A COMPARISON OF THE NUTRITIVE VALUE OF NORMAL AND OPAQUE-2 MAIZE FOR GROWING PIGS IN DIETS CONTAINING MEAT AND BONE MEAL WITH AND WITHOUT AMINO ACID SUPPLEMENTATION

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CHAPTER 1: AN INTRODUCTION

Pig meat production in New Zealand has traditionally been associated with the dairy industry, relying upon dairy by-products (principally skim-milk and whey) as major sources of protein and energy for pig feed. However, export market prospects for milk protein led to the diversification of dairy factory products, such as the ultra-filtration of whey protein and lactose extraction of whey. The prevailing economic climate also encouraged dairy farmers to change to whole milk tanker collection and large numbers of the supplementary pig enterprises were closed down. Despite fluctuations, market prices generally showed an increase and many farmers began investigating alternative food supplies in order to take advantage of these higher prices. Garbage and other edible waste provided only limited scope for expansion, and the main alternative appeared to be the use of cereal grains. The New Zealand pig industry, in the last 5-10 years, has therefore begun to move towards a specialised form of production based upon the use of diets containing a predominance of cereals, similar to what has prevailed in many overseas countries for a longer period. The local report of Kingma and Ryan (1971) illustrates the need for efficiency in the high cost system of production based upon meal feeding.

Where skimmilk is the principal ingredient in the ration, the supply of dietary protein is generally adequate due to the high nutritive value of the protein in this feedstuff. However, when the major source of nutrients comes from cereal grains, although these provide a concentrated source of energy, the poor balance of amino acids becomes limiting to the utilization of such rations. In New Zealand there is a restricted range of feeds high in protein and suitable for incorporation into pig rations. They include skimmilk powder, buttermilk powder, meat meal, meat and bone meal, liver meal and fish meal. Alternative sources of protein in soybeans, lupins, field beans and lucerne are being investigated.

High costs restrict the use of skimmilk powder to creep rations for suckling pigs and at present there are limited supplies of fish meal. Meat and bone meal remains an attractive substitute with its lower price, although there is a significant reduction in the quality of protein supplied. Therefore it would be an advantage to study the protein supplementation of maize diets for growing pigs and to investigate the use of meat and bone meal as a major supplementary component. This then becomes basically a problem of nutrition and production economics: of nutrition in a thorough knowledge of performance in relation to different ration formulations, and of production economics in finding that level of animal performance which is most desirable in economic terms.

The acreage of maize grown in New Zealand for grain production has shown a marked increase in recent years, and has been very rapid in some areas (Table A1.1). An important factor in this increase has been the demand for maize by the poultry industry.

With the increasing availability of maize grain, and the need for data relevant to the formulation of diets based on maize and used under local conditions, it was decided to embark upon a programme of investigation into the suitability of maize as a major ingredient in swine rations and how it could be incorporated into an efficient ration formulation procedure. A supply of opaque-2 maize also became available from seed supplied to the Massey University Agronomy Department from the United States.

The principal objectives of the study to be described may be enumerated as follows:

1. To provide a comparison between opaque-2 and normal hybrid maize in diets containing meat and bone meal as the single protein supplement.
2. To attempt to establish the essential amino acids which are limiting the performance of animals fed a maize/

meat and bone meal diet by supplementation with synthetic lysine, methionine and tryptophan. Also, whether the two maize types differed in this respect.

3. To investigate a possible relationship between growth performance and the retention of dietary nitrogen (N) on these experimental diets.

The investigations to be described are intended to be only a part of the work necessary to provide information for the efficient utilization of maize (and meat and bone meal) in rations for growing pigs.

CHAPTER 2: THE PROTEIN AND AMINO ACID NUTRITION OF THE GROWING PIG

2.1 INTRODUCTION

Research in the field of animal nutrition in the last century has yielded comprehensive information in qualitative and quantitative terms on the requirements of animals in various physiological states. One of the earliest and subsequently most useful attempts at chemically separating out feed constituents which related to animal requirements was introduced approximately 100 years ago by Henneberg and Stohmann, working at the Weede Experimental Station in Germany. It was termed the "Proximate Analysis of Feeds" and divided the diet into six fractions: moisture, ash, crude protein, ether extract, crude fibre and nitrogen free extract (by difference). However, it soon became apparent that although the Proximate Analysis was useful in some respects for comparing feeds, because of the heterogenous nature of these categories, more sophisticated descriptions became necessary. Not only have the various constituents of the diet, necessary for normal body function, been substantially described, but there has also developed an increasing awareness that these ingredients cannot be considered in isolation to each other. Dietary constituents often show inter-relationships which in certain situations become important in determining the nutritional adequacy of a diet.

The following discussion will be related specifically to the protein (and amino acid) requirements of the growing pig. The growth period may be defined as the period from weaning to approximately 100 kg live weight, with particular emphasis on the period up to 50 kg. Where necessary, this aspect of nutrition will be considered in relation to other dietary constituents. However, as a general assumption and unless otherwise stated, the performance of the animal as influenced by the supply of protein will be discussed in the

context of a dietary regime supplying all other necessary nutrients in amounts which allow optimum performance (however that may be defined).

2.2 PROTEIN AS A DIETARY CONSTITUENT

Different dietary proteins had long been known to influence the rate of live weight gain in young animals (Magendie, 1841a), but Osborne et al. (1919) were the first to standardise a method using this criterion as an index to compare the nutritive value of dietary proteins. They initially defined the "Protein Efficiency Ratio" (PER) as the amount of body weight gain per unit weight of protein ingested, with certain conditions specified. Thomas (1909) had earlier observed that the ability of dietary protein to maintain normal body function was related to the proportionate amount of N in the diet which was retained in the body. He introduced the term "Biological Value" (BV) as an index of protein quality. Thus the ability of dietary protein to influence the growth and N metabolism in the young animal was recognised in these early attempts to classify proteins. Both BV and PER have been modified since these early studies, and many additional methods have been presented, but to the present day they have played a major role in the development of understanding in protein nutrition. These two measures of protein quality are mentioned here as they form the basis of the criteria studied in the experiments to be described.

Variations in the nutritional value of dietary proteins was recognised by such early workers as Megandie (1841b), but it was not until Loewi (1902) first showed that fractions of proteins could replace dietary protein that investigations began into the composition of proteins in feed. Understanding of the role of protein in human and animal nutrition paralleled biochemical progress in determining the primary structure of proteins as chains of animo acids joined by peptide linkages.

The early methods of relating N intake to animal performance, such as BV and PER, recognised nutritional differences between proteins, but considered them as a single entity. Investigations into the amino acid make-up of proteins was begun by workers such as Hopkins (1916), Osborne and Mendel (1914), and Rose (1937, 1938, 1949). Abderhalden (1912) put forward an initial hypothesis that a feed protein became more favourable nutritionally to the animal with greater similarity between the feed protein and body protein. Osborne and Mendel (1914) modified this concept to put importance upon those amino acids which the animal was unable to synthesize and therefore had to be provided in the diet. Using rats as the experimental animals, tryptophan, lysine and histidine were the first shown to definitely indispensable components of feed, but as purified amino acids became available phenylalanine, leucine and isoleucine, threonine, methionine, valine and arginine were tentatively added to this list for the growing rat (Osborne and Mendel, 1914). These workers used zein extracted from maize endosperm as the protein fraction in the diet and showed that both lysine and tryptophan were needed as supplements to promote growth.

As techniques for the qualitative and quantitative measurement of amino acids became available, the pattern of amino acids in feed proteins was found to vary greatly, a notable example being zein, which was found to be very low in lysine and tryptophan relative to many other proteins (Osborne and Clapp, 1907-08). Amino acids were classified into dispensible and indispensible categories for the growing rat, and there followed a great deal of research effort directed at determining the minimum levels of each of the essential amino acids required in the diet for this species (Rose, 1937). It was pointed out that various factors such as age, weight, sex and other constituents in the diet can influence minimum requirements. An amino acid was defined as being indispensable when its exclusion from the diet would not allow for optimum growth. Growing rats showed an ability to survive on diets devoid of arginine, but as normal growth rates could not be achieved, this amino acid was classified as essential for the growing rat.

After these initial investigations had demonstrated that the monogastric animal assimilates dietary protein as the constituent amino acids and that several of these could not be synthesized by the rat, there were attempts to characterise the amino acids for other species and determining their minimum requirements. Initial investigations relating to the amino acid nutrition of the pig were conducted by Mertz and co-workers (1952). Since that time, the National Research Council (NRC) and the Agricultural Research Council (ARC) of Great Britain have reviewed the relevant literature at different times and published recommended nutrient requirements for pigs (NRC, 1944, 1950, 1953, 1959, 1964, 1968; ARC, 1967). Rerat and Lougnon (1968) have characterised the 19 common amino acids found in dietary proteins according to available evidence into essential, semi-essential, and non-essential categories (Table 2.2.1).

TABLE 2.2.1 Dietary amino acids characterised for the growing pig (taken from Rerat and Lougnon, 1968).

Essential Amino Acids	Semi-essential Amino Acids	Non-essential Amino Acids
Arginine		Alanine
Histidine		Aspartic acid
Isoleucine		Glutamic acid
Leucine		Glycine
Lysine		Hydroxyproline
Methionine----------	Cystine	Proline
Phenylalanine----------	Tyrosine	Serine
Threonine		
Tryptophan		
Valine		

The pig production systems of America, as compared to Europe and Britain, differ principally in that the former practice ad libitum feeding, while in the latter countries

pigs are usually restricted to a feeding scale based upon live weight for most of the growth period. With the consequent differences in production objectives, and also since the response of animals to dietary protein can be influenced by feed intake (Section 2.3.7), the published work related to restricted feeding systems, as is practiced in New Zealand, is given greater emphasis in the following review. The work published by the ARC (1967) is the most comprehensive review to date of the protein and amino acid requirements of the growing pig under restricted feeding systems. No effort will be made to expand on this thorough review, but particular problems relating to the assessment of protein and amino acid requirements will be high-lighted, with reference also to recent studies related to this aspect of pig nutrition.

2.3 PROBLEMS IN ASSESSING THE PROTEIN AND AMINO ACID NEEDS OF THE GROWING PIG

There are problems associated with assessing the nutrient requirements of animals and man (Cuthbertson, 1970). As research has continued into the protein nutrition of the growing pig, many factors have been described which influence the animals response to a dietary protein. Any factor which influences the requirement of an animal for a particular nutrient must necessarily interact with the metabolism of the body so that utilization is affected in some way. Therefore it is convenient to discuss such influences in the context of the difficulties which arise in attempting to describe protein and amino acid requirements.

2.3.1 Crude Protein as an Index of Dietary Protein Content.

The most common method of classifying the protein content of feedstuffs has been to multiply the nitrogen content by a factor related to the estimated N content of the proteins in the feed under consideration. Such an approach involves certain assumptions which are not always correct and overlooks the major nutritional function of feed proteins (to supply amino acids). In terms of estimating the amount of petide N,

the factor 6.25 is satisfactory for the common feedstuffs used in pig nutrition (Wallace, 1959). However there are instances where this should be varied (FAO, 1970). There is a tendency for the Kjeldahl method to underestimate N content (Martin and Skyring, 1962), and also, N apart from amino-N can be digested (Fleck and Munro, 1965; Bigwood, 1972). The principal nutritional value of protein to the animal is in supplying essential amino acids and N for the synthesis of the dispensable amino acids (Rose, 1938). An expression of the amino acid content as crude protein ignores the contribution that any particular protein makes to the supply of amino acids, especially the essential amino acids. Therefore it is not surprising that attempts to describe the crude protein requirement for the growing pig have shown widely varying conclusions (ARC, 1967).

The inadequacy of per cent crude protein as a description of the protein regime of a diet (Barber *et al.*, 1964) is clearly illustrated by the experiments of Kropf *et al.* (1959) and Woodman and Evans (1951). The same problem had been mentioned much earlier by Osborne and Mendel (1914). Kropf and co-workers were able to demonstrate that dietary crude protein levels can move quite independently of resultant animal performance (Table 2.3.1.1.).

TABLE 2.3.1.1. The effect of protein level and quality on pig performance over the range 18-68 kg live weight. (Adapted from Kropf *et al.*, 1959).

	Protein Level		
% Crude Protein	16%A	16%B	12%C
Amino Acid Balance[+]	"good"	"poor"	"good"
Days on exp. (mean)	72.5	98.0	73.0
Average daily gain (g)	648	476	644
Kg feed/kg gain (mean)	3.34	3.83	3.38
% lean cuts - barrows	50.98	49.52	48.81
- gilts	53.76	51.87	50.79
% fat cuts	32.57	33.69	34.86

[+]Based upon the recommendation of the NRC (1953).

Reducing the crude protein from 16% to 12%, but maintaining a "good" amino acid balance, resulted in very similar live weight gains and food conversion efficiencies. However the indices of carcass quality used show a widening of the fat/lean ratio in the carcass at the lower crude protein level. Maintaining the crude protein level at 16%, but using protein sources of lower quality, resulted in a lower growth rate and carcasses of poorer quality. Using the dietary levels of lysine, methionine, cystine and tryptophan quoted, Morgan and Robinson (1962) applied the method of Waddell (1958) for assessing amino acid balance and the results are shown in Table 2.3.1.2.

TABLE 2.3.1 2 Dietary amino acid balance of diets used by Kropf *et al*. (1959). (Taken from Morgan and Robinson, 1962).

Amino Acid (AA)	AA reqmt. % of diet[+]	Propns.of AA's (Tryp. =1)	AA content of diets.			Propns.of the AA's,% of reqmt.		
			A	B	C	A	B	C
L-lysine	1.0	5.0	0.78	0.41	0.53	91	63	90
DL-methionine +cystine	0.6	3.0	0.57	0.56	0.43	112	140	110
DL-tryptophan	0.2	1.0	0.17	0.13	0.13	100	100	100

[+]Recommendations of the NRC (1953).

This shows that the balance of amino acids in rations A and C is very similar and differs considerably from that in ration B. Waddell's method of calculation is probably an over-simplification of a complex situation (Morgan and Robinson, 1962), but it does serve to highlight the fact that ration B contains very low levels of lysine in comparison to the methionine plus cystine and tryptophan levels in this diet. Obviously the absolute levels of the essential amino acids, as well as their balance in relation to each other, will need to be considered in assessing the nutritive value of a dietary

protein. Despite this work, crude protein levels are still widely used to describe the protein fraction of pig diets, and the above discussion may be a partial explanation for some of the disparities between expected animal performance and dietary crude protein levels (ARC, 1967).

Recent reports on crude protein levels in pig diets include those of Cooke et al. (1972a), who investigated the response of growing pigs (23-59 kg l. wgt.) to protein level in diets of high digestible energy (DE) (3500 kcal DE/kg), when the amino acid balance was maintained relatively constant and feed intake was controlled to the same level for all diets. Using only gilts, they concluded that recommendations for dietary crude protein varied according to criteria (Section 2.3.2). With the prediction equation specified, they concluded that the best feed into lean efficiency occurred with a 22.5% crude protein (CP) diet, while best efficiency of dietary protein into carcass protein was on the diet with 17.5% CP. Maximum growth rate and feed conversion efficiency occurred at 17.5% CP, so the best efficiency of utilization of energy and protein appeared to be attained at higher values. However, in the economically more significant terms of maximum daily lean deposition and feed requirements per unit of lean, the trend was for the best performance on diets of 22.5% CP. Such deductions must be related to the sources of protein and the equations used, but do serve the useful purpose of considering the efficiency of using the major dietary ingredients in terms of the composition of the saleable carcass.

Jones et al. (1962) showed that two diets with a difference in crude protein level of 50%, but with similar energy contents (expressed as TDN),[+] produced similar live weight gains and carcass composition figures. Unlike the experiment described previously, there was no attempt to maintain amino acid balance and they related the similarity to the levels of lysine in these respective diets.

[+]TDN = Total Digestible Nutrients.

These experiments illustrate the complex situation which can occur when crude protein values are considered in relation to several indices of performance. Also, animal response can often be related more directly to the level of certain amino acids in the diet.

