Amino Acid Digestibility in Meat and Bone Meal for the Growing Pig: The Development of a Digestibility Assay Based on the Laboratory Rat

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Armstrong Donkoh

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CORRIGENDA

e 9, line 16 from the top should read "--- amino acids ---". On page 14, last line read "--- reference ---". On page 29, line 21 from the top should read "--- ginine ---". On page 33, line 34 from the top, "identical" should be "similar". On page 11 from the top should read "--- sampling time may vary with ---". On page 51, line the top should read "--- offered freely ---". On page 81 line 30 from the top should appears ---".

ABSTRACT

The study involved developing and applying a routine ileal amino acid digestibility assay, based on the sampling of digesta from the euthanased laboratory rat, with specific application to the digestion of meat and bone meal (MBM) protein by the growing pig. The work was conducted in six separate experiments.

1. The first experiment aimed to evaluate the slaughter method as a technique for sampling ileal digesta in the pig under defined sampling conditions. In a preliminary investigation, the influence of time after feeding (3, 4, 5, 7, 9 and 11 hours) on the amount of digesta obtained from the terminal 20 cm of ileum and apparent ileal nitrogen (N) digestibility was determined. Thirty-six 30 kg liveweight entire male pigs were given a semi-synthetic diet containing meat and bone meal (MBM) as the sole protein source and chromic oxide as an indigestible marker. The animals were given the MBM diet for 14 days and were killed by intracardial injection of a barbiturate on the 14th day. Digesta were flushed from the terminal ileum using deionised water. Secondly, the effect of site in the ileum for sampling digesta at 9 hours post-feeding was determined using 12 entire male pigs. The animals were equally and randomly allocated to two sampling sites (the terminal 0-20 cm or 0-40 cm of ileum). Further samples of ileal digesta were taken at regular 20 cm intervals up the final 140 cm of ileum of each pig. Sampling at 9 hours after the start of feeding resulted in the greatest and least variable sample size as well as the highest N digestibility. Sampling site within the terminal ileum had no significant ($P > 0.05$) influence on the apparent digestibility of nitrogen.

In the same study, comparison of apparent ileal amino acid digestibility in MBM, under the defined sampling conditions, was made between 8 pigs whose digesta were sampled from the terminal 20 cm of ileum at death 9 hours after the commencement of feeding, and 8 pigs with simple T-cannulas and with hourly collection of digesta over 10 hours on the final 2 days of the 14-day study. The simple T-cannulated animals were accepted as the control. There was no significant ($P > 0.05$) effect of digesta collection procedure on the apparent ileal N and amino acid digestibility coefficients. Faecal N and amino acid digestibility coefficients in intact pigs were identical to those in pigs fitted with a simple T-piece cannula in the distal ileum. Faecal digestibility values in both intact and cannulated pigs were, however, considerably ($P < 0.05$) higher than the corresponding ileal values. It was concluded that the slaughter technique is a viable alternative to simple T-cannulation for the determination of N and amino acid digestibility in the pig given a semi-synthetic MBM diet.

2. The second experiment determined the optimal digesta sampling conditions with the slaughter method applied to the laboratory rat. The effect of time after feeding (1, 2, 3, 4, 5 and 6 hours) on the amount of digesta obtained from the terminal 20 cm of ileum and apparent ileal N digestibility was investigated. Thirty-six 190 g male rats received a semi-synthetic diet whose sole protein source was meat and bone meal. Chromic oxide was

added to the diet an indigestible marker. The animals were given the MBM diet for 14 days and were killed by CO_2 asphyxiation on the 14th day. Digesta were sampled from the terminal ileum. Furthermore, the effect of site within the terminal ileum (0-5, 0-10, 0-15 and 0-20 cm) for sampling digesta 4 hours post-feeding was determined with 72 male rats. The optimal time for sampling digesta was 4 hours after the start of feeding, while 20 cm of ileum was the maximum length of ileum sampled without affecting apparent N digestibility. Significantly ($P < 0.05$) higher quantities of digesta were collected from the terminal 20 cm of ileum compared to the shorter ileum lengths.

3. In experiment three, the use of the rat as a model animal for allowing the determination of apparent ileal amino acid digestibility in the growing pig, using the slaughter method was evaluated. Sixteen male rats and 16 male pigs were fed diets containing chromic oxide and as the sole protein source, meat and bone meals which were expected to differ in their quality. Ileal contents from the terminal 20 cm of ileum were collected after slaughter of the rats and pigs, 4 and 9 hours after the start of feeding, respectively. Inter-species comparison made under the defined conditions, indicated close agreement between the rat and pig for the ileal digestibility of N and most of the amino acids in each of the two different meat and bone meals evaluated. It was concluded that the growing rat is a satisfactory model for the growing pig, for determining ileal amino acid digestibility in meat and bone meal. The measurement of digestibility at the terminal ileum indicated differences in amino acid digestibility between two meat and bone meals, however, the faecal approach which generated significantly higher digestibility coefficients than the ileal digestibility method, did not allow the differences in amino acid digestibility to be detected.

4. Experiment four was undertaken to compare a recently-developed peptide alimentation method and the protein-free and regression methods for determining endogenous ileal N and amino acid excretion in the rat, and was aimed at the development of a true ileal digestibility assay with application to meat and bone meal. Preliminary investigations determined the effect of the time of sampling of digesta from rats given a protein-free (PF) or an enzymically hydrolysed casein (EHC) based diet, on ileal digesta and endogenous N excretion. There was a significant ($P < 0.05$) effect of the time of sampling on the amount of digesta collected and the endogenous N excretion for both the EHC- and PF-fed rats. The amount of digesta collected from the terminal 20 cm of ileum and the endogenous N excretion for both the EHC- and PF-fed rats were least variable at 3 hours post-feeding.

In the main study, endogenous ileal amino acid excretions were determined in the growing rat fed an EHC-based diet and with subsequent treatment of the digesta using ultrafiltration ($n = 6$) or in 6 rats given a PF diet or by extrapolation from data for 30 animals given 5 diets which contained graded levels of MBM as the protein source. For the EHC treatment, the ileal digesta precipitate plus retentate was used to determine the endogenous flows. Such processing excludes unabsorbed dietary amino acids from the measure of endogenous loss. Chromic oxide was the reference marker in all the diets. The endogenous

flows determined by the protein-free and the regression method were similar but both significantly ($P < 0.01$) lower than those for rats fed the EHC-based diet. The mean (±SE) endogenous ileal N flows determined by the peptide alimentation method, the protein-free and regression approaches were 1866 (±30.8), 1103 (±22.6) and 1019 (±3.6) $\mu g\ g^{-1}$ FDMI, respectively. It was concluded that endogenous amino acid flows at the terminal ileum were underestimated when determined using the traditional protein-free or regression methods.

5. The effect of dietary protein content (25, 60, 95, 130, 165 and 200 g CP kg^{-1} diet) on the apparent and true ileal digestibility of N and amino acids was investigated. Semi-synthetic diets in which the protein content was varied by the inclusion of graded amounts of MBM (50 to 400 g kg^{-1} diet) at the expense of maize starch were fed to 36 growing rats for 14 days. On the 14th day, the rats were fed and euthanased 4 hours after the start of feeding and digesta were collected from the terminal 20 cm of ileum. Endogenous amino acid excretion was determined for 18 rats given an EHC-based diet. The EHC-fed rats were euthanased 3 hours after the start of feeding and digesta were collected from the terminal 20 cm of ileum. The true ileal digestibility values determined with reference to chromium as a marker, were higher than the corresponding apparent estimates. Apparent digestibility of N and amino acids significantly ($P < 0.001$) increased with increasing dietary protein level, however, dietary protein content had no significant ($P > 0.05$) effect on the true ileal digestibility of N and amino acids. The mean apparent ileal digestibility of N in MBM ranged from 65.6 to 75.3%. The corresponding range of true ileal digestibility of N was 76.9 to 78.2%

6. The final experiment generated data on the nutrient compositions of meat and bone meals collected from eight processing plants throughout New Zealand. The true ileal digestibility of the amino acids in the meat and bone meals were also determined using the rat assay developed in the previous studies. Endogenous amino acid excretion, used for the correction of apparent digestibility values to true estimates, was determined for rats given a protein-free or an enzymically hydrolysed casein diet. The meat and bone meals were variable in their nutrient composition and in the true ileal digestibility of protein and amino acids. The true nitrogen and amino acid digestibility coefficients based on endogenous flows determined by the EHC method were markedly higher than with the protein-free diet. The true ileal N and lysine digestibility coefficients based on the respective endogenous flows for rats fed the EHC diet ranged from 62.7 to 88.9% and 66.4 to 92.3%, respectively. Values determined with endogenous flows for rats fed the protein free diet ranged from 59.0 to 85.2% for N, and 63.2 to 88.9 for lysine. The variable ileal digestible N and amino acid contents of meat and bone meals emphasise the limitation of tabulated analytical values and the need for a routine relatively inexpensive digestibility assay.

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GENERAL INTRODUCTION

Food is the major item of expenditure in pig production and rate and composition of pig growth the major determinants of product value. Consequently, any decision regarding the feeding strategy to be adopted for growing pigs, is a key one influencing long term profitability. It is important to minimise feed cost and increase the efficiency of conversion of feed to animal protein.

In this respect, there has been much interest over recent years in developing technologies to assist in the formulation of diets for pigs. Linear programming has become an extensively used procedure and computerised mathematical models simulating pig growth are becoming increasingly important tools for specifying dietary nutrient requirements. These approaches require that consideration be given not only to the gross amino acid composition of the dietary ingredients but also to determination of the bioavailability of the amino acids.

Bioavailability refers to the amount of each amino acid in a feedstuff which can potentially be utilised for body protein synthesis and other anabolic processes, following the successive steps of digestion, absorption and metabolism. Despite considerable research effort having been devoted to the development of techniques for the routine assessment of the amino acid availability of pig feedstuffs, a satisfactory method has yet to emerge. In the absence of a routine measure of amino acid availability, methodology has concentrated on the measurement of amino acid disappearance from the gut, i.e. digestibility. There is now general agreement that digestibility measurements made at the end of the ileum of the pig are superior to the traditional faecal approach.

Apparent ileal amino acid digestibility values for feedstuffs used in formulating pig diets are now appearing in feed composition tables. However, these 'average' values are generally based on only a few individual values, which themselves are variable, while data are sparse or lacking for several important feedstuffs. In view of the *in vivo* measurement of ileal amino acid digestibility in pigs being expensive and labour demanding, the laboratory rat has been used as a model for the growing pig in determining protein (amino acid) digestibility at the terminal ileum. Preliminary results from this model approach have been sufficiently encouraging to warrant further research.

The main objective of this study was to further develop and evaluate a rat bioassay for determining the ileal amino acid digestibility of feedstuffs for the growing pig. The assay, developed here, was specifically for application to meat and bone meals and the refined technique was used to determine the digestion of protein in a range of commercial meat and bone meal samples. Meat and bone meal was chosen for study as it is of considerable value as a major protein source in pig and poultry diets in several countries but is known to be of variable quality.

Chapter 1

Review of Literature

1.1 INTRODUCTION

The overall function of the digestive process is to reduce food to a molecular size that will allow absorption to take place. However, several factors, including inaccessibility of the protein to proteolytic enzymes, resistance to enzymatic hydrolysis, inhibition of proteolytic enzymes or inhibition of amino acid absorption may alter the extent of protein digestion and/or absorption.

Comprehensive reviews on the physiology of digestion with emphasis on the pig have been published by Kidder and Manners (1978) and Low and Zebrowska (1989). Detailed discussion of the nervous and hormonal control of secretion and motility in the gastrointestinal tract have been adequately covered in several other reviews (Kidder and Manners, 1978; Walsh, 1981; Holst, 1986; Fioramonti and Bueno, 1988; Strombeck and Guilford, 1990) and mechanisms of amino acid absorption from the gastrointestinal tract have been reviewed by Adibi (1985), Silk *et al.* (1985), Steinhardt (1987), Friedrich (1989), Webb (1990) and Rerat and Corring (1991). Extensive reviews specifically on digestion and absorption of protein have also been presented (Snook, 1973; Rerat *et al.*, 1976; Kidder and Manners, 1978; Rerat, 1981; Hunt and Groof, 1990; Rerat and Corring, 1991).

In general, dietary protein sources differ in their susceptibility to digestive breakdown which implies the importance of being able to quantitate the degree of digestion and absorption of dietary amino acids. The present review considers the concept of availability and digestibility of protein and amino acids and focuses on the various approaches to their determination. The use of the laboratory rat as a model animal for digestion in the pig is introduced and reviewed.

1.2 THE CONCEPT OF AMINO ACID DIGESTIBILITY AND AVAILABILITY

The terms "digestibility" and "availability" are key facets of dietary protein quality and have been ascribed to amino acids to demonstrate their supply from feedstuffs. With the high cost and general world-wide shortage of protein-rich feedstuffs, it is becoming increasingly important that the proportion of each amino acid digested and absorbed for useful metabolism, is known rather than relying on gross amino acid content. This will help to ensure that animal diets are adequately and economically formulated and that the maximum effectiveness is obtained from a given feedstuff. Furthermore, the need for a rapid, simple and economical method to assess protein quality is heightened by the range and quantity of materials and the effects of processing and storage on amino acid digestibility. In addition, new protein sources are continuously being sourced and these need to be accurately evaluated for protein quality.

Although digestibility is clearly different from availability, the two terms are often used synonymously. Digestibility refers to the combined effects of digestion and absorption but provides no information as to the extent of utilization of the absorbed nutrient (McNab, 1976).

A nutrient can be defined as available if, on entering a living tissue, it can potentially be used for the metabolic functions of protein maintenance or new protein synthesis. By definition, therefore, the bioavailability of an amino acid in a food refers to the amount which is absorbed by the gut in a structural form that can potentially be used for protein synthesis or other anabolic processes. Absorption of a nutrient from the lumen of the gut is a precondition for, but not proof of availability. Digestibility values for nitrogen and amino acids may overestimate availabilty, especially in materials damaged by excess heat during processing (Carpenter, 1973).

1.2.1 Determination of Amino Acid Availability in Feedstuffs

Currently, there are different assays (Whitacre and Tanner, 1989), based on various assumptions, which are used to determine bioavailable amino acid concentrations in feedstuffs. The methods used fall into a number of categories: biological, enzymatic, chemical, microbiological, and others which mainly involve the chemical measurement of a biological effect, such as blood measurements and muscle assays.

1.2.1.1 In Vitro Assays to Predict Amino Acid Availability

Several *in vitro* assays have been developed which purport to measure one or more bioavailable amino acids in feedstuffs. There is considerable appeal in evaluating proteins by means of *in vitro* assays. Such assays are relatively simple, inexpensive and rapid, and high levels of precision are often achieved without resort to the use of animals. In addition many samples can be compared at the same time. One method which has found widespread application (Hurrell and Carpenter, 1981; Erbersdobler and Anderson, 1983; Finot and Hurrell, 1985) is the fluoro-dinitrobenzene (FDNB) method (Carpenter, 1960) for the determination of the amount of chemically available lysine.

The major drawback with the FDNB-reactive lysine method is that it does not detect structurally unaltered lysine units which may be resistant to digestion and absorption. There is evidence that the chemically available or reactive lysine units in heat treated proteins may not be completely absorbed and in consequence chemically available lysine values overestimate the uptake of the amino acid (Moughan, 1991). Moreover, hydrolysis of the protein is required before the lysine derivative can be detected and this can lead to loss of the derivatives (Hurrell and Carpenter, 1981).

The assays appear to be capable of providing useful quality control information, particularly in identifying heat damage in feedstuffs. The data obtained by chemical assays such as the FDNB one are more useful for ranking feedstuffs than for providing absolute

values. The correlation between such assays and pig performance is often low (Batterham *et al.*, 1979; Taverner and Farrell, 1981).

Although chemical methods may be used to determine available lysine, it is not possible to evaluate other amino acids, with the possible exception of methionine (Lipton and Bodwell, 1977; MacKenzie, 1977; Bos *et al.*, 1983), since suitable methodologies have not been developed.

Coon (1990) recently published an extensive review of the limitations of present methods for amino acid availability determination in poultry, pigs and non-ruminant animals. It was concluded that amino acid availability can be affected by factors other than the Maillard reaction in feeds, for example, the presence of protease inhibitors, tannins and β-glucans. There is considerable scope for critical re-evaluation of existing assays and also for new approaches to determining the availability of dietary amino acids.

1.2.1.2 In Vivo Measures of Availability

The baseline against which all the *in vitro* procedures should be compared is animal whole-body amino acid retention per unit dietary amino acid intake. First, the availability of amino acids *in vivo* can be determined by measurement of protein deposition, by measuring nitrogen (N) balance though this has its limitations (Duncan, 1966; Just *et al.*, 1982). A superior approach is to determine the retention of amino acids in the whole body after slaughter.

Availability can also be determined from blood amino acid levels (Morrison *et al.*, 1961; Zimmerman and Scott, 1965) or from blood urea (Munchow and Bergner, 1967; Eggum, 1970, 1973a). Blood amino acid concentrations, however, are influenced by physiological and pathological as well as dietary factors (Feigin *et al.*, 1967; Boomgardt and MacDonald, 1969; Ishibashi and Kametaka, 1974). The plasma free amino acid assay has been used to assess the effects of heating on the availabilities of some amino acids (Smith and Scott, 1965a,b; Erbersdobler *et al.*, 1972). Further work on the accuracy and precision of blood urea concentrations as a means of determining protein quality in pigs would seem worthwhile.

Rerat *et al.* (1980) described a method for the quantitative measurement of amino acids absorbed and transported into the hepatic portal vein. The method requires catheterisation of the portal vein and carotid artery and a procedure for measuring blood flow. The virtue of the method is that it provides a net absorption picture for nutrients absorbed throughout the gut. Nevertheless, the method is technically complex and time consuming and a large number of analyses are required to construct a profile of absorption. It is not, therefore, suitable for practical feed evaluation. Moreover, the method does not take into account the high rate of metabolism in the gastrointestinal tract, and differences in dietary and blood amino acids are obscured by different rates of removal of amino acids from the plasma to meet the requirements of the tissues.

Also, measures of amino acid availability can be based on urinary urea output (Brown and Cline, 1974) or free amino acid levels in muscle (Pion, 1973). The sensitivity of these tests as predictors of amino acid availability, needs to be investigated.

Growth assays have also been suggested as tests for amino acid availability, and these have been discussed in detail by Sibbald (1987). The growth assay provides a combined measure of digestibility and post-absorptive utilization of the amino acids. A refinement of the growth assay is the slope-ratio procedure which has been described by Batterham *et al.* (1979). A modification of the slope-ratio has been described by Leibholz (1986). Such assays, though sensitive tests of protein availability, have their limitations (Austic, 1983; Whitacre and Tanner, 1989). It is the aspect regarding the post-absorptive utilization of amino acids that imposes some limitations on this assay, because many factors other than the limiting amino acid may influence post-absorptive utilization. The potential difficulties of the growth assay are well illustrated by recent work in Australia (Batterham, 1987). In a collaborative study involving two Research Centres, considerable differences in the estimates of lysine availability, as determined by the slope-ratio assay with weaner and grower pigs given the same sample of cottonseed meal, were obtained. The results unexpectedly indicated much higher lysine availability (0.69) for weaner pigs (Leibholz, 1986) compared to grower pigs (0.27) (Batterham *et al.*, 1979; 1984).

The variation in the availability coefficient for a specific amino acid with the growth assay can be considerable, depending on whether availability is estimated from weight gain, daily carcass gain or from feed conversion. This was illustrated by Batterham *et al.* (1979) who measured the availability of lysine by a slope-ratio assay, relative to the utilisation of free lysine given eight times daily. The availability of lysine in cottonseed meal was 0.39 when measured by daily carcass gain, but 0.62 as measured by liveweight gain (refer Table 1.1).

TABLE 1.1
Availability of lysine (proportion of total) in protein concentrates, as determined using the slope ratio assay

	Criterion of response					
	Liveweight gain		Food conversion efficiency		Carcass gain	
Protein concentrate	Mean	SD[+]	Mean	SD	Mean	SD
Cottonseed meal	0.62	0.082	0.69	0.078	0.39	0.107
Fish meal	1.04	0.090	1.06	0.086	0.89	0.104
Meat and bone meal	0.66	0.082	0.67	0.078	0.50	0.098
Skim milk powder	0.88	0.086	0.86	0.081	0.88	0.103
Soybean meal	0.93	0.087	0.91	0.082	0.87	0.103

Adapted from Batterham *et al.* (1979);
[+] SD = standard deviation

Leibholz (1989) has also discussed how results may vary markedly dependent upon the response criterion adopted. The question as to the best response criteria for amino acid availability determinations remains open.

Differences in feeding method may also influence the estimates obtained by the dose/response assay. The pigs of Leibholz (1986) had unrestricted access to food, while in the experiments of Batterham *et al.* (1984) the level of feed intake was the same for all pigs. Under controlled feeding, all animals receive similar quantities of test proteins (on a liveweight basis), whereas with full feeding, there are differences in intake and this makes it more difficult to relate the responses to the test amino acid alone.

Another limitation with the assay is the impact of potential amino acid imbalances. Baker (1978) showed that when the amino acid pattern of the test material is ignored, undetected growth stimulations or depressions might affect the animal receiving the test protein, thus, over- or underestimating amino acid availability.

Also, large standard deviations occur in measurements of this type reflecting that the method is open to error from several sources (Austic, 1983; Sibbald, 1987). This means that it is difficult to assign absolute values for amino acid availability, as measured by slope-ratio assays. Furthermore, growth assays such as the slope-ratio assay can only evaluate one amino acid at a time, which makes it very expensive, time consuming and unsuited to routine application. Copelin *et al.* (1978), for example, used 236 individually-fed pigs to determine the availability of tryptophan, lysine and threonine in one sorghum hybrid. Unless such numbers of animals are used the assays are often imprecise because of the variation in growth response between individual animals. It is concluded that a satisfactory growth assay has yet to be developed (Sibbald, 1987).

Summary

The determination of amino acid availability in feedstuffs is a complex problem that has evoked considerable research effort. The various methods described do not completely fulfil the requirements for the reliable evaluation of dietary protein quality. There is still much uncertainty both in the understanding of the problem and in the acceptance of techniques. However, the growing interest in the use of by-products and novel feedstuffs in animal nutrition, and the extent to which protein and amino acid quality can be changed by processing underlines the need for a rapid, accurate and inexpensive method of assessment. In the absence of a reliable method for the screening of amino acid availability in various feedstuffs, research has concentrated on the quantitative measurement of absorption. Although a technique has been developed for the direct measurement of amino acid absorption in the pig (Rerat *et al.*, 1980), it is only suited for the research laboratory. For practical purposes methodology has concentrated on measurement of amino acid disappearance from the gut, that is, digestibility.

1.2.2 Measurement of Amino Acid Digestibility in Feedstuffs

1.2.2.1 In Vitro Determination of Digestibility: Enzymatic Methods

Many attempts have been made to simulate the *in vivo* processes of protein digestion using *in vitro* methods, and subsequently numerous proteolytic enzymes have been tested for their ability to release amino acids from the test material. Earlier methods for determining protein digestibility *in vivo* relied on single-enzyme digests (Rayner and Fox, 1976; McBee and Marshall, 1978; Holz, 1972; Pieniazek *et al.*, 1975) but more recently multi-enzyme assays, more closely simulating conditions found in the live animal have been developed (Furuya *et al.*, 1979; Pedersen and Eggum, 1981, 1983; Dierick *et al.*, 1985; Metz and Van der Meer, 1985; Lowgren *et al.*, 1989; Graham *et al.*, 1989).

Moughan *et al.* (1989a) compared three *in vitro* multi-enzymatic methods, those of Hsu *et al.* (1977), modified by Satterlee *et al.* (1979), of Taverner and Farrell (1981) and of Metz and Van der Meer (1985) for evaluation of meat and bone meals. The original Hsu *et al.* (1977) method was shown to be a particularly precise assay in spite of the high buffering capacity of the meat and bone meals. It was found that the best correlation with *in vivo* ileal protein digestibility was with pH measurement at 10 minutes ($r = \pm 0.75$; $P < 0.001$). The method has, however, been criticised for its sensitivity to the buffering capacity of the protein suspensions, pentosans, phenolic acids and other ionizable compounds (Hahn *et al.*, 1982; Pedersen and Eggum, 1983; Moughan *et al.*, 1989a).

Numerous authors have discussed the advantages and disadvantages of enzymatic methods for protein quality evaluation (Stahmann and Woldegiorgis, 1975; Hsu *et al.*, 1977; Bodwell and Marable, 1981). Enzymatic methods give a relative rather than an absolute measure of amino acid digestibility. On the basis of the information available, it appears that these methods may be useful for ranking feedstuffs in terms of their apparent digestibility of nitrogen, rather than providing values for diet formulation.

Even though such *in vitro* methods are rapid, relatively inexpensive and reproducible, they cannot mimic the complex and dynamic conditions in the digestive tract *in vivo*, where exogenous and endogenous amino acids are mixed and where concentration, competition, inhibition, microbial, neural and humoral effects interact (Low, 1982a). It is important to recognise that they do not imitate apparent digestibility, because endogenous secretions are either not included at all or only at low levels. A method must be developed to the point that is applicable to all types of feeds and correlated with biological or *in vivo* estimates.

1.2.2.2 In Vivo Determination of Digestibility

1.2.2.2 (a) Faecal Versus Ileal Digestibility Measurements

The commonly used procedure for determining amino acid digestibility for pigs has been the faecal index method that was first used by Kuiken and Lyman (1948), with rats as the test animals. While the overall apparent digestibility measurement (that is, using faecal collection) is not technically difficult, there are basic objections to this approach because of

the presence of undigested and unabsorbed endogenous protein in the faeces (Just, 1980), and possible microbial alterations of undigested and unabsorbed endogenous and exogenous N residues, in the large intestine. The hindgut microflora hydrolyse the nitrogenous compounds and most of the nitrogen is absorbed as amines (amides) and ammonia (Michel, 1966) instead of amino acids and is excreted in the urine. Further evidence of the intense fermentation that occurs in the large intestine of the pig is the predominance of bacterial N (62 - 76% bacterial N as a percentage of total N) in the faeces (Mason, 1984). As a result, the amino acid composition of faeces in pigs fed diets that differ widely in amino acid composition and digestibility is rather similar (Mason *et al.*, 1976). Although the effect of hindgut microbial metabolism on protein digestion does appear to be a rather general phenomenon across animal species (Moughan and Donkoh, 1991), it is not necessarily of practical significance in all cases. The extent of microbial activity depends on type and numbers of microorganisms present, the type of feedstuff and the time of residence of material in the hindgut. It is thus a function of both species of animal and diet.

From several studies, involving the determination of ileal and faecal N and amino acid digestibilities in pigs and employing a variety of methods for collecting ileal digesta, some general findings have emerged, indicating the superior predictive accuracy of ileal digestibility values. The differences between ileal digestibility and apparent digestibility measured in faeces do not appear to be constant. Depending on the amino acid and on the feedstuff considered, the digestibilities obtained with the faecal method overestimate (Low, 1982b; Sauer *et al.*, 1982a; Tanksley and Knabe, 1984) (which is usually the case) or underestimate those obtained by the ileal method. For example, Taverner (1984) compared a diet based on soyabean meal with one based on meat and bone meal and found that discrepancies between faecal digestibility and ileal amino acid digestibility were much greater for the meat and bone meal diet (Table 1.2)

TABLE 1.2
Differences between apparent faecal and ileal digestibility (%) of amino acids for the growing pig

Amino acid	Soyabean meal	Meat and bone meal
Lysine	+3.3	+14.7
Threonine	+7.1	+21.5
Methionine	-2.1	+9.3
Average of all amino acids	+2.9	+16.5

Adapted from Taverner (1984)

Large differences between ileal and faecal apparent digestibilities have typically been found for proteins of low digestibility (Zebrowska and Buraczewski, 1977; Jorgensen and

Sauer, 1982). Differences are lower for "high quality" proteins, such as, milk powder or casein. The review of Zebrowska (1978) further highlights the significance of differences between faecal and ileal estimates of the apparent digestibility of N and amino acids in feedstuffs used in practical dietary formulation.

Consequently, the terminal ileum appears to be a more appropriate site to assess the amounts of N and amino acids absorbed. The justification for this view comes from pig experiments showing that the ileal digestibility of amino acids, at least for a range of commonly used feedstuffs which have not been subjected to high temperatures during their processing, is a good indicator of amino acid availability (Laplace *et al*., 1985; Leibholz, 1985; Dierick *et al*., 1987; Green and Kiener, 1988; Laplace *et al*., 1989). However, recent studies in the pig (Fuller *et al*., 1981; Jagger *et al*., 1987; Batterham *et al*., 1990a,b) have shown that digestible amino acids are not always reliable indicators of amino acid availability. This is further discussed in section 1.3.3. Nevertheless, the ileal method for determining protein digestibility remains the method for providing the most meaningful availability values for amino acids.

Further justification for assessing the amounts of N and amino absorbed at the terminal ileum comes from studies indicating that proteins infused into the large intestine are largely degraded with a low utilisation, compared to oral administration (Zebrowska, 1973). Reports by other researchers (Zebrowska, 1975; Sauer, 1976; Hodgdon *et al*., 1977; Gargallo and Zimmerman, 1981; Just *et al*., 1981) clearly confirm that protein or amino acids infused in the large intestine make little or no contribution to the protein status of the pig.

In addition, other recent studies (Low and Partridge, 1984; Just *et al*., 1985; Leibholz, 1985; Moughan and Smith, 1985; Dierick *et al*., 1987) indicate close correlation between the apparent ileal digestibility of amino acids and animal performance (carcass retention, daily gain and feed conversion). In a recent review of ileal digestibility values, Tanksley and Knabe (1984) concluded that ileal digestible values offer great potential for increasing the precision of diet formulation for the growing pig.

Moreover, apparent ileal digestibility coefficients have been shown to be sensitive in detecting small differences in protein digestibility due to the processing of foods (Rudolph *et al*., 1983; Vandergrift *et al*., 1983; Van Weerden *et al*., 1985; Sauer and Ozimek, 1986; Knabe *et al*., 1989) compared with faecal estimates. The phenomenon of a distinct depressive effect of overheating on ileal amino acid digestibility, but not on faecal digestibility, has been observed by Schutte *et al*. (1987) who compared the digestibilities of protein and amino acids of *Phaseolus vulgaris* beans heat-treated in two different ways (105°C for 40 minutes, 105°C for 110 minutes) in intact cocks and birds cannulated at the terminal ileum. With the 40-minute autoclaved beans, the differences between faecal and ileal digestibilities were relatively small. However, with the beans heated for 110 minutes, the digestibilities were lower, especially the ileal digestibilities of threonine and tryptophan, confirming that ileal digestibility is more sensitive to the effects of heat treatment than the

faecal method.

The above discussion serves to highlight the inadequacies of the traditional faecal measure of amino acid digestibility. The ileal, rather than faecal analysis method, should be used to determine amino acid digestibilities (Rerat, 1981; Tanksley and Knabe, 1984; Laplace *et al.*, 1985; Sauer and Ozimek, 1986). To allow comparison between the different types of digestibility measurements, however, requires that methods be developed to allow adequate collection of ileal digesta, and this in itself has not been straightforward.

1.2.2.2 (b) Digesta Collection With Pigs

Considerable work has been done with ileal collection methods in pigs. Further studies are required, however, before drawing firm conclusions about which procedure should be adopted. The different methodologies for the measurement of digestion have been discussed in recent reviews (Sauer *et al.*, 1989a; Low, 1990; Fuller, 1991). These methods mainly involve the surgical implantation of cannulas.

The ileo-ileo and ileo-caecal re-entrant cannulation procedures described by Cunningham *et al.* (1963) and Hazem and Drochner (1976) involve total transsection of the small intestine, with the physiological complications that entails, and there are difficulties in their use with fibrous diets, such as blockages proximal to the ileal cannula (Sauer and Ozimek, 1986; Oslage *et al.*, 1987; Schroder *et al.*, 1989). In addition leakages around the cannulas have been reported by these authors.

Another technique, which allows for routine total collection of ileal digesta, is ileo-rectal anastomosis, whereby digesta pass directly from the ileum to the rectum bypassing the large intestine (Fuller and Livingstone, 1982; Picard *et al.*, 1984a; Darcy-Vrillon and Laplace, 1985; Souffrant *et al.*, 1985; Hennig *et al.*, 1986; Green and Kiener, 1989). There are several variations of this method which is also referred to as the "ileo-rectal shunt (IRS) procedure" and involves transection of the distal ileum anterior to the ileo-caecal valve. Pigs prepared with the IRS require much less time and effort to maintain than pigs fitted with re-entrant cannulas. Food intake can be maintained at normal levels and diets relatively high in fibre, which include many of the by-products, can be tested. However, there are serious doubts concerning the physiological normality of anastomised animals (Picard *et al.*, 1984a; Moughan, 1991).

A rapid but rather complicated assay for ileal measurement of amino acid digestibility, using a modification of the nylon bag technique for energy evaluation has been reported (Sauer *et al.*, 1983a; Leibholz and Gannon, 1987; Cherian *et al.*, 1989; Leibholz, 1991; Van der Poel, 1991). The method would seem to offer promise for rapid assessment of large numbers of samples of a given type of feedstuff, at least to provide a measure of relative protein quality, for thermally processed feedstuffs, such as beans and also to predict whole-tract protein digestibility of feedstuffs for pigs (Sauer *et al.*, 1989b). While there are considerable attractions with the mobile bag approach, there are grounds for concern

regarding the isolation of the test material from contact with the gut wall. Firstly, the material enclosed in a nylon bag is never able to come into contact with membrane-bound hydrolases. The second is that the MNBT does not allow for the effect of interaction between the antinutritional factors in samples (for example, lectins; trypsin inhibiting activity) and the digestive tract (Cherian *et al.*, 1988; Huisman *et al.*, 1988; Sauer *et al.*, 1989b), which in normal digestion would result in villus atrophy and increased endogenous nitrogen loss (Kik *et al.*, 1989). In the study of Huisman *et al.* (1988) ileal MNBT determinations did not predict the negative *in vivo* effects of antinutritional factors in soya and *Phaseolus vulgaris* beans. This method warrants further investigation.

