# The effect of dietary crude protein content on apparent and true ileal nitrogen and amino acid digestibilities

BY A. DONKOH AND P. J. MOUGHAN*

*Department of Animal Science, Massey University, Palmerston North, New Zealand*

(*Received 9 August 1993 – Revised 11 October 1993 – Accepted 2 November 1993*)

**The effect of dietary protein content (25, 60, 95, 130, 165 and 200 g crude protein (N × 6·25)/kg diet) on the apparent and true ileal digestibilities of N and amino acids in meat-and-bone meal given to the growing rat was investigated. Semi-synthetic diets in which meat-and-bone meal was the sole protein source were given to 180 g body-weight rats for 14 d. On the fourteenth day the rats were fed and then killed 4 h after the start of feeding and digesta were sampled from the terminal 200 mm ileum. Endogenous amino acid excretion was determined for eighteen rats given an enzymically hydrolysed casein (EHC)-based diet and with subsequent treatment of the digesta using ultrafiltration. The EHC-fed rats were killed 3 h after the start of feeding and digesta were collected from the terminal 200 mm ileum. True ileal digestibility values determined with reference to Cr as a marker were higher than the corresponding apparent estimates. Apparent digestibility values of N and amino acids increased significantly ($P < 0·001$) with increasing dietary protein level; however, dietary protein content had no significant ($P > 0·05$) effect on the true ileal digestibilities of N and amino acids. The mean apparent ileal digestibility of N in meat-and-bone meal ranged from 65·6 to 75·3%. The corresponding range for the true ileal digestibility of N was 76·9 to 78·2%. True ileal digestibility, unlike apparent digestibility, appears to be independent of dietary protein level and may allow feed ingredients to be compared accurately even if they are ingested in different quantities.**

**Ileal digestibility: Protein: Meat-and-bone meal: Rat**

The ileal measure of dietary amino acid digestibility is generally recognized (Tanksley & Knabe, 1984; Sauer & Ozimek, 1986) as a more acceptable approach than the traditional faecal method. The digestive tract and its exocrine glands secrete large amounts of endogenous protein and other N-containing compounds into the gut lumen during the course of digestion (Fauconneau & Michel, 1970; Low, 1982; Alpers, 1987) and these are mixed with the dietary proteins. Only part of this material is digested and reabsorbed (Buraczewski, 1980). At the same dietary dry matter intake but different crude protein (CP) intakes a similar amount of undigested endogenous material at the terminal ileum will have a disproportionate effect on the determination of apparent digestibility. For this reason apparent digestibility is influenced by the protein level in the test diet (Sauer *et al.* 1980; Furuya & Kaji, 1989). True digestibility coefficients are expected to be less affected by assay conditions than are apparent digestibility coefficients.

The traditional protein-free method for determining endogenous loss leads to an underestimation of the physiologically normal level of endogenous excretion. Thus in the present study the endogenous ileal N and amino acid flows used to adjust apparent digestibility values to true ones were determined by the recently developed enzymically hydrolysed casein (EHC)-ultrafiltration method (Moughan *et al.* 1990; Butts *et al.* 1991). With this approach the animal is given a semi-synthetic diet containing EHC as the sole N

* For reprints.

source (molecular weight (MW) < 5000 Da). Ileal digesta are collected and the nitrogenous fraction is separated physically by ultrafiltration. The high MW (> 10000 Da) fraction resulting from the ultrafiltration provides a measure of endogenous amino acid flow. With this method, endogenous free amino acids and small peptides (< 10000 Da) are discarded with the low MW fraction, but this leads to only a small degree of underestimation of endogenous amino acid loss.

In the present study comparison was made between the apparent and true ileal digestibilities of N and amino acids in meat-and-bone meal (MBM) determined over a range of dietary protein concentrations. The aim was to highlight the potential inaccuracies of apparent digestibility coefficients. MBM was chosen for study as it is of value as a major protein source in pig and poultry diets but is known to be of variable quality. The growing laboratory rat was used in the present work as a model animal for other single-stomached species.

## MATERIALS AND METHODS

### *Animals and housing*

Fifty-four Sprague–Dawley male rats (body weight 180 g) were selected at random from a group of animals which had been weaned at 4 weeks of age and were reared on a high quality diet. The rats were kept individually in raised stainless steel cages with wire mesh floors, and housed in a temperature-controlled room (21 ± 1°) with a 12 h light–dark cycle.

