ASPECTS OF DIETARY PROTEIN QUALITY FOR THE GROWING PIG

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ABSTRACT

A study of several aspects of dietary protein quality for the growing pig is described. The study was conducted in three parts.

Reservations regarding the interpretation of three recent empirical estimates of the ideal amino acid balance for the growing pig prompted the first part of the study. A diet (basal diet) was formulated in which enzymatically hydrolysed casein supplemented with synthetic amino acids formed the sole protein source. The balance of essential amino acids in the diet approximated the mean of the three published estimates.

Eight entire male pigs (boars) of 28 Kg initial liveweight, confined in metabolism crates, were fed the basal diet for 20 days and thereafter a protein-free diet for a further eight days. Mean daily excretion of urinary urea nitrogen over six-day collection periods was 93 $mg/Kg^{0.75}$ for pigs fed the basal diet and the corresponding value for the protein-free diet was 19 $mg/Kg^{0.75}$. Assuming that the difference between these values was attributable to deamination of amino acids from the basal diet, this corresponds to an efficiency of utilisation of dietary protein of 0.940. It was concluded that the amino acid pattern of the basal diet approximated an ideal balance.

Part two of the study entailed the determination and evaluation of estimates of the apparent ileal digestibility of crude protein and amino acids for the growing pig and included a comparison of protein digestibility in the rat and pig.

Samples of ileal digesta were collected from boars prepared with T-piece cannulae in the terminal ileum. Values for the digestibility of crude protein and amino acids in barley-meal, pea-meal, meat-and-bone-meal, fish-meal and a mixture of enzymatically hydrolysed casein and synthetic amino acids are cited.

In an evaluation of the determined digestibility values ten boars received a barley-, pea-, meat-and-bone-, fish-meal diet and ten a control diet containing enzymatically hydrolysed casein and synthetic amino acids as its sole protein source. The gross amino acid composition

of the latter diet equalled the determined apparent ileal digestible amino acid composition of the barley-based diet. Accepting that the control protein source was completely digestible and that the two feeding regimes were iso-caloric, the similar growth characteristics of pigs on the two diets suggested that apparent ileal amino acid digestibility coefficients are accurate measures of the degree of amino acid digestion and absorption in the growing pig.

Preliminary results showed close agreement between the rat and pig for the apparent ileal digestibility of crude protein in barley-, meat-and-bone- and fish-meal.

In the third part of the study a deterministic computer model which simulates the digestion and metabolism of dietary nitrogen in the growing pig was constructed. The model was based on the concept of a partitioning of daily dietary nitrogen intake in pig growth. Initial validation exercises demonstrated that results obtained from simulation were in close agreement with observations from experimentation with the live animal.

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INTRODUCTION

The concept of dietary protein quality defies succinct definition because the term 'protein quality' has several connotations, each associated with a particular method of protein evaluation. In a general sense, however, dietary protein quality is synonymous with the degree of utilisation of dietary protein during animal growth.

It is in this context, therefore, that insight may be gained into the nature of protein quality by considering the factors affecting the utilisation of protein by the growing animal. The influence of three factors is of primary importance:

(i) The requirement of the growing animal for dietary amino acids;

(ii) The amounts of amino acids digested and absorbed;

(iii) The interactive metabolism of protein and energy.

The central theme of this thesis is dietary protein quality for the growing pig, and as such embodies consideration of the above three factors.

The first aim of the study reported herein, was to establish an estimate of the pattern of amino acids required by the growing pig to maximise the efficiency of protein synthesis per unit of absorbed protein. This pattern of amino acids is referred to as an ideal balance of amino acids, and the requirement of the growing pig for amino acids is considered a requirement for an amount of ideally balanced protein. The second aim was to determine the ileal digestibilities of amino acids in several natural foodstuffs and appraise the accuracy of these estimates for measuring the digestion and absorption of amino acids in mixed diets. Thirdly, it was aimed to construct and validate a simulation model, encompassing the interaction in pig growth, between absorbed protein and energy.

The estimate of an ideal amino acid balance for growth along with estimates of apparent ileal amino acid digestibility were necessary for formulation of the simulation model, the overall objective of which was to enable the prediction of protein quality in mixed diets fed to the growing pig.

PART I

ESTABLISHMENT OF THE IDEAL AMINO ACID BALANCE FOR GROWTH IN THE PIG

CHAPTER 1

REVIEW OF LITERATURE

I.1.1 Introduction

Several recent studies, aimed at determining the amino acid requirements of the growing pig, have emphasised the concept of amino acid balance.

It is proposed that there is a balance or pattern of amino acids which can be regarded as ideal for growth in the pig. The concept of a requirement of the growing pig for amounts of individual amino acids is superceded by that of a requirement for an amount of ideally-balanced protein. The establishment of an ideal amino acid balance is an important aspect of a study of dietary protein quality, for the ideal pattern of amino acids plays a major role in dictating the amount of absorbed dietary protein available for protein synthesis.

The present review considers the problems associated with the conventional approach to determining amino acid requirements, discusses the concept of ideal amino acid balance and presents empirically derived estimates of the ideal amino acid balance for the growing pig. Finally, detailed consideration is given to the various experimental factors which may confound the determination of ideal amino acid balance.

I.1.2 The Determination of Amino Acid Requirements

The 'requirement' of an animal for a nutrient may be thought of as a point on a dose-response curve relating the level of intake of the nutrient and some measure of productivity of the animal. In the case of protein the 'requirement' is best considered as the requirement for individual amino acids rather than for the protein as a whole.

Although accurate estimates of the amino acid requirements for growth in the pig are necessary for efficient dietary formulation, considerable variation in the published recommendations is evident. The comprehensive reviews of Rérat and Lougnon (1968) and Rérat (1972)

demonstrate the degree of this variation. Also, Poppe (1976) has reviewed estimates of amino acid requirements for growing pigs determined in the European Socialist countries between 1963 and 1973. The estimates were extremely variable. Values for methionine plus cystine, for example, ranged from 0.23 to 0.74% air-dry weight of diet. The Agricultural Research Council (1981), in a technical review, emphasised the inadequacies of current information concerning amino acid requirements of the growing pig. Several estimates of requirements for amino acids were presented and some values are given in Table I.1.1.

Table I.1.1 Requirements of the growing pig for some amino acids (Agricultural Research Council, 1981).

Amino acid	Range of requirement (% air-dry weight of diet)
Methionine + cystine	0.32 - 0.67
Tryptophan	0.12 - 0.22
Threonine	0.47 - 0.60
Isoleucine	0.27 - 0.67

Differences in experimental factors such as age, sex, breed and genetic strain of pig, feeding level, dietary composition and methods used are undoubtedly responsible for a great deal of the variation shown in Table I.1.1.

The methods adopted and the relative importance of experimental factors in the determination of amino acid requirements have been discussed in detail by Rérat and Lougnon (1968) and Fuller (1978). Most estimates of amino acid requirements have been made from empirically derived dose-response relationships. The successful estimation of amino acid requirements using this approach is reliant upon the formulation of a basal diet deficient in the amino acid being studied but adequate in all other nutrients. Graded additions of the limiting amino acid are made to the basal diet and responses are measured. Three major problems are apparent. Firstly, to ensure adequate levels

of all nutrients excepting the amino acid being examined, implies that the requirements for all other nutrients are previously known. Unfortunately, this is not the case. To circumvent the problem, nutrients other than the one being examined may be supplied in generous excess of the best estimates of requirements. This practice, however, may lead to imbalance effects. The feeding of imbalanced mixtures of amino acids is likely to affect the growth rate of animals (Harper, Benevenga and Wohlhueter, 1970). Imbalance may affect the utilisation of the limiting amino acid and under conditions of *ad libitum* feeding a primary effect on growth due to decreased food intake may be observed. Secondly, there is no generally accepted single measurement of response and consequently the determined amino acid requirement will vary as to the type of response measured. Thirdly, some workers equate amino acid requirement with the input level corresponding to the point of maximum response whereas others choose the minimum input of the amino acid which produces a response not statistically significantly less than the maximum, as the required level. The latter approach is likely to lead to considerably lower estimates of amino acid requirements in cases whereby a large deviation of amino acid input from the input corresponding to maximum response, produces only a small alteration in response.

In view of these problems, it seems desirable to develop a method for estimating the amino acid requirements of the growing pig, which minimises the difficulties inherent in the dose-response approach. Recently much attention has been given to the importance of considering the balance between dietary amino acids in determining amino acid requirements, with the aim of obtaining more reliable estimations.

The concept of amino acid balance has been highlighted in studies concerning the amino acid requirements of the chicken (Lewis and Annison, 1974, and Fuller, Woodham and Henderson, 1979). A similar emphasis is emerging with respect to the amino acid requirements of the growing pig (Lewis and Cole, 1976; Fuller, 1978; Cole, 1979; Taylor, Cole and Lewis, 1979, and Low, 1981). It is also worthy of note that in a recent technical review of the nutrient requirements of pigs (Agricultural Research Council, 1981), considerable emphasis has been placed on the concept of an ideal amino acid balance.

I.1.3 Ideal Amino Acid Balance

Cole (1979, 1980) has defined an ideal protein as one which is 'perfectly' balanced in terms of its essential amino acid content and its supply of non-essential nitrogen. 'Perfect' balance implies the pattern of amino acids required by the animal to maximise the efficiency of protein synthesis per unit of absorbed protein. The protein requirements of the growing pig for various levels of production are then viewed as requirements for amounts of ideal protein.

Whereas amino acid balance is a long-established idea in nutritional studies, it is only recently that the concept has been re-emphasised (Fuller, 1980). Recognition of the importance of the proportions of amino acids in diets was discussed by Osborne and Mendel (1914). By the 1950's several workers (Mitchell, 1950; Price, Taylor and Russell, 1953; Fisher and Scott, 1954, and Williams, Curtin, Abraham, Loosli and Maynard, 1954) had suggested that the amino acid composition of an animal's tissue protein could give an indication of the correct dietary amino acid pattern. In 1955, the Food and Agriculture Organisation of the United Nations reported an estimate of a desirable pattern of essential amino acids for use in human nutrition. More recently the idea of optimal amino acid balance has been adopted for poultry diet formulation (Agricultural Research Council, 1975) and has been suggested for use in the pig (Cole, 1979, and Agricultural Research Council, 1981).

Determination of the composition of the ideal protein requires that a reference amino acid be chosen. The optimum levels of all other amino acids are then identified relative to this reference amino acid. The Food and Agriculture Organisation of the United Nations (1955), in deriving a provisionally desirable pattern of dietary amino acids for man, chose tryptophan as the reference amino acid. The level of tryptophan in a foodstuff, however, cannot be determined accurately (Williams, 1982). With regard to the pig it is preferable to choose lysine as the reference amino acid. Lysine is usually the first limiting amino acid in practical pig diets and consequently much information is available concerning the requirement of the growing pig for lysine.

Having established the ideal amino acid balance, the absolute level of the reference amino acid required may be adjusted in relation to dietary energy content, food intake and the potential performance standard of the animal. In his discussion on the usefulness of the concept of ideal amino acid balance, Boorman (1980) stated that by choosing the appropriate variables for dose and response it may be possible to establish a single relationship between the input of the reference amino acid and productive output.

I.1.4 Approaches to the Estimation of the Ideal Amino Acid Balance

Two approaches are evident in the determination of the composition of ideal protein for the growing pig. In the first instance, the pattern of amino acids in lean tissue or in whole body protein may be used as an estimate of the ideal pattern.

This approach is acceptable from a theoretical perspective and it appears that in the absence of more detailed information, the pattern of amino acids in the product may be used as a basis for the ideal pattern.

More precise information, however, may be obtained by the second approach which relies upon empirical methods. The latter, based upon the determination of maximum utilisation of dietary protein for growth, attempt to provide estimates of the balance of amino acids deemed to be ideal for the combined processes of protein maintenance, new protein synthesis and supply of precursors for other substances.

The possibility that age of the growing pig may affect the composition of the ideal protein has been indicated by the Agricultural Research Council (1981). The protein requirement for maintenance, however, is only a small fraction of the total protein requirement of the growing pig (Fuller, 1978) and as the amino acid composition of pig muscle does not vary with increasing liveweight (Williams *et al.*, 1954), the effect of age is likely to be minimal. Duée, Calmes and Desmoulin (1980) compared the amino acid composition of muscle crude protein in three breeds of pig (French Landrace, Belgian Landrace and Pietrain) at three slaughter weights (40, 60 or 80 Kg liveweight). The variation in composition due to slaughter weight was low and no

effect of genotype was observed.

The Agricultural Research Council (1981) has stated the desirability of expressing the ideal balance in units of absorbed amino acids. Composition of the ideal protein is usually presented in the form of total dietary amino acid levels relative to lysine, even though the balance of amino acids in the diet may not be the balance of amino acids absorbed by the animal after digestion has occurred.

Lately, several estimates of the amino acid composition of ideal protein have been derived, and these are considered in the next section.

I.1.5 Estimates of the Ideal Amino Acid Balance

Lewis and Cole (1976) have reported results from several experiments in which diets composed of barley, soyabean-meal and fish-meal were supplemented with various combinations of synthetic amino acids and fed to the growing pig. Several performance indices were recorded and an ideal balance between five amino acids has been proposed (Table I.1.2).

A further estimate of the ideal amino acid balance for the growing pig has been determined by Fuller, Livingstone, Baird and Atkinson (1979)†[1] using a barley-based diet supplemented with synthetic amino acids singly and in combinations. Minimum urinary nitrogen excretion was used as the criterion of adequacy of the amino acid balance and it was concluded that the protein, in the diet which minimised urinary nitrogen excretion, was a first approximation to an ideally balanced protein. This dietary protein had an estimated biological value of 0.93. The amino acid composition of ideal protein estimated by Fuller *et al.* (1979a) is presented in Table I.1.2.

Later, Fuller, Mennie and Crofts (1979)†[2] investigated the effects on pig growth of supplementing barley with L lysine and L threonine. The dietary lysine concentrations required for maximum daily gain, minimum food conversion ratio and minimum urinary nitrogen excretion, were similar. However, the dietary concentration of threonine required for maximum daily liveweight gain and minimum food conversion ratio wa:

†[1] Hereafter Fuller *et al.* (1979a). †[2] Hereafter Fuller *et al.* (197

proportionally higher (0.16) than that needed to minimise urinary nitrogen excretion. This discrepancy does not appear to be serious. In the growth trial, the accepted threonine level corresponded to maximum growth rate whereas when urinary nitrogen was minimised the optimal threonine concentration was taken to be that beyond which further additions of threonine produced no significant response. Also, measures of carcass leanness continued to respond to additions of synthetic amino acids above those levels supporting maximum growth rate. This observation implies that a slightly different balance between dietary amino acids may be required to maximise carcass leanness as opposed to maximising growth. This effect may be important in determining a balance between amino acids which would be optimal for maximising profit. Fuller *et al.* (1979a) and Fuller *et al.* (1979b) fed pigs diets with a crude protein concentration of approximately 100 g/Kg. The ideal amino acid pattern determined at this protein level may not be valid at higher dietary protein concentrations, whereby a greater level of lean deposition may be supported. The amino acid requirements for maintenance are not the same as those for growth. Also, when the protein concentration of a diet is raised, the efficiency of dietary protein utilisation may decrease and the decreased efficiency may not affect all amino acids equally. In reply to these considerations, Fuller (1978) noted that the rates of nitrogen retention and growth recorded in these experiments compared favourably with rates commonly found for pigs fed conventional diets.

Low, Pittman and Fulford (1980), using a similar method to that of Fuller *et al.* (1979a), fed a barley, wheatings, soyabean-meal diet to growing pigs and measured urinary nitrogen excretion. Four synthetic amino acids were added to the diet either singly or in combination. Based on the findings of this and similar studies, Low (1981) provided an estimate of the composition of ideal protein (Table I.1.2).

An estimate of the amino acid composition of pig whole body protein (Aumaitre and Duée, 1974), an estimate of the amino acid composition of pig muscle protein (Duée *et al.*, 1980) and the recently proposed ideal amino acid balances given by Cole (1980) and the Agricultural Research Council (1981) are also presented in Table I.1.2.

Table I.1.2 Amino acid composition of ideal protein for the growing pig (lysine = 100).

Amino acid	Empirical estimates			Pig muscle	Whole body†	Proposed balances	
	Lewis & Cole (1976)	Fuller *et al.* (1979a)	Low (1981)	Duée *et al.* (1980)	Aumaitre & Duée (1974)	Cole (1980)	Agricultural Research Council (1981)
Lysine	100	100	100	100	100	100	100
Methionine + cystine	48	53	55	48	43	50	50
Tryptophan	16	12	12	-	-	18	14
Histidine	-	32	31	47	38	34	33
Phenylalanine + tyrosine	-	96	105	91	96	100	96
Threonine	59	56	53	56	55	60	60
Leucine	-	84	90	98	101	100	100
Isoleucine	44	44	53	63	52	50	54
Valine	-	63	61	65	70	70	70
Non-essential amino acids	-	-	-	623	870	-	857

† determined on a four-week-old pig.

Examination of the contents of Table I.1.2 reveals that little account has been taken of the non-essential amino acids. Low (1981) assumed a 1.0 : 1.1 ratio of essential to non-essential amino acids in ideal protein, this being based upon the composition of pig lean tissue. Mitchell, Becker, Harmon, Norton and Jensen (1968) found that maximum nitrogen retention in the pig was achieved when the diet contained equal amounts of essential and non-essential amino acids. Henry and Rérat (1970) noted that the non-essential amino acids should be 0.60 of the total nitrogen supply to achieve maximum growth and the sparing of essential amino acids.

The three empirically derived estimates of ideal amino acid balance presented in Table I.1.2 are in reasonably good agreement. Before these estimates are accepted for practical application, however, consideration should be given to two important aspects relating to experimental procedure.

Firstly, natural ingredients (barley, soyabean-meal, fish-meal and wheatings) were used in the three studies. Consequently, the array of amino acids fed was not necessarily indicative of the array of amino acids digested and absorbed. For example, in the diets fed by Low *et al.* (1980) the determined apparent digestibility of nitrogen was 0.80. The digestibility coefficients for individual amino acids are likely to deviate from this value. Secondly, in each experiment, free synthetic amino acids were added to the basal diet. The synthetic amino acids may have been absorbed at a faster rate than the amino acids derived from digestion of the intact proteins, thereby impairing the efficiency of protein synthesis. The efficiency of utilisation of free amino acids in mixed diets has been reviewed (Batterham, 1980). It was concluded that supplements of free lysine are inefficiently utilised by growing pigs fed once daily. Batterham (1980) also pointed out that lysine is one of the more stable amino acids in terms of turn-over rate in the body and it is therefore likely that other added synthetic amino acids may be inefficiently utilised under limited feeding regimes. Referring to the study of Lewis and Cole (1976), Batterham (1980) considered that under the limited feeding system adopted, only 0.50 to 0.60 of the supplemental threonine may have been utilised, this resulting in an overestimation of the threonine requirement. Low *et al.* (1980) adopted twice daily feeding and concluded that efficient use of

free lysine resulted. The possibility, however, that other synthetic amino acids were not fully utilised was appreciated by the authors.

Differences in the rate and degree of amino acid digestibility and absorption may affect the interpretation of the empirical estimates of ideal amino acid balance shown in Table I.1.2. In the experiments used to derive these estimates, the dietary amino acid balance which afforded maximisation or minimisation of certain performance indices was accepted as the best estimate of ideal balance. It is postulated, however, that the patterns of amino acids provided by the diets used in these studies may have differed markedly from the amino acid patterns supplying the sites of protein synthesis within the animal. The pattern of amino acids at the site of protein synthesis, moreover, is the pattern of amino acids which must be considered as ideal.

Other factors may also interfere with the experimental determination of the ideal amino acid balance. The following sections give specific consideration to the effects of protein-free energy intake, dietary electrolyte levels, and deviation from an ideal balance, on the utilisation of dietary amino acids.

I.1.6 The Influence of Dietary Energy Intake on the Utilisation of Dietary Amino Acids

The interactive nature of protein and energy metabolism has been described comprehensively (Buttery and Boorman, 1976; Kielanowski, 1976, and Lindsay, 1976). It is generally accepted by these authors that increased efficiency of dietary protein utilisation accompanies increases in the amount of non-protein energy fed to the growing pig, although it is difficult to describe this effect quantitatively (Boorman, 1980).

Fuller and Crofts (1977) endeavoured to develop a quantitative relationship between the intakes of protein and non-protein energy for the growing pig. When starch was used to progressively increase dietary non-protein energy, nitrogen retention in the castrated male pig of 33 Kg liveweight increased with each increment of added starch. The protein-sparing effect of starch was greatest when daily protein intake

exceeded 220 g. At a high dietary protein intake, increases in nitrogen retention per unit increase in dietary starch decreased as the starch intake increased, so that at high starch levels the effect of added starch on nitrogen retention was small.

In a review of amino acid utilisation by the growing pig, Low (1981) referred to studies he had recently undertaken to investigate the protein-sparing effect of increasing the protein-free energy intake of a diet, whilst maintaining a constant amino acid intake. A standard grower diet was supplemented with maize oil or starch, but no differences were detected in the rate or efficiency of amino acid use by the growing pig.

To conclude, it appears that the addition of energy to a diet providing a high level of protein-free energy will at most lead to small increases in nitrogen retention. However, precise quantitative data concerning the effects of protein-free energy intake on nitrogen retention are lacking. In experiments designed to determine the ideal balance of amino acids required for the growing pig, the supply of protein-free energy must be adequate to minimise catabolism of dietary amino acids. In a situation whereby protein-free energy is limiting and amino acids are deaminated to provide energy, some amino acids may be catabolised at a greater rate than others, thus leading to overestimation of the required levels of these amino acids.

I.1.7 The Influence of Dietary Electrolytes on the Utilisation of Dietary Amino Acids

In a biological context, acid-base balance refers to the regulation of the concentration of the anions and cations in the body fluids of animals (Mongin, 1980). Because acid-base homeostasis is an important facet of the physiology of the growing animal, it is important to consider the dietary balance of electrolytes along with their absolute requirement levels (Fletcher, 1980, and Neathery, 1981).

Austic, Madubuike, Boyd, Klasing and Riley (1982) reviewed evidence suggesting that there is an interaction, in mammalian nutrition, between dietary electrolytes and basic amino acids but also remarked that the physiological mechanisms underlying the interaction are only

poorly understood.

Dietary electrolytes are major determinants of an animal's acidity input although endogenous acid production arising from the metabolism of dietary proteins and the solublisation of salts must also be considered. Endogenous acid production contributes to acid-base balance and this contribution varies with the protein source fed to the animal (Mongin, 1980), indicating the basis for an interaction between protein metabolism and acid-base balance. A steady state is apparent when the sum of net acidity intake plus endogenous acid production is equal to the net acidity excreted in the urine. For an animal to achieve steady state, anions and cations become involved in various metabolic pathways and may thereby be unavailable for the metabolic processes associated with growth (Mongin, 1980).

O'Dell and Savage (1966) found that the addition of potassium acetate to a purified casein diet containing graded levels of arginine hydrochloride improved the growth rate of chickens at each level of arginine supplementation. The increased growth occurred in the presence or absence of added lysine hydrochloride, demonstrating that the stimulated growth was not directly related to the level of chloride ion. Further, the addition of potassium acetate to a soyabean-meal diet adequate in lysine produced no effect whereas addition to a sesame-meal diet deficient in lysine tended to depress growth.

Miller and Kifer (1970) showed that the effect on chicken growth, due to supplementation of a fish-meal diet with glutamic acid, was either positive or negative depending upon whether glutamic acid was included in the free form or as the hydrochloride. The detrimental effect of the hydrochloride salt of glutamic acid was partially overcome by adding antiacids to the diet. Also, Miller (1970) found that the utilisation of fish-meal by chickens was greatly improved by the addition of various mineral mixtures.

An intracellular potassium ion deficiency in the pig (Liebholz, McCall, Hays and Speer, 1966) is counteracted by a diffusion of sodium ions into the cell and by an increase in the cellular concentration of free basic amino acids. Madubuike, Calvert and Austic (1980) concluded that the dietary level of potassium and perhaps the dietary level of sodium, may exert

a sparing effect on the lysine requirement of growing pigs. Increased growth in response to higher levels of dietary sodium and potassium was observed, however, only when lysine was limiting for growth (0.4 to 0.5% of the diet). No effect of sodium or potassium supplementation occurred at higher dietary lysine levels (0.8 to 2.0%).

In relation to the association between dietary electrolyte levels and the utilisation of dietary protein sources, Mongin (1980) considered that the establishment of amino acid requirements in experiments using synthetic amino acids may be in error. Response or lack of response to amino acid supplementation may in some cases be due to electrolyte effects.

Endogenous acid production cannot be readily controlled by dietary formulation, but the dietary mineral intake can be manipulated. It has been argued (Mongin, 1980) that the manipulation of dietary levels of sodium, potassium and chloride is practically most useful. The relationship between these ions is usually represented as the ($Na^+ + K^+ - Cl^-$) 'concentration factor' expressed in milliequivalents per 100 g of diet.

Investigations with poultry (Mongin and Sauveur, 1977, and Johns, 1981) have established an optimum level of the ($Na^+ + K^+ - Cl^-$) factor for growth in chickens of 25 m.eq. per 100 g of diet. According to Mongin and Sauveur (1977), differences in the ($Na^+ + K^+ - Cl^-$) factor ranging from 15 m.eq. to 30 m.eq. per 100 g of diet had little effect on growth rate. Rate of growth was markedly depressed, however, when the ($Na^+ + K^+ - Cl^-$) factor was below 15 or above 30 m.eq. per 100 g of diet.

Although several investigations concerning the effects of dietary electrolytes have been conducted with chickens, little information is available regarding the effects of electrolytes on pig growth (Fletcher, 1980). Differences in avian and mammalian renal physiology and structure are well documented (Gans, 1970) and in consequence extrapolation of results from poultry to the growing pig may be misleading. Overall, it should be realised that sufficient experimental evidence is available to suggest that the balance of dietary electrolytes may be of significance in the determination of amino acid

requirements. Quantification of the effects of electrolyte balance on pig growth requires investigation.

I.1.8 Deviation from an Ideal Amino Acid Balance

Amino acid imbalance, antagonism and toxicity are important aspects of a consideration of deviation from an ideal amino acid balance (Cole, 1979).

Dietary amino acid imbalance has been extensively reviewed (Harper *et al.*, 1970, and Rogers, 1976). Amino acid imbalance will generally cause decreased food intake for animals fed *ad libitum* and consequently a decline in growth rate. Decreased utilisation of the limiting amino acid in an imbalanced amino acid mixture seems unlikely, but rather enhanced utilisation may occur (Boorman, 1980).

Payne (1972a) and the Agricultural Research Council (1975) concluded that the classical effects of amino acid imbalance are unlikely to be encountered when practical mixed diets, providing mildly imbalanced amino acid mixtures, are fed to growing animals. Also, Cole (1979) indicated that amino acid antagonisms and toxicity are unlikely to affect the performance of pigs fed practical diets. Nevertheless, Tobin, Boorman and Lewis (1973) showed that young chickens fed an imbalanced diet (25g of an imbalancing amino acid mixture added per Kg of a 100 g crude protein/Kg control diet) exhibited faster growth relative to the control chickens, even though intake of the imbalanced diet was slightly depressed. It is notable that the protein content of the control diet was low and the observed result may not be verifiable at higher dietary protein concentrations.

An interesting experimental approach was undertaken by Waldroup, Mitchell, Payne and Hazen (1976). Broiler diets were formulated using commercially available foodstuffs. Dietary crude protein varied from 190 to 269 g/Kg and it was ensured that the chicken's minimum amino acid requirements were exceeded in each diet. No effect on growth or food consumption, due to minimisation of the levels of amino acids supplied excess to requirements, was demonstrated. It was also shown by Morris and Wethli (1978) that there was no observable effect upon food intake or production, caused by mild dietary amino acid

derangement, for hens fed diets in which the degree of derangement was maintained in constant proportion to the dietary protein level.

Further information, especially specifically related to the growing pig, is required to enable concise description of the effects of imbalanced dietary protein on nitrogen retention. In the absence of such information, it appears that the degree of imbalance encountered in practically formulated diets for growing pigs is unlikely to significantly influence nitrogen retention.

In experimental situations, however, whereby large amounts of free amino acids may be added to a basal diet, the possible effects of amino acid imbalance, antagonism or toxicity can not be discounted. Such effects may be particularly important in experiments of this nature if the removal of amino acids from the basal protein source is precluded. Finally, it should be appreciated that amino acid imbalance is a relativistic concept and the term 'imbalance' is only meaningful in relation to a reference pattern of amino acids. In experimentation designed to determine an ideal balance of amino acids, then, it becomes imperative to carefully define the criterion of balance adequacy, thus allowing precise establishment of the reference pattern.

I.1.9 Conclusion

The establishment of an ideal amino acid balance affords considerable refinement in the estimation of amino acid requirements for growth in the pig. Consequently, studies have been undertaken which provide empirical estimates of the ideal amino acid balance required by the growing pig.

In these studies the maximisation or minimisation of a number of performance indices were used as the criteria for attainment of an ideal balance. The amino acid balance of the diet which gave optimal performance was equated with the ideal amino acid balance. There is, however, a drawback to this approach.

The balance of amino acids supplying the sites of protein synthesis within an animal may not be the same as the balance of amino acids provided by the diet. Whereas it is accepted that in the studies

deriving empirical estimates of the ideal amino acid balance, the pattern of amino acids which reached the sites of protein synthesis was likely to approximate an ideal balance, it cannot be assumed that this pattern was the same as the one supplied by the diet. Further, the effects of factors such as protein-free energy supply, dietary electrolyte balance and amino acid imbalance, antagonism and toxicity may also confound the estimation of ideal amino acid balance.

In this case, establishment of the ideal amino acid balance for the growing pig, giving due attention to the various confounding experimental factors, is a justifiable research aim.

CHAPTER 2

THE ASSESSMENT OF A BALANCE OF AMINO ACIDS CONSIDERED TO BE IDEAL FOR THE GROWING PIG (20 to 80 Kg LIVEWEIGHT)

I.2.1 Introduction

The review of literature focusses attention upon several factors important to the experimental determination of an ideal amino acid balance. The design of the present study gives attention to these factors.

Of particular concern was the possibility that differences in the extent and rate of digestion and absorption of dietary amino acids may have influenced the results of studies aimed at determining ideal balance. The amino acid balances of the diets fed in these studies did not necessarily equate with the amino acid balances supplying the sites of protein synthesis within the animal.

The overall objective of the study described here was to assess the adequacy of an amino acid balance considered to be ideal for the growing pig based upon estimates from the literature. A more precise estimation of the ideal amino acid balance would be pursued if the proposed balance was deemed to be inaccurate.

A pilot trial was designed in which a basal diet (amino acid balance formulated with reference to empirical estimates of ideal amino acid balance) was to be fed to the growing pig and daily urinary nitrogen excretion recorded. Comparison of the urinary nitrogen excretion of pigs fed the basal diet with literature estimates of endogenous urinary nitrogen loss, served as a guide to the adequacy of the basal dietary amino acid balance. Dependent upon the relationship between the two estimates of urinary nitrogen excretion, it was planned to follow one of two strategies.