2.3.2. Criteria used in Making Recommendations

In stating a particular nutrient requirement, the criteria which are used to describe "adequate" performance must be carefully elucidated. Variability between experimenters and between countries in this regard will account for some of the differences in recommended requirements encountered by reviewers (ARC, 1967). Live weight gain (LWG), feed conversion efficiency (FCE, weight of feed used/unit weight of LWG) and measures of carcass quality (centred principally around the ratio of fat to lean meat in the carcass) have been the most common criteria used to investigate the protein and amino acid requirements for the growing pig. Nitrogen retention, as measured by the balance method or from carcass analysis has also been used.

One pattern which has become apparent from studies using LWG as the criterion is that maximum growth performance is approached asymptotically. With the attendant experimental errors involved, this makes the definition of a requirement in narrow terms very difficult. From the data presented, often an experimenter is justified in stating the requirement as a range only.

Where several criteria are being used, the most desirable performance as specified by such criteria may result in different recommendations (Rerat and Lougnon, 1968). The most common observation in experiments with growing pigs is a continued improvement in carcass quality with no concomitant increase in LWG or FCE as the nutritional value of the diet in terms of the supply of amino acids is improved (Robinson and Lewis, 1964; Cooke *et al.*, 1972a). That is, LWG and FCE can reach an optimum at a particular CP or amino acid

level, while carcass quality continues to improve with increases above this level. The work of Barber et al. (1964), Barber et al.(1968) and Braude et al.(1969) do not demonstrate these changes in carcass quality at higher nutrient intake levels, but no explanation for this discrepancy has been given (Cooke et al., 1972a). The decision on a particular stated requirement therefore becomes a decision on the weight which should be placed on the various criteria. For example, Cooke et al. (1972a) considered that maximum daily lean deposition and feed requirement per unit of lean (as calculated) was economically more significant than maximum LWG or FCE. A DE to CP ratio of 160 was therefore taken for their subsequent experiments on the inter-relationships between protein and energy. Measurements under scientific conditions may show an increase in the lean content of the carcass from a change in the protein or amino acid content of the ration, but unless this is reflected in the commercial returns to pig production there is no justification apart from the absolute values as such, in using such information to state requirements. Ultimately, with commercial pig meat production, requirements for individual food nutrients must be related to the production economics prevailing in a particular country or area.

Therefore it is of interest to note that Dent et al. (1970) used an economic analysis with multivariate regression and taking the joint objectives: total cost of production, speed of gain and quality of gain as a basis for the analysis of their results. Although the accuracy of their response relationships need further experimental support, this comprehensive work should provide a good basis for resolving the conflicts which have traditionally arisen in stating nutrient requirements for animal production systems.

Nitrogen metabolism studies by the nitrogen balance method (Jones et al., 1961; Jones et al., 1962; Carr and Dunkin, 1969) and by carcass analysis (Oslage, 1962a and b) have been used to supplement growth studies in growing pigs. The ARC (1967) have reviewed nitrogen balance studies related to protein and amino acid requirements. However, because

both methods are labour-demanding and expensive, they have not been widely used to determine protein and amino acid requirements in young pigs. When the efficiency of utilisation of dietary N from N balance experiments is compared with growth and carcass quality work, conflicts can arise in reaching the optimum for each of these criteria at a common nutrient intake. In the work of Robinson et al. (1964b), although not all the treatments used to obtain LWG, FCE and carcass quality results were represented in the N balance experiment, there is the suggestion that efficiency of N utilisation increases with decreasing crude protein level. This effect was more pronounced at heavier live weights, although a statistical analysis was not attempted. Crude protein levels of 14 and 20% were compared on the N balance experiment, and a higher growth rate, improved efficiency of feed conversion, with indications of an increased lean to fat ratio in the carcass occurred at the higher crude protein level in the growth experiment. Although Cooke et al. (1972) did not conduct balance experiments, on the basis of the calculations used, they concluded that the efficiency of protein (and energy) utilisation was highest on the two lower protein diets and declined with increasing dietary protein level. However growth rate continued to improve up to a crude protein level of 22.5%, although the increase above 17.5% were not significant. Total dissection of one side of the carcass also showed an improved ratio of lean to fat with increasing protein level. The calculations of Lucas and Miles (1970) suggest that the efficiency of conversion of crude protein and lysine into economically important lean tissue decreases as the protein level of the diet increases. These experiments again serve to illustrate that the most efficienct utilisation of dietary protein usually does not coincide with optimum performance according to other criteria.

An early method suggested for determining amino acid requirements was based upon tissue analysis (Mitchell, 1950; Williams et al., 1954). Ericson (1961) discusses the disadvantages of the tissue analysis method and they all arise

from the possibility that not all the essential amino acids present in the diet will be utilised for protein synthesis. Relating calculations to the estimated requirement for a single amino acid such as lysine from nutritional trials also introduces possible error due to differential utilisation between amino acids.

In estimating the requirements for individual amino acids, plasma amino acid levels have recently come into prominence as a method attempting to ascertain the adequacy of a particular amino acid intake. Early studies relating the concentration of plasma amino acids to the composition of dietary protein are reviewed by Longenecker and Hause (1959) and Longenecker (1963). Gitler (1964) however gives reasons why caution must be taken in relating plasma amino acid levels to the pattern of amino acids being absorbed from the diet. Thirumurthi and Longenecker (1966) discuss the Plasma Amino Acid ratio as a method for determining amino acid requirements, but suggest that reliability of the data obtained needs to be evaluated from growth studies. Comprehensive work has been conducted to find the most suitable method for sampling plasma (Nordstrom, 1964; Typpo, 1964; Windels, 1964; Stockland *et al.*, 1971). The work of Bravo *et al.*, (1970) and Sowers and Meade (1972) suggest that amino acid requirements derived from plasma levels of free amino acids agree closely with those calculated from growth rate and efficiency of feed conversion data. All the published work has been conducted in the U.S.A. under *ad libitum* feeding conditions. However the present evidence gives promise to the use of this method under a wider range of feeding conditions. Relationships between plasma amino acid levels, growth performance, nitrogen retention and carcass composition criteria will need to be more fully established before requirements based upon the broken line response curve for amino acid concentrations can be accepted with confidence.

2.3.3 Simultaneous Changes in Dietary Nutrient Levels.

The assessment of requirements according to some criteria is also complicated by the fact that often the proportion of one ingredient in the mixed diet is changed with the object of altering the animal's intake of a particular nutrient. However, because of the composition of many of these ingredients, the resultant change can be in quite a number of nutrients apart from the one under study.

This is particularly important in studies on the protein nutrition of the growing pig, where often a change in the protein N level of the diet is accompanied by a change in the quality of protein or balance of amino acids absorbed by the animal (ARC, 1967; Blair et al., 1969a). It is common practice for the crude protein level of the diet to be altered by a change in the amount of high protein concentrate relative to some cereal base. Since cereal protein is generally of lower quality than that of protein concentrates (usually of animal origin), an increased crude protein content of the diet is accompanied by an improvement in the balance of essential amino acids. Therefore the interpretation of treatment effects due to changes in crude protein levels is confounded with the changes in protein quality. For example, Jones et al. (1961) needed to assume little difference between the protein quality of ground barley and fine millers offals for the young pig, as the ratio of these two constituents varied between test diets as crude protein levels were altered.

Recent workers have attempted to overcome this problem by supplementing diets with synthetic amino acids to maintain the balance of the expected critical essential amino acids (Cooke et al., 1972a). This is done according to the analysed composition of the ingredients used and this involves certain assumptions which are outlined in a following section (Section 3.3.4). Realising the importance of certain essential amino acids which may be limiting in conventional

rations, it is difficult to understand why such workers should maintain the concept of crude protein as the only means of classifying the N content of rations and how such factors as energy influence N metabolism. Possibly a more meaningful approach in nutritional terms would be to examine the level of individual essential amino acids, particularly those predicted to be the most limiting to protein utilisation, and how environmental, nutritional and genetic factors may influence the animal's response to such levels (Bellis, 1961).

In addition to the quantitative changes which may occur in the dietary protein fraction as the proportion of a protein concentrate is altered, the following table shows some of the changes which occur as the proportion of meat and bone meal is increased on a ration based upon barley (Table 2.3.3.1.).

TABLE 2.3.3.1 Some changes in the nutrient composition of a barley-based diet as the proportion of meat and bone meal is increased.+

Component	Unit	Experimental Diets		
		1	2	3
Barley meal	%	82	76	70
Meat and bone meal	%	18	24	30
Crude protein (CP)	%	18	20.5	23
Lysine	%	0.7	0.83	0.96
Lysine/CP		3.89	4.05	4.17
Calcium (Ca	%	1.8	2.4	3.0
Phosphorus (P)	%	0.70	0.83	0.96
Ca/P		2.57	2.89	3.13

+Data from an experiment conducted at the Massey University Research Centre.

These figures show that as the crude protein content of the diets is increased, the relative content of lysine, which is likely to be an amino acid limiting to growth on such diets, (Braude and Lerman, 1970; Blair et al., 1970) is increased. This suggests a change in protein quality as the crude protein level changed.

Beames and Sewell (1969) recognised the problem of simultaneous changes of nutrients in their work on energy/protein ratios. The ARC (1967) present a review of literature investigating the influence of the Ca/P ratio on pig performance. Although the evidence is conflicting, they conclude that under certain circumstances, the absolute and relative amounts of these two elements can affect growth (particularly where the levels of Phosphorus or Zinc are low compared to requirements). Ca/P ratios are changing as the CP level is altered in the above table. Therefore there is the possibility that any difference between these diets may not be due solely to a change in the CP level. Essentially the only accurate interpretation of effects is in terms of a changing proportion between barley and meat and bone meal with several factors changing simultaneously, some of which have been mentioned but are of unknown importance.

2.3.4 The Interaction of Dietary Protein and Amino Acids with other Dietary Constituents

Specifying the conditions under which a particular protein or amino acid requirement is estimated includes the levels of other nutrients in the diet since several protein-nutrient interactions have been suggested.

2.3.4.1 The Relationship between Protein and Energy Intake

Protein synthesis is an energy dependent process and therefore energy intake can ultimately become limiting in the utilisation of dietary protein apart from the balance of amino acids in the protein. Munro (1964b) has discussed the influence of energy intake upon N utilisation. Feed protein can also be used as a source of energy via deamination

of amino acids. Factors which affect the balance of catabolism and anabolism when energy intake is restricted have been outlined as : the extent of the energy restriction,, the quantity and nutritional value of the dietary protein, the physiological state of the animal with respect to body reserves of protein and fat, the age or weight of the animal and in certain instances the dietary sources of energy (Swanson, 1959). When the animal is unable to consume sufficient energy yielding constituents apart from protein, dietary amino acids which could be used in protein anabolism under an adequate energy regime, can be deaminated and used as a source of energy. This is because of a preferential use of dietary components to supply energy needs before other requirements. The result is a reduced utilisation of dietary protein for body protein synthesis, and with the usual consequent lowering of growth rate in young animals, the ability of the dietary protein to support growth is reduced. Therefore it becomes necessary to supply adequate energy yielding constituents such as carbohydrate and fat to allow for maximum utilisation of the amino acids in the feed. The detrimental effect an insufficient supply of energy has upon dietary protein utilisation is to be distinguished from that fraction of the protein which is used as an energy source in the animal's metabolism under an adequate energy regime. Dietary amino acids will be absorbed into the bloodstream and arrive at the sites for protein synthesis in proportions which usually do not allow complete utilisation of all the amino acids. As the amount and balance of essential amino acids which arrive at the anabolic sites becomes more aligned to the animal's requirements for these given amino acids, then the proportionate utilisation of dietary protein for energy purposes is likely to decrease.

It is now well established that under normal dietary conditions, animals regulate their feed intake according to caloric needs (Clawson et al., 1962; Morrison, 1964). This is demonstrated by an alternation in intake when animals are fed ad libitum diets of changing energy density so that

energy intake remains approximately constant (ARC, 1967). Therefore there is a sound biological basis for relating requirements for essential nutrients to caloric intake (Cole *et al.*, 1967; Crampton, 1964).

In reviewing the literature on protein-energy relationships in animal nutrition up to that time, Costain and Morgan (1961) comment that the information available for pigs was still "rather fragmentary" although the large volume of work in protein nutrition for poultry had been reviewed (Gutridge, 1958; Coombs, 1962). The reports involving the energy-protein relationships in pigs were without exception using *ad libitum* feeding conditions. There were conflicting reports of protein-energy interactions being significant, with younger pigs showing a greater tendency for significant interactions. In discussing their own experiment, Costain and Morgan (1961) point out the simultaneous alteration in variables which occurred in the formulation of the experimental diets so that interpretation of the results was made more complex. They deduce from the data that the lysine level in the ration was a better indicator of animal performance, (in terms of growth rate), than the ratio of energy to protein over the 23-45 kg live weight period. Similar conclusions were reached by Robinson *et al.* (1964a).

Cooke *et al.* (1972a) attempted to minimise any variation in protein quality which occurred with changes in crude protein levels by supplementing with synthetic amino acids in their study of protein/energy relationships. Their results did not suggest a protein-energy interaction exists within the normal range of energy and protein intakes, although there was a trend for maximum growth rate and feed conversion efficiency to be achieved with higher crude protein levels as the energy level was increased. In experiments with the finishing pig (45-92 kg), Robinson and Lewis (1964) found no significant differences between diets containing four different protein levels and two energy levels with a common feeding scale, in terms of LWG and FCE. The expected deleterious effect of high energy diets upon

carcass quality could be partially offset by an increase in the crude protein level. In suggesting that best performance was recorded with a finishing diet containing 16% crude protein with 2950 kcal DE/kg and 0.83% lysine, they took a compromise between the maximum LWG and the best performance in terms of carcass quality.

Almquist (1968) has pointed out that where the supply of protein is adequate, or more so, diets with big variations in the DE/CP ratio may yield equivalent biological results because the protein used for energy purposes is nearly equivalent to the carbohydrate displaced by the protein. Since dietary protein is an expensive commodity to supply, there appears to be a need to relate the influence of changes in protein supply with the economic implications from making such alterations.

2.3.4.2 The Influence of Crude Fibre upon Protein Utilisation

The ARC (1967) review experiments involving the addition of fibre to pig diets as a diluent to reduce the energy concentration per unit weight of feed. However, apart from any affect on the energy status of the feed, the level of indigestible fibre in the diet can affect the utilisation of feed protein (ARC, 1967; Dammers, 1964; Swaminathan, 1967) possibly by modifying the digestive processes in the gut through an effect on the action of proteolytic enzymes (Munro, 1964; Glover and Dougall, 1961). However, although the influence of dietary fibre on N utilisation is likely to be negligible where a fibrous material is not used as an energy diluent, some workers have taken special precautions to minimise differences in the crude fibre content of experimental rations (Jones *et al.*, 1962).

2.3.4.3 Protein and Amino Acid: Cofactor Interactions

It has been established that the supply of several vitamins and minerals in the diet is essential to the efficient

utilisation of dietary protein (Munro, 1964a; Rerat and Lougnon, 1968). Deficiencies of vitamins and minerals probably do not affect protein synthesis directly, but mediate their effect through an alteration in endocrine activity (Munro, 1964a).

The supply of nicotinic acid (niacin) is of particular importance in cereal-based diets for growing pigs. Tryptophan is a precursor of this vitamin and where the intake of nicotinic acid is below requirements, tryptophan appears to be preferentially used for niacin synthesis (ARC, 1967). Tryptophan is potentially the limiting amino acid in many ration formulations, and therefore any diversion for the synthesis of niacin may have a significant affect upon dietary protein utilisation. Furthermore, it has been well established that the niacin in many cereals, particularly maize, is in a bound form completely unavailable to the pig (ARC, 1967). Consequently it is possible for diets containing a high proportion of maize and/or of low protein content to produce signs of niacin deficiency in pigs (ARC, 1967). However, there need not be clinical signs of deficiency for the supply of niacin to influence protein metabolism. In experiments reported by Batterham (1970a), he suggests that the larger response to a mineral-vitamin-antibiotic (MVA) supplement in the absence of tryptophan supplementation, an interaction which was significant in one experiment involving maize/meat and bone meal diets, may have been due to a niacin deficiency. That is, the reduced utilisation of dietary protein due to the shortage of niacin, and perhaps other cofactors, was reflected in a lower growth rate than when a MVA supplement was used.