The post-valvular ileo-colic fistulation or the ileo-colic post-valve procedure (Darcy *et al.*, 1980a), post-valvular T-caecum cannulation (PVTC) (van Leeuwen *et al.*, 1988), and the simple T-cannulation all have the distinct advantage that the functional integrity of the small intestine and of the ileo-caecal valve is maintained. When simple T-cannulation of the ileum is adopted the surgery is less invasive than for the other two approaches but with this technique digesta can be collected only by spot-sampling. This requires the use of an indigestible marker compound such as chromic oxide or titanium oxide. An extensive review of the use of markers in nutritional studies has been published by Kotb and Luckey (1972). As a modification of the PVTC cannulation, the so-called steered ileo-caecal valve (SICV) cannulation has been reported by Mroz *et al.* (1991). However, Mroz *et al.* (1991) have suggested a limited application of this technique of about 6-8 weeks due to proliferation of fibrous tissue and dilation of the distal ileum by muscular hypertrophy.

Although the post-valve T-caecum technique (van Leeuwen *et al.*, 1988) would appear to be the current method-of-choice, its superiority over the simple T-cannulation of the ileum remains to be demonstrated. The potential impact of any form of cannulation on the normal physiological functioning of the animal, however, should not be overlooked. Livingstone and McWilliam (1985) reported that pigs with simple T cannulas placed in the terminal ileum had similar voluntary feed intakes to their non-cannulated counterparts but exhibited lower growth rates and less efficient feed utilisation. Also, Wenham and Wyburn (1980) in a radiological investigation with sheep, found that several forms of cannulation, including simple T-piece cannulation, caused some disruption to the normal digesta flow. Furthermore, the use of cannulated pigs for the routine determination of amino acid digestibilities is costly, labour intensive and time consuming.

The simplest method for the assessment of amino acid digestibility up to the terminal ileum involves collecting the digesta from the ileum under anaesthesia before sacrifice of the animal (Payne *et al.*, 1968; Kies *et al.*, 1986; Moughan and Smith, 1987; George *et al.*, 1988; van Barneveld *et al.*, 1991). The digestibility of amino acids can then be measured with reference to an indigestible marker given with the test feedstuff. The method involves minimal disruption of normal digestive function and samples of digesta for any type of diet may be obtained from several parts of the digestive tract. The main criticism of this method

concerns the potential difficulty of obtaining representative samples of digesta. However, digestibility data obtained using this technique (coupled with a frequent feeding regime or sampling at a predetermined optimal time) are not necessarily any more variable than those obtained from cannulated animals (Moughan, 1991). In addition, it can be expected that the shedding of mucosal cells into the intestinal lumen at death (Badawy *et al.*, 1957; Fell, 1961) may have an influence on digestibility measurement of the nitrogenous compounds. Therefore digesta samples have to be obtained by avoiding mucosal shedding. This can be done under anaesthesia or by euthanasia with a barbiturate such as sodium pentobarbitone (Badawy, 1964). This approach is, however, expensive when applied to large species (Fuller, 1991). The method is suited to small animal (e.g. rat and chick) digestibility assays in which large numbers of animals can be used for treatment replication.

Although the methods involving cannulation and other surgical operations may be appropriate for research purposes, they are unlikely to be appropriate for routine use in feed evaluation. To a considerable extent the staple feedstuffs used for pigs are also used for other monogastric animals such as poultry. It would therefore be very useful in practice if their nutritive values were demonstrated to be similar in both species. It would also be an advantage if nutritive values measured in small animals such as the rat could be applied to pigs and to poultry. Ileal digesta samples can be obtained quickly and easily from the rat after slaughter and this species lends itself to relatively inexpensive, well controlled experimentation with large numbers of animals being able to be studied at any one time. An overall objective of the work reported in this thesis was to evaluate a rat digestibility assay, based on sampling of digesta from euthanased animals, in determining ileal amino acid digestibility in meat and bone meal for the growing pig. Consequently, the use of the laboratory rat as a nutritional model for the growing pig in digestibility studies is discussed in detail in section 1.4.

1.2.2.3 Limitations of the Ileal Method

It is now generally agreed (Low, 1980a; Rerat, 1981) that the ileal method is preferred to the faecal one as a means of determining the digestion and absorption of dietary amino acids in animals. However, the ileal assay currently being used to determine the nutrient potential of feeds has been criticised by Rerat (1990) both from a quantitative point of view and in terms of the true course of nutrient disappearance from the gut. Also, digestibility does not provide complete information about differences in the bioavailability of amino acids (Batterham *et al.*, 1990a). It is possible that digestibility coefficients for lysine and perhaps in a similar manner those for methionine, cystine and tryptophan are somewhat inaccurate, for feedstuffs having undergone the Maillard reaction. Maillard compounds are considered as digested sources of amino acids with the ileal method, but being excreted in the urine, are not available for protein synthesis. With the early stages of the Maillard reaction, the deoxyketosyl derivative (Amadori compound) formed may revert back to lysine during the

acid hydrolysis employed in amino acid analysis. Thus, the actual lysine present in feeds or ileal digesta from an animal fed the processed feedstuff may be overestimated. There may also be a generally lowered *in vivo* digestibility of all amino acids due to the formation of enzyme resistant cross-linkages and a possible direct effect of the advanced Maillard compounds on the digestive enzymes (Hurrell and Finot, 1985; Oste *et al.*, 1986).

Digestibility, however, should be a useful overall criterion of bioavailability for feeds not having undergone significant heat-damage, and for processed foods, digestibility coefficients for amino acids other than lysine, tryptophan and the sulphur amino acids should be meaningful. Also, and for the key dietary amino acids lysine, tryptophan and the sulphur amino acids in processed feeds, ileal digestibility coefficients may well be satisfactory for practical dietary formulation purposes. The degree of error caused by amino acid reversion during hydrolysis is unknown. Austic (1983) concluded that for most processed feedstuffs the relative quantity of absorbed lysine which is unavailable is small and does not pose a major disadvantage for the ileal digestibility assay.

A further potential criticism of the ileal measure is that nutrients may be destroyed or modified in the digestive tract as a result of the action of the microflora in some segments of the proximal gut such as the stomach (Schneider and Bolduan, 1985) and the terminal ileum where a slowing down of transit occurs (Rerat, 1990). The *in vitro* and *in vivo* studies of Dierick *et al.* (1986a,b) indicate that there may be a small but measurable catabolism of amino acids by the flora in the upper digestive tract of the pig.

The duration of transit of a feed in the proximal digestive tract ranges from 4-5 hours to 14-16 hours according to whether the initial or the terminal fractions of the feeds arriving at the ileo-caecal junction are being considered (Darcy *et al.*, 1980b). Consequently, digestibility measurements in pigs are affected by the frequency and total period of sampling. In the case of simple ileal or caecal cannulation studies most authors agree that regular sampling of large amounts of digesta throughout 12-hour periods, replicated on several successive days, will provide representative samples, provided that the pigs are fed at regular intervals. In the case of re-entrant cannulation, collections have usually lasted for 24-72 hours, and collections can be taken from pigs with ileo-rectal shunts for periods of many days. Circadian rhythms are important in determining sampling frequency from simple cannulas, as described by Livingstone *et al.* (1980) and Graham and Aman (1986). With the use of the slaughter method, sampling of digesta at a predetermined optimal time may generate reliable digestibility estimates.

Digestibility measurements determined by the slaughter or simple T-piece cannulation methods also depend upon the representativeness of the samples collected and the validity of the marker used. As already pointed out, a wide range of markers is used and all have some shortcomings. It is generally accepted that comparisons between the digestibility of different feedstuffs can be made within a trial using the same marker, but it must be recognised that the behaviour of a marker may be influenced by the physico-chemical

nature of the materials with which it moves. There is the need for further systematic and comparative studies on the behaviour of markers used for digestibility measurements in pigs.

Nevertheless, it remains that digestibility is an important factor influencing availability since undigested amino acids represent an often large source of amino acid loss. Expression of an amino acid requirement and diet composition in terms of ileal digestible amino acids should be an improvement over traditional approaches.

1.2.2.4 Factors Influencing the Accuracy of Determination of Dietary Ileal Amino Acid Digestibility

Various factors may influence the ileal digestibility of amino acids in pigs. Within study comparisons of digestibility are therefore often more useful than the absolute values obtained, which might have been different if they had been determined with different pigs or under different conditions.

1.2.2.4 (a) Effect of Digesta Collection Method

Since different methods have been used to sample ileal digesta, it is important to know whether they give rise to similar digestibility coefficients. Some comparisons of the different methods have been performed to investigate their specific effects on digestibility (Zebrowska *et al.*, 1978; Taverner *et al.*, 1983; Schroder, 1988; Kohler *et al.*, 1990, 1991; Fuller, 1991). Picard *et al.* (1984b) compared results from four preparations, which are shown in Table 1.3. These show, for the most part, good agreement.

TABLE 1.3.
Mean apparent digestibility of lysine, threonine and methionine for seven raw materials, estimated in intact and caecectomised cockerels, in cannulated pigs and in rats with ileo-rectal anastomosis

	Lysine	Threonine	Methionine
Intact cockerel	0.66	0.72	0.73
Caecectomised cockerel	0.68	0.60	0.80
Cannulated pig	0.70	0.73	0.81
Anastomised rat	0.70	0.70	0.83

Adapted from Picard *et al.* (1984b)

Moreover, Leterme *et al.* (1990a) found only minor differences in the digestibility of the amino acids in peas between anastomised and T-cannulated pigs. On the other hand, Darcy-Vrillon and Laplace (1990) reported lower apparent digestibility of total N and amino acids in a semi-synthetic diet containing beet pulp, with ileo-rectal anastomised pigs compared with an ileo-colic post-valve cannulation which was considered as a refernce

method.

Kohler *et al.* (1991) conducted studies to compare different techniques for collection of ileal digesta in pigs. Digestibilities of crude protein and lysine of three different feedstuffs (maize, groundnut meal expeller and sunflower meal) and measured in PVTC cannulated pigs were comparable with corresponding results from the literature (Jorgensen *et al.*, 1984; Just *et al.*, 1985; Green *et al.*, 1987; Knabe *et al.*, 1989; Van Leeuwen *et al.*, 1991) in which re-entrant cannulae, or simple T-cannulae or ileorectal anastomosis were used. It was concluded that PVTC cannulation is an appropriate alternative for digestibility measurements at the distal ileum of pigs.

Overall, it appears that post-valvular cannulation of the caecum using a simple T-piece cannula, the traditional T-piece cannulation of the terminal ileum or the slaughter method are likely to yield the most reliable results (Moughan and Donkoh, 1991). George *et al.* (1988) compared ileal digestibilities of N by the slaughter technique with those obtained by cannulation methods and found no differences. Moughan and Smith (1987) and van Barneveld *et al.* (1991) have also compared ileal digestibility of amino acids from the slaughter method with those from T-piece cannulated animals and these show good agreement. However, variability of data was generally greater using the slaughter method. Therefore, greater replication may be required if the slaughter technique is to be used.

1.2.2.4 (b) Effect of Food Intake

Studies by Sauer *et al.* (1982b) did not show any effect of level of food intake (0.84, 1.26 and 1.68 kg dry matter per day) on the apparent ileal digestibility of amino acids in a barley meal diet, determined with growing pigs fitted with ileo-caecal re-entrant cannulas. Van Leeuwen *et al.* (1987) fed a corn-soybean meal diet at levels of 2.4 and 3.2 times the metabolizable energy required for maintenance (this corresponded with 1.7 and 2.3 kg feed per day, respectively). There was no significant effect of feeding level on apparent ileal digestibility. They suggested that the level of feed intake has no effect on nutrient digestibility if highly digestible diets are used, but when less digestible diets are used, there may be a decline in apparent nutrient digestibility as the level of food intake is increased.

1.2.2.4 (c) Effect of Dietary Fibre

When the amount of crude fibre in the diet is increased, the overall digesta transit time is reduced (Fioramonti and Bueno, 1980; Kuan *et al.*, 1983). Den Hartog *et al.* (1985), however, found a decrease in rate of passage in the small intestine when the amount of fibre in the diet was increased from 5.2 to 9.2%. The delay in rate of passage in the small intestine may be caused by a slower stomach emptying after an increase of the fibre content of the diet. The composition of the diet is not only important in relation to the rate of passage and digestibility of nutrients, but also influences the endogenous N. Increasing dietary fibre may result in sloughing off of mucosal cells and an increased mucus production

(Schneemann *et al.*, 1982).

Fibre can physically hinder the access of the proteolytic enzymes due to interference from the thick cellulosic cell walls. *In vitro* studies suggest that fibre may also adsorb trypsin and chymotrypsin, as shown by a decrease in the activities of these enzymes after incubation of pancreatic juice with different types of fibre (Schneemann, 1978). The ileal digestibility of amino acids in sows of 120 kg, however, was not influenced by the inclusion of 5 or 7.5% ground straw in the diet (Den Hartog *et al.*, 1988a). Possibly, the enzyme secretion in older pigs is sufficient to overcome an increase in fibre content of the diet, at least up to a certain level of inclusion.

Soluble fibre fractions forming a gel may obstruct the access of the digestive enzymes. The inclusion of 5% pectin in a diet that contained 20% soya protein decreased the apparent ileal digestibilities of the essential amino acids by 3.5 - 16.5% in experiments on pigs (Dierick *et al.*, 1983a). Fibre is capable of adsorbing amino acids and peptides and withholding them from absorption, and the extent of such adsorption depends on the degree of lignification (Bergner *et al.*, 1981).

1.2.2.4 (d) Effect of Dietary Protein Concentration

In general, a positive relationship between protein level in the diet and the apparent ileal digestibility of amino acids has been found, because the endogenous fraction which is primarily related to the dry matter intake remains fairly constant while the undigested dietary protein fraction increases, with an increase in protein level (Eggum, 1973b; Sauer *et al.*, 1980; Bell *et al.*, 1983; Furuya and Kaji, 1989; Keith and Bell, 1991). Theoretically, the ileal apparent digestibility of protein will increase and reach a plateau with increasing protein concentration of the diet. However, Buraczewska and Horaczynski (1983) reported that increasing the protein content of the diet from 10 to 20% had no effect on the apparent ileal digestibility of the amino acids. Level of inclusion of soyabean meal (20 or 40% at the expense of corn) had no effect on apparent amino acid digestibility in soyabean meal, irrespective of whether this was determined according to the ileal or faecal analysis method (Van Leeuwen *et al.*, 1987).

It seems that true ileal digestibility is independent of dietary protein concentration (Taverner, 1979; Green, 1987; McNab, 1989; Furuya and Kaji, 1989; Zuprizal *et al.*, 1991).

1.2.2.4 (e) Effect of Dietary Fat

Generally, it appears that the quantity and type of fat may influence protein digestibility (Nielsen *et al.*, 1985a,b; Ozimek *et al.*, 1985). With changing fat contents of the diet, other components of the diet are also altered. Fat may delay stomach emptying and subsequently affect the ileal digestibility of amino acids. Sauer *et al.* (1980), found a 3% unit increase in the ileal digestibility of crude protein and amino acids when the dietary animal fat concentration was increased from 4.5 to 26.8% of diet dry matter.

1.2.2.4 (f) Effect of Antibiotics

In general, some antibiotics added to the diet may increase the ileal digestibility of amino acids, while there is no real effect on the disappearance rate of amino acids from the large intestine. Just *et al.* (1980) reported that addition of nebacetin to the diet increased the apparent ileal digestibility of dietary amino acids, with the exception of histidine, isoleucine, and phenylalanine.

Avoparcin (an antibiotic produced by a strain of *Streptomyces candidas*) has been shown to increase the apparent absorption of amino acids from the ovine and porcine small intestine (Livingstone *et al.*, 1982; Macgregor and Armstrong, 1984). Parker *et al.* (1984) found that avoparcin stimulated dipeptidase activity in the small intestinal mucosa of the rat and thus possibly enhances the uptake of dietary peptides. Moughan *et al.* (1989b), however, found no significant effect of avoparcin or zinc bacitracin addition on the apparent ileal digestibility of N and amino acids in pre-ruminant milk-fed calves.

1.2.2.4 (g) Effect of Anti-Nutritional Factors

The presence of antinutritive factors (food chemical components, particularly those from plants) can interfere with the digestion and absorption of dietary proteins (or to a lesser extent with the breakdown of other food components) either directly, by reacting with food proteins and making them less digestible or indirectly, by reacting with gut cells and affecting their digestive, absorptive, secretory, or protective functions.

Tannins (polymeric phenolic substances) may bind to proteins forming complexes resistant to proteolytic enzymes, or may bind directly to enzyme proteins (Eggum and Christensen, 1975; Krogdahl, 1987); thus affecting protein digestion. Cousins *et al.* (1981) studied the apparent ileal digestibility of amino acids in low- and high-tannin sorghum and found that the digestibility of all amino acids, with the exception of methionine, was lower in high-tannin sorghums.

Protease inhibitors (proteins with molecular weights between 6000 and 25000 Daltons) may bind to the proteolytic digestive enzymes (trypsin and chymotrypsin) of animals and thus affect the digestion of proteins in the small intestine. The negative effect of trypsin inhibitor on the ileal digestibilities of amino acids in soyabean has been shown by Vandergrift *et al.* (1983) and Ozimek and Sauer (1985) and recently with peas by Leterme *et al.* (1990b).

Lectins or phytohemagglutinins (glycoproteins with molecular weights ranging from 91000 to 120000 Daltons) decrease the digestibility and availability of dietary amino acids because their action causes a loss of absorptive surface area in the intestine (Jaffe, 1980).

1.2.2.4 (h) Effect of Processing

The processing of feedstuffs may also have a major effect on protein and amino acid

digestibility within the small intestine. For example, the ileal digestibilities of amino acids were higher in finely ground than in cracked wheat (Sauer *et al.*, 1977b) while decreasing the particle size of sorghum improved amino acid digestibilities (Owsley *et al.*, 1981). A depressive effect of over-heating on ileal amino acid digestibility in beans, determined with cannulated birds, has been reported by Schutte *et al.* (1987). During the processing of feedstuffs such as meat and bone meal, materials may be subjected to variable and high degrees of pressure and temperature for varying lengths of time and this will affect the digestibility and availability of the protein content in the feedstuff produced. The Maillard reaction (or non-enzymic browning) may be responsible for reducing protein and amino acid digestion and utilization. The reaction occurs when reducing carbohydrates combine with free amino groups of proteins. The Maillard reaction is greatly influenced by moisture content, pH, temperature and the type of reducing compounds present (Hurrell and Carpenter, 1978). Maillard reaction may occur between amino acids and amines, with sugars, aldehydes and ketones (Hurrell and Carpenter, 1978). Digestive enzymes do not cleave peptide bonds adjacent to an amino acid which has a carbohydrate moiety attached to it. Since trypsin is specific for peptide bonds containing a carboxyl group from lysine or arginine and an alpha amino group from another amino acid, the carbohydrate that is bound to the free epsilon amino group of lysine interferes with the ability of trypsin to break the peptide bond. The inability to cleave lysine peptide bonds by trypsin results in a decreased protein digestion and reduced availability of lysine and other amino acids.

1.3 TRUE AND APPARENT DIGESTIBILITY

Accepting that amino acid digestibility should be based on measurements made at the terminal ileum of monogastric animals, it needs to be recognised that ileal digesta are derived both from dietary and endogenous sources. Endogenous amino acid loss is used to correct apparent digestibility coefficients to true values.

True amino acid digestibility has the advantage over apparent digestibility in that it is a fundamental property of a feed ingredient regardless of the dietary conditions under which the ingredient is fed (McNab, 1976). For a given amino acid, apparent digestibility increases exponentially with the ingested quantity because endogenous excretion, as a percent of total excretion, decreases proportionally (Sauer *et al.*, 1980; Bell *et al.*, 1983; Furuya and Kaji, 1989; Keith and Bell, 1991). By contrast, several studies (Taverner, 1979; Hopkins, 1981; Sarwar and Peace, 1986; Green, 1987; McNab, 1989; Furuya and Kaji, 1989; Zuprizal *et al.*, 1991) indicate that true amino acid digestibility is not affected by the level of dietary protein intake. Therefore, using true rather than apparent digestibility allows raw materials to be accurately compared, even if they are ingested in different quantities.

The argument as to whether apparent or true digestibility values are preferred for practical dietary formulation is also inextricably linked to the approaches adopted in estimating amino acid requirements for growth. In the formulation of diets for pigs, it is

assumed 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. For feedstuffs with a lower level in one amino acid, their apparent ileal digestibility would be reduced by the influence of the endogenous ileal contributions (Sauer and Ozimek, 1986; Furuya and Kaji, 1989). As true ileal amino acid digestibility is corrected for the endogenous ileal amino acid, true rather than apparent digestibility values would be expected to be more additive (Taverner *et al.*, 1981a). Green *et al.* (1987) and Furuya and Kaji (1991) concluded that when calculating the amino acid digestibility in feed formulation, true rather than apparent amino acid digestibility should be used. However, the difficulty of accurately determining endogenous excretion (Kidder and Manners, 1978), has prompted many researchers to recommend the use of apparent amino acid digestibility values for the formulation of diets (Low, 1982b; Austic, 1983; Sauer *et al.*, 1983b).

Low (1980a) claimed that for practical purposes, apparent digestibility coefficients are more meaningful than true digestibility coefficients because the former indicates the net loss which results from feeding a test diet, without taking into account the origin of the amino acids. This might be important for estimating the digestibility of complete feeds formulated to satisfy requirements which do not include the endogenous amino acid excretion from the digestive tract. However, for the purpose of evaluating individual feedstuffs for diet formulations, true digestibility values may be a better alternative. In support, the Agricultural Research Council (1981) observed that there was a trend towards expressing the composition of feed ingredients in terms of truly absorbed nutrients while allowing for endogenous loss in the calculation of nutrient requirements.

Also, with increasing interest in the use of computer models to simulate biological aspects of growth in animals such as the pig and chicken, an accurate account of the animal's metabolism of protein is required, including the estimation of endogenous protein excretions in the terminal ileum. There is an increasing trend to define amino acid requirements at the tissue level using computerised simulation models and in this case true coefficients of amino acid digestibility are most meaningful for dietary formulation. An increased need for dietary amino acids by the animal to offset a heightened loss of amino acids from the gut due to components of a mixed diet such as anti-nutritional factors or complex non-starch polysaccharides can be accounted for when modelling the amino acid transactions.

1.3.1 Endogenous Protein Secretion Into The Gastrointestinal Tract

Exogenous dietary proteins provide over half the peptides and amino acids eventually absorbed from the gastrointestinal tract of the monogastric animal (Smith, 1990). The contents of the gastrointestinal tract are composed not only of undigested residues from ingested protein, but also of products of endogenous origin (digestive enzymes, mucoproteins, desquamated epithelial cells, urea, amino acids produced by cellular

catabolism and albumin) and other non-dietary, but not strictly endogenous, materials such as bacteria and ingested hair, which dilute the exogenous residues (Rerat *et al.*, 1976). The amounts of protein contributed by each component differ. The endogenous N secretion into the porcine digestive tract in relation to N intake is shown in Table 1.4

TABLE 1.4
Endogenous nitrogen secretion into the digestive tract of the growing pig, as a percentage of food nitrogen intake

	%
Salivary + Gastric secretion	5.0 - 8.0
Pancreatic secretion	4.0 - 15.6
Bile secretion	4.5 - 6.5
Small intestinal secretion	22.0 - 26.5
Sloughed cells	2.5 - 3.5
Entire endogenous secretion	38.0 - 60.1

Adapted from Auclair (1986)

The data in Table 1.4 indicate that the total endogenous N secretion to the end of the ileum is 38 - 60% of the total N intake, with secretion from the small intestine accounting for 22 - 27% of total N intake. The N from sloughed cells was 3 - 4% of total N intake. In contrast to the latter values, Potten (1986) observed that approximately half of the total endogenous protein entering the small intestine comes from replacement of epithelial cells lining the intestinal lumen (10^9 cells weighing 1 g being produced every 16 min in the human, and 4.5 days in the mouse intestine). This high rate of cell turnover is presumably needed in order to maintain the barrier function of the epithelium and enable the intestine to adapt rapidly to changes in the local environment (Smith, 1990).

The composition of endogenous ileal digesta from the pig determined under dietary protein-free conditions was determined by Taverner *et al.* (1981b). The four most abundant amino acids in endogenous ileal digesta were identified as proline, glycine, glutamic acid and aspartic acid in order of decreasing abundance, with serine and threonine being the next most abundant. These six amino acids are also reported to occur most abundantly in mucin protein (Horowitz, 1963). Taverner (1979) and Zebrowska (1982) have also shown that amino acid composition of the endogenous protein at the terminal ileum is similar to that of the muco-proteins. Hashimoto *et al.* (1963) found that muco-proteins are moderately resistant to proteolytic enzymes. Thus, it appears likely that muco-proteins, such as those present in the mucosal secretions, are a major source of endogenous protein N appearing in the ileum. This was supported by Fauconneau and Michel (1970) who reported that 75% of endogenous excretion was likely derived from the intestinal mucosa in the form of muco-

protein and the protein present in the sloughed-off epithelial cells.

Digestion of the endogenous protein secretions occurs along the entire length of the intestine and is probably a continuous process. All of these proteins are assumed to be digested and absorbed in a similar way to those arriving from the diet, though it is realised that the digestibility of individual proteins varies widely (Alpers, 1987). Fauconneau and Michel (1970) and Zebrowska and Buraczewska (1972) considered the digestion of endogenous protein to be much slower than that of exogenous protein, with the majority of endogenous protein digestion occurring in the ileum and caecum by the joint action of bacterial and endogenous enzymes. In contrast, other workers (Nasset *et al.*, 1973; Romero and Canolty, 1979) have shown endogenous and exogenous proteins to be digested and absorbed at similar rates. Taverner (1979) considered after reviewing the available literature, that most of the digestive enzymes secreted into the gut would be digested and reabsorbed without contributing significantly to the overall net endogenous excretion of protein from the gut.

Estimates of the quantity of endogenous protein digested and reabsorbed, reported in the literature, vary markedly. According to Souffrant (1991), data on the total amount of N secreted into the digestive tract during the passage of digesta vary between 16 and 33 g depending on the literature consulted. This endogenous N is reabsorbed, at least partially. Rerat (1990) using a number of different techniques calculated that 46 g of endogenous amino acids were digested and absorbed from a total secretion of 54 g (i.e. 85% of total N secreted is recycled). The estimates of Rerat *et al.* (1976) and Low (1982c) indicate that approximately half (54%) of the total endogenous N secreted is digested and absorbed after secretion into the gastrointestinal tract.

Using the estimates of Rerat *et al.* (1976) and Low (1982c), Butts (1991) calculated that approximately 8 g of endogenous N and 38 g of endogenous amino acids secreted into the gastrointestinal tract remained undigested and unabsorbed at the end of the ileum, while using the estimate of Rerat (1990) gave 2.8 g of endogenous N and 12 g of endogenous amino acids remaining undigested and unabsorbed at the end of the ileum. The values of 3 - 6 and 0.7 - 7.3 g of N per day leaving the terminal ileum of the pig reported by Low and Zebrowska (1989) and Souffrant (1991), respectively, are in agreement with the latter estimate.

1.3.2 Factors Affecting Endogenous Secretion

Dietary protein and peptides have been shown to influence the secretion of specific digestive enzymes (Green *et al.*, 1973; Corring, 1980). Schneeman (1982) and Temler *et al.* (1983) found that in the rat and other mammalian species, dietary peptides or proteins are much more potent stimulators of pancreatic secretion than are free amino acids. Rerat *et al.* (1976) provided evidence that the pancreatic secretions increased in proportion to increases in the protein component of the diet, and Snook (1965) suggested that dietary protein can

affect the endogenous N output both quantitatively and qualitatively through modifying secretion and increasing the stability of the enzymes. Different protein sources or differently treated proteins influenced the N secretion by porcine pancreas. However, conflicting views do exist and Green and Nasset (1983) found no difference in pancreatic enzyme activity between rats fed intact casein- or synthetic amino-acid-based diets. Corring (1980) observed that a higher dietary protein content resulted in an increase in protease activity in pancreatic juice but not in a significant change in the amount of N secreted.

The content of antinutritional factors is also an important factor that affects the endogenous excretion of nitrogen. Trypsin inhibitors and tannins appear to increase the output of endogenous proteins (Green *et al.*, 1973; Rostango *et al.*, 1973).

Inclusion of fibre in the diet has been shown to increase sloughing-off of intestinal mucosal cells (Bergner *et al.*, 1975) and to enhance mucus production (Schneeman *et al.*, 1982), resulting in increased losses of amino acids. Sauer *et al.* (1977a) also found that endogenous ileal amino acid excretion, determined with protein-free diets, was markedly increased as dietary fibre was increased with additions of cellulose as the fibre source. An increase of dietary crude fibre content from 2.0 to 6.4% resulted in an increase of daily N secretion in pancreatic juice from 2.5 to 3.0 g (Zebrowska, 1985).

The dietary fat content also affects the amount of N in the pancreatic juice of the pig. Ozimek *et al.* (1985) observed an increase of 0.4 mg of N secreted at a dietary fat content of 15% compared to a fat-free diet.

Another factor capable of affecting the amount of endogenous N in ileal chyme is reabsorption of endogenous nitrogen. Souffrant *et al.* (1986) reported that for a N intake of 23.6 g, a total amount of 7.4 g N had been secreted endogenously, with a reabsorption rate of 70% up to the terminal ileum and 82% up to the rectum.

1.3.3 Determination Of The Endogenous Excretion Of Protein

Although the need for correction of apparent amino acid digestibility values for endogenous excretion is recognised, there are problems in attempting to apportion amino acids appearing in ileal digesta to dietary or endogenous origin. Various approaches to the estimation of endogenous amino acids have been employed. These include:

1.3.3.1 The Protein-Free Method: The traditional method for determining endogenous N and amino acid excretion involves feeding the animals a protein-free diet and then measuring the N and amino acids in digesta collected at the terminal ileum (Sauer *et al.*, 1977a; Taverner *et al.*, 1981b; Leibholz, 1982; Darcy and Laplace, 1984; Moughan, 1984; Kies *et al.*, 1986; Skilton *et al.*, 1988; de Lange *et al.*, 1989a,b; Furuya and Kaji, 1989; Wang and Fuller, 1989; Leterme *et al.*, 1990a; Furuya and Kaji, 1991; Hennig *et al.*, 1991).

Literature values for the endogenous N and amino acid excretion (expressed as mg kg^{-1} dry matter intake) from the terminal ileum of the growing pig are given in Table 1.5.

TABLE 1.5

Summary of literature values for endogenous ileal amino acid excretion (g kg^{-1} dry matter intake) in the pig determined under protein-free alimentation

Amino acid	Reference												
	1	2	3	4	5	6	7	8	9	10	11	12	Mean
Lysine	0.27	0.53	0.25	0.47	0.27	0.48	0.53	0.63	0.26	0.38	0.23	0.46	0.40
Methionine	0.06	0.17	0.10	0.10	0.06	0.18	0.16	0.22	0.14	0.11	0.13	0.12	0.13
Cystine	-	0.30	-	0.19	0.13	-	-	-	-	0.19	0.12	0.14	0.18
Histidine	0.14	0.26	-	0.18	0.12	0.18	0.22	0.26	0.15	0.41	0.21	0.13	0.21
Phenylalanine	0.23	0.40	-	0.38	0.23	0.45	0.60	0.79	0.25	0.36	0.57	0.30	0.41
Tyrosine	0.13	0.38	-	0.33	0.21	0.36	0.41	0.47	0.30	0.35	0.35	0.19	0.32
Threonine	0.39	0.97	0.53	0.48	0.35	0.43	0.65	0.91	0.46	0.87	0.69	0.34	0.59
Leucine	0.39	0.76	0.34	0.64	0.40	0.72	0.60	0.77	0.41	0.62	0.62	0.49	0.56
Isoleucine	0.21	0.79	0.19	0.34	0.24	0.60	0.36	0.47	0.23	0.28	0.31	0.33	0.36
Valine	0.31	0.94	0.33	0.51	0.35	0.67	0.48	0.65	0.34	0.45	0.45	0.37	0.49
Alanine	0.42	0.69	0.44	0.46	0.36	-	0.59	0.73	0.42	-	1.01	0.50	0.56
Aspartic acid	0.56	1.17	0.70	0.79	0.48	-	1.01	1.24	0.50	-	0.90	0.62	0.80
Arginine	0.49	0.43	0.48	0.41	0.25	0.34	0.73	0.62	0.37	0.45	0.39	0.30	0.44
Serine	0.38	1.56	0.50	0.42	0.30	-	0.70	0.85	0.46	-	0.58	0.30	0.61
Glutamic acid	0.71	2.26	0.72	0.89	0.64	-	1.16	1.39	0.61	-	1.11	0.82	1.03
Glycine	1.39	1.02	1.51	0.62	0.46	-	1.94	1.44	1.23	-	0.57	0.45	1.06
Proline	4.74	1.65	2.29	0.41	0.35	-	6.22	3.64	-	-	0.55	0.79	2.29
Total amino acids	10.95	14.47	8.83	7.58	5.20	-	16.36	15.08	6.13	-	9.28	-	10.38
Nitrogen	2.05	2.97	1.81	1.36	-	1.12	3.17	2.96	1.71	-	1.38	1.35	0.91

1. Sauer *et al.* (1977a), n = 6, 45-70 kg
2. Van Weerden *et al.* (1980), n = 8, 45 kg
3. Taverner *et al.* (1981b), n = 5, 86 kg
4. Darcy *et al.* (1982), n = 5, 59 kg
5. Green *et al.* (1987), n = 4, 20-25 kg
6. Leibholz and Mollah (1988), n = 6, 25 kg
7. De Lange *et al.* (1989a), n = 6, 60 kg
8. De Lange *et al.* (1989b), n = 4, 55 kg
9. Furuya and Kaji (1989), n = 4, 42 kg
10. Wang and Fuller (1989), n = 8
11. Leterme *et al.* (1990a), n = 6, 50 kg
12. Hennig *et al.* (1991), n = 8, 144 kg

The values presented in this table are from studies with the growing pig given protein-free diets containing 3 - 6% dietary fibre. The endogenous amino acid excretions are highly variable between studies, particularly for proline. Serine, threonine, proline, glycine, aspartic and glutamic acids are predominant in endogenous ileal amino acid excretions determined under protein-free alimentation. These amino acids constitute a large proportion of the mucus glycoproteins (Hashimoto *et al.*, 1963; Bella and Kim, 1972, Cetta *et al.*, 1972). De Lange *et al.* (1989b) cited evidence for the high proline secretion under protein-free alimentation being the result of large quantities of glutamine from muscle breakdown being metabolised to proline in the intestinal tract. This may be enhanced by the reduction of intestinal transport of proline and other amino acids under protein-free alimentation (Karasov *et al.*, 1987). Taverner *et al.* (1981b) suggested that the high levels of proline and glycine in endogenous ileal excretion following protein-free feeding were the result of their absorption as constituents of small peptides and their reflux back into the lumen as free amino acids, following intracellular digestion.