Firstly, if the results from the pilot trial indicated that daily urinary nitrogen excretion of pigs fed the basal diet was significantly greater than the estimates of daily endogenous urinary nitrogen loss, then a series of metabolism studies would be conducted to investigate

the effect on urinary nitrogen excretion of manipulation of the pattern of amino acids in the basal diet. The successive addition or removal of synthetic amino acids (either singly or in combinations) would be made to and from the basal diet until minimum urinary urea nitrogen excretion was achieved.

In the second case, if the results from the pilot trial indicated that daily urinary nitrogen excretion of pigs fed the basal diet was close to or lower than the literature estimates of daily endogenous urinary nitrogen loss, then a further metabolism study would be conducted to verify the ideal nature of the basal amino acid balance. A comparison of urinary urea nitrogen excretion of growing pigs fed the basal diet with that of the same pigs fed a protein-free diet would serve to quantify the adequacy of the balance of the basal diet.

Before conducting the pilot trial it was considered necessary to investigate the influence of the electrolyte and protein-free energy levels of the basal diet on the nitrogen metabolism of the growing pig. Consequently, two preliminary trials were undertaken.

There are several aspects of experimental design directly relevant to the preliminary experiments, pilot trial and main experiment. It is pertinent to outline these aspects before presenting specific details of the respective experiments.

I.2.2 Aspects of Experimental Design

(i) Animals and housing.

Landrace X Large White entire male pigs (boars) of approximately 20 Kg liveweight were selected at random from a weaner pool at the Massey University Pig Research Centre. The boars were individually penned for five days before being transferred to metabolism crates whereby their movement was restricted. The metabolism crates were designed to ensure complete urine collection from male pigs. The crates were housed in a controlled-environment room, the ambient temperature being maintained at 21° ± 1°C.

(ii) Protein component of the basal diet.

It was desirable that the source of dietary protein allow complete digestion and absorption of its constituent amino acids and that the absorbed amino acids be transported to the sites of protein synthesis at similar rates.

The assumption that mixtures of crystalline amino acids are completely absorbed by growing animals has led to their use for determining amino acid requirements in the rat (Nasset, 1957), the chicken (Fisher and Johnson, 1956) and man (Rose, 1957). Crystalline amino acids were fed as the sole source of nitrogen for the baby pig (Dudley, Becker, Jensen, Terrill and Norton, 1962) and adequate performance was obtained when young pigs received a mixture of synthetic amino acids and ammonium salts as the sole nitrogen source (Mertz, Beeson and Jackson, 1952).

Even though the use of synthetic amino acids allows precise standardisation of the dietary amino acid pattern (Rérat and Lougnon, 1968) provision of the levels required by the growing pig is very expensive. Furthermore, substantial evidence is accumulated (Das and Radhakrishnan, 1976) to suggest that the absorption of peptides is considerable. In this case, the possibility of incomplete or relatively slow absorption of some amino acids, when synthetics comprise the sole source of dietary protein, must be realised.

It seemed more appropriate to simulate the natural products of digestion by feeding pigs a compound such as enzymatically hydrolysed casein. The latter is a mixture of oligopeptides and free amino acids.

Evidence supporting virtually complete digestion and absorption of the amino acids from intact casein has been provided from studies with the rat (Chen, Rogers and Harper, 1962, and Buraczewski, Porter, Rolls and Zebrowska, 1971), and the pig (Carlson and Bayley, 1970, and Low, 1979). Further to this, the jejunal absorption of amino acids from an enzymatic hydrolysate of casein was shown to be greater and more even than from an amino acid mixture simulating casein, when administered to normal human subjects (Silk, Clark, Marrs, Addison, Burston, Matthews and Clegg, 1975).

Enzymatically hyrolysed casein (New Zealand Pharmaceuticals Ltd., Palmerston North, New Zealand) was chosen, therefore, as the primary dietary protein source. Synthetic amino acids were added to the enzymatically hydrolysed casein to produce the protein source for the basal diet. The composition of the casein, amino acid mixture suggests the facility for removal or addition of specific individual amino acids. The effects of amino acid imbalance, especially amino acid antagonisms and toxicities are unlikely to be problematical in an experimental design allowing for removal and addition of free amino acids.

Support for the assumption that the protein content of the basal diet was completely digested and absorbed by the growing pig was provided by results from a digestibility study in which determinations were made of the true ileal and faecal digestibilities of nitrogen in the basal diet. Details of this study are given in part two of the thesis. Mean values of 0.996 true ileal digestibility and 1.010 true faecal digestibility were recorded for the nitrogen component of the basal diet.

(iii) Formulation of the basal diet.

The balance between the essential amino acids in the basal diet was formulated to be close to the average of the three published empirical estimates of ideal amino acid balance (Lewis and Cole, 1976; Fuller *et al.*, 1979a, and Low, 1981). The non-essential amino acid component of the diet was formulated to comprise 0.55 of the total dietary protein content, this being based on the findings of Mitchell *et al.* (1968) and Henry and Rérat (1970).

The levels of addition of synthetic amino acids to the enzymatically hydrolysed casein were based on a determined value for the amino acid composition of enzymatically hydrolysed casein.

The amino acid composition of the enzymatically hydrolysed casein and the levels of synthetic amino acids (L isomers) added to produce the protein source for the basal diet are given in Table I.2.1. The ingredient, nutrient and amino acid compositions of the basal diet are presented in Tables I.2.2, I.2.3 and I.2.4 respectively. The

amino acid composition of the basal diet (Table I.2.4) was confirmed by chemical analysis (I.2.2.vii).

(iv) Feeding level of the basal diet.

It was important to supply dietary protein to the growing pig at an amount adequate to allow a high level of body protein deposition but not at a level exceeding the animal's ability to deposit protein. An estimate of total obligatory nitrogen loss of 0.23 g/ $Kg^{0.75}$ /d was taken from Berschauer, Gaus and Menke (1980) and an upper limit to protein retention in the entire male pig (20 to 80 Kg liveweight) of 130 g/d was proposed, this being based on the observations of Kielanowski (1969). Assuming complete efficiency of use of the casein, amino acid nitrogen for maintenance and growth and based on an average protein deposition rate of 100 g/d a scale of food intake was calculated and is shown in Table I.2.5.

(v) Measurement of the efficiency of utilisation of dietary protein.

Methods of evaluating the efficiency of utilisation of dietary protein by growing animals have been reviewed by Eggum (1976) and Delort-Laval (1976). It was concluded that urinary total nitrogen and urea excretions serve as accurate measures of the extent of utilisation of dietary proteins. Brown and Cline (1972a) demonstrated the usefulness of urinary urea as a measure of the utilisation by the growing pig of maize protein supplemented with lysine and tryptophan. In a further supplementation experiment (Brown and Cline, 1972b) good agreement was found between the results obtained from a technique measuring urinary urea production and those from a standard growth assay.

The collection of urine from growing pigs and the determination of urinary total nitrogen or urea are relatively straightforward and allow several measurements of response to changes in dietary amino acid balance to be made on the same animal. The minimisation of either urinary total nitrogen or urea excretion was adopted, therefore, as the criterion for attainment of maximum utilisation of dietary protein.

Table I.2.1 The determined amino acid composition of enzymatically hydrolysed casein and amounts of synthetic amino acids added to provide the sole protein source of the basal diet.

Amino acid	Amount in casein (mg/g 'as fed')	Addition to casein (mg/g 'as fed')
Lysine	63.70	27.10 †[2]
Methionine + cystine	20.80	28.60 †[3]
Tryptophan	9.10 †[1]	2.30
Histidine	20.70	9.80
Phenylalanine + tyrosine	80.20	11.30 †[4]
Threonine	31.70	21.70
Leucine	75.40	4.95
Isoleucine	41.92	0
Valine	51.50	8.50
Non-essential amino acids	433.70	195.30 †[5]

†[1] Calculated value from Silk *et al.* (1975).

†[2] Added as 33.64 mg L lysine monohydrochloride.

†[3] Added as 22.88 mg D L methionine and 5.72 mg L cystine.

†[4] Added as 8.48 mg L phenylalanine and 2.82 mg L tyrosine.

†[5] Non-essential amino acids added were: L glycine 43.40 mg; L glutamic acid 86.80 mg; L aspartic acid 43.40 mg; L arginine 21.70 mg.

Table I.2.2 Ingredient composition of the basal diet.

Ingredient	(g/Kg air-dry weight)
Casein, amino acids	95.5
Maize oil	30.0
Purified cellulose	33.2
Sucrose	66.6
Maize starch	736.8
Mineral, vitamin supplement †	37.9

† Supplied (mg/Kg diet): Vitamin A 5.42, Vitamin D_3 0.833, Vitamin E 5.42, Vitamin B_2 3.75, Vitamin K 5.0, Vitamin B_{12} (10%) 3.33, Vitamin B_1 2.50, Vitamin B_6 3.33, Calcium-d-pantothenate 14.99, Nicotinic acid 15.83, Biotin (2%) 2.5, Folic acid 0.833, Para amino benzoic acid 19.99, Vitamin C 32.49, Inositol 193.26, Choline chloride 420, Magnesium oxide 0.833, Sodium fluoride 8.33, Copper sulphate (25%) 17.49, Cobalt sulphate (21%) 12.91, Ferrous sulphate (31%) 223.57, Manganese sulphate 87.60 and Zinc oxide 66.70; (g/Kg diet): Potassium carbonate 4.50, Calcium phosphate 20.82, and Sodium chloride 5.0.

Table I.2.3 Nutrient composition of the basal diet.

Crude protein (g/Kg air-dry weight)	-	83.0 †[1]
Crude fibre (" " " ")	-	33.2 †[1]
Gross energy (MJ/Kg air-dry weight)	-	15.62 †[2]
Apparent digestible energy (MJ/Kg air-dry weight)	-	15.00 †[3]

†[1] Calculated value.

†[2] Determined value (refer p.40).

†[3] Determined in an independent study (Moughan and Smith, unpublished).

Table I.2.4 The amino acid composition of the basal diet.

Amino acid †	(g/Kg air-dry)	(Relative to lysine = 100)
Lysine	6.589	100
Methionine + cystine	3.585	54
Tryptophan	0.827	13
Histidine	2.213	34
Phenylalanine + tyrosine	6.640	101
Threonine	3.875	59
Leucine	5.831	89
Isoleucine	3.042	46
Valine	4.354	66
Non-essential amino acids	45.642	693

† Based upon determined amino acid composition of enzymatically hydrolysed casein (Table I.2.1) and known additions of synthetic amino acids.

Table I.2.5 Feeding level of the basal diet.

Pig liveweight (Kg)	Food intake (Kg/d)	Protein intake † (g/d)
30	1.43	118.4
35	1.45	120.7
40	1.48	122.9
45	1.51	125.0
50	1.53	127.0

† Based on a diet with approximately 83.0 g crude protein/Kg air-dry weight.

(vi) Urinary collection.

Urine was collected over acid (10% sulphuric acid at 0.025 urine volume). The volume and weight of daily urine excretion were recorded and the urine then stored in deep freeze (-20°C). Representative samples (500 cm^3) were taken from each pig's total urinary excretion for the respective test period. The samples were chilled while awaiting analysis.

(vii) Analytical methods.

(a) Dry matter. Duplicate determinations were performed on food and faeces. Samples of approximately 2.0 g were placed in pre-weighed porcelain crucibles and weighed to the nearest 0.1 mg. The samples were oven-dried at 80°C to a constant weight. After cooling in a dessicator, the crucibles and contents were again weighed. Dry matter content was expressed as a proportion of the weight of the original sample.

(b) Organic matter. Duplicate determinations were made on oven-dried samples of food and faeces. Samples of approximately 2.0 g were placed in pre-weighed porcelain crucibles, weighed to the nearest 0.1 mg and then transferred to a cold muffle furnace. The temperature of the furnace was increased slowly until burning was complete, when it was quickly elevated to 450°C. The samples were left at this temperature for six hours before being removed to a dessicator and weighed when cool. Organic matter was calculated as the difference in weight between the initial sample and the ash remaining after combustion, and was expressed as a proportion of the weight of the original sample.

(c) Gross energy. The gross energy content was determined on duplicate samples of food, faeces and urine. Analysis was performed on an adiabatic bomb calorimeter (A. Gallenkamp and Co. Ltd., Christopher St., London). The air-dry food samples were pelleted before combustion whereas fresh faecal material and freeze-dried urine were wrapped in cellophane to enhance combustion. Gross energy values were expressed in megajoules (MJ) per unit weight of sample.

(d) Total nitrogen. Duplicate determinations were made on food, faecal and urinary samples. Total nitrogen content was determined using the Kjeldahl technique (Hiller, Plazin and Van Slyke, 1948). The material was digested in hot concentrated sulphuric acid in the presence of mercuric sulphate as a catalyst. This was followed by the distillation of ammonia into boric acid (Meeker and Wagner, 1933) and subsequent titration against standardised 0.1N sulphuric acid using methyl red as an indicator.

(e) Urea. The concentration of urea in urine samples was measured colorimetrically on an autoanalyser (Technicon Instruments Corp., Tarrytown, New York, U.S.A.). The method is based on the reaction of urea and diacetyl monoxime in the presence of thiosemicarbazide under acid conditions and has been described in detail by Marsh, Fingerhut and Miller (1965). The concentration of urea in each test sample was determined with reference to a standard curve. The latter was derived by analysing a series of urea solutions of known concentration. Initial studies with the autoanalyser demonstrated that the repeatability between samples was such as to obviate the need

for duplication.

(f) Creatinine. The concentration of urinary creatinine was measured colorimetrically by the Jaffe reaction on an autoanalyser (Technicon, methodology N-30, Technicon Instruments Corp., Tarrytown, New York, U.S.A.). Initial trials with the autoanalyser demonstrated that the repeatability between samples was such as to obviate the need for duplication.

(g) Amino acids. Samples of enzymatically hydrolysed casein peptone and the basal (casein/amino acid) diet were analysed for amino acid contents using ion exchange chromatography (Moore and Stein, 1963, and Hamilton, 1967). Amino acid analyses were carried out on a Beckman 119 B.L. amino acid analyser using 0.2 mg duplicate samples (on an 'as fed' basis). Protein was hydrolysed in 0.2 cm^3 of 5.8M glass-distilled hydrochloric acid in tubes sealed under a vacuum of 0.01 mm mercury. The hydrolyses were conducted at 110° ± 2°C for 24, 48 and 72 hours respectively and consistent values were found for all amino acids at these hydrolysis intervals. Tryptophan, being destroyed during acid hydrolysis, was not determined. Performic acid treatment was used to oxidise methionine to methionine sulphone and cysteine to cysteic acid (Moore, 1963).

(h) Chloride ion. The chloride ion concentrations of two diets, one based on barley and the other on enzymatically hydrolysed casein (basal diet) were determined using a combination chloride electrode (Orion Research Inc., Blackstone St., Cambridge, Massachusetts, U.S.A.).

A representative sample of food was ground to pass through a 1 mm sieve. Approximately 5 g of sample was weighed to the nearest 0.1 mg, transferred to a volumetric flask and made up to 250 cm^3 by the addition of 0.1% (v/v) acetic acid. The solution was shaken vigorously, left standing for approximately 12 hours and then thoroughly shaken again. The flask contents were centrifuged (2000 r.p.m.) and 2 cm^3 of a 5M sodium nitrate solution were added to 100 cm^3 of the supernatant liquid. The molar concentration of chloride ion in this solution was read directly off a microprocessor (Orion Research Microprocessor, Ionanalyser 901, Orion Research Inc., Blackstone St.,

Cambridge, Massachusetts, U.S.A.). The ionanalyser had been previously calibrated using standard solutions of sodium chloride in 0.1% acetic acid. Measures adopted to avoid contamination of the test solutions with chloride ions comprised the use of deionized water for the cleaning of glassware and in the preparation of solutions, and the storage of solutions in polythene containers. Six samples of each diet were analysed and average values for chloride ion concentration were calculated and expressed in g chloride per 100 g sample ('as fed').

(i) Sodium and potassium. The levels of sodium and potassium were determined in duplicate samples of a barley based diet and a casein based diet, using flame emission spectrophotometry (Perkin Elmer, 306 atomic absorption spectrophotometer, Perkin Elmer Corp., Main Avenue, Norwalk, U.S.A.). The dietary levels of sodium and potassium were expressed in g per 100 g sample ('as fed').

I.2.3 Preliminary Study One: The Influence of the Dietary $(Na^+ + K^+ - Cl^-)$ Factor on the Energy and Nitrogen Metabolism of the Growing Pig

(i) Introduction.

If optimum performance is to be attained the growing animal must be provided with a balanced supply of dietary electrolytes (Fletcher, 1980). The dietary levels of sodium, potassium and chloride may be manipulated to achieve electrolyte balance (Mongin, 1980) and the relationship between these minerals is conveniently described by the $(Na^+ + K^+ - Cl^-)$ concentration factor.

It is clear from the review of literature (I.1.7) that although the balance between dietary electrolytes may be particularly important in experiments designed to determine amino acid requirements, little is known regarding the effect of alteration of the $(Na^+ + K^+ - Cl^-)$ factor on pig growth.

The objective of the present experiment was to determine the effect of variation of the dietary $(Na^+ + K^+ - Cl^-)$ factor on the energy and nitrogen metabolism of the growing pig when a diet was

fed to pigs for the duration of a metabolism trial. It was decided to vary the ($Na^+ + K^+ - Cl^-$) factor in a high quality cereal-based pig grower diet. On the basis of the results obtained a decision was made as to the relevance of investigating the effect of manipulating the ($Na^+ + K^+ - Cl^-$) factor in the basal (casein, amino acid) diet.

(ii) Experimental procedure.

(a) Treatments. A barley-based diet known to have supported satisfactory growth in the pig served as a control. The ingredient and nutrient compositions of this control diet are given in Table I.2.6. Two test diets were also formulated which differed from the control diet only in that 0.10 and 0.50 of the cellulose in the latter were replaced by calcium chloride dihydrate and potassium carbonate, respectively.

In each diet the concentrations of sodium, potassium and chloride were expressed in milliequivalents (m.eq.) per 100 g of air-dry diet. The diets provided three distinct levels of the ($Na^+ + K^+ - Cl^-$) factor, namely 7.2, 4.5 and 21.8 m.eq./100 g diet in the control and two test diets respectively.

(b) Animals and housing. Twelve boars each of approximately 30 Kg liveweight were used in a metabolism study which comprised two replicates each of six animals. The boars were confined in metabolism crates (I.2.2.i).

(c) General conduct of study. For each replicate two boars were allocated at random to each of the three dietary treatments. The daily meal allowance was fixed at 0.11 of metabolic body weight ($W^{0.75}$) and feeding was twice daily at 08.30 h and 16.30 h. The meal was mixed with water before feeding (2.2 cm^3 water/g) and no extra water was given. All spillages of food were recorded and meal intakes were adjusted accordingly. The trial comprised a seven-day accustoming period, followed by a six-day faecal and urinary collection period. Faecal and urinary outputs of each boar were weighed daily and then stored in deep freeze (-20°C) until required for chemical analysis. The boars were weighed prior to the commencement of the accustoming period and at the beginning and end of the collection

Table I.2.6 The ingredient and nutrient compositions of the control diet.

Ingredient	Composition (g/Kg air-dry weight)
Maize	400.4
Barley	324.0
Soyabean meal	99.0
Skim milk powder	80.0
Blood meal	20.0
Bone flour	29.0
Limestone	13.0
Purified cellulose	20.0
D L methionine	0.4
Lysine monohydrochloride	1.2
Sodium chloride	3.0
Vitamin, mineral supplement †[1]	8.0
Choline chloride (50%)	2.0

Nutrient	Composition (g/Kg air-dry weight)
Crude protein	168.0
Lysine	10.0
Calcium	17.5
Phosphorus	8.1
Sodium †[2]	1.9
Potassium †[2]	5.2
Chloride †[2]	5.1
Apparent digestible energy (MJ/Kg air-dry weight)	13.7

†[1] Tasmix; Pig grower, vitamin mineral premix (Tasman Vaccines Ltd., Auckland, New Zealand.)

†[2] Determined value.

periods.

Prior to chemical analysis the faecal output of each pig was thawed, thoroughly mixed and a representative sample was taken. Samples of all diets were taken at each feeding time over the final eight days of the trial and bulked. Representative sub-samples of each diet were kept in deep freeze (-20°C) until required for chemical analysis.

Food and faecal samples were analysed for dry matter, organic matter, total nitrogen and gross energy and samples of urine were analysed for total nitrogen, urea and gross energy (refer I.2.2.vii).

The following variables were determined:

1. Apparent digestibility values for dietary dry matter, organic matter, total nitrogen and gross energy.
2. Metabolisability values for dietary total nitrogen and gross energy.
3. Daily urinary urea and total nitrogen outputs expressed on a basis of metabolic bodyweight, $W^{0.75}$ (mean liveweight over the six days of urine collection).

(d) Statistical analysis. In deciding upon the expression of urinary urea and nitrogen excretions on a liveweight basis, data from all the experiments reported in part one of this thesis were analysed to determine the most appropriate exponent of liveweight. Urinary nitrogen and urea excretion data and the respective animal liveweights were transformed by taking natural logarithms. The transformed data were subjected to linear regression analysis (Pfaffenberger and Patterson, 1977) and the slopes of the regression lines along with their standard deviations were calculated. The liveweight ranges were very narrow, however, and the error of liveweight measurement is relatively large. Correlation coefficients were calculated which showed that, at least for the present sets of data, there was very little relationship between urinary urea or nitrogen excretion and liveweight. This result was not unexpected and indicates that a larger range of liveweight would be required to establish the exponent of liveweight relevant for the expression of urinary

excretion.

In this case, and with the desire to be able to readily compare the data with determinations made by other workers, the commonly-accepted exponent of 0.75 was chosen.

The digestibility and metabolisability data along with urinary total nitrogen and urea outputs were subjected to analysis of variance (Snedecor and Cochran, 1980). One example of this analysis is given in Appendix I.1.

(iii) Results.

There were no food refusals in either replicate of the trial and all pigs appeared healthy.

No statistically significant interactions between replicates and treatments were demonstrated for any of the recorded variables. Also, there were no statistically significant differences between replicates for the variables, excepting daily urinary nitrogen and urea excretions. Thus, results presented for all variables other than daily urinary nitrogen and urea excretions are average values over the two replicates, for pigs on the same dietary treatment.

Digestibility values for dietary dry matter, organic matter, total nitrogen and gross energy are given in Table I.2.7, and the metabolisability values of dietary total nitrogen and gross energy are presented in Table I.2.8.

There was no statistically significant effect of dietary electrolyte level on the digestion of dry matter, organic matter, total nitrogen and gross energy, nor was there any statistically significant effect on the metabolism of total nitrogen and gross energy.

Daily urinary total nitrogen and urea excretions are given in Tables I.2.9 and I.2.10. As the differences between replicates for these variables were statistically significant ($P < 0.05$) the results are presented as mean values of two pigs per treatment in each of the replicates.

Table I.2.7 Mean apparent faecal digestibility values determined at three dietary levels of the ($Na^+ + K^+ - Cl^-$) factor.

	($Na^+ + K^+ - Cl^-$) factor (m.eq./100 g diet)			Pooled S.E. of treatment means	Significance level †
	4.5	7.2	21.8		
Dry matter	0.824	0.821	0.823	0.0061	NS
Organic matter	0.848	0.848	0.845	0.0056	NS
Total nitrogen	0.848	0.867	0.843	0.0084	NS
Gross energy	0.825	0.834	0.824	0.0073	NS

† In this and subsequent tables, NS = not significant;

* $P < 0.05$;

** $P < 0.01$;

*** $P < 0.001$.

Table I.2.8 Mean metabolisability values† determined at three dietary levels of the ($Na^+ + K^+ - Cl^-$) factor.

	($Na^+ + K^+ - Cl^-$) factor (m.eq./100 g diet)			Pooled S.E. of treatment means	Significance level
	4.5	7.2	21.8		
Total nitrogen	0.551	0.567	0.553	0.0011	NS
Gross energy	0.803	0.809	0.799	0.0071	NS

† $\dfrac{\text{Intake of metabolite - faecal output of metabolite - urinary output of metabolite}}{\text{Intake of metabolite}}$

Table I.2.9 Mean values of daily urinary total nitrogen excretion ($g/Kg^{0.75}$) at three dietary levels of the ($Na^+ + K^+ - Cl^-$) factor.

Replicate	($Na^+ + K^+ - Cl^-$) factor (m.eq./100 g diet) 4.5	7.2	21.8	Pooled S.E. of treatment means	Significance level (between treatments)
1	0.84	0.85	0.82	0.020	N S
2	0.75	0.83	0.78		

Table I.2.10 Mean values of daily urinary urea excretion ($g/Kg^{0.75}$) at three dietary levels of the ($Na^+ + K^+ - Cl^-$) factor.

Replicate	($Na^+ + K^+ - Cl^-$) factor (m.eq./100 g diet) 4.5	7.2	21.8	Pooled S.E. of treatment means	Significance level (between treatments)
1	1.50	1.60	1.53	0.050	N S
2	1.28	1.44	1.36		

Dietary electrolyte level had no statistically significant effect upon urinary total nitrogen or urea excretion. There was a tendency, however, for the excretion of urinary total nitrogen and urea to be higher at the intermediate value of the dietary electrolyte factor.

(iv) Discussion.

Although no statistically significant treatment effects were demonstrated, it should be appreciated that the diets were only fed to the pigs for 13 days and effects may become apparent over longer feeding periods. A growth study with chickens (Johns, 1981) in which an optimum dietary ($Na^+ + K^+ - Cl^-$) factor was established, entailed a

28-day feeding period.

Of particular significance are the relatively lower excretions of urinary urea and total nitrogen recorded in the second replicate of the study. As it was endeavoured to maintain a constant environment between replicates and the experimental animals for both replicates were taken at random from a large weaner pool, this result suggests that urinary urea and total nitrogen excretions were sensitive to apparently minor variation in environmental and/or animal factors.

Overall, it would appear that variation of the $(Na^+ + K^+ - Cl^-)$ factor between 4.5 and 21.8 m.eq./100 g of a cereal-based diet does not affect energy or nitrogen metabolism in the growing pig when the diet is fed to pigs for the duration of a short-term metabolism trial. Unfortunately data on the influence of the $(Na^+ + K^+ - Cl^-)$ factor on pig performance are sparse. Austic *et al.* (1982), however, who investigated variation in the $(Na^+ + K^+ - Cl^-)$ factor ranging from -10 to +50 m.eq./100 g diet on the performance of growing pigs over a 35-day period reported no difference in the rate or efficiency of liveweight gain. These workers concluded that a $(Na^+ + K^+ - Cl^-)$ factor of +10 m.eq./100 g diet provided a safe level of dietary electrolytes for the growing pig.

An association between the dietary level of potassium and the utilisation of lysine in pig growth has been shown (Liebholz *et al.*, 1966, and Madubuike *et al.*, 1980) but the effects of potassium ion supplementation were observed only at low dietary lysine levels.

The basal diet (casein, amino acid) which was proposed for use in the main investigations of the present study supplied adequate lysine and had a determined $(Na^+ + K^+ - Cl^-)$ factor of 16.8 m.eq./100 g diet. In accordance with the findings of this preliminary study it was assumed that electrolytic changes associated with amino acid supplementation of the basal diet would not affect the short-term nitrogen metabolism of the growing pig.

I.2.4 Preliminary Study Two: The Influence of the Protein-free Energy Level of the Basal Diet on Urinary Urea Excretion in the Growing Pig.

(i) Introduction.

As discussed in the review of literature (I.1.6) there is a fundamental relationship between protein and energy metabolism in the growing pig. The form of this relationship is complex and has not been adequately quantified (Boorman, 1980). Moreover, there is an increased requirement for energy to maintain positive nitrogen balance associated with diets containing high levels of synthetic amino acids as opposed to bound proteins (Rose, Coon and Lambert, 1954, and Leveille and Fisher, 1958).

It was proposed, therefore, to undertake a study to ascertain whether the protein-free energy level supplied by the basal (casein, amino acid) diet was adequate to minimise the catabolism of dietary amino acids. Urinary urea excretion was measured because urea production is directly related to the rate of amino acid deamination.

(ii) Experimental procedure.

(a) Treatments. The basal diet was formulated as described (I.2.2.iii) and one of a higher energy content (hereafter referred to as the experimental diet) was also prepared in which part of the maize starch in the basal diet was replaced with maize oil and sucrose. The ingredient compositions of the basal and experimental diets are given in Table I.2.11.

The diets were prepared in a Hobart dough mixer. The gross energy contents of the basal and experimental diets, as determined by bomb calorimetry, were 15.62 and 16.20 KJ/Kg air-dry diet respectively. As the two diets were isonitrogenous, two distinct levels of protein-free energy were supplied.

(b) Animals and housing. Six boars each initially weighing approximately 35 Kg liveweight were confined in metabolism crates (I.2.2.i).

Table I.2.11 Ingredient compositions of the basal and experimental diets.

Ingredient	Composition (g/Kg air-dry weight)	
	Basal diet	Experimental diet
Casein, amino acids	95.5	95.5
Maize oil	30.0	60.0
Purified cellulose	33.2	33.2
Sucrose	66.6	86.6
Maize starch	736.8	686.8
Mineral, vitamin supplement †	37.9	37.9

† Refer Table I.2.2.

(c) General conduct of study. Three boars were allocated at random to each of the two dietary treatments. After weighing, the boars were fed the diets for an accustoming period of four days followed by a further four-day period during which urine was collected. Individual liveweights were recorded at the commencement and termination of the urine collection period. Feed intake was fixed throughout the study and was apportioned according to metabolic liveweight (I.2.2.iv). The daily food allowance was split-fed at 08.30 h and 16.30 h. Each meal was mixed with water (500 cm^3) before feeding and was followed by 1000 cm^3 of water after the consumption of each meal.

Daily urine outputs were collected and bulked over each consecutive two-day period (I.2.2.vi) and representative samples were analysed for their urea contents (I.2.2.vii). Mean liveweights of the boars over the four-day urine collection period were calculated and daily urinary urea excretions relating to each two-day period were expressed per Kg of metabolic liveweight ($W^{0.75}$).

(d) Statistical analysis. Urinary urea excretion data were subjected to analysis of variance as for a split plot design (Federer, 1955). An example of the analysis of variance is given in Appendix I.2.

(iii) Results

The diets were eaten readily by the pigs and no spillages or refusals occurred. Mean daily urinary urea excretions on the two diets are presented in Table I.2.12. As urinary urea output differed significantly ($P < 0.01$) between the two consecutive collection periods the mean result of three pigs per treatment is given for each period.

Table I.2.12 Mean daily urinary urea excretion† ($mg/Kg^{0.75}$) of pigs fed at two dietary gross energy levels.

Period	Basal diet (15.62 MJGE/Kg)	Experimental diet (16.20 MJGE/Kg)	Significance level
1	378.34	348.21	NS
2	307.61	296.10	NS

† The difference between the treatment means (pooled over periods) was 20.82 mg urea/$Kg^{0.75}$/d. The standard error of the difference was 91.57.
The difference between the period means (pooled over treatments) was 61.67 mg urea/$Kg^{0.75}$/d. The standard error of the difference was 16.12.