This recent study emphasises the importance of supplying essential dietary factors involved in the utilisation of protein in adequate amounts relative to the animal's requirements. Otherwise the performance of the individual may be the result of an inadequate utilisation of dietary protein due to some factor apart from the supply or balance of essential amino acids.

2.3.4.4 The Influence of Added Antibiotics

Any feed additive which influences the performance of an animal will affect estimations of protein and amino acid requirements according to the level of addition. Antibiotics assume particular importance because of their widespread use (Rerat and Lougnon, 1968). This discussion of Rerat and Lougnon, (1968) indicates that both the influence of antibiotics and interpretations of their actions are varied. Use of these substances introduces difficulties of comparison between experiments on protein and amino acid requirements.

2.3.5 Interrelationships Between the Amino Acids

In seeking to establish the requirements of growing animals for the individual essential amino acids, it became apparent that the levels of certain other amino acids could influence the amount of a particular amino acid required for optimum performance. Such relationships again amphasise that protein nutrition is essentially amino acid nutrition.

Methionine is an indispensible amino acid for the growing pig. However, where the cystine intake is low, the methionine requirement can be increased due to an irreversible transformation of methionine into cystine. There is therefore a "saving" in the requirement for metionine as cystine levels are increased from sub-optimal levels and it is generally assumed that cystine may supply up to half the methionine requirement (ARC, 1967; NRC, 1964; Dammers, 1955). Because of this interrelationship, the requirement for methionine is often incorporated into a total value for sulphur containing amino acids (methionine and cystine).

A similar situation exists with phenylalanine, an indispensable amino acid, and tryosine which is not essential as a component of the diet for satisfactory growth. Available evidence suggests that where tryosine is present in the

diet, the requirement for phenylalanine can be reduced by approximately 30% (NRC, 1964; Dammers, 1955).

Another specific effect of an amino acid upon the requirement for another has been termed "amino acid antagonism", (Harper, 1964) as unique effects of amino acids present in excess. From growth studies in the rat (Harper et al., 1955), an excess of leucine was found to depress the utilisation of the structurally similar isoleucine and valine, even though neither of the latter were limiting amino acids. However Rosenberg et al. (1960), working with rats, considered it was unnecessary to postulate an isoleucine-leucine antagonism in order to explain the results of their experiments. The responses observed appeared to be due to imbalance, although they suggested that further work was necessary. Growth rate and feed per unit gain were the only criteria studied. With young pigs, Oestemer et al. (1972) were able to detect decreases in plasma-free isoleucine and valine from increased dietary supplementation with leucine, which supports work done with rats (Rogers et al., 1962). There were significant decreases in LWG with increases in dietary leucine so it appears that the pig may react to an alteration in the ratio of isoleucine to leucine in a similar way to the rat. Harper (1964) comments that the corn protein zein is relatively high in leucine and so diets containing a high proportion of corn are most likely possibilities for a leucine-isoleucine antagonism to occur. He also reviews experiments conducted with rats which, although by no means conclusive, do not rule out the possibility of a growth depressing effect of excess leucine in diets where corn protein is a major source of dietary protein.

A further amino acid antagonism which has been described is that between lysine and arginine as found for the chick (Jones, 1962a) and rat (Jones, 1962b). Lysine is often the limiting amino acid in pig diets and many studies have involved supplementation with synthetic lysine, several of

which are cited by Ostrowski (1969). However, it is not known whether the lysine-arginine antagonism operates in the pig, or to what extent such a possibility exists in lysine supplemented diets. Recently, Buraczewski et al. (1970) have obtained evidence that the absorption of lysine and arginine from the duodenum of the pig is influenced by the dietary concentrations of arginine and lysine respectively.

Other evidence for the level of an amino acid affecting the requirement of another is suggested by Hegsted (1969). In discussing the comprehensive experiments of Rosenberg and Eckert (1961) and more specifically the contour lines of growth response in rats with the addition of threonine and lysine to a basal rice diet, Hegsted points out that, assuming the graph is a reasonably accurate description of the data, the concept of the limiting amino acid is not clear from the results. The levels of lysine and threonine appear to influence the performance, and hence the requirement, of the animal to supplementation with threonine and lysine respectively. For example, an absolute requirement for threonine at any level of total lysine cannot be identified from the graph. He comments:

"Within limits there is no 'absolute requirement'...."

Bressani (1969), in reviewing experiments involving amino acid supplementation of corn diets with children, dogs and pigs, concludes there may be a close interrelationship between the four essential amino acids methionine, valine, isoleucine and threonine. The addition of methionine or valine to lysine and tryptophan supplemented diets can depress N retention; an effect which is alleviated by adding threonine and/or isoleucine. Therefore the apparent requirement for the latter two amino acids is increased with methionine or valine supplementation. Bressani suggests that samples of corn containing larger amounts of leucine, methionine and/or valine could require the addition of isoleucine and threonine to improve their nutritive value.

However it seems more likely that supplementation rather than biological variation in amino acid content could be responsible for these effects on requirements.

2.3.6 The Effect of Age or Body Weight Upon Protein and Amino Acid Requirements

As the growing animal increases in body weight, there are accompanying changes in the relative proportions of the constituents making up the animal body (McMeekan, 1940; O'Grady, 1966). Of particular interest in the growing pig, as a meat producing animal, are the absolute and relative amounts of muscle and fat; as these are commercially important variables in carcass composition (Harrington, 1959). The main nutritional value of dietary protein is in the supply of essential amino acids and N for the synthesis of non-essential amino acids and the production of body protein. Age, with the consequent change in body size, has an important influence upon the requirements for proteins and amino acids because of the relative importance of protein formation in body metabolism.

Oslage and Fliegel (1965) present data which show that under their particular experimental conditions (including a near maximal feed intake), absolute N retention (NR) in grams per day obtained from N balance trials, changes very little over the growth period 20-50 kg. Assuming that the principal use of the N retained is for protein synthesis, that the balance of amino acids used for protein anabolism remains essentially constant and that the efficiency of N utilisation is unaffected by age, the requirement for essential amino acids as a unit weight of intake per day will remain approximately constant over the growing period (approximately 20-50 kg). However, as animals increase in body weight, they require greater amounts of feed of a given energy density to satisfy the increased demands for maintenance and to produce the increments in weight gain which are more expensive in terms of energy cost due to rapid

increase in the rate of fat deposition (Oslage and Fliegel, 1965). Given a diet of constant amino acid composition, a smaller proportion of the essential amino acids will be used for protein synthesis as the animal ages, because of the approximately constant rate of deposition of lean muscle tissue and increasing amounts of feed being injested under free feeding conditions. Allowing for adequate levels of the amino acids, this reduced utilisation will be partially offset by the increased demand for essential amino acids in the repair and maintenance of the increasing total muscle mass. Under the conditions specified, it can therefore be expected that, with a constant dietary N content, there will be a decrease in the utilisation of N, with the N retained expressed as a function of the N digested. This is confirmed in large measure by the results of several workers (Thorbek, 1969; Nielsen, 1971; Poppe and Wiesemuller, 1968). Apart from treatment effects, other workers have shown that daily NR probably does stay approximately constant over much of the growing period (Carr and Dunkin, 1969; McConnell *et al.*, 1971; Cunningham *et al.*, 1962). The data of Robinson *et al.*, (1964a and b) suggests that this applies to diets of lower crude protein; at higher intakes of crude protein there appears to be a more pronounced decline in NR (g/day) as the animal increases in body weight. Hornicke (1962) also records a decline in daily NR from a maximum at 50 kg live weight. Rerat and Henry (1964) found NR stayed approximately constant at 12% CP, but decreased linearly with time at 16% CP, and was curvilinear with maximum retention at 65 kg with an 8% CP diet.

In a regression analysis, Dent *et al.* (1970) showed, according to their data, that for the growth period 23-50 kg live weight over the four crude protein and feed intake levels studied, there was an almost linear increase in LWG/day as lysine intake (weight ingested/day) was increased. This same relationship was seen for rate of lean deposition per day. In later growth periods these two relationships

were essentially horizontal, suggesting that at heavier weights the diets used were adequate in lysine, irrespective of feeding or crude protein level.

These demonstrated changes in requirements with increases in body weight, in what ever terms they are expressed, suggest that the composition of the ration should be changing on a continuing basis to give the maximum utilisation of dietary components. In practice, the compromise approach is to determine requirements over specified growth periods so that an average requirement is estimated. Assuming these estimated requirements are accurate, and that rations can be formulated which closely represent these values, this procedure will generally mean there is an excess of protein or amino acids supplied at the start of the growth period, developing into a deficiency later. To achieve statistically reliable results, it has become common practice to divide the growing/finishing period for commercial pig meat production into the growing phase (approximately 20-50 kg live weight) and the finishing phase (approximately 50-100 kg live weight) and determine ration formulations for these two periods (ARC, 1967).

However, there have been studies investigating the use of a single diet through the growing and finishing stages and also the effect of varying the weight at which the changeover to a lower protein diet occurs (Bellis, 1965; Braude and Rowell, 1968; Lucas and Miles, 1970).

Robinson et al. (1964b) investigated the effect of changing the live weight at which the changeover to a lower protein diet occurred with two general protein levels involved. There was the now well recognised effect of a higher protein level producing more rapid gains during the growing period with these differences being non-significant in the finishing period. The same changes occurred with the efficiency of feed conversion. Carcass quality measurements showed a significant improvement with the higher protein level. However, there was no significant effect on LWG from altering the live weight at which the change to a finisher ration

occurred. Feeding higher protein levels beyond 45 kg tended to reduce the per cent of lean in the carcass, but at the moderate dietary protein level, lean content continued to increase as the weight at changeover from grower to finisher ration was delayed until 65 kg. This interaction was unexpected but did not reach significance. Nitrogen balance studies were conducted with some of the treatments, but did not give any consistent information on the effect of changeover weight or the protein level.

Quoting results achieved at Banger, Chamberlain (1972) suggests the use of single protein levels in practical diets, with the obvious advantage of greater simplicity in formulations. Overall utilisation of protein and energy appeared to be unaffected by the use of a single diet.

Although in strictly nutritional terms, alterations in the protein content rations to allow for changing requirements with age would appear to be necessary, in practice such alterations may not be necessary to achieve production objectives.

2.3.7 The Influence of Feed Intake upon Protein and Amino Acid Requirements

It has been mentioned previously that the principal factor determining intake is normally energy requirements (ARC, 1967). Where the animal is fed restricted amounts of feed below appetite, the supply of energy is consequently limited. There is a simultaneous reduction in the absolute intake of other nutrients and therefore the various concentrations of these in the diet becomes important in relation to the animal's requirements.

The dietary protein intake may be increased either by increasing the protein content of the diet at a given level of feed intake or by raising the level of feed intake. Increasing the level of protein results in only small differences in energy intake, while a higher level of feed intake results in an increase in the intake of energy as well as protein and other nutrients (Chamberlain, 1972).

Rerat and coworkers (Rerat, 1961; Rerat and Henry, 1964; Rerat and Lougnon, 1966; Rerat, 1971c) have stressed the importance of differentiating between ad libitum and restricted feeding in recommending crude protein contents of growing pig diets. This discussion will be limited to a consideration of the effect of various levels of feed restriction below appetite and different protein levels in these situations.

Effects of variations in protein quality and quantity and feed intakes upon animal performance appear to be inter-related (Blair et al., 1969b). Using several degrees of feed restriction, these workers found interactions involving protein level, lysine level and feed intake. Lysine was taken as an index of protein quality since it was considered likely to be limiting protein utilisation in the diets used. There appears to be a general trend for protein level and feed intake to have opposing effects. An increase in the protein level at a given energy intake usually improves growth performance and carcass quality (Blair et al., 1969 a and b; Cooke et al., 1972; Robinson et al., 1964b; Barber et al., 1972), while an increase in feed intake generally results in faster LWG, but a deterioration in FCE and carcass quality (Blair et al., 1969a; Barber et al., 1972).

An effect of feed intake upon an animal's response to different protein levels would be evident from an interaction of intake and protein on those parameters measured in an appropriately designed experiment. Blair et al.,(1969a), in a large factorial experiment, were not able to detect a significant protein by feed intake level interaction on growth rate or efficiency of feed conversion at any growth stage from 23-110 kg live weight. However, several significant interactions were present in the carcass composition data (Blair et al., 1969b). For example, with animals slaughtered at 45 kg live weight, there was a marked reduction in the % lean in the dressed side as intake was increased at low protein levels, but this trend was much less at higher

protein levels. Again, at 70 kg live weight, with a 12% CP diet, an increase in intake significantly reduced the proportion of ham in the side, while at 18% CP an increase in the level of intake did not significantly change this criteria. These results are in agreement with the calculations of Chamberlain (1972). Figure 2.3.7.1 shows that the absolute amount of carcass lean gain (g/day) increases as the level of feeding increases at 60 kg live weight. However in terms of the efficiency of utilising dietary protein, increasing the level of feed intake has a deleterious effect (Figure 2.3.7.2). Davies and Lucas (1972) present a graphical description of their data which shows that in per cent terms, an increase in the level of feeding increases the proportion of carcass fat and decreases the proportion of carcass lean, (see also Holder et al., 1969). Therefore although the absolute amount of lean deposition increases with increased feeding levels, the ratio of lean to fat in the carcass is decreasing, an undesirable result in terms of carcass grading.

These experiments serve to illustrate that the general feeding level may significantly influence animal response to changes in protein nutrition. Therefore conclusions relating to suggested requirements for protein and amino acids must incorporate the intake parameter. Expressing nutrient requirements as an absolute intake per day is possible under restricted feeding regimes, and in view of the interrelationships involved, it would appear desirable to consider protein and energy requirements concurrently and on the same quantitative basis (Blair et al., 1969a) Where dietary concentrations are used, and this is still common, such recommendations must be related to the intake regime.

2.3.8 The Influence of Sex Upon Protein and Amino Acid Requirements

This discussion will be restricted to a comparison between gilts and castrate males since entire males are not

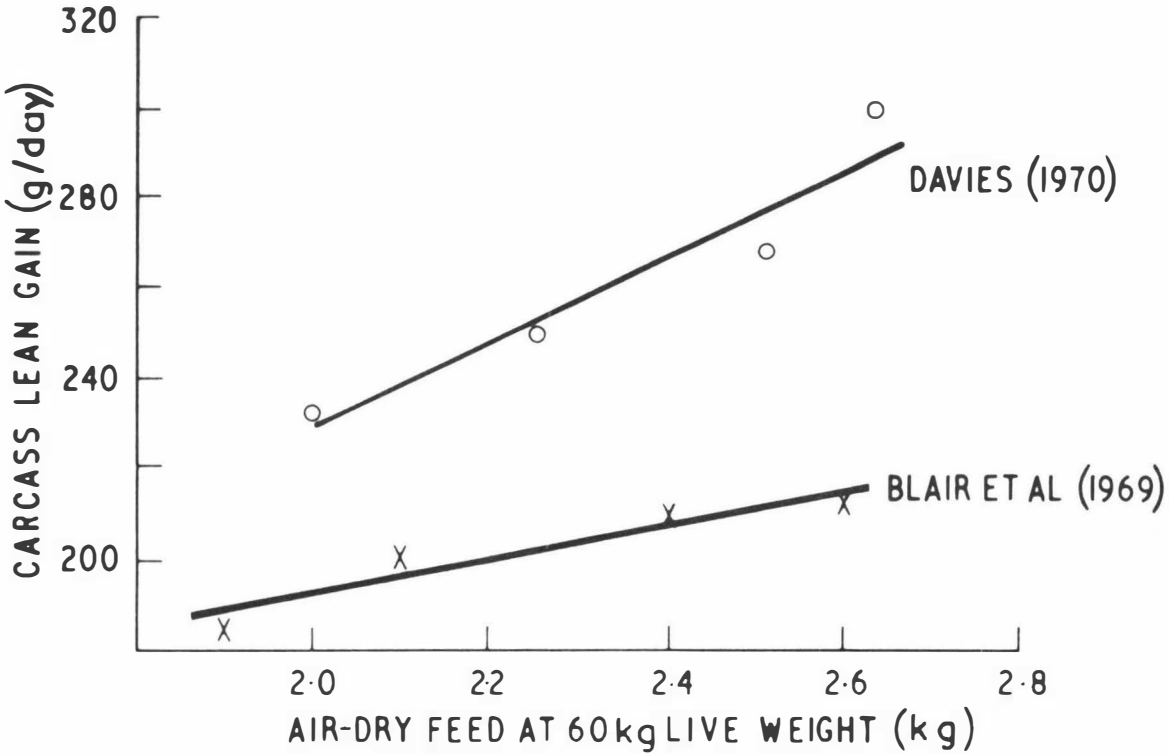

FIG. 2.3.7.1 *Effect of plane of nutrition on daily carcass lean gain.* X *Blair* et al. *(1969),* ○ *Davies (1970)* (Taken from Chamberlain, 1972).