Sauer (1982) interpreted true digestibility coefficients determined using a protein-free diet of greater than unity, in particular for proline and glycine, to be due to an overestimation of these amino acids under protein-free alimentation.

The protein-free method has been criticised (Low, 1982c; Sauer, 1982) due to the possible influence the addition of fibre, often added to protein-free diets, has on excretion and due to the absence of dietary protein. It is not possible to investigate quantitatively or qualitatively relations between protein feeding and endogenous losses.

The protein-free method has also been criticised because of the possible inducement of physiologically abnormal metabolism with unknown consequences on endogenous protein excretion (Low, 1980a). When animals are deprived of dietary protein and enter negative body N balance, their rate of whole-body protein synthesis falls (Millward *et al.*, 1976). Therefore, the overall endogenous excretion of protein into the gut would be expected to decrease, probably by way of reduced pancreatic secretions and mucus production. Given that N-free feeding is not physiologically normal and could evoke specific reactions in the animal body, it is not clear if data obtained with this method can be extrapolated to protein feeding.

The validity of determining endogenous amino acid excretion following protein-free alimentation has, however, received support from recent work where the endogenous ileal amino acid flows of protein-free fed animals were similar to those found in animals given either a synthetic amino acid diet devoid of certain non-essential amino acids (Skilton *et al.*, 1988) or a protein-free diet with simultaneous intravenous infusion of amino acids (de Lange *et al.*, 1987; 1989b). From the results of these two studies, it does not appear that the protein-deplete state *per se* affects endogenous amino acid loss at the distal ileum of animals. It remains possible, however, that dietary peptides exert a direct stimulatory effect on gut endogenous protein secretion.

It has been demonstrated with the growing pig (Buraczewska, 1979) and the rat (Darragh *et al.*, 1990; Butts *et al.*, 1991) that the presence of dietary protein or peptides in the gut lumen increases endogenous excretion of amino acids at the distal ileum. Therefore, an alternative technique for determining endogenous amino acid flows is needed whereby the endogenous excretion of protein into the digestive tract can be measured under conditions of peptide and protein alimentation.

1.3.3.2 The Regression Method: Another traditional method for determining endogenous N and amino acid excretion, adopting protein-containing diets, is the regression method. The regression procedure adopted by several workers (Carlson and Bayley, 1970; Taverner *et al.*, 1981b; Leibholz, 1982; Moughan *et al.*, 1987; Leibholz and Mollah, 1988; Furuya and Kaji, 1989) involves feeding a range of diets containing graded levels of a protein source, measuring the ileal protein flow for each diet and extrapolating back to zero protein intake by linear regression, to give an estimate of the endogenous protein excretion. This technique has an advantage in that the influence of dietary protein and fibre is accounted for. Also the regression method makes it possible to assess the effects of protein quality and specific protein components, for example, antinutritional factors (Souffrant, 1991). However, the linear regression method for estimating endogenous excretion has been criticised, because the estimation of endogenous loss is constrained by a mathematical function which may not be a suitable descriptor of the real biological phenomenon (P.J. Moughan, personal communication). The increase in amino acid flow with increasing protein intake is attributed entirely to increased amounts of undigested food protein, assuming that there is no change in the amount of endogenous amino acid excretions. There is evidence, however, that the rate of excretion into the intestine does vary with the amount of protein given (Snook and Meyer, 1964a,b; Temler *et al.*, 1983; Ozimek *et al.*, 1984). Consequently, some of the increase in amino acid flow with increased dietary protein intake is probably the result of enhanced secretion of endogenous proteins. Souffrant (1991) also reported that it is unlikely that a linear relation exists between feed intake and endogenous N or amino acids in digesta or faeces. An increase in the protein level of the feed used with the regression method is always associated with changes in the composition of the other crude nutrients and other feed ingredients, therefore hindering the interpretation of the results regarding causes and effects.

Literature values for endogenous amino acid excretions at the terminal ileum of the pig determined by the regression method are shown in Table 1.6. There is some variation across the different studies which is probably due to differences in digesta collection methods and animal liveweights. These values determined by the regression method are similar to those found for pigs given a protein-free diet. This can be seen more clearly in those studies where both methods have been used to determine the flow of amino acids at the terminal ileum of the pig (Taverner *et al.*, 1981b; Leibholz and Mollah, 1988; Furuya and

TABLE 1.6
Summary of literature values for endogenous ileal amino acid excretion (g kg^{-1} dry matter intake) in the pig determined by the regression method

Amino acid	Reference								
	1 Wheat	1 Barley	1 Overall	2 Milk	2 Cottonseed meal	3 Casein	4 Milk proteins	5 Barley	Mean
Lysine	0.33	0.28	0.32	0.37	0.37	0.26	1.05	0.32	0.41
Methionine	0.14	0.16	0.13	0.16	0.12	0.14	0.30	0.08	0.49
Cystine	-	-	-	-	-	-	0.30	-	0.30
Histidine	-	-	-	0.15	0.13	0.14	0.33	-	0.19
Phenylalanine	-	-	-	0.36	0.40	0.25	0.54	0.39	0.39
Tyrosine	-	-	-	0.34	0.36	0.29	0.46	0.29	0.35
Threonine	0.58	0.51	0.60	0.45	0.39	0.42	1.06	0.33	0.54
Leucine	0.33	0.43	0.33	0.64	0.67	0.41	1.14	0.58	0.57
Isoleucine	0.15	0.14	0.15	0.61	0.59	0.23	0.80	0.28	0.37
Valine	0.55	0.28	0.42	0.56	0.69	0.33	1.41	0.35	0.57
Alanine	0.62	0.59	0.58	-	-	0.41	1.22	0.28	0.62
Aspartic acid	0.72	0.83	0.82	-	-	0.47	1.81	0.47	0.85
Arginine	0.62	0.58	0.53	0.29	0.40	0.37	1.44	0.25	0.56
Serine	0.62	0.60	0.63	-	-	0.46	1.60	0.33	0.71
Glutamic acid	0.82	0.91	0.85	-	-	0.55	2.24	1.07	1.07
Glycine	2.27	2.12	1.71	-	-	1.23	2.76	0.25	1.72
Proline	3.67	4.26	1.73	-	-	-	-	-	3.22
Total amino acids	11.45	11.69	8.80	-	-	5.96	18.46	5.27	10.30
Nitrogen	2.51	3.00	2.29	0.94	0.89	-	3.44	-	2.11

1. Taverner *et al.* (1981b), n = 2,2,7, 86 kg
2. Leibholz and Mollah (1988), n = 6,6, 25 kg
3. Furuya and Kaji (1989), n = 4, 42 kg
4. Leibholz (1982), n = 20, 4kg
5. Moughan *et al.* (1987), n = 11, 26 kg

Kaji, 1989). Also, different proteins do not appear to significantly affect the determined endogenous amino acid flows (Taverner *et al.*, 1981b; Leibholz and Mollah, 1988).

The regression method was used by Moughan *et al.* (1987) for rats and pigs fed barley-meal, but the majority of the intercepts were not significantly different from zero. Skilton (1986) in reviewing the previous authors' results, noted that the coefficients of determination, particularly for the pigs, were low (R^2 =0.18 - 0.62, n = 11). The low correlations exemplify the problems associated with the variability of endogenous protein excretion (Snook, 1973) and the large number of animals required to obtain meaningful results.

Philosophically, the regression method would appear to be better than the protein-free method for determining endogenous N and amino acids in ileal digesta. This, however, does not appear to be the case in practice.

1.3.3.3 Peptide Alimentation Method: More recent studies (Darragh *et al.*, 1990; Moughan and Rutherfurd, 1990; Butts *et al.*, 1991) indicate that endogenous amino acid loss from the rat's small intestine is higher under peptide alimentation than under protein-free or synthetic free amino acid feeding. Further, Moughan and Rutherfurd (1990) have shown that the endogenous flow of lysine at the terminal ileum of rats fed a diet containing guanidinated protein was significantly higher than that of rats fed a protein-free diet.

The approach of Darragh *et al.* (1990), however, in which endogenous ileal amino acid excretion was determined after feeding rats an hydrolysed casein based diet, relied on the assumption that dietary amino acids were completely absorbed. The enzymically hydrolysed casein (EHC), a mixture of free amino acids and oligopeptides, was assumed to be completely digested and absorbed by the end of the small intestine of the rat.

Although the latter seems likely, it is difficult to establish unequivocally. Consequently, a method for determining endogenous loss has been proposed (Moughan *et al.*, 1990) which removes the need to make assumptions concerning the completeness of absorption of the dietary amino acids. In this method the animal is fed a semi-synthetic diet containing EHC as its sole N source. Ileal digesta are collected and the nitrogenous fraction separated physically using large volume disposable Centriprep-10 ultrafiltration devices (Amicon, W.R. Grace and Co., Danvers, Massachussetts). The high molecular weight (M.W. >10,000 Daltons) fraction resulting from the ultrafiltration provides a measure of endogenous amino acid flow. If some of the dietary amino acids and small peptides are not absorbed, they will be removed in the low molecular weight fraction. In addition to the unabsorbed dietary amino acids and peptides, the low molecular fraction will contain non-protein N and may contain endogenous free amino acids and small peptides. The latter if present, are expected to be at a low concentration (Butts *et al.*, 1991). Nevertheless, their removal in the low molecular weight fraction may lead to some underestimation of the actual endogenous loss of amino acids. Butts *et al.* (1991) applied this new method with growing rats, and their

results, some of which are presented in Table 1.7, provide further evidence that dietary peptides have a stimulatory effect on endogenous amino acid secretion and loss from the small intestine

Table 1.7 Mean endogenous amino acid flows[1] (±SE) at the terminal ileum of the growing rat given an enzymically hydrolysed casein based diet and with treatment of the digesta , or a protein-free diet

Amino acid	Endogenous flow		
	Hydrolysed casein	Protein-free	Statistical significance
Lysine	293 (24)	219 (16)	*
Histidine	159 (16)	140 (7)	NS
Threonine	612 (64)	393 (25)	**
Valine	498 (42)	238 (14)	***
Isoleucine	437 (32)	152 (11)	***

Adapted from Butts *et al.* (1991).
[1]Micrograms per gram diet freeze dried dry matter

1.3.3.4 Homoarginine Method: The homoarginine method which allows a more definitive study of the effect of peptides and protein on endogenous amino acid loss, has recently been developed (Hagemeister and Erbersdobler, 1985; Siriwan and Bryden, 1987; Siriwan *et al.*, 1987; Moughan and Rutherfurd, 1990). This approach is able to distinguish between exogenous and endogenous protein and allows the determination of endogenous lysine flow in the small intestine of mammals and birds. The method involves transforming lysine units in dietary protein to homoarginine units by the guanidination reaction with O-methylisourea (MIU). Body proteins do not contain homoarginine; therefore, any unabsorbed homoarginine must have come from the diet rather than the endogenous secretions. In contrast with other markers (^{15}N, ^{13}C, ^{14}C), it is not incorporated into endogenous protein, so intestinal scales and pancreatic protein are free of homoarginine. After absorption, homoarginine is partially reconverted into lysine by arginase, an enzyme mainly found in the liver, with subsequent release of urea. With regard to the latter assumption, Schuttert *et al.* (1991) in an *in vitro* experiment did not detect any arginase activity in the small intestine of the growing rat. Provided that guanidinated protein and native protein are broken down enzymatically at similar rates in the digestive tract and that homoarginine is absorbed in a similar manner as the other protein-bound feed amino acids, the endogenous proportion of N in ileal chyme can be calculated on the basis of the amount of homoarginine that disappears during the chymes's passage up to the terminal ileum.

Rutherfurd and Moughan (1990) who studied the effects of pH and protein level on the extent of guanidination of casein, gelatin and soya protein isolate, reported that complete

conversion of lysine to homoarginine was not achieved for any of the proteins studied. Nevertheless, with gelatin, which has a low natural lysine content, a maximum conversion of lysine to homoarginine of 95% was achieved, indicating that guanidinated gelatin is a suitable protein source for directly determining the endogenous excretion of lysine in the mammalian gut.

Hagemeister and Erbersdobler (1985, 1987) used the homoarginine method to investigate the ileal digestibility of casein and soya isolate in pigs and found that the true digestibility for both feed N and endogenous N was 99.5% and that more than 90% of the ileal N was of an endogenous origin. Siriwan and Bryden (1987) and Siriwan *et al.* (1987) who used the homoarginine technique with chickens fed guanidinated casein, reported that 90% of the amino acids appearing in the ileum were of endogenous origin. Moughan and Rutherfurd (1990) completely guanidinated gelatin protein and in a study with growing rats demonstrated that when protein is present in the gut, endogenous lysine loss from the terminal ileum is considerably enhanced above that found with protein-free alimentation.

Souffrant (1991) concluded that the homoarginine method is an appropriate method available to estimate proportions of endogenous protein and amino acids in ileal chyme. The method makes it easy to assess the effects of protein content and quality on endogenous N and amino acid levels in the chyme at the terminal ileum. The major disadvantage of the method, however, is that it provides direct information only for endogenous lysine flow. Further disadvantages include the possible accumulation of homoarginine in the body over time due to the slow rate of conversion of homoargine to lysine. Again, homoarginine may interfere with the urea cycle leading to an accumulation of ammonia in the body.

1.3.3.5 Radioactive Isotope or Tracer Technique: The use of radioactive isotopes or tracers (^{15}N, ^{14}C, ^{13}C, ^{35}S, ^{75}Se) to label dietary protein or the whole animal body protein prior to the trial, in order to distinguish between the exogenous and endogenous amino acids appearing in the terminal ileum after feeding a protein-containing diet, has been carried out by many workers (Nasset and Ju, 1961; Ochoa-Solano and Gitler, 1968; Kohler *et al.*, 1978; Buraczewska *et al.*, 1979; Bergner *et al.*, 1980, 1983, 1984; Gebhardt *et al.*, 1978; De Lange *et al.*, 1990). The most successful application to date has been the ^{15}N tracer (De Lange *et al.*, 1990; Krawielitzki *et al.*, 1990).

Using the technique of labelling the animal's body protein with radioactive or ^{15}N stable isotopes, De Lange *et al.* (1990) showed that endogenous nitrogen flow at the terminal ileum is considerably enhanced when natural protein-containing feeds are given as opposed to protein-free diets.

Souffrant *et al.* (1982) and Souffrant (1991) have reviewed the use of the ^{15}N dilution technique and outlined some of the difficulties with this method. The practical aspects of the technique which require further critical analysis include the method of labelling the animal's N pool, and the selection of the pool with a labelling level equal to that of total

endogenous nitrogen. Choice of precursor pool has a significant effect on the dilution factor (Moughan *et al.*, 1992). The ^{15}N method can be used to determine the endogenous N in digesta and faeces, but not the proportions of endogenous amino acids. The latter means that a constant assumed endogenous amino acid composition is used to determine true amino acid digestibility coefficients. This constant amino acid composition is usually that determined following feeding of the animal a protein-free diet, which as discussed earlier has its own inherent difficulties. Although the use of labelled dietary protein and animal body protein may give a good measure of endogenous protein excretion, the technique involves considerable expense and specialised equipment, which may limit its use even as a baseline measurement. More work needs to be done on the suitability of alternative precursor pools.

Further research into methods for determining ileal endogenous amino acid excretion is required, but true ileal amino acid digestibility data should ultimately provide more meaningful data on amino acid absorption in the pig.

1.4 THE GROWING LABORATORY RAT AS A NUTRITIONAL MODEL FOR OTHER MONOGASTRIC ANIMALS

Ileal digestibility studies with pigs (Low, 1980a) are expensive both in terms of time and materials, and are prone to difficulties which lead to the premature loss of animals. Further, small numbers of observations per treatment have been characteristic of digestibility studies involving cannulated and slaughtered pigs (Low, 1980b), so economic considerations dictate their replacement by simpler and cheaper methods. Nevertheless, it seems unlikely that animal experiments can be totally eliminated in the forseeable future, but the dependence on pigs can be minimised by choosing a sensitive model animal. In this respect the growing laboratory rat has proved a valuable model for investigations into basic mammalian processes of nutrition and metabolism (Waddell and Desai, 1981), and tests with laboratory rats have been used in the development of methods designed to replace the use of live animals, such as the growing pig.

The rat has prominence over most other species in nutrition research for many reasons (Yang and Mickelsen, 1974). The rat is omnivorous and can be fed the same (nutritionally adequate) diet for most of its lifetime. It is easily handled and cared for, and makes minimal maintenance demands, being docile, hardy and thriving well in small areas. Rats are also notable for high fecundity: they breed easily all year round, they are sexually mature at 6-7 weeks, and litter sizes average 14 (Universities Federation for Animal Welfare, 1976). As a result several generations may be studied in a few weeks. The rat is intelligent and can learn quite complicated manipulations.

The rat is considered to be in a continual state of growth during its lifetime (Dunn *et al.*, 1947). The latter is probably one of the more important reasons for its widespread use in nutritional studies which requires the use of animals that continue to gain weight. The rat, at

birth, weighs about 6 gm; it is weaned at 3 weeks when it weighs about 7 times its birth weight (Yang and Mickelsen, 1974). At puberty, which occurs when the rat is about 6 weeks of age, it weighs more than 25 times its weight at birth.

There are, however, drawbacks to the use of laboratory rats in nutritional research. First, many strains of rats are susceptible to chronic respiratory disease (Lindsey *et al.*, 1971), middle ear disease, pinworm, and scabies. Another problem with the use of rats in nutritional research is the fact that they practice coprophagy. This recycling of faeces can occasionally result in unexpected findings. The rat normally consumes 50 - 60% of its faeces (Barnes *et al.*, 1957), which probably explains why the adult does not require dietary folic acid, biotin, or vitamin K (Yang and Mickelsen, 1974). Most nutrition research requires the prevention of coprophagy. However, the fecundity, hardiness, low cost and size of the rat, coupled with the ease of collection of ileal digesta after slaughter appear to compensate for many of these drawbacks.

1.4.1 Comparative Digestive Anatomy and Physiology of the Rat and Pig

Since rats have long been used in nutritional studies, a great deal is known about their anatomy, physiology, and metabolism. The pig and rat are two examples of non-ruminants (monogastrics). The digestive processes of the rat and pig are anatomically very similar (Davenport, 1977) with the exception of the large intestines. The rat does not have a gall bladder. Bile is produced in the liver and passed directly to the duodenum via the bile duct. The process of protein digestion in rats is very similar to that in pigs. The pig has a long but simple small intestine, a moderate-sized caecum, and a sacculated large intestine. The pig and (man) are classified as colonic digesters (Stevens, 1977). In comparison, the rat has a relatively shorter but simple small intestine, an enlarged caecum, and an unsacculated large intestine (classed as a caecal fermentor). Both of these species depend on hindgut fermentation to varying degrees, the pig having fermentation in both the caecum and colon, while most of the fermentation in the rat's gastrointestinal tract occurs in the caecum.

The amino acid profile of the animal's body has been suggested (Fuller, 1978) as an indicator of the required profile in the diet. A comparative composition presented by Eggum (1964) indicated that the amino acid composition of the two species is not markedly different. However, the lysine and cystine contents in rats were somewhat higher than in pigs, whereas the opposite is the case with methionine. The relatively high cystine concentration in rats is presumably due to the high concentration of this amino acid in hair (Jorgensen and Eggum, 1971). The higher cystine concentration, when considered in conjunction with the probable higher requirement for methyl groups as a result of the high metabolic rate of small animals (Kleiber, 1961), would suggest that the needs of the rat for methionine plus cystine would be greater than the needs of the pig.

Kennedy *et al.* (1974) compared rats and pigs on diets containing increasing concentrations of meat and bone meal, and collagen. Both animal species reacted in a

similar negative fashion to these increases. The results of the study showed a linear ($P < 0.01$) reduction in the average daily gain and feed conversion efficiency in growing pigs and rats as the levels of meat and bone meal in the diet increased. In work with 9 Nigerian protein feeds, Fetuga *et al.* (1974) obtained no consistent differences in biological values between rats and pigs.

1.4.2 Comparative Digestibility Studies With Rats and Pigs

Digestibility is a very important criterion in feed evaluation, but is quite cumbersome to measure in domestic animals. Markedly less effort is required in the use of laboratory animals as models. Rats have an advantage over several domestic animal species in that they can be induced to consume a nutritionally inadequate diet for a considerable period of time. Thus trials can be carried out with isolated feedstuffs.

Published evidence indicates much promise for the rat as a suitable model for monogastric animals in digestibility studies. Support for this view comes from the findings of many studies including that of Likuski *et al.* (1961), Just *et al.* (1977) and Furuya *et al.* (1981) of a similar digestibility of energy, in a range of feeds, when growing rats and pigs were compared. Smith *et al.* (1987) in a comparative study concluded that for the major cereals (ground barley, maize, oats, triticale and wheat) used in pig feeding, the rat is a useful model of bioavailable energy content for the pig. There are also several reports (Atkinson and Carpenter, 1970; Ruane *et al.*, 1971; Kennedy *et al.*, 1974) in which close agrement has been found between the relative results of the rat and pig in feeding experiments. Also, research findings indicate that the growing rat is a useful model for the growing pig for protein (amino acid) digestibility at the terminal ileum.

Generally, there are similarities between the rat and pig for the ileal digestibility of dietary protein. Moughan *et al.* (1984) conducted a small-scale trial and found no significant differences between apparent ileal protein digestibility determined in the rat and pig with fishmeal, meat and bone meal and barley meal, however, there was a species difference for peas. Batterham (1983) used the slope-ratio assay to determine lysine availability in a variety of protein-rich feedstuffs, and found close agreement between lysine availabilities for rats and pigs for all the feedstuffs assayed, with the exception of another type of legume (lupin). Apparently, the rat and the pig utilised dietary lysine with similar efficiencies.

Picard *et al.* (1984b) conducted a comparative amino acid digestibility trial using five (5) animal models (intact cockerel, caecectomised cockerels, rats deprived of large intestine, piglet with an ileo-caecal cannulation, and piglet with an ileo-rectal shunt) and reported that the true digestibility values of amino acids were very similar between these monogastric animals.

Comparison of rat and pig apparent ileal digestibility values by Moughan *et al.* (1987) indicated agreement for all essential amino acids in barley except methionine, phenylalanine and most of the non-essential amino acids. When the results were expressed as true ileal

digestibilities, the differences between the rat and the pig were generally reduced. It is possible that at least some of the between-species differences observed were the result of insufficiently similar experimental conditions being applied, rather than intrinsic differences in the way pigs and laboratory rats digest proteins.

In a recent study, Smith *et al.* (1990) determined the apparent ileal digestibilities of several amino acids in a mixed diet containing barley meal, fish meal, meat and bone meal, and pollard as the protein sources, using the growing laboratory rat and pig. The apparent digestibilities of the essential amino acids were not significantly different for the two species, but those of the non-essential amino acids glutamic acid and proline were relatively higher in the rat. In view of their significant role in the endogenous excretion, however, this species difference may have been less if comparisons for glutamic acid and proline were related to true ileal amino acid digestibility.

It appears from the present discussion that the digestibilities of energy and protein determined in rats are close to values obtained with pigs.

1.5 INFERENCES FROM THE LITERATURE REVIEW

The measure of amino acid digestibility is a useful component measure of dietary amino acid availability. The digestibility of amino acids in feeds is highly variable. Accurate data on the digestibility of amino acids in feeds is needed, therefore, to allow the animal's daily requirement for individual dietary amino acids to be met precisely and economically. Because of the microbial action in the hindgut, and that at least for most species of animals amino acids released in the hindgut do not become available for body protein synthesis, faecal digestibility coefficients, are likely to be misleading. Measurement of amino acid flow and digestibility at the end of the ileum is now generally recognised as a more acceptable approach, at least theoretically. Ileal amino acid digestibility appears to be the method of choice for determining amino acid absorption from the gastrointestinal tract of the pig. True ileal amino acid digestibility coefficients are likely to be more useful indicators of amino acid absorption than apparent values. True ileal amino acid digestibility has particular application to feedstuffs in which the constituent amino acids have not undergone structural changes during processing or storage and, for the processed feedstuffs, to amino acids other than lysine, methionine, cystine and tryptophan. However, the drawback, particularly with pig ileal assays, is their cost. The growing rat is the most frequently used laboratory animal in nutritional studies and the physiology of digestion appears to be similar between the two species. The laboratory rat may be a useful model animal for digestibility studies in the growing pig. Where comparative studies have been made using identical diets and techniques, the results have been promising. However, more developmental work is required before a reliable rat assay can be established.

CHAPTER 2

Influence of time after feeding and site in the ileum for sampling digesta from euthanased pigs and comparison of the slaughter method and simple T-piece cannulation of the terminal ileum for determining ileal amino acid digestibility in the growing pig

2.1 INTRODUCTION

The ileal measure of dietary amino acid digestibility is generally considered (Rerat, 1981; Low, 1982b; Fuller, 1988) to be more accurate than that based on faeces and has been shown to be more sensitive in detecting small differences in the digestibility of amino acids in protein concentrates (Ivan and Farrell, 1976a; Sauer *et al.*, 1981). Recently, a relatively routine apparent ileal amino acid digestibility assay has been developed based on the sampling of digesta from the euthanased laboratory rat (Moughan *et al.*, 1987; Smith *et al.*, 1990; Skilton *et al.*, 1991). An overall objective of the work reported in this thesis was to evaluate the rat digestibility assay in determining ileal amino acid digestibility in meat and bone meal (MBM) for the growing pig. To allow a valid inter-species comparison, sampling of ileal digesta from the animal at slaughter was used in both the rat and pig.

The latter technique as applied to the pig has been criticised (Fuller, 1991) due to a potential difficulty in obtaining representative digesta samples, as only one sample of terminal ileal digesta can be obtained per animal. Also there may be a shedding of mucosal cells into the gut lumen at death which interferes with the digesta nitrogen (N) content (Badawy *et al.*, 1957; 1958; Fell, 1961). To avoid cell shedding the digesta must be sampled from the terminal ileum of animals while under anaesthesia or after death with a barbiturate (Badawy, 1964). Despite these potential difficulties, the "slaughter method" does have the advantage of causing minimal interference with the animal's digestive tract prior to the time of sampling and allows digesta to be taken from several parts of the digestive tract.

In digestibility assays where a single sample of digesta is taken, as is the case with the slaughter method, it is important that the sample be representative of the digesta from the whole meal. There is, however, no general agreement in the literature as to an optimal time after feeding for sampling ileal contents in the pig and the optimal time for sampling may vary with diet among other factors (Cunningham *et al.*, 1963; Buraczewski *et al.*, 1971; Rerat, 1972). Further, in most pig and rat ileal digestibility studies (Leibholz, 1982; Moughan *et al.*, 1987; Skilton *et al.*, 1988; Smith *et al.*, 1990) where the slaughter method has been applied, samples of digesta have been collected from the terminal 20 cm of the ileum immediately anterior to the ileo-caecal junction, but whether this is the optimal sampling site is not known. It may be that the optimal sampling site varies with protein source.

The present study included two preliminary trials the objectives of which were to determine: (i) the optimum time from the start of feeding for sampling of ileal digesta and (ii) the optimal site of sampling within the terminal ileum, for pigs given a semi-synthetic diet containing MBM as the sole protein source. Having ascertained the optimal sampling conditions, the main study allowed evaluation of the slaughter method as a technique for sampling digesta in the pig. In the present study, comparison of amino acid digestibility in MBM was made between pigs whose digesta were sampled at death and pigs fitted with simple T-cannulas. Simple T-piece cannulation of the terminal ileum of the pig is a widely-used and accepted methodology and thus served as a control in the present study.

2.2 MATERIALS AND METHODS

2.2.1 Animals, housing and diet

Twelve-week-old 30 kg bodyweight entire male Large White X Landrace pigs were randomly selected from a weaner pool at the Pig Research Unit, Massey University. The pigs were penned individually in smooth-walled metabolism crates and housed at 21±1°C. The ingredient composition of the experimental diet (containing MBM as the sole protein source) is given in Table 2.1. The determined nutrient composition of the diet is given in Table 2.2. Chromic oxide was added to the diet as an indigestible marker.

TABLE 2.1
Ingredient composition of a semi-synthetic meat and bone meal based diet

Ingredient	Composition (g kg^{-1} air dry weight)
Maize starch	621.0
Meat and bone meal	200.0
Sucrose	80.0
Maize oil	50.0
Purified cellulose[1]	30.0
Mineral, vitamin premix[2]	15.0
Chromic oxide	4.0

[1]Avicel, Asahi Chemical Industry Company Limited, Tokyo, Japan.

[2]Tasmix pig grower vitamin-mineral premix (Tasman Vaccines Ltd, Auckland, New Zealand). Provided the following per kg diet: 5000 IU vitamin A; 500 IU vitamin D_3; 22 IU vitamin E; 2 mg vitamin K; 3 mg riboflavin; 1.2 mg thiamin; 2 mg pyridoxine; 20 mg niacin; 9 mg pantothenic acid; 20 µg vitamin B_{12}; 0.8 mg folic acid; 20 mg betaine; 40 mg manganese; 100 mg zinc; 100 mg iron; 10 mg copper; 0.5 mg cobalt; 0.5 mg iodine; 0.3 mg selenium; 520 mg choline; 1.6 g chlorine; 1.3 g sodium; 0.5 g magnesium; 0.4 g sulphur; 3.0 g potassium.

TABLE 2.2
Mean determined nutrient composition[1] of a semi-synthetic meat and bone meal based diet

Nutrient	Composition (g kg^{-1} air dry weight)
Dry matter	957.0
Crude protein	124.2
Ash	54.2
Ether extract	67.2
Calcium	20.2
Phosphorus	9.9
Lysine	7.8
Methionine	2.0
Cystine	1.1
Histidine	3.5
Phenylalanine	5.2
Tyrosine	3.8
Threonine	5.2
Leucine	10.2
Isoleucine	3.7
Valine	7.5
Alanine	8.9
Aspartic acid	10.3
Arginine	9.6
Serine	5.7
Glutamic acid	15.5
Glycine	13.6
Proline	6.9
Digestible energy[2] (MJ kg^{-1})	15.9

[1]Determined following standard Association of Official Analytical Chemists (1980) procedures. Amino acids were determined as described in section 2.2.2.1 (a)

[2]Calculated based on tabulated values for dietary ingredients

2.2.2 Experimental procedure

2.2.2.1 Preliminary study 1: The effect of time of sampling of digesta on apparent ileal nitrogen digestibility

Thirty-six pigs were allocated equally and at random to 6 ileal digesta sampling times (3, 4, 5, 7, 9 and 11 hours after the start of the feeding period). The pigs were given a commercial grower meal for 2 days following penning, after which they received the MBM diet for 14 days. The diet was offered as a wet mash for a single 3-hour period (08.30 - 11.30 h) each day at a set intake (0.10 metabolic body weight, kg0.75). The diet was mixed with water (1:1; w/v) immediately prior to feeding and fresh water was freely available between meals.

Feeding times were staggered on the day of slaughter and also the previous 2 days, so

as to facilitate the killing of the pigs within the designated times. The pigs were anaesthetised using halothane gas (Fluothane, Imperial Chemical Industries Limited, Cheshire, England) and euthanased by a 10 ml intracardial injection of sodium pentobarbitone (Anathal 60 mg/ml, V.R. Laboratories Australia Pty Limited, Thornleigh, New South Wales, Australia). The digestive tract was exposed by an incision along the mid-ventral line and the final 20 cm of ileum (directly anterior to the ileo-caecal valve) was removed immediately, and carefully cleaned with water. The ileal contents were slowly flushed-out using deionized water from a syringe and were frozen (-20°C). Butts *et al.* (1992a) established that storage at this temperature did not influence the proportions of nitrogen-containing compounds in rat ileal digesta. Samples of ileal digesta and diet were freeze-dried, finely ground and stored at -20°C for the determination of nitrogen and chromium.

2.2.2.1 (a) Chemical analysis

The diet and ileal digesta were analysed in duplicate for total nitrogen (N) using the Kjeldahl method (Association of Official Analytical Chemists, 1980). The chromium contents of six 150 mg samples of the MBM diet and duplicate 15 mg samples of ileal digesta were determined by the method of Costigan and Ellis (1987).

The amount of freeze-dried matter (FDM) collected from the terminal ileum of each pig was determined after freeze drying the samples for 4 days (< 70 Pa) to constant weight.

2.2.2.1 (b) Data analysis

Estimates of apparent ileal N digestibility were calculated from the dietary ratio of N to chromium relative to the corresponding ratio in the ileal digesta. The data were subjected to a one-way analysis of variance and differences between means were examined using Duncan's multiple range test. Before the conduct of analysis of variance the variances were tested for homogeneity using Bartlett's test (Snedecor and Cochran, 1989). Statistical analysis was carried out using the computerised statistical package, REG (Gilmour, 1990).

2.2.2.2 Preliminary study 2: The effect of sampling site on apparent ileal nitrogen digestibility

Twelve 30 kg bodyweight entire male Large White X Landrace pigs were equally and randomly allocated to two sampling sites (the terminal 0-20 cm or 0-40 cm of ileum). Further samples of ileal digesta were taken at regular 20 cm intervals up the final 140 cm of ileum of each pig. The diet and experimental procedure were as detailed in sections 2.2.1 and 2.2.2.1, except that all pigs were euthanased on day 14 at 9 hours after commencement of the meal. The digesta collected were freeze-dried, and along with samples of the experimental diet, were analysed for chromium and nitrogen. Chemical and data analyses were as described in sections 2.2.2.1 (a) and 2.2.2.1 (b), respectively.

2.2.2.3 Main study: Comparison of the slaughter method and simple T-cannulation of the terminal ileum

2.2.2.3.1 Animals, housing and surgery

Sixteen entire male Large White X Landrace pigs of approximately 35 kg live weight were randomly selected from a weaner pool at the Massey University Pig Research Unit. Eight of the pigs were allocated at random for cannulation (simple T-piece cannula implanted in the distal ileum 20 cm from the ileo-caecal valve) while 8 were allocated to the slaughter method. The cannulas were constructed of medical grade Silastic tubing (internal diameter, 10 mm; outer diameter, 16 mm; Silkomed, Rusch, West Germany). The length of the cannula barrel was 85 mm and the foot measured 75 mm wide by 6 mm deep. For surgery, the pigs were sedated and placed in left lateral recumbency under halothane/oxygen (Fluothane, Imperial Chemical Industries Ltd, Cheshire, England) anaesthesia administered via a face mask. A 5-6 cm vertical incision was made into the body wall, 3-4 cm behind the last rib just above the midline. The small intestine was exteriorised via blunt dissection. A 2-2.5 cm incision was made along the anti-mesenteric side of the small intestine 10-15 cm anterior to the ileo-caecal junction. A Murphy's purse string suture was made around the incision and the cannula inserted through this incision. The free ends of the purse string suture were gently pulled and secured tightly around the barrel of the cannula. The latter was further secured by a purse string suture placed 2-3 mm below the Murphy's suture. The cannula was exteriorised via a stab wound approximately 1 cm in diameter, 3-4 cm anterior to the initial incision. The intestine was secured to the peritoneum and fascia with discontinuous sutures. The initial incision was closed with continuous sutures in the deep muscle layers and peritoneum, discontinuous sutures in the subcutaneous muscle, and discontinuous mattress sutures in the skin. The cannulated pigs were given antibiotic injections daily for 3-5 days post surgery. The pigs regained consciousness 4-5 hours following surgery and their appetites were normal within 48 hours of surgery.