Although urinary urea excretion tended to be lower for the high-energy diet there was no statistically significant effect of dietary energy level on the excretion of urinary urea.

(iv) Discussion

Even though mean urinary urea excretion on the two diets was not statistically significantly different, there was nevertheless a large and statistically significant difference in urea excretion between the two urinary collection periods. Also of note is that the difference in urinary urea output between the treatment means in period one was much greater compared with that in period two. These results indicate that the duration of the accustoming period was too short for

the animals to completely adjust to the synthetic diets and also suggest that had the experiment been continued, the difference between the treatment means may have decreased further.

Low (1981) found no change in the rate or efficiency of amino acid use as adjudged by growth characteristics, when a standard pig grower diet was supplemented with either maize oil or starch. The gross energy intakes for pigs fed the grower diet were similar to those for pigs fed the basal diet in the present study. Also, compared to conventional diets the protein-free energy component of the basal diet was highly digestible (0.960 apparent faecal digestibility of gross energy; refer Table I.2.3).

Consideration of the present findings and those of Low (1981) led to the conclusion that the energy level provided by the basal diet was adequate to minimise the catabolism of dietary amino acids.

I.2.5 Pilot Trial: The Estimation of the Urinary Total Nitrogen Excretion of Growing Pigs Fed the Basal Diet.

(i) Introduction.

Having established the adequacy of the electrolyte balance and protein-free energy content of the basal (casein, amino acid) diet, a pilot trial was conducted to ascertain the urinary total nitrogen excretion of pigs fed this diet. Consideration of the degree of relationship between the urinary nitrogen excretion of pigs fed the basal diet and literature estimates of endogenous urinary nitrogen loss permitted a decision as to the direction of further experimental work.

(ii) Experimental procedure.

(a) Animals and housing. Six boars each initially weighing approximately 35 Kg liveweight were confined in metabolism crates (I.2.2.i).

(b) General conduct of study. The six boars were fed the basal diet for a seven-day accustoming period. Food intake was apportioned according to metabolic liveweight (I.2.2.iv) and the pigs were split-fed their daily ration at 08.30 h and 16.30 h respectively, water (1500 cm^3) being given along with the meal at each feeding time. Following the accustoming period the boars were reweighed (although food intake was not adjusted) and placed on test. Daily urine excretion was collected over a four-day period and the boars were weighed at the end of this period. Representative urine samples were analysed for their total nitrogen content (I.2.2.vii). Individual mean liveweights over the four-day collection period were calculated and daily urinary total nitrogen excretions were expressed per Kg of metabolic liveweight ($W^{0.75}$).

(iii) Results.

One boar was removed from test suspected to be suffering from a mild enteritis condition. The remaining five boars consumed the diet readily and no food refusal or spillage was encountered. The mean urinary total nitrogen excretion of five boars fed the basal diet

is given in Table I.2.13.

Table I.2.13 The mean urinary total nitrogen excretion ($mg/Kg^{0.75}/d$) of pigs fed the basal diet.

Number of pigs	Urinary nitrogen excretion	
	Mean	S.D.
5	221.03	19.91

(iv) Discussion.

The mean estimate of urinary total nitrogen excretion of boars fed the basal diet (Table I.2.13) may be compared with estimates of endogenous urinary total nitrogen loss taken from the literature. Some recent estimates of endogenous urinary nitrogen loss are presented in Table I.2.14.

Table I.2.14 Estimates of the endogenous urinary nitrogen loss of growing pigs.

Endogenous urinary nitrogen ($mg/Kg^{0.75}/d$) †	Source of estimate
150	Fuller & Crofts (1977)
170	D'Mello, Peers & Whittemore (1976)
180	Whittemore & Fawcett (1976)
210	Whittemore, Tullis & Hastie (1978)

† Approximate values calculated from the published estimates

The 95% confidence limits for the urinary total nitrogen excretion of boars fed the basal diet in the present trial are 196.31 and 245.80 $mg/Kg^{0.75}/d$. This implies that the interval 196.31 to 245.80 includes the true mean with a long run frequency of 95%. The estimate of endogenous urinary nitrogen loss (Whittemore *et al.*, 1978) is also covered by this interval. It may be concluded, therefore, that the mean urinary nitrogen excretion of pigs fed the basal diet in the present study is unlikely to be statistically significantly different from at least the latter estimate of endogenous urinary nitrogen excretion.

Further, the average dietary protein intake for a boar (42.7 Kg liveweight) in the present trial was 122 g/d. Assuming a value for endogenous urinary nitrogen excretion of 177.5 $mg/Kg^{0.75}/d$ (mean of the literature estimates, Table I.2.14) it is calculated that 0.037 of dietary amino acid intake was deaminated. It is considered therefore that the basal dietary crude protein was utilised with an efficiency of around 0.96. The difference between the urinary nitrogen excretion of pigs fed the basal diet and any of the literature estimates of endogenous urinary nitrogen excretion is small in relation to the level of dietary nitrogen intake (a maximum of 0.05 of the basal dietary protein may have been deaminated). It is also likely that any imbalance will pertain to several of the essential amino acids rather than one amino acid in particular. Thus the level of urinary nitrogen excretion associated with such minor degrees of imbalance would be very difficult to detect experimentally.

It is concluded that catabolism of the basal dietary amino acids was very low and the excretion of urinary nitrogen for pigs fed the basal diet was largely derived from the catabolism of body protein. The amino acid balance of the basal diet is assumed to be close to ideal. For these reasons, experimentation involving the addition or removal of amino acids to and from the basal diet in an attempt to obtain a more precise estimate of ideal amino acid balance, cannot be justified.

Due to the nature of a pilot trial the above conclusion must be interpreted with a certain amount of caution. In this light it was considered thorough to further verify these initial findings with a more detailed study.

I.2.6 Main Study: Determination of the Irreducible Minimum Urinary Urea Nitrogen Loss of the Growing Pig

(i) Introduction.

Following on from the previously reported pilot trial, a major investigation was undertaken in which an estimate of the urinary urea nitrogen excretion of pigs fed the basal diet was compared with an estimate of the urinary urea nitrogen excretion of the same pigs fed a protein-free diet. The excretion of urinary urea nitrogen on a protein-free diet was accepted as synonymous with 'endogenous' urinary urea nitrogen loss.

The term endogenous urinary expenditure usually refers to the relatively static period of nitrogen excretion for animals fed a protein-free diet that succeeds catabolism of the labile proteins and is related to that of the mobilisable proteins (Peret and Jacquot, 1972). It has been suggested, however, (Waterlow and Stephen, 1966, and Waterlow, 1968) that the concept of labile protein reserve has no physiological basis and that the losses or gains in nitrogen observed when intake is altered rather represent a lag in metabolic adjustments. Moreover, the excretion of urinary nitrogen for animals fed a protein-free diet is not constant but declines with time in an approximately exponential manner (Holmes, 1965). Endogenous expenditure should be viewed as a conventional idea, relying upon measurement following an empirically defined time period (Brody, 1945).

Of primary interest in the present study was the excretion of urinary nitrogen in the actively growing pig, derived from sources other than the catabolism of dietary amino acids. The term "irreducible minimum urinary nitrogen loss" (Fuller *et al.*, 1979a) seems preferable to that of "endogenous loss" for describing such excretion.

Recent research on laboratory animals and man (Millward, Garlick, James, Sender and Waterlow, 1976) suggests that the irreducible minimum urinary nitrogen loss may depend not only on lean body mass but also on daily protein intake which influences the rate of protein turnover. Thus, the amount of nitrogen excreted daily by pigs at zero nitrogen intake may underestimate the amount which would

inevitably be lost from the tissues of growing animals.

Nevertheless, in the face of a lack of reliable alternate methods, it was decided in the present study to estimate the irreducible minimum urinary urea and total nitrogen excretion by feeding the growing pig a protein-free diet for a short period of time. The difference between the urinary urea nitrogen excretion of pigs fed the basal diet and irreducible minimum urinary urea nitrogen excretion was taken as a measure of the adequacy of the basal dietary amino acid balance. With respect to the possible underestimation of irreducible minimum urinary urea nitrogen excretion, the estimate of the efficiency of utilisation of the basal protein may be regarded as a minimum value.

(ii) Experimental procedure.

(a) Animals and housing. Eight boars each initially weighing approximately 28 Kg liveweight were confined in metabolism crates (I.2.2.i).

(b) Diets and feeding level. The basal diet was formulated as described in Section I.2.2.iii and a protein-free diet was also formulated by removing the casein, amino acid component from the basal diet and adding a small amount of di-calcium phosphate. The ingredient composition of the protein-free diet is given in Table I.2.15. Both diets were prepared in 25 Kg batches following the weighing of ingredients and their subsequent mixing in a Hobart dough mixer.

Table I.2.15 Ingredient composition of the protein-free diet.

Ingredient	Composition (g/Kg air-dry weight)
Maize oil	33.1
Purified cellulose	36.6
Maize starch	812.2
Sucrose	73.1
Di-calcium phosphate	3.0
Mineral, vitamin supplement †	42.0

(† Refer Table I.2.

The feeding scale of the basal diet was the same as in the pilot trial (refer I.2.2.iv) and that of the protein-free diet such as to ensure provision of the same quantities of all nutrients, except protein, as in the basal diet.

(c) General conduct of study. Once habituated to the metabolism crates, the pigs were introduced to the basal diet and this was fed for a 14-day accustoming period. In an attempt to randomise uncontrolled environmental effects, which had influenced the outcome of urinary urea and nitrogen excretions in the first preliminary trial (I.2.3), the starting time for the accustoming period was phased over eight days, with each of the eight boars commencing the period on a different day.

At the mid-point of its accustoming period each boar was weighed and intake of the basal diet was adjusted according to liveweight. At the termination of the accustoming periods liveweights were again recorded and the pigs were placed on test for six-day periods with no adjustment of the feeding level of the basal diet. During the six-day experimental periods each boar's urine output was collected daily, weighed, bulked and stored as described previously (I.2.2.vi).

Immediately following the termination of the urine collection period each boar was weighed and thereafter fed a protein-free diet for eight days, during which urine excretions were again collected daily, weighed and stored separately. Each boar was weighed upon conclusion of its eight-day experimental period. Throughout the study the daily dietary allowances were mixed with water (1500 cm^3) and split-fed at 08.30 h and 16.30 h. Provision was made for the collection of spillages and refusals, but neither presented a problem.

Representative samples of urine were analysed for their contents of urea, total nitrogen and creatinine (I.2.2.vii). The coefficient of variation of daily creatinine excretion for each pig was calculated from data relating to the 14 days of urinary collection, and the mean coefficient of variation pertaining to the eight pigs was taken as an indication of the completeness of urine collection (Pierro and Johnson, 1970, and Das and Waterlow, 1974).

Mean daily excretions of urinary urea and urinary urea and total nitrogen on the basal diet were calculated and expressed per Kg of metabolic liveweight ($W^{0.75}$). Urinary urea nitrogen output was determined from the corresponding urinary urea excretion on the basis that one g urea contains 0.467 g nitrogen. The mean daily excretions of urinary urea and urinary urea and total nitrogen on the protein-free diet were calculated from urine outputs over days two to seven inclusive, of the eight-day collection period, and these were expressed per Kg of metabolic liveweight ($W^{0.75}$).

(d) Statistical analysis. Differences between mean daily excretions of urinary urea and urinary urea and total nitrogen on the basal and protein-free diets were subjected to a paired t-test (Pfaffenberger and Patterson, 1977).

(iii) Results.

Individual liveweights of the boars at the beginning, mid-point and end of the 14-day urinary collection periods are given in Table I.2.16.

Liveweight change was positive for all pigs over the six-day period they received the basal diet, and thereafter the pigs maintained or slightly increased liveweight during the eight days they were fed a protein-free diet.

Mean daily urinary creatinine excretion for the eight boars over the total urinary collection period was 105 mg/$Kg^{0.75}$ and the coefficients of variation for individual pigs over this period ranged from 7.8 to 13.5%. The mean coefficient of variation of daily urinary creatinine excretion over all pigs was 10.4%.

Mean daily excretions of urinary urea and urinary urea and total nitrogen on the basal and protein-free diets are given in Table I.2.17.

Table I.2.16 Liveweights of the boars during the urinary collection periods.

Pig. No.	Liveweight (Kg) Day 1	Day 7	Day 14
1	34.0	37.5	38.5
2	38.0	41.3	42.6
3	33.5	38.5	39.0
4	36.5	40.0	40.0
5	40.0	43.0	43.1
6	40.0	44.0	44.9
7	45.2	46.5	47.0
8	36.5	40.2	40.2

Table I.2.17 Mean daily urinary excretions ($mg/Kg^{0.75}$) of urea, urea nitrogen and total nitrogen for pigs fed the basal and protein-free diets.

	Diet: Basal		Protein-free	
	Mean	SE	Mean	SE
Urea	198	19.0	42	6.0
Urea nitrogen	93	8.8	19	2.8
Total nitrogen	213	9.9	109	6.0

Differences between the mean excretions on the basal and protein-free diets were statistically significant ($P < 0.001$) for each of the urinary metabolites shown in Table I.2.17.

(iv) Discussion.

In accordance with the objective of the present experiment it was critical that all urine voided be collected. To this end the boars were kept in metabolism crates which had been adapted to ensure, as far as was possible, complete urine collection. If urinary collection was complete it is expected that the day-to-day variation in urinary creatinine excretion would be low. Calculation of the coefficient of variation of daily urinary creatinine excretion thus provides a check for the completeness of urine collection.

The present estimate of daily urinary creatinine excretion is in close agreement with the published estimates of Aulstad (1970), Duggal and Eggum (1978) and Bate and Hacker (1981). Furthermore, in accordance with the findings of Kumar, Land and Boyne (1959), Waterlow, Neale, Rowe and Palin (1972), Das and Waterlow (1974) and Rand, Scrimshaw and Young (1981), the mean coefficient of variation of daily urinary creatinine excretion calculated over the eight pigs in the present study, suggests complete urine collection.

Between-animal variation in urinary urea excretion within treatments was high, which was also observed by Fuller *et al.* (1979a) when determining the optimal amino acid supplementation of barley for the growing pig. They reported a mean rate of urinary nitrogen excretion, after progressive dietary supplementation, of 270 mg $N/Kg^{0.75}/d$ which can be compared with that of 213 mg $N/Kg^{0.75}/d$ for pigs fed the basal diet in the present study.

The present estimate of daily urinary nitrogen excretion (109 mg/$Kg^{0.75}$) for pigs fed the protein-free diet is low in comparison with values of 150 and 170 $mg/Kg^{0.75}$ reported by Fuller and Crofts (1977) and D'Mello *et al.* (1976), respectively. Mean daily urinary urea nitrogen excretion of pigs fed the protein-free diet in the present study (19 $mg/Kg^{0.75}$) is also lower than the value of 31 $mg/Kg^{0.75}$ given by D'Mello *et al.* (1976). Furthermore, urea nitrogen excretion comprised 0.43 of total nitrogen excretion for pigs fed the basal diet in the present study and declined to 0.18 of total nitrogen excretion following their transfer to a protein-free diet.

Under a protein-free feeding regime for the growing pig D'Mello *et al.* (1976) also noted that urinary urea nitrogen was 0.18 of urinary total nitrogen while according to Platt and Heard (1958) the proportion of urinary total nitrogen present as urea nitrogen is less than 0.30 in malnourished human subjects.

The low excretion of urinary urea nitrogen for pigs fed a protein-free diet after receiving a well-balanced one is not contrary to expectation. If ideal protein is fed to pigs, at a suitably low level, then by definition urinary urea excretion reflects the deamination of amino acids derived from the net difference between the rates of body protein catabolism and resynthesis. Most amino acids liberated by body protein breakdown are reutilised (Millward *et al.*, 1976) and it is expected therefore, that liver enzymes involved in deamination reactions will have a low rate of activity when balanced protein is fed to the growing pig. Upon inhibition of the balanced protein intake (protein-free diet) the rates of activity of the enzymes will decrease further and the initially low urea excretion will fall resultant from a "shutting down" in the urea cycle (Millward *et al.*, 1976). Further to this, Schmidt-Nielsen (1977) demonstrated that the mammalian kidney has the ability to return a greater fraction of filtered urea to the blood when plasma urea levels are low, as on a low protein diet, than when the levels are high.

It is also postulated that under the feeding of ideal protein the rate of activity of activating enzymes which catalyse the first step of protein synthesis will be high. There is evidence (Gaetani, Paolucci, Spadoni and Tomassi, 1964, and Stephen, 1968) that the rate of activity of these activating enzymes in the liver increases when protein intake is reduced. In addition, the high energy levels of the basal and protein-free diets will exert a protein-sparing effect. Fuller and Crofts (1977) predict that at a maximum attainable starch intake for the growing pig the reduction in urinary nitrogen excretion is 0.60 of fasting nitrogen loss.

In the present study, the differences in mean daily urinary total nitrogen excretion between pigs fed each of the two diets was 104 mg/$Kg^{0.75}$ and the difference in daily urinary urea nitrogen excretion was 74 mg/$Kg^{0.75}$. In this instance the estimated decrease in daily

urinary total nitrogen excretion unexplained by a decrease in urinary urea nitrogen excretion was 30 mg/$Kg^{0.75}$ which although being statistically significant ($P < 0.01$) represents only 0.29 of the decrease in urinary total nitrogen excretion. The major decrease in urinary total nitrogen excretion (0.710) was attributable to a decrease in urea nitrogen excretion, and urea production, moreover, is directly related to the rate of amino acid deamination.

Accepting, for the moment, that the urea nitrogen excretion of the pigs fed the protein-free diet is representative of the irreducible minimum urinary urea nitrogen excretion, it can be calculated that 74 mg N/$Kg^{0.75}$/d may have been voided in the urine derived from the deamination of amino acids from the basal diet. It is pertinent to enquire if further reduction of urinary urea nitrogen excretion could have been attained by alteration of the dietary amino acid balance.

For a boar of 40 Kg liveweight (mean liveweight in present trial), a daily urinary urea nitrogen excretion of 74 mg/$Kg^{0.75}$ is assumed to be derived from the deamination of 7.36 g protein, which equates with 0.060 of the daily dietary protein intake. This implies that dietary basal protein was utilised with an efficiency of 0.940. Recognising, however, that the present value for irreducible minimum urinary urea nitrogen loss may be underestimated, then the efficiency factor for the utilisation of the basal protein (0.940) should be regarded as a minimum value. Whittemore (1983) stressed the need for a higher estimate of irreducible minimum urinary nitrogen loss than that obtained by feeding a protein-free diet and suggested a value of at least 210 mg/$Kg^{0.75}$/d. The latter estimate was obtained by feeding a protein maintenance ration to the growing pig (Whittemore *et al.*, 1978). If this estimate (210 mg N/$Kg^{0.75}$/d) is used to correct the urinary total nitrogen excretion of a 40 Kg boar fed the basal diet employed in the present study, it is predicted that dietary protein was utilised with an efficiency of 0.997.

It appears, then, that the basal protein was utilised for growth with an efficiency of 0.940 or greater. On this basis it is concluded that the amino acid balance of the basal diet approximates an ideal amino acid balance for the growing pig.

The above discussion highlights the need for a more precise method of determining the irreducible minimum urinary nitrogen loss of the growing pig. A seemingly more appealing approach to that of feeding pigs a protein-free diet is the estimation of urinary nitrogen excretion at zero protein intake from a linear regression equation relating urinary nitrogen excretion to dietary protein intake (Berdanier, Brush and Fisher, 1967). This approach, however, also poses certain theoretical and practical problems (Holmes, 1965).

It must also be stressed that a key assumption in the present study is that maximum utilisation of absorbed protein for growth, determined by minimum urinary nitrogen excretion, implies ideal dietary amino acid balance. This assumption may be challenged for it is possible that essential amino acids may be transformed into non-essential amino acids if the basal non-essential level is less than ideal. Supplementation of the basal diet with non-essential amino acids coupled with successive removal of essential amino acids from the basal diet is a suggested procedure meriting further investigation.

I.2.7 Concluding Discussion

The findings of the main metabolism study (I.2.6) confirmed the results of the pilot trial (I.2.5) in demonstrating that the protein source of the basal (casein, amino acid) diet was utilised for growth in the pig, with near-complete efficiency.

In proposing the present study, some recently-published estimates of ideal amino acid balance for the growing pig were queried. In spite of this it was not considered possible to improve the amino acid balance of a diet in which the essential amino acid balance was formulated around the empirically derived balances of Lewis and Cole (1976), Fuller *et al.* (1979a) and Low (1981), and the non-essential amino acid component was supplied in accordance with the findings of Mitchell *et al.* (1968) and Henry and Rérat (1970). It thus appears that the combined effect of differences in the degree and rate of digestion and absorption of amino acids in the diets used in the above mentioned studies did not cause the pattern of amino acids supplying the sites of protein synthesis within the animal to deviate greatly from the pattern in the diet. The ideal nature of the amino acid balance, proposed to be ideal based upon empirical estimates given in the literature, is confirmed.

Comparison can be made between the present estimate of ideal balance and that recommended by the Agricultural Research Council (1981). As shown in Table I.2.18, values for tryptophan, histidine and threonine are in close agreement. The determined values for methionine plus cystine and phenylalanine plus tyrosine are greater than those recommended whereas the determined values for leucine, isoleucine and valine are lower than the recommendations. Of particular note is the discrepancy between the present determined value and that recommended by the Agricultural Research Council (1981) for the non-essential amino acid component, albeit the recommended value is a maximum.

The present estimate of the ideal level of non-essential amino acids must be interpreted with caution, however, for it is possible that essential amino acids are transaminated to non-essential amino acids if the non-essential amino acid component is limiting. It is assumed in the present study that transamination of amino acids was

minimal. Also, unless one amino acid was transaminated to a greater extent than others then the balance between the essential amino acids would not be affected by transamination reactions.

According to the recommendation of the Agricultural Research Council (1981), isoleucine is the first limiting amino acid in the presently-derived ideal amino acid balance. Accepting that isoleucine is limiting to the extent indicated by the Agricultural Research Council (1981), it can be shown (Appendix I.3) that a boar of 40 Kg liveweight fed 1480 g/d of the basal diet would excrete 2590 mg urinary urea nitrogen daily derived from the deamination of imbalanced dietary amino acids. The findings of the main study (I.2.6) indicate, however, that a boar of identical liveweight and nutritional circumstance excreted a maximum 1177 mg of urinary urea nitrogen daily derived from the catabolism of basal dietary amino acids. This suggests that the ideal level of isoleucine for the growing pig as recommended by the Agricultural Research Council (1981) is an overestimate.

Further support for the estimate of ideal amino acid balance found in the present study is provided by Fuller, Cadenhead and Chen (1983). These workers attempted to validate the ideal amino acid balance recommended by the Agricultural Research Council (1981) by measuring the biological value of a casein-based diet before and after supplementation with various synthetic amino acids. The results showed that the Agricultural Research Council's estimate of the ideal level of methionine plus cystine is low and that the recommended ideal balance is also likely to be marginally limiting in one or more other amino acids. Comparison of the ideal amino acid balance determined in the present study with that recommended by the Agricultural Research Council (1981) indicates that the level of methionine plus cystine is low in the Agricultural Research Council's balance and suggests that the level of phenylalanine plus tyrosine may also be limiting in the recommended balance. It is interesting to note that the recommended level of threonine (Agricultural Research Council, 1981) appeared to be adequate (Fuller *et al.*, 1983) which is also expected on the basis of the present findings.

Table I.2.18 Comparison of the estimate of ideal amino acid balance found in the present study with that recommended by the Agricultural Research Council (1981).

Amino acid	Ideal amino acid balance	
	Present study	Agricultural Research Council recommendation
Lysine	100	100
Methionine + cystine	54	50
Tryptophan	13	14
Histidine	34	33
Phenylalanine + tyrosine	101	96
Threonine	59	60
Leucine	89	100
Isoleucine	46	54
Valine	66	70
Non-essential amino acids	693	857

The present estimate of ideal amino acid balance bears a close resemblance to the pattern of amino acids in pig muscle protein (Table I.2.19). This similarity is understandable as although 0.80 of amino acid flux in the growing animal is related to body protein breakdown (Millward *et al.*, 1976), the resynthesis of body protein is likely to be very efficient. The amino acid requirements for growth will be dictated, therefore, by the requirements for new protein synthesis and a considerable propórtion of new protein synthesis consists of the synthesis of lean tissue.

Data for the growing rat published by Millward, Brown and Odedra (1981), however, indicate that the liver and gastrointestinal tract together and muscle and skin each contribute about a quarter of total whole body protein synthesis. Also, the liver combined with the gastrointestinal tract comprises a significant proportion of the

Table I.2.19 Comparison of the estimate of ideal amino acid balance found in the present study with the amino acid patterns of pig muscle, liver and intestinal tissues.

Amino acid	Amino acid pattern			
	Muscle†[1]	Liver†[2]	Intestine†[2]	Ideal balance
Lysine	100	100	100	100
Methionine + cystine	48	50	44	54
Tryptophan	-	-	-	13
Histidine	47	33	31	34
Phenylalanine + tyrosine	91	118	107	101
Threonine	56	55	55	59
Leucine	98	118	105	89
Isoleucine	63	62	-	46
Valine	65	77	74	66
Non-essential amino acids	623	578	613	693

†[1] Duée *et al.* (1980)

†[2] from Munro and Fleck (1969).

bodyweight of a mature mammal (Munro, 1969). These considerations imply that the amino acid patterns of skin, liver and gastrointestinal tract may influence the pattern of amino acids regarded as optimal for animal growth as predicted from the pattern of amino acids in muscle protein.

The level of methionine plus cystine in the presently-estimated ideal balance is higher than the level in muscle protein and this may be explained by the high concentration of cystine in skin and hair (Munro and Fleck, 1969) and the continuous losses of skin and hair from the body of the growing pig. The high level of phenylalanine plus tyrosine in relation to the level in pig muscle may be explained

by the relatively high levels of these amino acids in liver and intestinal tissue (Table I.2.19). An analagous relationship is not apparent, however, in respect of leucine and isoleucine although the level of isoleucine in pig intestinal tissue is not given in Table I.2.19.

The ideal estimate for histidine is significantly lower than the level in pig muscle but is close to levels found in liver and intestinal tissue. Boorman (1980) has speculated that since the amino acid composition of muscle does not differ greatly among animal species, the ideal amino acid balance may be similar for different animal species.

It is concluded that the studies reported here provide an estimate of the ideal amino acid balance for the growing pig. The ideal balance is given in units of truly absorbed amino acids relative to lysine and may be used with confidence for predicting the amounts of balanced protein supplied by various dietary amino acid mixtures. The ability to make the latter prediction is centrally important in estimating dietary protein quality.

PART II

THE DIGESTIBILITY OF AMINO ACIDS IN PROTEINS FED TO THE GROWING PIG

CHAPTER 1

REVIEW OF LITERATURE

II.1.1 Introduction

Digestibility is a key facet of dietary protein quality. 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 process of digestion and absorption of protein in the growing pig and the methods used for measuring the digestibility of amino acids are reviewed in this chapter.

II.1.2 The Digestion and Absorption of Protein

The role of endogenous enzymes in the digestion of protein in the growing pig has been the subject of detailed review (Rérat, Corring and Laplace, 1976, and Kidder and Manners, 1978). Further, the important function of the brush-border oligopeptidases and the intracellular peptidases of the mucosal cells in integrated digestion and absorption has been described by Das and Radhakrishnan (1976) and transport systems involved in the absorption of amino acids have been adequately discussed by Davenport (1977).

In addition to the action of endogenous enzymes in protein digestion, the proteolytic, deaminative and decarboxylative activities of the intestinal microflora must be considered. The alimentary tract of the pig is densely populated with microorganisms which undoubtedly interfere with the digestive process. Bacterial activity is considered to be mainly concentrated in the large intestine, this being in accordance with the hindgut having the most dense bacterial population (Rérat, 1978).

Although a lower population of microorganisms is apparent in the upper digestive tract, the possible effect that this may have on digestion cannot be discounted especially in view of the rapid turnover of bacterial cells (Boorman, 1976). Bacterial proteases may enhance the digestion of dietary protein in the small intestine (Coates, 1976), although no substantial differences for the net protein values of a range of dietary proteins were observed when they were fed to germ-free

and conventional chickens (Salter and Fulford, 1974). Also, it is possible that microbial fermentation in the stomach and small intestine of the pig results in the disappearance of amino acids from the ileum (Cranwell, 1968). There is evidence of bacterial degradation of amino acids in the small intestine of the chicken (Skurray and Cumming, 1975).

Seemingly of greater nutritional significance, however, is the considerable effect that the microflora of the large intestine can have on protein remaining undigested at the terminal ileum. Nitrogen entering the large intestine consists of undigested dietary nitrogen and products of endogenous origin (digestive enzymes, mucoproteins, desquamated cells, urea, amino acids produced by cell catabolism, serum albumen and microorganisms) (Rérat, 1978). This nitrogen may be acted upon by the hindgut bacteria leading to net appearance or disappearance of amino acids between the ileum and the rectum (Rérat, 1981).

In certain cases (Holmes, Bayley, Leadbeater and Horney, 1974; Mason, Just and Bech-Anderson, 1976, and Low, 1979) the faecal amounts of some amino acids have been higher than the amounts measured at the terminal ileum, indicating that net bacterial synthesis has occurred. Such synthesis may be particularly significant for methionine (Just, 1980). The assimilation of nitrogenous materials into microbial cell components is based principally on ammonia (Mason, 1980), although peptides and amino acids can be utilised directly by some bacterial species (Payne, 1975).

The microorganism population of the hindgut is capable of intense metabolic activity (Coates, 1976) and so it is not surprising that Mason *et al.* (1976) found that in excess of half the nitrogen in the faeces of pigs, fed various diets, was contained in microbial cells. Further, Stephen and Cummings (1980) observed that approximately half of the dry-matter and two-thirds of the nitrogen in human faeces were of microbial origin. Microbial protein is eventually excreted in the faeces and in the case of the growing pig is of no nutritional value to the host.

Mason (1980) has cited evidence demonstrating the proteolytic, deaminative and decarboxylative activities of caecal bacteria and the capability of these bacteria to digest amino acid-sugar complexes,

bacterial mucoproteins, mucosal residues, mucus, uric acid and urea. The degradative activities of the bacteria lead to the formation of ammonia (Michel, 1966; Castell and Moore, 1971, and Hoover and Heitmann, 1975) though amine production may also be significant (Hill, Kenworthy and Porter, 1970, and Milne and Asatoor, 1975).