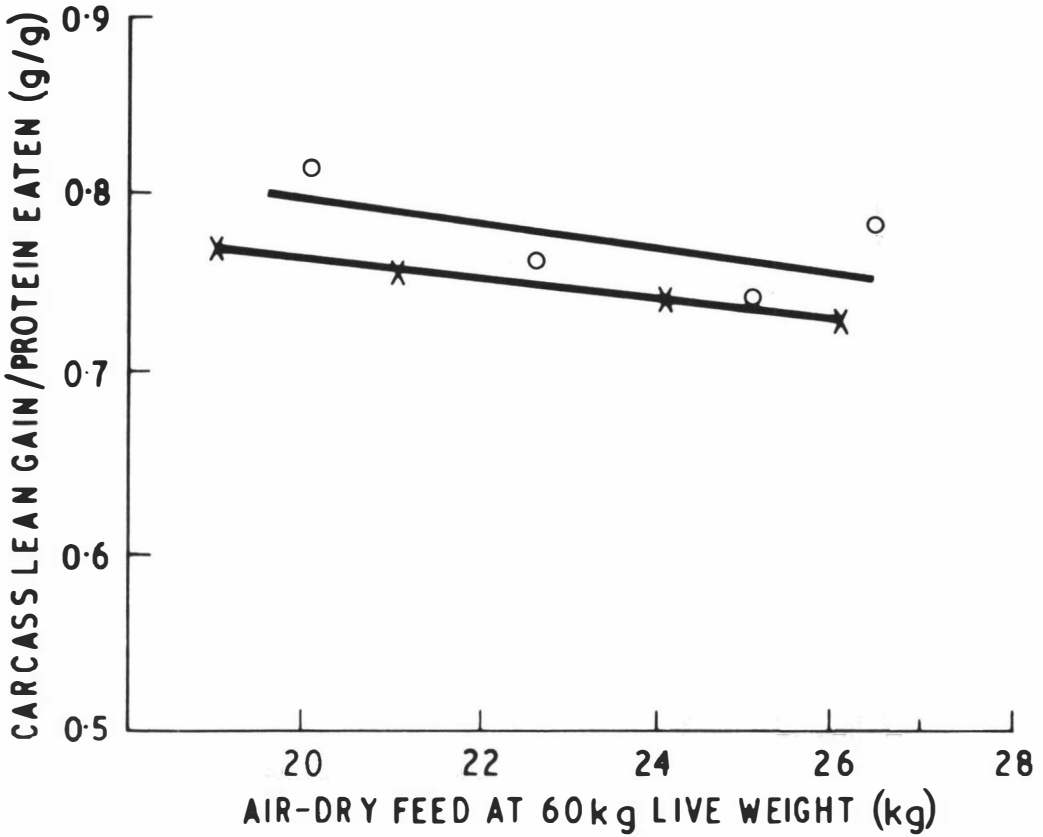

FIG. 2.3.7.2 *Effect of plane of nutrition on efficiency of conversion of dietary protein to lean meat.* × *Blair* et al. *(1969).* ○ *Davies (1970)*

(Taken from Chamberlain, 1972)

generally accepted as meat producing animals at the present time. The results of recent work have confirmed that under similar restricted feeding conditions, gilts generally have significantly leaner carcasses than castrates (Blair, et al., 1969b; Robinson, et al., 1964b; O'Grady, 1966; Davies and Lucas, 1972). In contrast, Lodge et al. (1972b) found no significant differences between the two sexes, which probably reflected the live weight at which these animals were slaughtered (59 kg). Blair et al. (1969b) found that indices of carcass composition showed a greater disparity between gilts and castrates at heavier slaughter weights.

The effect of sex upon growth rate and feed conversion efficiency has not been well defined. Most workers have reported little difference between gilts and castrates under restricted feeding in terms of growth rate and efficiency of feed conversion (Lodge et al., 1972b; Robinson et al. 1964b; Robinson and Lewis, 1964; Blair and English, 1965), although there is some evidence that gilts are superior in these criteria (Jones et al., 1962; Carr, 1967; Holder et al., 1969). Under ad libitum feeding conditions however, castrates consume more feed and consistently show higher growth rates (Bowland and Berg, 1959; Lodge and Day, 1967; Kemm and Ras, 1972), with the higher voluntary feed intake being more pronounced at heavier live weights (Blair and English, 1965).

A consistently greater proportion of lean in the carcass of gilts under similar dietary conditions would suggest that they are able to utilise the protein portion of the diet more efficiently than castrated males. Nielsen (1971) showed that females deposit more nitrogen daily and that this difference is greater at heavier weights. Babatunde et al. (1967) found that gilts had higher concentrations of protein and water and less fat in the carcass than castrated males with ad libitum and restricted feeding. From nitrogen balance and carcass dissection studies, Jung and Piatkowski (1967) reported a significant relationship

between nitrogen deposition and edible protein in the half-carcass (r = +0.89), area of *longissimus dorsi* muscle behind the thirteenth rib (r = +0.76) and fat content of the carcass estimated from the dry matter of the ham (r = -0.79). At 110 kg live weight, the edible protein content of the carcass was greater in gilts than in castrated males. In an earlier experiment (Piatkowski and Jung, 1966) they found nitrogen retention was 12% greater in gilts than in castrates.

The effects of level of feed intake, protein intake and amino acid levels on performance are complicated by significant influences due to sex. Blair *et al*. (1969 a and b) record several significant interactions between these parameters and sex. For example, over the 23-45 kg stage, females showed a more marked change in the ratio of feed intake to live weight gain as the protein level increased, with a larger increase in the feed conversion ratio from the third to the highest intake level. Their results suggest that at low levels of feed intake, gilts make more efficient use of protein in terms of increase in live weight per unit of feed eaten. Performance between the sexes was very similar with varying protein levels at the highest level of intake. Irrespective of treatment, gilts generally had higher feed conversion efficiencies and converted feed to lean meat more efficiently than males. During the 45-70 kg live weight period, a greater "sensitivity" of females to protein nutrition was seen with the greatest response to increasing lysine levels being at 12% CP for castrates but at 18% CP for gilts. Over the 23-90 kg period, these workers record no fewer than six significant interactions involving sex on lean meat gain and ratio of feed intake to lean meat gain. Feed intake had a greater effect on lean meat gain and the feed required per unit of lean meat gain in males, but females were more responsive to changes in protein and lysine levels in these parameters. Interactions at slaughter weights of 70 and 110 kg suggest that although castrates may be more responsive to increases in feed intake, there is a

correspondingly greater deposition of fat in the carcass. Again, gilts showed greater responses to increases in the quantity of protein ingested and to improved quality (lysine level), both in terms of growth performance and carcass lean content. These conclusions are supported by the more recent work of Davies and Lucas (1972). They also found that shoulder fat thickness was reduced less with castrates than gilts when the daily feed allowance was lowered (animals slaughtered at approximately 90 kg live weight). This is a significant finding since castrates consistently show greater backfat measurements than gilts and are therefore the major target in trying to improve carcass quality through a restriction in feed intake.

Ostrowski (1969), using diets based on dry potato meal with and without additional lysine and menthionine fed to pigs from 30-90 kg live weight, found significant interactions involving sex. Gilt carcasses having less fat but showing greater differences due to treatment. Robinson and Lewis (1964) observe similar effects over the 45-92 kg live weight period and comment: "....(this differential response) will influence the level of lysine to be recommended and will be different for hogs and gilts." Robinson (1966) also found significant differences and interactions involving sex with lysine and methionine supplementation of barley meal/fish meal diets fed to pigs over the 18-120 kg live weight period. During the growing phase (18-50 kg), there was a highly significant sex by supplementation interaction on growth performance and supports the hypothesis that castrates have lower requirements for protein and amino acids than gilts.

The experimental results discussed appear to conflict to some degree with the data of Lodge *et al*. (1972b), who found no significant differences between the sexes in either growth rate, efficiency of feed conversion or carcass measurements (except backfat thickness and eye muscle area) with increasing protein levels. According to their results, barrows respond more to increases in protein level than

gilts. The authors discuss the lack of sex differences and consider that the light slaughter weight (59 kg) is perhaps the most obvious reason. In addition, they point out that the methods of assessing carcass lean may be deceiving and tissue composition of the back region may not be representative of the whole carcass.

There is now a considerable body of information available indicating that, apart from other factors, the utilisation of dietary amino acids is affected by the sex of the animal. According to various criteria, most workers agree that females show a greater sensitivity to changes in protein intake, both in quantity and quality. Dent et al. (1970) conclude from the regression analysis of a comprehensive factorial experiment that generally, females"... require smaller quantities of both dietary lysine and energy intake at any crude protein level to sustain the same performance in terms of live weight and lean meat gain than males. Females are thus more efficient in converting lysine and energy into both live weight and lean meat."

Objectives will need to be clearly defined before the known influence of sex is allowed to determine separate ration formulations.

2.3.9 Genetic Influences on Protein and Amino Acid Requirements

The acceptability of pig meat cuts to the consumer is influenced to a major extent by the ratio of lean meat to fat (Harrington, 1959). As this ratio increases, the demand for such cuts improves and higher prices are possible. There has been systematic breeding in many pig producing countries, one of the objectives being to breed an animal with a low propensity for fat production. In the discussion on the effect of age upon the metabolism of the growing pig (section 2.3.6), it has been pointed out that the absolute deposition of fat becomes greater as the animal increases in body weight while the rate of muscle deposition appears

to remain approximately constant over a wide weight range. Greater feed restriction at heavier weights has been a common technique used to restrict the amount of fat deposited (Oslage, 1962b), but this has undesirable effects on growth rate. Some workers have suggested that an improved lean to fat ratio may be agreeably accomplished by increasing the protein level in finishing diets (Robinson et al., 1964b), or by feeding a single diet through the growing and finishing periods (Hardy and Lewis, 1968/69).

The experiments of Bowland and Berg (1959), Bayley and Summers (1968), Clawson et al. (1972) all suggest that the strain or breed of pig can influence the response to changing energy, protein and amino acid levels in terms of growth rate or carcass composition.

In an attempt to maintain growth rate with a high feeding level and yet produce carcasses of economically acceptable composition, breeding programmes for the establishment of "lean-type" swine have been undertaken. Significant progress has been made in several countries and Oslage (1962a) suggests that the influence of breeding a leaner type of market pig has been in maintaining nitrogen retention through the growing and finishing periods, since there is normally a decline during the latter period. The more recent work of McConnell et al. (1971,1972) also suggests this effect of breeding on nitrogen retention. Hetzer and Harvey (1967) reported that after ten generations of selection, high and low-fat Duroc lines differed in backfat thickness by 2.6 cm or 68% of the initial mean. For two selected Yorkshire lines, the difference after 8 generations was 1.4 cm or 44%. It could perhaps be pointed out that the selection in this case was in both directions so that the difference between the two selected lines would be greater than if each was compared to a control population.

Henning and Kleeman (1967) found substantial differences between improved German Landrace pigs and Vietnamese pigs in nitrogen retention and utilisation of dietary nitrogen and

energy. German Landrace pigs deposited 76 g protein per day over the period considered while Vietnamese pigs deposited only 56 g protein per day. Utilisation of crude protein was 21 and 14% respectivley while the utilisation of gross energy was in the opposite order, 30 and 35% respectively. Greater amounts of crude protein from the feed were retained in edible parts of the carcass of German Landrace pigs, while the Vietnamese breed showed a higher retention of gross energy from the feed in edible parts of the carcass.

In examining the influence of protein intake upon the performance of fat and lean-type swine, Davey and Morgan (1969) indicated that lean-type pigs retained more dietary protein than fat-type pigs when the entire growth period was considered. Whereas fat-type pigs had similar amounts of separated fat and lean with 12 and 20% CP diets, lean-type pigs showed a large increase in lean as the protein level increased. Lysine and methionine were added to these diets so that these two amino acids were in approximately the same ratio with respect to total protein in each diet. These workers suggest that this supplementation may have altered the amino acid balance, particularly in the 12% CP diet, but the sensitivity of the breed lines to changes in amino acid balance has not been clearly established. They conclude "... a difference exists among the lines of swine used in this study in their need for dietary protein and in their ability to make use of this protein to form body tissue."

In a recent study of nutrient digestibility and nitrogen metabolism with fat and lean-type swine fed two levels of protein, McConnell et al. (1971) found several important differences between these two types. Although type and protein level had no significant effect on the digestibility of dry matter, gross energy or crude protein, differences in nitrogen utilisation within individual trials due to type were apparent. The data suggests that the carcass characteristics of fat-type pigs were little affected

by protein level while the effect of breeding for a lean-type of swine was to increase the level of protein required for optimum performance. In a more recent experiment involving fat and lean-type swine, McConnell *et al*. (1972) found NR remained approximately constant from 31-53 kg body weight with a decrease during the 53-91 kg stage for lean-type swine. However fat-type pigs showed a progressive decline in NR for the three periods studied, although the two types were similar in this respect at the 31 kg stage. Carcass composition data supported the NR results with correlation coefficients of approximately r = +0.7 for indices from physical separation and chemical analysis of tissues in two later trials (at 53 and 91 kg live weight).

Braude (1970) summarises over 30 years of research in pig breeding at the National Institute for Research in Dairying and graphically shows the progress which has been made in improving live weight gain and feed conversion efficiency. With this greater potential for growth being realised, there may be a need for altering the daily allowances for protein and essential amino acids. Clausen and Gerwig (1958) present tables showing the progress which has been made through progeny testing in various European countries. Generally, live weight gains, efficiencies of feed conversion and measurements of carcass quality have improved. This suggests that daily requirements for protein and amino acids need to be modified as breeding programmes increase the performance of pig populations.

With the recorded differences between breeds and strains in their ability to retain dietary nitrogen and respond to different protein and amino acid regimes, recommendations involving dietary nitrogen should under some circumstances, take such variations into account (Richter, 1969).

2.3.10 The Influence of Protein Level upon Amino Acid Requirements

As research was conducted to estimate the requirements of several species for individual amino acids, it became apparent that the general level of protein influenced these determined requirements. Several authors have concluded that an increase in the dietary nitrogen level produces an increase in individual amino acid requirements (Grau, 1948; Munaver and Harper, 1959; Brinegar et al., 1950).