The pigs were housed singly in smooth-walled metabolism crates at 21$\pm$1°C. The cannulated and intact animals were given a standard barley-based diet for 21 days before the start of a 14-day experimental period. At the commencement of the experimental period pig liveweights were around 45 kg.

2.2.2.3.2 Experimental procedure

During the experimental period, the pigs were given a semi-synthetic diet, the sole protein source of which was meat and bone meal (Table 2.1). The level of feeding and feeding procedure were as described in section 2.2.2.1. For the cannulated pigs and after 10 days on trial, total faeces were collected for 2 days, followed by a 2-day collection of ileal digesta. Digesta were spot-sampled hourly after the commencement of feeding for 10 hours (0830 to 1830 hours) each day. Digesta were bulked for each pig.

For the intact pigs the total faeces were also collected over days 11 and 12 and the ileal digesta collection procedures were the same as described in section 2.2.2.1 except that on day 14, each animal was euthanased at 9 hours after the start of the meal and the digesta were collected from the terminal 20 cm of ileum. Faeces and ileal digesta were frozen (-20°C) immediately upon collection. Ileal digesta samples for each pig were thawed, mixed and sub-sampled as were total faeces. The sub-samples were again immediately frozen (-20°C) and subsequently freeze-dried.

Representative samples of the freeze-dried ileal digesta and faeces, together with a sample of the diet, were finely ground and stored at -20°C for the determination of chromium and N as described in section 2.2.2.1 (a). Amino acids were determined following acid hydrolysis using a Beckman 119 BL amino acid analyzer. Duplicate samples (5-7 mg) were hydrolysed in 500 µl of 6M HCl with 1% added phenol for 24h at 110±1°C in glass tubes sealed under vacuum. For the determination of methionine and cystine, separate duplicate samples were oxidised with performic acid prior to acid hydrolysis. Tryptophan, being destroyed during acid hydrolysis, was not determined.

At the end of the experiment the 8 cannulated pigs were euthanased whilst under halothane anaesthesia, by an intracardial injection of sodium pentobarbitone. A post-mortem examination was conducted on each pig to determine the position of the cannula in the ileum and the extent of any gastrointestinal adhesions.

2.2.2.3.2 (a) Data analysis

The proportion of chromic oxide recovered in the ileal digesta from each pig was calculated from the amount of chromic oxide determined in the sample of ileal digesta relative to that fed to the pig.

Estimates of apparent ileal and faecal N and amino acid digestibilty were calculated from the dietary ratio of N or amino acid to chromium relative to the corresponding ratio in the ileal digesta or faeces, respectively. A linear model which included terms for method of digesta collection (method, slaughter or cannulation), digestibility type (type, faecal or ileal) and the first order interaction, was fitted to the apparent digestibility data and reduction in sums of squares was used to determine levels of statistical significance. Analyses were carried out using the computerised statistical package REG (Gilmour, 1990).

2.3 RESULTS

2.3.1 Preliminary study 1: The effect of time of sampling

The pigs readily consumed their meals within the 3-hour feeding period and gained in weight over the 14-day trial period. For the 6 pigs killed at 3 and 4 hours after the start of feeding respectively, the terminal 20 cm of ileum was devoid of digesta and consequently could not be sampled. The variances around the other treatment means were homogeneous. There were no significant ($P > 0.05$) differences between the slaughter times

of 5, 7, 9 or 11 hours after the start of feeding for the amount of digesta collected from the terminal 20 cm of ileum (Table 2.3). Significant effects of slaughter time on the digesta N:chromium ratios and on apparent ileal N digestibility were observed. Apparent ileal N digestibility increased by 9% units with sampling at 9 rather than 5 hours but there was no further change in digestibility over the next two hour period.

2.3.2 Preliminary study 2: The effect of length of ileum sampled

The amount of digesta collected from the terminal 40 cm of ileum was significantly ($P < 0.05$) greater than that obtained from the terminal 20 cm (Table 2.4), however there was no significant difference between sampling at the terminal 20 or 40 cm of ileum for apparent N digestibility. Furthermore, there did not appear to be an effect of sampling site on apparent N digestibility determined at 20 cm intervals over the final 140 cm of the small intestine (Fig. 2.1). The highest mean difference in N digestibility between sites was 2.3% units and there was little difference (1.8% units) between mean measurements made at 20 and 140 cm.

2.3.3 Main study: Comparison of amino acid digestibility determined using the slaughter method or simple T-cannulation of the ileum

The cannulated and intact animals consumed the MBM-based diet readily, remained in apparent good health and gained in weight (500 $\pm$ 5 g/d, mean $\pm$ SE) over the 14-day study. There were no significant ($P > 0.05$) differences in weight gain between the intact and cannulated animals. There were no food refusals and the overall average daily food intake ($\pm$SE) was 1728 $\pm$ 4g per day for the intact and cannulated pigs. No pathological changes to the ileum or the gastrointestinal tract were detected upon post-mortem examination of the cannulated pigs. There were no adhesions, and digesta flow through the cannula appeared to be unimpeded

Fourteen percent (14.1 $\pm$ 0.88%), (mean $\pm$ SE) of the total daily dietary chromium intake was recovered from the terminal 20 cm of ileum for the intact pigs and 33.2 $\pm$ 1.35% for the pigs fitted with simple T-cannulas at the terminal ileum. The data for chromium recovery were somewhat more variable for the intact pigs (coefficient of variation, CV = 17.6%) than for the cannulated pigs (CV = 11.5%).

The apparent ileal and faecal N and amino acid digestibilities for the cannulated and intact animals given the MBM based diet are given in Table 2.5. There was no method X type interaction for nitrogen or amino acid digestibility. The apparent ileal and faecal digestibilities, respectively were not significantly different ($p > 0.05$) for cannulated compared with intact pigs. Ileal N and amino acid digestibilities, however, were all markedly lower ($P < 0.05$) than their faecal counterparts. A comparison of the between animal variation for apparent ileal N and amino acid digestibility, for the intact and cannulated pigs respectively, is presented in Table 2.6. The variation about the means was low and of similar magnitude for cannulated and intact pigs.

TABLE 2.3

Effect of the time of sampling of digesta[1] for pigs given a meat and bone meal based diet on the amount of ileal digesta, its nitrogen (N) to chromium ratio, and apparent N digestibility

	Time of sampling (h)				Level of significance[2]
	5	7	9	11	
Amount of digesta (g)[3]	6.9 (±2.51)	7.0 (±1.32)	7.1 (±0.42)	6.8 (±1.48)	NS
N : chromium ratio[3]	1.6^{a} (±0.07)	1.4^{b} (±0.05)	1.2^{c} (±0.04)	1.1^{c} (±0.14)	*
Ileal N digestibility (%)[3]	70.9^{a} (±4.3)	75.9^{b} (±1.2)	79.6^{c} (±0.6)	79.5^{c} (±1.4)	*

[1]defined as the time from start of feeding to slaughter

[2]NS = non significant; * = P< 0.05

[3]Mean values (±SE); n = 6

[a,b,c] Means within a row with different superscripts are significantly different (P< 0.05)

TABLE 2.4

Effect of sampling site in the ileum on the amount of digesta, its nitrogen (N) to chromium ratio, and apparent N digestibility for growing pigs given a meat and bone meal-based diet

	Length of ileum (cm)		Level of significance[1]
	0-20	0-40	
Amount of digesta (g)[2]	3.1 (±0.39)	4.8 (±0.50)	*
N : chromium ratio[2]	1.2 (±0.03)	1.3 (±0.04)	NS
Ileal N digestibility (%)[2]	79.5 (±0.72)	78.5 (±0.83)	NS

[1]NS = non significant; * = P< 0.05

[2]Mean values (±SE); n = 6

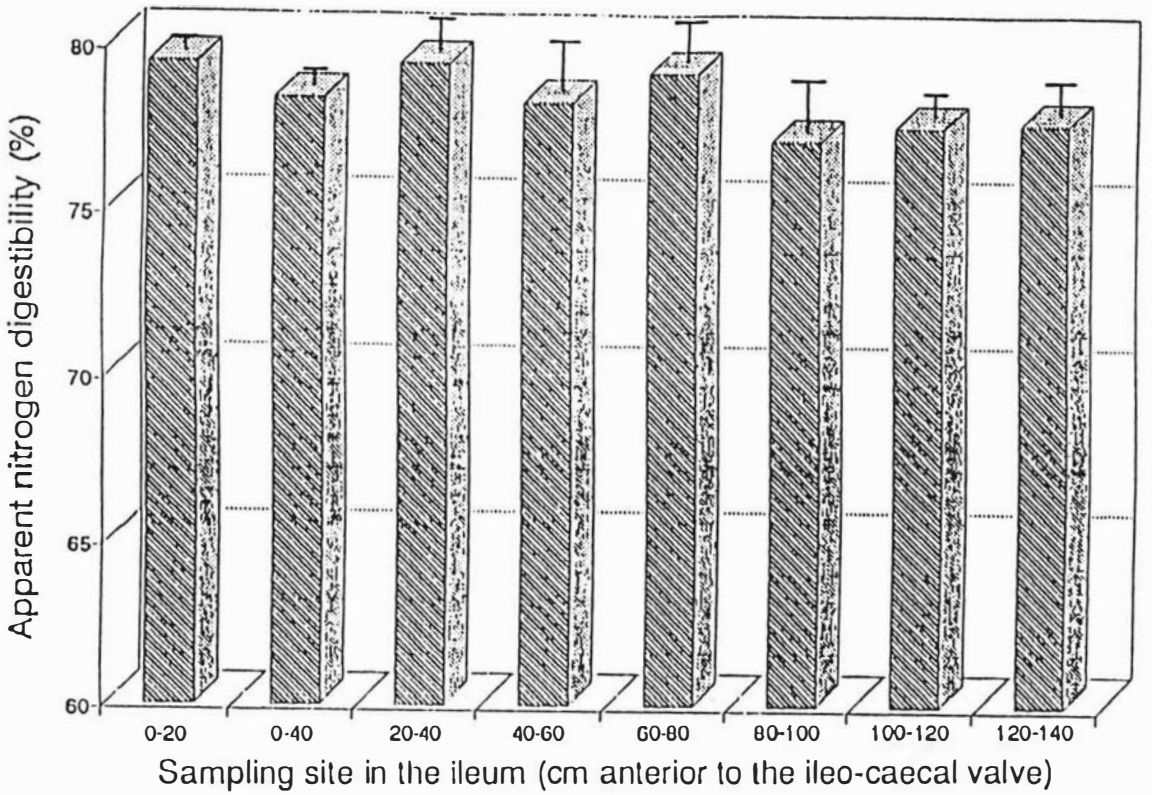

Fig. 2.1 Effect of sampling site in the ileum on the mean (±SE) apparent ileal nitrogen digestibility for pigs given a meat and bone meal based diet

TABLE 2.5

Mean apparent ileal (I) and faecal (F) digestibility (%) of nitrogen and amino acids in meat and bone meal for cannulated and intact growing pigs

	Method						
	Cannulation		Slaughter		Overall	Level of significance[1]	
Nutrient[2]	I	F	I	F	SE	Method	Type (Ileal/faecal)
Nitrogen	73.1	83.8	72.9	83.5	3.03	NS	*
Amino Acid							
Lysine	81.6	90.0	81.7	90.7	2.52	NS	*
Methionine	84.0	91.5	83.6	91.0	2.16	NS	*
Cystine	72.2	78.9	70.4	77.1	2.00	NS	*
Histidine	75.9	84.7	75.1	84.1	3.05	NS	*
Phenylalanine	82.0	89.4	82.9	88.2	1.86	NS	*
Tyrosine	78.2	82.9	78.8	83.4	1.35	NS	*
Threonine	73.0	86.0	72.9	85.8	3.74	NS	*
Leucine	79.1	86.2	77.9	86.0	2.21	NS	*
Isoleucine	82.6	90.0	81.2	89.3	2.26	NS	*
Valine	81.4	90.8	80.3	91.3	2.96	NS	*
Alanine	82.1	92.0	81.2	92.6	3.08	NS	*
Aspartic acid	67.0	84.0	67.4	82.5	4.64	NS	**
Arginine	81.7	93.0	84.2	92.4	2.86	NS	*
Serine	74.0	83.5	73.5	84.4	2.95	NS	*
Glutamic acid	72.1	88.1	71.3	87.7	4.68	NS	*
Glycine	75.7	89.2	75.1	91.3	4.31	NS	*
Proline	77.9	91.0	78.2	90.4	3.65	NS	*

[1] NS = non significant; * = $P < 0.05$; ** = $P < 0.01$

[2] Mean values; n = 8

TABLE 2.6
Comparison of coefficients of variation for apparent ileal nitrogen and amino acid digestibility (%) as determined with cannulated or intact growing pigs given a meat and bone meal based diet

	Cannulated pigs	Intact pigs
Nitrogen	4.2	5.4
Amino acid		
Lysine	2.8	3.1
Methionine	3.4	4.4
Cystine	6.3	5.6
Histidine	4.1	3.4
Phenylalanine	1.0	2.1
Tyrosine	3.3	2.9
Threonine	3.5	4.7
Leucine	1.8	3.3
Isoleucine	3.1	3.8
Valine	4.9	6.3
Alanine	3.5	2.4
Aspartic acid	3.0	3.8
Arginine	3.8	5.4
Serine	2.3	3.1
Glutamic acid	4.3	4.0
Glycine	10.5	11.7
Proline	7.6	7.2

2.4 DISCUSSION

2.4.1 The effect of time of sampling of ileal digesta

The time of sampling of ileal digesta in the euthanased animals had no significant influence on the amount of digesta collected, though slaughter at 9 hours after the start of feeding gave the least variable sample size. Ivan and Farrell (1976b) reported that peak flow of digesta at the ileum of pigs given a soft-wheat diet and fitted with re-entrant cannulas occurred 9 hours post-feeding. The rate of gastric emptying is controlled by receptors located in the duodenum which are sensitive to various factors such as the chemical composition and texture of the diet (Auffray *et al.*, 1967; Rerat, 1972). Given that no digesta were found in the terminal ileum at 4 hours post-feeding, it appears that the first material leaving the stomach reached the terminal ileum around 5 hours after feeding. The lipid content (Hunt and Knox, 1968; Ivan and Farrell, 1976b; Davenport, 1977), type of protein (Den Hartog *et al.*, 1989) and type and level of carbohydrate in the diet can all affect the pattern of stomach emptying and the flow of digesta at the terminal ileum (Cunningham *et al.*, 1963; Rogers and Harper, 1964; Buraczewski *et al.*, 1971). The relatively high lipid content of the diet used in this study may have delayed emptying.

There was an effect of sampling time on the N:chromium ratio in the ileal digesta and on apparent ileal N digestibility. A relatively greater contribution of endogenous N to total digesta N may have led to the significantly higher ileal N:chromium ratio at the 5 and 7-hour sampling times. Apparent digestibility increased from 5 to 7 hours post-feeding and reached a maximum of 80% at 9 and 11 hours post-feeding. This increase in determined digestibility was most likely caused by changes in the relative concentration of endogenous N, as true digestibility would not be expected to increase as the flow of material in the intestine increased from 5 to 11 hours. Nitrogen digestibility was least variable at 9 hours and this time was accepted as the optimal sampling time for the growing pig given a semi-synthetic MBM based diet. Although 9 hours after feeding was considered as the optimal sampling time for this type of diet, the optimum sampling time will may with the type of diet. Consequently, the optimal sampling time after feeding, for ingredients making up diets of different composition needs to be determined, when the slaughter technique is adopted.

2.4.2 The effect of sampling site

In the present study, site of sampling within the ileum of the pig (0-140 cm from the ileo-caecal valve) appeared to have little influence on the apparent digestibility of N in MBM. This is in contrast to the observations of Buraczewska *et al.* (1975) and Poppe and Meier (1977) who with pigs given soya bean meal and casein-wheat or casein based diets, respectively found a significant effect of sampling site within the small intestine on apparent ileal N digestibility. The studies of Poppe and Meier (1977), in which pigs were fitted with re-entrant fistulas at the terminal ileum at a distance of 40, 35 or 10 - 15 cm from the caecum, indicated that it is necessary to specify the site at which the fistula is inserted and to take this into consideration when interpreting the results. The present result, however, confirms that of Kies *et al.* (1986) who showed that small intestinal sampling site (0 - 140 cm from ileo-caecal valve) had no effect on the apparent digestibility of N in lactic casein for the growing pig. This is also supported by the recent work of Van Barneveld *et al.* (1991), who using the slaughter technique, showed that sampling in the final 0-140 and 0-15 cm of ileum of the pig had little influence on the apparent ileal digestibility of lysine in heat-treated field peas. The results of Zebrowska (1980) and Green (1988) also provide evidence that amino acid digestion and absorption is almost complete before the end of the ileum. Leibholz (1982) concluded that the digestion and absorption of milk protein was largely completed by the end of the jejunum in the pig, which also suggests that the site of sampling within the ileum may not be a critical factor in the measurement of N digestibility. Site of sampling within the ileum in small animals, such as the laboratory rat, is nevertheless critical in respect of sample size, if digesta are to be analysed independently for several chemical components, for example, dry matter, total nitrogen, and all essential amino acids including methionine + cystine and tryptophan which being partially destroyed during acid hydrolysis, require specific analytical procedures.

2.4.3 Comparison of the slaughter method and simple T-cannulation

Simple T-cannulation has been widely accepted by workers as a means of sampling ileal digesta in pigs and has been shown to be an acceptable technique for digesta collection, at least when non-bulky diets are used (den Hartog *et al.*, 1988b; Kohler *et al.*, 1990). Pigs fitted with simple T-cannulas at the terminal small intestine were used in the present study as controls to evaluate a method whereby ileal digesta were collected from the euthanased animal. When simple T-cannulation of the ileum is adopted the surgery is less invasive than with other cannulation approaches and the procedure has fewer adverse effects on the physiology of the alimentary tract. Jorgensen *et al.* (1985) found that simple T-cannulated pigs grew at a similar rate to intact pigs and Livingstone and McWilliam (1985) reported similar voluntary food intakes between T-cannulated and normal pigs but a slightly lower (7%) mean growth rate with the cannulated animals. A number of studies have compared apparent faecal nutrient digestibility between T-cannulated and unoperated pigs. Generally, there appears to be little effect of cannulation on nutrient digestion determined over the entire digestive tract (Furuya *et al.*, 1974; Sauer *et al.*, 1977a,b; Huisman *et al.*, 1984; Metz *et al.*, 1985), though some studies (Laplace and Borgida, 1976; Sauer *et al.*, 1979) have demonstrated a small adverse effect of cannulation. In the present work there were no statistically significant differences in apparent faecal N or amino acid digestibility between T-cannulated or intact pigs, which is further support for the minimal disturbance caused by simple T-cannulas implanted in the terminal ileum. Jorgensen *et al.* (1985) conducted a comprehensive study on the effects of cannulation in the pig. From a comparison of 46 T-piece cannulated and 156 normal pigs given 38 different diets varying widely in ingredient and nutrient composition, it was concluded that T-cannulation did not affect growth rate but may result in a slight increase in apparent faecal nutrient digestibility (around 0.5, 1.8 and 1.3% units for dry matter, N and lysine, respectively). The greatest effect of cannulation on digestibility was observed with high-fibre diets and those including potato starch.

Various other techniques, such as re-entrant cannulation, ileo-colic post valve fistulation or ileo-rectal anastomosis, have been used for the quantitative collection of ileal digesta from pigs. These techniques markedly alter the motility or normal functioning of the gastrointestinal tract (Laplace and Darcy, 1980; Low, 1980b; Hennig *et al.*, 1989; Kohler *et al.*, 1990; Fuller, 1991). Recently, van Leeuwen *et al.* (1988) described the post valvular T-caecum cannulation (PVTC) technique which is supposed to enable almost complete collection of digesta from pigs and allows normal function of the ileo-caecal valve and colon. The PVTC technique has the advantage that during collection most of the digesta pass through the cannula because the ileo-caecal valve protrudes directly into the cannula. When using this technique, however, there is the possibility that collected ileal digesta may be contaminated with colonic digesta, particularly at the later stages of pig growth, when the gastrointestinal tract is larger and the ileo-caecal valve does not protrude directly into the

cannula (Mroz *et al.*, 1991). However, recent comparative studies with simple T-piece cannulas and post-valve caecal simple cannulas (den Hartog *et al.*, 1988b; Kohler *et al.*, 1990) showed that the digestibilities of dry matter and N determined with the T-cannula were comparable to results obtained with the post-valve T-caecum method. It is concluded that, at least for refined diets and with frequent spot-sampling of digesta, T-cannulated pigs should give reliable observations on the digestibility of dietary nitrogen.

Regardless as to whether digesta are sampled following euthanasia or by means of a simple T-cannula, an indigestible marker must be included in the diet. In the present study, the marker chromic oxide was used. Chromic oxide has been shown to be a suitable marker for dietary ileal digestibility in pigs (Sauer *et al.*, 1981; Tavernier *et al.*, 1983; Kohler *et al.*, 1990). A positive correlation between chromium and dry matter concentration in both duodenal and ileal digesta has been reported by different authors (Partridge *et al.*, 1985; Graham amd Aman, 1986). This means that chromic oxide will generally follow the flow of dry matter through the gastro-intestinal tract. There was a reasonably high recovery of marker for both the cannulated pigs and those sampled at death in the present study, though the recovery was higher for the cannulated pigs. The fourteen percent recovery of ingested marker for the euthanased pigs was considered satisfactory to provide a representative sample of ileal digesta.

The surgery required for simple T-cannula implantation is costly, and with high fibre diets cannulas are susceptible to blockage (Schroder, 1988; Potkins *et al.*, 1991). Further, cannulation may lead to discomfort in the animal and physiological effects due to the cannula may become important as the cannulated animals age. For these reasons, it would be useful to develop an alternative method of digesta collection and it appears from the present results that the slaughter approach may be a satisfactory alternative. In the present study, where the apparent ileal digestibility of N and amino acids in meat and bone meal were determined with recently cannulated animals given adequate post-surgical recovery and which were shown at post-mortem to have not developed adhesions, similar digestibilities were obtained for intact and cannulated animals. Moreover, the similar magnitude of the coefficients of variation of the apparent ileal N and amino acid digestibility values demonstrated that digestibility was not consistently more variable with the slaughter method in comparison with cannulation. That the apparent ileal amino acid digestibility values for MBM determined by the slaughter method were comparable to those obtained by simple T-cannulation, is in agreement with the observation of Moughan and Smith (1987) who found close agreement between the ileal digestibilities of the amino acids in ground barley as determined with intact and cannulated pigs.

The slaughter technique, as outlined in this study, has the distinct advantage of involving minimal disruption to normal digestive function in the animal and allows samples of digesta to be taken from several parts of the digestive tract. In addition, different types of diets can be fed to the animal without interfering with the sampling procedure. The main technical

criticism of this method concerns the potential difficulty of obtaining representative samples of digesta, thereby increasing the variability of digestibility estimates. Based on the present study, however, if digesta are sampled at the optimal time after feeding the pig, this does not appear to be a major difficulty.

Another factor considered to possibly influence the accuracy of digestibility estimates determined with the slaughter method is the sloughing of epithelial cells into the gut lumen, with effect on the N content of the digesta (Badawy *et al.*, 1957; 1958; Fell, 1961). This is a time related phenomenon and in the current study should have been minimised by the short interval of 4-5 min that elapsed between death of the animals and sampling of the ileal contents. Thorpe and Thomlinson (1967) found that epithelial cell shedding in the pig increased with time after death. In their work in which pigs were killed with chloroform by inhalation 2 hours post-feeding, no macroscopic lesions were found in any of the animals at the initial sampling within 5 and 10 min after death. However, gastrointestinal tympany developed in all pigs commencing with moderate gaseous distension of the stomach and small intestine from 90 min after death. Thorpe and Thomlinson (1967) also found that cell shedding commenced later at the ileum than at the duodenum. At 90 min after death the ileal mucosa showed the best preservation of the surface epithelium of any of the small intestinal sites examined in the pigs. Similar results for epithelial cell shedding were noted by Pearson and Logan (1978) using exsanguinated calves in which samples were taken from 1 to 30 min after severing the carotid arteries. Epithelial cell separation was minimal up to 10 min after death. Pearson and Logan (1978) also took samples from the proximal, middle and distal sections of the small intestine and found epithelial cell shedding took longer to begin at the distal section of the small intestine than at the proximal section (15 vs 5 min). In the present study, samples were collected from the ileum immediately after slaughter. On the basis of the above evidence, cell shedding at the terminal ileum should not have begun or should have been minimal. Further, the relatively low inter-animal variation for apparent ileal amino acid digestibility suggests that epithelial cell shedding did not occur to any significant extent in the present study. Sampling of digesta from the terminal ileum of animals while under anaesthesia or after death with a barbiturate minimises mucosal cell shedding (Badawy, 1964).

One other potential concern with the slaughter technique is the possibility of agonal spasms with an accompanying movement of digesta between different parts of the gastrointestinal tract. Struggling of the animal at death may influence the passage rate of digesta in the gut and interfere with the digesta N content. Such an effect has been reported by Bolton (1964) who used a cervical dislocation slaughter procedure which caused agonal spasm of the fowl's intestine with consequent digesta movement within the gut. The slaughter of pigs under anaesthesia in the present study, however, precluded struggle and there did not appear to be contractions of the intestinal tract. The procedure adopted in this study led to rapid immobilization and the animals lapsed into unconsciousness quietly.

A further possible criticism of the method adopted in this study is the use of deionised water rather than physiological saline to flush the ileal contents. Recently, however, Butts *et al.* (1992a) compared collection of ileal digesta with distilled water or physiological saline and observed that the use of distilled water had no effect on the determination of endogenous protein excretion in the growing rat.

In conclusion, the slaughter of animals at predetermined intervals after the start of feeding and with subsequent sampling of the ileal digesta, is technically straightforward and involves no long-term manipulation of the digestive system. When sampling is carried out with care the method provides digestibility data which are no more variable than those obtained following simple T-cannulation. The slaughter method reduces the time of animals on experiment, has a lower labour requirement and is possibly ethically more acceptable. There are no restrictions as to type of diet that can be fed to the pig. This makes the slaughter technique a viable alternative to simple T-cannulation for the determination of N and amino acid digestibility in the pig. The present results give support to using the slaughter technique with the growing pig, to allow comparison of the ileal digestion of MBM protein, using the same digesta collection technique with the laboratory rat.

CHAPTER 3

Influence of time after feeding and site in the ileum for sampling digesta from euthanased rats

3.1 INTRODUCTION

A recently developed rat ileal digestibility assay (Moughan *et al.*, 1987; Skilton *et al.*, 1991) shows promise for the routine determination of amino acid digestibility in feedstuffs such as meat and bone meal (MBM), which may vary markedly in quality with source and time of year. To establish a reliable, rapid and inexpensive routine method, however, some procedural aspects of the present rat assay need further investigation.

In the previous study (refer chapter 2), optimal ileal digesta sampling conditions for the slaughter method with the growing pig were determined and it was established that the 'slaughter' technique is a satisfactory method for obtaining ileal digesta samples in digestibility studies. The overall aim of the present study, was to determine the optimal ileal digesta sampling conditions for the slaughter method with the growing rat. This would allow comparison of the ileal digestion of MBM protein in the growing pig and laboratory rat based on the same digesta collection technique for each species.

The present study involved two trials the specific objectives of which were to determine: (i) the optimum time from the start of feeding for sampling of ileal digesta and (ii) the optimal site of sampling within the terminal ileum, for rats given a semi-synthetic diet containing MBM as the sole protein source.

3.2 MATERIALS AND METHODS

3.2.1 Animals, housing and diet

Male Sprague-Dawley rats of around 7 weeks of age and 190 g bodyweight were selected at random from a group of animals which had been weaned at 4 weeks of age and reared on a high quality diet at the Small Animal Production Unit, Massey University. The rats were kept individually in raised stainless steel cages with wire-mesh floors, in a temperature-controlled room (21$\pm$1°C) with a 12-hour light/dark cycle.

The ingredient composition of the experimental diet (containing MBM as the sole protein source) is given in Table 3.1. and its determined nutrient composition is given in Table 3.2. Chromic oxide was added to the diet as an indigestible marker.

TABLE 3.1
Ingredient composition (g kg^{-1} air-dry weight) of the experimental diet for the growing rat

Ingredient	Composition
Maize starch	621.0
Meat and bone meal	200.0
Sucrose	80.0
Maize oil	50.0
Purified cellulose[1]	30.0
Mineral and vitamin premix[2]	15.0
Chromic oxide	4.0

[1]Avicel, Asahi Chemical Industry Company Limited.

[2]Tasmix Special Mouse Premix (Tasmix, Pfizer Laboratories, Auckland, New Zealand).
Supplied the following per kg diet: 5250 IU vitamin A; 750 IU vitamin D; 37.5 IU vitamin E; 1.5 mg vitamin K; 3.0 mg vitamin B_1; 3.75 mg vitamin B_2; 4.5 mg Vitamin B_6; 0.04 mg vitamin B_{12}; 21.0 mg pantothenic acid; 0.08 mg biotin; 15 mg niacin; 0.75 mg folic acid; 0.75 g choline; 60 mg iron; 37.5 mg zinc; 37.5 mg manganese; 3.75 mg copper; 0.38 mg iodine; 0.53 mg cobalt; 0.11 mg selenium; 30 mg inositol; 2.75 g potassium; 0.3 g magnesium; 0.38 g sodium.

3.2.2 Experimental procedure

3.2.2.1 Trial 1: The effect of time of sampling of digesta on apparent ileal nitrogen digestibility

Thirty-six rats were equally and randomly allocated to six slaughter times (1, 2, 3, 4, 5 and 6 hours after the commencement of feeding). The rats were initially fed a casein-based diet for 2 days and were then given the MBM based diet (Table 3.1) for 14 days. The diet was freely offered in stainless steel feeders fitted with anti-spill devices similar to those described by Thomsen (1981).

The rats were trained to consume the MBM diet over a single 3-hour period (08.30 to 11.30 h) each day, and this training was achieved within 7 days. Water was available *ad libitum*.

On day 14, the rats were asphyxiated in carbon dioxide gas and decapitated (immediately ceasing all neural stimulation to the gut) at the set slaughter times. 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 days to accustom the rats to the change in procedure. The abdomen was opened by an incision along the mid-ventral line and the skin and musculature were folded back to expose the viscera. The final 20 cm of the ileum was immediately dissected from the body, and the intestinal surface cleaned using absorbent tissue paper, taking care not to apply pressure to the intestine. The digesta were slowly flushed out into a plastic bag, with 10 ml deionised water from a plastic syringe and frozen (-20°C) immediately. The digesta samples were freeze-dried, finely ground and

TABLE 3.2
Mean determined nutrient composition[1] of a semi-synthetic meat and bone meal based diet

Nutrient	Composition (g kg^{-1} air dry weight)
Dry matter	957.0
Crude protein	124.2
Ash	54.2
Ether extract	67.2
Calcium	20.2
Phosphorus	9.9
Lysine	7.8
Methionine	2.0
Cystine	1.1
Histidine	3.5
Phenylalanine	5.2
Tyrosine	3.8
Threonine	5.2
Leucine	10.2
Isoleucine	3.7
Valine	7.5
Alanine	8.9
Aspartic acid	10.3
Arginine	9.6
Serine	5.7
Glutamic acid	15.5
Glycine	13.6
Proline	6.9
Digestible energy[2] (MJ kg^{-1})	15.9

[1]Determined following standard Association of Official Analytical Chemists (1980) procedures. Amino acids were determined as described in section 3.2.2.1 (a)
[2]Calculated based on tabulated values for the dietary ingredients

stored at -20°C (along with samples of the diet) for the determination of total nitrogen (N) and chromium. The stomach contents were inspected for signs of faecal contamination resulting from coprophagy.

3.2.2.1 (a) Chemical analysis

The diet and ileal digesta were analysed in duplicate for N using the Kjeldahl method (Association of Official Analytical Chemists, 1980). The chromium contents of six 150 mg samples of the MBM diet and duplicate 15 mg samples of ileal digesta were determined by the method of Costigan and Ellis (1987). The diet was analysed for amino acids following acid hydrolysis using a Beckman 119 BL amino acid analyser. Duplicate samples of the diet (5-7 mg) were hydrolysed in 500 µl of 6M HCl with 1% added phenol, for 24 hours at 110 ±

1°C in glass tubes sealed under vacuum. For the determination of methionine and cystine, separate duplicate samples were oxidised with performic acid prior to hydrolysis. Tryptophan, which is partly destroyed during acid hydrolysis, was not determined.

The amount of freeze-dried matter (FDM) collected from the terminal ileum of each rat was determined after freeze drying the samples for 3 days (< 70 Pa) to a constant weight.

3.2.2.1 (b) Data analysis

Apparent estimates of ileal N digestibility were calculated from the dietary ratio of N to chromium relative to the corresponding ratio in the ileal digesta.

The data were subjected to a one-way analysis of variance and differences between means were examined using Duncan's multiple range test. Before the conduct of analysis of variance the variances were tested for homogeneity using Bartlett's test (Snedecor and Cochran, 1989). Statistical analysis was carried out using the computerised statistical package, REG (Gilmour, 1990).

3.2.2.2 Trial 2: The effect of sampling site on apparent ileal nitrogen digestibility

Seventy-two rats were allocated equally and at random to four ileum lengths to be sampled (terminal 0-5, 0-10, 0-15 and 0-20 cm of the ileum). Housing, feeding and ileal digesta collection procedures were the same as described above (refer sections 3.2.1 and 3.2.2.1), except that on day 14 the rats were fed and then slaughtered 4 hours after the start of feeding and the digesta were collected from the specified sites. Ileal digesta from randomly drawn trios of rats in each treatment group were pooled to obtain 6 samples of digesta for each sampling site. Pooling was necessary to ensure that sample sizes were adequate for chemical analysis particularly with digesta obtained from the shorter lengths of ileum.