### *Experimental diets and feeding*

The ingredient compositions of six diets containing graded levels of a MBM as the sole protein source are shown in Table 1. MBM was incorporated into the diets to provide 25, 60, 95, 130, 165 and 200 g CP/kg. This was achieved by progressively replacing maize starch with MBM. An EHC-based diet, used in the determination of endogenous ileal amino acid flow, was also formulated (Table 1) to contain all the ingredients, except MBM, included in the MBM diets. The same level of purified cellulose was included in all of the diets. Variable amounts of mineral supplements were included to ensure that mineral levels were constant across diets. $Cr_2O_3$ was added to each diet as an indigestible marker.

Thirty-six rats were equally and randomly allocated to six dietary treatment (MBM) groups and eighteen rats were allocated to the EHC diet to allow determination of ileal endogenous N and amino acid flows. The animals were given unrestricted access to the diets from stainless steel feeders fitted with anti-spill devices, similar to those described by Thomsen (1981), for 3 h (8.30–11.30 hours) each day for 14 d. Fresh water was available at all times.

### *Slaughter, collection and treatment of ileal digesta*

On day 14, 4 h after the start of feeding, the MBM-fed rats were asphyxiated with $CO_2$ gas and decapitated (Donkoh *et al.* 1993), while those given the EHC-based diet were killed 3 h after the start of feeding (Donkoh, 1993). It has been demonstrated previously (Donkoh, 1993) that 4 and 3 h are optimal sampling times for rats given MBM- and EHC-based diets respectively. To facilitate the killing of the rats within 10 min of the designated times, feeding times were staggered on the day of slaughter and also on the previous 2 d to accustom the rats to the change in procedure. The abdomen was opened by an incision along the mid-ventral line and skin and musculature were folded back to expose the viscera. The terminal 200 mm of the small intestine (directly anterior to the ileo-caecal valve) was immediately removed. The terminal ileal contents were flushed out with deionized water from a syringe. Digesta samples from the MBM-fed rats were immediately frozen (−20°) and later freeze-dried. To minimize the activity of digestive enzymes the pH of the digesta

Table 1. *Ingredient composition (g/kg air-dry weight) of the experimental diets*

| Ingredient | Enzymically hydrolysed casein diet | Meat-and-bone-meal diet Dietary crude protein content (g/kg diet) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 25 | 60 | 95 | 130 | 165 | 200 |
| Maize starch | 687·0 | 753·0 | 690·6 | 624·8 | 556·4 | 487·8 | 420·4 |
| Purified cellulose* | 30·0 | 30·0 | 30·0 | 30·0 | 30·0 | 30·0 | 30·0 |
| Maize oil | 50·0 | 50·0 | 50·0 | 50·0 | 50·0 | 50·0 | 50·0 |
| Sucrose | 80·0 | 80·0 | 80·0 | 80·0 | 80·0 | 80·0 | 80·0 |
| Sodium chloride | 4·0 | 1·0 | 0·8 | 0·8 | 0·6 | 0·4 | 0·2 |
| Potassium carbonate | 4·0 | 1·0 | 0·8 | 0·6 | 0·4 | 0·2 | 0·2 |
| Dicalcium phosphate | 24·0 | 15·0 | 8·0 | 4·0 | 3·0 | 2·0 | — |
| Magnesium sulphate | 2·0 | 1·0 | 0·8 | 0·8 | 0·6 | 0·6 | 0·2 |
| Vitamin-mineral mix† | 15·0 | 15·0 | 15·0 | 15·0 | 15·0 | 15·0 | 15·0 |
| Chromic oxide | 4·0 | 4·0 | 4·0 | 4·0 | 4·0 | 4·0 | 4·0 |
| Enzymically hydrolysed casein‡ | 100·0 | — | — | — | — | — | — |
| Meat-and-bone meal | — | 50·0 | 120·0 | 190·0 | 260·0 | 330·0 | 400·0 |

* Avicel, Asahi Chemical Industry Company Limited, Tokyo, Japan.

† Tasmix special mouse premix, Pfizer Laboratories, Auckland, New Zealand. Supplied the following per kg diet: vitamin A 1·806 mg; vitamin D 0·019 mg; vitamin E 37·5 mg; vitamin K 1·5 mg; thiamin 3·0 mg; riboflavin 3·75 mg; pyridoxine 4·5 mg; cyanocobalamin 0·04 mg; pantothenic acid 21·0 mg; biotin 0·08 mg; niacin 15 mg; pterolymonoglutamic acid 0·75 mg; choline 0·75 g; Fe 60 mg; Zn 37·5 mg; Mn 37·5 mg; Cu 3·75 mg; I 0·38 mg; Co 0·53 mg; Se 0·11 mg; inositol 30 mg; K 2·75 g; Mg 0·3 g; Na 0·38 g.