Using the results of an experiment to determine the influence of protein level upon the isoleucine requirement of the growing pig, Becker (1958) has calculated the requirements for other essential amino acids at different protein levels. For example, the requirement for lysine is estimated at 0.63% of the diet at a protein level of 12%, but increases to 0.96% at 21% crude protein. Rerat and Lougnon (1968) discuss possible reasons for this observation. First, either the protein level or an individual amino acid could be limiting growth, so that supplementing the diet with either could improve performance. They point out that this is not strictly an increased requirement, since tissue synthesis is increasing with higher protein or amino acid levels. Secondly, the creation of amino acid imbalance using a protein deficient in an essential amino acid increases both the relative requirement of an amino acid as per cent of the diet and also the absolute amount of lysine necessary to obtain maximum growth (Munaver and Harper, 1959). That is, by some means, an imbalance situation results in the increased requirement for an amino acid. Thirdly, the nitrogen level can be expected to influence amino acid requirements where the supply of nitrogen for the synthesis of non-essential amino acids is critical. This is only likely to occur where synthetic amino acid mixtures are used to determine requirements, since dietary proteins contain at least 40% of the dispensable amino acids (Harper, 1959). However, Henry and Rerat (1970) consider that with proteins of good amino acid balance, the proportion of dispensable

amino acids should be approximately 60% of the nitrogen supply to minimise destruction of the indispensable amino acids.

In a discussion on the influence of dietary protein level upon amino acid requirements, Harper (1964) points out the limitation of expressing such requirements as a per cent of the diet since it is not obvious how the absolute amounts of each amino acid required for a given rate of body weight gain are altered. There is also the close relationship which exists between the methods of determining the effect of the dietary level of protein on amino acid requirements and those used to study amino acid imbalance. From the evidence considered, Harper (1964) concludes that increased "... amino acid requirements with increasing quantities of protein in the diet are actually examples of the effect of amino acid imbalance." Harper et al. (1970) points out the influence that feed intake will have upon the absolute amounts of amino acids ingested and the resultant efficiency of utilisation. They quote experiments with rats which show that the efficiency with which the limiting amino acid is used may not be decreased and may even be increased when food intake of the rats fed the imbalanced diet and those fed the control diet are equalised. This can be accomplished by limiting the intake of the control group or by stimulating the intake of the group fed the imbalanced diet by insulin injections, force-feeding or exposing the latter group to a cold environment. However where intake is increased by supplementing the imbalanced diet with the limiting amino acid, the efficiency of utilisation of this amino acid appears to be adversely affected; although the results are not consistent and appear to be influenced by the experimental conditions. In a recent experiment examining the effect of protein level upon the threonine requirement of the pig, Sowers and Meade (1972) show that the protein level appears to affect requirements according to the plasma-free threonine criteria in a linear manner. However increasing the protein level using crystalline amino

acids in constant ratios to each other, with threonine excluded, appears to induce an imbalance situation (as outlined by Harper, 1964).

The influence of protein level upon the utilisation of individual amino acids may be distinguished from the effect of protein level on the metabolism of protein N. Barnes et al. (1946) showed that biological value as an index of protein utilisation was influenced to a significant extent by the level of intake of various proteins. Figure 2.3.10.1 gives a graphical description of their experimental results and calculations for whole egg protein. The curves for two samples of soyflour and wheat gluten were similar although displaced downwards on the Y axis, showing they were proteins of lower quality to whole egg. An almost perfect distribution of amino acids in whole egg protein for the growing rat up to 40 g of protein absorbed is shown by biological values approaching 100. At higher intakes, a surplus of amino acids occurs, with consequent deamination of amino acids, increased urinary N output and heat increment ("waste") loss (Crampton and Harris, 1969). The decline in biological value is then due to a reduced proportion of the protein intake being used for maintenance and body protein synthesis. Lower quality proteins differed from whole egg protein with biological values not only being lower at a given intake, but also declining more rapidly from a maximum at low levels of protein intake. Similar work by Barnes and Basshardt (1946) indicated that protein level could affect the PER as an index of protein utilisation, with the influence of intake varying according to the quality of the protein. Therefore, although the total amount of dietary protein which is used for body tissue synthesis can be improved by increasing the protein intake, Figure 2.3.10.1 shows that a maximum is reached followed by a decline in protein gain. Increasing the intake of a protein will supply more of the limiting amino acid and so allow greater muscle deposition: however Rerat (1969) presents evidence that an increased intake of a low quality protein cannot

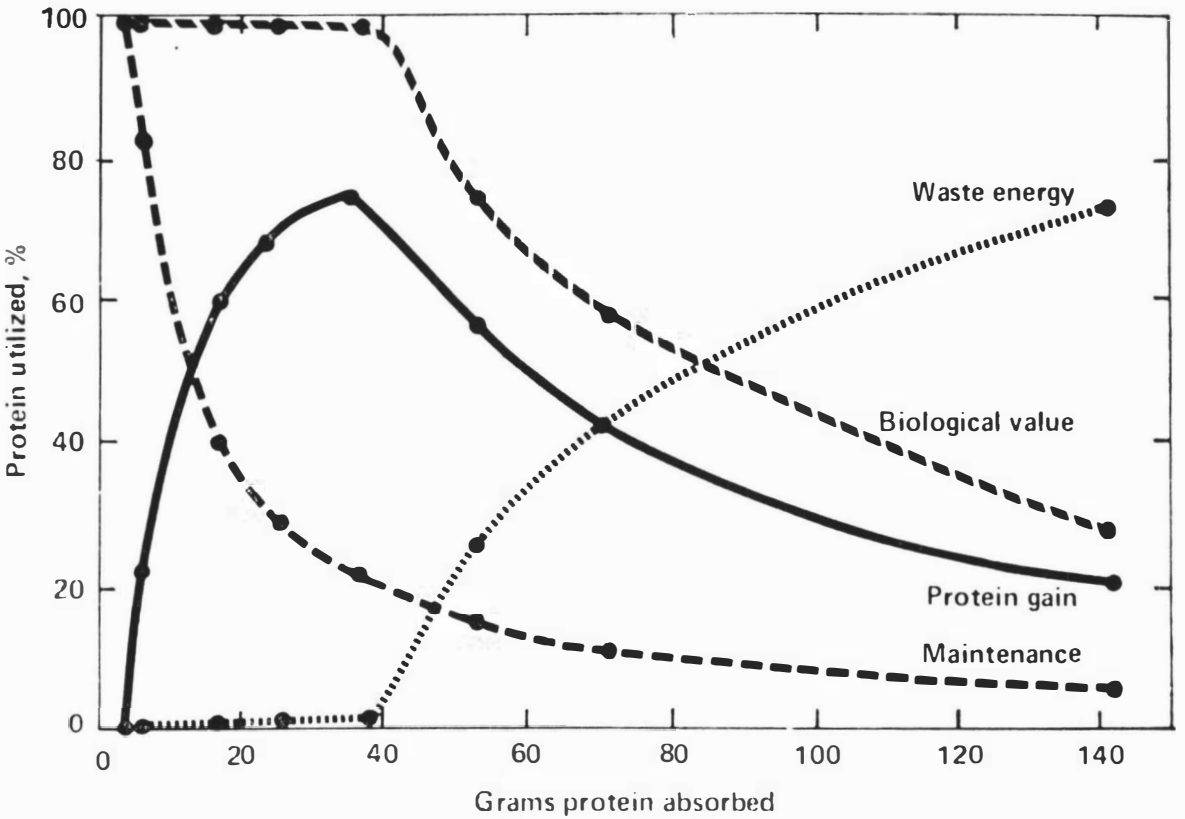

FIG. 2.3.10.1 Effects of amount of protein ingested on its use. (Adapted from Barnes et al., 1946). (Taken from Meade, 1972).

entirely replace a good quality protein as maximum body weight gains may be lower when using the former.

The presence of an amino acid imbalance may increase the requirement for a particular amino acid. However, where the protein level of the diet is increased using sources of reasonable quality, several workers have demonstrated that the concentration of the critical amino acids in the dietary protein may be decreased with optimum performance being maintained (McWard et al., 1959; Klay, 1964; Becker, 1958). Chamberlain (1972) has combined the results of these studies in a graph which is shown as Figure 2.3.10.2. It shows close agreement in the trend for lower required concentrations of lysine in the protein as the dietary protein intake increases for optimum growth. However, only dietary concentrations are quoted, with no clear indication how daily quantitative intakes are affected.

In view of the influence of dietary protein level upon the concentrations of essential amino acids required in the protein or feed for a given level of performance, and the uncertainty of the methods used in establishing the effect of protein level on requirements, stated amino acid needs should be examined carefully. Where they are quoted as concentrations of the protein or diet, then food intake is an important parameter which must be controlled or defined and the energy concentration of the diet assumes greater significance.

2.3.11 The Influence of the Environment upon Requirements for Protein and amino acids

Recent reviews on the influence of the environment upon pig performance include those of Mount (1968, 1972) and Sainsbury (1972). Only a brief discussion with reference to protein nutrition is warranted here.

Recent studies both with groups of pigs (Moustgaard et al., 1960) and pigs kept individually (Fuller, 1964), show that environmental temperature can influence nitrogen

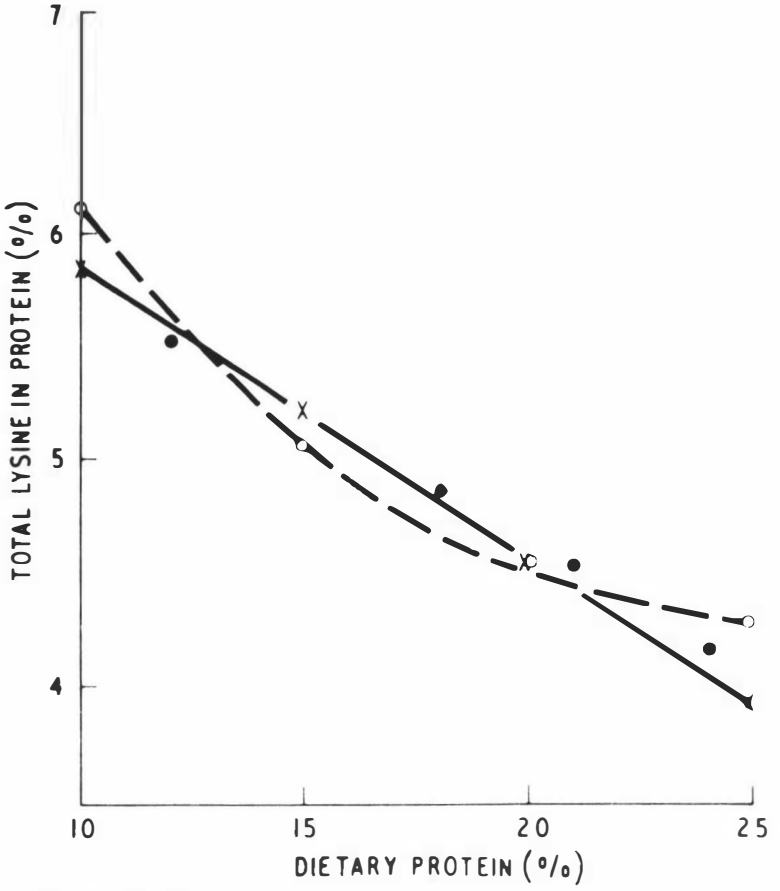

FIG. 2.3.10.2

The optimal concentration of lysine in dietary crude protein. X (—) *McWard (1959),* ○ (— —) *Klay (1964),* ● *Becker (1958)*
(Taken from Chamberlain, 1972).

retention. Corresponding differences in growth rate and food conversion are also recorded. Fuller (1965) has shown that carcass composition is also influenced by temperature, the deposition of protein being less reduced by variations in temperature outside the thermoneutral range than that of fat. Therefore pigs were fattest in a thermoneutral environment. departures from this zone producing leaner carcasses. The effects of humidity upon animal performance apart from an influence on the incidence of disease, need to be more clearly defined (Sainsbury, 1972).

Environmental conditions will influence the estimation of protein and amino acid requirements by affecting the criteria which are used to determine such requirements (Rerat and Lougnon, 1968). For greater accuracy, stated supplies of nutrients considered to produce optimum performance according to various criteria should be placed within the context of a particular set of environmental conditions.

2.4 CONCLUSIONS

The previous discussion has outlined the many factors which may influence the nitrogen metabolism of the growing pig. In any particular situation, the relative order of importance of these influences is unlikely to be the same. As nutritionists have become more aware of how dietary and animal factors may affect the animal's response to a particular dietary regime, so descriptions of nutritional needs have been modified. Since dietary protein has the major role of supplying indispensible amino acids, crude protein is now well recognised as an inadequate description of the efficacy with which the nitrogen content of the diet can meet the demands of the animal for compounds containing this element.

Although the concept of a limiting amino acid has been questioned recently, (Hegsted, 1969), the bulk of evidence suggests that the degree to which dietary protein is utilised for protein synthesis is related to the supply of those

essential amino acids in shortest supply relative to the animal's need. In recent years there has been a trend for diets to be described in terms of crude protein but also to include the levels of one or more of those indispensable amino acids likely to be critical to the use of dietary protein.

Research workers continue to use different means for arriving at suggested requirements. There is a need for uniformity in the basis upon which decisions are made and the comprehensive analysis of Dent et al. (1970) may prove to be a significant step in this direction. The ARC reviewers (1967) in their concluding remarks on protein and amino acid requirements state:

> "....there has not been to date a sufficiently systematic or integrated approach to the problem of estimating the protein requirements of the pig.. We conclude that experiments are needed of the type which provide response curves, instead of estimates of levels which provide maximal response. This is so, because maximal responses are very ill-defined, because the response to any particular nutrient is likely to depend on the concentrations of others in the diet and because only response curves of this type can provide the basis for the type of information required at the practical level. Moreover before any such experiments are undertaken it is most important that the criteria by which responses are to be measured should be agreed."

As a greater volume of data becomes available, the response of the animal to alterations in various factors simultaneously can be predicted with greater confidence. Calculated requirements can then be more closely related not only to the basic body metabolism but also to the commercial production of pig meat. At the present time there appears to be a lack of reliable information translating treatment differences under experimental conditions into effects in the commercial system.

The importance of energy intake in relation to the utilisation of dietary protein has been discussed. Rerat and Lougnon (1968) are careful to draw distinction between ad libitum and restricted methods of feeding in their

conclusions on amino acid requirements for the growing pig. In estimating these needs, their review is significant in that they relate estimated absolute daily intakes of individual amino acids to energy intake. Their figures for restricted feeding under the assumptions specified are presented in Table 2.4.1.

TABLE 2.4.1 Amino acid requirement (g/mcal digestible energy) of the growing pig with restricted feeding. Adapted from Rerat and Lougnon (1968).

Weight (kg)	20	40	75[1]
Lysine	2.9	2.8	2.2
Methionine + cystine	2.0	2.2	1.7
Tryptophan	0.7	0.7	0.5
Threonine	2.0	1.9	1.5
Isoleucine	2.2	2.5	1.9
Leucine	3.0	3.0	2.3
Valine	2.0	1.9	1.5
Histidine	0.9	0.9	0.7
Phenylalanine + tyrosine	1.7	1.6	1.3
Arginine	?	?	?

(1) Values strictly tentative as the evidence is less certain for live weights greater then 50 kg. Based upon the calculated requirement for lysine.

Implicit in the above figures are the following conditions. The feeding level was specified and based upon recommendations from Reading. Intake of digestible energy varied from 3000 kcal at 20 kg to 5000 kcal at 40 kg and 6950 kcal at 60 kg. Diets were estimated to contain 2900 kcal DE/kg.

During the discussion they are careful to point out that the decisions made for several of the amino acids are

based upon very little evidence and thus are of doubtful reliability. They also state: "The values calculated from data found in the literature and which we related to the energy of the diet, should be considered only as suggestions, the accuracy of which must be verified by experiments." However their calculations may be considered an improvement upon the recommendations of the ARC (1967), where requirements are specified only in percentage terms with no clear indication of the feeding level or the energy concentration of the diet.

With the wide range of experimental conditions possible, differing objectives, and a multitude of factors which may influence the nitrogen metabolism of the growing pig, the large variations in estimated requirements which have been encountered by reviewers (ARC, 1967; Rerat and Lougnon, 1968) is to be expected.

In this chapter, the animal as an experimental unit has been discussed. The following sections include some of the problems relating to the description of diets and how these relate to meeting the nutritional needs of the growing pig.