Chemical and data analyses were as detailed in section 3.2.2.1 (a) and 3.2.2.1 (b), respectively.

3.3 RESULTS

3.3.1 Trial 1: The effect of time of sampling

The rats remained healthy, consumed the diet readily and were fully accustomed to the feeding procedure after 7 days. On average the animals consumed 11.0 g of diet daily.

None of the rats used in the experiment showed evidence of ingested faeces at slaughter and, therefore, coprophagy was not considered to be of significance. The variances for all of the data sets were found to be homogeneous. The amount of digesta collected from the terminal 20 cm of ileum, the N:chromium ratio in the digesta and apparent N digestibility, determined at each sampling time are given in Table 3.3. For the 6 rats killed 1 hour after the start of feeding, the terminal 20 cm of ileum was devoid of digesta and consequently

TABLE 3.3

Effect of the time of sampling digesta[1] for rats given a meat and bone meal based diet on the amount of ileal digesta, its nitrogen (N) to chromium ratio, and apparent N digestibility

	Time of sampling (h)					Level of significance[2]
	2	3	4	5	6	
Amount of digesta (mg)[3]	93.3[a] (±19.1)	130.6[b] (±15.4)	170.4[c] (±8.2)	100.2[a] (±14.1)	148.4[d] (±21.3)	*
Nitrogen (N): chromium ratio[3]	0.9 (±0.16)	0.9 (±0.13)	1.2 (±0.05)	1.2 (±0.07)	1.2 (±0.07)	NS
Ileal N digestibility (%)[3]	84.2 (±2.55)	84.1 (±2.12)	79.8 (±0.88)	80.6 (±1.17)	80.3 (±1.20)	NS

[1]defined as time from start of feeding to slaughter

[2]NS = non significant; * = $P < 0.05$

[3]Mean values (±SE); n = 6

[a,b,c] Means within a row with different superscripts are significantly different ($P < 0.05$)

could not be sampled. Significantly ($P < 0.05$) higher quantities of freeze-dried ileal digesta were obtained from rats killed 4 hours after the start of feeding compared with the other sampling times. Sampling time did not significantly affect the N:chromium ratios in digesta nor apparent N digestibility.

3.3.2 Trial 2: The effect of sampling site in the ileum

The amounts of freeze-dried ileal digesta, N:chromium ratios in the digesta and estimates of apparent N digestibility, at different sampling sites within the ileum of the rat are given in Table 3.4. Significantly ($P < 0.05$) higher quantities of digesta were collected from the terminal 20 cm of ileum compared to the shorter ileum lengths. Sampling site did not significantly ($P > 0.05$) affect the N:chromium ratios or apparent N digestibility.

3.4 DISCUSSION

3.4.1 The effect of time of sampling of ileal digesta in the rat

Time of sampling had a significant influence on the amount of digesta collected from the terminal 20 cm of rat ileum. This is contrary to the earlier study with pigs (refer chapter 2) which indicated no significant effect of sampling time on the amount of digesta collected from the terminal 20 cm of ileum. Slaughter at 4 hours after the start of feeding gave the largest and least variable amount of sample. This is in contrast to the findings of Skilton et al. (1991) for rats fed a MBM based diet and slaughtered at 2, 3, 4, 5 and 6 hours after the start of feeding, which indicated that slaughter at 3 hours gave the largest and least variable amounts of digesta at the terminal 20 cm of ileum. Close observation of the feeding behaviour of rats in the course of this study showed that they were actively eating their food at the end of the first hour of the feeding period, and not eating much more until 3 hours after the start of the feeding period. This pattern of feeding may explain the absence of digesta in the terminal ileum of rats killed at 1 hour, and the least variability in peak digesta flow at 4 hours, with a more variable peak at 6 hours after the commencement of feeding. However, there were no significant differences between the sampling times for N digestibility in the rat. Nitrogen digestibilities were fairly constant between 4 and 6 hours after the start of feeding. This agrees with Skilton et al. (1991) who found that the N digestibility of a MBM based diet varied from 66% at 2 hours to 54% at 6 hours but were fairly constant between 3 and 6 hours after the start of feeding. The data presented by Skilton *et al.* (1991) indicate that the greatest flow of digesta and nitrogen coincides approximately with the average digestibility of nitrogen. As the amount of digesta recovered in the present study was maximum and the N digestibilities were least variable at the 4-hour sampling time, this was chosen as the optimal sampling time for rats given meat and bone meal as the sole dietary protein source.

TABLE 3.4

Effect of sampling site in the ileum on the amount of digesta, its nitrogen (N) to chromium ratio, and apparent N digestibility for the growing rat given a meat and bone meal based diet

	Sampling site (cm)				Level of significance[1]
	0-5	0-10	0-15	0-20	
Amount of digesta (mg)[2]	130.3[a] (±15.3)	250.6[b] (±20.1)	318.2[c] (±18.4)	364.4[d] (±10.3)	*
Nitrogen (N): chromium ratios[2]	0.9 (±0.06)	1.4 (±0.33)	0.9 (±0.07)	0.9 (±0.05)	NS
Ileal N digestibility (%)[2]	81.7 (±1.15)	81.0 (±1.30)	81.2 (±1.34)	81.6 (±1.04)	NS

[1] NS = non significant; * = $P < 0.05$

[2] Mean values (±SE); n = 6

[a,b,c] Means within a row with different supersripts are significantly different ($P < 0.05$)

3.4.2 The effect of sampling site in the ileum of the rat

In the present study, site of sampling within the final 20 cm of rat ileum had no influence on the apparent digestibility of N in MBM. Maccoll and James (1988) collected digesta from successive segments of the distal ileum of rats fed diets containing either casein or wheat as the sole protein sources to determine the apparent digestibilities of protein and found no diet X segment interaction; but there were significant effects of diet and segment. Although the work of Maccoll and James (1988) showed a significant effect of segment of ileum, the actual differences between the 0-10 cm and 0-20 cm segments were small and likely to be of little practical relevance. Various studies (Zebrowska, 1980; Leibholz, 1982; Kies *et al.*, 1986; Green, 1988; Van Barneveld *et al.*, 1991) suggest that the site of sampling within the ileum may not be a critical factor in the measurement of N digestibility. However, for protein feedstuffs of low digestibility and with a wider range of digestibilities over the length of the ileum, the sampling site may be of practical significance (Payne *et al.*, 1968; Raharjo and Farrell, 1984). In the present study, it was concluded that the terminal 20 cm of the ileum was satisfactory for the sampling of digesta from the rat given a semi-synthetic MBM based diet.

A possible criticism of the method adopted in this study is the use of CO_2 to asphyxiate the rats. In the earlier study with pigs (refer chapter 2), animals were anaesthetised and killed with a barbiturate to avoid mucosal cell shedding. However, asphyxiation with CO_2 followed by decapitation to cease all neural stimulation to the gut is a commonly used and accepted technique with rats. It leads to rapid immobilisation of the rat, and was retained in the present study.

CHAPTER 4

Evaluation of the laboratory rat as a model animal for determining ileal amino acid digestibility in meat and bone meal for the growing pig

4.1 INTRODUCTION

Although determination of dietary amino acid digestibility at the terminal ileum of the pig is now generally recognised as a more acceptable approach than the traditional faecal method (Tanksley and Knabe, 1984; Sauer and Ozimek, 1986), a drawback with the ileal assay, particularly when applied to large animals such as the growing pig, is its high cost. This limits its use in the routine evaluation of feedstuffs. An alternative approach is to develop an ileal assay with a smaller monogastric animal such as the laboratory rat for application to the pig. The growing laboratory rat can be housed and reared relatively inexpensively, consumes only small amounts of food, and lends itself to ease of collection of a sample of ileal digesta using the slaughter method. Preliminary studies (Picard *et al.*, 1984b; Moughan *et al.*, 1984; Moughan *et al.*, 1987; Smith *et al.*, 1990) indicate that overall the growing rat is a suitable model for the pig for the determination of protein (amino acid) digestibility at the terminal ileum. Where species differences have been reported, it is possible that they have resulted from insufficiently similar experimental conditions being applied to both species, rather than intrinsic differences in the way that pigs and rats digest protein. The method of sampling ileal digesta adopted, in particular, may affect the results obtained.

The optimal sampling conditions to be used with the slaughter method for the determination of total nitrogen (N) and amino acid digestibilities in the growing pig and laboratory rat have been determined in earlier studies (refer chapters 2 and 3, respectively). It was also shown that for the growing pig the slaughter technique is a viable alternative to simple T-cannulation in the determination of N and amino acid digestibilities.

The aim of the present study was to undertake a refined inter-species comparison of the apparent ileal digestibility of N and amino acids in meat and bone meal (MBM) using a common and defined ileal digesta sampling procedure for the growing pig and laboratory rat. The study allowed comparison between pigs and rats for apparent ileal and faecal amino acid digestibility in two meat and bone meals differing in the digestibility of their protein.

4.2 MATERIALS AND METHODS

4.2.1 Meat and bone meals and their processing

A 200 kg sample of MBM was collected from each of 2 rendering plants situated in the lower North Island of New Zealand. Each sample was built up over a continuous 8-hour period during the production of the meal and frozen (-20°C) within 24 hours of collection to minimize any possible effects of storage. The two meat and bone meals which differed in crude protein content (621.0 and 496.0 g crude protein kg^{-1} air dry weight) were designated as MBM1 and MBM2, respectively.

The raw material for MBM1 consisted of sheep and lamb offals and blood. Viscera were cut and washed before rendering and the blood was stored overnight before being added to the rest of the raw material prior to rendering. The Meat Industry Research Institute of New Zealand (MIRINZ) low temperature rendering system (Fernando, 1982) was used in the processing of MBM1. After rendering, the solids were dried in a rotadisc drier, ground and immediately bagged.

The raw material for MBM2 generally consisted of sheep, pig and cattle offals but did not include blood. Before rendering the viscera were cut and washed. The rendering plant consisted of a batch dry rendering melter operated with a 20 to 30 min pressure cycle at the beginning of a 2-hour cooking cycle. After rendering, the cracklings were left to drain in a percolating pan, and subsequently the fat was removed using a basket centrifuge. Once cool, the meal was ground in a hammer mill and then bagged.

Based on previous work (Skilton *et al.*, 1991) with meals from rendering plants in New Zealand it was expected that MBM1 would be representative of a high-quality meal and MBM2 of a lower quality meat and bone meal. The two extreme meals were chosen to provide a comprehensive test of the rat as a model for the pig. The determined chemical composition of the meat and bone meals is given in Table 4.1.

4.2.2 Animals, diets and procedure

Sixteen male rats (190 g body weight) and 16 Large White X Landrace entire male pigs (30 kg body weight) were each equally and randomly allocated to the 2 experimental diets. The ingredient compositions of the rat and pig diets (each containing 200 g kg^{-1} of one of the 2 meat and bone meals as the sole protein source) were as given in Tables 4.2 and 4.3, respectively.

The rats were initially fed a casein-based diet for 2 days and were then given the MBM based diets for 14 days. The rats were trained to consume the MBM diets over a single 3-hour period (08.30 to 11.30 h) each day, and this training was achieved within 7 days. Water was available *ad libitum*.

TABLE 4.1
Mean determined chemical compositions[1] of meat and bone meals (MBM) obtained from two rendering plants

Nutrient	Composition (g/kg air dry weight)[2] MBM1	MBM2
Crude protein	621.0	496.0
Crude fat	85.8	111.4
Ash	271.1	354.1
Amino Acids		
Lysine	39.1	28.0
Methionine	9.8	7.9
Cystine	5.6	4.1
Histidine	17.3	8.9
Phenylalanine	26.2	18.8
Tyrosine	19.1	13.1
Threonine	26.0	18.1
Leucine	51.1	35.4
Isoleucine	18.7	16.6
Valine	37.5	24.3
Alanine	44.6	38.5
Aspartic acid	51.5	41.0
Arginine	48.1	39.0
Serine	28.4	22.7
Glutamic acid	77.7	69.3
Glycine	67.8	61.4
Proline	34.7	29.8

[1]Determined following standard Association of Official Analytical Chemists (1980) procedures. Amino acids were determined as described in section 4.2.2
[2]Values are means of duplicate analyses

On day 14, the rats were asphyxiated in carbon dioxide gas and decapitated (immediately ceasing all neural stimulation to the gut) at 4 hours from the start of feeding. 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 days to accustom the rats to the change in procedure. The abdomen was opened by an incision along the mid-ventral line and the skin and musculature were folded back to expose the viscera. The final 20 cm of the ileum was immediately dissected from the body, and the intestinal surface cleaned using absorbent tissue paper, taking care not to apply pressure to the intestine. The digesta were slowly flushed out into a plastic bag with 10 ml deionised water from a plastic syringe and frozen (-20°C) immediately. There was no pooling of digesta across rats. The digesta samples were freeze-dried, finely ground and stored at -20°C (along with samples of the diet) for the determination of N and chromium.

TABLE 4.2
Ingredient composition (g kg^{-1} air-dry weight) of the experimental diet for the growing rat

Ingredient	Composition
Maize starch	621.0
Meat and bone meal	200.0
Sucrose	80.0
Maize oil	50.0
Purified cellulose[1]	30.0
Mineral and vitamin premix[2]	15.0
Chromic oxide	4.0

[1]Avicel, Asahi Chemical Industry Company Limited, Tokyo, Japan

[2]Tasmix Special Mouse Premix, Pfizer Laboratories, Auckland, New Zealand).
Supplied the following per kg diet: 5250 IU vitamin A; 750 IU vitamin D; 37.5 vitamin E; 1.5 mg vitamin K; 3.0 mg vitamin B_1; 3.75 mg vitamin B_2; 4.5 mg vitamin B_6; 0.04 mg vitamin B_{12}; 21.0 mg pantothenic acid; 0.08 mg biotin; 15 mg niacin; 0.75 mg folic acid; 0.75 mg choline; 60 mg iron; 37.5 mg zinc; 37.5 mg manganese; 3.75 mg copper; 0.38 mg iodine; 0.53 mg cobalt; 0.11 mg selenium; 30.0 mg inositol; 2.75 g potassium; 0.3 g magnesium; 0.38 g sodium.

TABLE 4.3
Ingredient composition (g kg^{-1} air-dry weight) of the experimental diet for the growing pig

Ingredient	Composition
Maize starch	621.0
Meat and bone meal	200.0
Sucrose	80.0
Maize oil	50.0
Purified cellulose[1]	30.0
Mineral and vitamin premix[2]	15.0
Chromic oxide	4.0

[1]Avicel, Asahi Chemical Industry Company Limited, Tokyo, Japan.

[2]Tasmix pig grower vitamin-mineral premix (Tasman Vaccines Ltd, Auckland, New Zealand).
Provided the following per kg diet: 5000 IU vitamin A; 500 IU vitamin D_3; 22 IU vitamin E; 2 mg vitamin K; 20 mg niacin; 3 mg riboflavin; 1.2 mg thiamin; 2 mg pyridoxine; 9 mg pantothenic acid; 20 µg vitamin B_{12}; 0.8 mg folic acid; 20 mg betaine; 40 mg manganese; 100 mg zinc; 100 mg iron; 10 mg copper; 0.5 mg cobalt; 0.5 mg iodine; 0.3 mg selenium; 520 mg choline; 1.6 g chlorine; 1.3 g sodium; 3.0 g potassium; 0.5 g magnesium; 0.4 g sulphur.

The stomach contents were inspected for signs of faecal contamination via coprophagy. The sampling time and the length of terminal ileum sampled were considered optimal for growing rats given a semi-synthetic MBM-based diet (refer chapter 3).

The pigs were penned individually in smooth-walled metabolism crates and housed at 21 ± 1°C. The animals were given a commercial grower meal for 2 days following penning, after which they were given the MBM diets for 14 days. The diet was offered as a wet mash for a single 3-hour period (08.30 - 11.30 h) each day at a set level of intake (0.10 metabolic body weight $kg^{0.75}$). Each meal was mixed with water (1:1; w/v) prior to feeding. Fresh water was provided on an *ad libitum* basis between meals. On day 14, and 9 hours after the start of the meal the pigs were anaesthetised using halothane gas (Fluothane, Imperial Chemical Industries Limited, Cheshire, England) and euthanased by a 10 ml intracardial injection of sodium pentobarbitone (Anathal 60 mg/ml, V.R. Laboratories Australia Pty Ltd., Thornleigh, New South Wales, Australia). Digesta were collected from the terminal 20 cm of ileum. The sampling time and the length of terminal ileum sampled were considered optimal for growing pigs given a semi-synthetic MBM-based diet (refer chapter 2)

Total faeces voided were collected over the final 2 days of the trial for each species and faecal amino acid digestibility was calculated based on chromic oxide as a marker. Faeces, ileal digesta and diets were stored frozen (-20°C) until freeze-drying and chemical analysis.

Ileal, faecal and diet samples were analysed for N content using the Kjeldahl method (Association of Official Analytical Chemists, 1980). Chromium was determined according to the procedure of Costigan and Ellis (1987). Amino acids were determined following acid hydrolysis using a Beckman 119 BL amino acid analyser. Duplicate samples of diet, digesta and faeces (5-7 mg) were hydrolysed in 500 µl of 6M HCl with 1% added phenol, for 24 hours at 110 ± 1°C in glass tubes sealed under vacuum. For the determination of methionine and cystine, separate duplicate samples were oxidised with performic acid prior to hydrolysis. Tryptophan, which is partly destroyed during acid hydrolysis, was not determined.

Estimates of apparent ileal and faecal (N) and amino acid digestibility were calculated from the dietary ratio of N or amino acid to chromium relative to the corresponding ratio in the ileal digesta or faeces, respectively. A linear statistical model which included terms for animal species (species, rat or pig), digestibility type (type, ileal or faecal) and the first order interaction was fitted to the apparent digestibility data and reduction in sums of squares was used to determine levels of significance. Simple linear regressions were computed in which N digestibility was the independent variable and ileal amino acid digestibility was the dependent one. All statistical analyses were carried out using the General Linear Models procedure of REG (Gilmour, 1990).

4.3 RESULTS

The apparent digestibility values for the two MBM-based diets, determined with rats and pigs are given in Tables 4.4 and 4.5.

TABLE 4.4

Mean[1] apparent ileal (I) and faecal (F) digestibilities (%) of nitrogen and amino acids in a high quality meat and bone meal (MBM1) determined with the rat or pig

	Rat		Pig		Overall	Level of significance[2]	
	I	F	I	F	SE	Species	Type (Ileal/faecal)
Nitrogen	73.1	81.1	72.9	83.5	2.73	NS	*
Amino Acid							
Lysine	80.6	88.9	81.7	90.7	2.54	NS	*
Methionine	83.4	90.1	83.6	91.0	2.05	NS	*
Cystine	69.7	75.4	70.4	77.1	1.83	NS	*
Histidine	73.9	83.2	75.1	84.1	2.66	NS	*
Phenylalanine	81.1	87.9	82.9	88.2	1.79	NS	*
Tyrosine	77.9	82.6	78.8	83.4	1.37	NS	*
Threonine	72.6	84.0	72.9	85.8	3.53	NS	*
Leucine	78.1	84.8	77.9	86.0	2.15	NS	*
Isoleucine	79.7	88.0	81.2	89.3	2.40	NS	*
Valine	78.4	90.0	80.3	91.3	3.65	NS	*
Alanine	79.0	90.7	81.2	92.6	3.39	NS	*
Aspartic acid	66.7	80.9	67.4	82.5	4.24	NS	*
Arginine	83.9	91.3	84.2	92.4	2.26	NS	*
Serine	72.9	82.6	73.5	84.4	3.00	NS	*
Glutamic acid	78.7	88.3	71.3	87.7	4.05	*	*
Glycine	73.9	90.8	75.1	91.3	4.93	NS	*
Proline	76.8	88.9	78.2	90.4	4.38	NS	*

[1] Mean values; n = 8

[2] NS = non significant; * = $P < 0.05$

TABLE 4.5

Mean[1] apparent ileal (I) and faecal (F) digestibilities (%) of nitrogen and amino acids in a low quality meat and bone meal (MBM2) determined with the rat or pig

	Rat		Pig		Overall	Level of significance[2]	
	I	F	I	F	SE	Species	Type (Ileal/faecal)
Nitrogen	64.1	79.0	65.7	80.6	4.33	NS	*
Amino acids							
Lysine	73.7	88.1	76.7	89.9	4.05	NS	*
Methionine	73.0	89.4	75.8	90.9	4.59	NS	*
Cystine	59.8	74.8	61.0	76.2	4.38	NS	*
Histidine	60.9	82.7	62.5	83.5	6.19	NS	**
Phenylalanine	78.0	87.5	79.6	87.9	2.59	NS	*
Tyrosine	63.6	81.9	63.9	82.3	5.27	NS	**
Threonine	61.7	83.8	60.6	84.6	6.66	NS	**
Leucine	66.1	84.0	67.5	85.7	5.23	NS	**
Isoleucine	75.0	87.4	76.5	85.8	3.16	NS	*
Valine	68.0	89.7	72.1	90.3	5.82	NS	*
Alanine	76.2	90.8	78.1	92.1	4.16	NS	*
Aspartic acid	52.6	79.8	52.8	80.9	8.15	NS	**
Arginine	79.2	90.6	80.0	91.8	3.36	NS	*
Serine	61.9	81.9	63.4	83.1	5.74	NS	**
Glutamic acid	73.8	87.8	62.8	86.2	5.86	*	*
Glycine	66.7	88.2	65.4	90.1	6.90	NS	**
Proline	66.1	88.4	66.9	89.1	6.43	NS	**

[1]Mean values; n = 8

[2]NS = not significant; * = $P < 0.05$; ** = $P < 0.01$

There was close agreement between the species for the ileal digestibility of N and for the amino acids in each of the two meat and bone meals, except for the non-essential amino acid glutamic acid, the digestibility of which was significantly ($P< 0.05$) higher for the rat than the pig. There was no species X type (ileal or faecal) interaction for N or amino acid digestibilities, except for glutamic acid in each of the two meat and bone meals. The apparent ileal N and amino acid digestibility values for both species were higher for MBM1 compared with MBM2.

A comparison of ileal and faecal apparent digestibilities indicated a significant net disappearance of N and all determined amino acids during passage through the large intestine in both species. Faecal digestibilities of N and amino acids were generally higher ($P< 0.05$) than the corresponding ileal values and were also slightly higher though not significantly so, in the pig than the rat. Ileal, faecal differences for the lower quality MBM (MBM2) were higher compared to those for MBM1.

Linear regression equations for the prediction of apparent ileal amino acid digestibility from apparent ileal N digestibility, based on all the digestibility determinations (n = 32; 16 rat and 16 pig observations combined) for N or each amino acid, are given in Table 4.6.

TABLE 4.6
Linear regression equations for the prediction of apparent ileal amino acid digestibility (AAd) from apparent nitrogen digestibility (Nd) in meat and bone meal

	Prediction equation	rsd[1]	R^2
Lysine	40.42 + 0.55 Nd	2.11	0.73
Methionine	27.08 + 0.75 Nd	4.06	0.53
Cystine	5.45 + 0.87 Nd	3.04	0.71
Histidine	-5.99 + 1.07 Nd	4.67	0.61
Phenylalanine	63.63 + 0.24 Nd	2.63	0.29
Tyrosine	-15.81 + 1.26 Nd	4.64	0.68
Threonine	-4.68 + 1.04 Nd	4.01	0.66
Leucine	8.49 + 0.93 Nd	4.54	0.58
Isoleucine	52.10 + 0.38 Nd	3.01	0.37
Valine	25.29 + 0.71 Nd	3.77	0.58
Alanine	59.41 + 0.28 Nd	2.34	0.38
Aspartic acid	-34.33 + 1.37 Nd	4.01	0.77
Arginine	47.77 + 0.50 Nd	2.05	0.62
Serine	0.71 + 0.98 Nd	3.47	0.70
Glutamic acid	45.26 + 0.45 Nd[3]	2.81	0.80
	26.80 + 0.58 Nd[4]		
Glycine	6.30 + 0.92 Nd	2.97	0.74
Proline	1.13 + 1.03 Nd	3.29	0.74

[1]Residual standard deviation; R^2 = coefficient of determination
[3,4]Prediction equations for rats and pigs, respectively

The coefficients of determination (R^2) of the prediction equations ranged from 0.29 for phenylalanine to 0.80 for glutamic acid, with an average R^2 of 0.56.

4.4 DISCUSSION

For an interspecies comparison of digestibility to be valid, the species should be examined under physiologically comparable conditions. There appear to be major similarities between rats and pigs in digestive anatomy and physiology (Davenport, 1977; Church and Pond, 1988), requirements for dietary energy (Garlick *et al.*, 1976; Andersen and Just, 1979) and amino acids (National Research Council, 1978, 1979) and relative growth rates (Yang and Mickelsen, 1974; Pullar and Webster, 1977), especially when comparison is made at a physiologically comparable age. Care was taken during the design of this study to ensure comparability.

The bodyweights of both rats and pigs were selected to correspond to the period of growth after weaning. The body weights of the rats at 7 weeks of age (190 g) corresponded to 35% of mature body weight (550 g; National Research Council, 1978) while those for the pigs at 12 weeks corresponded to 15% of mature weight (200 kg; Pond and Houpt, 1978). Although the weights as proportions of mature weight were different, the rats (National Research Council, 1962) and pigs (Headley *et al.*, 1961) should have been in their linear phase of growth and so there should not have been any major differences in the stage of development of their digestive systems. This is supported by the work of Pelletier *et al.* (1983) with rats and pigs which indicated that the proteolytic activity of the gastric mucosa of both species increased progressively from birth and had virtually levelled off reaching adult levels by the time they were 4- and 8-weeks-old, respectively.

Further, the level of feed intake in the present study was chosen so that relative food intake was comparable between the species. The meal intakes of the 190 g growing rats (11.0 g/day) corresponded to 0.73 of the *ad libitum* digestible energy intake (National Research Council, 1978) and was sufficient to maintain substantial growth rates over the trial period. The same level of meal intake (0.73 of the *ad libitum* digestible energy intake, 1.28 kg day^{-1}), equivalent to 0.10 metabolic weight $kg^{0.75}$, was offered to the pigs.

For both rats and pigs, the amounts of dietary N and amino acids digested and absorbed at the terminal ileum were lower than the values determined over the entire digestive tract. The differences between faecal and ileal N and amino acid digestibility for the low quality meal (MBM2) were greater compared with the differences for MBM1. Apparently, the lower the ileal digestibilities of N and amino acids, the greater is the difference between ileal and faecal digestibilities. This is in agreement with the findings of other authors (Zebrowska and Buraczewski, 1977; Jorgensen and Sauer, 1982). With diets containing highly digestible proteins, most protein is absorbed before the digesta enter the large intestine, whereas with protein sources of lower quality there are larger residues to undergo disappearance between the terminal ileum and rectum. Breakdown of N and amino acids in the hindgut of

rats and pigs to non-utilisable products of absorption results in the faecal method considerably overestimating N and amino acid absorption. According to Zebrowska (1978) the amount of amino acids disappearing in the large intestine usually varies from 5 to 35% of the total amino acids ingested and amino acids disappearing from the large intestine have been shown to be of little nutritional value to simple-stomached animals (Zebrowska, 1973; 1975; Gargallo and Zimmerman, 1981; Just *et al.*, 1981; Wunsche *et al.*, 1982; Just *et al.*, 1985). It is noteworthy that whereas the measurement of digestibility at the end of the small intestine indicated major differences in amino acid digestibilities for the two meat and bone meals, such differences were not detected with the faecal approach. The faecal digestibility values for MBM1 and MBM2 were similar. This is in agreement with several studies which indicate that the ileal analysis, as compared to the traditional faecal approach, is more sensitive for detecting small differences in amino acid digestibilities (Ivan and Farrell, 1976a; Sauer *et al.*, 1981).

There were considerable differences, in the present study, in digestibility among the amino acids within each protein source. Also, the digestibility values for N were substantially different from those of the amino acids, particularly for the key amino acids, lysine and methionine. Most of the amino acids were digested to a greater extent than the total N. This is consistent with the findings of Sauer *et al.* (1980), Moughan and Smith (1985) and Green (1989) and may be explained by the crude protein containing non-protein nitrogen compounds which have lower digestibilities than the amino acids.

The simple linear regression analyses indicate a moderate relationship (R^2, 0.53 to 0.80) between apparent ileal amino acid digestibilities and apparent ileal N digestibility, with the exception of phenylalanine, isoleucine and alanine. The data obtained in the present study suggest that the apparent ileal digestible amino acids in MBM cannot be predicted, with a high degree of certainty, from the apparent ileal N digestibility value. Knabe *et al.* (1989) also observed that neither ileal nor faecal N digestibility could be used with a high degree of accuracy to predict ileal amino acid digestibilities. In a recent study by Skilton *et al.* (1991) to predict amino acid digestibility from N digestibility in MBM, the regression coefficients of the derived equations indicated that the apparent ileal digestibilities of several of the indispensable amino acids, particularly lysine and methionine, were generally higher than apparent N digestibility whereas those for threonine and most of the dispensable amino acids were generally lower. Regression equations relating the apparent and true ileal digestibility coefficients of amino acids to apparent and true ileal N digestibility, respectively derived by Skilton *et al.* (1991) had residual standard errors ranging from 0.04 to 0.10 depending upon the amino acid under consideration. This agrees with the present findings and those of Fuller (1988) that the accuracy in estimating apparent ileal amino acid digestibilities from apparent N digestibility is likely to be unacceptably low.

For most of the essential amino acids, particularly in MBM2, low ileal digestibility coefficients were obtained. The relatively low digestibility of amino acids in meat and bone

meals has been reported by several other workers (Tanksley and Knabe, 1980; Fuller *et al.*, 1981; Jorgensen and Sauer, 1982; Moughan and Smith, 1985; Skilton *et al.*, 1991). The differences in apparent digestibility of N and amino acids between the two extreme sources of MBM may have been due to differences in the amount of protein intake as well as in the quality of the meals. Various studies (Sauer *et al.*, 1980; Shah *et al.*, 1982; Furuya and Kaji, 1989; Keith and Bell, 1991) provide evidence that at the same dietary dry matter intake, apparent digestibility coefficients are likely to be influenced by the level of protein included in the test diet.

Accepting the need for a routine assay for protein evaluation of feedstuffs, the findings of the present study support the use of the growing rat as a model for the growing pig in terms of the apparent ileal digestibility of N and amino acids in MBM. A finding of relevance to the present work is that of Moughan *et al.* (1984), who also recorded close similarities between the rat and pig for the apparent ileal digestibility of crude protein in meat and bone meal. These findings support those of Picard *et al.* (1984) who reported close similarities between the rat and the pig for the apparent and true ileal digestibilities of amino acids in several protein sources. Also, Taverner (1979) demonstrated close agreement between the rat and the pig for the true ileal digestibility of N in wheat. The interspecies agreement for protein digestibility in MBM in the present study is consistent with the similarities in digestive physiology found between rats and pigs. The apparent digestibility of glutamic acid was significantly different between the species. Glutamic acid is a major component of endogenous amino acid excretion in the rat (Moughan *et al.*, 1987) and pig (Taverner, 1979). Accordingly, the species difference in apparent ileal glutamic acid digestibility found in the present study for both meals may reflect differences in endogenous ileal amino acid excretion. In view of its significant role in the endogenous excretion, the species difference for glutamic acid may be reduced if comparison was based on true ileal amino acid digestibility.

Although other studies have also shown similarities between rats and pigs for ileal amino acid digestibility coefficients, the rat may not be a useful model for all feedstuffs. Of particular relevance here may be the legumes. Moughan *et al.* (1984) found significant differences between apparent ileal pea protein digestibility determined in the rat and pig. Recent studies by Huisman *et al.* (1991) to compare the sensitivity of various animal species (pigs, rats, chickens and mice) to antinutritional factors in legume seeds (beans, *Phaseolus vulgaris* and peas, *Pisum sativum*) demonstrated that the rat and pig respond differently. Piglets were more sensitive to antinutritional factors in legume seeds than rats, mice and chickens.

To conclude, in the present study where the apparent digestibility of N and amino acids were determined under standardised conditions and for two extreme samples of MBM, good agreement was found between the growing rat and pig. The laboratory rat assay as described here offers an accurate and relatively inexpensive, rapid technique for

determining the apparent ileal digestibility of N and amino acids in meat and bone meal for the growing pig. Based on these and other findings (Picard *et al.*, 1984b; Moughan *et al.*, 1987; Smith *et al.*, 1990) the growing laboratory rat is accepted as a suitable model animal for determining ileal amino acid digestibility in meat and bone meal for the growing pig.

CHAPTER 5

Towards the establishment of a rat true ileal amino acid digestibility assay: A comparison of methods for the determination of endogenous amino acid flow at the terminal ileum of the growing rat

5.1 INTRODUCTION

The laboratory rat is a suitable model animal for the growing pig, to allow determination of meat and bone meal (MBM) protein digestibility (refer chapter 4). The rat assay offers a more rapid and less expensive approach to determining ileal amino acid digestibility. A further aspect of digestibility assays warranting consideration, however, is the influence of endogenous amino acid excretion at the terminal ileum. The rat assay developed previously (refer chapter 4) is an apparent digestibility assay and as such its validity is open to question in view of the influence of endogenous protein excretion.

The digestive tract and its exocrine glands secrete considerable quantities of amino acids and other nitrogen-containing compounds into the gut lumen (Fauconneau and Michel, 1970; Snook, 1973; Low, 1982c; Alpers, 1987) and only part of this material is digested and reabsorbed (Buraczewski, 1980). Nitrogen-containing material found in terminal ileal digesta may be from bacteria, hair, enzymes, mucoproteins, desquamated cells, serum albumin, endogenous peptides, endogenous free amino acids, amines and urea (Fauconneau and Michel, 1970). Correction of ileal digesta flows for the non-dietary component to give true rather than apparent digestibility coefficients, is expected to lead to a more accurate description of dietary protein digestibility.

In the past the main approach adopted for the routine determination of endogenous protein loss from the ileum has been the protein-free method (Sauer *et al.*, 1977a; Taverner *et al.*, 1981b; Leibholz, 1982; Darcy-Vrillon and Laplace, 1984., Kies *et al.*, 1986; De Lange *et al.*, 1989a; Skilton *et al.*, 1991) though the regression technique has also been applied (Carlson and Bayley, 1970; Leibholz, 1982; Moughan *et al.*, 1987; Leibholz and Mollah, 1988; Furuya and Kaji, 1989). It appears, however, that determining endogenous loss under protein-free alimentation leads to a considerable underestimation of the physiologically normal level of endogenous excretion (Darragh *et al.*, 1990; de Lange *et al.*, 1990; Moughan and Rutherfurd, 1990; Butts *et al.*, 1991). The regression method has also been criticised on the basis that the estimation of endogenous loss is constrained by a linear function which may not be a suitable descriptor of the real biological phenomenon (P.J. Moughan, personal communication). Furthermore, in using the regression technique it is assumed that endogenous loss remains constant with increasing levels of protein intake.