The quantitative importance of the hindgut microfloral digestion in the pig has been a matter of some contention. Poppe and Meier (1977), who fed highly digestible casein and wheat/casein diets to pigs fistulated at the terminal ileum and observed no marked difference between ileal and faecal digestibility coefficients, concluded that the effects of microbial digestion in the hindgut were only very slight. Furthermore, these authors proposed that any observed increase in nitrogen digestibility in the hindgut is mainly related to technical problems concerning the location of the ileal cannulae, with ileal and faecal digestibilities becoming more similar the closer the placement of the cannula to the ileo-caecal junction. In direct contrast to these findings, Zebrowska and Buraczewski (1977), feeding casein-, soyabean- and meat-and-bone-meal-based diets to pigs fitted with simple cannulae at the terminal ileum, concluded that the digestion of nitrogen in the large intestine was considerable. This study demonstrated that the difference between ileal and faecal digestibility estimates was greater the lower the digestibility of dietary nitrogen in the small intestine. Such an observation had been noted earlier by Ivan and Farrell (1976) and was confirmed by Sauer, Stothers and Phillips (1977)† and Tanksley and Knabe (1980). It is now generally agreed (Rérat, 1978; Just, 1980; Mason, 1980; Rérat, 1981, and Low, 1982) that the effect of the hindgut microflora on protein digestion in the pig is quantitatively significant.

Despite protein digestion in the hindgut being significant, there is little evidence that the mucosa of the large intestine of the growing pig is capable of transporting amino acids (Tanksley and Knabe, 1980). Binder (1970) showed that mammalian colonic mucosa does not have significant absorptive capacity and according to Wrong, Edmonds and Chadwick (1981) the true active transport of amino acids across the large bowel has not been convincingly demonstrated for any amino acid in any adult animal. The proximal colon of the newborn piglet, on the other hand, has the ability to actively transport methionine (James and Smith, 1976), and Olszewski and Buraczewski (1978) provided evidence that

† Hereafter Sauer *et al.* (1977a).

asparagine, serine, threonine, tyrosine, arginine, histidine, lysine and aspartic acid were absorbed to varying degrees from isolated pig caecum examined *in situ*, whereas the remaining ten determined amino acids were not absorbed.

It seems (Rérat, 1981) that the nitrogenous compounds disappearing from the hindgut enter the blood mainly as products of amino acid breakdown, these being poorly used by the animal for anabolic purposes. Infusion experiments (Zebrowska, 1975; Sauer, 1976, and Just, Jorgensen and Fernández, 1981) have demonstrated that nitrogen although well absorbed by the large intestine is almost entirely excreted in the urine. Rérat (1978) has suggested that a proportion of the absorbed ammonia may be utilised in the synthesis of non-essential amino acids to meet the maintenance protein requirement of adult animals, but agrees that evidence indicates that nitrogenous substances absorbed in the large intestine have little nutritional value for the growing animal.

II.1.3 The Measurement of Protein Digestibility

The term 'digestibility' refers to the combined effects of digestion and absorption but provides no information as to the extent of utilisation of the absorbed nutrients (McNab, 1976). The terms 'digestibility' and 'availability' are often used synonymously but availability should strictly apply to the digestion, absorption and subsequent metabolism of a nutrient (Low, 1982).

Digestibility values for amino acids may overestimate their availability, especially in materials damaged by excess heat during processing (Carpenter, 1973). The latter author provides the example of heated milk powders, but theorises that in materials which do not contain reducing sugars, digestibility measurements should closely equate with estimates of availability. The absorption of non-utilisable forms of amino acids, moreover, is not a major limitation of the digestibility assay (Austic, 1983) as urinary losses represent only a small fraction of amino acid intake in feedstuffs which have been deliberately damaged by heat treatment.

Low (1982) has distinguished between three types of digestibility

measurement. Apparent digestibility may be defined as the difference between the amount of an amino acid in the diet, and in ileal digesta or faeces as a proportion of that in the diet. True digestibility is defined similarly except that the amounts of endogenous amino acids in faeces or ileal digesta are subtracted from the total amount of amino acids in the faeces or ileal digesta. Real digestibility is calculated in the same manner as apparent digestibility but applies when the difference between the intake and output of faecal or ileal amino acids is measured using nitrogen isotopes.

The estimation of amino acid digestibility in dietary protein sources has often been made following the method outlined by Kuiken and Lyman (1948). In the traditional approach, the amount of an amino acid truly absorbed from the alimentary canal is estimated as the difference between the amount of the amino acid consumed in the diet and that voided in the faeces, after correction of the latter for the amount presumed to be excreted in the metabolic faecal nitrogen. Because of difficulties in measuring the excretion of metabolic faecal nitrogen (Mason, 1980), apparent faecal digestibility is frequently measured.

Although the faecal method is highly reproducible (Eggum, 1977), its accuracy has been criticised due to the modification of nitrogenous compounds by the hindgut microflora. Taverner (1979) conjectured that the inability of faecal analysis to account for differences in hindgut microbial activity may explain the difficulties experienced in reconciling pig growth data with faecal nitrogen digestibility data, in experiments with various processing techniques, with oats (Crampton and Bell, 1946), wheat (Lawrence, 1967) and barley (Cole, Dean and Luscombe, 1970).

It was argued by Payne, Combs, Kifer and Snyder (1968) that ileal analysis should provide a better index of amino acid digestibility. In accordance with the conclusion (Terpstra, 1977) that bacterial amino acid breakdown occurs at a greater rate than synthesis, most workers have found that amino acid digestibility estimates based on ileal analysis are lower than those based on analysis of the faeces.

Sauer, Just, Jorgensen, Fekadu and Eggum (1980) and Tanksley and

Knabe (1980) referred to numerous studies conducted over the last decade where the apparent ileal digestibilities for most amino acids were lower than digestibilities measured over the entire digestive tract. Large differences between ileal and faecal apparent digestibility values have been typically found for proteins of low digestibility (Zebrowska and Buraczewski, 1977).

The study of Jorgensen and Sauer (1982) is of particular interest in that the digestibilities of amino acids in protein sources commonly used in pig diets were investigated. Large differences were found between ileal and faecal digestibilities of amino acids in soyabean-, sunflower-, fish- and meat-and-bone-meal, differences being greatest for threonine, phenylalanine and lysine and least for arginine and methionine. These workers also demonstrated considerable variation among the ileal digestibilities of amino acids within a protein source and concluded that in correcting gross to digestible amino acid levels, the use of a common correction factor for amino acids (eg. protein digestibility) may not be appropriate. The review by Zebrowska (1978) further highlights the significance of the differences between faecal and ileal estimates of apparent digestibility of nitrogen and animo acids in foodstuffs used in practical dietary formulation.

Despite general agreement that the measurement of ileal amino acid digestibility is theoretically superior to that of faecal digestibility, there are still reservations (Just, 1980) regarding the practical advantages of adopting the ileal technique. Two recent and comprehensive reviews (Low, 1980a, and Rérat, 1981) agreed, however, that the determination of apparent digestibility of amino acids at the terminal ileum is the more acceptable method. Low and Partridge (1980a) in summarising a recent discussion on the use of ileal as opposed to faecal digestibility estimates, noted that ileal digestibility is more difficult to measure than faecal digestibility, and surgery and implantation of cannulae may affect the outcome. Some workers have found relatively greater variation between pigs and between measurements on the same pig for ileal measurement although others disputed that this was so. The suggestion was made that part of the variability of apparent ileal digestibility may be due to variation in endogenous secretion. No conclusion could be drawn, however, regarding the practical significance of using true ileal digestibilities as opposed to apparent values. The

discussion concluded in agreement that ileal measurement of amino acid digestibility is likely to be more meaningful than faecal measurement.

Despite the importance of correcting apparent ileal digestibility estimates for the contribution of amino acids of endogenous origin, no suitable method for determining the amount of endogenous protein remaining undigested at the terminal ileum is currently available (Low, 1980a). Two approaches have been used to quantify endogenous levels of amino acids appearing at the terminal ileum. These are; analysis of ileal digesta from animals given a protein-free diet, and the feeding of graded amounts of a single protein source followed by extrapolation to zero intake of amino acids of the linear regression of ileal amino acid output on dietary amino acid intake. Both these methods are open to strong criticisms (Boorman, 1976; McNab, 1976; Szentmihályi, 1977, and Low, 1980a).

It is well established that digestive secretions are influenced by the quantity and quality of dietary protein (Partridge, Low, Sambrook and Corring, 1979, and Corring, 1980). The amount of nitrogen excreted from the small intestine by animals fed a protein-free diet may be considerably lower than the amount of endogenous ileal nitrogen excreted on a diet containing protein (Buraczeska, 1980). In an attempt to overcome this limitation of protein-free diets some researchers have supplemented protein-free diets with small amounts of a highly digestible protein source (Mason, 1980).

Extrapolation from diets containing graded levels of a single protein source, being closer to the practical situation, might be expected to provide a more reliable estimate of endogenous amino acids at the terminal ileum than the use of protein-free diets. In the one comparable study reported for pigs (Taverner, Hume and Farrell, 1981) the quantities of amino acids present at the terminal ileum were different for the two methods of determination, but whether the extrapolation method quantifies endogenous ileal amino acid output accurately remains doubtful. It appears that the amino acid composition as well as the quantity of endogenous secretion are influenced by the method of determination. Relatively high levels of glycine and proline found in the ileal digesta of pigs seem to be characteristic of protein-free or low-protein diets (Taverner, 1979). There is evidence that with both

methods currently used for the determination of endogenous ileal amino acid output, the level of dietary fibre influences the outcome (Taverner *et al.*, 1981), probably through an effect on mucin production.

True digestibility is a fundamental property of the ingredient regardless of the dietary conditions under which that ingredient is fed to the animal (McNab, 1976). True digestibilities of dietary amino acids (albeit from faecal analysis on rats) have been shown to be directly related to the amino acid digestibilities of the individual protein components of the mixed diet (Eggum and Jacobsen, 1976). This additivity seems unlikely to apply, however, with apparent amino acid digestibilities which will only be meaningful under strictly standardised conditions (Eggum, 1977). Taverner (1979), in studies with growing pigs, found that apparent ileal amino acid digestibility was influenced by the protein level of the diet but not so true ileal digestibility. Apparent digestibility increases curvilinearly with increasing dietary protein concentration (Eggum, 1977). Taverner (1979) and Sauer *et al.*, (1980) also noted that the effect of level of endogenous protein on the apparent digestibility of protein was greater at lower dietary protein levels. In fact, Haydon, Tanksley and Knabe (1980) found no effect of the level of crude protein on apparent ileal nitrogen and amino acid digestibility when a diet containing 160 g/Kg crude protein was fed to growing pigs at daily intakes exceeding 0.03 of bodyweight.

It has sometimes been argued (Just Nielsen, 1968, and Low, 1982) that on a practical basis, apparent digestibility coefficients are more relevant than true digestibility coefficients since both undigested dietary and endogenous amino acids are lost to the animal and thus have to be accounted for in diet formulation. The endogenous excretion of an amino acid, however, is accounted for in the estimation of the requirement for that amino acid and there is also a trend (Agricultural Research Council, 1981) towards expressing amino acid requirements for pig growth in units of truly absorbed amino acids. Hopkins (1981) expressed the desirability of using true as opposed to apparent digestibility data in human nutrition.

In spite of the possible limitations of estimates of apparent amino acid digestibility, however, and with a view to the difficulties in determining true amino acid digestibilities, it seems that estimates of the

apparent ileal amino acid digestion and absorption may be useful in practical dietary formulation (Low and Partridge, 1980a, and Austic, 1983). Nevertheless, experimental information concerning the validity of apparent ileal digestibility coefficients for predicting the level of digestion and absorption of amino acids in the pig is greatly needed (Braude, 1980; Just, 1980, and Agricultural Research Council, 1981). Although some validation trials have been undertaken (Tanksley and Knabe, 1980, 1982; Fuller, Baird, Cadenhead and Aitken, 1981, and Low, Partridge, Keal and Jones, 1982), the findings have been contradictory.

II.1.4 Ileal Analysis as a Measure of Amino Acid Digestibility

Although apparent ileal digestibility estimation is probably the most acceptable technique, currently available, for the measurement of digestion and absorption of amino acids in foodstuffs, the choice of methodology for ileal analysis is not straightforward. With small animals it is feasible to collect ileal digesta at slaughter, and this technique has been used in the rat (Buraczewski *et al.*, 1971, and Taverner, 1979) and in the chicken (Payne *et al.*, 1968; Soares and Kifer, 1971, and Varnish and Carpenter, 1971).

Horszczaruk (1972a) and Cho and Bayley (1972) collected ileal digesta following the slaughter of pigs. Their method, however, is open to the criticism that shedding of mucosal cells into the gut lumen may occur at the time of death. This receives the support of Horszczaruk (1972a, b, c) who found considerably more nitrogen in the small intestine of slaughtered pigs as compared with those cannulated at the terminal ileum. A major shedding of epithelial cells into the gut lumen of sheep has been shown to occur when the animals were slaughtered by using a humane killer (Badawy, Campbell, Cuthbertson and Fell, 1957) but if the gut of the sheep was removed under anaesthesia there was no mucosal cell shedding. A further limitation of the slaughter technique is that only one observation at one point in time is possible per animal (Low, 1982).

Due to the difficulties and cost of using the slaughter method with pigs the sampling of ileal digesta from pigs fitted with permanent

cannulae has become a widely adopted technique. The use of gut cannulae allows repeated observations to be made on a single conscious pig. Re-entrant cannulae have been used for the total collection and subsequent sampling of ileal digesta whereas 'simple' or 'T-piece' cannulae have been employed in conjunction with indigestible markers for the spot-sampling of ileal digesta.

Sauer *et al.* (1980) have listed several problems which may arise in experiments involving cannulation Among these, site and effect of the cannula, particle size of the diet, feeding frequency, collection procedure and marker recovery are important.

The surgical implantation of gut cannulae may interfere with the normal digestive and absorptive processes. Macrae and Wilson (1977) compared intact sheep with sheep prepared with rumen cannulae and subsequently fitted with either simple T-shaped or re-entrant cannulae at the duodenum or ileum. There was little difference in voluntary food intake, dry matter digestibility, nitrogen balance, blood constituents and marker retention times, but sheep fitted with re-entrant cannulae had lower wool growth rates than the intact animals. Wenham and Wyburn (1980) carried out radiological examinations on sheep prepared with intestinal cannulae. All the cannulations caused some disruption to the normal flow of digesta. The simple T-piece cannula caused the least disturbance whereas the 'Ash' re-entrant cannula in the transverse duodenum, jejunum or ileum caused gross abnormalities of propulsion and flow. Also, Laplace and Borgida (1976) found that pig ileal musculature was seriously disturbed by re-entrant cannulation. The problems associated with re-entrant cannulation of the ileum have been summarised by Laplace (1980). The disturbance to motility can be partly overcome by ileo-caecal cannulation. This involves bypass of the ileo-caecal valve, however, which has a sphincter-like action for a purpose which is not well understood. Furthermore, ileo-caecal cannulation may lead to an invasion of the small intestine by a caeco-colic-type microflora.

Sauer *et al.* (1977a) and Taverner (1979) demonstrated similar faecal digestibility values in non-cannulated and ileo-caecal cannulated pigs, whereas Sauer, Aherne and Thacker (1979) noted that the apparent faecal digestibilities of all amino acids and protein were significantly higher for pigs fitted with ileo-caecal cannulae than in intact animals.

Furuya, Takahashi and Omori (1974) detected no significant differences in growth rates or faecal digestibility coefficients between non-fistulated pigs and those prepared with T-piece cannulae although Livingstone (1982) reported reduced food intake and rate and efficiency of growth in pigs with simple cannulae in the terminal ileum.

Since re-entrant cannulation is labour intensive and because the re-entrant cannulae may be prone to blockage leading to hypertrophy of the gut and digesta leakage (Low, 1980b), the use of simple cannulae is preferred by an increasing number of workers studying digestibility in the pig. Cannulation of the ileum with a simple T-piece cannula allows the satisfactory collection of faeces, the feeding of bulky foods and causes minimal digesta leakage or loss of appetite (Low and Partridge, 1980b).

Collection of digesta through a simple T-piece cannula adopts a spot-sampling technique and relies therefore on a reference marker(s) that passes through the gut at the same rate as the nutrients being measured. According to Low (1982) chromic oxide is the most commonly-used marker in digestion studies with pigs although recent attention has been given to the use of rare earth metals such as ruthenium. Kotb and Luckey (1972) reviewed the use of markers in animal nutrition and concluded that chromic oxide is non-toxic and has been demonstrated to be virtually completely recovered from the faeces of man and other animals and can therefore be used as an inert marker in digestibility studies. A detailed description of cannula types and the methodologies involved in working with cannulated pigs has been presented by Low (1980a).

II.1.5 Conclusion

The digestibilities of amino acids in various protein sources fed to the growing pig differ due to the effects of many factors, both animal and dietary. It is important, therefore, to be able to measure the digestion and absorption of amino acids.

Because of the action of the hindgut microflora of the growing pig it is most appropriate to measure the digestibility of dietary amino acids at the terminal ileum.

The seemingly most satisfactory technique currently available for this purpose is the measurement of the apparent digestibility of amino acids at the terminal ileum of pigs fitted with simple T-piece cannulae and fed diets which include an indigestible marker.

Reservation, partly concerning the use of cannulated animals and partly concerning the appropriateness of the apparent estimate of protein digestibility, however, prompts an evaluation of the practical application of estimates of apparent ileal digestibility.

CHAPTER 2

THE DETERMINATION AND ASSESSMENT OF APPARENT ILEAL DIGESTIBILITY COEFFICIENTS FOR CRUDE PROTEIN AND AMINO ACIDS IN SOME FOODSTUFFS FOR THE GROWING PIG (20 TO 80 Kg LIVEWEIGHT).

II.2.1 Introduction

Although the determination of digestibility coefficients for amino acids in foodstuffs is an integral part of protein quality evaluation, reliable data are sparse. In the past studies have been made to determine the faecal digestibility of crude protein and amino acids in many foods. It is apparent from the review of literature (II.1), however, that there is doubt regarding the interpretation of faecal values. Ileal analysis offers promise as an alternative technique in digestibility studies.

The following chapter describes an experimental programme the objectives of which were three-fold. The primary aim of the study was the determination of apparent ileal digestibility coefficients for amino acids and crude protein in several foodstuffs for the growing pig.

In view of difficulties experienced in working with cannulated pigs the second objective was to contribute to the development of a more routine method for determining ileal digestibility. To this end a comparison of the apparent ileal digestibility of dietary crude protein was made between the rat and the pig.

Finally, it was intended to evaluate the accuracy of apparent estimates of ileal amino acid digestibility for predicting the digestion and absorption of amino acids in the growing pig.

II.2.2 Determination of the Digestibility of Dietary Crude Protein and Amino Acids in Protein Sources for the Growing Pig

(i) Introduction

The purpose of this study was to obtain estimates of the degree of digestion and absorption of crude protein and amino acids in several foodstuffs for growing pigs.

It was critical to the design of the experiments reported in part one of this thesis that the protein source of the basal diet (refer I.2.2.iii) be completely digested and absorbed. Accordingly, an initial study was conducted to determine the digestibility of the protein component of the basal diet. Upon completion of this study the apparent ileal digestibilities of amino acids in barley-, pea-, meat-and-bone- and fish-meal were determined.

(ii) The digestibility of crude protein and amino acids in a casein , amino acid based (basal) diet

(a) Experimental procedure.

Animals. Nine Landrace X Large White entire male pigs each of approximately 25 Kg liveweight were selected at random from a group of pigs at the Pig Research Centre, Massey University. The pigs were penned individually for seven days prior to undergoing surgery to effect permanent implantation of T-piece ileal cannulae. Three animals died post-operatively and in two cases post-mortem examination revealed adhesions between parts of the intestines and the area of cannula insertion. Six boars of 35 Kg average liveweight had resumed normal food intake and appeared in good health three weeks following surgery. These boars were used in the present study.

Design of T-piece cannulae. Intestinal T-shaped cannulae were handmade from rigid polyvinylchloride plumbing fittings. The base was adapted from a three-way connector of 15.9 mm outer diameter and 12.7 mm inner diameter. The stem was a 45 mm length of tubing of 18.9 mm outer diameter and 15.9 mm inner diameter. The design and method of construction of the cannulae were similar to those described

by Gargallo and Zimmerman (1980).

Surgery and animal care. The surgical procedure was similar to that described by Gargallo and Zimmerman (1980). Fasted pigs (24 hours) were lightly sedated with azaperone (Stresnil; Ethnor, Sydney, Australia) (40 mg/20 Kg liveweight) and anaesthesia was induced and maintained with halothane (Fluothane; I.C.I., New Zealand). The pigs were placed in left lateral recumbency and the surgical area was shaved and disinfected. A seven cm incision was made starting four cm below the transverse process of the fifth lumbar vertebra and proceeding in a ventral direction. The abdominal muscles were incised parallel to the direction of their fibres. The terminal part of the ileum was located and exteriorised, and a two cm incision on the antimesenteric side of the ileum was made approximately 15 cm cranial to the ileo-caecal valve. The cannula was inserted into the ileum and a double purse-string suture was placed around the stem. The ileal cannula was exteriorised through a stab wound in the right abdominal wall close to the incision site. A broad spectrum antibiotic (Streptopen; Glaxo, New Zealand) was administered intramuscularly (5 cm^3) immediately following surgery and the dose was repeated two days after surgery. The skin sutures were removed eight days post-surgery.

The cannulated animals were housed in smooth-walled metabolism crates of a modified design which allowed easy side access, and were kept in a controlled environment at an ambient temperature of 21 ± 1°C.

An initial liquid diet (reconstituted milk for three days) was followed by the feeding of a barley-based pig grower diet. The area surrounding the cannula of each pig was washed daily with warm water and disinfectant and the skin around the cannula was treated with an antibiotic cream.

Diets. A diet was formulated similar to the basal diet described in part one of this thesis (refer I.2.2.iii). The composition of the diet is given in Table II.2.1.

The diet contained chromic oxide as an indigestible marker but did not contain maize oil as it was considered that this may interfere with an even distribution of the marker throughout the diet. Maize

Table II.2.1 Ingredient composition of a casein, amino acid based (basal) diet.

Ingredient	(g/Kg air-dry weight)
Enzymatically hydrolysed casein plus amino acids †[1]	95.50
Purified cellulose	33.20
Sucrose	66.60
Maize starch	764.30
Chromic oxide	2.50
Mineral, vitamin supplement†[2]	37.90

†[1] Refer Table I.2.4 for the amino acid composition of the basal diet.

†[2] Refer Table I.2.2 for the composition of the mineral, vitamin supplement.

oil was supplied to the pigs separately from the diet. A protein-free diet was also formulated, the composition of which is given in Table II.2.2.

General conduct of study. Each of the two diets was fed to three pigs, the feeding levels being as previously described (I.2.6.ii.b). A pig was fed its respective experimental diet for ten days, this comprising an eight-day dietary accustoming period followed by a two-day ileal collection period. Each meal was given with water and no extra water was supplied to the pigs.

The pigs were split-fed their daily meal allowance at 08.00 h and 20.00 h, respectively. Samples of ileal digesta (100 cm^3) were collected at two, four, six, eight and ten hours after the morning feed. The collection of digesta was facilitated by attaching a rubber balloon to the stem of the cannula. The ileal contents were frozen immediately following their collection. The total faecal output of each

Table II.2.2 Ingredient composition of a protein-free diet.

Ingredient	(g/Kg air-dry weight)
Maize starch	842.80
Purified cellulose	36.60
Sucrose	73.10
Chromic oxide	2.50
Dicalcium phosphate	3.00
Mineral, vitamin supplement†[1]	42.00

†[1] Refer Table I.2.2 for the composition of the mineral, vitamin supplement.

pig was collected over the final two days of the dietary accustoming period and was thoroughly mixed and subsampled. The frozen ileal digesta samples for each pig were subsequently thawed, mixed and a representative sub-sample taken. Samples of faeces and ileal digesta were freeze-dried and along with samples of the diets were finely ground and stored at -20°C prior to the determination of dry matter, chromium, nitrogen and amino acids. The apparent faecal and ileal digestibility coefficients for crude protein and the apparent ileal digestibility coefficients for amino acids were calculated. An estimate of faecal and ileal endogenous excretion (protein-free diet) allowed the calculation of true estimates of digestibility.

Analytical methods. Dry matter analyses were performed in duplicate, as described earlier (refer I.2.2.vii.a). Total nitrogen was determined in duplicate samples following the Kjeldahl method (refer I.2.2.vii.d). Crude protein content was calculated as nitrogen multiplied by the factor 6.25. Amino acid compositions were determined on duplicate samples using ion-exchange chromatography. The method of analysis was essentially the same as that described in part one of the thesis (refer I.2.2.vii.g). Each hydrolysis was conducted for 24 hours only, however, and the performic acid oxidation of methionine and

cystine was not undertaken. Tryptophan was not determined. Chromium contents were determined in duplicate following the method outlined by Fisher and Lee (1982). This method involves fusion of the sample with sodium peroxide followed by determination of chromium content by inductively-coupled argon plasma emission spectrometry.

(b) Results

Due to food refusals, ileal digesta samples were obtained from only two pigs fed the casein , amino acid-based diet and from one pig fed the protein-free diet whilst faecal samples were obtained from three pigs fed the basal diet and from one pig given the protein-free diet. The faecal and ileal crude protein digestibility coefficients are given in Table II.2.3.

Table II.2.3 The digestibility of crude protein in the casein , amino acid based (basal) diet.

	Apparent digestibility		True digestibility	
Pig	Faecal	Ileal	Faecal	Ileal
1	0.969	0.891	1.010	0.997
2	0.966	-	1.008	-
3	0.963	0.892	1.004	0.998
Mean	0.966	0.892	1.007	0.998

The data in Table II.2.3 exhibit extremely close agreement between individual pigs for each measure of digestibility. There was a large difference between the mean estimates of apparent digestibility though only a negligible difference between the mean estimates of true digestibility of crude protein.

The coefficients of ileal amino acid digestibility are given in Table II.2.4.

Table II.2.4 The ileal digestibility of amino acids in the casein, amino acid based (basal) diet.

Amino acid	Apparent digestibility			True digestibility		
	Pig 1	Pig 3	Mean	Pig 1	Pig 3	Mean
Lysine	0.947	0.958	0.953	0.984	0.995	0.990
Methionine †[1]	0.957	0.961	0.959	0.975	0.980	0.978
Histidine	0.871	0.901	0.886	0.944	0.974	0.959
Phenylalanine	0.892	0.910	0.901	1.014	1.032	1.023
Tyrosine	0.830	0.830	0.830	1.036	1.040	1.038
Threonine	0.906	0.930	0.918	0.984	1.008	0.996
Leucine	0.929	0.944	0.937	0.983	0.997	0.990
Isoleucine	0.886	0.909	0.898	0.950	0.973	0.962
Valine	0.904	0.923	0.914	0.968	0.986	0.977
Aspartic acid	0.872	0.871	0.872	0.945	0.944	0.945
Serine	0.818	0.864	0.841	0.913	0.960	0.937
Glutamic acid	0.907	0.926	0.917	0.936	0.955	0.946
Proline	0.898	0.914	0.906	1.077	1.093	1.085
Glycine	0.895	0.821	0.858	1.178	1.104	1.141
Alanine	0.833	0.869	0.851	0.985	1.021	1.003
Arginine	0.945	0.949	0.947	1.012	1.016	1.014
Overall mean			0.899			0.999

†[1] The methionine data should be interpreted with caution due to the possible partial loss of methionine during the acid hydrolysis.

The figures given in Table II.2.4 demonstrate very good agreement between the two pigs within each measure of digestibility. The values for serine and glycine, however, were notably different for the animals. The mean apparent digestibility ranged from 0.830 for tyrosine to 0.959 for methionine, and mean true digestibility ranged from 0.937 for serine to 1.141 for glycine.

(c) Discussion

On the basis of these results it seems that the protein component of the basal diet, which comprised a mixture of enzymatically hydrolysed casein and synthetic amino acids, was virtually completely digested and absorbed. This finding is supported by Clegg, Smith and Eggum (1980), who, using the rat, found a very high digestibility for the nitrogen in enzymatically hydrolysed casein and in a mixture of synthetic amino acids simulating the enzymatic hydrolysate. The latter workers reported respective values for true faecal digestibility of 0.977 and 1.003. Further, Low (1980a) has reported high values (0.895, 0.910) for the apparent ileal digestibility of crude protein in casein fed to the growing pig.

It is interesting to note that the present value for the apparent faecal digestibility of dietary crude protein was markedly greater than the corresponding ileal value yet the true faecal digestibility of dietary crude protein was almost the same as the true ileal value. The data suggest that virtually all of the dietary nitrogen was absorbed anterior to the terminal ileum and consequently there was no bacterial degradation of dietary nitrogen in the large intestine. It seems that endogenous nitrogen remaining undigested at the end of the ileum was largely broken down by the hindgut bacteria. Similarity between the faecal and ileal estimates of true digestibility of crude protein for the highly digestible casein diet is consistent with the findings of Poppe and Meier (1977). This similarity is understandable as with diets containing highly digestible protein most is absorbed before the digesta enter the large intestine whereas with protein sources of lower digestibility there are larger residues to undergo a disappearance of protein between the terminal ileum and rectum.

It is well established (Taverner, 1979, and Bressani, Torún, Elias, Navarrete and Vargas, 1981) that at low dietary protein intakes the apparent digestibility of protein increases with increasing dietary protein intake. This effect is considered to be due to the relatively greater contribution of endogenous protein sources at lower dietary protein levels. Taverner (1979) noted that as the protein content of a wheat-based diet was increased from 64 to 96 g/Kg, the apparent ileal digestibility of lysine increased from 0.690 to 0.770. The basal diet used in the present study contained only 83 g crude protein/Kg which may explain the lower values obtained for the apparent ileal digestibility of nitrogen and amino acids.

Because of the possible effect of endogenous protein secretion on the determination of the apparent estimates of digestibility in the present study it seems most appropriate to place emphasis on the true digestibility values. The present estimates of true digestibility, however, were based upon a correction for endogenous loss derived by feeding a protein-free diet, an approach which is open to criticism (refer II.1). The work described here may be further criticised in that the measurement of endogenous excretion was made on only one animal. It is encouraging, however, that the present estimate of the amount of nitrogen voided endogenously was very similar to a mean value based on the studies of Taverner (1979) and Sauer, Stothers and Parker (1977)† (refer Appendix II.1).

The amino acid composition of the endogenous secretion of the growing pig is influenced by the feeding of a protein-free diet (Taverner, 1979). It seems that relatively high levels of proline and glycine are characteristic of the protein-free regime. In this respect it is significant that the presently determined values for the true ileal digestibility of glycine and proline markedly exceed unity. Sauer (1982) also noted problems in the interpretation of true estimates of amino acid digestibility in studies in which the endogenous ileal amino acid levels were determined by the use of protein-free diets. It was reasoned (Sauer, 1982) that if endogenous values of proline and glycine are overestimated by feeding a protein-free diet, then endogenous values of all other amino acids are also probably overestimated. Taverner (1979) argued, however, that the overestimation of the ileal levels of proline and glycine was an artefact of the protein-

† Hereafter Sauer *et al.* (1977b).

free feeding regime and typical only of these amino acids. There is evidence in fact noted by Moughan and Smith (1982) which suggests that the excretion of ileal endogenous protein may be underestimated following the feeding of a protein-free diet. The study of Taverner (1979) also clearly demonstrated an effect of the level of cellulose in protein-free diets on the ileal excretion of endogenous nitrogen and amino acids. In the present study, however, the daily intake of cellulose was the same for animals fed the protein-free and basal diets.