CHAPTER 3: PROTEIN NUTRITION IN RELATION TO CERTAIN FEEDSTUFFS, RATION FORMULATION AND NITROGEN BALANCE STUDIES

The purpose of this chapter is to consider maize and meat and bone meal as dietary ingredients supplying protein and to discuss protein and amino acid nutrition in relation to ration formulation. Only those topics considered to be necessary as an introduction will be included: nutritional trials with particular reference to the growing pig and involving the feedstuffs mentioned will be discussed in relation to the experimental results presented.

3.1 MAIZE AS A MAJOR INGREDIENT IN RATIONS FOR GROWING PIGS

Extensive agricultural use of the cereals as sources of food since ancient times has been largely due to their storability, high content of carbohydrate and high yields (Wall, 1964). Cereal grains provide an important source of energy in both human and livestock diets. However, although the protein fraction compared to many other products is of lower quantity and quality per unit weight of material, because of the significant proportions in which cereal products are often added to human foods and animal rations, the cereal protein often contributes the major part of the dietary amino acid supply (Harper and De Muelenaere, 1961; Parman, 1968). Boyne et al. (1961) have commented: "The staple cereals that provide most of the energy also provide between one third and two thirds of the protein needs of the stock, the remainder of the protein being usually provided by a mixture of two or more protein concentrates." Therefore the protein fraction of cereal grains is nutritionally important, quite apart from the major role of supplying energy for body metabolism.

There have been several extensive reviews on various aspects of cereal proteins. In a discussion on plant proteins

in human nutrition, Swaminathan (1967) includes individual cereals and the factors which affect the nutritive value of plant proteins. Bigwood (1972) emphasises the low levels of lysine in cereal proteins but also points out that the wide variations in tryptophan, lysine and methionine between different feedstuffs is likely to be an important factor in changes in protein quality among various foods and feeds. Harper and De Muelenaere (1961) pay particular attention to amino acid availability in their discussion on the nutritive value of cereal proteins.

The comparative data of Block and Bolling (1951) show that, with cereal proteins, maize protein is relatively low in lysine, methionine and tryptophan. From a summary of the literature, the FAO (1970) consider that lysine is the limiting amino acid in maize protein by the Chemical Score method and expressing the amino acid contents as mg/g N, while tryptophan becomes the critical amino acid when the data is expressed as mg/100 g of food.

Wall (1964) reviews the biochemical nature of cereal proteins and mentions that their generally low nutritional value is due to a deficiency of basic amino acids (more particularly lysine and tryptophan), in the prolamine fraction. Prolamines (and also the glutelines) are storage proteins located in the seed endosperm as "protein bodies". This latter fraction is the predominant proportion of maize cereal protein and is present almost exclusively in the endosperm as apart from the germ; although the germ has a very high content of protein, mainly of the globulin and albumin types. The zein content of normal maize (included in the prolamine fraction) is approximately 54% of the total soluble protein (Jimenez, 1966). An intensive investigation of the zein fraction of maize protein is reported by Mosse (1961). The nutritional inadequacy of zein as the sole source of protein for the growing rat and the stimulatory effects of tryptophan supplementation upon growth has been known since the beginning of this century (Willcock and Hopkins. 1906). This pioneering work illustrated the importance of individual amino acids in protein nutrition and stimulated research on these

compounds in nutrition. Later work (Osborne and Mendel, 1914) showed that maize proteins were also deficient in lysine. Growth responses with the addition of several other amino acids indicated that there are several amino acids which are deficient in maize protein (Sauberlich et al., 1953; Benton et al., 1955). Waddell (1958) lists the limiting amino acid in several cereal products and lysine is the most limiting in 10 out of 12 cases, with chemical and biological evidence supporting each other.

There is therefore now considerable evidence that the proteins of cereal grains, including maize, are nutritionally unbalanced for the young growing animal. Extensive research has been conducted in attempts to improve the protein value of maize by agronomic procedures (Sauberlich et al., 1953a and b; MacGregor et al., 1961; Kurtz and Smith, 1966; Keeney, 1970), breeding (Dobbins et al., 1950; Woodworth et al., 1952; Eggert et al., 1953; Alexander, 1968), protein supplementation (Maner et al., 1971) and the addition of synthetic amino acids (Batterham, 1970; Lawrence, 1972). However, the use of various agronomic procedures, such as increased fertiliser application, and breeding to produce higher crude protein contents in maize grain have been frustrated by the general observation that the increased nitrogen content is due almost entirely to an increase in the zein fraction (Hansen et al., 1946; Mitchell et al., 1952; Schneider et al., 1952; Dimler, 1966). Therefore there has not been any significant increase in protein quality when crude protein levels have been increased by these means.

3.1.1 Improvements in Maize Grain Amino Acid Balance by Mutation

With the lack of success in improving the quality of maize grain protein by the procedures mentioned above, and because of the importance of maize as a source of energy, and protein in many human and animal populations, the announcement of genetic mutants which favourably altered the amino acid balance of maize grain protein (Mertz et al., 1964; Nelson et al., 1965) was greeted with a great deal of

interest. The opaque-2 (op-2) mutant gene had been described much earlier by Emerson et al. (1935), but no biochemical investigations were conducted until Mertz and co-workers, in the course of extensive studies on the protein fraction of the maize kernel, became interested in possible reasons for the pronounced opacity of the endosperm in the op-2 and other mutants. This characteristic was in contrast to the translucence of normal maize endosperm. Nutritionally, the most important mutation reported apart from op-2 is floury (fl-2). Generally the characteristics of fl-2 are intermediate between those of normal hybrid and op-2 maize (Mosse, 1966). The major exceptions to this are a higher proportion of non-protein-nitrogen and an increase in the methionine content of fl-2 as compared to op-2 and normal hybrid maize. The latter effect has been shown to have significance in poultry nutrition (Cromwell et al., 1968). However, the fl-2 mutation has not been investigated as much as op-2 because the lysine levels are not as high and are more variable in the former, with a more marked influence of the genetic background. In addition, the mode of inheritance makes it more difficult to produce homozygous fl-2 lines from heterozygous plants. Finally, the op-2 mutant is usually nutritionally superior to fl-2, although the latter is superior to normal maize (Nelson, 1969). Greater attention will be paid to the op-2 mutation because of its relevance to the experiments conducted.

Preliminary reports concerning op-2 suggested dramatic changes in the lysine content of the endosperm (Mertz, 1963), which appeared to be due to a reduction in the ratio of zein to glutelin. Changes in amino acid balance due to the op-2 mutation (Mertz et al., 1964) were similar to a comparison between zein and glutelin (Lloyd and Mertz, 1958). Subsequent studies have confirmed that the effect of the op-2 mutation is to change the relative proportions of different protein fractions in the grain without any significant effect upon total crude protein levels or the composition of individual proteins (Mertz et al., 1966; Watson and Yahl, 1967; Jimenez, 1966 and 1968; Mosse, 1966). The following table illustrates some of the biochemical changes which occur when the op-2 gene is incorporated into the normal maize genotype.

TABLE 3.1.1.1 Relative amounts of protein fractions in a normal hybrid and an opaque-2 maize sample. (Adapted from Mosse, 1966).

Extract	Fraction	Maize	
		$^{1}_{260}$seed	Opaque-2
Aqueous	NPN (1)	4.6 (3)	13.0
	PN (2)	14.2	24.7
	Total	18.8	37.7
Alcoholic	Total (Zein)	48.4	17.2
Residue	(Glutelins)	31.2	41.3
Total Recovered		98.4	96.2

(1) Non-protein-nitrogen
(2) Protein nitrogen
(3) g N/100 g defatted corn N.

This shows a big reduction in the total alcoholic fraction (zein) and also a significant increase in the proportion of non-protein nitrogen in the aqueous fraction of op-2. Nelson (1966) has suggested that the op-2 locus may have a regulatory function, influencing the synthesis of the alcohol-soluable proteins, with corresponding changes in other protein fractions.

Previous mention was made of breeding for higher crude protein levels in maize grain. Although there was no corresponding improvement in the nutritional value of the grain, attempts have been made to incorporate the op-2 mutation into high protein maize strains (Nelson, 1966 and 1968). The following table (Table 3.1.1.2) is adapted from the report of Nelson (1966) and shows that for some nutritionally important essential amino acids, (tryptophan values not given), the incorporation of the op-2 mutation produces a very similar amino acid balance of the op-2 developed in the normal hybrid

strains. However there is more than twice as much protein in the mutations from the high protein maize, so there is a much greater supply of amino acids per unit weight of the grain.

TABLE 3.1.1.2 Amino Acid content (expressed as g/100 g of protein) in the defatted endosperm of Illinois High Protein (IHP), an opaque-2 (op-2) stock and an op-2 recovery for the cross (IHP x op-2) x IHP. (Adapted from Nelson, 1966).

Amino Acid	IHP	Recovery 329-8	op-2
Lysine	1.3	3.6	3.6
Methionine	2.0	2.0	2.1
Isoleucine	4.5	3.9	3.8
Threonine	3.7	3.6	3.7
% Crude Protein	20.4	17.4	7.9

Nutritional studies with rats, reported by Nelson (1966), indicate that the high protein op-2 mutant (15% CP) is superior to the low protein stock (11.6% CP); the former giving equivalent growth rates to a 15% casein diet (14.8% casein plus 0.2% cystine). At present however there is a problem of increasing the yield of these high protein op-2 varieties before they are suitable for commercial use (Johnson et al., 1970).

The kernels of op-2 maize appear to have a lower density (Watson and Yahl, 1967). However this does seem to be affected by the genetic background, and it may be possible to select for lines in which op-2 kernels have higher densities (Nelson, 1966; Johnson et al., 1970).

Initially, greater attention was paid to the influence of the op-2 mutation on increasing the lysine content of maize protein, since studies with normal maize diets had suggested that lysine was the first limiting amino acid for

growing pigs (Acker et al., 1959; Clawson et al., 1963; Cahilly et al., 1963; Vipperman et al., 1963), all under ad libitim feeding conditions. Consequently it has been termed "high lysine" maize, and the major increase in nutritional value was thought to be due to the increase in lysine. However, Mattern (1969) suggests that the increased tryptophan concentration in the op-2 endosperm may also be of significance in the nutrition of monogastric animals.

Tables A3.1a and A3.1b list published protein and amino acid values for normal hybrid and opaque-2 maize respectively and may be compared with the analytical values obtained from samples of the consignments used in the experiments to be reported. Considerable variability exists in values for individual amino acids, but there is a clear trend for lysine and tryptophan values to be higher in the opaque-2 varieties. Histidine and arginine values are usually higher in opaque-2 maize, while leucine, tryosine and phenylalanine show a decrease with the mutation.

3.2 MEAT AND BONE MEAL AS A PROTEIN SUPPLEMENT IN RATIONS FOR GROWING PIGS

The previous discussion has highlighted the biochemical basis for the nutritional inadequacy of the maize grain protein as a sole source of amino acids for the young growing animal. In order to achieve an improved balance of essential amino acids absorbed, it has become common practice to supplement cereal diets with one or more feedstuffs containing relatively high concentrations of protein of improved quality. Meat and bone meal, as a by-product of the meat processing industry, has been widely used as a protein supplement in pig rations.

The Stock Food Act (1946 and Amendments) states: "If the stock food is a meat meal containing less than 60 per cent of crude protein (allowing one per cent of nitrogen as equivalent to six and one quarter per cent of protein) it shall be deemed to be meat and bone meal...." They commonly contain

approximately 50% crude protein (Crampton and Harris, 1969; McDonald et al., 1966; Carr, 1971; Pritchard and Smith, 1957).

Although meat and bone meals contain high levels of crude protein relative to cereals, there is now a considerable body of evidence to suggest that the protein is of low nutritional value (Boyne et al., 1961). Using Gross Protein Value as the measure of protein quality, Duckworth (1955) reviews the results from the literature for several animal products and there is a clear indication that the values for meat and bone meals are lower than those for fish meals, whale meat meals, dried skimmilk or dried buttermilk powder. This is supported by the work of Atkinson et al. (1970 a, 1970b), who also suggest that digestibility is not the major factor influencing this characteristic. In addition, meat and bone meals are extremely variable in composition and Underwood et al. (1950) found that Biological Values varied between 29 and 62 with a mean of 46. In a review of the literature, the FAO (1970) gives a chemical score value of 40 from the column chromotography method and 43 from microbial assays. The limiting amino acids are given as methionine plus cystine and isoleucine by the two methods respectively. One reference quoted gives a determined Net Protein Utilisation Value of 24, which may be compared to a range of 63.5 to 84.0 for fish meals. Using purified diets, Terrill et al., (1954) showed meat and bone scrap protein was inferior to soybean oil meal or dried skimmilk for growing pigs.

This relatively low value as a protein source may be partly explained by the large and variable proportions of connective tissue, principally collagen, which has a very low nutritive value (Hoaglund and Snider, 1926) with a BV of only 25 (Mitchell et al., 1927). Lysine is present at low levels in connective tissue protein (Neuman, 1949) and the inferiority of meat meals for poultry has been remedied by lysine supplements (March et al., 1950; Patrick, 1953). The high ash content of meat and bone meals (Carr, 1971) is due to the inclusion of bone, the protein of which is very poor quality since it is almost entirely collagen (Eastoe

and Eastoe, 1954). The amount of collagen protein in relation to total protein in meat and bone meals has been estimated from hydroxyproline and hydroxylysine values and is approximately 60% (Eastoe and Long, 1960). These authors, in comparing collagen with muscle protein, show that collagen has low levels of lysine, even lower levels of methionine plus cystine and is almost completely devoid of tryptophan. Therefore collagen must be considered as a low quality protein, and although digestibility values have not been determined with any great certainty, the lysine values of meat and bone meal may alleviate marginal deficiencies of this amino acid which may occur in livestock rations (Eastoe and Long, 1960). Gelatin (which may be considered as a purified form of collagen due to a similar amino acid pattern), has been shown to have this effect when added to diets where white flour supplied the only source of dietary protein for rats (Chick and Slack, 1945). It may be noted that Eastoe and Long (1960) give zero values for tryptophan in their samples since this amino acid is destroyed under normal conditions of acid hydrolysis for amino acid determinations. Other references quoted, using microbiological assays, do indicate the presence of small amounts of tryptophan.

Pritchard and Smith (1957) conclude from a comprehensive analysis of meat meal and meat and bone meal samples that the calcium and phosphorus contents, the levels of several vitamins of the B complex and the presence of all the essential amino acids with high levels of lysine, make these feedstuffs valuable ingredients for balancing rations. However there is no account taken of any differential availabilities that may occur between the microbiological assays used and the livestock to which the meals are to be fed; or of the low levels of tryptophan in their meat and bone meal samples. They found satisfactorily low levels of non-amino acid nitrogen (ammoniacal nitrogen). As a result of his studies with a large number of samples, Waterworth (1964) comments:

"...among the meat meals, the availability of amino acids was generally low, as were the biological values....." (compared to fish meals and whale meat meals using a micro-biological assay).

Lyman et al. (1956) have stated: "The decided tryptophan deficiency of .... meat and bone scraps ... should prompt the use of supplements high in tryptophan with (this product)". The low levels of tryptophan and methionine which have been reported may indicate that meat and bone meal protein has a limited supplemental value in diets where a predominance of the protein is supplied from cereals which are also low in these amino acids. Table A3.2 contains some published values for the essential amino acids in meat and bone meals.

3.3 RATION FORMULATION WITH PARTICULAR REFERENCE TO AMINO ACID NUTRITION

One of the basic objectives in nutritional studies with growing animals is the accumulation of reliable data which will allow for the formulation of rations consistent with the desired production objectives. Information on both the nutritional needs of the animal and the composition of possible dietary ingredients is necessary so that requirements and the corresponding supply may be equated. Under commercial production systems, a cost may be assigned to the supply of each nutrient and therefore for efficiency in both nutritional and economic terms, requirements and feedstuffs composition should be accurately defined so that dietary formulations will result in the minimum waste of dietary constituents. For a good discussion of the role of the production economist in formulating rations the reader is referred to a paper given by Townsley (1972).