A new method for determining ileal endogenous amino acid excretion that involves

feeding the animal peptides (enzymically hydrolysed casein, EHC), followed by ultrafiltration of the ileal digesta has been proposed (Moughan *et al.*, 1990) and evaluated (Butts *et al.*, 1991). The latter approach is not subject to the criticisms of the protein-free or regression methods. The hydrolysed casein mainly consists of small (< 5000 Da molecular weight) peptides, which apparently maintain physiologically normal levels of endogenous nitrogen excretion. The high molecular weight (MW > 10,000 Daltons) fraction resulting from the ultrafiltration of the ileal digesta provides a measure of endogenous amino acid loss. The method has been applied to the rat (Butts *et al.*, 1991) and the pig (Butts *et al.*, 1992b) and it has been shown to give considerably higher estimates of ileal endogenous amino acid loss than the protein-free method. The method is particularly suited for application to feedstuffs, such as meat and bone meals, which do not contain anti-nutritional factors or appreciable amounts of plant fibre.

The present study was undertaken to compare the new peptide alimentation method, protein-free and regression approaches in determining endogenous ileal amino acid excretion in the rat and was aimed at the development of an assay for the true ileal digestibility of amino acids in meat and bone meal. Preliminary investigations to determine the optimum time for sampling ileal digesta from the laboratory rat given the protein-free and the enzymically hydrolysed casein-based diets were also undertaken.

5.2 MATERIALS AND METHODS

5.2.1 Animals and housing

Male Sprague-Dawley rats of around 7 weeks of age and 190 g bodyweight were selected at random from a group of animals which had been weaned at 4 weeks of age and reared on a high quality lactic casein-based diet at the Small Animal Production Unit, Massey University. The rats were kept individually in stainless steel wire-bottom cages at 21$\pm$1°C and with a 12-hour light/dark cycle.

5.2.2 Experimental diets

A protein-free (PF) diet, an enzymically hydrolysed casein (EHC) based diet and 5 other diets containing graded levels of meat and bone meal (MBM) were prepared. The ingredient compositions of the PF and EHC diets are shown in Table 5.1., while those of the MBM diets are given in Table 5.2. The EHC (Sigma Chemical Company, St Louis, U.S.A. Type I bovine milk. Total nitrogen (N) = 12.7%, Amino nitrogen = 6.3%; M.W. < 5000 Daltons) comprised free amino acids and small peptides. The crude protein contents of the MBM based diets were: 80, 100, 120, 140 and 160 g crude protein kg^{-1}. Chromic oxide was added to each diet as an indigestible marker to allow calculation of ileal digesta flows.

TABLE 5.1
Ingredient composition (g kg^{-1} air dry weight) of the enzymically hydrolysed casein (EHC) and protein -free (PF) diets for rats

Ingredient	EHC	PF
Maize starch	687	787
Purified cellulose[1]	30	30
Maize oil	50	50
Sucrose	80	80
Mineral, vitamin premix[2]	15	15
Chromic oxide	4	4
Enzymically hydrolysed casein	100	-
Sodium chloride	4	4
Magnesium sulphate	2	2
Potassium carbonate	4	4
Dicalcium phosphate	24	24

[1]Avicel, Asahi Chemical Industry Company Limited, Tokyo, Japan.

[2]Tasmix special mouse premix (Tasmix, Pfizer Laboratories, Auckland, New Zealand). Supplied the following per kg diet: 5250 IU vitamin A; 750 IU vitamin D; 37.5 IU vitamin E; 1.5 mg vitamin K; 3.0 mg vitamin B_1; 3.75 mg vitamin B_2; 4.5 mg vitamin B_6; 0.04 mg vitamin B_{12}; 21.0 mg pantothenic acid; 0.08 mg biotin; 15 mg niacin; 0.75 mg folic acid; 0.75 g choline; 60 mg iron; 37.5 mg zinc; 37.5 mg manganese; 3.75 mg copper; 0.38 mg iodine; 0.53 mg cobalt; 0.11 mg selenium; 30 mg inositol; 2.75 g potassium; 0.3 g magnesium; 0.38 sodium.

TABLE 5.2
Ingredient composition (g kg^{-1} air dry weight) of the semi-synthetic meat and bone meal based diets for rats

	Dietary crude protein level (g kg^{-1} diet)				
	80	100	120	140	160
Maize starch	661	621	581	541	501
Purified cellulose[1]	30	30	30	30	30
Maize oil	50	50	50	50	50
Sucrose	80	80	80	80	80
Vitamin/mineral mix[2]	15	15	15	15	15
Chromic oxide	4	4	4	4	4
Meat and bone meal	160	200	240	280	320

[1]Avicel, Asahi Chemical Comapny Limited, Tokyo, Japan.

[2]Tasmix special mouse premix, Pfizer Laboratories, Auckland, NZ.
Provided the same amounts of nutrients per kg diet as shown in Table 5.1

5.2.3 Experimental procedure

5.2.3.1 Preliminary study: The effect of time of sampling on endogenous nitrogen flows at the terminal ileum of growing rats

In each of two preliminary studies, thirty rats were equally and randomly allocated to five slaughter times (2, 3, 4, 5 and 6 hours after the commencement of feeding). For a period of 13 days prior to collection of ileal digesta the rats were trained to consume their daily intakes of either of two experimental diets (offered in stainless steel feeders) within a single 3-hour period (08.30 - 11.30 h) each day. The rats were initially fed a casein-based diet for 7 days and were then given either the PF or EHC-based diet (Table 5.1) for a further 7 days. Water was available at all times. These diets were offered to the rats on days 8-14 inclusive.

On day 14, the rats were asphyxiated with CO_2 gas and then decapitated (ceasing all neural stimulation to the gut) at the set slaughter times. To facilitate the killing of rats within 10 min of the designated times, feeding times were staggered on the day of slaughter and also the previous 2 days of the trial to accustom the rats to the procedure. The abdomen was opened by an incision along the mid-ventral line and the skin and musculature were folded back to expose the viscera. The final 20 cm of the ileum were immediately dissected out from the body, and the dissection rinsed with distilled water to remove any traces of blood and hair, and dried with absorbent tissue paper, taking care not to apply pressure to the intestine. The digesta were slowly flushed out with 10 ml deionised water from a plastic syringe into a plastic bag. The digesta from each of the rats fed the PF diet were frozen (-20°C), freeze-dried, finely ground and analysed for N and chromium. The pH of digesta samples obtained from rats fed the EHC-based diet was adjusted to pH 3.5 by the addition of 9M H_2SO_4, to reduce bacterial and enzyme activity, and the samples then immediately frozen. Digesta samples were subsequently thawed and subjected to centrifugation and ultrafiltration according to the procedure detailed by Butts *et al.* (1991).

5.2.3.1 (a) Centrifugation and ultrafiltration of digesta samples from animals fed EHC diets

The ileal digesta samples obtained from animals fed the EHC-based diets were centrifuged at 2500 rpm (1450 g) for 45 min at 0°C. The supernatant was gently poured off into a separate container. The precipitate was washed with 5.0 ml of deionised water and centrifuged again for 30 min at 2500 rpm (1450 g) at 0°C. The second supernatant was gently poured off and added to the first supernatant. The weight of the precipitate was measured and recorded.

The supernatants were ultrafiltered in Centriprep-10 concentrators (Amicon, W.R. Grace Company, Danvers, Massachusetts, U.S.A.) according to the manufacturer's instruction (Amicon, 1987). The high molecular weight fraction (M.W. > 10,000 Daltons) following ultrafiltration was added to the precipitate. The precipitates were frozen immediately, freeze-dried, finely ground and then analysed for N and chromium. The weights of the total

amounts of freeze-dried digesta were recorded before chemical analyses.

5.2.3.1 (b) Chemical analysis

Diets, ileal digesta and ileal digesta precipitates plus ultrafiltrates were analysed in duplicate for total nitrogen (N) using the Kjeldahl method (Association of Official Analytical Chemists, 1980) on a Kjeltec 1030 auto-analyser (Tecator, Sweden). The chromium contents of six 100 mg samples of the experimental diets and duplicate 10-15 mg samples of ileal digesta precipitate plus ultrafiltrate and total ileal digesta were determined on an Instrumentation Laboratory Atomic Absorption Spectrophotometer using the method of Costigan and Ellis (1987).

5.2.3.1 (c) Data analysis

Endogenous flows of nitrogen (N) at the terminal ileum related to the ingestion of 1 g freeze dry matter (FDM) were calculated using the following equation:

$$\text{Nitrogen flow } (\mu g\ g^{-1}\ FDM) = \text{N concentration in ileal digesta } (\mu g\ g\ FDM^{-1}) \times \frac{\text{Dietary chromium } (mg\ g^{-1}\ FDM)}{\text{Ileal chromium } (mg\ g^{-1}\ FDM)}$$

Endogenous N flows for the animals given the EHC diet were calculated based on the nitrogen content of the precipitate plus high molecular weight fraction following centrifugation plus ultrafiltration treatments.

Data were subjected to analysis of variance and differences between means were examined using a multiple range test (Snedecor and Cochran, 1989). Statistical analyses were carried out using the computerised statistical package REG (Gilmour, 1990).

5.2.3.2 Main study: Determination of endogenous amino acid flows

Twelve male rats were equally and randomly allocated to the PF and EHC treatments. For the regression method of determining endogenous amino acid loss, 30 male rats were equally and randomly allocated to 5 experimental MBM diets.

Housing, feeding, ileal digesta collection and treatment, and other experimental procedures were the same as described in the preliminary study (sections 5.2.1, 5.2.3.1, and 5.2.3.1 (a)) except that on day 14, the rats given the PF and EHC based diets were slaughtered 3 hours after the start of feeding while those fed the MBM based diets were euthansed at 4 hours post-feeding. Freeze-dried samples of ileal digesta and precipitates were finely ground and analysed for N and chromium as described previously in section 5.2.3.1 (b). Amino acids were determined following acid hydrolysis, using a Beckman 119 BL amino acid analyser. Duplicate samples (5-7 mg) were hydrolysed in 500 µl of 6M HCl with 1% added phenol for 24 hours at 110 $\pm$ 1°C in glass tubes sealed under vacuum. For the determination of methionine and cystine concentrations, separate samples were

oxidised with performic acid at 0°C for 10 hours followed by neutralisation with hydrogen bromide. Tryptophan, being destroyed during acid hydrolysis, was not determined.

5.2.3.2 (a) Data analysis

Endogenous ileal amino acid excretions for rats ($\mu g\ g^{-1}$ freeze dry matter intake) given the PF diet were calculated using the ratio of chromium in the diet to chromium in the ileal digesta.

Endogenous ileal amino acid excretions for animals given the EHC diet were calculated based on the amino acid content of the precipitate plus high molecular fraction following centrifugation plus ultrafiltration treatments.

The flows of endogenous amino acids from the terminal ileum of animals given the MBM-based diets were determined by extrapolation to zero intake of linear regressions of ileal amino acid output (mg g^{-1} FDMI) on dietary amino acid intake (mg day^{-1}). Regression equations which provided the estimates of endogenous flow were derived for nitrogen and each determined amino acid.

Apparent digestibilities of N and amino acids in MBM at each level of dietary nitrogen and amino acid intake were calculated from the ratio of N or amino acid to chromium in the ileal digesta relative to the corresponding ratio in the diet.

Endogenous amino acid flows for the EHC-fed rats (after centrifugation plus ultrafiltration of the digesta) and the protein-free fed rats were subjected to analysis of variance for each amino acid. The effect of dietary intake of each amino acid on apparent ileal digestibility for the rats fed the MBM diets was examined using analysis of variance. Comparison of the respective means was carried out, where appropriate, using Tukey's test. Statistical analyses were carried out using the computerised statistical package REG (Gilmour, 1990).

5.3 RESULTS

The rats consumed the EHC, MBM and PF diets readily and appeared healthy throughout the study. In the main study, daily feed intakes stabilised by about the tenth day of the experiment. The mean intakes determined over the last 7 days of trial were 13.3 (EHC), 11.0 (MBM) and 10.6 (PF) g day^{-1} were more variable for rats receiving the MBM diets (CV = 20.7%) compared with those on the EHC-based (CV = 13.8%) or PF (CV = 10.3%) diet . Growth rates (mean $\pm$ SE) determined over the last 7 days of trial were 3.17 ($\pm$0.14), 2.96 ($\pm$0.09) and 0.36 ($\pm$0.03) g day^{-1} for rats on the EHC, MBM and PF treatments. At slaughter, faeces were not detected in the gastric digesta, indicating that coprophagy had not occurred.

The data given in Table 5.3 relate to the preliminary experiments conducted to determine the effect of time of sampling of ileal digesta after the start of feeding on the endogenous excretion of N at the terminal ileum of rats fed the PF or EHC-based diets.

TABLE 5.3

Effect of the time of sampling digesta[+] for rats given a protein-free or an enzymically hydrolysed casein (EHC) based diet on the amount of ileal digesta and endogenous nitrogen excretion

	Time from start of feeding (h)					
	2	3	4	5	6	Statistical significance[1]
Protein-free method						
Freeze dried matter (mg)[2]	113^{a} (17.2)	218^{b} (8.1)	146^{c} (25.4)	177^{d} (18.3)	201^{b} (20.5)	*
Endogenous N excretion ($mg\ g^{-1}$ FDMI)[2]	0.99^{a} (0.12)	1.08^{b} (0.09)	1.03^{ab} (0.18)	1.04^{ab} (0.16)	1.06^{ab} (0.12)	*
EHC/ultrafiltration method						
Freeze dried matter (mg)[2]	136^{a} (21.6)	241^{b} (11.4)	161^{a} (18.3)	195^{c} (23.4)	234^{b} (19.1)	*
Endogenous N excretion ($mg\ g^{-1}$ FDMI)[2]	1.72^{a} (0.13)	1.88^{b} (0.07)	1.80^{ab} (0.12)	1.85^{b} (0.10)	1.82^{b} (0.16)	*

[+]defined as the time from start of feeding to slaughter

[1]* = significant $P < 0.05$

[2]Mean values ($\pm$SE); n = 6

[a,b,c,d]Means within a row with different superscripts are significantly different ($P < 0.05$)

FDMI = freeze dry matter intake

Endogenous N excretion increased from 2 to 3 hours from the start of feeding and then remained relatively constant from 3 to 6 hours. The amount of digesta collected and the endogenous N excretion were least variable at the 3 hours post-feeding. Accordingly, the EHC- and the PF-fed rats in the present series of experiments were slaughtered and digesta sampled at 3 hours from the start of feeding.

The regression equations for ileal N and amino acid flow as a function of dietary crude protein or amino acid intake, as determined in the main study, are given in Table 5.4.

TABLE 5.4
Linear regression relationships between ileal amino acid flow and dietary amino acid intake for rats fed diets containing meat and bone meal

	Regression equation[1]	rsd	R^2
Nitrogen	y = 0.30x + 1.019	0.186	0.93
Amino acid			
Lysine	y = 0.28x + 0.168	0.086	0.86
Methionine	y = 0.19x + 0.052	0.048	0.87
Cystine	y = 0.40x + 0.057	0.030	0.97
Threonine	y = 0.26x + 0.301	0.015	0.97
Valine	y = 0.26x + 0.228	0.179	0.87
Isoleucine	y = 0.34x + 0.153	0.097	0.93
Leucine	y = 0.20x + 0.250	0.167	0.93
Tyrosine	y = 0.30x + 0.154	0.059	0.72
Phenylalanine	y = 0.20x + 0.203	0.095	0.53
Histidine	y = 0.36x + 0.131	0.100	0.92
Arginine	y = 0.18x + 0.209	0.312	0.88
Aspartic acid	y = 0.42x + 0.623	0.272	0.91
Serine	y = 0.30x + 0.366	0.122	0.88
Glutamic acid	y = 0.23x + 0.699	0.387	0.87
Glycine	y = 0.29x + 0.758	0.188	0.97
Alanine	y = 0.22x + 0.209	0.040	0.94
Proline	y = 0.32x + 0.573	0.120	0.88

[1] y = ileal amino acid flow (mg g^{-1} freeze dry matter intake); x = dietary nitrogen or amino acid intake (mg day^{-1})
R^2 = Coefficient of determination; rsd = residual standard deviation

Significant linear relationships were found between ileal amino acid flow and dietary amino acid intake for all amino acids. All intercepts and slopes were positive. Coefficients of determination (R^2) obtained for rats by regressing ileal N and amino acid flows on dietary N and amino acid intakes were generally high, ranging from 0.87 to 0.97 with the exception of phenylalanine and tyrosine which had R^2 values of 0.53 and 0.72, respectively. The intercepts of the regression equations were estimates of endogenous ileal N and amino acid flows.

The endogenous ileal N and amino acid flows determined by the EHC/ultrafiltration technique and with animals given the protein-free diets are given in Table 5.5. For comparison, the endogenous N and individual amino acid flows determined using the regression method are also given.

TABLE 5.5
Mean (±SE) endogenous amino acid flows[1] at the terminal ileum of the growing rat determined using the enzymically hydrolysed casein/ultrafiltration (EHC), protein-free (PF) or regression (R) analysis methods

	Endogenous amino acid flow[2]		
	EHC[3]	PF	R
Nitrogen	1866 (30.8)	1103 (22.6)	1019 (3.6)
Amino acid			
Lysine	275 (7.1)	172 (5.3)	168 (0.07)
Methionine	127 (2.3)	53 (0.7)	52 (0.03)
Cystine	142 (2.4)	56 (1.0)	57 (0.02)
Histidine	223 (5.0)	133 (3.2)	131 (0.08)
Phenylalanine	237 (5.6)	212 (3.3)	203 (0.08)
Tyrosine	179 (2.8)	161 (2.8)	154 (0.05)
Threonine	525 (12.3)	311 (6.0)	301 (0.10)
Leucine	386 (7.1)	256 (4.2)	250 (0.13)
Isoleucine	313 (8.2)	159 (2.0)	153 (0.08)
Valine	341 (10.7)	234 (3.4)	228 (0.14)
Alanine	349 (4.3)	213 (3.2)	209 (0.03)
Aspartic acid	748 (14.5)	636 (7.4)	623 (0.22)
Arginine	274 (6.5)	217 (5.4)	209 (0.25)
Serine	759 (17.2)	374 (4.2)	366 (0.01)
Glutamic acid	1366 (28.5)	701 (10.3)	699 (0.31)
Glycine	796 (16.1)	765 (22.3)	758 (0.15)
Proline	493 (7.4)	584 (6.6)	573 (1.00)

[1] μg g freeze dry matter^{-1} intake (n = 6 for EHC and PF; n = 30 for R)
[2] The EHC endogenous flows were significantly ($P < 0.01$) higher than for the protein-free fed rats except for tyrosine and glycine
[3] Digesta were centrifuged and ultrafiltered; flows were based on amino acid contents of precipitate plus retentate (M.W. > 10,000 Daltons)

Endogenous N and ileal amino acid flows determined by the EHC/ultrafiltration method were significantly ($P< 0.01$) higher than those for rats on the PF diets, with the exception of endogenous tyrosine and glycine flows. The endogenous ileal amino acid flows for rats on the PF diet were similar to those calculated using the regression method.

The mean apparent N and amino acid digestibilities at five levels of dietary crude protein intake of a MBM diet, as determined from data collected in the study of the regression method of determining endogenous loss, are shown in Table 5.6.

TABLE 5.6

The mean apparent ileal digestibility (%)[1] of nitrogen and amino acids in a meat and bone meal-based diet fed to growing rats at 5 levels of dietary crude protein (CP) intake

	Dietary crude protein intake (mg day^{-1})					Overall	Statistical
	880	1100	1320	1540	1760	SE	significance[2]
Nitrogen	62.8	63.3	64.8	66.1	67.9	0.93	**
Amino acid							
Lysine	65.8	66.4	67.4	69.2	70.9	0.94	**
Methionine	70.4	72.8	73.9	75.1	76.3	1.01	**
Cystine	53.8	54.6	55.2	56.4	57.1	0.60	*
Histidine	63.9	65.8	66.4	67.0	67.8	0.66	*
Phenylalanine	75.1	76.3	77.2	78.8	79.5	0.80	*
Tyrosine	64.9	65.5	67.2	68.0	68.7	0.72	*
Threonine	63.1	64.9	66.2	68.4	69.8	1.20	**
Leucine	69.2	70.8	71.6	73.2	74.1	0.87	**
Isoleucine	68.8	69.9	71.9	73.7	74.9	1.14	**
Valine	70.2	71.2	72.6	74.7	75.4	0.99	**
Alanine	73.1	74.6	76.2	78.1	79.8	1.20	**
Aspartic acid	50.9	52.1	53.6	55.8	56.8	1.10	**
Arginine	78.8	80.7	81.3	81.9	82.1	0.59	*
Serine	65.7	66.8	68.3	70.1	71.8	1.10	***
Glutamic acid	68.4	70.8	71.2	72.0	72.6	0.72	**
Glycine	60.6	64.2	68.4	70.0	72.8	2.16	***
Proline	58.8	62.8	63.7	66.8	68.9	1.74	***

[1] n = 6 rats per dietary crude protein intake level.

[2] NS = non significant, * = significant $P < 0.05$, ** = significant $P < 0.01$, *** = significant $P < 0.001$

Dietary crude protein intake had a significant ($P< 0.05$) effect of on apparent ileal N and amino acid digestibility of MBM, there being a marked increase in digestibility as dietary crude protein intake increased over the range 880 to 1760 mg day^{-1}.

5.4 DISCUSSION

The endogenous ileal amino acid flows determined in the present study using the EHC/ultrafiltration method were close to other comparable values reported in the literature for rats (Butts *et al.*, 1991). The EHC (centrifugation followed by ultrafiltration) amino acid flows were significantly higher than the corresponding protein-free and extrapolated values. This is further evidence that peptides either administered *per os* or derived from the natural digestion of dietary protein have a considerable influence on the loss of endogenous amino acids from the digestive tract. This confirms earlier findings with the growing rat (Darragh *et al.*, 1990; Moughan and Rutherfurd, 1990; Butts *et al.*, 1991). A similar effect has been found with growing pigs (De Lange *et al.*, 1989b; Butts *et al.* 1992b). In support of these findings, lower proteolytic enzyme activities in the pancreas and intestine have been reported (Kimura *et al.*, 1977; Schneeman, 1982) for animals fed a PF diet while the presence of dietary peptides in the gut has been reported to be more effective than free amino acids in stimulating the rates of trypsin and chymotrypsin synthesis (Temler *et al.*, 1983; Puigserver *et al.*, 1986). Bioactive peptides formed during the digestion of foods have been isolated and may play a role in stimulating gut secretory processes (Schlimme *et al.*, 1989).

The mean endogenous amino acid flows for the PF method, found in this study, are close agreement with those reported for rats by Skilton *et al.* (1988), Darragh *et al.* (1990), Moughan and Rutherfurd (1990) and Butts *et al.* (1991). Although, the use of a PF diet is the method usually employed to determine gut endogenous amino acid losses, this approach has nevertheless been frequently criticised as being unphysiological. When animals are deprived of dietary protein and enter negative body N balance, their rate of whole-body protein synthesis falls (Millward *et al.*, 1976) and this may affect the entry of protein into the gut lumen. Studies have recently been conducted to test the hypothesis that the state of body N balance *per se* affects endogenous amino acid loss. Skilton *et al.* (1988), with growing rats given either a PF diet or a similar one but containing synthetic free amino acids as the source of dietary N, found that the negative body N balance caused by the PF regime, which was presumably accompanied by a fall in the rate of protein synthesis, did not lead to a lowered endogenous loss of amino acids from the ileum. Also, de Lange (1989b) with growing pigs fed a PF diet while intravenously infusing half the animals with physiological saline and the remainder with a balanced mixture of synthetic amino acids, found no significant differences in ileal endogenous amino acid excretion between the pigs in positive N balance and those receiving the PF diet with intravenous infusion of saline, except that proline loss was significantly ($P< 0.05$) lower for animals given the amino acid

infusion. Apparently, the protein-deplete state *per se* does not affect endogenous amino acid loss at the distal ileum of animals. It appears, however, that dietary peptides exert a direct stimulatory effect on gut endogenous protein secretion.

Various workers (Taverner, 1979; Leibholz, 1982; Bielorai *et al.*, 1985; Moughan *et al.*, 1987) have adopted the regression procedure (also used in the present study) for determining endogenous ileal amino acid flow, assuming that the values obtained reflect those occurring when protein-containing feeds are given to animals. In the study reported here, the flows of endogenous amino acid from the terminal ileum were similar irrespective of whether they were determined using a PF diet or obtained by extrapolation from data in animals given the diets containing graded levels of meat and bone meal. This similarity of endogenous amino acid flows determined using either the PF or regression analysis receives the support of comparable studies with growing pigs (Taverner *et al.*, 1981b; Leibholz and Mollah, 1988; Furuya and Kaji, 1989).

In general, high coefficients of determination resulted from regressing ileal N and amino acid flow on dietary N and amino acid intakes, respectively. The intercepts (dietary N or amino acid intake = 0 mg day^{-1}) of the regression lines provided measures of endogenous N and amino acid flows at the terminal ileum. Similarly, Leibholz and Mollah (1988) obtained R^2 values between 0.96 and 0.99 for a cottonseed meal diet. For diets containing milk, the correlation between intake and flow to the terminal ileum was low for phenylalanine (0.61) and tyrosine (0.43). Moughan *et al.* (1987) also found significant linear relationships between ileal amino acid flow and dietary amino acid intake for rats given barley diets.

The increase in amino acid flow with increasing dietary protein intake, as found in several studies, is attributed entirely to increased amounts of undigested food protein, the assumption being that there is no change in the amounts of the endogenous amino acid secretions. Nevertheless, there is evidence that the rate of protein secretion into the intestine varies with the amount of protein consumed (Snook and Meyer, 1964 a,b; Corring and Saucier, 1972; Lavau *et al.*, 1974; Temler *et al.*, 1983; Ozimek *et al.*, 1984). Consequently, some of the increase in amino acid flow which occurs with increased dietary protein intake is possibly the result of enhanced secretion of endogenous proteins. Philosophically, the regression method appear to be superior to the PF method for determining endogenous nitrogen and amino acids in ileal digesta. There may not, however, be a linear relationship between level of feed intake and the amount of endogenous N or amino acids in digesta, and any increase in protein level in the feed is always associated with other changes in dietary composition which complicates the interpretation of the results.

In view of present findings which support those of others, the EHC/ultrafiltration method was considered to be an improvement over the PF and regression techniques for the routine estimation of endogenous ileal amino acid excretion, Nevertheless, this method may also have some limitations. Butts *et al.* (1991) observed that the mean endogenous amino acid flows after the centrifugation plus ultrafiltration treatments of rat digesta were generally

lower than the corresponding total digesta amino acid flows. The estimates of endogenous flow after centrifugation and ultrafiltration may be an underestimate because of the possible presence of endogenous free amino acids and small peptides in the digesta which are discarded in the low molecular weight ultrafiltrate. The degree of underestimation of endogenous amino acid flow consequent upon discarding any endogenous free amino acids and small peptides has recently been shown in general to be of only minor significance (Moughan *et al.*, 1990; Butts *et al.*, 1991).

It is also possible that estimates of endogenous loss determined with the peptide alimentation method may be influenced in some way by the enzymic hydrolysate of casein itself and thus be an artefact of this particular dietary treatment. In support of the technique, however, are the results from a number of studies using other detailed approaches to estimating endogenous loss such as the isotope dilution method (Souffrant *et al.*, 1981; Bergner *et al.*, 1983; de Lange *et al.*, 1990; Moughan *et al.*, 1992) or the guanidination technique (Hagemeister and Erbersdobler, 1985; Moughan and Rutherfurd, 1990). It has also been shown using these methods that the presence of protein in the diet leads to a higher flow of ileal endogenous protein in comparison to protein-free alimentation. Moreover, Moughan and Ruthefurd (1990) and Butts *et al.* (1991) found very similar flows for endogenous ileal lysine using the guanidination and EHC methods, respectively. The EHC method generates estimates of endogenous loss, applicable only to the correction of ileal flows for protein sources which do not contain fibre and/or antinutritional factors, such as meat meals, fish meals, milk powder, etc. This is not of a problem for this study.

Based on this study and other results cited, apparent N and amino acid digestibilities are likely to be influenced by the dietary conditions. In the present study, crude protein intake had a significant influence on its apparent digestibility when diets of different protein content were given at similar levels of dry matter intake. This is in agreement with several workers (Taverner, 1979; Bressani *et al.*, 1981; Bielorai *et al.*, 1985; Furuya and Kaji, 1989) who reported increases in the apparent digestibility of dietary crude protein and amino acids with increasing dietary protein intake. Contrary to this, however, are the findings of Buraczewska and Horaczynski (1983) and Van Leeuwen *et al.* (1987) who found no effect of dietary protein level on apparent ileal digestibility of amino acids in pigs.

It is concluded that the protein-free method for determining endogenous loss leads to considerable underestimation of endogenous ileal amino acid excretion in the rat, and possibly simple-stomached animals generally. Further, and given that the regression technique generates similar values to those obtained after feeding animals a PF diet, use of the former method may also lead to error. The higher flows of endogenous N and amino acids observed after feeding the animal EHC as the sole dietary protein source, indicate that the presence of dietary peptides in the gut enhances the excretion of amino acids at the terminal ileum of the animal. The peptide alimentation method whereby the animal is fed a semi-synthetic diet containing hydrolysed casein as the sole nitrogen source with

subsequent centrifugation and ultrafiltration of the ileal digesta following collection, would appear to be an improvement over existing methodology for the routine estimation of endogenous ileal protein and amino acid excretion and consequently for determining the true digestibility coefficients of dietary proteins and amino acids. It is considered to be suitable for application to feeds such as meat and bone meal and should lead to more accurate estimates of its true N and amino acid digestibilities.

CHAPTER 6

Effect of dietary crude protein content on the apparent and true ileal digestibility of nitrogen and amino acids in meat and bone meal for the growing rat

6.1 INTRODUCTION

The generation of meaningful digestibility data for accurate diet formulation is a priority in applied nutrition. The calculation of apparent digestibility does not take into account endogenous secretion and so consequently is not a true measure of digestibility. True digestibility is obtained by correcting the apparent digestibility value for endogenous loss. It is anticipated that true, rather than apparent digestibility, will be more accurate for diet formulation.

True digestibility coefficients are fundamental measures of the animal's ability to absorb dietary amino acids and as such are less affected by assay conditions than are apparent coefficients. Apparent digestibility coefficients are likely to be influenced by the level of protein included in the test diet as was observed in the previous study and in those of Sauer *et al.* (1980) and Furuya and Kaji (1989). At the same dietary dry matter intake but different crude protein intakes, a similar amount of undigested endogenous material at the terminal ileum will have a disproportionate effect on the determination of apparent digestibility. True amino acid digestibility, although being more independent of dietary conditions than apparent digestibility may not, however, be completely independent of them (McNab, 1976). Recent studies using a variety of techniques (isotopic dilution, guanidinated protein, peptide alimentation) have provided evidence that dietary factors, in particular an elevation of the protein content, may increase the secretion of endogenous protein-rich substances into the gut, with a resultant increase in their quantity in the total ileal digesta. Nevertheless, true digestibility estimates determined under conditions of peptide alimentation, would seem to offer a considerable advance in accuracy over apparent digestibility measures.

In the present study comparison was made between the apparent and true ileal digestibility of nitrogen (N) and amino acids in a meat and bone meal (MBM) for the growing rat, determined over a wider range of dietary protein concentrations (25 to 200 g crude protein kg^{-1} diet) than the 80 to 160 g crude protein kg^{-1} range used in the previous study (refer chapter 5) for application of the regression technique for the measurement of endogenous protein excretion. The aim was to highlight the potential inaccuracies of apparent digestibility coefficients. Recognising the limitations of the traditional protein-free method of determining endogenous excretion, the endogenous ileal N and amino acid flows used to adjust apparent digestibility values to true ones were determined by the enzymically hydrolysed casein (EHC)/ultrafiltration method (Butts *et al.*, 1991) which was evaluated in an

earlier study (refer chapter 5). In this method the animal is fed a semi-synthetic diet containing EHC as its sole N source (M.W. <5000 Daltons). Ileal digesta are collected and the nitrogenous fraction is separated physically using large-volume disposable ultrafiltration devices. The high molecular weight (M.W. > 10000 Daltons) fraction resulting from the centrifugation and ultrafiltration provides a measure of endogenous amino acid flow.

6.2 MATERIALS AND METHODS

6.2.1 Animals and housing

Fifty-four male Sprague-Dawley rats of approximately 7 weeks of age and 180 g bodyweight were selected at random from a group of animals which had been weaned at 4 weeks of age and reared on a high quality diet at the Small Animal Production Unit, Massey University. The rats were kept individually in raised stainless steel cages with wire mesh floors, and housed in a temperature-controlled room (21$\pm$1°C) with a 12-hour light/dark cycle.

6.2.2 Diets

Six diets containing graded levels of a MBM as the sole protein source were prepared (Table 6.1). The MBM was incorporated into the diets to provide 25, 60, 95, 130, 165 and 200 g crude protein kg^{-1}. This was achieved by changing the proportions of maize starch and MBM. The chemical composition of the meat and bone meal (MBM) used in the study is shown in Table 6.2. An enzymically hydrolysed casein (EHC) based diet, used in the determination of endogenous ileal amino acid flow, was also formulated (Table 6.1) to contain all the ingredients included in the other diets, excepting meat and bone meal. 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. Chromic oxide was added to the diets as an indigestible marker.

6.2.3 Experimental procedure

Thirty-six rats were weighed at the beginning of the trial and were then equally and randomly allocated to six groups. Each group was given one of the six experimental diets differing only in MBM content. A group of 18 similar rats was used to determine ileal endogenous N and amino acid flows. This group was fed the EHC-based diet. All the rats were given unrestricted access to the various diets for 3 hours (0830 - 1130 h) each day and fresh water was available at all times.

On the fourteenth day of the experiment, 4 hours after the start of feeding, the rats fed the MBM based diets were asphyxiated with carbon dioxide gas and decapitated (ceasing all neural stimulation to the gut); while those fed the EHC-based diet were euthanased 3 hours after the start of feeding (refer chapters 3 and 5, respectively).