‡ Sigma Chemical Company, St Louis, MO, U.S.A. Type I from bovine milk. Total N 127 g/kg, amino N 63 g/kg; free amino acids + peptides (< 5000 Da).

samples from the rats given the EHC-based diet was adjusted to 3·5 by adding $H_2SO_4$ (9 M) and the samples were frozen immediately. The latter were subsequently thawed and subjected to centrifugation and ultrafiltration for the determination of endogenous amino acid loss.

Digesta samples from the eighteen EHC-fed rats were rapidly thawed to 4° and samples from sets of three rats drawn at random were pooled to obtain six samples for laboratory processing using the centrifugation–ultrafiltration technique (Butts *et al.* 1991). The digesta samples were centrifuged at 1450 *g* for 45 min at 0°. The supernatant was decanted and retained. The precipitate was washed with 5 ml distilled water and centrifuged for a further 30 min at 1450 *g* at 0°. The combined supernatants were subjected to ultrafiltration using Centriprep-10 concentrators (MW exclusion limit 10000 Da; Amicon; W. R. Grace and Co., Danvers, IL, USA). The retentate (high MW fraction, MW > 10000 Da) was added to the precipitate, and the material was freeze-dried, finely ground and stored at −20° for chemical analysis.

*Chemical analysis*

Samples of ileal digesta and the experimental diets were analysed for total N, amino acids and Cr. Amino acid analysis on the ileal digesta samples from the rats given the MBM diets was restricted to the 25, 60 and 200 g/kg dietary CP treatments. The N content of duplicate MBM (100 mg) and ileal digesta (30 mg) samples and six 100 mg samples of each diet were determined by the Kjeldahl method (Association of Official Analytical Chemists, 1980). The Cr contents of six 100 mg samples of each diet and duplicate 15 mg samples of ileal digesta were determined by atomic absorption spectrophotometry using the method outlined by Costigan & Ellis (1987). Amino acids were determined following acid hydrolysis using a Beckman 119 BL amino acid analyser. Duplicate samples of MBM,

experimental diets and ileal digesta (5–7 mg) were hydrolysed in 500 $\mu$l 6 M glass-distilled HCl with added phenol (10 mg/ml) for 24 h at 110 ± 1° in glass tubes sealed under vacuum. For the determination of methionine and cystine the hydrolysis was preceded by performic acid oxidation at 0° for 10 h followed by neutralization with HBr. Tryptophan, which is destroyed by acid hydrolysis, was not determined.

*Data expression and analysis*

Endogenous N and amino acid (AA) flows at the terminal ileum relative to the ingestion of 1 g freeze dry matter (FDM) were calculated using the equation:

$$\text{AA flow } (\mu\text{g/g FDM}) = \text{AA concentration in ileal digesta } (\mu\text{g/g FDM}) \times \frac{\text{diet Cr (mg/g FDM)}}{\text{ileal Cr (mg/g FDM)}}.$$

Endogenous ileal amino acid flows for the EHC-fed rats were calculated based on the amino acid and Cr concentrations of the precipitate plus high MW fraction following centrifugation and ultrafiltration.

The apparent and true ileal amino acid digestibility coefficients were calculated using the following equations:

$$\text{apparent AA digestibility (\%)} = \frac{\text{dietary AA } (\mu\text{g/g FDM intake (FDMI))} - \text{ileal AA } (\mu\text{g/g FDMI})}{\text{dietary AA } (\mu\text{g/g FDMI})} \times \frac{100}{1}$$

$$\text{true AA digestibility (\%)} = \frac{\begin{pmatrix}\text{dietary AA}\\(\mu\text{g/g FDMI})\end{pmatrix} - \begin{pmatrix}\text{ileal AA}\\(\mu\text{g/g FDMI})\end{pmatrix} - \begin{pmatrix}\text{endogenous ileal AA}\\(\mu\text{g/g FDMI})\end{pmatrix}}{\text{dietary AA } (\mu\text{g/g FDMI})} \times \frac{100}{1}.$$

The apparent and true digestibility data were subjected to a one-way analysis of variance for each amino acid singly.

## RESULTS

In the digestibility assay the rats consumed the experimental diets readily. The mean body weights of the rats on the seven dietary treatments at the beginning and end of the 14 d feeding period along with the mean food intakes on the final day of study are presented in Table 2. There were no significant ($P > 0{\cdot}05$) treatment differences in body weight at the start of the feeding period, but the rats on the lower protein diets (25, 60 and 90 g CP/kg) were lighter in weight ($P < 0{\cdot}01$) at the end of the feeding period compared with their counterparts on the EHC- and the higher protein MBM-based diets. There were, however, no differences in mean food intake on the final day of study when ileal digesta were sampled. Daily feed intakes stabilized by the seventh day of the experiment. There was no evidence of ingested faeces in the gastric contents when these were examined after slaughter of the animals, indicating that coprophagy had not occurred.