Because of the inadequacies of the faecal measurement of digestibility and with view to the uncertain nature of the correction for endogenous levels of amino acids, the best measure of the digestibility of the basal dietary protein is the true ileal digestibility of crude protein. In this respect the present data, accepting that they are based on a limited number of observations, indicate that the protein component of the basal diet was fully digested and absorbed and thus support the use of the basal diet in the experimental approach reported in part one of this thesis.

(iii) The apparent ileal digestibility of amino acids in barley-, pea-, meat-and-bone- and fish-meal

(a) Experimental procedure

Animals. Four Landrace X Large White entire male pigs, each cannulated at the terminal ileum with a T-piece cannula, were used. Individual pig liveweights on introduction to the test diets, ranged from 40 to 50 Kg. The cannula design, surgical procedure and animal care were as described in the previous section (II.2.2.ii.a).

Diets. Four diets were prepared, the ingredient compositions of which are given in Table II.2.5.

Barley-, pea-, meat-and-bone- and fish-meal were each used as sole sources of dietary protein. The diets were formulated, using determined values for the chemical compositions of the ingredients, to contain the same levels of crude protein (92.20 g/Kg air-dry weight) and acid detergent fibre (82.30 g/Kg air-dry weight). Chromic oxide was added to each diet as an indigestible marker for the determination

Table II.2.5 The ingredient compositions (g/Kg air-dry weight) of the diets.

	Barley	Peas	Meat-and-bone	Fish
Barley-meal	950.0	-	-	-
Meat-and-bone-meal	-	-	169.4	-
Fish-meal	-	-	-	148.4
Pea-meal	-	485.0	-	-
Maize starch	-	404.7	638.3	660.3
Sucrose	-	50.0	100.0	100.0
Cellulose	24.3	33.3	82.3	82.3
Chromic oxide	2.5	2.5	2.5	2.5
Mineral, vitamin supplement†[1]	2.5	2.5	2.5	2.5
Sodium chloride	2.0	2.0	2.0	1.0
Potassium carbonate	-	-	3.0	3.0
Di-calcium phosphate	18.7	20.0	-	-

†[1] Tasmix, pig grower, mineral vitamin premix (Tasman Vaccines Ltd., Auckland, New Zealand).

of ileal digestibility values. Maize oil was supplied as an extra-dietary supplement to avoid problems with obtaining an even distribution of chromic oxide in the feed.

General conduct of study. The experiment was planned as a 4 (pigs) x 4 (diets) Latin-square design. Daily food intake was restricted to 0.10 of metabolic liveweight ($W^{0.75}$). Samples of ileal digesta were collected from the pigs. The feeding and ileal digesta collection procedures were as described for the previous study (II.2.2.ii.a). Representative samples of ileal digesta were freeze-dried and along with samples of the diets were finely ground and stored at -20°C. prior to the determination of their amino acid, nitrogen and chromium contents. The apparent ileal digestibility coefficients for amino acids and crude protein were calculated.

Analytical methods. Refer to Section II.2.2.ii.a for the methods of determination of total nitrogen and chromium. Amino acid compositions were determined using ion-exchange chromatography. The analyses were conducted on an L.K.B. 4150 amino acid analyser. Duplicate samples of material (20 mg) were each hydrolysed in 4 cm^3 of 6.8M HCl at 110° ± 2°C for 22 hours in tubes sealed under vacuum. Because of destruction under acid hydrolysis, tryptophan, methionine and cystine were not determined. Proline was also not determined as this amino acid could not be detected by the single-channel integrator connected to the amino acid analyser. Duplicate samples of barley- and pea-meal were analysed for acid detergent fibre following the method of Van Soest (1963).

Statistical analysis. A linear model which included terms for diet, amino acid, and diet x amino acid was fitted to the data. As the data set was unbalanced reductions in sums of squares were used to determine levels of significance.

(b) Results

In several instances food refusals were encountered and consequently the 4 x 4 Latin-square design was not accomplished. Whenever necessary, trials were repeated until four samples of ileal digesta had been obtained for each of the four diets. Chemical analysis

showed that five of the ileal digesta samples had abnormally high protein to chromium ratios and so the estimates of digestibility pertaining to these samples were not included in the statistical analysis. Data were available for analysis from three pigs fed the barley diet, four fed the pea diet, two fed the meat-and-bone diet and two fed the fish diet.

The statistical analysis of the data showed a significant ($P < 0.001$) first order (diet x amino acid) interaction. The coefficient of determination (R^2) indicated that 0.93 of the variation in ileal digestibilities was explained by fitting the least squares model. The mean coefficients of apparent ileal amino acid digestibility are given in Table II.2.6. The mean estimates of the apparent ileal digestibility of crude protein, which are not given in Table II.2.6, were 0.707 for the barley diet, 0.689 for the pea diet, 0.658 for the meat-and-bone diet and 0.820 for the fish diet.

The apparent ileal amino acid digestibility coefficients for barley-meal ranged from 0.632 for glycine to 0.838 for glutamic acid. The corresponding range for pea-meal was from 0.633 for glycine to 0.768 for phenylalanine; for meat-and-bone-meal was from 0.548 for threonine to 0.753 for arginine and for fish-meal was from 0.786 for glycine to 0.893 for leucine.

(c) Discussion.

The data obtained in this study supply valuable information on the digestibilities of amino acids in four important protein sources for the growing pig. The statistically significant first order interaction indicates that the differences among digestibility coefficients for amino acids within a diet were not the same from diet to diet. The residual variation after having fitted the linear model was small which shows that the variation between animals within diets for ileal digestibility was relatively low.

There were considerable differences in digestibility among amino acids within a protein source which suggests the unsuitability of using crude protein digestibility as a common factor for indicating the digestibility of dietary amino acids. Also, it is interesting to note

Table II.2.6 Mean coefficients of apparent ileal amino acid digestibility in four dietary protein sources for the growing pig.

Protein source	Amino acid †[1]														
	Lys.	Hist.	Phen.	Tyr.	Thre.	Leuc.	Iso.	Val.	Asp.	Ser.	Glu.	Gly.	Ala.	Arg.	S.E.
Barley-meal	0.744	0.774	0.816	0.809	0.737	0.788	0.781	0.771	0.727	0.754	0.838	0.632	0.744	0.797	0.0155
Pea-meal	0.734	0.731	0.768	0.735	0.659	0.739	0.701	0.697	0.737	0.708	0.746	0.633	0.691	0.761	0.0134
Fish-meal	0.892	0.821	0.890	0.881	0.798	0.893	0.882	0.863	0.793	0.820	0.882	0.786	0.874	0.885	0.0189
Meat-&-bone-meal	0.654	0.720	0.752	0.647	0.548	0.656	0.643	0.676	0.591	0.608	0.671	0.713	0.733	0.753	0.0189

†[1] Lys. = lysine
Hist. = histidine
Phen. = phenylalanine
Tyr. = tyrosine
Thre. = threonine
Leuc. = leucine
Iso. = isoleucine
Val. = valine
Asp. = aspartic acid
Ser. = serine
Glu. = glutamic acid
Gly. = glycine
Ala. = alanine
Arg. = arginine

that most of the amino acids were digested and absorbed to a greater extent than the crude protein. This is consistent with the findings of Sauer *et al.* (1980) and may be explained by the crude protein possibly containing non-protein nitrogen compounds which have lower digestibilities than the amino acids. In the future, it may be possible with sufficient data on specific foodstuffs, to develop relationships between the digestibility of crude protein and that of individual amino acids.

For most of the essential amino acids, ileal digestibilities were highest in fish-meal followed by barley-meal and pea-meal, and were lowest in meat-and-bone-meal. The relatively low digestibility of amino acids in meat-and-bone-meal has also been reported by several other workers (Tanksley and Knabe, 1980; Fuller *et al.*, 1981, and Jorgensen and Sauer, 1982). Further, relatively high values for the digestibility of amino acids in fish-meal have been recorded (Fuller *et al.*, 1981, and Jorgensen and Sauer, 1982). Sauer, Kennelly and Aherne (1979) determined the apparent ileal digestibility of amino acids in various barley cultivars and found very similar values to those reported in the present study.

Having established estimates of the apparent ileal digestibility of amino acids in single protein sources it was of interest to ascertain whether these estimates were 'additive'. It is possible that there are effects on amino acid digestibility consequent upon the mixing of different protein sources (associative effects). It is important to know whether the levels of digested amino acids determined separately in different feed ingredients remain the same if these ingredients are combined into a complete diet.

There was sufficient food remaining after the determination of the digestibility of amino acids in the single protein sources to allow preparation of a diet containing equal proportions of the barley-, pea-, meat-and-bone- and fish-meal diets. The mixed diet was fed to one cannulated boar and ileal digesta were collected.

Predicted and determined values for the apparent ileal digestibility of amino acids in the mixed diet are presented in Table II.2.7.

Table II.2.7 Predicted†[1] and determined apparent ileal amino acid digestibilities in a mixed diet containing four protein sources.

Amino acid	Apparent digestibility	
	Predicted	Determined
Lysine	0.774	0.756
Histidine	0.764	0.739
Phenylalanine	0.809	0.809
Tyrosine	0.776	0.745
Threonine	0.690	0.686
Leucine	0.778	0.761
Isoleucine	0.765	0.702
Valine	0.757	0.720
Aspartic acid	0.721	0.707
Serine	0.726	0.699
Glutamic acid	0.795	0.779
Glycine	0.703	0.697
Alanine	0.770	0.749
Arginine	0.784	0.783

†[1] Predicted digestibilities are based on the data presented in Table II.2.6 and assume additivity of the respective digestibility coefficients.

The determined amino acid digestibilities in the mixed protein diet were very close to the predicted values which were calculated from a knowledge of the amino acid digestibilities in barley-, pea-, meat-and-bone- and fish-meal. The maximum difference between predicted and determined values was 0.063 for isoleucine and there was no difference for phenylalanine. The mean difference between predicted and actual values

was 0.020.

The present result provides no evidence, in terms of amino acid digestibility, of associative effects between the four protein sources in the mixed diet and it is thus concluded that the apparent estimates of amino acid digestibility behaved in an additive manner. It is noted, however, that the feeding level of the mixed-protein diet was the same as that employed in the determination of the digestibility of the amino acids in the single-protein diets. It may also be misleading to place emphasis on the results from one animal. Nevertheless, the present result is in agreement with Sauer, Cichon and Ozimek (1983) who found that the apparent ileal digestibilities of amino acids in barley- and canola-meal were additive. These initial findings encourage further research in this area.

II.2.3 A Comparison of the Apparent Digestibility of Dietary Crude Protein in the Rat and Pig as Determined at the Terminal Ileum and over the Entire Digestive Tract

(i) Introduction

It became obvious, in the initial stages of the previous trial (II.2.2) that the measurement of ileal digestibility using cannulated pigs is not straightforward. It was also noted by Low (1980a) that studies with cannulated animals are labour-intensive and prone to difficulties which lead to the premature loss of animals. Small numbers of observations per treatment have been characteristic of digestibility studies involving cannulated pigs (Low, 1980b).

The rat, considered to be suitable as a general mammalian model for nutritional research (Waddell and Desai, 1981), may serve as a useful model for the pig in the nutritional evaluation of feed ingredients. The main advantage of using the rat for the determination of protein digestibility is the ease of collection of ileal contents following slaughter of the animal.

The present trial, which was of a preliminary nature, was undertaken to investigate the suitability of the growing rat as a model for the digestion and absorption of dietary protein in the growing pig. The work reported here was conducted as an adjunct to study II.2.2.iii.

(ii) Experimental procedure

(a) Animals

The pigs used in the trial were described in the previous experiment (II.2.2.iii.a).

Male Sprague Dawley weaned rats (Small Animal Production Unit, Massey University) of 45-50 g body weight, were housed individually in raised stainless steel cages. The temperature of the animal house was maintained at 21°± 1°C and lighting was controlled by a 12-hour light-dark cycle.

(b) Diets

Four diets were each fed to rats and pigs. The ingredient compositions of the pig diets were as given in Table II.2.5. The ingredient compositions of the rat diets are presented in Table II.2.8. Within a species, all the diets were formulated using determined values for the chemical compositions of the ingredients, to contain the same levels of crude protein (rat diets, 84.0 and pig diets, 92.2, g/Kg air-dry weight) and acid detergent fibre (rat diets, 75.0 and pig diets, 82.3, g/Kg air-dry weight). Chromic oxide was added to each diet as an indigestible marker for the determination of ileal and faecal digestibility values. Maize oil was supplied to the pigs as an extra-dietary supplement to avoid problems with obtaining an even distribution of chromic oxide in the feed.

(c) General conduct of study

The experimental procedure for the pigs was as described in the previous study (II.2.2.iii) excepting that in addition to the collection of ileal digesta, the faecal output for each pig was collected over the final two days of the eight-day dietary accustoming period. Representative samples of the pig faeces and ileal digesta were freeze-dried and along with samples of the diets were finely ground and stored at -20°C prior to the determination of nitrogen and chromium.

At weaning all rats were fed a starter diet containing lactalbumin

Table II.2.8 The ingredient composition (g/Kg air-dry weight) of four diets for the growing rat.

	Barley	Peas	Meat-and-bone	Fish
Barley-meal	865.0	-	-	-
Meat-and-bone-meal	-	-	154.0	-
Fish-meal	-	-	-	135.0
Pea-meal	-	442.0	-	-
Maize starch	0.5	415.5	658.5	677.5
Maize oil	50.0	50.0	50.0	50.0
Cellulose	22.0	30.0	75.0	75.0
Chromic oxide	2.5	2.5	2.5	2.5
Mineral, vitamin supplement †	60.0	60.0	60.0	60.0

† The composition of the mineral, vitamin supplement was as described by James and Treloar (1984).

(99.5 g crude protein/Kg) until they had reached 80 g body weight. The rats were then randomly allocated in a 4 (diets) x 18 (rats/diet) experimental design and fed the test diets for eight days. Food and water were available *ad libitum*. Individual body weights and food intakes were recorded every two days. Total faeces were collected over the final two days of the trial, and following slaughter (asphyxiation in carbon dioxide gas followed by decapitation) digesta from the terminal 20 cm of ileum was flushed out with water using a syringe. Faeces and ileal digesta from three rats were each pooled to provide six samples per diet and these along with samples of the diet were freeze-dried and stored at -20°C prior to the determination of nitrogen and chromium.

The apparent ileal and faecal digestibility coefficients for crude protein, in the rat and pig, were calculated. The original intention in this trial was to measure the digestibility of protein in six dietary protein sources. Because of a lack of acceptance by the pig of two of the six protein sources, however, it was possible to study only four protein sources in this species. The rat readily consumed the six ingredients which explains why there are six rat samples per diet, for ileal digesta and faeces respectively.

(d) Analytical methods.

Refer to section II.2.2.ii.a for the methods of determination of total nitrogen and chromium and refer to section II.2.2.iii.a for the method of determination of acid detergent fibre.

(e) Statistical analysis

A linear model which included terms for species, diet, digestibility type and interactions was fitted to the data. To account for the unbalanced nature of the data reductions in sums of squares were used to determine levels of significance.

(iii) Results

As reported previously (II.2.2.iii.b) faecal and ileal digestibility data were available for analysis from three pigs fed the barley diet, four fed the pea diet, two fed the meat-and-bone diet and

two fed the fish diet. A complete set of data (six replicates per diet) was available from the rat.

Statistical analysis of the data revealed a significant ($P < 0.001$) second order interaction (diet x species x digestibility type) and further analysis indicated that this had arisen as a result of the pea data alone. It is notable that the mean ileal digestibility of crude protein in peas for the rat was higher than the corresponding faecal value (rat ileal = 0.810, rat faecal = 0.774; pig ileal = 0.689, pig faecal = 0.852). Reanalysis of the data after excluding peas gave two significant first order interactions (digestibility type x diet, $P < 0.01$) and (digestibility type x species, $P < 0.05$). These interactions are not unexpected. Accordingly the ileal and faecal digestibility data were analysed separately. The coefficients of apparent digestibility of crude protein in fish-meal, meat-and-bone-meal and barley-meal are given in Table II.2.9.

The coefficients of determination (R^2) indicated that 0.97 of the variation in faecal digestibilities and 0.77 of the variation in ileal digestibilities were explained by fitting the least squares model.

When the data were analysed within species a significant ($P < 0.05$) first order interaction (digestibility type x diet) was apparent for both species. As shown in Table II.2.9 the coefficients of faecal digestibility of crude protein were greater than the corresponding ileal coefficients and the differences between the digestibility types were greater for the pig than the rat.

(iv) Discussion

The present findings suggest that the growing rat is a satisfactory model for the growing pig in terms of the apparent ileal digestibility of crude protein in fish-, meat-and-bone- and barley-meal. The differences in ileal digestibility between the species were not statistically significant. Also, the recorded differences were small from a practical perspective. The present results support those of Taverner (1979) who found close agreement between the rat and the pig for the true ileal digestibility of nitrogen, in wheat. Also the initial findings of Picard, M. (pers. comm., 1983) suggest a close similarity between the

Table II.2.9 The mean apparent digestibility of crude protein (± S.E.) in three dietary ingredients as measured on ileal digesta or faeces in the rat and the pig.

	Fish-meal		Meat-and-bone-meal		Barley-meal		Level of significance (between species within diets)
	Rat	Pig	Rat	Pig	Rat	Pig	
Ileal digestibility	0.818 ± 0.0169	0.820 ± 0.0293	0.691 ± 0.0169	0.658 ± 0.0293	0.663 ± 0.0169	0.707 ± 0.0239	N S
Faecal digestibility	0.876 ± 0.0069	0.920 ± 0.0012	0.775 ± 0.0069	0.814 ± 0.0012	0.670 ± 0.0069	0.752 ± 0.0098	***

rat and pig for the apparent and true ileal digestibilities of amino acids in several protein sources. Further, it was noted by Goranzon, Forsum, Hambraeus, Rundgren and Thilen (1980) that the utilisation of protein in two mixed diets was the same for the pig and the rat and Batterham (1983) using the slope-ratio assay showed general agreement between the pig and the rat for the availability of lysine in cottonseed-, fish-, meat-, soyabean- and sunflower-meal.

Agreement between the present values of rat and pig ileal digestibility of crude protein was not close in the case of peas. In this respect it is notable that Batterham (1983) reported a poor relationship between the rat and the pig for the availability of lysine in another type of legume (lupin). Furthermore, the present data show that the apparent faecal digestibility of crude protein in peas was lower than the ileal value for the rat. The latter finding is difficult to explain although it is possible that bacterial fermentation in the rat is inhibited by some factor present in the peas. This study indicates that further investigation of the digestibility of pea protein in the pig and the rat is warranted. In general, values for the apparent faecal digestibility of crude protein were higher than the corresponding ileal values which has been observed in numerous other studies (Sauer *et al.*, 1980).

The differences between the rat and pig for the apparent faecal digestibility of crude protein in fish-, barley- and meat-and-bone-meal were statistically significant. The faecal digestibility of crude protein being greater in the pig than the rat is in agreement with the findings of Poppe and Meier (1972) and Slump, van Beek, Janssen, Terpstra, Lenis and Smits (1977) and accords with the relatively greater size of the large intestine in the pig (Wrong *et al.*, 1981).

The estimates of apparent ileal digestibility of crude protein were more variable than the faecal values. The same conclusion has been reache by several other workers (Just, 1980). Part of this variability may be du to variation in endogenous secretion. The determination of true ileal dig estibility may thus afford greater accuracy of prediction of the digestibility of dietary crude protein.

The findings reported here certainly encourage further investigation with the rat as a nutritional model for the pig. It may well be

that use of the rat will provide an inexpensive and routine method for the determination of the ileal digestibility of dietary proteins for pigs.

II.2.4 An Assessment of the Accuracy of Apparent Ileal Amino Acid Digestibility as a Measure of the Digestion and Absorption of Amino Acids by the Growing Pig

(i) Introduction

There is reservation concerning the accuracy of apparent ileal amino acid digestibility values for predicting the degree of digestion and absorption of amino acids during pig growth.

Research is needed to establish the reliability of estimates of apparent ileal amino acid digestibility for predicting the ability of a foodstuff to supply amino acids for protein synthesis in the pig (Low, 1982). This also appears to be the case for the chicken (Parsons, 1981).

Although several studies have been undertaken to evaluate the application of apparent amino acid digestibility coefficients in dietary formulation the results are not conclusive. Poppe and Meier (1977) demonstrated that formulating a diet on the basis of truly digested (faecal) amino acid levels as opposed to gross amino acid levels permitted a more precise prediction of rat growth. Further, the preliminary results of Low *et al.* (1982) suggest that measurement of the apparent ileal digestibility of the limiting amino acid in a diet may be a satisfactory predictor of its deposition in the pig carcass. Also, in studies conducted by Tanksley and Knabe (1980, 1982) the use of apparent ileal amino acid digestibility values allowed the replacement of highly digestible dietary protein sources by ingredients of lower digestibility, without adversely affecting pig performance.

In contrast to the findings of the above-mentioned studies, however, Fuller *et al.* (1981) found no increased precision in dietary formulation by using values for the apparent digestibility of amino acids at the terminal ileum of the pig. The latter study involved a metabolism trial in

which nitrogen balance was measured. Synthetic amino acids were added to each of several food ingredients such that the total intakes of amino acids for each ingredient were considered to be 'ideal' (Agricultural Research Council, 1981) on either an apparently digestible or gross basis. When the supplementation was based on apparent ileal digestibility, nitrogen retention was not consistently higher than when it was based on gross amino acid content. As noted by Low (1982), however, whether the result was due to the unsuitability of the ileal measurements or to shortcomings in the Agricultural Research Council's recommended ideal amino acid balance, remains unclear.

Although the majority of investigations reported to date have shown that estimates of amino acid digestibility permit increased efficiency in dietary formulation the degree of accuracy with which apparent estimates of ileal amino acid digestibility predict the levels of absorbed amino acids has not been clearly established.

The present trial was designed to determine whether estimates of apparent ileal amino acid digestibility are accurate measures of the degree of amino acid digestion and absorption in the growing pig.

(ii) Experimental procedure

(a) Animals and housing. Twenty Landrace X Large White entire male pigs (boars) of about 15 Kg liveweight were selected at random from a weaner pool at the Pig Research Centre, Massey University. The boars were individually penned in a controlled-environment building maintained at 21° ± 1°C and were equally and randomly allocated to one or other of the two dietary regimes.

(b) Diets. A diet was prepared which contained barley-, pea-, meat-and-bone- and fish-meal as the only sources of protein. The latter ingredients were part of the same batch of materials fed to the pigs in Trial II.2.2 for the determination of apparent ileal amino acid digestibility coefficients. Whilst awaiting the determination of amino acid digestibilities and subsequent use in the present investigation the ingredients were kept in deep freeze (-20°C). The ingredient and determined nutrient compositions of this diet (barley-based diet) are given in Table II.2.10. As shown in Table II.2.11, with reference to

Table II.2.10 The ingredient and nutrient compositions of the barley-based diet.

Ingredient	Composition (g/Kg air-dry weight)
Barley-meal	841.31
Meat-and-bone-meal	70.00
Fish-meal	50.00
Pea-meal	34.00
Vitamin, mineral supplement †[1]	2.50
Sodium chloride	2.00
Antioxidant (Ethoxyquin)	0.19

Nutrient †[2]	Composition (g/Kg air-dry weight)
Lysine *	8.92
Methionine + cystine *	6.70
Tryptophan	1.60
Histidine *	3.90
Phenylalanine + tyrosine *	13.10
Threonine *	6.50
Leucine *	12.40
Isoleucine *	6.30
Valine *	8.60
Non-essential amino acids *	100.70
Calcium	12.70
Phosphorus	8.60
Sodium	1.60
Chloride	2.90
Potassium	5.80
Crude fibre	26.00
Apparent digestible energy* (MJ/Kg air-dry weight)	13.22

†[1] Tasmix, Pig grower, vitamin mineral premix (Tasman Vaccines,Ltd. Auckland, New Zealand).

†[2] Values marked with an asterisk were determined. All other values were calculated.

Table II.2.11 The amino acid balance of the barley-based diet compared with an estimate of ideal amino acid balance.

Amino acid	Amino acid balance (relative to lysine = 100)	
	Barley-based diet	Ideal (refer Table I.2.4)
Lysine	100	100
Methionine + cystine	75	54
Tryptophan	18	13
Histidine	44	34
Phenylalanine + tyrosine	147	101
Threonine	73	59
Leucine	139	89
Isoleucine	71	46
Valine	96	66
Non-essential amino acids	1129	693

a proposed ideal amino acid balance for the growing pig lysine was clearly the first limiting amino acid in the barley-based diet.

A second diet (control diet) the protein source of which comprised enzymatically hydrolysed casein and synthetic amino acids was formulated so that its gross amino acid composition equalled the determined apparent ileal digestible amino acid composition of the barley-based diet. Determination of the absorbed levels of amino acids in the barley-based diet was achieved by using the previously determined apparent ileal amino acid digestibility coefficients (refer Table II.2.6) for each of the four protein sources comprising the barley-based diet. In the absence of apparent ileal digestibility values for methionine, cystine and tryptophan these were assumed equal to the mean of the determined apparent ileal amino acid digestibilities.

The digestibility of the non-essential amino acid component was taken as the mean of the apparent ileal digestibilities of aspartic acid, serine, glutamic acid, glycine, alanine and arginine. The ingredient and nutrient compositions of the control diet are given in Table II.2.12.

The apparent digestible energy contents of the barley-based and control diets were determined in a separate metabolism trial (total faecal collection) and the results of this trial are presented in Appendix II.2.

(c) General conduct of study. The pigs were gradually introduced to their respective diets. When liveweight approximated 20 Kg the animals were weighed to the nearest 0.1 Kg and placed on trial. The feeding regime was a fixed feed/fixed time one and comprised 40 days, during which time each pig received 50.04 Kg of its respective diet in accordance with a pre-determined feeding scale (Table II.2.13). The pigs fed the barley-based diet, which had a lower digestible energy content than the control one received a supplement of maize starch at each feeding time so as to ensure that apparent digestible energy intakes were equal for pigs on the two diets. Each boar was split-fed its respective daily meal allowance at 08.30 h and 16.30 h. Both diets were given to the pigs mixed with water (in the ratio of 1:2, w/w) and water was also available *ad libitum* from drinkers in the pens.

Table II.2.12 The ingredient and nutrient compositions of the enzymatically hydrolysed casein, synthetic amino acid control diet.

Ingredient †[1]	Composition (g/Kg air-dry weight)
Casein, amino acids	147.28
Maize oil	30.00
Purified cellulose	30.00
Sucrose	60.00
Maize starch	682.03
Vitamin, mineral supplement †[2]	2.50
Antioxidant (Ethoxyquin)	0.19
Potassium carbonate	6.00
Di calcium phosphate	40.00
Sodium chloride	2.00

Nutrient †[3]	Composition (g/Kg air-dry weight)
Lysine	6.87
Methionine + cystine	5.08
Tryptophan	1.25
Histidine	2.97
Phenylalanine + tyrosine	10.52
Threonine	4.56
Leucine	9.67
Isoleucine	4.90
Valine	6.56
Non-essential amino acids	76.82
Calcium	9.40
Phosphorus	8.30
Sodium	3.10
Chloride	2.20
Potassium	3.40
Crude fibre	30.00
Apparent digestible energy (MJ/Kg air-dry weight)	14.88

(Legend overleaf)

Legend to Table II.2.12.

†[1] A daily supplement of magnesium oxide was provided with the diet.

†[2] Tasmix, Pig grower, vitamin mineral premix (Tasman Vaccines Ltd., Auckland, New Zealand).

†[3] The dietary amino acid composition (excepting tryptophan content) was based upon a determined value for the amino acid composition of enzymatically hydrolysed casein and known additions of synthetic amino acids. The tryptophan content was based on a tabulated value for the level of tryptophan in enzymatically hydrolysed casein and the known addition of synthetic tryptophan. The digestible energy content was determined. All other values are calculated.

On the morning (08.00 h) after completion of its 40-day feeding period, each pig was weighed then slaughtered on the premises using a captive-bolt pistol followed by exsanguination. Immediately following slaughter the ham joint of the left-hand side of the carcass was removed by a transverse cut taken at right angles to the anterior edge of the *symphysis pubis*. The hind foot was removed from the ham joint at the distal end of the tibia, fibula bones. The ham, sealed in plastic, was then placed in deep freeze (-20°C) until required for physical dissection. On removal from deep freeze each ham was physically separated into skin, bone, muscle, subcutaneous fat and intermuscular fat. The total muscle is hereafter referred to as lean content while values for the subcutaneous and intermuscular fat contents were combined to provide a measure of total dissected fat content. One skilled dissector, using fine-boning and skinning knives, did all the sample joint dissections.

(d) Analytical methods. Amino acid compositions were

Table II.2.13 The feeding scale†[1] for pigs fed the barley-based and control diets.

Days	Daily meal intake (g)	Maize starch supplement (g/d) †[2]
1 - 7	858	101
8 - 14	1000	118
15 - 21	1150	136
22 - 28	1400	165
29 - 35	1540	182
36 - 40	1680	198

†[1] The feeding level was kept low to avoid food refusals, and to ensure that the daily supply of balanced protein reaching the sites of protein synthesis was less than an estimate of the potential rate of protein deposition of the entire male pig.

†[2] Maize starch (14.1 MJDE/Kg air-dry weight) was given as a supplement to the barley-based diet to ensure iso-caloric intakes on the two diets.

determined on duplicate samples of each of the feed ingredients using ion-exchange chromatography. The method of analysis was essentially the same as that described in section I.2.2.vii .g, excepting that each hydrolysis was conducted for 24 hours only. Samples of diets and faeces from the metabolism trial were analysed for gross energy (refer I.2.2.vii.c) to enable determination of the digestible energy contents of the two diets.

(e) Statistical analysis. Performance and carcass data were subjected to analysis of covariance (Snedecor and Cochran, 1980),

liveweight at the start of test being the covariate.

(iii) Results

There were no food refusals during the trial, but one pig fed the control diet developed enteritis and was removed from test. Mean growth rates of the pigs and the sample joint dissection data are given in Table II.2.14.

Table II.2.14 Mean (± S.E.) daily liveweight gains and lean and total fat contents of the ham joint.

	Diet		
	Barley-based	Control	Significance of difference
Number of pigs	10	9	
Daily liveweight gain (g)	602.3 (±9.80)	607.3 (±10.42)	N S
Lean in ham joint (g)	1912.1 (±59.99)	1840.6 (±63.81)	N S
Total fat in ham joint (g)	548.8 (±21.17)	591.4 (±22.51)	N S

There was a very small and statistically non-significant difference in daily liveweight gain between the pigs on the barley-based and control diets. The ham joints of the pigs fed the barley-based diet were slightly leaner and contained less total fat than those of the control pigs but these differences were not statistically significant.

(iv) Discussion

The design of the current trial was such that if the coefficients of apparent ileal amino acid digestibility, as previously determined for the ingredients comprising the barley-based diet, were accurate measures of amino acid digestion and absorption in the growing pig, the levels of amino acids absorbed from the casein control diet should have equated with those digested and absorbed from the barley-

based diet. More importantly (as lysine was the first-limiting amino acid in the barley-based diet) the gross level of lysine in the control diet should have equalled the level of lysine digested and absorbed from the barley-based diet.