TABLE 6.1
Ingredient composition (g kg^{-1} air-dry weight) of the experimental diets

Ingredient	Enzymically hydrolysed casein diet	Meat and bone meal diet					
		Dietary protein level (g CP kg^{-1} 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[1]	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[2]	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[3]	100.0	----	----	----	----	----	----
Meat and bone meal	-----	50.0	120.0	190.0	260.0	330.0	400.0

[1]Avicel, Asahi Chemical Industry Company Limited, Tokyo, Japan.

[2]Tasmix special mouse premix, Pfizer Laboratories, Auckland, New Zealand. Supplied the following per kg diet: 5250 IU vitamin A; 750 IU vitamin D; 37.5 IU vitamin E; 1,5 mg vitamin K; 3.0 mg vitamin B_1; 3.75 mg vitamin B_2; 4.5 mg vitamin B_6; 0.04 mg vitamin B_{12}; 21.0 mg pantothenic acid; 0.08 mg biotin; 15 mg niacin; 0.75 mg folic acid; 0.75 choline; 60 mg iron; 37.5 mg zinc; 37.5 mg manganese; 3.75 mg copper; 0.38 mg iodine; 0.53 mg cobalt; 0.11 mg selenium; 30 mg inositol; 2.75 g potassium; 0.3 g magnesium; 0.38 sodium.

[3]Sigma Chemical Company, St. Louis, U.S.A. Type I from bovine milk. Total nitrogen = 12.7%, Amino nitrogen = 6.3%; Free amino acids + peptides (< 5000 Daltons)

TABLE 6.2
Chemical composition of a meat and bone meal used in the digestibility study

Nutrient[1]	Composition (g per 100 g air dry weight)[2]
Crude protein	50.3
Moisture	6.1
Ash	23.2
Ether extract	10.3
Amino acid	
Lysine	3.63
Methionine	0.96
Cystine	0.87
Histidine	1.93
Phenylalanine	2.14
Tyrosine	1.56
Threonine	2.11
Leucine	4.21
Isoleucine	1.70
Valine	2.76
Alanine	4.23
Aspartic acid	4.83
Arginine	4.88
Serine	2.56
Glutamic acid	7.37
Glycine	7.11
Proline	4.68

[1]Determined following standard Association of Official Analytical Chemists (1980) procedures. Amino acids were determined as described in section 6.2.3 (a)
[2]Mean values (n = 2)

The terminal 20 cm of the small intestine (directly anterior to the ileo-caecal valve) were immediately removed and cleaned of any blood or adhering hair. The terminal ileal contents were flushed out with deionised water from a syringe. Digesta samples from the MBM fed rats were immediately frozen (-20°C) and later freeze-dried. To minimize the activity of digestive enzymes the pH of the digesta samples from the rats given the EHC-based diet was adjusted to 3.5 by adding H_2SO_4 (9M) and the samples immediately frozen. The latter were subsequently thawed and subjected to centrifugation and ultrafiltration. Digesta samples from the 18 EHC-fed rats were rapidly thawed to 4°C and samples from 3 rats drawn at random were pooled to obtain 6 samples for laboratory processing using the centrifugation, ultrafiltration technique (Butts *et al.*, 1991). The digesta samples were centrifuged at 1450 x g for 45 min at 0°C. The supernatant was decanted and retained. The precipitate was washed with 5 ml of distilled water and centrifuged for a further 30 min at

1450 x g at 0°C. The combined supernates were subjected to ultrafiltration using Centriprep-10 concentrators (molecular weight (MW) exclusion limit 10,000 Daltons; Amicon, W.R. Grace and Co., Danvers, U.S.A.). The retentate (high molecular weight fraction, M.W. > 10,000 Daltons) was added to the precipitate, and the material freeze-dried, finely ground and stored at -20°C for chemical analyses.

6.2.3 (a) Chemical analysis

Samples of ileal digesta and the experimental diets were analysed for nitrogen (N), amino acids and chromium. Amino acid analysis on ileal digesta samples from the rats given the MBM diets was restricted to the 25, 60 and 200 g kg^{-1} dietary crude protein treatments. Total 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 chromium 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 and 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 µl of 6 M glass-distilled HCl with 1% added phenol, for 24 hours at 110±1°C in glass tubes sealed under vacuum. For the determination of methionine and cystine contents, the hydrolysis was preceeded by performic acid oxidation at 0°C for 10 hours followed by neutralization with hydrogen bromide. Tryptophan, which is destroyed by acid hydrolysis, was not determined.

6.2.3 (b) Data analysis

Endogenous N and amino acid flows at the terminal ileum relative to the ingestion of 1 g of freeze dry matter (FDM) were calculated using the equation: Amino acid flow (µg g^{-1} FDM) =

$$\text{Amino acid concentration in ileal digesta } (\mu g\ g^{-1}\ FDM) \times \frac{\text{Diet chromium } (mg\ g^{-1}\ FDM)}{\text{Ileal chromium } (mg\ g^{-1}\ FDM)}$$

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

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

$$\text{Apparent amino acid (AA) digestibility (\%)} = \frac{\text{Dietary AA } (\mu g\ g^{-1}\ FDMI) - \text{AA flow } (\mu g\ g^{-1}\ FDMI)}{\text{Dietary AA } (\mu g\ g^{-1}\ FDMI)}$$

True amino acid (AA) digestibility (%) =

$$\frac{\text{Dietary AA } (\mu g\ g^{-1}\ \text{FDMI}) - (\text{AA flow } (\mu g\ g^{-1}\ \text{FDMI}) - \text{endogenous AA flow } (\mu g\ g^{-1}\ \text{FDMI}))}{\text{Dietary AA } (\mu g\ g^{-1}\ \text{FDMI})}$$

The apparent and true digestibility data were subjected to a one-way analysis of variance for each amino acid singly and the comparison of means was by Tukey's test. All statistical analyses were carried out using the General Linear Models Procedure of REG (Gilmour, 1990).

6.3 RESULTS

In the digestibility assay the rats consumed the experimental diets readily and appeared healthy. The mean bodyweights of the rats on the seven dietary treatments at the beginning and end of the 14-day feeding period along with the mean food intakes on the final day of study are presented in Table 6.3. There were no significant ($P > 0.05$) treatment differences in bodyweight at the start of the feeding period, but the rats on the lower protein diets (25, 60 and 90 g crude protein kg^{-1}) were lighter in weight ($P < 0.01$) 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 stabilised by about 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 N and amino acid flows for rats, determined by the EHC/ultrafiltration method are presented in Table 6.4. The effect of dietary crude protein concentration on apparent and true N digestibility in MBM is shown in Table 6.5 and is illustrated in Fig. 6.1. Dietary crude protein level significantly ($P < 0.05$) influenced apparent ileal N digestibility with values increasing as dietary crude protein was increased from 25 to 200 g kg^{-1}. There was a highly significant ($P < 0.001$) positive correlation ($r = 0.88$) between level of dietary crude protein and apparent ileal N digestibility. There was no effect of diet crude protein content on true ileal N digestibility.

The apparent and true ileal digestibilities of amino acids in meat and bone meal determined at three dietary protein levels (25, 60 and 200 g kg^{-1}) are shown in Table 6.6. As dietary protein level was increased from 25 to 60 to 200 g kg^{-1} of diet, there were significant ($P < 0.05$) increases in the apparent ileal digestibilities of all the amino acids examined. In contrast to the apparent ileal digestibility coefficients, the true amino acid digestibility values were not influenced by crude protein content of the diet.

TABLE 6.3

Mean bodyweights at the beginning and end of the feeding period and food intakes on the last day of study, for rats given an enzyme-hydrolysed casein diet or meat and bone meal based diets containing different levels of crude protein

	Meat and bone meal diet						Enzyme-hydrolysed casein	Overall SE	Level of significance[1]
	Dietary crude protein level (g CP kg^{-1} diet)								
	25	60	95	130	165	200			
Bodyweight (g)[2]									
Start of feeding	177.7	177.5	177.6	177.1	176.6	177.2	176.6	0.39	NS
End of feeding	194.2^{a}	194.7^{a}	196.7^{a}	200.6^{b}	201.0^{b}	201.1^{b}	199.4^{b}	0.26	**
Food intake									
g day^{-1}	14.2	13.6	13.3	13.6	13.1	13.8	14.0	0.10	NS

[1] * = $P < 0.05$; ** = P 0.01; NS = Non-significant

[a,b] Means with different supercripts are significantly different

[2] Mean values (n = 6 rats per dietary treatment)

TABLE 6.4
Mean (±SE) endogenous nitrogen and amino acid excretion (μg g^{-1} freeze dry matter intake) at the terminal ileum of the growing rat (n = 6) determined under peptide alimentation and following centrifugation and ultrafiltration of the digesta

Nitrogen	1806 (25)
Amino acid	
Lysine	286 (14)
Methionine	79 (5)
Cystine	85 (3)
Histidine	165 (9)
Phenylalanine	151 (11)
Tyrosine	189 (8)
Threonine	606 (13)
Leucine	468 (18)
Isoleucine	419 (8)
Valine	475 (14)
Alanine	295 (14)
Aspartic acid	726 (21)
Arginine	169 (12)
Serine	955 (32)
Glutamic acid	1533 (85)
Glycine	867 (7)
Proline	486 (15)

TABLE 6.5

Mean[1] apparent and true rat ileal digestibility (%) of nitrogen in meat and bone meal based diets containing different levels of crude protein

	Dietary crude protein level (g kg^{-1})						Overall SE	Level of significance[2]
	25	60	95	130	165	200		
Apparent nitrogen digestibility	65.6	69.9	71.5	73.2	74.7	75.3	0.29	***
True nitrogen digestibility	76.9	77.1	77.8	78.0	78.0	78.2	0.26	NS

[1]Mean values (n = 6 rats per dietary treatment)

[2]NS = Non-significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

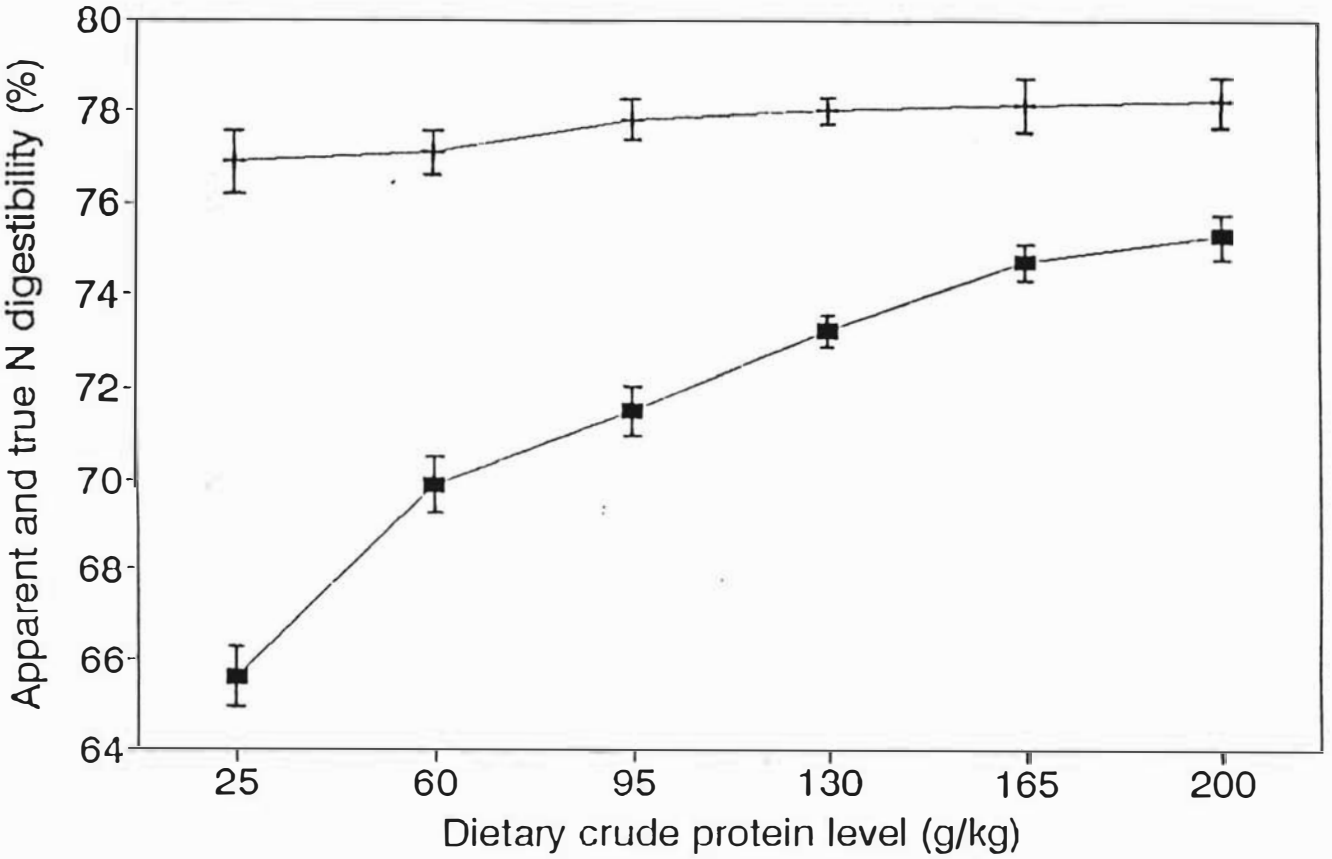

Fig. 6.1 Effect of dietary protein level on mean (±SE) apparent (■) and true (+) ileal nitrogen digestibility for rats given a meat and bone meal based diet.

TABLE 6.6

Mean[1] apparent and true rat ileal digestibility (%) of amino acids in meat and bone meal based diets containing different levels of crude protein (CP) (25, 60 or 200 g kg^{-1})

	Apparent digestibility					True digestibility				
	Dietary CP (g kg^{-1})			Overall SE	Level of significance[2]	Dietary CP (g kg^{-1})			Overall SE	Level of significance[2]
	25	60	200			25	60	200		
Lysine	71.5	77.6	82.0	0.39	***	86.1	85.8	86.9	0.38	NS
Methionine	70.0	76.9	80.9	0.47	***	84.3	85.1	85.8	0.31	NS
Cystine	60.2	66.1	70.7	0.41	***	72.5	73.1	73.0	0.46	NS
Histidine	72.7	78.1	83.2	0.41	***	87.6	86.4	88.1	0.53	NS
Phenylalanine	62.9	69.6	72.6	0.53	***	75.8	77.0	76.4	0.57	NS
Tyrosine	63.8	69.8	74.1	0.48	***	76.9	77.2	77.9	0.51	NS
Threonine	56.8	61.2	64.5	0.43	**	68.4	67.7	69.2	0.44	NS
Leucine	62.5	68.6	72.2	0.43	***	75.3	75.9	74.9	0.34	NS
Isoleucine	61.2	67.0	71.5	0.40	***	73.7	74.1	73.2	0.48	NS
Valine	61.0	67.1	72.2	0.47	***	73.5	74.2	74.9	0.41	NS
Alanine	64.2	69.5	74.1	0.38	***	77.3	76.9	78.9	0.35	NS
Aspartic acid	53.3	60.9	66.2	0.40	***	66.3	67.3	67.9	0.35	NS
Arginine	69.8	76.2	82.7	0.36	***	84.1	84.3	85.5	0.30	NS
Serine	62.1	67.0	71.7	0.42	***	74.8	74.1	75.4	0.37	NS
Glutamic acid	54.5	59.8	63.3	0.41	**	65.6	66.1	67.0	0.44	NS
Glycine	50.2	55.0	59.0	0.48	**	60.5	60.8	61.6	0.35	NS
Proline	71.0	78.0	83.2	0.48	***	85.5	86.3	87.1	0.41	NS

[1]Mean values (n = 6 rats per dietary treatment)

[2]NS = Non-significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

6.4 DISCUSSION

A significant effect of dietary crude protein content on the apparent ileal digestibility of N and amino acids was found in the present study. Increasing the dietary protein content in equidistant amounts from 25 g kg^{-1} to 200 g kg^{-1} resulted in increases in apparent ileal N and amino acid digestibility. The increase in apparent N digestibility with increasing dietary protein was greatest at the three lowest protein levels and negligible at the two highest protein levels (Fig. 6.1). This result is similar to that reported by Dammers (1964), who illustrated with soybean meal that apparent protein digestibility can be markedly influenced by the protein level of the diet. It was shown theoretically that the apparent digestibility of protein will increase and reach a plateau with increasing dietary protein concentration. Eggum (1973b) observed that apparent digestibilities increase curvilinearly with dietary protein concentration. The present results are in support of other reported studies investigating the effect of dietary protein level on apparent ileal N and amino acid digestibility in the growing pig (Sauer *et al.*, 1980; Bell *et al.*, 1983; Bell and Keith, 1989; Furuya and Kaji, 1989; Keith and Bell, 1991), in the growing rat (Shah *et al.*, 1982; Sarwar and Peace, 1986) and in humans (Bressani *et al.*, 1981; Hopkins, 1981).

Den Hartog *et al.* (1989) also reported a general positive relationship between protein level in the diet and apparent ileal digestibility of N and amino acids. The explanation of their findings was that the endogenous fraction, which is primarily influenced by the dry matter intake of the animal, remains relatively constant with an elevation of dietary protein level, while there is an increase in the undigested fraction. In contrast, Buraczewska and 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 ileo-caecal re-entrant cannulas, found no effect of the level of inclusion of soybean 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 level on apparent digestibility 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 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. 6.1), and that feedstuffs differ widely in their protein content, from cereal grains as low as 7 - 8% protein to protein supplements of around 90%, then apparent digestibility coefficients for protein and amino acids, and corresponding estimates of digestible crude protein 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 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 this study, two major factors known to influence the magnitude of endogenous N and amino acid excretions namely, dry matter (DM) intake and the type and level of fibre in the diet, were controlled. Thus, a constant level of purified cellulose, as the sole dietary fibre source, was included in all of the diets while similar food intakes were recorded across diets on the last day of study.

The mean ileal endogenous excretions of amino acids determined in the present study are in close agreement with data obtained using the same method (EHC technique) reported in chapter 5 and by Butts *et al.* (1991). This accords with recent experiments which have demonstrated that the protein-free technique leads to a considerable underestimation of the physiologically normal level of endogenous excretion, which is not experienced with proteinaceous diets (De Lange *et al.*, 1989a; Darragh *et al.*, 1990; De Lange *et al.*, 1990; Moughan and Rutherfurd, 1990; Butts *et al.*, 1991).

The digestibility coefficients presented here indicate that the correction of apparent N and amino acid digestibility for endogenous excretion, as determined by the EHC technique, results in true ileal N and amino acid digestibilities which are markedly higher (about 12% for cystine and threonine and up to 14% for lysine and methionine) than corresponding apparent estimates particularly at the lower dietary protein levels (25 g protein kg^{-1} 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 of 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 and Peace, 1986), the chicken (Green, 1987; McNab, 1989; Zuprizal *et al.*, 1991) and in the pig (Eggum, 1973b; Taverner, 1979; Furuya and Kaji, 1989). It seems that true ileal digestibility is independent of the dietary protein concentration.

In this study, it is evident that only at the dietary protein content of 165 g kg^{-1} 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 digestibility decreased as dietary protein content increased. In this range (165 to 200 g kg^{-1} crude protein),

increasing dietary protein content caused only small changes in the apparent estimates. This is because at the higher levels of dietary protein, the endogenous nitrogen loss makes up a smaller proportion of the total flow at the terminal ileum.

In general, there is controversy over the use of apparent or true amino acid digestibility in the formulation of pig diets. 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. On the other hand, 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 standardised conditions. The digestibility of feedstuffs can be satisfactorily compared using apparent digestibilities provided that the dietary protein content of test diets is similar. Evidently, the endogenous protein excretion contributes to variation in apparent ileal digestibility data and this should be recognised in any system which attempts to estimate the bioavailable amino acid values of feedstuffs. Given that true ileal digestibilities appear to be independent of dietary protein level, however, their adoption may allow feed ingredients to be more accurately compared thus leading to greater accuracies in diet formulation.

CHAPTER 7

Assessment of the true ileal digestibility of amino acids in several meat and bone meals - The laboratory rat as a model animal for digestion in the growing pig

7.1 INTRODUCTION

It is now well accepted that for more reliable dietary formulation and for a greater control over diet quality, information on the relative ability of different feedstuffs to supply digestible rather than total amino acids, is desirable. The ileal measure of dietary amino acid digestibility has been shown to be superior to the faecal method in detecting small differences in the digestibility of amino acids, especially for less digestible protein sources (Ivan and Farrell, 1976a; Sauer *et al.*, 1981). The work described in chapter 4 of this thesis also established, that whereas the measurement of digestibility at the end of the small intestine indicated differences in amino acid digestibilities for the two differently processed meat and bone meals, the faecal approach did not detect these differences. Moreover, since apparent amino acid digestibility is influenced by the dietary crude protein content, as shown earlier (refer chapter 6), apparent values are comparable only under strictly standardised conditions and true rather than apparent digestibility values are thus considered more accurate for diet formulation (Eggum, 1973a; Furuya and Kaji, 1989).

The purpose of the present study was to determine and compare the true digestibilities of nitrogen and amino acids in a range of meat and bone meals. Meat and bone meal (MBM) is of considerable value as a major protein source in pig and poultry diets and additionally provides a source of energy and some minerals. Information on the chemical composition and nutrient digestibility of MBM indicates that it is a highly variable product (Hegedus *et al.*, 1983; Parsons, 1986; Batterham *et al.*, 1986; Knabe *et al.*, 1989; Skilton *et al.*, 1991). Few studies have been conducted to characterise the variation in digestible nutrient content of meat and bone meal. In particular, there is a paucity of information on the true ileal digestibility of amino acids in MBM for pigs. The novel aspect of the presently described work was the application of the recently developed enzymically hydrolysed casein (EHC)/ultrafiltration method (described in chapter 5) for estimating endogenous loss, to determine the true digestibility of amino acids in this commercially important protein source. The values obtained here, were compared with true digestibility estimates derived using endogenous amino acid flows from the traditional protein-free method. The present investigation was facilitated by using the laboratory rat as a model animal for digestion in the growing pig.

7.2 MATERIALS AND METHODS

7.2.1 Meat and bone meals

Eight commercially processed meat and bone meal samples (hereafter referred to as A-H) were collected from eight different rendering plants situated in both the North and South Islands of New Zealand and were stored at -20°C within 24 hours of collection. Each sample was built up over an 8-hour period during the production of the meal. The meals collected represent the major rendering processes for meat and bone meals used in New Zealand.

Meal A was processed by the traditional batch dry rendering system operated on a 20-30 min pressure cycle at the beginning of a 2-hour cooking cycle, with removal of fat by centrifugation after rendering. The raw material was a mixture of sheep and lamb offals with inclusion of bones but not blood. After rendering the meal was dried, ground and immediately bagged. The raw material for MBM B consisted of cattle, sheep and lamb offals and bones. Before rendering the viscera were cut and washed. The continuous dry rendering (Keith) system which uses a single, steam-jacketed cooker to heat and dry the raw material was used in the processing of MBM B. The material was heated for 2 hours and was discharged at 125 to 130°C. This process is the same as dry batch, except that a continuous cooker is used instead of a batch cooker. After rendering, the meal was ground and immediately bagged. The rendering system used for the manufacture of MBM C was as described for MBM A but the raw material comprised sheep, lamb and cattle offals and a small quantity of bones but not blood. The Meat Industry Research Institute of New Zealand low temperature (below 100°C) rendering (MLTR) system was used in the processing of meals D, E, F and G. After rendering, the solids were dried in a rotadisc drier, ground and bagged (MBM D and E) or stored in bulk silos (MBM F). The raw material used for MBM D generally consisted of cut-outs of lamb, venison and goat and a limited amount of offals and blood were included in the raw material mix. On the other hand, the raw material for MBM E was a mixture of sheep and lamb offals and blood was included in the raw material, but carcasses were not boned-out on site, while the raw material for MBM F comprised cattle and sheep offals with the inclusion of blood and a small quantity of bones in the mixture. MBM G consisted of the viscera of cattle and sheep and bone but not blood. Meal H was processed using the batch rendering system under a pressure of 300 kPa and a temperature of 115 to 130°C, followed by removal of the fat through a drain valve by the internal pressure in the digester. The solid material was then removed, dried and ground to produce the meal. The raw material processed was a mixture of sheep and cattle offals with the inclusion of bones, but blood was not included in the final product.

7.2.2 Animals, housing and diets

Sixty Sprague-Dawley male rats (190 g bodyweight) were selected and kept individually in raised stainless steel cages with wire mesh floors, in a temperature-controlled room (21±1°C) with a 12-hour light/dark cycle. Eight diets were prepared (Table 7.1), each

containing 200 g kg^{-1} of one of the meat and bone meals as its sole protein source. A protein-free (PF) diet and an enzymically hydrolysed casein (EHC) based diet were also formulated. Chromic oxide was included in each diet as an indigestible marker.

TABLE 7.1
Ingredient composition (g kg^{-1} air-dry weight) of the experimental diets

	Meat and bone meal[1]	EHC	Protein-free
Maize starch	621.0	687.0	787.0
Meat and bone meal	200.0	--	--
Sucrose	80.0	80.0	80.0
Maize oil	50.0	50.0	50.0
Purified cellulose[2]	30.0	30.0	30.0
Mineral vitamin premix[3]	15.0	15.0	15.0
Enzymically hydrolysed casein(EHC)[4]	--	100.0	--
Chromic oxide	4.0	4.0	4.0
Sodium chloride	--	4.0	4.0
Magnesiun sulphate	--	3.0	3.0
Potassium carbonate	--	3.0	3.0
Dicalcium phosphate	--	24.0	24.0

[1]One of 8 meat and bone meal samples collected from different rendering plants in New Zealand was included in each diet.

[2]Avicel, Asahi Chemical Industry Company Limited, Tokyo, Japan.

[3]Tasmix special mouse premix, Pfizer Laboratories, Auckland, New Zealand. Supplied the following per kg diet: 5250 IU vitamin A; 750 IU vitamin D; 37.5 IU vitamin E; 1.5 mg vitamin K; 3.0 mg vitamin B_1; 3.75 mg vitamin B_2; 4.5 mg vitamin B_6; 0.04 mg vitamin B_{12}; 21.0 mg pantothenic acid; 0.08 mg biotin; 15 mg niacin; 0.75 mg folic acid; 0.75 g choline; 60 mg iron; 37.5 mg zinc; 37.5 mg manganese; 3.75 mg copper; 0.38 mg iodine; 0.53 mg cobalt; 0.11 mg selenium; 30 mg inositol; 2.75 g potassium; 0.3 g magnesium; 0.38 sodium.

[4]Sigma Chemical Company, St. Louis, U.S.A. Type I from bovine milk. Total nitrogen = 12.7%, Amino nitrogen = 6.3%., free amino acids and peptides (M.W. < 5000 Daltons).

7.2.3 Experimental procedure

The rats were randomlly allocated to the 10 experimental diets such that there were 6 rats per diet. Each rat had free access to its respective diet for a 3-hour period (0830 - 1130 h) each day for a 14-day period. Water was available *ad libitum*.

On the 14th day, the rats fed the protein-free and EHC based diets were killed (asphyxiation in carbon dioxide gas followed by decapitation) 3 hours from the start of feeding, while those fed the MBM based diets were killed 4 hours from the start of feeding, and digesta were removed from the terminal 20 cm of ileum. It has been shown previously (refer chapter 5) that 3 hours is an optimal sampling time for rats fed the protein-free or EHC

based diets, while 4 hours is an optimal sampling time for rats fed a semi-synthetic MBM diet (refer chapter 3). It has also been established (refer chapter 3) that 20 cm of ileum is a suitable length for sampling digesta for rats given the MBM-based diet.

The ileal digesta samples were immediately frozen (-20°C) and subsequently freeze-dried and finely ground. The ileal digesta from rats fed the EHC based diet were pH-adjusted (3.0 - 3.5) by addition of 9M H_2SO_4 and stored frozen (-20°C) for no longer than 96 hours. The pH was lowered to minimize the activity of digestive enzymes. Prior to laboratory analysis, the digesta samples from the EHC fed rats were rapidly thawed to 4°C and then centrifuged at 1450 x g for 45 min at 0°C. The supernatant was decanted and retained. The precipitate was washed with 5 ml of distilled water and centrifuged for a further 30 min at 1450 x g at 0°C. The combined supernates were subjected to ultrafiltration using Centriprep-10 concentrators (molecular weight (MW) exclusion limit 10,000 Daltons (Da); Amicon, W.R. Grace and Co., Danvers, U.S.A.). The ultrafiltration process provides molecular separation of proteins (M.W > 10,000 Da) from peptides and free amino acids (M.W < 10,000 Da). The high molecular weight fraction (retentate; M.W > 10,000 Da) was added to the precipitate, and the total precipitate plus retentate weighed prior to freeze-drying. After freeze-drying, the precipitate was finely ground and stored at -20°C for subsequent analysis of nitrogen and amino acids.

7.2.3 (a) Chemical analysis

The total nitrogen (N) contents of triplicate MBM (100 mg) and duplicate ileal digesta (30 mg) samples and six (100 mg) samples of each diet were determined using the Kjeldahl technique (Association of Official Analytical Chemists, 1980) performed on a Kjeltec 1030 auto-analyzer (Tecator, Sweden). The ash content of the MBM was obtained after heating duplicate 10 g samples in a furnace at 500°C for 24 hours. Ether extract (fat) was determined according to the method outlined by the Association of Official Analytical Chemists (1980). The chromium (Cr) contents of six 100 mg samples of each diet and duplicate 15 mg samples of ileal digesta were analysed using atomic absorption spectrophotometry (Costigan and Ellis, 1987).

The amino acid composition of the MBM samples, the diets and ileal digesta were determined on duplicate 6 mg samples using a Beckman 119 BL amino acid analyser. Protein was hydrolysed in 6M glass-distilled HCl (containing 1% phenol) for 24 hours at 110 $\pm$ 1°C in glass tubes sealed under vacuum. For the determination of methionine and cystine, the hydrolysis was preceeded by performic acid oxidation at 0°C for 10 hours followed by neutralization with hydrogen bromide. Tryptophan, which is destroyed during acid hydrolysis, was not determined.

7.2.3 (b) Data analysis

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

$$\text{Amino acid flow } (\mu g\ g^{-1}\ FDM) = \text{Amino acid concentration in ileal digesta } (\mu g\ g^{-1}\ FDM) \times \frac{\text{Diet chromium } (mg\ g^{-1}\ FDM)}{\text{Ileal chromium } (mg\ g^{-1}\ FDM)}$$

Endogenous ileal amino acid flows for the rats fed the EHC-based diet were determined on the precipitate (after centrifugation) plus retentate (M.W >10,000 Daltons) after ultrafiltration.

True ileal amino acid digestibility coefficents were calculated using the following equation:

True amino acid (AA) digestibility (%) =

$$\frac{\text{Dietary AA } (\mu g\ g^{-1}\ FDMI) - (\text{AA flow } (\mu g\ g^{-1}\ FDMI) - \text{endogenous AA flow } (\mu g\ g^{-1}\ FDMI))}{\text{Dietary AA } (\mu g\ g^{-1}\ FDMI)}$$

The endogenous amino acid flows for the EHC-fed rats (after centrifugation plus ultrafiltration of the digesta) and the protein-free fed rats were subjected to a one-way analysis of variance for each amino acid singly. The true ileal amino digestibilities were also subjected to a one-way analysis of variance. All statistical analyses were performed using the General Linear Models Procedure of REG (Gilmour, 1990).

7.3 RESULTS

The chemical compositions of the eight meat and bone meals are shown in Table 7.2. Despite the non-homogeneous nature of meat and bone meal, the various chemical components analysed appeared to be reasonably consistent within a sample. For example, where triplicate analyses of meals for N and duplicate analyses for moisture, ash, ether extract and lysine were performed, mean errors of 5.5, 3.3, 2.3 and 6.5% (largest difference expressed as percentage of the mean), respectively were obtained (maximum error = 10%). The compositions were quite variable between meals particularly for moisture, ash, ether extract and most of the indispensable amino acids. The crude protein contents of meals C, D, E and F were much higher and these meals contained significantly higher amounts of the indispensable amino acids, lysine, methionine, cystine, histidine, tyrosine, threonine, phenylalanine and leucine, than the other meals.

TABLE 7.2

Mean chemical compositions (g 100 g^{-1} air-dry weight) of eight meat and bone meals

Chemical component	Meat and bone meal							
	A	B	C	D	E	F	G	H
Crude protein[1]	49.1	47.8	55.8	56.5	59.3	51.0	49.3	49.7
Moisture[2]	5.8	5.2	5.9	6.1	5.6	5.5	5.8	5.4
Ether extract[2]	10.2	9.1	9.8	11.1	9.3	9.6	12.3	11.5
Ash[2]	31.1	30.9	22.5	19.1	15.8	28.9	28.6	29.4
Amino acid[2]								
Lysine	2.75	2.46	2.95	3.56	3.89	2.68	2.66	2.45
Methionine	0.76	0.73	0.80	0.98	0.86	0.69	0.73	0.69
Cystine	0.61	0.54	0.83	0.95	0.95	0.54	0.56	0.57
Histidine	0.85	0.79	0.82	1.36	1.64	0.83	0.82	0.75
Phenylalanine	1.81	1.64	1.87	2.52	2.58	1.69	1.64	1.54
Tyrosine	1.25	1.24	1.49	1.86	1.62	1.05	1.14	1.04
Threonine	1.72	1.62	2.24	2.39	2.56	1.54	1.76	1.54
Leucine	3.29	2.96	3.67	4.97	4.85	3.02	3.06	2.85
Isoleucine	1.56	1.38	1.65	1.85	1.56	1.27	1.36	1.31
Valine	2.21	1.89	2.43	3.02	3.24	2.10	2.03	1.88
Alanine	3.62	3.46	3.76	3.92	4.27	3.78	3.56	3.51
Aspartic acid	4.00	3.53	4.23	4.73	5.13	3.58	3.66	3.48
Arginine	3.94	3.85	4.42	4.14	4.55	3.91	3.94	3.86
Serine	2.11	2.01	2.73	2.66	2.82	1.96	2.10	1.93
Glutamic acid	6.61	6.05	7.21	7.72	7.38	5.82	6.34	5.97
Glycine	6.61	6.79	6.94	5.33	6.65	7.39	6.77	6.85
Proline	4.04	3.95	5.09	5.49	6.18	4.20	4.29	4.11

[1] Based on triplicate analyses
[2] Based on duplicate analyses

In the digestibility assay the rats consumed the experimental diets readily and appeared healthy. The food intakes (mean ± SE) of the animals on the final day of study, when ileal digesta were sampled, were 11.2 ± 0.6 g, 12.1 ± 0.4 g and 9.6 ± 0.9 g for the MBM, EHC and PF treatments, respectively. The food mean intake was not significantly different across all the MBM diets. There was no evidence of faeces particles in the stomach contents when these were examined after slaughter of the animals, indicating that the practice of coprophagy had not occurred, at least on the last day of trial.