The mean endogenous ileal N and amino acid flows for rats determined by the EHC/ultrafiltration method are presented in Table 3, and the effect of dietary CP concentration on the apparent and true ileal N digestibility values for MBM is illustrated in Fig. 1. Dietary CP content significantly ($P < 0{\cdot}05$) influenced apparent ileal N digestibility with values increasing as dietary CP was increased from 25 to 200 g/kg. There was a highly significant ($P < 0{\cdot}001$) positive correlation ($r$ 0·88) between level of dietary CP

Table 2. *Mean body weights at the beginning and end of a 14 d feeding period and food intakes on the last day of study for rats given an enzyme-hydrolysed casein-based diet or meat-and-bone-meal-based diets containing different levels of crude protein*†

(Mean values for six rats on each meat-and-bone-meal diet, and eighteen rats on the casein diet)

| | Meat and bone meal diet Dietary crude protein content (g/kg diet) | | | | | | Enzyme-hydrolysed casein | Pooled SE | Statistical significance of treatment effects |
|---|---|---|---|---|---|---|---|---|---|
| | 25 | 60 | 95 | 130 | 165 | 200 | | | |
| Body wt (g) | | | | | | | | | |
| Start of feeding | 178 | 178 | 178 | 177 | 177 | 177 | 177 | 0·39 | NS |
| End of feeding | 194[a] | 195[a] | 197[a] | 201[b] | 201[b] | 201[b] | 199[b] | 0·26 | ** |
| Food intake (g/d) | 14·2 | 13·6 | 13·3 | 13·6 | 13·1 | 13·8 | 14·0 | 0·10 | NS |

NS, not significant.
[a, b] Mean values with unlike superscript letters were significantly different ($P < 0·05$).
** $P < 0·01$.
† For details of diets and procedures, see Table 1 and p. 60.

Table 3. *Mean endogenous N and amino acid excretion rates at the terminal ileum of the growing rat determined under peptide alimentation and following centrifugation and ultrafiltration of the digesta**

(Mean values with their standard errors for six pooled samples)

| | Endogenous loss (μg/g freeze-dry matter intake) | |
|---|---|---|
| | Mean | SE |
| N | 1806 | 25 |
| Amino acid | | |
| Lys | 286 | 14 |
| Met | 79 | 5 |
| Cys | 85 | 3 |
| His | 165 | 9 |
| Phe | 151 | 11 |
| Tyr | 189 | 8 |
| Thr | 606 | 13 |
| Leu | 468 | 18 |
| Ile | 419 | 8 |
| Val | 475 | 14 |
| Ala | 295 | 14 |
| Asp | 726 | 21 |
| Arg | 169 | 12 |
| Ser | 955 | 32 |
| Glu | 1533 | 85 |
| Gly | 867 | 7 |
| Pro | 486 | 15 |

* For details of diets and procedures see Table 1 and pp. 60–62.

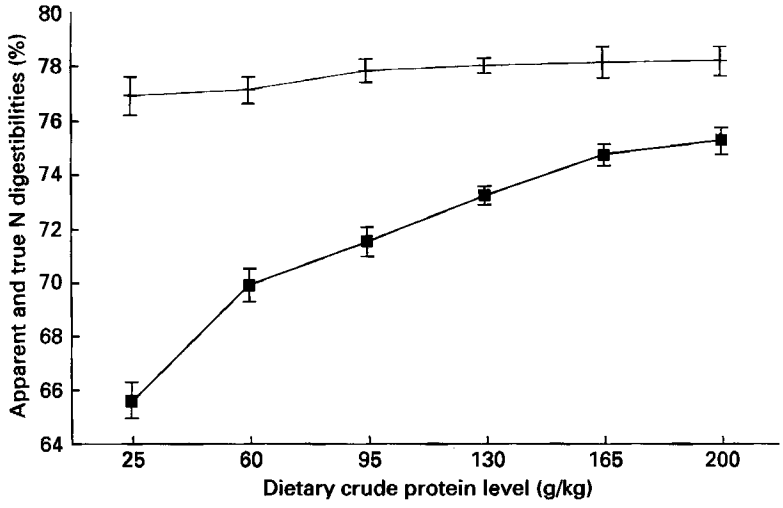


Fig. 1. Effect of dietary protein level on mean apparent (■) and true (+) ileal N digestibility values for rats given a meat-and-bone-meal-based diet.

