA was used as a reference compared to the total faeces collection method in humans and pigs. It is important to take into account that use of the marker inclusion method to measure the nutrient digestibility profile of feed in pigs provides an accurate and reliable estimate. In regard to environmental issues, however, further investigations in the use of natural reference substances will provide important information relevant to future studies.

Overall, the present article suggest that the AID analysis is the most important estimate of the nutritive value of feeds for pigs that has been accepted and practiced world-wide.

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