The mean endogenous flows of nitrogen and amino acids for the EHC (processed digesta) and protein-free fed rats are presented in Table 7.3.

TABLE 7.3
Mean endogenous nitrogen and amino acid excretion[1] at the terminal ileum of the growing rat determined under protein-free or peptide (enzymically hydrolysed casein) alimentation.

	Endogenous flow		Overall SE	Statistical significance[3]
	EHC[2]	Protein-free		
Nitrogen	1843	1124	23	**
Amino acid				
Lysine	275	232	14	*
Methionine	77	53	10	*
Cystine	82	62	11	*
Histidine	146	138	12	NS
Phenylalanine	173	153	5	*
Tyrosine	179	161	3	*
Threonine	591	402	28	**
Leucine	429	256	17	**
Isoleucine	419	149	21	**
Valine	467	281	15	**
Alanine	291	213	13	**
Aspartic acid	748	636	16	*
Arginine	167	217	8	*
Serine	899	341	50	***
Glutamic acid	1341	701	87	***
Glycine	836	805	18	NS
Proline	463	534	13	*

[1]Mean; $\mu g\ g^{-1}$ dry matter intake; n = 6
[2]Digesta were centrifuged and ultrafiltered; flows were based on amino acids in the precipitate plus retentate (MW > 10,000 Da).
[3]NS = Non significant (P> 0.05); * P< 0.05; ** P< 0.01; *** P< 0.001.

The endogenous amino acid flows for the EHC fed rats were significantly higher (P< 0.05) than those for the protein-free fed animals except for histidine and glycine. Endogenous arginine and proline excretions were significantly greater for the protein-free fed rats.

The true digestibilities (Tables 7.4 and 7.5) were calculated using the endogenous amino acid flows from either the EHC (processed digesta) or protein-free methods.

TABLE 7.4

Mean true (EHC[1] method) ileal digestibilities (%) of nitrogen and amino acids in eight different meat and bone meals

Chemical component	Meat and bone meal A	B	C	D	E	F	G	H	Overall SE	Statistical significance[2]
Nitrogen	78.5	73.4	76.0	83.6	88.9	87.0	68.8	62.7	2.99	••
Amino acids										
Lysine	84.1	76.5	80.7	89.3	92.3	91.2	74.2	66.4	3.02	••
Methionine	86.6	80.2	84.7	91.8	94.3	93.8	76.9	68.8	2.96	••
Cystine	72.2	65.6	68.6	76.7	82.5	79.6	59.0	53.7	3.32	•••
Histidine	84.3	74.9	72.8	85.9	91.0	89.8	65.3	60.6	3.77	•••
Phenylalanine	82.2	74.7	74.0	82.1	83.8	79.3	61.4	57.5	3.28	••
Tyrosine	72.5	69.1	66.9	76.1	77.2	73.7	47.9	47.4	3.95	•••
Threonine	79.0	68.1	71.3	87.0	88.0	86.3	56.0	53.2	4.57	•••
Leucine	88.4	80.3	80.0	89.1	91.8	91.7	78.5	71.3	2.46	•
Isoleucine	79.0	80.5	81.0	88.7	90.2	90.7	76.9	73.8	2.13	•
Valine	83.0	78.5	67.3	81.3	86.4	82.3	64.9	58.9	3.35	••
Alanine	71.9	72.7	77.9	80.5	85.8	85.0	70.6	61.9	2.67	••
Aspartic acid	63.7	53.8	45.7	77.6	82.1	82.7	34.5	39.2	6.43	•••
Arginine	78.8	68.6	83.8	79.4	84.0	83.8	75.9	65.8	2.32	•
Serine	79.7	65.9	70.8	79.4	85.0	85.2	53.4	54.6	4.23	•••
Glutamic acid	84.4	72.5	78.3	85.7	89.7	89.3	67.2	62.3	3.42	••
Glycine	39.6	41.3	58.5	56.3	65.6	69.0	60.5	50.3	3.53	•••
Proline	69.8	64.1	73.2	72.2	79.9	80.3	63.6	57.3	2.67	••

[1]Calculated using the mean endogenous amino acid flows for rats given the EHC diet

[2]NS = Non significant (P> 0.05); • P< 0.05; •• P< 0.01; ••• P< 0.001

TABLE 7.5

Mean true (protein-free[1]) ileal digestibilities (%) of nitrogen and amino acids in eight different meat and bone meals

Chemical component	Meat and bone meal								Overall SE	Statistical significance[2]
	A	B	C	D	E	F	G	H		
Nitrogen	75.0	69.9	72.9	80.1	85.2	83.3	63.7	59.0	3.06	**
Amino acids										
Lysine	80.9	73.9	78.2	85.9	88.9	88.0	71.6	63.2	2.96	**
Methionine	82.4	76.2	80.7	87.6	90.1	89.6	73.1	64.7	2.94	**
Cystine	67.9	61.6	64.7	72.4	78.2	75.3	54.9	49.8	3.27	***
Histidine	80.9	72.7	70.5	84.1	87.3	86.5	63.1	58.3	3.63	***
Phenylalanine	79.5	72.6	71.8	79.7	81.2	76.8	59.3	55.5	3.20	**
Tyrosine	69.4	66.2	64.0	73.0	74.1	70.6	44.9	44.6	3.95	***
Threonine	71.9	62.5	65.9	80.0	80.9	79.1	50.6	47.8	4.31	***
Leucine	82.5	75.8	75.6	83.5	85.9	86.0	71.9	66.8	2.33	*
Isoleucine	71.4	71.7	72.2	81.1	82.6	83.1	68.1	65.0	2.29	*
Valine	79.9	76.7	65.5	78.2	83.3	79.2	63.1	57.1	3.16	**
Alanine	69.2	70.9	76.1	77.8	83.1	82.3	68.8	60.1	2.57	**
Aspartic acid	60.0	50.9	42.8	74.1	78.4	79.0	31.6	36.3	6.32	***
Arginine	79.4	69.2	84.4	80.0	84.6	84.4	76.5	66.0	2.35	*
Serine	71.7	59.7	64.6	71.4	77.0	77.2	47.2	48.4	3.95	***
Glutamic acid	77.6	67.0	72.8	78.9	82.9	82.5	61.7	56.8	3.23	**
Glycine	38.4	39.8	57.0	55.1	64.4	67.8	59.0	48.8	3.56	***
Proline	70.4	64.6	73.7	72.8	80.5	80.9	64.1	57.8	2.68	**

[1]Calculated using the mean endogenous amino acid flows for rats given the protein-free diet

[2]NS = Non significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

The true digestibilities of nitrogen and amino acids based on the respective endogenous flows for EHC-fed rats were generally markedly greater than those based on the respective protein-free endogenous flows. The overall mean true ileal amino acid digestibilities determined using the endogenous amino acid flows from the EHC (processed digesta) fed rats (except arginine and proline) exceeded the corresponding true digestibilities calculated using the protein-free based endogenous flows, by 1.8 (alanine) to 7.1% units (serine).

The mean true ileal N digestibility coefficients based on the endogenous flows determined by the EHC/ultrafiltration method for the eight MBM samples ranged from 63 to 89% with an overall mean of 77%. The mean true ileal lysine digestibility ranged from 66 to 92% with an overall mean of 82%. Methionine, leucine, isoleucine and lysine tended to be the most digestible indispensable amino acids, across all MBM samples based on the true measure of digestibility calculated using the EHC/ultrafiltration method, while the least digestible indispensable amino acids were tyrosine, cystine, threonine and phenylalanine.

7.4 DISCUSSION

The meat and bone meals evaluated in the study were variable in their nutrient composition and in the digestibility of nitrogen and amino acids. The gross lysine contents ranged from 2.5 to 3.9 g 100 g^{-1} air dry weight over a crude protein range of 48 to 60 g 100 g^{-1} air dry weight. The results are similar to the ranges of nutrient composition reported in the literature (Lambden and Averill, 1978; Batterham *et al.*, 1980; Skilton *et al.*, 1991). The variation in nutrient composition of MBM may have resulted from differences in raw material used (i.e. differences in proportions of bone, soft tissue, blood and hair in the raw materials) or processing method or a combination of these two factors. Meals D, E and F contained coagulated blood in the raw materials used for MBM manufacture which probably contributed to the higher protein contents in the MBM. Blood is a relatively rich source of the essential amino acids, particularly lysine, valine and histidine, which would explain the high levels of these amino acids in these meals, particularly meals D and E. In contrast, meals A, B, G and H contained considerable amounts of bone in the raw material used for MBM manufacture which resulted in meals low in protein and essential amino acids, but high in ash. Processing method also appears to affect nutrient composition (Knabe *et al.*, 1989; Skilton *et al.*, 1991). The batch and continuous dry methods apply more heat than the low temperature rendering (MLTR) process. In general, the nitrogen content averaged for the meals processed by the low temperature rendering method was about three percentage units higher than the averages for the batch and continuous dry methods.

As discussed earlier (refer chapters 5 and 6) a potential criticism of the use of apparent values is that significant quantities of endogenous amino acids are present in the digesta at the terminal ileum of the animal and correction needs to be made for the endogenous component. Thus, in the present study with meat and bone meals, total ileal N and amino acid flows were adjusted for endogenous amino acid excretion determined by the recently

developed EHC/ultrafiltration technique and compared with those determined using the traditional protein-free method.

The mean endogenous amino acid flows presented here for the EHC and protein-free fed rats compared closely with those reported previously in chapter 5 of this thesis and similar results have been reported by other authors for rats fed EHC diets (Butts *et al.*, 1991) and those for protein-free fed animals (Skilton *et al.*, 1988; Darragh *et al.*, 1990; Moughan and Rutherfurd, 1990; Butts *et al.*, 1991). In the present study, there were no significant differences between the endogenous histidine and glycine flows determined by both the EHC and protein-free methods and the endogenous arginine and proline excretions were markedly higher for the protein-free fed rats. Significant endogenous proline excretion has been observed in the rat and pig by other workers (Taverner *et al.*, 1981b; Skilton *et al.*, 1988; De Lange *et al.*, 1989a; Moughan and Rutherfurd, 1990) and may be an artefact associated with the protein-deplete state. In general, it appears that the traditional protein-free method underestimates endogenous ileal amino acid excretion in the growing rat (Darragh *et al.*, 1990; Butts *et al.*, 1991) and pig (De Lange *et al.*, 1989a; Butts *et al.*, 1992b).

The method for determining endogenous amino acid excretion at the terminal ileum of rats significantly influenced the estimation of true digestibility of amino acids in the different meat and bone meals. The endogenous ileal amino acid flows following protein-free alimentation generally yielded true amino acid digestibility coefficients lower than did the endogenous flows determined by feeding an EHC-based diet followed by centrifugation plus ultrafiltration of digesta. This was particularly marked for threonine, isoleucine, glutamic acid, serine and leucine.

The variability shown in the nutrient composition and in the protein digestibility of meat and bone meals is of particular importance nutritionally and economically. A summary of the different meat and bone meals evaluated in the present study, their processing methods, N content and true ileal digestibilities are presented in Table 7.6. Although detailed comparison is difficult, it appears that the raw material used and the processing method should be considered when purchasing meat and bone meals. A considerable part of the variation in the true ileal N digestibilities of the 8 MBM samples was probably due to variation in the composition of the raw material used in MBM manufacture. For example, the highest N digestibilities were associated with samples which had blood included in the meal, while some of the lower digestibilities were found for those samples which had appreciably higher amounts of bones included in the meal. Although MBM G was manufactured by the low temperature rendering system (MLTR), its true N digestibility was lower than those of meals D, E and F manufactured by the same system. Published data using pigs (Batterham *et al.*, 1986; Knabe *et al.*, 1989) and rats (Skilton, 1988) suggest that dried blood meal protein can be highly digestible.

TABLE 7.6
A summary of the different meat and bone meals evaluated, their processing methods, mean nitrogen content (g $100g^{-1}$ air-dry weight) and mean true ileal nitrogen digestibilities (%)

Sample identification	Raw material mix	Processing method	Nitrogen content	True ileal nitrogen digestibility[1]
MBM A	Sheep and lamb offals plus bones	Traditional batch dry rendering	49.1	78.5
MBM B	Cattle, sheep and lamb offals plus bones	Continuous dry rendering (Keith)	47.8	73.4
MBM C	Sheep, lamb and cattle offals plus bones	Traditional batch dry rendering	55.8	76.0
MBM D	Cut-outs of lamb, venison plus blood and limited amounts of offals	New Zealand Meat Industry Research Institute low temperature rendering (MLTR)	56.5	83.6
MBM E	Sheep and lamb offals plus blood	MLTR	59.3	88.9
MBM F	Cattle and sheep offals plus bones and blood	MLTR	51.0	87.0
MBM G	Viscera of cattle and sheep plus bones	MLTR	49.3	68.8
MBM H	Sheep and cattle offals plus bones	Batch rendering	49.7	62.7

[1]Calculated using the mean endogenous nitrogen flows for rats given the EHC diet.

In addition to the differences in raw material being processed, the rendering system in operation and how efficiently it is operated can greatly influence protein digestibility. For example, the batch dry rendering which was used for the manufacture of meals A, C and H and the continuous dry rendering used for MBM B, involved more severe heat treatment (between 125 and 130°C) and a long residence time (120 min), compared with the low temperature MLTR system (at below 100°C for 6 min) used in the manufacture of meals D, E and F. This was however, not so in all the meals evaluated. For instance, meal A was manufactured using the traditional batch dry rendering system yet its true ileal N digestibility was higher compared with meal G which was manufactured by the MLTR system. Other factors may also be important. There is the likelihood of an interaction between raw material and processing method. As such, soft offal protein though highly digestible with mild heat treatment, is more susceptible to heat damage than hard offal protein (Haughen *et al.*, 1985). Maillard reactions may occur between amino acids and sugars, aldehydes and ketones during processing of protein feedstuffs, such as MBM. There may be a generally lowered *in vivo* digestibility of amino acids due to the formation of enzyme-resistant cross-linkages and a possible direct effect of the advanced Maillard compounds on the digestive enzymes (Hurrell and Finot, 1985; Oste *et al.*, 1986). In some cases, however, amino acids (particularly for lysine which reacts with reducing sugars to form lysine-sugar complexes) may be absorbed from the small intestine but cannot be completely utilised by the animal (Carpenter, 1973). Thus, ileal digestibility may overestimate the availability of amino acids. As yet the magnitude of the difference has not been established, although Austic (1983), on the basis of some indirect evidence, concluded that the effect was small.

Since feedstuffs often have a highly variable digestible amino acid content and pig ileal assays are expensive, a routine relatively inexpensive assay, such as the present rat ileal assay, is required if protein supplements such as meat and bone meals are to be used efficiently in pig diet formulation.

It is concluded that the digestibility of amino acids varies greatly among different meat and bone meals. True digestibility is independent of the dietary conditions (e.g. fibre and protein level) under which it is determined, thus the use of true amino acid digestibility should improve the precision of dietary formulation. The choice of method for determining endogenous amino acid flow strongly influences the resultant true amino acid digestibility coefficients. The present findings give support to the practical application of a recently proposed method for the determination of total endogenous ileal amino acid excretion based on peptide alimentation.

SUMMARY AND GENERAL CONCLUSIONS

Current assay procedures for assessing the quality of protein sources in monogastric animals have been discussed and distinction between the measures of amino acid availability and digestibility is stressed. Overall, a reliable routine method for assessing the availability of amino acids in feedstuffs for pigs has yet to be developed. For practical purposes, methodology has by and large concentrated on the determination of amino acid digestibility. Because of the microbial activity in the hindgut, and that at least for most species of animals, amino acids from the hindgut do not become available for body protein synthesis, faecal digestibility coefficients are likely to be misleading. The ileal method is the preferred way of measuring amino acid digestibility particularly for more poorly digested feedstuffs. It appears that use of the laboratory rat may allow routine inexpensive determination of ileal protein digestibility for pigs. Results obtained with the laboratory rat in nutritional studies are, in most cases, directly applicable to pigs. Considerable developmental work is required to establish a reliable routine rat ileal digestibility assay and consequently, studies were conducted with the growing rat and pig to investigate various methodological aspects. The results of these studies have been reported in the body of this thesis. In this section, the observations will be summarised and their implications discussed more generally. Recommendations for further research will be made in view of the findings, to help create a better understanding of digestion and absorption of amino acids in feedstuffs for monogastric animals.

To allow a valid inter-species comparison, sampling of ileal digesta from the animal at slaughter was used in both the rat and pig. Since each ileal digesta collection technique involves a specific manipulation of the gastrointestinal tract, digestibility measurement may differ depending on the technique used. For this reason an experiment was first carried out to compare the slaughter method and simple T-cannulation of the terminal ileum of growing pigs as approaches to determining the digestibility of nitrogen (N) and amino acids in meat and bone meal (MBM), (chapter 2). A preliminary study was designed to establish the optimum time of sampling after the start of feeding and site in the ileum for sampling digesta from pigs, using the slaughter method. The study showed that ileal N digestibility coefficients were relatively constant over sampling times of 9 and 11 hours with the greatest and least variable value at the 9-hour sampling time. The quantity of ileal digesta obtained from the pig was numerically greatest at the 9-hour sampling time, for the MBM based diet and for these reasons, 9 hours represented the preferred sampling time.

Sampling site had little influence on apparent digestibility of N in pigs suggesting that ileal samples could be collected further up within the terminal ileum. As a precautionary measure against incidental source of error or bias, however, it is suggested that the length of terminal ileum selected should be kept as short as that consistent with obtaining sufficient digesta.

Consequently, the terminal 20 cm of the ileum was considered as the preferred site for sampling digesta from pigs.

In the main study, apparent ileal N and amino acid digestibility measurements in intact pigs, using the slaughter method under the defined sampling conditions, were compared with those for pigs fitted with simple T-cannulas (control animals) and given a semi-synthetic MBM-based diet. It was considered important that the study be undertaken with animals that are in a normal physiological state and that are subjected to minimal levels of stress as a result of environmental or experimental conditions. In the present study, where the apparent ileal digestibility of N and amino acids in MBM were determined with recently cannulated animals given adequate post-surgical recovery and which were shown at post-mortem to have not developed adhesions, similar digestibilities were obtained for intact and cannulated animals. Moreover, the variation about the means, as a measure of the precision of the digesta collection procedures, was similar for both methods and not consistently high with the slaughter technique. Based on the results of this study, the slaughter method is a viable alternative to simple T-cannulation for the determination of N and amino acid digestibility in the pig.

In the future it would be of interest to attempt to establish the consistency over time post-surgery, of the ileal digestibility coefficients for a given protein source for pigs prepared with simple T-cannulas. Further investigations are still necessary to standardise the collection procedures as well as the use of markers to improve the comparability of digestibility results measured at different institutes.

Preliminary investigations (chapter 3) evaluated the influence of time (1, 2, 3, 4, 5 and 6 hours) after start of feeding and site within the terminal ileum (0-5, 0-10, 0-15 and 0-20 cm from the ileo-caecal valve) for sampling digesta from euthanased rats given a MBM-based diet. The ileal N digestibility coefficients were relatively constant over sampling times of 3 to 6 hours with the greatest and the least variable value being found at the 4-hour sampling time. The amount of ileal digesta obtained from the rat was numerically greatest at the 4-hour sampling time and for these reasons this represented a preferred sampling time. Ideally, the optimum slaughter time should be determined for each type of diet fed, by a simple preliminary trial where rats are slaughtered at different times (for example, 2 to 4 hours). Similar to the pig study described in chapter 2, sampling site had little influence on apparent digestibility. Consequently, the terminal 20 cm of the ileum was considered as the preferred site for sampling digesta from rats.

Following on from the studies described in chapter 2 and 3, the main interest of the study described in chapter 4 was focused on the use of the rat as a model animal for determining ileal amino acid digestibility in the growing pig when the slaughter method was used for the collection of digesta. When the necessary inter-species comparison was made under the comprehensively defined conditions using the slaughter method, there was close agreement between the rat and pig for the apparent ileal digestibility of N and amino acids in each of

two different meat and bone meals tested, with the exception of glutamic acid which had a relatively higher digestibility in the rat. It was also found that the ileal digestibility method was more sensitive than the faecal approach for detecting differences in MBM digestibility. In view of its significant role in the endogenous excretion, the species difference observed in the present study for glutamic acid may be reduced if comparison was based on true ileal amino acid digestibility. The findings of this study give comprehensive support for the use of the rat as a model animal for the growing pig in terms of the apparent ileal digestibility of N and amino acids in MBM, but this needs to be thoroughly investigated for a number of different ingredients. To be of general practical significance, a method to determine amino acid digestibility must be able to generate values across the broad range of feedstuffs used in the feed industry. If the present assay can be applied successfully for a wider range of feedstuffs, then the use of the rat may afford an inexpensive, rapid and routine method for determining the ileal digestibility of amino acids in the pig.

In an effort to develop a reliable routine rat assay, it is important to take into consideration the selective eating habits of the rat. Whilst it is recognised that simplicity of assay procedure is an important feature of routine digestibility assays and feeding by free access offers considerable advantages in labour, effort and time, particle size separation and/ or selection of test diets may give rise to a design bias and lower the sensitivity and reliability of the assay technique. This problem could be avoided by finely grinding the ingredients, such as MBM, before feeding to the rats, but the grinding process *per se* may affect protein digestibility. Further research is needed to determine whether the feeding behaviour of rats results in particle selection when animals are given free access to diets designed to differ in particle size, and whether there is any subsequent effect on protein and amino acid digestibility.

One of the main criticisms of the slaughter method concerns the potential difficulty of obtaining representative samples of digesta. A desirable objective for assays using the slaughter technique is to maintain a uniform flow rate of digesta. In the studies reported here, animals were trained to eat their daily meal within a restricted period of time. Alternatively, a frequent feeding regime could have been adopted. There is the need for further comparative studies to determine the effect of single meal intake within a specified period versus a frequent feeding regime, on digesta flow rate and on digestibility measurements.

Evidence in the literature (Waddell and Desai, 1981) has warned against the likely effects of coprophagy on nutritional studies with rats. In the present study, there was no evidence of faecal particles in the stomach contents when these were examined after slaughter of the animals, thus indicating that the practice of coprophagy was minimal. However, coprophagy still remains a potential problem and therefore the stomach contents should always be monitored or checked during sampling of the ileal digesta.

The rat assay developed previously (chapter 4) is an apparent digestibility assay and the

validity of apparent digestibility coefficients may be questioned due to the influence of endogenous protein excretion. To obtain a 'true' estimate of the unabsorbed dietary amino acid flow, correction needs to be made for the endogenous component. However, the determination of endogenous ileal amino acid excretion is a technical problem that has not been completely resolved. Thus the study reported in chapter 5 was carried out to compare different approaches to determining endogenous ileal amino acid excretion in the rat to develop a rat assay for the true ileal digestibility of amino acids in meat and bone meal. The recently-developed centrifugation / ultrafiltration technique (Butts *et al.*, 1991) was used with laboratory rats to determine the endogenous ileal amino acid flows under peptide alimentation, and these flows were compared with those obtained with the often-used protein-free method, or with the regression method. The results from applying the technique of feeding animals an enzyme hydrolysed casein (EHC)-based diet followed by centrifugation and ultrafiltration of digesta for determining endogenous amino acids, supports other evidence that the traditional protein-free method leads to considerable underestimation of the endogenous flow of amino acids at the terminal ileum of the growing rat (Butts *et al.*, 1991) and pig (Butts *et al.*, 1992b). Further, and given that the regression technique generates similar values to those obtained after feeding animals a protein-free diet, as observed in the present study and that of other authors (Leibholz and Mollah, 1988; Furuya and Kaji, 1989), the use of this method may also lead to underestimation of the endogenous amino acid excretion at the terminal ileum of animals.

The use of EHC in the determination of endogenous excretion may also have limitations. The main limitation of the method is that it may underestimate endogenous ileal amino acid excretion, because endogenous free amino acids and peptides are discarded in the low molecular weight ultrafiltrate. A further limitation is that the method does not allow the effect of protein *per se* on endogenous loss or the effect of different amino acid compositions of dietary proteins to be investigated. Besides, it is possible that estimates of endogenous loss determined with the peptide alimentation method may be influenced in some way by the enzymic hydrolysate of casein itself and thus be an artefact of this particular dietary treatment. The effects of hydrolysates of other proteins and the degree of hydrolysis of the dietary nitrogen on the quantity and amino acid composition of endogenous ileal amino acid excretion need to be determined to ensure the present results are not artefacts due to the enzymically hydrolysed casein.

There is a possibility that there is binding of the undigested dietary free amino acids and peptides to the endogenous proteins in ileal digesta, which could result in an overestimation of endogenous loss using the EHC method. The binding of free amino acids and peptides to plasma proteins has been reported to occur in blood (Ohara and Ariyosha, 1979). There is indirect evidence from the study of Butts *et al.* (1992b), however, that shows that this does not occur in digesta to any appreciable extent. In their study, the endogenous ileal amino acid flows for pigs fed a synthetic amino acid diet were not higher than those for protein-free

fed pigs, indicating that the dietary free amino acids were not bound to endogenous proteins. It is worth noting that the studies of Moughan and Rutherfurd (1990), De Lange *et al.* (1990) and Butts *et al.* (1991) using the homoarginine, ^{15}N-tracer and the EHC/ultrafiltration methods, respectively, corroborate the present findings that the presence in the gut of dietary protein or peptides enhances endogenous ileal nitrogen excretion. A further possibility is that the ultrafiltration devices did not adequately separate digesta amino acids and proteins. However, from results of the preliminary studies conducted by Butts *et al.* (1991) this appears unlikely. This possibility could be investigated by quantitatively adding free amino acids (for example, homoarginine) and/or peptides to ileal digesta and measuring the recovery of the added material following centrifugation and ultrafiltration.

Although the protein-free diet contained only purified ingredients, some contamination was inevitable and the diet was found on analysis, to contain about 5 g kg^{-1} protein. Possible bacterial contamination of the diets and the bacteria contained in the small intestine should not have markedly affected the results as they should be reasonably consistent across all treatments. Ileal digesta sampled from rats were found to contain fine hairs ingested as a result of the rats grooming themselves. The hairs are a component of the non-dietary endogenous excretion and thus can be accounted for in true protein digestibility studies. In apparent digestibility studies they may result in considerable error and are likely to increase the variability of the data in nutritional studies. The presence of the hair in ileal digesta may thus cause some problems in a routine apparent digestibility assay with rats, although it may be possible to separate them out by sieving if necessary. More research is needed to justify the various methods mentioned, to give a better understanding of the excretion of endogenous amino acids at the terminal ileum of animals, but true ileal amino acid digestibility data should ultimately provide more meaningful data on amino acid absorption in the animal.

Data on endogenous amino acid flow at the terminal ileum, for 45 kg liveweight pigs cannulated at the terminal ileum and fed a semi-synthetic EHC based diet (A. Donkoh, unpublished), allowed a comparison here of true amino acid digestibility between rats and pigs given the MBM based diets. This provides a further inter-species comparison of digestibility. The latter endogenous excretion data for pigs and those for rats (refer chapter 5) were used to correct apparent digestibility coefficients to true coefficients. The rat true ileal digestibility data are compared with pig true ileal digestibilities in Table 8.1. Although the comparison is somewhat limited in that the pigs were cannulated whereas rat digesta were collected at slaughter, they do indicate, once again, close agreement between rats and pigs for their ability to digest MBM protein. No species difference in digestibility for glutamic acid was observed as was the case when the apparent digestibility coefficients were compared.

TABLE 8.1
True ileal digestibility of amino acids in meat and bone meal for the growing rat and pig

	True ileal digestibility[1]		Statistical significance[2]
	Rat	Pig	
Nitrogen	75.9 (0.20)	76.4 (0.27)	NS
Amino acid			
Lysine	78.2 (0.12)	78.9 (0.33)	NS
Methionine	81.1 (0.20)	82.5 (0.29)	NS
Cystine	61.1 (0.11)	62.4 (0.19)	NS
Histidine	71.2 (0.19)	72.9 (0.25)	NS
Phenylalanine	83.7 (0.28)	84.1 (0.31)	NS
Tyrosine	73.4 (0.17)	74.0 (0.22)	NS
Threonine	75.0 (0.29)	75.6 (0.17)	NS
Leucine	79.2 (0.21)	79.7 (0.24)	NS
Isoleucine	78.6 (0.16)	79.4 (0.20)	NS
Valine	78.3 (0.25)	79.0 (0.52)	NS
Alanine	85.2 (0.15)	85.8 (0.37)	NS
Aspartic acid	62.8 (0.24)	63.4 (0.31)	NS
Arginine	87.0 (0.19)	88.6 (0.16)	NS
Serine	78.9 (0.29)	79.2 (0.32)	NS
Glutamic acid	80.2 (0.13)	79.3 (0.18)	NS
Glycine	77.1 (0.18)	77.5 (0.25)	NS
Proline	72.0 (0.38)	72.8 (0.42)	NS

[1]True digestibility values based on endogenous flow values (rat: μ g^{-1} freeze dry matter intake; pig: mg kg^{-1} freeze dry matter intake) from EHC/ultrafiltration. Values are means ($\pm$SE). n = 30 for rat, n = 30 for pig.
[2]NS = Non significant

A criticism of apparent digestibility measurements is that the values are likely to be influenced by the level of protein included in the test diet as was observed in the study reported in chapter 5. In the study reported in chapter 6, comparison was made between the apparent and true digestibility of N and amino acids in a MBM for the growing rat, determined over a wide range of dietary crude protein concentrations (25, 60, 95, 130, 165 and 200 g CP kg^{-1} diet). The aim was to highlight the potential inaccuracies of apparent digestibility coefficients. Recognising the limitations of the traditional protein-free method of determining endogenous excretion, the endogenous ileal N and amino acid flows used to adjust apparent digestibility values to true ones were determined by the new peptide alimentation method. Under the conditions of this study, apparent ileal digestibility of protein and amino acids significantly increased with increasing dietary protein levels, while very little difference in the true ileal digestibility of protein and amino acids was found with increasing dietary protein concentration. Therefore, for feedstuffs with a lower level in one amino acid,

their apparent digestibility would be lower especially if such an amino acid had a high endogenous ileal output. The observations in the present study further highlight the deficiencies of using apparent estimates. It may be argued that because endogenous excretion is difficult to measure accurately (Low, 1982b; Austic, 1983) then the correction for endogenous excretion is not worthwhile. However, the independence of true digestibility of the dietary protein levels under which it is determined, makes true digestibility a better measure of amino acid availability for feed formulation purposes, than apparent digestibility values. Moreover, methods for determining endogenous ileal amino acid excretion are continually being developed and refined. True rather than apparent digestibility estimates, may allow feed ingredients to be accurately compared even if they are ingested in different quantities during the assay.

Perhaps another key factor which must be considered is the influence of age or liveweight on endogenous secretions. If there are differences due to liveweight, these could contribute to inappropriate apparent digestibility coefficients being applied from adult to young animals and vice versa. This would seem to be a compelling argument for the use of true amino acid digestibility, if correct, but further studies are required on this subject.

The last study, reported in chapter 7 of this thesis, involved investigation into the differences in chemical composition and true ileal digestible N and amino acid content of meat and bone meals sourced from eight rendering plants in New Zealand. The true ileal digestibility of nitrogen and amino acids from each of the rendering plants were determined using the validated rat assay. The new peptide alimentation method, and the protein-free approaches for determining endogenous ileal amino acid excretion were applied and compared. There was considerable between-plant variation for most of the chemical components, and particularly for the amino acids. This variability was mainly attributed to differences in the raw material mix that MBM is produced from and the processing conditions under which it was produced. The endogenous excretions of amino acids determined by the protein-free or the EHC/centrifugation plus ultrafiltration methods in the present study were in most instances similar to those determined by Butts *et al.* (1991). When an enzymically hydrolysed casein (EHC) diet was fed to rats, followed by centrifugation and ultrafiltration of the digesta, the resulting true digestibilities were greater than those obtained by reference to the protein-free method. It is important to note that whatever the magnitude of the differences between true digestibilities, depending on the method used for estimating endogenous excretions, it is worthwhile determining true digestibility coefficients if endogenous excretions can be routinely measured. This is because the inclusion of amino acids of endogenous origin in the undigested portion is a systematic error rather than a random error of the apparent digestibility assay and therefore should be corrected if possible.

Overall, the variable ileal digestible amino acid content of meat and bone meals emphasises the limitations of tabulated gross analytical values and the need for a routine

relatively inexpensive digestibility assay, such as the present rat assay, if meat and bone meal is to be used efficiently as a protein supplement. It must be stressed, that because MBM is a protein source that has been subjected to heating during its processing, the digestibilities reported here for lysine, methionine, cystine and tryptophan may be subject to error. However, and in the absence of better information they may be used for dietary formulation purposes. Particular attention in research needs to be given to these latter amino acids in heat-treated foods, and a specific availability assay should be developed.

Although amino acid digestibility values have generally been determined by the *in vivo* ileal analysis method, the determination is not only tedious but time-consuming. Also, the large variation often observed among different samples of the same feedstuff, such as MBM, is of concern and illustrates the need to further study other simple approaches that can accurately and rapidly predict the *in vivo* digestibility of amino acids in feed ingredients. Firstly, attention should be directed towards the development of multiple regression equations, based on chemical determinations such as, the ash, lipid and protein contents, and physical determinations, such as solubility, to predict amino acid digestibilities. Secondly, prediction equations based on *in vitro* digestibility methods must be developed. Recent reports also indicate that Near Infrared Reflectance (NIR) spectroscopy may be an alternative for measurement of the ileal digestibility of protein (Gives *et al.*, 1990; Harrison *et al.*, 1991). NIR spectroscopy is a rapid technique which may be used for analysing the contents of nutrients in feedstuffs (Van Lonkhuijsen and Jansen, 1987). The principle of the technique is that compounds with similar chemical groupings absorb infrared radiation at characteristic wavelengths. NIR has the potential to be developed into a reliable method for predicting apparent and true amino acid digestibilities. Before any such methods can be applied with confidence, however, reliable *in vivo* digestibility data must be available. The present work is a contribution towards this end point.

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