Table 4. *Mean apparent and true rat ileal digestibilities* (%) *of amino acids in meat-and-bone-meal-based diets containing different levels of crude protein* (*CP*)†

(Mean values for six rats per dietary group)

| | Apparent digestibility Dietary CP (g/kg) | | | | Statistical significance of treatment effects | True digestibility Dietary CP (g/kg) | | | | Statistical significance of treatment effects |
|---|---|---|---|---|---|---|---|---|---|---|
| | 25 | 60 | 200 | SE | | 25 | 60 | 200 | SE | |
| Lys | 71·5 | 77·6 | 82·0 | 0·39 | *** | 86·1 | 85·8 | 86·9 | 0·38 | NS |
| Met | 70·0 | 76·9 | 80·9 | 0·47 | *** | 84·3 | 85·1 | 85·8 | 0·31 | NS |
| Cys | 60·2 | 66·1 | 70·7 | 0·41 | *** | 72·5 | 73·1 | 73·0 | 0·46 | NS |
| His | 72·7 | 78·1 | 83·2 | 0·41 | *** | 87·6 | 86·4 | 88·1 | 0·53 | NS |
| Phe | 62·9 | 69·6 | 72·6 | 0·53 | *** | 75·8 | 77·0 | 76·4 | 0·57 | NS |
| Tyr | 63·8 | 69·8 | 74·1 | 0·48 | *** | 76·9 | 77·2 | 77·9 | 0·51 | NS |
| Thr | 56·8 | 61·2 | 64·5 | 0·43 | ** | 68·4 | 67·7 | 69·2 | 0·44 | NS |
| Leu | 62·5 | 68·6 | 72·2 | 0·43 | *** | 75·3 | 75·9 | 74·9 | 0·34 | NS |
| Ile | 61·2 | 67·0 | 71·5 | 0·40 | *** | 73·7 | 74·1 | 73·2 | 0·48 | NS |
| Val | 61·0 | 67·1 | 72·2 | 0·47 | *** | 73·5 | 74·2 | 74·9 | 0·41 | NS |
| Ala | 64·2 | 69·5 | 74·1 | 0·38 | *** | 77·3 | 76·9 | 78·9 | 0·35 | NS |
| Asp | 53·3 | 60·9 | 66·2 | 0·40 | *** | 66·3 | 67·3 | 67·9 | 0·35 | NS |
| Arg | 69·8 | 76·2 | 82·7 | 0·36 | *** | 84·1 | 84·3 | 85·5 | 0·30 | NS |
| Ser | 62·1 | 67·0 | 71·7 | 0·42 | *** | 74·8 | 74·1 | 75·4 | 0·37 | NS |
| Glu | 54·5 | 59·8 | 63·3 | 0·41 | ** | 65·6 | 66·1 | 67·0 | 0·44 | NS |
| Gly | 50·2 | 55·0 | 59·0 | 0·48 | ** | 60·5 | 60·8 | 61·6 | 0·35 | NS |
| Pro | 71·0 | 78·0 | 83·2 | 0·48 | *** | 85·5 | 86·3 | 87·1 | 0·41 | NS |

NS, not significant.
** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.
† For details of diets and procedures, see Table 1 and pp. 60–62.
‡ Based on values for endogenous loss determined after feeding rats on an enzyme-hydrolysed, casein-based diet and with ultrafiltration of the digesta.

and apparent ileal N digestibility. There was no effect of dietary CP content on true ileal N digestibility.

The apparent and true ileal digestibilities of amino acids in MBM determined at three dietary protein levels (25, 60 and 200 g/kg) are given in Table 4. As dietary protein content

was increased from 25 to 60 to 200 g/kg there were significant ($P < 0.05$) increases in the apparent ileal digestibilities of all of the amino acids examined. In contrast to the apparent ileal digestibility coefficients, the true amino acid digestibility values were not influenced by CP content of the diet.

## DISCUSSION

A significant effect of dietary CP content on the apparent ileal digestibilities of N and amino acids was found in the present study. Increasing the dietary protein content of a MBM-based diet in equal amounts from 25 to 200 g/kg resulted in higher apparent ileal N and amino acid digestibility coefficients. The increase in apparent ileal N digestibility with increasing dietary protein was greatest at the three lowest protein levels and negligible at the two highest protein levels (Fig. 1). The present results are in support of other reported studies investigating the effect of dietary protein level on apparent ileal N and amino acid digestibilities in the growing pig (Dammers, 1964; Eggum, 1973; Sauer *et al.* 1980; Bell *et al.* 1983; Bell & Keith, 1989; Den Hartog *et al.* 1989; Furuya & Kaji, 1989; Keith & Bell, 1991), in the growing rat (Eggum, 1973; Shah *et al.* 1982; Sarwar & Peace, 1986) and in humans (Bressani *et al.* 1981; Hopkins, 1981).