Three assumptions, however, were critical to the design of this experiment, namely: the protein content of the basal diet was completely digested and absorbed; the daily amount of balanced protein absorbed by each pig fed the barley-based diet, was below that required to attain the animal's potential daily body-protein deposition; the digestible energy intakes of the pigs fed the two diets were equal.

Evidence for the complete digestibility of the protein in a mixture of enzymatically hydrolysed casein and synthetic amino acids has been presented (II.2.2). Also the use of a computerised simulation model of growth in the pig (refer part III of thesis) indicated that the daily protein deposition of pigs fed the barley-based diet in the present trial was well below their potential protein deposition rate. The model also showed that the dietary energy supply was adequate to avoid excess catabolism of amino acids. Finally, the digestible energy contents of the respective experimental diets were determined before commencement of the trial. The lower digestible energy intake of pigs fed the barley-based diet was corrected by feeding some maize starch, the digestible energy content of which had been determined in an earlier and independent study (Moughan and Smith, unpublished).

The mean growth rates of the pigs, fed equal amounts of the two diets, were very similar in both magnitude and variance. They did not differ significantly and the standard error of the difference between the means was a very small proportion (0.022) of the mean gain. These results indicate that similar amounts of lysine were digested and absorbed from the two diets.

The deposition of body lean is likely to be more closely related to the amounts of absorbed dietary protein than liveweight gain. Further the composition of the ham of the pig is a reasonably accurate predictor of total carcass composition (Evans and Kempster, 1979) and the ham is quickly and easily dissected into its respective tissue components. In this case it was informative to compare the average lean contents of

the hams of the two groups of pigs. The mean value for the pigs fed the barley-based diet was proportionally greater (0.037) than that for pigs fed the control diet. This small difference was not statistically significant and the result confirms that the levels of lysine digested and absorbed from the two diets were similar. The data relating to the total fat contents of the hams suggest that the hams of the pigs fed the control diet were somewhat fatter than those of pigs fed the barley-based diet but once again the difference was not statistically significant.

Strictly, liveweight gain and carcass lean content are measures of dietary amino acid availability rather than digestibility. It is possible that the digestibility of lysine from the barley-based diet may have been greater than predicted but that a proportion of the absorbed lysine was not available for growth. A recent review of the subject (Austic, 1983) indicates, however, that the excretion of non-utilisable forms of absorbed amino acids is not likely to be a major factor in diets composed of practical feed ingredients.

At least for the ingredients examined here, the results of this trial show that apparent ileal amino acid digestibility should be useful in practical dietary formulation for indicating the levels of dietary amino acids available for growth in the pig, and also suggest that apparent ileal digestibility coefficients are accurate measures of the degree of amino acid digestion and absorption in the growing pig.

The present findings need to be verified in further trials using a wider range of feed ingredients.

II.2.5 Summary and Conclusions

In the first instance, the study reported here has provided evidence for the complete digestibility of a protein source composed of enzymatically hydrolysed casein and synthetic amino acids. Although the finding is based upon a limited number of observations the agreement between pigs was very high. The virtually complete digestibility of protein in enzymatically hydrolysed casein suggests distinct advantages for using this compound in the design of nutritional experiments with the growing pig.

Secondly, the apparent ileal digestibilities of crude protein and amino acids in barley-, pea-, fish- and meat-and-bone-meal were determined. Furthermore, the present results support the application of apparent ileal amino acid digestibility coefficients for allowing an accurate prediction of the levels of dietary amino acids digested and absorbed by the growing pig. In the future it would be of interest to attempt to verify the latter results by adopting a different experimental technique such as measuring the removal of amino acids in the portal blood flow consequent upon feeding pigs the test and control diets.

Thirdly, and stressing that the results reported here are only of a preliminary nature, it seems that the growing rat may be used to obtain estimates of the apparent ileal digestibility of amino acids for application in the formulation of pig grower diets. If the present results can be verified over a wider range of food ingredients then use of the rat may afford an inexpensive, rapid and routine method for determining the ileal digestibility of amino acids in the pig.

The apparent estimates of ileal amino acid digestibility determined in this study find application in the validation of a simulation model of the digestion and metabolism of dietary nitrogen in the growing pig (the model is discussed in part III of this thesis). The express purpose of designing the simulation model was to enable quantification of dietary protein quality for the growing pig. The apparent ileal digestibility of dietary amino acids is an important component of this model.

PART III

THE PREDICTION OF DIETARY PROTEIN QUALITY BASED ON A MODEL OF THE DIGESTION AND METABOLISM OF NITROGEN IN THE GROWING PIG

CHAPTER 1

REVIEW OF LITERATURE

III.1.1 Introduction

The overall objective of the studies reported in this thesis was to develop present knowledge concerning three key aspects of dietary protein quality for the growing pig. Two of these aspects have already been considered. In the third instance, it was intended to describe the interactive metabolism of protein and energy by way of constructing a simulation model. Such description afforded achievement of the final aim of the study which was to enable prediction of the quality of the protein component of compounded pig grower diets.

The following review discusses approaches used in the determination of dietary protein quality, introduces the concept of simulation modelling and describes several simulation models which have been developed for use in animal nutrition.

III.1.2 Protein Quality: An Appraisal of Methods of Determination

The importance of protein quality evaluation in animal nutrition is reflected by the numerous studies on aspects of protein quality which have been conducted over the past 100 years (Payne, 1972b).

Essentially there are two purposes for developing methods of protein quality evaluation (Harper, 1973). In the first case a procedure is necessary for ranking single protein sources according to their efficiency of utilisation under some set of standard conditions. Secondly, it is necessary to be able to predict the efficiency of utilisation of protein mixtures in meeting amino acid requirements for growth.

The methods involved in protein quality evaluation have been the subject of extensive review (Allison, 1964; Dingle, 1972; Payne, 1972a; Delort-Laval, 1976, and Evans and Witty, 1978). Most of the methods which have been developed involve measurement under standardised conditions and are useful, therefore, for ranking single protein sources

according to nutritive value (Payne, 1972b). These methods are concerned with determining the maximum potential nutritive value of a protein (Harper, 1973) and as such adopt protein intakes which are considerably lower than those found in the practical situation whereby high growth rates are desired.

In a very comprehensive critique of the uses of bioassays in protein evaluation, McLaughlan (1972) concluded that the estimates of protein quality obtained are dependent upon the level of protein fed. For example, Henry and Kon (1957) demonstrated that the biological value of whole egg protein declined from 0.94 to 0.83 when the protein level of the diet was increased from 80 to 120 g/Kg, while Henry (1965) noted a decrease in net protein utilisation (NPU) of egg and casein between dietary protein levels of 80 and 200 g/Kg of diet.

A major problem stemming partly from the dependence of protein quality estimation upon feeding level and partly from the complementary effect of combining single protein sources, is that protein quality estimates for individual sources are not additive (Evans and Witty, 1978). Platt and Miller (1959) addressed this problem in distinguishing between NPU (standardised) which aims to compare the qualities of different protein sources and NPU (operative) aiming to assess the utilisation of proteins in mixed diets.

NPU (standardised) is measured using a sole protein source incorporated in a purified test diet, with maximum potential nutritive value being determined by adopting a low level of protein intake. The second measurement entails feeding a mixture of proteins at the level encountered in practice. Although NPU (operative) provides a means of predicting the efficiency of utilisation of protein mixtures in meeting amino acid requirements for animal growth, its determination involves biological assay, which in the face of an infinity of dietary regimes, limits its application.

Delort-Laval (1976) and Poppe (1976) have concluded that bioassays in general are of little value for routinely assessing the protein quality of compound foods used in animal production. Bender (1982) also alluded to the problem of evaluating the quality of protein in mixed foods and further criticised conventional measures from the standpoint

that the attempt has been made to express protein quality by a single figure, which does not allow suitable description of the factors influencing protein quality.

A treatise of the factors affecting the efficiency of protein utilisation in animal growth has been given by the Agricultural Research Council (1981). Protein utilisation is a multi-dimensional factor mainly depending upon the requirement for a balanced array of amino acids, amino acid availability, nitrogen and energy intakes, genetic and environmental influences and age, weight, sex and physiological state of the animal. The protein quality of a diet may be regarded as synonymous with the degree of utilisation of the dietary protein component, and protein quality can thus be viewed as a variable determined by a complex production system.

The use of computers in agricultural research has allowed complex production systems to be simulated by the construction of models (Wright, 1971). A model may be constructed containing certain essential components of a system and representing interaction among the components. Quantification of the model allows the statement of complex hypotheses and the extension of these to relevant practical situations (Spedding, 1981).

III.1.3 Simulation Modelling

Shannon (1975) defined simulation as being the process of construction of a model and the subsequent analytical use of the model. Wright (1971) discussed the procedures involved in systems simulation and divided these into three phases; modelling, validation and experimentation. Modelling involves formalisation of the current knowledge of a system in the form of equations and solution of these by use of a computer.

Confusion exists in the literature concerning the distinction in meaning between the terms validation and verification. The terminology advocated by Mihram (1972) is adopted in this thesis. Validation refers to testing the agreement between the behaviour of the simulation model and the real system, whereas verification implies ensuring that the model functions in the intended manner. In the experimental phase of simulation a model allows the experimenter to achieve perfect homogeneity

of experimental medium, allowing treatments to be compared under identical conditions. Further, Wright (1971) has listed the possible objectives of experimentation with simulation models and has made the point that even if a model is not sufficiently realistic to give a close estimate of the absolute level of system performance, it may be suitable for estimating the relative merits of different alternatives.

Baldwin (1976), in discussing the development of models used in ruminant nutritional studies, found it convenient to categorise models into three main types. Balance models are simple input-output models constrained by metabolic pathways. Secondly, optimisation models include some optimising objective function and both balance and optimisation models being static in nature may be considered separately from dynamic models. Whittemore (1981) has drawn the distinction between models which are empirically based and those which are essentially deductive or theoretical.

Several models of nutrient assimilation in growing animals have been developed.

III.1.4 Models in Animal Nutrition

Fowler (1978) commented on the large range of model types prevalent in the study of animal growth and development and suggested that this diversity reflects the difficulties encountered in attempting to build complex observations into a logical framework. Critical evaluations of nutritional models have been presented by Taylor (1978) and Fawcett (1978). According to the former author most models have been devoid of parameters affected by the nutritional history of the growing animal and there is a dearth of models which incorporate adaptive control processes and impose limits. The model of Whittemore and Fawcett (1976) was cited as an exception.

The model of Whittemore and Fawcett (1976) simulates protein and lipid growth in the pig and allows daily growth to be maximised in the face of various constraints, by the use of a recursive linear programming algorithm. The model is also notable in that total protein synthesis is apportioned between protein resynthesis and new protein

synthesis and the daily energy maintenance cost is corrected for the energy cost of protein maintenance.

Another model incorporating complex adaptive control mechanisms and imposing limits to growth has been built by Stombaugh and Oko (1980). Their dynamic model was designed to evaluate production alternatives and nutritional-environmental interactions in the growing pig. The model has a theoretical base and entails description of certain physiological processes and their control. The model of Schulz (1978), simulating energy metabolism in the simple-stomached animal, represents the animal as a pseudo-steady-state system and describes metabolic pathways in terms of biochemical equations, thereby enabling estimation of adenosine triphosphate yield and utilisation.

A growth model, developed primarily for use in poultry nutrition but relevant to animal growth on an *ad libitum* feeding regime generally, has been presented by Emmans (1981). This model is of particular interest, being based on the idea that an animal has a potential rate of growth and seeks to consume an amount of food which will enable the potential growth rate to be achieved.

Moe and Tyrrell (1973) reviewed the use of simple energy partitioning models in ruminant nutrition and Black (1974) described an empirically-based model partitioning metabolisable energy intake between maintenance, protein gain, wool gain and fat gain for growing lambs. The model was subsequently used to predict the effects of nutrition on lamb growth and body composition. The partitioning of dietary energy between different physiological functions in the case of the growing pig has been reviewed by Fowler (1978) and the shortcomings of the statistical approach to partitioning were noted.

According to Wright (1971) the starting point in the process of constructing a model should be a simple input-output model and the initial model may be expanded in detail by successive identification of (i) major subsystems, (ii) components and relationships within subsystems and (iii) links between subsystems. An excellent example of model development is provided by the work of Whittemore and Fawcett (1974, 1976). The first version of a pig growth model (Whittemore and Fawcett, 1974) was a simple balance-type model based largely on findings

of an empirical nature, whereas the later-developed model (Whittemore and Fawcett, 1976) included a much greater theoretical content and was structurally more complex.

III.1.5 Conclusion

The quality of dietary protein fed to a growing animal is determined by the interaction of many factors and because of this, conventional measures of protein quality have inherent inadequacies. There is no satisfactory method for determining the quality of protein mixtures in compound diets. The technique of simulation modelling offers a means whereby complex systems may be studied quantitatively and several simulation models describing the assimilation of nutrients in animal growth have been developed. Reference to these models provides insight into the varying degrees of complexity by which the description of nutrient assimilation may be approached.

CHAPTER 2

THE DEVELOPMENT OF A MODEL SIMULATING THE DIGESTION AND METABOLISM OF DIETARY NITROGEN IN THE GROWING PIG (20 TO 80 Kg LIVEWEIGHT)

III.2.1 Introduction

In spite of its theoretical and practical importance, no suitable method is available for routinely estimating the quality of the protein component of mixed diets fed to growing animals. The aim of this study was to provide an objective procedure for predicting the protein quality of diets fed to the growing pig. Although the term 'protein quality' is sometimes used with strict reference to dietary amino acid pattern (eg. chemical score) it is regarded here, in line with the discussion of Payne (1972b) and Mauron (1973), as being synonymous with the degree of dietary protein utilisation in growth.

Considerable variation in the ingredient and chemical compositions of pig grower diets is evident in practice (Carr, 1974, and Houeix, Latimier, Poilpre and Saulnier, 1981). Thus, it is important to be able to estimate the relative efficiencies with which practical pig grower diets support protein synthesis and indicate ways in which the efficiency of dietary protein utilisation may be improved. From a theoretical perspective, it is pertinent to ascertain the effects on dietary protein quality of changes in dietary and animal factors. Furthermore, the ability to determine the effects on pig growth of changes in diet and animal factors has important implications for the teaching of nutritional principles and aids also in the rationalisation of priorities for research in nutrition.

The problems encountered in assessing the protein quality of mixed diets are largely due to the complex interactive nature of the components characterising protein quality. Simulation modelling allows the quantified description of a complex system and experimentation with the system under controlled conditions. Many models have been used in the study of animal nutrition and some of these were discussed briefly in the review of literature. No model has yet been developed, however, with the exclusive objective of predicting the quality of the protein in a

mixed diet fed to the growing pig.

The design and evaluation of a model simulating the digestion and metabolism of dietary nitrogen in the growing pig (20 to 80 Kg live-weight) is described in this chapter. There were two objectives involved in the design of the model. Firstly, it was intended that the model derive estimates of protein quality by quantitatively predicting the utilisation of the protein component of a mixed diet fed at a given level to the growing pig. Secondly, the model should enable determination of the influence of certain dietary and animal factors on protein utilisation.

III.2.2 Design of the Model

(i) Introduction

Baldwin (1976) urged the need for systematic development in model construction and provided an outline of the steps involved (Table III.2.1).

Table III.2.1 A suggested procedure for the construction of a model (Baldwin, 1976).

1. Set the modelling objective.
2. Prepare a block diagram representing the central elements of the system and the interactions among them.
3. Convert the concepts represented in the block diagram to mathematical statements.
4. Formulate the required numerical inputs based on literature data, experimental data or statistical estimation.
5. Evaluate solutions and/or validate the model.

   Return to steps 2, 3 or 4 if the evaluation indicates inadequacies.

The final step suggested by Baldwin (1976) implies that model construction is an evolving process and that successive changes may need to be incorporated in models to achieve the initial modelling objective.

The procedure outlined in Table III.2.1 was followed to develop a model simulating nitrogen metabolism in the growing pig. This model was regarded as the initial stage in a developmentary process leading to a suitably robust model of nitrogen metabolism for this species. The developmentary nature of model building is regarded by Shannon (1975) as being inevitable and desirable:

> "One begins with a very simple model and attempts to move in an evolutionary fashion toward a more elaborate model that reflects the complex situation more clearly. The process of elaboration or enrichment involves a constant interaction and feedback process between the real world situation and the model. There is a constant interplay between the modification of the model and a confrontation with the data generated."[1]

In this context, the aim guiding the present model design was to describe the assimilation of dietary nitrogen by the growing pig in a simplified manner. A model was formulated around the concept of nitrogen partitioning in growth given by Miller and Payne (1961). These workers considered nitrogen intake (I) to be diverted into three metabolic pathways; namely nitrogen, for maintenance (Im), for growth (Ig) and for the provision of energy (Ie); (I = Im + Ig + Ie).

The model simulates growth within a 24-hour period and can be regarded as a static model as daily tissue deposition is not summated over time. The model describes the partitioning of nutrients in a single pig of specified liveweight and sex, with a defined set of physiological parameters, living in a thermoneutral environment. This approach provides the experimenter with perfect homogeneity of experimental medium.

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[1] Shannon, R.E. (1975): *Systems Simulation, the Art and Science.* Publ. Prentice-Hall Inc., Englewood Cliffs, New Jersey, U.S.A. p.19-20.

A model simulating the digestion and metabolism of dietary protein and energy by the pig also based on the concept of a simple partitioning of nutrients during growth has been developed (Whittemore and Fawcett, 1974; 1976). Although the objective underlying the construction of the latter model was to enable the prediction of growth characteristics such as rate and efficiency of liveweight gain and carcass composition, the model is nevertheless capable of generating estimates of the efficiency of dietary protein utilisation.

Several aspects of the approach of Whittemore and Fawcett (1976) may be refined. Dietary protein intake, for instance, is described in units of digestible crude protein (apparent, faecal). Yet it seems that the accuracy of prediction of the amounts of dietary amino acids digested and absorbed by the pig may be enhanced by the use of apparent ileal digestibility coefficients. The significance of adopting the ileal estimates of amino acid digestibility is likely to be greatest for diets comprising high levels of relatively indigestible ingredients (Moughan and Smith, 1982).

In the model of Whittemore and Fawcett (1976), the amount of imbalanced dietary protein is determined by use of either the essential amino acid index or chemical score. In the first instance the dietary amino acid pattern is compared with the amino acid pattern of whole egg protein and in the case of chemical score the dietary pattern is compared with the amino acid pattern of pig whole body protein. Neither the amino acid pattern of egg nor the amino acid pattern of body protein necessarily constitutes an 'ideal' balance of amino acids. It is considered more appropriate here to identify the limiting amino acid and calculate the degree of dietary amino acid imbalance by comparing the amino acid balance of the diet with an empirically-derived estimate of ideal amino acid balance. Whittemore and Fawcett (1976) do not allow for the situation whereby the dietary level of non-essential amino acids limits growth. In the event of the latter, essential amino acids are converted to non-essentials (Rérat and Lougnon, 1968).

Also, the Whittemore and Fawcett (1976) model estimates the level of urinary nitrogen excretion resulting from an imbalanced dietary amino acid supply by dividing the amount of imbalanced protein by the factor 6.25. It cannot be assumed, however, that the mixture of amino acids

deaminated due to dietary amino acid imbalance, contains 0.16 nitrogen. Each amino acid has a characteristic nitrogen content. Phenylalanine, for example, contains 0.08 nitrogen whereas histidine contains 0.27 nitrogen. The excretion of nitrogen derived from the deamination of imbalanced dietary amino acids is often a considerable proportion of urinary total nitrogen excretion and so the approach of Whittemore and Fawcett (1976) may lead to an inaccurate prediction of urinary nitrogen excretion in certain cases. It seems preferable to convert the dietary amino acid inputs to units of elemental nitrogen before calculating the amount of imbalanced nitrogen supply. The correction factor (6.25) may be employed for estimating the urinary nitrogen loss derived from deamination of the portion of balanced protein not used for protein synthesis (McDonald, Edwards and Greenhalgh, 1973).

Again, Whittemore and Fawcett (1976) described the protein metabolism of the growing pig in terms of body protein turnover and protein accretion. Although their approach is undoubtedly theoretically correct the model relies upon data obtained from studies with the growing rat to describe the protein flux of the pig. Such extrapolation, while understandable in the absence of relevant data for the pig, may be misleading. Furthermore, the energy cost of protein deposition was derived by calculating the number of moles of adenosine triphosphate required for protein synthesis. Estimates of the number of high energy bonds required for the formation of a single peptide bond, however, are very variable (Agricultural Research Council, 1981 and Tess, 1981).

The estimate of the energy cost of protein deposition preferred in the present study is that of Pullar and Webster (1977). The latter workers confirmed the recommended value for simple-stomached species given by Kielanowski (1976), in an experiment which enabled the cost to be measured in a way that was free from assumptions as to the energy cost of maintenance. The data of Reeds, Cadenhead, Fuller, Lobley and McDonald (1980), further support the application of Pullar and Webster's estimate in the case of the growing pig.

The above suggested refinements to the description of protein digestion and metabolism in the growing pig were included in the present model.

(ii) Description of the model

A flow diagram representing the important elements of the model and the interactions among them is given in Figure III.2.1. This is followed by details of the input data required to run the model and an explanation of the steps shown in the flow diagram.

A. Model input.

(a) NI : It is assumed that dietary nitrogen is ingested entirely in the form of amino acids (lysine, methionine plus cystine, tryptophan, histidine, phenylalanine plus tyrosine, threonine, leucine, isoleucine, valine and the non-essential amino acid component). The weight of each amino acid ingested from each dietary ingredient is calculated from a knowledge of daily food intake, the ingredient composition of the diet, the essential amino acid composition of each ingredient and the crude protein content of each ingredient.

(b) Dix : The apparent ileal digestibility coefficient of each amino acid in each dietary ingredient. (Where ileal amino acid digestibility coefficients are unknown, preferably an estimate of the ileal digestibility or alternatively the faecal digestibility, of nitrogen may be used.)

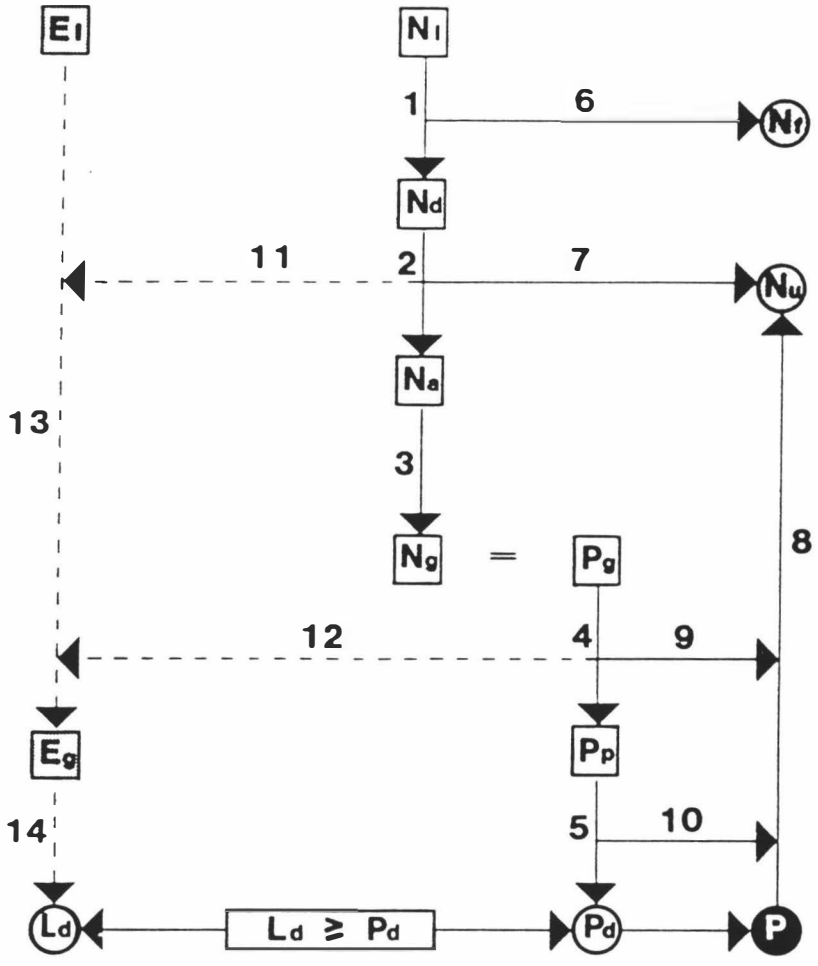
(c) EI : Calculated using daily food intake, the ingredient composition of the diet, the apparent digestible energy content of each ingredient and the digestible crude protein intake, assuming a value for the energy content of crude protein.

(d) W : Liveweight of the pig.

(e) The sex of the pig (entire male, castrated male or female).

B. Steps represented in the flow diagram

Step 1. The model calculates the daily digestible amino acid intake of lysine, methionine plus cystine, tryptophan, histidine, phenylalanine plus tyrosine, threonine, leucine, isoleucine,



Key to notation in flow diagram

Symbol	Meaning	Symbol	Meaning
NI	nitrogen ingested	EI	Protein-free energy intake
Nd	digested nitrogen	Eg	Energy available for growth
Na	nitrogen available for growth and maintenance	Ld	lipid deposited
Ng	nitrogen available for growth	□	Stages of nitrogen digestion and metabolism
Pg	protein available for growth		
		□	Stages of energy metabolism
Pp	protein possibly deposited within dietary and physiological constraints	○	tissue accumulation(g/d)
Pd	protein deposited	○	excretion of waste products (g.N/d)
P	total body protein	◆—□—▶	applied constraint
		——▶	flow of nitrogen
Nu	nitrogen excreted in urine	- - ▶	flow of energy
Nf	nitrogen excreted in faeces	●	total body protein

Fig. III.2.1 Flow diagram of a model to simulate the daily partitioning of nitrogen in pig growth.

valine and the non-essential amino acid component. Digestible amino acid intakes are converted to units of elemental nitrogen by use of molecular weight conversion factors (refer Appendix III.1) and the dietary balance of amino acids (lysine = 100) is determined.

Step 2. The dietary array of amino acids and an array of amino acids considered ideal for growth in the pig are inputs to a linear programming algorithm (simplex) which identifies the limiting dietary amino acid and maximises the daily amount of nitrogen available for growth and maintenance. The algorithm allows the conversion of excess essential amino acids to non-essentials if the non-essential amino acid component is limiting in relation to the level in the ideal balance. The ideal balance of amino acids (Epsilon array) is the experimentally-derived balance presented in Table I.2.4 of this thesis. The amino acid levels in the balance have been converted to grams of elemental nitrogen and the balance has been expressed with lysine equal to 100 units.

Step 3. The daily amount of nitrogen available for growth and maintenance is corrected for the daily maintenance nitrogen requirement to give the daily amount of nitrogen available for growth. The daily maintenance nitrogen requirement is assumed to be 0.17 g/ $W^{0.75}$,($Kg^{0.75}$) based on an estimate of endogenous urinary nitrogen loss (D'Mello *et al.*, 1976). Daily nitrogen available for growth is converted to units of daily protein by implementation of the average conversion factor 6.25 (McDonald *et al.*, 1973).

Step 4. The amount of daily protein available for growth is compared with the maximum daily rate of protein deposition physiologically possible and the amount of protein that can possibly be deposited due to dietary and physiological influences is calculated. The model assumes that the maximum daily rate of protein deposition physiologically possible is constant over the liveweight range 20 to 80 Kg and based on the findings of Piatkowski and Jung (1966) and Kielanowski (1969) adopts the following values: castrated male = 100 g/d, female = 115 g/d, and entire male = 130 g/d.

Step 5. Lipid retention is often regarded as a secondary process depending upon metabolisable energy intake, the maintenance

energy requirement and the energy requirement for accretion of body protein. Such a view is only acceptable if it is considered that adipose tissue is solely involved in the passive role of caloric storage and heat regulation. Adipose tissue, however, has a variety of metabolic and structural roles (Dauzier, 1976) and therefore at least a proportion of lipid deposition must be regarded as physiologically essential and associated with lean deposition. In this case the model imposes a constraint such that the amount of lipid deposited daily must be greater than or equal to the amount of protein deposited daily. This constraint is based on the observation that the minimum, lipid to protein ratio in pig body gain appears to be 1:1 (Fowler, 1978). The daily rate of protein deposition is calculated with regard to this constraint (refer Step 14).

Step 6. The difference between the daily nitrogen intake and the daily amount of apparently digested nitrogen predicts the total daily excretion of nitrogen in the faeces. (Note: It is recognised that the use of ileal digestibility coefficients overestimates the faecal nitrogen excretion and underestimates the urinary nitrogen excretion. A varying proportion of nitrogen remaining undigested at the terminal ileum of the pig will be degraded in the hindgut and will ultimately be excreted in the urine.)

Step 7. The linear programming algorithm (refer Step 2) calculates the daily amount of nitrogen excreted in the urine due to deamination of imbalanced dietary amino acids.

Step 8. Daily endogenous urinary nitrogen loss is calculated (refer Step 3).

Step 9. If the daily amount of protein available for growth is greater than the daily rate of protein deposition physiologically possible then the daily amount of protein deaminated and excreted as urinary nitrogen is calculated.

Step 10. Whenever dietary protein is deaminated in order that the lipid, protein deposition constraint be met, the level of daily urinary nitrogen excretion is calculated.

Step 11. There is a net yield of energy when protein is deaminated. The metabolisable energy yield from deaminated protein is assumed to be 11.5 MJ/Kg (Whittemore and Fawcett, 1976) (72 MJ/Kg protein nitrogen). The net yield of metabolisable energy from the deamination of imbalanced dietary amino acids (Step 7) is calculated and added to the dietary protein-free energy intake.

Step 12. The net yield of metabolisable energy from the deamination of protein (Step 9) is calculated and added to the dietary protein-free energy intake.

Step 13. The dietary protein-free energy intake together with the net energy contributed from protein deamination is corrected for the energy cost of maintenance to give the energy available for growth. The energy cost of maintenance includes the energy cost of protein turnover and a value of 0.460 MJ ME/$W^{0.75}$/d is assumed, based on the findings of Kotarbinska (1971), Davies and Lucas (1972), Verstegen, Close, Start and Mount (1973), Kielanowski (1976), Fowler, Fuller, Close and Whittemore (1980) and Vangen (1980).

Step 14. The energy content of the daily amount of protein which can possibly be deposited due to dietary and physiological influences is calculated. It is assumed that the energy content of protein is 23.6 MJ/Kg (Whittemore and Fawcett, 1976). This energy is added to the energy available for growth and total energy is apportioned between lipid and protein deposition facing the constraint that daily lipid deposition must be equal to or greater than daily protein deposition. The metabolisable energy requirements to deposit 1 KJ of protein and 1 KJ of lipid are taken to be 2.25 and 1.36 KJ respectively (Pullar and Webster, 1977). These estimates correspond to an energy cost of depositing one gram of protein or lipid of 53 KJ ME.