The discussion in Chapter 2 has highlighted the difficulties which arise in accurately defining the protein and amino acid needs of the growing pig. In this section, corresponding difficulties in the description of feedstuffs and the principles involved in the formulation of diets with particular reference to protein and amino acids nutrition will be discussed.

3.3.1 The Concept of Amino Acid Balance

It has now been well established that the growing pig must be supplied with ten essential amino acids as part of the dietary intake for maximum growth to be possible. Furthermore, although many factors can influence the specific values, individual essential amino acids are required in certain absolute amounts determined principally by the age or body weight of the animal. From this arises the concept of a theoretical spectrum of essential amino acids which, when absorbed into the bloodstream and transported to the sites of protein anabolism, will be completely utilised for the synthesis of body protein. A well-balanced dietary protein will be one which upon digestion and absorbtion of the individual amino acids, supplies the animal with the required amounts of the essential amino acids for optimum growth (Harper, 1959). Proteins of high nutritional quality will not only supply all of the essential amino acids, but in amounts allowing near optimum rates of protein synthesis when included at suitable levels in the diet and associated with a given intake. Since few dietary proteins of animal or vegetable origin fulfil such a demand, the nutritionist is confronted with the problem of exercising means by which the supply of essential amino acids to the animal is the most desirable in terms of resultant performance. He must also think in terms of the efficiency of utilising dietary nitrogen, as the protein fraction of the diet is invariably a relatively expensive component to supply.

3.3.2 Problems in the Description of the Protein and Amino Acid Contents of Feedstuffs

3.3.2.1 Limitations of the Crude Protein Value

It is now well recognised that expressing the protein content of a feedstuff on the basis of a chemical analysis for nitrogen is an inadequate method of stating its usefulness in supplying amino acids to the animal (Section 2.3.1). Although non-amino acid nitrogen is difficult to assess, the total amino and amide nitrogen by Kjeldahl analysis (from

the acid hydrolysis of glutamine and asparagine), is usually about 92% of the total nitrogen in cereal grains, with a range of 87.5 to 98.5% (Bigwood, 1960, 1963). However, a Kjeldahl analysis gives no indication of the amino acid balance of the protein fraction.

3.3.2.2. Variability of analytical values

Bigwood (1972) discusses the history of how techniques for amino acid analysis were developed. An examination of the published values for the crude protein and amino acid contents of various feedstuffs shows that there can be considerable variability, even when comparing results using the same method. The FAO (1970) have done a statistical analysis of the data they had available for various feedstuffs. With normal hybrid maize, the coefficient of variation for most of the amino acids is 8 to 10%, while for lysine, methionine and cystine, which are likely to be critical to the utilisation of protein in cereal-based rations, the values are 13.3, 19.05 and 56.5% respectively.

Although the usual method of column chromotography does give highly repeatable results, with coefficients of variation of the order of 1 to 2% for a given sample (with the exception of tryptophan, methionine and cystine), there does occur wide variations between laboratories in the accuracy of analysis. Environmental, processing and strain differences may affect the amino acid content of a feedstuff. Therefore there may be considerable error in using published values to describe the amino acid content of a given ingredient, particularly in the case of cystine, methionine and tryptophan (FAO, 1970).

3.3.2.3 Amino acid availability

The amino acid content of a feedstuff is usually determined chemically. However the animal is only able to utilise for protein synthesis those amino acids which are absorbed from the alimentary tract and made available

at the sites for protein synthesis in the body. Errors in the evaluation of the nutritive value of the dietary protein will occur where the absolute and relative amounts of the essential amino acids described by chemical analysis do not accurately reflect the products absorbed from digestion. The greatest effect is likely to be where the availability of those essential amino acids limiting the utilisation of dietary protein is impaired in some way.

Some methods have been developed which assess the quality of a particular protein by a calculation based upon one or more of the essential amino acids. They include the Chemical Score method of Block and Mitchell (1946-7) and the Essential Amino Acid Index of Oser (1951). This was an important step in understanding the biochemical basis for variations in the nutritive value of different food proteins and attempting to numerically describe these differences. However in addition to establishing a suitable reference pattern of amino acids used to calculate chemical scores (Payne, 1972), there is the difficulty of using analytical values for amino acid content, rather than those quantities available to the animal. It has been established that the availability of amino acids likely to be critical to protein utilisation can be affected by heat treatment, processing, etc. (Donoso et al., 1962; NRC, 1950), and therefore chemical scores may be seriously in error because availability is not accounted for. However, in many cases the relationship between biological nutritive value and chemical score is good (Block and Mitchell, 1946-7), suggesting amino acid availability is not significantly influencing protein quality in most common foods.

There have been several recent comprehensive reviews published dealing with the biological availability of amino acids (Guttridge, 1962; Harper and De Muelenaere, 1963; Bressani et al., 1972; Morrison and McLaughlan, 1972; Meade, 1972). Only a brief discussion of specific points is warranted here.

The term "availability" is perhaps best defined as that portion of an amino acid present in a protein which is used for growth, development and maintenance of an animal in so far as it is dependent on the digestibility of the protein; presence of enzyme-resistant peptide linkages, enzyme-inhibiting substances, and the rate of release of the amino acid in the intestinal tract. It is now commonly recognised that vegetable proteins are less digestible than animal proteins (Block and Mitchell, 1946-7), although the reason for this has not been clearly established (Bigwood et al., 1972).

The nutritive value of feedstuff proteins can vary widely independent of crude protein and amino acid content (Boyne et al., 1961). This is particularly evident in processed vegetable and animal products, and much of the variation can be explained in terms of altered animo acid biological availability, and except for cystine, with little destruction of the amino acids themselves, (Miller et al., 1965).

3.3.2.3.1 Methods for estimating availability

The methods used to determine amino acid availability have been recently reviewed (Meade, 1972; Morrison and McLaughlan, 1972). These authors closely examine the many techniques available and discuss their particular usefulness and accuracy and a full enumeration will not be attempted here, although it should be noted that the term "availability" may have a different meaning according to the method used (De Muelenaere et al., 1967a; Harper and De Muelenaere, 1963), and that some take better account of the variables involved than others (Gupta and Elveljem, 1957). Despite the range of methods available, Meade (1972) concludes: "There continues to be a need for development of a rapid method for determination of availability of amino acids, particularly as values for available amino acids are used in computer formulation of diets for poultry and swine." Morrison and McLaughlan (1972) also state: "It is difficult to escape the conclusion that recommendations regarding

the most suitable availability test must apply only to a given product processed in a specific manner."

3.3.2.3.2 Availability of amino acids in maize and meat and bone meal

There is little published information on the estimated availability of amino acids in these two feedstuffs. Ousterhoult et al. (1959), using the chick growth assay method, quoted the following per cent amino acid availabilities in their meat and bone meal samples: arginine 71.4, histidine 75, lysine 103.7, tryptophan 62.5, methionine plus cystine 79.2. Using enzyme hydrolysis followed by microbiological assay, Waterworth (1964) gave the following figures: arginine 28, histidine 56, methionine 33, isoleucine 48, leucine 33, valine 42. There appears to be little agreement between these two methods and further work is obviously needed. From the analyses of over 750 samples of meat and bone meal, Pritchard et al. (1964) found the majority of samples had available lysine values (Carpenter's method) of between 3.5 and 4.5 available lysine (g/16 g N) irrespective of CP content. The average value of all samples examined was 4.06 g/16 g N. Total lysine values were not quoted, so per cent availabilities are not shown. Meade (1969) and Stockland et al. (1970), using the levels of free amino acids in plasma, suggest there are qualitative differences in amino acid availability from meat and bone meals but the data did not allow any quantitative estimations to be made.

For maize also, there is little published information and results show little agreement (De Muelenaere et al., 1967a). Harper and De Muelenaere (1963) have pointed out that little information exists on the availability of amino acids in cereal proteins largely because suitable and reliable procedures have yet to be worked out. The comparatively low availability values obtained for maize by growth methods indicates that factors apart from the concentration of the limiting amino acid in the diet are having an effect. De Muelenaere and Feldman (1960), using the fecal analysis

method, reported availability figures of: isoleucine 92.8, lysine 89.5, methionine 95.3, and threonine 88.8. The value for lysine can be contrasted with those quoted by Gupta et al. (1958) of 49 and 51% using the growth assay method. In estimating the availability of isoleucine from zein, De Muelenaere and Feldman (1960) give values of 89% by the fecal analysis method, while a value of 30% was obtained by the growth method (Desphande et al., 1957). Linkswiler et al. (1960) in nitrogen balance studied with man, and Ousterhout et al. (1959) using a growth assay, both concluded that the isoleucine in maize protein was highly available. However the data of Benton et al. (1955), using the rat growth assay, suggested the isoleucine in maize had low availability.

De Muelenaere et al. (1967a) present evidence that amino acid imbalance is often operating to decrease the apparent availability as the protein level is raised by an amino acid mixture free of one amino acid. One comment they make is the following: "If a protein is severely unbalanced, as is the case with lysine and tryptophan in most ceral products, the estimate of availability may be low." A varying ratio of available lysine to true protein digestibility in their results suggests that there are different factors influencing these two parameters. In a similar series of experiments investigating the availability of other amino acids in cereal products, De Muelenaere et al. (1967b) also found several factors influencing the values calculated. In maize, threonine availability was consistently higher by the fecal abalysis method (range 77.5-99.4) than by the growth method (range 45.3-70.0), suggesting good absorption but poor utilisation of threonine from maize protein. Availability values for isoleucine appeared to be influenced by a leucine/isoleucine antagonism; as omitting leucine from the basal amino acid mixture increased the availability of isoleucine from 54.1 to 73.9% by the growth method. The authors consider that such antagonisms are not likely to occur in practical diets containing a mixture of proteins, so such effects in the methods used

should be removed as much as possible.

Using the net amino acid utilisation method, Harper and De Muelenaere (1963) gave per cent availability figures of: lysine 88.1, threonine 98.8, isoleucine 72.0, valine 87.4 in maize gluten; with a value of 64.5 for isoleucine in zein. They state "....the information currently available indicates that the amino acids of the major cereal grains are quite highly available." However, zein protein gives consistently low figures, which may be due in part to factors other than the amino acid content. Since zein is the predominant fraction in normal hybrid maize grain protein (see Section 3.1.1.3), amino acid availability may be lower in this cereal compared to others, either in real terms to the animal or by the current methods of estimating availability. Since the opaque-2 mutation drastically alters the zein content of the endosperm protein, the improved nutritive value of the mutant may be due partially to increased amino acid availability as well as an improved amino acid balance (Section 3.1.1.3). Pick and Meade (1970) found the lysine in opaque-2 maize appeared to be as equally available, with isoleucine less so, than a reference diet containing synthetic amino acids, fish meal and soybean meal. However the plasma amino acid levels used and the experimental design did not allow quantitative estimations nor a comparison with normal hybrid maize.

It may be concluded from a review of the literature that differential amino acid availability is likely to occur both within and between animal feedstuffs. However the inconsistency in results by different methods and workers does not allow a confident assessment of availability in any particular sample.

3.3.3 Improvement of Amino Acid Balance through Protein Supplementation

During the following discussion on protein and amino acid supplementation, it will be assumed that energy supply is not limiting protein utilisation. The energy content

of the diet has been shown to have a significant effect upon indices of protein quality (Swanson, 1959; Morrison, 1964a; Lewis, 1969).

In their classical studies of the biochemical basis for protein quality, Block and Mitchell (1946/7) recognised that the nutritive value of a mixture of proteins was not simply the arithmetical mean of the individual proteins. No single value for the nutritive value of a protein was appropriate when mixed with others. Much earlier, Mitchell (1924b) had indicated that the supplementary nature of proteins invalidated the concept of individual Biological Values for individual components of the diet.

A recent review of Bressani et al. (1972) outlines the general principles of protein and amino acid supplementation. The concept of protein supplementation involves an effort to meet the animal's protein requirements, both in terms of quantity and quality, by including a mixture of proteins in the diet (Ellinger, 1958). There are few sources available which contain an adequate level and balance of the essential amino acids, and since these are often too highly priced for normal animal production systems, a combination of lower priced poorer quality proteins is used to try and achieve the same result. Protein supplementation can be differentiated from the use of synthetic amino acids in that the former produces an increase in the quantity of protein as well as well as an increase in the level of certain amino acids which occurs with the latter (Bressani et al., 1972). Lewis (1972) presents a list of the essential amino acids in an order depending upon the amount of a hypothetical protein supplement required to satisfy the individual requirement in a cereal-based diet.

Protein complementation involves the step-wise replacement of the N supplied from one source by another, usually at a fixed total N content in the diet. The aim is to relate animal performance to the differing ratios and see if there is any ratio where response is optimum

and perhaps higher than can be achieved with either protein alone. From investigations with varying proportions of cereals and protein concentrates, Bressani and Elias (1968) classified four ways in which BV can vary with changes in dietary composition. True complementation is achieved in one type, where the BV from a certain combination of two protein sources exceeds that for each of the two alone. This suggests that each was able to correct specific deficiencies in the other to provide a total amino acid balance more closely aligned to the animals's requirements. Bressani et al. (1972) maintain that the evidence from human nutrition studies strongly supports the suggestion that the content and proportions of the essential amino acids, together with their availability, are the most important factors dictating the value of different protein mixtures. Morrison (1964b) considered that supplementary relationships can often be deduced from amino acid composition. However, since analyses do not take account of availability, there remains a need for biological tests to show supplementary effects. Henry and Kon (1958) came to a similar conclusion, but also review evidence which suggests that the supplemental value of different protein sources may be dependent upon them being eaten at the same time.

Bigwood (1972) gives graphs showing how mixtures of protein sources vary in the amounts required to maintain N balance in adult humans. A combination of two proteins in a particular ratio often produced a significant drop in the total amount of protein necessary to produce balance, suggesting a complementary action in the terminology of Bressani et al. (1972). Small substitutions of wheat or maize protein for egg protein slightly reduced the required amount to maintain balance from egg protein alone, so this beneficial combination is possible even with high quality proteins. They maintain that the straight line relationships do not agree with the concept of a single limiting amino acid.

The results of Sihombing *et al*. (1969) would suggest that a slightly greater protein complementary effect occurs with normal maize and soybean meal than is evident with opaque-2 maize. Although normal maize remains inferior with lower levels of soybean supplementation, at a ratio of 25/75 normal maize/soybean meal, the growth performance is superior to an opaque-2 maize based diet at the same ratio. Figure 3.3.3.1 illustrates the effect of altering the ratio of the two protein sources, and subjective lines drawn through the points present a very similar picture to one case presented by Bressani *et al*. (1972, Type III) of true complementation.

Block and Mitchell (1946-7) suggest that no supplementation occurs where the limiting amino acids is the same for two proteins. This will indeed be the case where the relative concentrations of this amino acid does not differ greatly, otherwise some supplementary effect may occur due to an increased supply of this amino acid from one protein source as it replaces another.

In ration formulation for commercial animal production, protein supplementation has been used in an effort to supply an adequate amino acid balance at least cost. Luce *et al*. (1964) discovered that meat and bone meal could replace part of the soybean meal as a protein supplement, providing a greater degree of total supplementation was allowable. Meade (1969) also found that meat and bone meal could replace part of the soybean meal with no reduction in ADG. With adequate pricing differentials between these two protein concentrates, there may be a financial advantage from this research information through providing an adequate diet with a lower total cost of protein supplementation.