In contrast, Buraczewska & Horaczynski (1983) observed no effect on the apparent ileal digestibility of amino acids when the protein content of the diet was increased from 10 to 20%. Similarly, Van Leeuwen *et al.* (1987), in studies with pigs fitted with ileal–caecal re-entrant cannulas, found no effect of the level of inclusion of soya-bean meal (20 or 40% of the diet) on amino acid digestibilities, irrespective of whether these were determined according to the ileal or faecal analysis method.

The positive effect of dietary protein content on apparent digestibilities of N and amino acids in MBM, as observed in the present study, is considered to be mainly due to the greater proportion of endogenous protein present at the terminal ileum, relative to protein of dietary origin, at low dietary protein levels (Taverner, 1979; Sauer *et al.* 1980). For the comparison of apparent digestibility between protein sources such as MBM, which is known to differ widely in protein content, the experimental diets are usually formulated to be isonitrogenous, but it is impossible for each individual amino acid to be adjusted. Thus, for meals with a lower content of one particular amino acid the apparent ileal digestibility would be expected to be lower due to the influence of endogenous ileal contributions, which are primarily related to the level of food dry matter intake. This would be especially so if such an amino acid had a relatively high endogenous ileal output. In practice, this effect would be particularly relevant for apparent digestibility values determined with compounds such as MBM having a variable protein content, or feedstuffs of low protein content. Given the relationship between apparent N and dietary protein levels (Fig. 1), and that feedstuffs differ widely in their protein content, from cereal grains as low as 70–80 g protein/kg to protein supplements of around 900 g/kg, then apparent digestibility coefficients for protein and amino acids, and corresponding estimates of digestible CP and amino acid contents, may be applicable only within the context of the type of diet used in the digestibility trial. The implication of this is that apparent digestibility coefficients determined at low dietary protein levels, for example in cereal diets, would not be applicable to balanced diets with higher levels of proteins. It is important, therefore, that apparent digestibility be corrected for endogenous excretions to achieve reliable values.

In the present study, two major factors known to influence the magnitude of endogenous N and amino acid excretion, namely dry matter intake and the type and level of dietary fibre in the diet, were controlled. There was a constant level of purified cellulose, as the sole dietary fibre source, and similar food intakes were recorded across diets on the last day of study.

Further, a new approach was used in the present study to correct for endogenous ileal

N and amino acid excretion. The mean ileal endogenous excretion rates of amino acids determined in the present study are in close agreement with values obtained using the same method (EHC technique) reported by Butts *et al.* (1991) and Donkoh (1993).

The digestibility coefficients presented here indicate that the correction of apparent N and amino acid digestibilities for endogenous excretion, as determined by the EHC technique, results in true ileal N and amino acid digestibilities which are markedly higher (12 percentage units for cystine and threonine and up to 14 percentage units for lysine and methionine) than corresponding apparent estimates, particularly at the lower dietary protein levels (25 g CP/kg diet). In contrast to the effect with apparent ileal digestibility, dietary protein content had no significant effect on the true ileal digestibility of amino acids in the meals tested in the present study. Confirmation of the absence of an effect of dietary protein content on true digestibility is provided by several authors for the rat (Sarwar & Peace, 1986), the chicken (Green, 1987; McNab, 1989; Zuprizal *et al.* 1991) and in the pig (Eggum, 1973; Taverner, 1979; Furuya & Kaji, 1989). It seems that true ileal digestibility is independent of the dietary protein concentration.

In our study it is evident that only at the dietary protein content of 165 g/kg does the apparent digestibility of protein approach the true digestibility of the MBM protein. At the higher dietary protein levels the disparity between true and apparent digestibilities decreased as dietary protein content increased. In this range (165–200 g CP/kg diet), increasing dietary protein content caused only small changes in the apparent estimates.