The model is described most succinctly by the use of mathematical equations:

C. Mathematical equations of the model

(1) $C_x = \%C_x \cdot I$ (for x = 1 to n)

(2) $A_{ix} = \%A_{ix} \cdot C_x$ (for i = 1 to 9 and x = 1 to n)

(3) $A_i = \sum_{x=1}^{n} A_{ix}$ (for i = 1 to 9)

(4) $CP_x = \%CP_x \cdot C_x$ (for x = 1 to n)

(5) $\%CP = \frac{(\sum_{x=1}^{n} CP_x)}{I} \cdot \frac{100}{1}$

(6) $A_{10x} = CP_x - \sum_{i=1}^{9} A_{ix}$ (for x = 1 to n)

(7) $A_{10} = \sum_{x=1}^{n} A_{10x}$

(8) $\gamma_{ix} = A_{ix} \cdot D_{ix}$ (for i = 1 to 10 and x = 1 to n)

(9) $Z_i = \sum_{x=1}^{n} \gamma_{ix}$ (for i = 1 to 10)

(10) $\%d_i = \frac{Z_i}{A_i} \cdot \frac{100}{1}$ (for i = 1 to 10)

(11) $\alpha_i = Z_i \cdot M_i$ (for i = 1 to 10)

(12) $\beta_i = \frac{\alpha_i}{\alpha_1} \cdot \frac{\varepsilon_1}{1}$ (for i = 1 to 10)

A linear programming algorithm†[1] (simplex) is used to calculate Ne and Na.

(13) $No = 0.17(W^{0.75})$

(14) $Ng = Na - No$

(15) $Pg = 6.25 \cdot Ng$

(16) If $Pg > Pr$, $Pp = Pr$

If $Pg < Pr$, $Pp = Pg$

(17) $$Np = \frac{(Pg - Pp)}{6.25}$$

(18) $$E = \sum_{x=1}^{n} (C_x \cdot 10^{-3} \cdot DE_x)$$

(19) $$DCP = \left(\sum_{i=1}^{10} Z_i \right) \cdot 10^{-3}$$

(20) $$EPF = E - (23.6 \cdot DCP)$$

(21) $$E1 = 72(Ne \cdot 10^{-3})$$

(22) $$E2 = 72(Np \cdot 10^{-3})$$

(23) $$Ea = EPF + E1 + E2$$

(24) $$Em = 0.460(W^{0.75})$$

(25) $$Eg = Ea - Em$$

(26) $$Ep = 23.6\ (Pp \cdot 10^{-3})$$

(27) $$Et = (Eg + Ep)10^{3}$$

(28) $$Epd_1 = Pd_1 \cdot Ecp$$

whence $Pd_1 = Pp$

(29) $$E\ell_1 = Et - Epd_1$$

(30) $$Ld_1 = \frac{E\ell_1}{Ec\ell}$$

Successive iterations ($Pd_2 = Pp - 1$) are performed until the constraint $Ld \geqslant Pd$ is met.

(31) $$Nz = \frac{(Pp - Pd)}{6.25}$$

(32) $Nu = (Ne + No + Np + Nz)$

(33) $$Nf = \frac{\left(\sum_{i=1}^{10} A_i - \sum_{i=1}^{10} Z_i\right)}{6.25}$$

(34) $CP = \%CP \cdot I$

(35) $$Pe = \frac{Pd}{CP} \cdot \frac{100}{1}$$

Symbols used in the equations:

C_x = Weight of the xth dietary ingredient (g/d).

$\%C_x$ = Percentage of the xth dietary ingredient in the diet.

I = Dietary intake (g air-dry weight/d).

A_{ix} = Weight of the ith amino acid ingested from the xth dietary ingredient (g/d).

$\%A_{ix}$ = Percentage of the ith amino acid in the xth dietary ingredient.

A_i = Intake of the ith amino acid (g/d).

CP_x = Crude protein intake of the xth ingredient (g/d).

$\%CP_x$ = Percentage crude protein of the xth ingredient.

$\%CP$ = Percentage crude protein of the diet.

A_{10x} = Intake of the non-essential amino acid component in the xth ingredient (g/d).

A_{10} = Intake of the dietary non-essential amino acid component (g/d).

Y_{ix} = Weight of the digested ith amino acid in the xth ingredient (g/d).

D_{ix} = The apparent digestibility (%) of the ith amino acid in the xth ingredient.

Z_i = Total digestible ith amino acid (g/d).

$\%d_i$ = The digestibility (%) of each amino acid in the diet.

α_i = Intake of the ith digestible amino acid corrected by a factor, M_i (see below) (g N/d).

M_i = Molecular weight conversion factor:

$$M_i = \frac{\text{Molecular weight of nitrogen in ith amino acid}}{\text{Total molecular weight of ith amino acid}}$$

β_i = The level of the ith amino acid (α_i) relative to lysine = 100.

ε_1 = The level of lysine in an ideal amino acid balance (set equal to 100).

Ne = Urinary nitrogen loss due to dietary amino acid imbalance (g N/d).

Na = Dietary nitrogen available for growth and maintenance (g N/d).

No = Endogenous urinary nitrogen loss (g N/d).

$W^{0.75}$ = Metabolic liveweight of the pig (Kg).

Ng = Nitrogen available for growth (g N/d).

Pg = Protein available for growth (g protein/d).

Pp = The amount of protein that can possibly be deposited due to dietary and physiological influences (g protein/d).

Pr = The maximum rate of protein deposition which is physiologically possible (g protein/d).

Np = Nitrogen excreted in urine due to the supply of protein available for growth exceeding the maximum amount of protein deposition physiologically possible (g N/d).

E = Total digestible energy intake (MJ DE/d).

DE_x = The digestible energy content of the xth ingredient (MJ DE/Kg).

DCP = Total dietary digestible crude protein intake (Kg/d).

EPF = Protein-free energy intake (MJ ME/d).

E1 = Energy yield from deamination of imbalanced dietary amino acids (MJ ME/d).

E2 = Energy yield from deamination of amino acids supplied in excess of the biological potential protein deposition rate (MJ ME/d).

Ea = Energy available for growth and maintenance (MJ ME/d).

Em = Maintenance energy requirement (MJ ME/d).

Eg = Energy available for growth (MJ ME/d).

Ep = The energy content of the protein which can possibly be deposited due to dietary and physiological influences (MJ ME/d).

Et = Total energy to be apportioned to the deposition of lipid and protein in growth (MJ ME/d).

Epd = Energy required for possible protein deposition (KJ ME/d).

Ecp = The total energy cost for depositing one gram of protein (KJ ME/g).

Pd = The weight of protein deposited (g/d).

Eℓ = Energy available for lipid deposition (KJ ME/d).

Ld = The weight of lipid deposited (g/d).

Ecℓ = The total energy cost for depositing one gram of lipid (KJ ME/g).

Nz = Nitrogen excreted in the urine from deamination of dietary protein to supply energy for lipid deposition (g N/d).

Nu = Total urinary nitrogen excretion (g N/d).

Nf = Faecal nitrogen from dietary and endogenous protein sources (g N/d).

CP = Crude protein intake (g/d).

i = A subscript denoting the ith amino acid:

- i = 1 = lysine
- i = 2 = methionine plus cystine
- i = 3 = tryptophan
- i = 4 = histidine
- i = 5 = phenylalanine plus tyrosine
- i = 6 = threonine
- i = 7 = leucine
- i = 8 = isoleucine
- i = 9 = valine
- i = 10 = non-essential amino acid component

x = A subscript denoting the xth dietary ingredient.

$\dot{1}$[1] Description of the use of a linear programming algorithm to determine Na and Ne.

Activity = ε_I, 'ideal' nitrogen balance.

$$\varepsilon_I = \{a_{iI}\}$$

$N_I = \sum_i a_{iI}$, Available nitrogen per unit of ideal activity.

Let, X_I be units of ideal activity.

T_i be a transfer activity; nitrogen from ith amino acid to the non-essential amino acid total.

x_i be level of ith transfer activity

and α is the Nitrogen intake vector

so $\sum_i \alpha_i$ is total nitrogen intake.

The objective function = Max. $X_I N_I$

subject to; $\alpha \geq X_I \epsilon_I + xT$

and $(X_I, x) \geq 0$

Then, $Na = X_I N_I$

$$Ne = \sum_i \alpha_i - Na$$

D. Computerisation of the model

The mathematical equations given above were programmed on to an Apple II microcomputer using the Applesoft II basic language (refer, Applesoft II Basic Programming Reference Manual, 1978. Apple Computer Inc., 10260 Bandly Drive, Cupertino, California, U.S.A.). A complete listing of the computer programme is lodged with the Department of Animal Science, Massey University, and may be made available on request.

III.2.3 Evaluation of the Model

Errors occurring in simulation can, according to Shannon (1975), be classified as those associated with design, programming, input data, use of the model and interpretation of the results.

The present model was scrutinised for errors arising due to its programming. The procedure used was that of exploring the model boundaries by choosing a wide range of combinations of model inputs, calculating the model output by hand, and comparing this with output generated by the computer.

Error messages have been incorporated in the model structure which aid in the proper use of the model. An example of misuse would be the analysis of a diet fed to a growing pig of 18 Kg liveweight. The latter input information would elicit an error message indicating that the liveweight range of the model was exceeded. Furthermore, the input data are listed on a print-out which allows checking for the accuracy of the input information. A sample of model print-out is enclosed in the back cover of the thesis.

The process of model validation incorporates three philosophical approaches; rationalism, empiricism and pragmatism. The approach of the rationalist was implicit in the validation of the presently-described model. The concept of nitrogen partitioning during growth was accepted as a valid first assumption, thus allowing an examination of the logic pertaining to the model structure. Further to this, model components were accepted on the basis of the empirical evidence construing to their estimation. Baldwin (1976), who has discussed the pragmatic approach to validating models of animal performance, stressed the need, in this context, for clearly-conceived modelling objectives. Following pragmatism, a model is deemed valid, if it is capable of accurate prediction regardless of the internal structure and underlying logic of the model. The presently-described model was validated in the positivist sense by comparing output generated by the model with output derived from experimentation with the growing pig. The aim of the validation exercises was to evaluate the accuracy of the model as a predictor of the body protein retention resultant upon various combinations of dietary nutrient inputs.

Validation Exercise One.

The ability of the model to predict urinary nitrogen excretion and body protein retention was investigated using the results from a series of nitrogen balance trials conducted with growing pigs (Holmes, Carr and Pearson, 1980). Even though the balance trial is considered to underestimate urinary nitrogen excretion and overestimate body nitrogen retention (Agricultural Research Council, 1981) the data of Holmes *et al.* (1980) were deemed to be useful for model validation because their study included a wide range of experimental factors. Because of the imprecise nature of the nitrogen balance trial, this validation exercise was not a strict test of the predictive accuracy of the model but sought rather to establish whether the model predictions were sensible.

Holmes *et al.* (1980) fed boars and gilts four diets (composed of varying proportions of maize, barley, soyabean-meal, skim milk powder, blood-meal, synthetic methionine and synthetic lysine) at two levels of intake. The pigs were housed in a thermoneutral environment and nitrogen balances were conducted at liveweights of 30 and 50 Kg. Determined amino acid values and digestible energy contents of the diets were given.

Ileal amino acid digestibility coefficients were not given so cited values for apparent faecal digestibilities of dietary nitrogen were used. Input data pertaining to 24 separate nitrogen balance trials, each involving one animal, were computed. The relevant input data for each balance trial and the accompanying actual and predicted urinary nitrogen excretions are given in Table III.2.2.

Predicted urinary nitrogen excretion was in close agreement with the actual excretion in several of the balance trials (*viz.* Expts. 1, 3, 7, 9, 13, 14, 20, 24). There were several trials, however, in which the agreement between the simulated and actual values was poor (*viz.* Expts. 8, 10, 12, 16, 18, 21, 22, 23). There does not appear to be any single reason relating to model function, as to why the simulated results disagree with the actual values. The greatest disagreement was observed in Trial 21. It is notable that the boar in this trial was on a low level of food intake and the model predicted considerable deamination of amino acids to supply energy for lipid synthesis (Nz = 5.28 g/d). This result raises some doubt concerning the appropriateness of the minimum lipid to protein deposition ratio of 1:1 used in the model. The predicted values of Nz for the gilts in Trials 13 and 14, however, were also high (Nz = 5.44 g/d), yet the simulated and actual levels of urinary total nitrogen excretion agreed closely in both trials. It may well be that the minimum ratio between body, lipid and protein retention is lower for entire male pigs as opposed to gilts or castrated males. It is interesting to compare the results of Trials 15 and 16. The pig in Trial 16 had a lower crude protein intake than its counterpart in Trial 15 yet the model predicts that the urinary nitrogen excretion in Trial 16 was relatively higher. The protein of the diet fed in Trial 16 was, however, very imbalanced [Ne (Trial 15) = 7.81 g N/d; Ne (Trial 16) = 10.56 g N/d].

If the simulation model predictions ($\hat{y}$) were equivalent to predictions obtained by fitting a regression of the observed dependent variable (y) on some set of independent variables, then the regression of y on $\hat{y}$, $y = \alpha + \beta\hat{y} + e$, would give $\hat{\beta} = 1$, $\hat{\alpha} = 0$. When this regression model was estimated $\hat{\alpha} \pm$ S.E. $= 0.149 \pm 0.823$ and $\hat{\beta} \pm$ S.E. $= 0.864 \pm 0.043$. It was shown using the t test statistic that α was not significantly different from zero but β was significantly different from one. Actual urinary nitrogen was thus proportionally less (0.13 to 0.14) than that predicted by the

Table III.2.2 Urinary nitrogen (N) excretion; actual (balance experiment †[1]) vs. predicted (simulation model).

Balance experiment	1	2	3	4	5	6	7	8	9	10	11	12
Sex †[2]	G	G	G	G	G	G	G	G	G	G	G	G
Liveweight (Kg)	31.7	32.9	31.3	29.2	31.0	30.5	33.1	30.2	63.8	56.8	53.8	57.3
Food intake (g/d)	1560	1560	1560	1440	980	980	1060	980	2520	2300	2300	2360
Diet crude protein (%)	21.58	17.67	13.33	11.62	20.15	18.00	14.11	11.59	21.63	17.68	13.71	11.94
Actual urinary nitrogen (g/d)	23.76	13.32	9.62	8.41	12.82	11.05	8.51	5.84	47.05	23.74	14.60	14.81
Predicted urinary nitrogen (g/d)	24.79	15.59	9.06	10.09	15.84	13.43	8.37	7.66	51.85	31.69	18.15	18.91
Balance experiment	13	14	15	16	17	18	19	20	21	22	23	24
Sex	G	G	G	G	B	B	B	B	B	B	B	B
Liveweight (Kg)	52.7	50.1	51.2	51.1	33.8	36.8	32.1	28.8	31.5	31.5	35.6	30.4
Food intake (g/d)	1460	1420	1420	1420	1680	1680	1560	1440	980	980	1120	980
Diet crude protein (%)	22.01	18.67	14.10	12.62	16.46	18.75	11.79	11.67	18.14	17.61	11.45	11.38
Actual urinary nitrogen (g/d)	25.95	19.65	13.72	10.30	14.70	14.54	10.09	10.09	9.93	9.18	6.64	7.07
Predicted urinary nitrogen (g/d)	28.96	20.73	11.07	13.81	10.52	18.93	12.20	11.34	14.03	12.23	8.95	7.22

†[1] Holmes *et al.* (1980). †[2] G = Gilt, B = Boar.

simulation model.

With view to the fact, however, that nitrogen balance experiments underestimate nitrogen excretion, it is concluded that the values predicted by the model are realistic.

The possibility has been raised that the model is imprecise in the prediction of protein retention in cases where dietary protein is deaminated to supply energy for lipid synthesis. To test this conjecture more closely, predicted and actual body protein retention rates were compared at low levels of food intake using further data from Holmes *et al.* (1980). The findings of Fuller and Boyne (1971) and Just Nielsen (1971) indicate that nitrogen balance experiments overestimate body nitrogen retention by a proportion of approximately 0.18. On this basis it was decided to correct the actual values of body nitrogen retention for the expected degree of overestimation. The relevant input data for each balance trial and the accompanying corrected actual and predicted rates of body protein deposition are given in Table III. 2.3. The components of the predicted urinary nitrogen loss are also included in Table III.2.3 to indicate the relative importance of the different sources of urinary nitrogen loss in each balance trial.

The data in Table III.2.3 generally demonstrate a close relationship between the actual and predicted rates of body protein deposition. Also, there is no indication that the prediction of body protein deposition is any less accurate for the balance trials in which Nz comprised a considerable proportion of total urinary nitrogen loss. It is notable, however, that in the experiment where the predicted value of Pd deviated most from the actual value (Trial b), a boar was predicted to have deaminated a relatively large amount of protein to supply energy for lipid synthesis (Nz = 4.48 g/d). The latter observation further supports the earlier statement that the minimum, lipid to protein ratio of 1:1 must be viewed with caution in the case of the entire male pig. In interpreting the data presented in Table III.2.3 it must be borne in mind that although a constant correction factor (0.18) was used to adjust the actual values, the extent to which the nitrogen balance trial overestimates body nitrogen retention is very variable (Just, Fernández and Jorgensen, 1982).

Table III.2.3 Body protein deposition (Pd); actual (balance experiment †1) vs. predicted (simulation model).

Balance experiment	a	b	c	d	e	f	g	h	i	j	k
Sex †2	B	B	C	C	G	G	G	G	G	G	G
Liveweight (Kg)	30	30	30	30	30	30	30	50	50	50	50
Food intake (g/d)	966	954	1007	963	973	977	968	1406	1433	1437	1383
Diet crude protein (%)	11.61	17.72	11.61	17.72	11.61	14.02	17.72	17.72	21.24	11.61	14.02
Actual Pd (g/d)	33	68	36	60	37	53	69	76	94	53	70
Predicted Pd (g/d)	31	56	33	58	31	56	58	82	84	46	78
% Difference †3	-6.1	-17.6	-8.3	-3.3	-16.2	+5.7	-15.9	+7.9	-10.6	-13.2	+10.3
Ne (g/d)	5.82	6.10	6.07	6.16	5.85	5.43	6.19	9.00	10.38	8.66	7.68
No (g/d)	2.18	2.18	2.18	2.18	2.18	2.18	2.18	3.20	3.20	3.20	3.20
Np (g/d)	0	0	0	0	0	0	0	1.34	7.90	0	0
Nz (g/d)	0	4.48	0	4.32	0	0	4.32	5.28	4.96	0	0

†1 Data from Holmes *et al.* (1980) corrected for the expected overestimation of nitrogen retention (0.18). Values for each balance experiment are the means for two pigs.

†2 G = Gilt; B = Boar; C = Castrate.

†3 % difference = $\left(\frac{\text{Actual Pd - Predicted Pd}}{\text{Actual Pd}} \times \frac{100}{1} \right)$.

Although the subjective nature of this validation exercise limits any conclusion regarding the predictive accuracy of the model, the exercise did demonstrate quite definitely that the simulation model generates values which are consistent with actual pig growth.

Validation Exercise Two.

Henderson, Whittemore, Ellis, Smith & Laird (1980) have given whole body protein and lipid deposition rates for growing boars based upon the chemical analyses of 24 genetically-improved pigs. Their data indicate a protein deposition rate of 129.0 ± 9.80 g/d ($\bar{x}$ ± s.d.) and a lipid deposition rate of 185 ± 18.6 g/d ($\bar{x}$ ± s.d.). The boars were housed in a controlled environment and an average liveweight boar (60 Kg) was fed 2050 g/d of a pelleted diet (Table III.2.4) which contained 13.8 MJ DE/Kg and 180 g/Kg crude protein, on an air-dry basis. The apparent faecal digestibility of nitrogen in this diet was 0.74 (Smith, W.C., 1982, pers. comm.). The amino acid profile of the diet was calculated using tabulated values for the amino acid composition of the ingredients.

These input data were computed (simulation model) and values for whole body protein deposition (Pd) and lipid deposition (Ld) of 130.0 g/d and 176.2 g/d respectively were derived. The simulated value of Pd is proportionally higher (0.008) than the mean actual value and the simulated value of Ld is proportionally lower (0.048) than the mean actual value.

The agreement between the simulated and actual values is close, especially when viewed against the inaccuracies inherent in the measurement of the model inputs (liveweight, dietary energy level, etc.). Both predicted values lie within the range of plus and minus one standard deviation of the mean actual values.

The present simulation model is deterministic, yet growth is stochastic in nature. Mihram (1972) noted the problems with validating a deterministic model of an essentially stochastic phenomenon and suggested the use of a one-sample statistical test. It may be hypothesised that the simulated output (S) is equal to the true population mean (μ); Ho : (μ - S) = 0. The value of μ is estimated by drawing a random sample from the population and the calculation of the t-statistic constitutes a test of the null hypothesis. The t-test failed to reject the null

Table III.2.4 Ingredient composition†[1] of the diet fed in the study of Henderson *et al.* (1980).

Ingredient	% air-dry weight
Ground barley	55.00
Wheatings	25.00
White fish-meal	10.00
Extracted soya bean-meal	5.00
Molasses	5.00
Added per tonne	**Kg**
Di-calcium phosphate	13.6
Zinc carbonate	0.2
Sodium chloride	0.2
Vitamin premix †[2]	2.3
Mineral premix †[3]	2.3

†[1] (Smith, W.C., 1982, pers. comm.)
†[2] (Drivite, Boots Pure Drug Co. Ltd.)
†[3] (Pig Mindif, Boots Pure Drug Co. Ltd.)

hypothesis in the case of protein deposition rate but the null hypothesis was rejected in the case of lipid deposition rate. The difference between the actual and predicted values of daily lipid deposition was just significant at $P = 0.05$. (Note: In rejecting the null hypothesis there is a risk of being wrong in one in twenty times or less.)

Adoption of the one-sample statistical test is a rather rigorous approach to the problem of model validation. The statistically significant difference between the actual and predicted values of lipid deposition does not refute the simulation model but rather indicates that the prediction of lipid retention be viewed with a certain amount of caution.

The acceptance or rejection of a simulation model ultimately coincides with a judgement concerning the suitability of the model for

meeting the modelling objectives (Wright, 1971). The present model was designed to assist in comparison of the protein quality of diets for pigs. It was intended that the model represent a single pig, the metabolic functioning of which would be akin to pig growth in general. Because the components of the model are described as constants, however, the model can not be expected to completely accurately represent all populations of pigs, nor is it necessary that it should do so. In its application to the particular sample of pigs referred to here, the model gave an estimate for protein deposition which was very close to that achieved, and for lipid deposition an estimate which although being significantly different from the sample mean in a statistical sense, was within one standard deviation of the mean and hence representative of a not inconsiderable proportion of the pigs in that population.

If the model constants had been estimated using the population of pigs from which the sample was drawn, then a closer agreement would be expected between the sample means and the estimates from the model. It can be demonstrated, using the model, that if the maintenance energy requirement for the particular population was in fact 440 rather than 460 MJ ME/$Kg^{0.75}$/d (the latter value is that of the model parameter) then the predicted rate of lipid deposition would be 185 g/d. Further, if the energy requirement for lipid deposition was in fact 50.5 rather than 53.0 KJ ME/g then the predicted rate of lipid deposition would also become 185 g/d. In conclusion, comparison of the actual and predicted data does not in any way suggest that the model is unsuitable for the purposes for which it was developed.

Because the simulated value of Pg was greater than 130.0 g/d (133.4 g/d) it may seem that the test of the accuracy of prediction of Pd becomes a test of the suitability of the model parameter Pr. It would be concluded that a value of Pr = 130.0 g/d is acceptable. The latter conclusion, however, relies on the assumption that the model prediction of Pg is accurate. Pg may be overestimated by simulation and the actual value of Pr may in fact be higher than 130.0 g/d. Once again, it is possible, although unlikely, that balanced protein was deaminated during actual growth to supply energy in response to a need for lipid synthesis, resulting in 130.0 g protein/d being available for deposition, regardless of the maximum physiologically possible rate of

protein retention. It can only be concluded that on average the actual values of Pr and Pg were $\geqslant$ 129.0 g/d. The above points clearly demonstrate an important feature of the validation process. Validation of a model in the positivist sense establishes that a model is satisfactory in predicting the output of a system for given levels of input. It does not provide proof of the veracity of the model components. The present validation merely demonstrates that, at least for this sample of pigs, the model is accurate in the prediction of protein and lipid deposition rates.

Validation Exercise Three.

Neither of the previous two validation exercises has made use of apparent ileal amino acid digestibility values the application of which should increase the accuracy of the model predictions. Also, the pigs of Henderson *et al.* (1980) referred to in the second validation exercise supported high protein deposition rates. It was considered of value, therefore, to validate the model in a situation whereby the protein deposition rate of the pigs was well below the expected maximum.

Twenty Landrace X Large White entire male pigs (boars) of about 15 Kg liveweight were selected at random from a weaner pool at the Pig Research Centre, Massey University. The boars were individually penned in a controlled-environment building maintained at 21° ± 1°C and fed a diet (refer Table II.2.10) composed of barley-, pea-, fish- and meat-and-bone-meal (barley-based diet). Ten of the pigs, selected at random, were killed using a captive bolt pistol followed by exsanguination, as soon as they reached 20 Kg liveweight. After slaughter, the blood of each pig was collected and frozen. Digesta from the intestinal tract of each pig and bladder urinary contents were removed and discarded. The complete body of each pig was frozen (-20°C) and later ground and mixed. A representative sample of the mixed material was freeze-dried and subsequently finely ground and re-mixed. The freeze-dried samples of whole body tissue were analysed for nitrogen and fat (ether-extract). Samples of the un-dried and freeze-dried material were analysed for dry matter content. The methods for the analysis of dry matter and nitrogen were as described earlier (I.2.2.vii). The extraction of fat using petroleum ether followed the method outlined by the Association of Official Analytical Chemists (1975).

On attaining a liveweight of 20 Kg each of the remaining ten boars was fed a pre-determined amount of the barley-based diet for a further 40 days. Each animal was split-fed its respective daily allowance at 08.30 h and 16.30 h. The diet was fed to the pigs mixed with water. Extra water was supplied to the pigs *ad libitum*. The initial and final liveweights of the pigs were recorded. As well as the barley-meal each animal received a daily supplement of maize starch (refer Table II.2.13).

The average liveweight of a boar during the 40-day period of the trial was 30.35 Kg and the average daily intake of the barley-based meal was 1251 g. Upon conclusion of the 40-day growth period, each boar was slaughtered and samples of whole body tissue were prepared (as described) for the analysis of fat, nitrogen and dry matter contents.

The determined crude protein, amino acid and digestible energy contents of the dietary ingredients were available along with their determined apparent ileal amino acid digestibility coefficients. The relevant input data were computed (simulation model) and predicted values for Pd of 89.24 g/d and for Ld of 128.58 g/d were obtained. Actual values of Pd and Ld calculated on the basis of whole body composition and initial and final pig liveweights were 87.02 ± 6.58 g/d ($\bar{x}$ ± s.d.) and 133.34 ± 16.85 g/d ($\bar{x}$ ± s.d.) respectively. Both predicted values lie within the range of plus and minus one standard deviation of the mean actual values. The predicted value of Pd was proportionally greater (0.026) than the mean actual value whilst the predicted value of Ld was proportionally less (0.036) than the mean actual value. Statistical analysis (Students t test) showed that the differences between the respective predicted and actual means were not significant. It is concluded that the simulation model accurately predicted the protein and lipid deposition rates for the group of pigs in the present validation trial.

III.2.4 Discussion

A deterministic model based on empirically-derived components and constructed around a theoretical concept of nutrient partitioning in growth has been described. Although the model apportions nutrients during pig growth in a simplified manner the initial validation exercises indicate that the model is suitably accurate for predicting protein and lipid deposition rates for various levels of nutrient inputs.

Dent and Blackie (1979) have commented that even if adequate model performance is demonstrated by one or a series of validation tests, the validation procedure should not cease. These authors envisage validation as a continuing process closely allied to model development.

As validation continues further refinements will be made to the model. The implementation of sensitivity analysis (Wright, 1971) will aid in detection of the model components of greatest importance and consideration of the functional effectiveness and possible improvement of these components will ensue. The model programme allows ready manipulation of its elements by the operator and such facility permits sensitivity analysis to be conducted rapidly.

Although the model has been validated mainly from a positivist perspective and good agreement has been demonstrated between predicted and actual values, this does not necessarily support the case that the model is a close representation of reality. However, following the argument of Shannon (1975), even though rationalist and empiricist approaches should be involved in model validation the validator must finally evaluate the adequacy or validity of the model in relation to the purpose for which it was created. Therefore, considering that the main objective which guided model construction was to develop a method for quantitatively predicting the utilisation of dietary protein, the predictive accuracy of the model is the parameter of primary importance.

The model meets the design objectives in allowing a ranking of mixed diets fed to growing pigs according to protein quality and by providing the means whereby the relevant importance of several factors

affecting protein quality may be assessed.

Most of the classical methods of protein quality evaluation (protein efficiency ratio, biological value, net protein utilisation etc.) attempt to measure the amount of dietary protein retained by an animal (Bender, 1982). The model variable Pe estimates the efficiency of utilisation of daily dietary crude protein during protein growth and Pe can thus be considered a fundamental measure of protein quality. The estimation of Pe is supported by the argument of Bender (1982) that it is necessary in the evaluation of protein quality to be able to determine the available level of each essential amino acid in a food and thence predict the value of the food under a variety of conditions. The value of Pe should be considered in conjunction with the absolute level of protein deposition (Pd). It is possible for diets to demonstrate similar Pe values but yet support widely different rates of protein deposition. Calculation of Pd per unit of the maximum rate of protein deposition physiologically possible (Pr) is often useful.

Hegsted (1973) urged the development of a measure 'f' such that the amount of dietary protein multiplied by f equals the protein available to the animal. The variable Pg estimates the daily amount of protein available for growth and can thus be used to generate Hegsted's f value ($f=\frac{Pg}{CP}$). The model output also includes an estimate of the daily digestible crude protein intake (DCP), based on the apparent digestibility of dietary amino acids at the terminal ileum of the growing pig.

The model predicts the effect on protein utilisation of manipulating several dietary factors. The first limiting amino acid in growth is identified and the effects of changing factors such as feeding level, dietary amino acid balance or protein to energy ratio are readily calculated. The effects of changes in the liveweight and sex of the animal may also be evaluated.

Despite the usefulness of the model, however, for estimating the protein quality of a mixed diet and in allowing an analysis of the effects of various factors on protein quality, the model is subject to certain limitations. The model includes assumptions and ignores the influence of several factors. It is assumed, for example, that the daily maintenance energy requirement is the same for all sexes, yet the evidence of Fuller, Gordon and Aitken (1980) indicates that boars and

gilts may have substantially higher maintenance energy requirements than castrates. Moreover, the model accepts the statistical concept of maintenance energy requirement as given by Kielanowski (1965) which does not provide a direct description of the energetics of protein turnover (Fawcett, 1978). In the statistical sense the metabolisable energy requirement for maintenance becomes a residual term and may be regarded as the metabolisable energy intake not accounted for by the energy cost for deposition of protein and fat. The statistical concept of maintenance has no strict physiological meaning but can be generally associated with the energy required for vital body functions such as circulation, respiration and body tissue turnover. Tess (1981) has argued that it is more appropriate to relate the maintenance energy requirement to lean body mass rather than body weight. Whittemore and Gibson (1983) have suggested that the pig growth model of Whittemore and Fawcett (1976) could be refined by expressing the energy cost of maintenance as a function of body protein mass.