In general, there is controversy over the use of apparent or true amino acid digestibilities in the formulation of diets for farm animals. Just-Nielsen (1968) and Sauer (1976) have both argued that for practical purposes apparent digestibility coefficients are more relevant than true digestibility coefficients because both dietary and endogenous amino acids are lost to the animal and must be accounted for in diet formulation. However, this approach assumes that the supply of digestible amino acids in a mixture of feedstuffs is equal to the sum of the supply based on the digestibility values determined for the single ingredients. This additivity seems unlikely to be generally applicable to apparent ileal digestibility values. As true ileal amino acid digestibility is adjusted for the endogenous amino acid, true rather than apparent digestibility coefficients should be additive. True ileal amino acid digestibility coefficients should ultimately provide more meaningful data on amino acid absorption in the animal.

From the results of the present study it can be concluded that dietary protein concentration is an important factor which may have a major influence on the magnitude of the apparent ileal digestibility of crude protein and amino acids. Apparent digestibility values should only be compared under strictly standardized conditions. The digestibility of feedstuffs can be satisfactorily compared using apparent digestibilities provided that the dietary protein content of test diets is similar. Given that true ileal digestibilities appear to be independent of dietary protein content, their adoption may allow feed ingredients to be more accurately compared, thus leading to greater accuracies in diet formulation. A new, more physiological approach for determining endogenous ileal N and amino acid excretion rates applied here allows generation of true digestibility coefficients for feedstuffs not containing plant fibre or anti-nutritional factors.

The authors are indebted to G. Pearson, R. Watson and C. Flyger for their skilled technical assistance and to the New Zealand Pork Industry Board for financial support.

## REFERENCES

Alpers, D. H. (1987). Digestion and absorption of carbohydrates and proteins. In *Physiology of the Gastrointestinal Tract*, 2nd ed., vol. 2, pp. 1469–1487 [L. R. Johnson, editor]. New York: Raven Press.

Association of Official Analytical Chemists (1980). *Official Methods of Analysis*, 13th ed. Washington, DC: AOAC.

Bell, J. M. & Keith, M. O. (1989). Factors affecting the digestibility by pigs of energy and protein in wheat, barley and sorghum diets supplemented with canola meal. *Animal Feed Science and Technology* **24**, 253–265.

Bell, J. M., Shires, A. & Keith, M. O. (1983). Effect of hull and protein contents of barley on protein and energy digestibility and feeding value for pigs. *Canadian Journal of Animal Science* **63**, 201–211.

Bressani, R., Torun, B., Elias, L. G., Navrrete, D. A. & Vargas, E. (1981). A short-term procedure to evaluate protein quality in young and adult human subjects. In *Protein Quality in Humans: Assessment and In Vitro Estimation*, pp. 98–117 [C. E. Bodwell, J. D. Adkins and D. T. Hopkins, editors]. Westport, CT: AVI Publishing Co.

Buraczewska, L. & Horaczynski, H. (1983). Influence of dry matter intake on ileal nitrogen output and of protein intake on digestibility of amino acids in pigs. In *Protein Metabolism and Nutrition. Les Colloques de l'INRA*, no. 16, pp. 381–384 [R. Pion, M. Arnal and D. Bonin, editors]. France: Clermont-Ferrand.

Buraczewski, S. (1980). Aspects of protein digestion and metabolism in monogastric animals. In *Proceedings of the Third European Association for Animal Production Symposium on Protein Metabolism and Nutrition*, pp. 179–195 [H. J. Oslage and K. Rhor, editors]. Braunschweig, Germany: Institute of Animal Nutrition.

Butts, C. A., Moughan, P. J. & Smith, W. C. (1991). Endogenous amino acid flow at the terminal ileum of the rat determined under conditions of peptide alimentation. *Journal of the Science of Food and Agriculture* **55**, 175–187.

Costigan, P. & Ellis, K. J. (1987). Analysis of faecal chromium derived from controlled-release marker devices. *New Zealand Journal of Technology* **3**, 89–92.

Dammers, J. (1964). Digestibility research in pig feeding. The influence of different factors on the digestion of feed components and the digestibility of amino acids. PhD Thesis, University of Leuven, Belgium.

den Hartog, L. A., Verstegen, M. W. A. & Huisman, J. (1989). Amino acid digestibility in pigs as affected by diet composition. In *Absorption and Utilization of Amino Acids*, pp. 201–216 [M. Friedman, editor]. Boca Raton, Florida: CRC Press.

Donkoh, A. (1993). Amino acid digestibility in meat and bone meal for the growing pig. The development of a digestibility assay based on the laboratory rat. PhD Thesis, Massey University, New Zealand.

Donkoh, A., Moughan, P. J. & Smith, W. C. (1994). The laboratory rat as a model animal for determining ileal amino acid digestibility in meat and bone meal for the growing pig. *Animal Feed Science and Technology* (In the Press).

Eggum, B. O. (1973). *A Study of Certain Factors Influencing Protein Utilization in Rats and Pigs. Report of the National Institute of Animal Science, Copenhagen*, no. 406. Copenhagen: National Institute of Animal Science.

Fauconneau, G. & Michel, M. C. (1970). The role of the gastrointestinal tract in the regulation of protein metabolism. In *Mammalian Protein Metabolism*, vol. 4, pp. 481–522 [H. N. Munro, editor]. New York: Academic Press.

Furuya, S. & Kaji, Y. (1989). Estimation of the true ileal digestibility of amino acids and nitrogen from the apparent values for growing pigs. *Animal Feed Science and Technology* **26**, 271–285.

Green, S. (1987). *Digestibilities of Amino Acids in Foodstuffs for Poultry and Pigs. Digestibility Report* 8/87, pp. 2–8. France: Rhone Poulenc Nutrition Laboratories.

Hopkins, D. T. (1981). Effects of variation in protein digestibility. In *Protein Quality in Humans: Assessment and In Vitro Estimation*, pp. 169–193 [C. E. Bodwell, J. S. Adkins and D. T. Hopkins, editors]. Westport, CT: AVI Publishing Co.

Just-Nielsen, A. (1968). Digestibility of amino acids in relation to digestibility of nitrogen in bacon pigs. In *Royal Veterinary and Agricultural College Yearbook, 1968*, pp. 79–88. Copenhagen: Royal Veterinary and Agricultural College.

Keith, M. O. & Bell, J. M. (1991). Composition and digestibility of canola press cake as a feedstuff for use in swine diets. *Canadian Journal of Animal Science* **72**, 879–885.

Low, A. G. (1982). Endogenous nitrogen evaluation from absorption studies. In: *Physiologie Digestive Chez Le Porc*, pp. 189–198 [J. P. Laplace, T. Corring and A. Rerat, editors]. Paris: Institute National de la Recherche Agronomique.

McNab, J. M. (1989). Measuring availability of amino acids from digestibility experiments. In *Proceedings of the Seventh European Symposium on Poultry Nutrition, 9–21 June, Girona, Spain*, pp. 45–53. World's Poultry Science Association.

Moughan, P. J., Darragh, A. J., Smith, W. C. & Butts, C. A. (1990). Perchloric and trichloroacetic acids as precipitants of protein in endogenous ileal digesta from the rat. *Journal of the Science of Food and Agriculture* **52**, 13–21.

Sarwar, G. & Peace, R. W. (1986). Comparisons between true digestibility of total nitrogen and limiting amino acids in vegetable proteins fed to rats. *Journal of Nutrition* **116**, 1172–1184.

Sauer, W. C. (1976). Factors affecting amino acid availabilities for cereal grains and their components for monogastric animals. PhD Thesis, University of Manitoba, Canada.

Sauer, W. C., Just, A., Jorgensen, H. H., Fekadu, M. & Eggum, B. O. (1980). The influence of diet composition on the apparent digestibility of crude protein and amino acids at the terminal ileum and overall in pigs. *Acta Agriculturae Scandinavica* **30**, 449–459.

Sauer, W. C. & Ozimek, L. (1986). Digestibility of amino acids in swine: results and their practical applications. A review. *Livestock Production Science* **15**, 367–388.

Shah, N. O., Atallah, M. T., Mahoney, R. R. & Pellett, P. L. (1982). Effect of dietary fibre components on endogenous nitrogen excretion and protein utilization in growing rats. *Journal of Nutrition* **112**, 658–666.

Tanksley, T. D. & Knabe, D. A. (1984). Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. In *Recent Advances in Animal Nutrition*, pp. 75–95 [W. Haresign and D. J. A. Cole, editors]. London: Butterworths.

Taverner, M. R. (1979). Ileal availability for pigs of amino acids in cereal grains. PhD Thesis, University of New England, Australia.

Thomsen, L. (1981). Bowl for feeding powdered diets to rats. *Laboratory Animals* **15**, 177–178.

Van Leeuwen, P., Sauer, W. C., Huisman, J., van Weerden, E. J., van Kleef, D. & den Hartog, L. A. (1987). Methodological aspects for the determination of amino acid digestibilities in pigs fitted with ileo–caecal re-entrant cannulas. *Journal of Animal Nutrition and Animal Physiology* **58**, 122–133.

Zuprizal, Larbier, M., Chagneau, A. M. & Lessire, M. (1991). Effect of protein intake on true digestibility of amino acids in rapeseed meals for adult roosters force fed with moistened feed. *Animal Feed Science and Technology* **34**, 255–260.

*Printed in Great Britain*