It is also assumed that the rate of lipid deposition can not be lower than the rate of protein deposition. By way of example, this constraint means that if a daily ration of 580 grams of barley is fed to a 35 Kg liveweight gilt (providing a digestible energy intake close to maintenance requirement) the model predicts that lipid is deposited at 0.73 g/d and protein at 0.47 g/d. The data of Close, Mount and Brown (1978) and Close and Stanier (1980), however, suggest that at low levels of food intake the growing pig loses stored lipid to support protein accretion. If the model contained an estimate of total body composition, it would seem more appropriate to express the minimum lipid constraint in terms of body lipid composition rather than daily lipid deposition rate. Also, the findings of Kemm (1980) show that the minimum lipid to protein ratio may be lower than 1:1. Whittemore (1983) has noted that recent research indicates that the body weight gain of the entire male pig is typified by a lower ratio of lipid to protein, than is the case for the gilt or castrated male. In face of the arbitrariness concerning the choice of the magnitude of the constraint between lipid and protein deposition rates, the small loss of energy associated with protein deamination (Step 10) is not calculated in the present model.

A further point relevant to the prediction of nutrient assimilation at low food intakes is that the model assumes a constant 'ideal' balance of amino acids required for growth, whereas this pattern may vary between

levels of protein intake. Finally, the model disregards the possible interactive effect on growth due to factors such as environmental temperature, genotype, disease, heat-damaged proteins, large degrees of amino acid imbalance and amino acid toxicity, the presence of anti-nutritive substances eg. tannins and the levels of other dietary substances, eg. minerals, vitamins, fats and fibre.

The limitations imposed by model design must be regarded in the interpretation of model output. In spite of these limitations, however, evidence accumulated to date suggests that the model allows rapid and accurate assessment of the protein quality of dietary regimes commonly encountered in practical pig production. An important attribute of the model is the speed at which the protein quality of a mixed diet can be determined. A protein quality score for a diet can be estimated (48 K memory Apple II computer) within approximately three minutes, thus allowing the prediction and comparison of protein quality scores for a large number of dietary regimes.

In order to illustrate the use of the simulation model, dietary formulations for the growing pig typical of those currently being employed on New Zealand farms, were studied. The respective dietary formulations are given in Table III.2.5. The nutrient compositions of the ingredients listed in Table III.2.5 were taken from tabulated values, and estimates of the apparent ileal digestibility of amino acids in the ingredients were based upon published estimates. It was assumed that the diets were fed to 50 Kg liveweight gilts at three levels of daily food intake. These input data were computed using the simulation model and output relating to the utilisation of dietary protein is presented in Table III.2.6.

At the two lower levels of feeding, the protein of diet five was utilised with the greatest efficiency and that of diet four with the least efficiency. At the high feeding level, diet six gave the greatest efficiency and diet four the lowest. The maximum value of Pe was 42.08% and the minimum value 18.82%. The difference between the highest and lowest efficiencies of protein utilisation, at each feeding level, decreased as food intake increased.

The values of Pe for the various diets rank differently at each

Table III.2.5 The ingredient compositions (g/Kg air-dry weight) of some commercial pig grower diets[†1].

Ingredient	Diet 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Maize	-	720	-	200	790	-	405	440	708	200	50	-	-	-	-
Barley	740	-	415	613	-	745	344	440	-	390	640	870	790	820	830
Wheat	-	-	275	-	-	200	-	-	-	170	100	-	-	-	-
Skim milk powder	-	50	-	-	110	25	-	-	75	-	-	-	-	-	-
Meat-and-bone-meal	100	175	100	-	70	-	-	70	-	-	-	100	100	110	100
Lucerne	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-
Peas	120	-	200	-	-	-	-	-	-	-	-	-	60	-	-
Pollard	-	-	-	125	-	-	51	-	50	100	100	-	-	-	-
Meat-meal	-	-	-	50	-	-	101	-	100	125	100	-	-	-	-
Blood-meal	30	-	-	-	20	-	38	40	40	-	-	25	40	30	35
Fish-meal	-	-	-	-	-	-	51	-	20	-	-	-	-	30	25
Casein	-	-	-	-	-	25	-	-	-	-	-	-	-	-	-
Tallow	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-
Synthetic lysine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
Vitamins & minerals	10	5	10	12	10	5	10	10	7	10	10	5	10	10	9.5

[†1] Source of information - The New Zealand Pork Industry Board.

Table III.2.6 Model prediction of the utilisation of dietary crude protein at three levels of food intake.

Model output	Diet †4														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DCP (g/d) †1	193	184	180	147	181	153	237	167	243	198	182	174	196	194	195
Pg (g/d)	86.40	73.61	68.77	37.99	96.38	59.41	108.66	65.68	127.86	58.75	52.81	64.28	87.73	83.50	92.98
Pd (g/d)	77.40	73.61	68.77	37.99	96.38	59.41	80.66	65.68	92.00	58.75	52.81	64.28	75.73	73.50	74.98
Pe (%)	29.23	28.83	27.66	18.82	41.88	30.63	26.21	30.86	30.00	21.90	21.09	26.66	28.44	27.35	28.15
DCP (g/d) †2	229	219	214	175	215	182	281	198	288	235	216	206	232	231	231
Pg (g/d)	106.34	91.15	85.41	48.86	118.20	74.30	132.78	81.74	155.58	73.51	66.46	80.08	107.93	102.90	114.16
Pd (g/d)	106.34	91.15	85.41	48.86	115.00	74.30	110.00	81.74	115.00	73.51	66.46	80.08	106.93	102.90	105.16
Pe (%)	33.82	30.07	28.93	20.38	42.08	32.26	30.01	32.34	31.58	23.08	22.35	27.97	33.82	32.25	33.24
DCP (g/d) †3	304	291	284	232	285	242	374	263	382	312	287	274	309	306	307
Pg (g/d)	147.71	127.55	119.92	71.41	163.45	105.17	182.80	115.05	213.08	104.13	94.77	112.85	149.82	143.14	158.09
Pd (g/d)	115.00	115.00	115.00	71.41	115.00	105.17	115.00	115.00	115.00	104.13	94.77	112.85	115.00	115.00	115.00
Pe (%)	27.55	28.57	29.34	22.44	31.70	34.40	23.70	34.28	23.79	24.62	24.01	29.69	27.40	27.15	27.38

†1 50 Kg liveweight gilt fed 1440 g meal/d.

†2 50 Kg liveweight gilt fed 1710 g meal/d.

†3 50 Kg liveweight gilt fed 2270 g meal/d.

†4 Refer Table III.2.5.

level of feeding. In several cases (eg. diet one) the maximum efficiency of utilisation of dietary protein was found at the intermediate feeding level whereas for other diets (eg. diet three), the efficiency of utilisation increased in accordance with feeding level. The fact that the value of Pe for a given diet changes with the level of feeding points to an inadequacy of describing dietary protein quality by the common lysine/energy ratio. Also, at any particular feeding level it can be readily shown using the data in Table III.2.6 that the lysine/energy ratio is a poor indicator of the efficiency of utilisation of dietary protein.

There are several examples in Table III.2.6 of diets the Pe values of which are similar yet the values for protein deposition (Pd) are very different (eg. diets eight and nine at 1440 g meal/d). This reiterates the need to interpret the values for Pe in the light of the values of Pd or alternatively to compare the quality of diets at equal crude protein intakes. With reference to the data in Table III.2.4 it can be shown that on average 0.43 of the daily digestible crude protein intake was available to the pig for growth. The proportion of digestible crude protein available for growth fell to a minimum value of 0.26 (diet four, 1440 g meal/d) indicating a considerable degree of amino acid imbalance for this diet. The importance of an adequate protein-free energy supply is exemplified by comparison of diets five and nine at the low level of food intake (1440 g/d). Although diet nine has a higher value for Pg (127.86 vs 96.38 g/d) diet five supports a greater level of protein deposition (96.38 vs 92.00 g/d) indicating considerable deamination of protein from diet nine.

The model provides the means for studying the effects of changes in dietary and animal factors on the utilisation of dietary protein. By way of example, if diet four was supplemented with synthetic lysine and this diet was fed to the 50 Kg liveweight gilt at 1440 g/d then the model indicates that with 0.06% added lysine, Pd increases from 37.99 to 48.30 g/d and Pe from 18.82 to 23.84%. With 0.10% added lysine, Pd increases to 55.22 g/d and Pe increases to 27.17%.

If diet four was fed (1440 g/d) to a 40 Kg liveweight gilt as opposed to a 50 Kg animal, Pd increases from 37.99 to 41.07 g/d and Pe from 18.82 to 20.35%, although the ratio of lipid to protein in

daily gain also increases considerably (2.95 to 3.30). The model indicates that lysine is the first limiting amino acid for growth in most of the diets studied. Methionine plus cystine, however, was shown to be first limiting in diet one. The model demonstrates that the addition of synthetic methionine (0.05%) to diet one at the low level of feeding would lead to insignificant changes in Pg and Pd, thus indicating that methionine plus cystine is only marginally limiting in this diet. A combined supplementation of lysine (0.06%) and methionine (0.04%) gives small increases in Pd and Pe (Pd: 77.40 to 78.55 g/d, Pe: 29.23 to 29.50%). Following the latter supplementation, however, Pg increases from 86.40 to 97.65 g/d. In this case, the model predicts that the replacement of some barley in diet one with peas (2% of diet) and tallow (3% of diet) in conjunction with the addition of lysine and methionine allows Pd to increase from 77.40 to 89.06 g/d and Pe from 29.23 to 33.65%.

Presentation of the above analysis demonstrates the considerable utility of the simulation model. Further, although the present model was developed to enable a rapid comparison of protein quality among differing nutritional regimes the model may find other applications. The model has already proved an effective aid for the teaching of nutritional principles and it is envisaged that an approach similar to the one adopted here, may afford application in the nutrition of other mammalian species including man.

ADDENDUM

Since completion of this study some pertinent information has been published concerning the nature of protein, energy interaction during pig growth. The data of Campbell, Taverner and Curic (1984) afforded a further test of the adequacy of the simulation model described in Part III of the thesis.

Campbell *et al.* (1984) fed eight diets of similar energy content but ranging in crude protein concentration from 95 to 256 g/Kg to entire male pigs growing from 45 to 90 Kg liveweight. Each of the diets was given at two levels of feeding to provide either 2.5 or 3.2 times the energy required for maintenance. Rates of body protein and lipid deposition were determined and are presented in Table 1.

Information pertaining to the latter study served as input for the model. Determined gross amino acid, ileal amino acid digestibility and digestible energy values for the dietary ingredients were not available so tabulated values were used. The predicted rates of protein and lipid deposition are also given in Table 1.

Although the nutrient data entered to the model programme were only estimates, there is close agreement between the actual and predicted values for tissue deposition rate.

Campbell *et al.* (1984) interpreted their data set as indicating an interdependent effect of dietary protein and energy on protein deposition rate. The determined response of protein deposition to dietary protein intake is shown in Figure 1. Reference to Figure 1 shows that when dietary protein was limiting for growth, rate of protein deposition was linearly related to protein intake but independent of energy intake. For pigs given adequate levels of crude protein, rate of protein deposition was related to energy intake and independent of crude protein.

Figure 1 also demonstrates that the simulated pattern of protein deposition rate follows the actual pattern very closely. The model output confirms that at lower protein intakes, protein available for growth (Pg) was less than the maximum rate of protein deposition

physiologically possible (Pr) and that energy available for tissue deposition (Et) was not limiting protein deposition. Further, at the three higher levels of protein intake on the low level of feeding the model indicates that Pg was greater than Pr but that Et limited protein deposition (note that Ld : Pd = 1.0). Finally, at the five higher levels of protein intake on the high feeding regime the model indicates that Pg was greater than Pr and that Et did not limit the rate of protein deposition (note Ld : Pd >1.0). It appears that at these levels of protein and energy intake, Pr imposed a limit on the rate of body protein deposition.

Table 1. A comparison of actual† rates of daily tissue deposition with values generated by a model simulating growth in the pig.

Feeding level	Protein intake (g/d)	Rate of tissue deposition (g/d)				Ratio of lipid to protein in gain	
		Protein		Lipid			
		Act.*	Pred.‡	Act.	Pred.	Act.	Pred.
Low	175	56	51	216	208	3.86	4.08
	217	77	70	207	192	2.69	2.74
	260	92	89	191	175	2.08	1.97
	303	117	107	159	155	1.36	1.45
	342	127	125	142	134	1.12	1.10
	389	125	130	138	131	1.10	1.00
	427	124	126	134	128	1.08	1.00
	474	125	127	136	127	1.09	1.00
High	226	76	74	302	325	3.97	4.39
	280	99	98	297	302	3.00	3.08
	336	124	122	283	281	2.28	2.30
	389	142	141	249	253	1.75	1.79
	445	145	141	247	252	1.70	1.79
	504	147	141	240	250	1.63	1.77
	557	137	141	238	244	1.74	1.73
	612	134	141	232	239	1.73	1.70

* Actual ‡ Predicted.

† CAMPBELL, R.G., TAVERNER, M.R. and CURIC, D.M. (1984). Effect of feeding level and dietary protein content on the growth, body composition and rate of protein deposition in pigs growing from 45 to 90 Kg. *Animal Production 38:* 233-240.

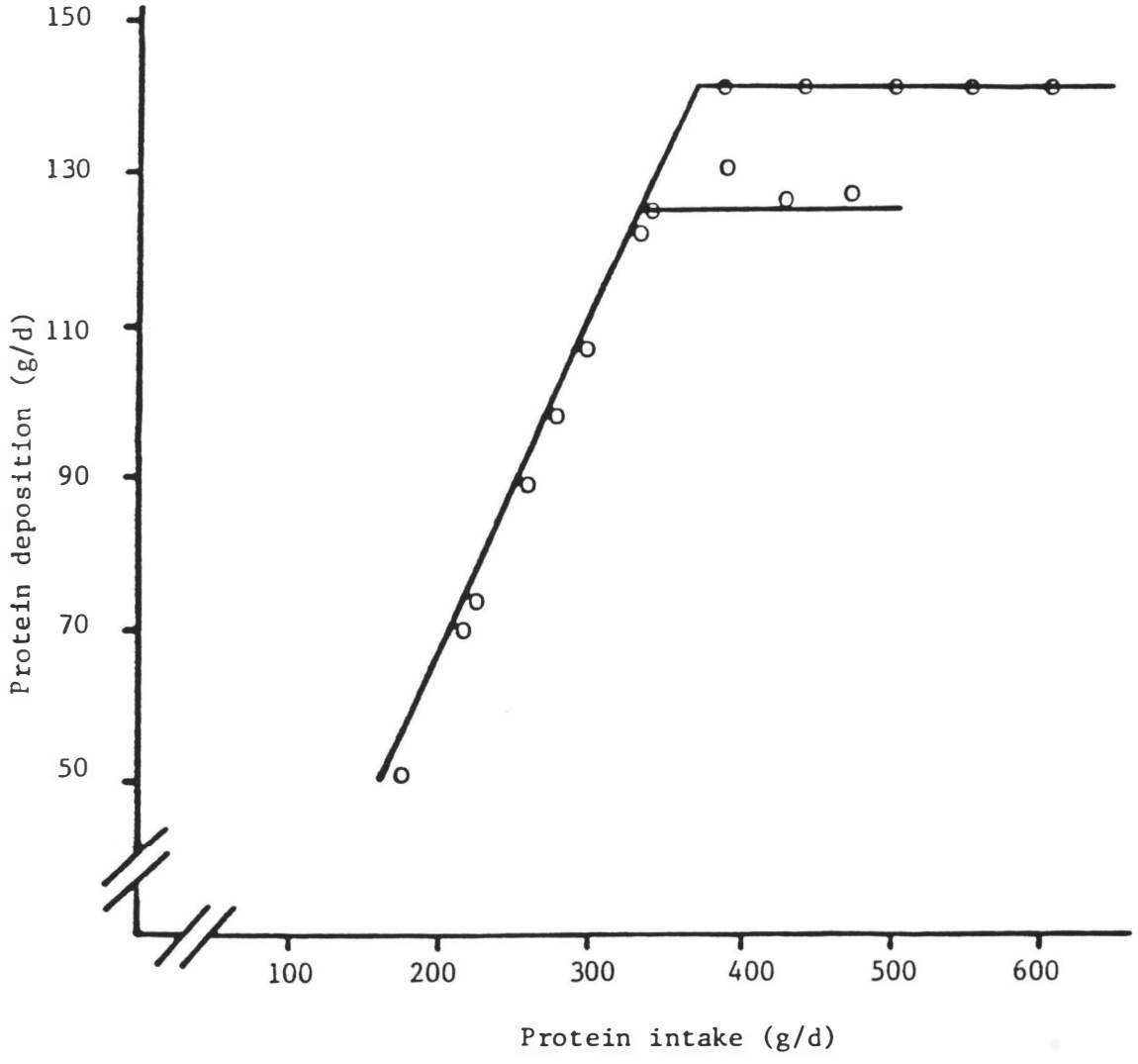


Fig. 1. Relationship between dietary protein intake and body protein deposition rate in the growing pig. Comparison of simulated values (O) with actual response (—), (Campbell *et al.*, 1984).

Appendix I.1 The analysis of variance of dry matter digestibility data pertaining to preliminary study one (Section I.2.3).

The experiment included three dietary treatments and was conducted in two replicates with six pigs per replicate.

Treatments and replicates are regarded as fixed variables.

Digestibility values for dietary dry matter

Replicate	t_1	t_2	t_3
1	0.827	0.818	0.810
	0.835	0.835	0.838
2	0.827	0.807	0.824
	0.808	0.824	0.819

ANOVA Table

Source of variation	d.f.	M.S.	F	Significance level
Treatment	2	0.000010	0.07	N S
Replicate	1	0.000251	1.67	N S
Treatment x replicate	2	0.000036	0.24	N S
Error	6	0.000150		
Total	11			

The overall mean = 0.823

The standard error of the overall mean = $\frac{\sqrt{0.000150}}{\sqrt{12}}$ = 0.0035

The pooled standard error of the treatment means = $\frac{\sqrt{0.000150}}{\sqrt{4}}$

= 0.0061.

Appendix I.2 The analysis of variance of urinary urea excretion data pertaining to preliminary study two (Section I.2.4).

The experiment included two dietary treatments (3 pigs per treatment) and involved the measurement of urinary urea excretion over two consecutive two-day periods.

Treatments and periods are regarded as fixed variables.

Urinary urea excretion ($mg/Kg^{0.75}/d$)

Treatment	1			2		
Pig	1	2	3	1	2	3
Period 1	351.61	421.99	271.04	378.25	317.70	439.07
Period 2	273.57	359.51	255.22	271.75	259.70	391.38

ANOVA Table

Source of variation	d.f.	M.S.	F	Significance level
Treatment	1	1300.21	0.16	N S
Main plot error	4	8384.83		
Period	1	11317.86	22.25	* *
Treatment x Period	1	259.94	0.51	N S
Sub-plot error	4	508.66		
Total	11			

The 1% critical F value with 1 and 4 degrees of freedom is 21.02.

The standard errors of the differences between means were calculated following Federer (1955).

The difference between the treatment means (pooled over periods) is (342.98 - 322.16) = 20.82 mg urea/$Kg^{0.75}$/d.

The standard error of this difference equals $\sqrt{\frac{2(8384.83)}{2}}$

= 91.57.

The difference between the period means (pooled over treatments) is 363.28 - 301.86 = 61.42 mg urea/$Kg^{0.75}$/d.

The standard error of this difference equals $\sqrt{\frac{2(259.94)}{2}}$

= 16.12.

Appendix I.3 Calculation of the level of urinary urea nitrogen excretion of a 40 Kg liveweight boar fed an imbalanced pattern of amino acids.

A computerised model simulating the digestion and metabolism of nitrogen in the growing pig (refer Part III of thesis) was used to predict the urinary urea nitrogen excretion of a boar of 40 Kg liveweight fed the basal diet (I.2.2.iii). Prediction of the urinary urea nitrogen excretion derived from the deamination of imbalanced basal dietary amino acids was made at two proposed 'ideal' levels of isoleucine, namely that in the basal diet (I.2.6) and that recommended by the Agricultural Research Council (1981). The basal diet supplied 46 units of isoleucine per 100 units of lysine whereas the Agricultural Research Council (1981) recommended 54 units of isoleucine per 100 units of lysine. The Agricultural Research Council's estimate of ideal amino acid balance indicates that isoleucine is the first limiting essential amino acid in the basal diet. In this case comparison was made between the following amino acid balances.

	Amino acid balance		
Amino acid	Basal diet	'Ideal' (1)	'Ideal' (2)
Lysine	100	100	100
Methionine + cystine	54	54	54
Tryptophan	13	13	13
Histidine	34	34	34
Phenylalanine + tyrosine	101	101	101
Threonine	59	59	59
Leucine	89	89	89
Isoleucine	46	46	54
Valine	66	66	66
Non-essential component	693	693	693

Computer input:

Name of diet - Basal.

Crude protein content of diet	8.3%
Apparent digestible energy content of diet	15 MJ/Kg diet
Digestibility of lysine	100%
Daily food intake	1480 g
Liveweight	40.0 Kg

Amino acid composition of diet (%)

Lysine	0.6589
Methionine + cystine	0.3585
Tryptophan	0.0827
Histidine	0.2213
Phenylalanine + tyrosine	0.6640
Threonine	0.3875
Leucine	0.5831
Isoleucine	0.3042
Valine	0.4354

Computer output

(a) Comparison between the basal dietary amino acid balance and 'ideal' amino acid balance (1).

Output:

Urinary urea nitrogen excretion due to imbalanced dietary amino acids	= 0 mg/d
Nitrogen available for growth and maintenance	= 18.77 g/d
Body protein deposited	= 100 g/d.

(b) Comparison between the basal dietary amino acid balance and 'ideal' amino acid balance (2).

Output:

Isoleucine is the first limiting amino acid.

Urinary urea nitrogen excretion due to imbalanced dietary amino acids = 2590 mg/d

Nitrogen available for growth and maintenance = 16.18 g/d

Body protein deposited = 84.24 g/d.

Appendix II.1 The endogenous output of nitrogen at the terminal ileum of the growing pig.

Ileal endogenous output (mg N/Kg dry matter intake) of growing pigs fed protein-free diets.

Taverner(1979)†[1]	Taverner(1979)†[2]	Sauer *et al*(1977b)†[3]	Mean†[4]	Experiment II.2 2.ii†[5]
1210	1810	1314	1445	1422

†[1] Diet did not contain cellulose.

†[2] Diet contained 50 g cellulose/Kg air-dry weight.

†[3] Diet contained 50 g cellulose/Kg air-dry weight.

†[4] Mean value based on the estimates of Taverner (1979) and that of Sauer *et al.*, (1977b).

†[5] Diet contained 36.60 g cellulose/Kg air-dry weight.

Appendix II.2 The determined apparent digestible energy contents (MJ/Kg air-dry weight) of two diets (Trial II.2.4) fed to growing pigs (30 Kg liveweight).

	Diet Barley-based		Diet Casein control
Pig 1	13.22	Pig 4	14.84
Pig 2	13.37	Pig 5	14.93
Pig 3	13.07	Pig 6	14.88
Mean	13.22	Mean	14.88

Appendix III.1 Derivation of molecular weight conversion factors (M_i).

Molecular formulae derived from structural data (McDonald *et al.*, 1973).

Lysine	=	$N_2C_6H_{14}O_2$
Threonine	=	$N_1C_4H_9O_3$
Methionine + cystine	=	$N_1C_5H_{11}O_2S_1$ + $N_2C_6H_{12}O_4S_2$
Isoleucine	=	$N_1C_6H_{13}O_2$
Tryptophan	=	$N_2C_{11}H_{12}O_2$
Histidine	=	$N_3C_6H_9O_2$
Phenylalanine + tyrosine	=	$N_1C_9H_{11}O_2$ + $N_1C_9H_{11}O_3$
Leucine	=	$N_1C_6H_{13}O_2$
Valine	=	$N_1C_5H_{11}O_2$
Glycine	=	$N_1C_2H_5O_2$
Alanine	=	$N_1C_3H_7O_2$
Serine	=	$N_1C_3H_7O_3$
Aspartic acid	=	$N_1C_4H_7O_4$
Glutamic acid	=	$N_1C_5H_9O_4$
Arginine	=	$N_4C_6H_{14}O_2$
Proline	=	$N_1C_5H_9O_2$

	Total molecular weight		$\frac{\text{Atomic weight nitrogen}}{\text{Total molecular weight}}$
Lysine	146		0.19 = M_1
Threonine	119		0.12 = M_2
Methionine + cystine	389		0.11 = M_3
Isoleucine	131		0.11 = M_4
Tryptophan	204		0.14 = M_5
Histidine	115		0.27 = M_6
Phenylalanine + tyrosine	346		0.08 = M_7
Leucine	131		0.11 = M_8
Valine	117		0.12 = M_9
Glycine	75)		
Alanine	89)		
Serine	105)		
Aspartic acid	133)	N.E.A.A.	0.17 = M_{10}
Glutamic acid	147)		
Arginine	174)		
Proline	115)		

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PIG PROTEIN

THIS IS A SIMULATION MODEL DESIGNED
BY F.J.MOUGHAN,MASSEY UNIVERSITY,4/11/81.

THE MODEL SIMULATES PROTEIN METABOLISM
WITHIN THE GROWING PIG (20 - 80 KG LIVEWEIGHT)
AND IS INTENDED FOR THE ASSESSMENT OF
DIETARY PROTEIN QUALITY

DIET AND ANIMAL CHARACTERISTICS

FOOD INTAKE 1280 G/PIG/DAY

LIVEWEIGHT 40 KG
METABOLIC LW. 15.91 KG

ACTUAL DIET IS -

MAIZE	40 %
BARLEY	35 %
MEAT&BONE	15 %
BLOOD	3 %
PEAS	5 %
OTHERS	2 %

AMINO ACID	ALPHA(G/N)	BETA	EPSILON	% IN DIET
LYSINE	1.51	100	100	.82
METH & CYST	.56	37	32	.51
TRYPTOPHAN	.23	15	9	.17
HISTIDINE	1.04	69	48	.35
PHEN & TYR	.97	64	43	1.17
THREONINE	.69	46	37	.6
LEUCINE	1.71	113	51	1.48
ISOLEUCINE	.54	36	27	.5
VALINE	1.01	67	42	.84
N.E.A.A	18.32	1210	620	11.41

AMINO ACID	INTAKE-A(G)	DIGESTIBLE-Z(G)	%DI
LYSINE	10.53	7.97	75.69
METH & CYST	6.46	5.05	78.19
TRYPTOPHAN	2.14	1.67	78.11
HISTIDINE	4.52	3.85	85.03
PHEN & TYR	15.01	12.12	80.72
THREONINE	7.71	5.77	74.79
LEUCINE	18.89	15.52	82.16
ISOLEUCINE	6.44	4.91	76.25
VALINE	10.71	8.45	78.89
N.E.A.A	146.05	107.74	73.77

CRUDE PROTEIN	17.85 %
AVERAGE DIGEST.FACTOR	75.74 %
DIGESTIBLE CRUDE PROTEIN	173 G
TOTAL DIGESTIBLE ENERGY	16.95 MJ.DE/DAY
PROTEIN FREE ENERGY	12.87 MJ.DE/DAY

MODEL VARIABLES

AMINO ACID	EPSILON
LYSINE	100
METH & CYST	32
TRYPTOPHAN	9
HISTIDINE	48
PHEN & TYR	43
THREONINE	37
LEUCINE	51
ISOLEUCINE	27
VALINE	42
N.E.A.A	620

NO .17 G.N/KG^.75/DAY
PR 115 G.PROTEIN/DAY
EM .46 MJ.ME/KG^.75/DAY
ECP 53 KJ.ME/GRAM PROTEIN
ECL 53 KJ.ME/GRAM LIPID
LD:PD = 1

3

OUTPUT
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AMINO ACID 1 IS LIMITING
VALUE OF OBJ. FN. = 1.51

NE	11.31	PG	78.58
NO	2.7	PP	78.58
NF	0	E1	.81
NZ	.32	E2	0
NU	14.33	EA	13.68
NF	8.87	EG	6.36
NA	15.28	ET	8217

LD 78.46 G.LIPID/DAY

PD 76.58 G.PROTEIN/DAY

PD/CP = 33.52 %

ABBREVIATIONS
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BETA - ACTUAL DIETARY AMINO ACID BALANCE (RELATIVE TO LYSINE @ 100)
CP - CRUDE PROTEIN INTAKE
%DI - PERCENTAGE DIGESTIBILITY OF EACH AMINO ACID IN THE DIET

E1 - ENERGY (MJ) FROM DEAMINATION OF IMBALANCED AMINO ACIDS
E2 - ENERGY (MJ) FROM DEAMINATION OF AMINO ACIDS SUPPLIED OVER AND ABOVE THE BIOLOGICALLY POTENTIAL PROTEIN DEPOSITION RATE
EA - ENERGY (MJ) AVAILABLE FOR GROWTH AND MAINTENANCE
ECL - ENERGY COST PER GRAM OF LIPID DEPOSITED (KJME/GRAM)
ECP - ENERGY COST PER GRAM OF PROTEIN DEPOSITED (KJME/GRAM)
EG - ENERGY (MJ) AVAILABLE FOR GROWTH
EM - ENERGY REQUIREMENT FOR MAINTENANCE (MJME)
ET - TOTAL ENERGY (KJ) AVAILABLE FOR THE DEPOSITION OF LIPID AND PROTEIN

EPSILON - IDEAL DIETARY AMINO ACID BALANCE (RELATIVE TO LYSINE @ 100)

LD - LIPID DEPOSITION RATE (G/DAY)
NA - NITROGEN AVAILABLE FOR GROWTH AND MAINTENANCE (G.N/DAY)
NE - URINARY NITROGEN LOSS DUE TO AA IMBALANCE (G.N/DAY)
NF - FAECAL NITROGEN FROM DIETARY PROTEIN + ENDOGENOUS PROTEIN SOURCES (G.N/DAY)
NO - ENDOGENOUS URINARY NITROGEN LOSS (G.N/DAY)
NP - NITROGEN EXCRETED IN URINE DUE TO DIETARY SUPPLY OVER AND ABOVE PIG'S GENETIC ABILITY TO DEPOSIT PROTEIN (G.N/DAY)
NU - TOTAL URINARY NITROGEN EXCRETION (G.N/DAY)
NZ - NITROGEN EXCRETED IN URINE DUE TO DEAMINATION OF DIETARY PROTEIN TO SUPPLY ENERGY FOR LIPID DEPOSITION (G.N/DAY)

PD - PROTEIN DEPOSITION RATE (G.PROTEIN/DAY)
PG - DIETARY PROTEIN AVAILABLE FOR DAILY GROWTH (G.PROTEIN/DAY)
PP - AMOUNT OF PROTEIN (G) THAT CAN BE DEPOSITED DAILY DUE TO DIETARY AND GENETIC INFLUENCES
PR - THE MAXIMUM RATE OF PROTEIN DEPOSITION BIOLOGICALLY POSSIBLE (G)