Maner *et al*.(1971) investigated the supplementary relationships between opaque-2 maize and various sources of protein concentrate. The responses varied greatly, but similar to that discussed above, an inferior protein source

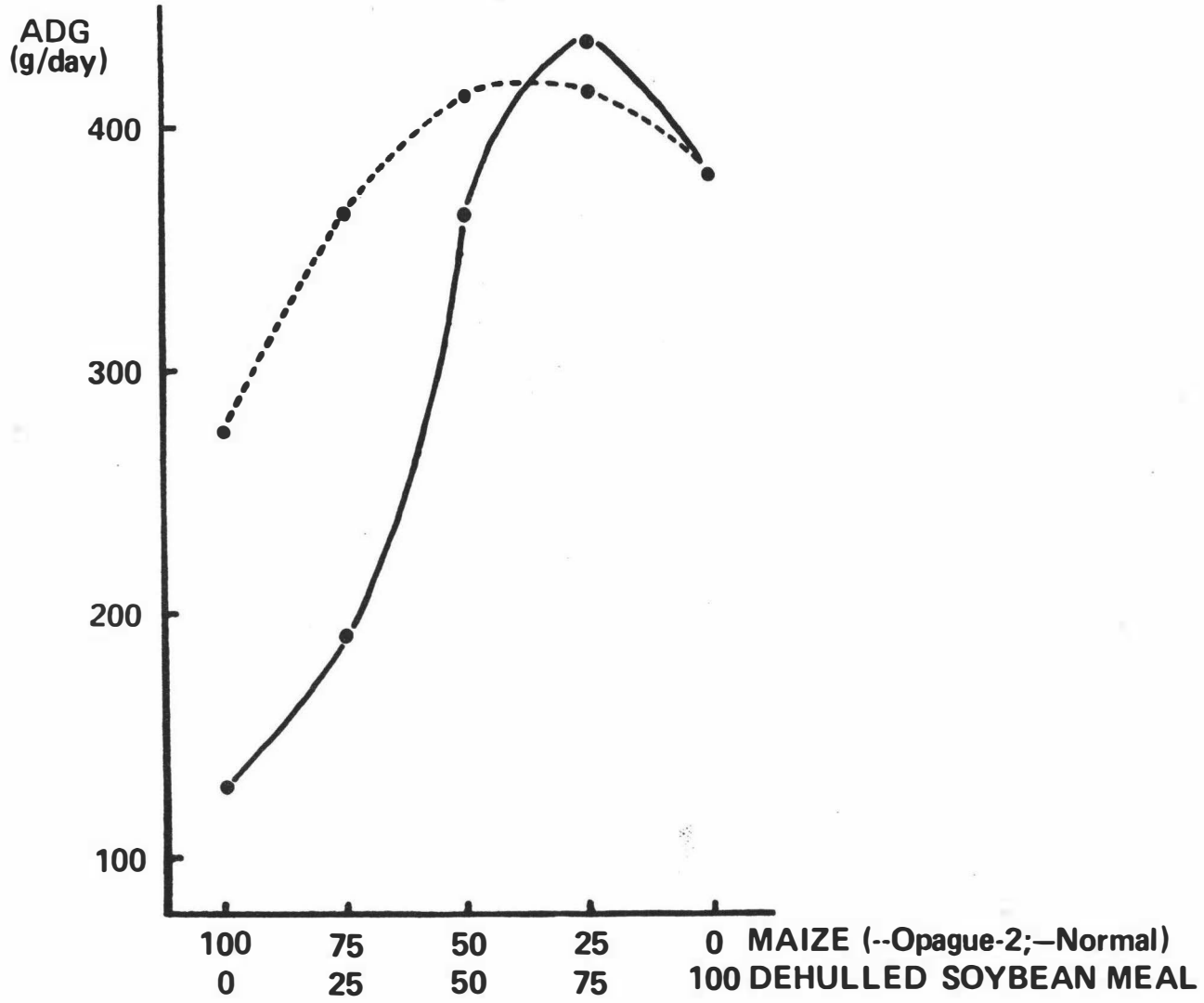

FIG. 3.3.3.1 Example of a complementary effect with two protein sources. (Taken from the data of Sihombing *et al.*, 1969).

(cotton-seed meal) could replace a portion of a high quality protein (fish meal) with the same total protein content and still maintain growth rate and feed conversion efficiency. However other combinations showed no benefits from the combination of several proteins. For example, the performance on an opaque-2/meat meal diet was similar to that for an opaque-2/meat meal/cotton-seed meal diet.

Although there are significant differences between many natural sources of protein, the widest diversity in amino acid patterns and nutritive value occurs between the animal and plant kingdoms (Bigwood, 1972). Thus in considering protein supplements, these two groups are often discussed separately or contrasted (Crampton and Harris, 1969). The latter authors, in comparing amino acid patterns of several feedstuffs within these groups, emphasise the common lysine deficiency of plant protein concentrates (except soybeans) and the relatively low levels of the sulphur amino acids in many products of animal origin. Therefore there is often very limited supplementary effects in the combination of plant protein concentrates and cereal grains, as the latter are also deficient in lysine relative to the animal's requirements (Section 3.1). There is also the added complication that some processes for deriving plant protein concentrates involve heating, which, if not carefully controlled, may reduce the supplemental value. This occurs principally through the loss of available lysine (Carpenter, 1958; Donoso et al., 1962), but other amino acids may be significantly affected.

Extensive studies have been conducted to examine animal responses to the ingestion of widely disproportionate spectrums of amino acids relative to the animal's requirements. This work has been comprehensively reviewed (Harper et al., 1970; Rerat, 1971). The validity of assuming normal physiological responses to dietary treatments involving imbalances or antagonisms resulting from amino acid supplementation, and the possibility of such reactions occurring with natural protein mixtures, has been questioned (Bigwood, 1972b). However, where certain natural or

processed protein sources have been combined in animal feeds, the often exaggerated spectrum of amino acids from the sum of the several protein sources would suggest that such imbalance or antagonisms could occur. For example, evidence of a leucine-isoleucine antagonism in cereal proteins (Harper et al., 1955; De Muelenaere et al., 1967b; Oestemer et al., 1972) would suggest that the addition of blood meal, with a very wide ratio of leucine to isoleucine, would aggravate this situation in high-cereal diets. Experiments with poultry (Mathur et al., 1971) and rats (Ferrando et al., 1962) show blood meal is a suitable protein supplement at low levels in the diet or only when certain amino acids are also added. McDonald et al. (1971) point out the tendency for animals to scour on diets containing more than 10% dried blood. These observations support the suggestion that blood meal may induce imbalances or antagonisms in certain circumstances.

The common tryptophan deficiency of meat and bone meal and maize grain (Sections 3.1, 3.2) probably produce an imbalance resulting in decreased feed intake and live weight gain with increasing levels of meat and bone meal (Peo and Hudman, 1962; see also Table A4.2). Therefore, in addition to the possibility of no beneficial effect from the combination of two or more proteins, there may also be a deleterious effect on animal performance.

It remains difficult to escape the conclusion that although tables or analysed values of amino acid content may be very useful in predicting relationships between dietary proteins, the resultant animal performance from feeding the combination is still necessary as the final step.

3.3.4 Improvement of Amino Acid Balance through Amino Acid Supplementation

While protein supplementation aims to improve the amino acid balance of a diet relative to the animal's requirements by the combination of feedstuffs containing different types and concentrations of protein, there may

be nutritional inefficiencies due to large excesses of some amino acids in an attempt to supply adequate amounts of those considered most limiting. Supplementation with synthetic amino acids has the potential for a more efficient utilisation of dietary protein (Lewis, 1966) by modifying essential amino acid balance through the use of only one or more essential amino acids (Bressani, 1969).

Several workers have discussed particular problems associated with amino acid supplementation (Flodin, 1953, 1957; Rosenberg, 1957; Howe,1958; Scrimshaw et al., 1958; Waddell, 1958). Waddell presents a cautious appraisal of the method of amino acid supplementation and comments: "The guiding principle in the supplementation of any diet (or foodstuff) should be to add the most limiting amino acid only in the amount needed to bring the total into balance with the amount available of the second limiting amino acid; if it is decided to supplement also with the second limiting amino acid, then both the first and second should be brought into balance with the third limiting amino acid." He indicates the danger of adding synthetic amino acids with little or no regard to the absolute levels of other essential amino acids present in the diet. Excessive supplementation can reduce the utilisation of protein or certain amino acids even when lower levels may have been beneficial (Harper et al., 1955; Bressani and Elias, 1968; Allen et al., 1970). There is also evidence that an excessive intake of some essential amino acids can increase the requirement for other nutrients such as vitamins (Sauberlich, 1961). Bigwood (1972b) points out that although amino acid imbalances are unlikely to occur in the combination of proteins, there is a hazard when one or more individual free amino acids are added which are not the limiting ones.

There is now evidence that amino acids must be in the L configuration to be incorporated into proteins by animal synthesising systems. Those occurring in the diet in the D form must be first converted to the L form to be used in protein synthesis. Work with rats shows that individual

amino acids vary in the extent to which the inversion can take place upon digestion and absorption (Berg, 1959). For example, the D and L forms of methionine appear to be utilised to an equal extent, while L-lysine shows no evidence of inversion in the rat. The growing pig appears to respond in a similar manner (Rerat and Lougnon, 1968) and therefore the isomerism of the synthetic amino acids to be added should be a consideration.

Cannon et al. (1947) review the evidence that there should be simultaneous availability of the essential amino acids at the sites of protein synthesis for maximum utilisation, since absorbed amino acids are not stored at the anabolic sites to any significant extent. A time interval any greater than one hour between the ingestion of an incomplete amino acid mixture and its supplementation with the missing amino acids resulted in failure of tissue synthesis. A time interval in the arrival of amino acids at the sites of protein synthesis could occur due to an uneven digestion and absorption of protein fractions or differential rates of absorption of amino acids derived from synthetic forms in the diet and those released from dietary proteins. Porter and Rolls (1971) investigated proteolysis in the small intestine of the rat and concluded that there can be enzyme-resistant peptides which are broken down at a slower rate. If the composition of these resistant peptides does not reflect that of the original protein but concentrate certain amino acids, this could have an important effect on protein utilisation. These authors also point out that if there is any marked discrepancy between the rates of absorption of the amino acids of the protein and the supplemental amino acid, the efficiency of utilisation of the supplement may be reduced. This could results in over-estimation of the requirement for this amino acid (Rerat and Lougnon, 1968). Rolls (1970) found that free amino acids were generally absorbed more rapidly than protein-bound amino acids in the rat. From the above discussion it may be concluded that supplementation of naturally occurring protein diets with synthetic amino acids

may have a lower effectiveness than theoretically estimated.

In experiments supplementing groundnut meal rations for growing pigs with synthetic amino acids, Jones et al., (1961) considered that more rapid absorption of added free lysine caused a slight imbalance of amino acids and reduced the effectiveness of supplementation at higher crude protein levels. However, Bressani et al. (1972) review studies which show that dietary protein level can influence the degree to which the diet is limiting in a particular amino acid (also Section 2.3.10). This occurred only where energy was not limiting the utilisation of dietary protein. These authors conclude that in the practical application of amino acid supplementation there is a need to consider the level of protein in the diet. This is in support of the comments of Waddell (1958) concerning the importance of absolute and relative levels of amino acids in supplemented diets, and similar conclusions are also made by Dent et al. (1970). Bressani (1969) has indicated a further danger in the supplementation of cereal diets with amino acids. Even small additions of other proteins to cereal grain diets may alter amino acid balance so that supplementation with amino acids showing improvement with cereals alone may even decrease the protein quality of the mixed diet.

Ostrowski (1969) reviews the evidence for lysine being the first limiting amino acid in most growing pig diets. Braude et al. (1972), as an introduction to their work, state: "...the greatest value of lysine supplementation may lie in the possible replacement of protein concentrates, since the latter are by far the most expensive dietary components." They do not attempt an economic appraisal of their results, but consider that the high cost of animal protein should provide an incentive to further study of the supplementation of cereal protein with synthetic amino acids. Through the accurate use of synthetic amino acids, it may be possible to reduce the overall protein level of the diet

and still meet animal requirements (Mertz et al., 1952; Evans, 1962; Gallo et al., 1968; Gipp and Cline, 1972). However a point may be reached where the level of total N becomes critical. Work with infants has suggested that the minimum protein requirement is much greater than the sum of the requirements for essential amino acids (Holt, 1959), and other workers have suggested that the ratio of non-essential to essential amino acid N should be approximately 1/1 (Mitchell et al., 1968; Stucki and Harper, 1962). However Lewis (1972) concluded from work with chicks that: "...there would appear to be no case for including non-amino nitrogen as a supplement in the diet until a very substantial part of the essential amino acid needs were met by adding free synthetic amino acids."

Despite the many aspects which must be considered, and the possible deleterious effects which have been outlined above, Lewis (1966) concludes: "This work (Robinson and Lewis, 1963) has demonstrated the practical possibility of directly supplementing pig rations with amino acids."

3.4 THE USE OF THE NITROGEN BALANCE TRIAL WITH GROWING PIGS

The basic principle of a balance trial involves measuring the intake and output from the body of a particular nutrient or element and calculating the amount deposited or depleted from the tissues of the animal by difference (Duncan, 1966). Thomas (1904) was the first to standardise a method evaluating the quality of dietary protein using a balance measuring N input and output from the body. The proportion of absorbed N which is actually retained is taken as an indication of the nutritive value of the dietary protein and which Thomas called Biological Value. Although ingested N may be used for purposes other than protein synthesis (Bigwood, 1972b), the N balance trial assumes that the principal function of dietary N is to form muscle protein in the young growing animal. The initial method of Thomas has been greatly modified, extended (Mitchell, 1924a;

Mitchell et al., 1926; Bender et al., 1953) and criticised (Hegsted, 1969) over the ensuing years, but the technique has provided much valuable information on protein metabolism (Duncan, 1966). The principal object of this section is to critically examine the N balance method, and highlight the problems associated with these studies in growing pigs. Standard procedures and calculations involved in N balance work have been adequately reviewed (Munro, 1964a; Allison, 1964; Den Hartog and Pol, 1972).

Crampton and Harris (1969) list the two most serious limitations of the N blanace method as: difficulties in estimating endogenous faecal N and metabolic urinary N to calculate True Biological Values, and also the significant effect that level of protein intake has upon biological values. The former may be overcome by accepting calculated values or using "apparent" values for NR and BV, assuming an insignificant effect of dietary treatment upon these forms of N output. Although there are several factors which can influence these forms of N excretion (Den Hartog and Pol, 1972; Peret and Jacquot, 1972), this latter assumption would seem to be reasonable for many experiments on protein nutrition where treatments do not differ to any great extent in proximate analysis. To help in this problem, two rapid techniques for estimating endogenous losses have been recently developed (Rerat, 1971).

Experimenters have acknowledged the effect of level of protein or feed intake upon biological values by standardising these parameters for comparisons between different proteins.

Often it may be of interest to know the distribution of N deposition in the body or test for possible redistribution of body protein (Munro, 1964c), which the N balance in itself is unable to examine.

Nitrogen retention, estimated by the difference of input to output, should give similar results to other

methods giving the same parameter such as in vivo estimations and cohort analysis. Duncan (1966) discusses some of the earlier work which showed often significant differences between balance and carcass analysis methods and the possible reasons for these discrepancies. It was noted that these differences were usually greater at higher protein intakes. More attention to detail in the balance experiments, particularly in relation to reducing errors in the collection and analysis of excreta, often produced a much closer agreement between the two methods. However, errors in the cohort analysis method have also been suggested as possible reasons for disparity. Calculating NR by difference allows for the magnification of errors in the analysis of feed and excreta (Waterlow et al., 1960). The Kjeldahl method of analysing for N is routinely used, but there is evidence that it may underestimate N content in some cases (Duncan, 1966; Martin and Skyring, 1962). Due to losses of material or N, intakes are generally overestimated and output underestimated. Also, some losses of N, such as in hair and skin, may not be measured at all. All these errors combine to exaggerate NR, but providing standard methods are used and N intakes are equal, comparisons remain valid (Waterlow, 1960).

Jacquot and Peret (1972) discuss the influence of the test animal upon a biological assay of protein quality, in this case PER with particular reference to the rat. Strain, age and sex have been shown to influence protein and amino acid nutrition in the growing pig (Sections 2.3.6, 2.3.8, and 2.3.9). The first two variables can often be standardised, but the influence of sex usually remains a problem. Since it is necessary to collect dung and urine separately for digestibility and utilisation data, female pigs present difficulties in accurate separation. Without a surgical operation this cannot be achieved in practice, and most researchers resort to the exclusive use of castrated male stock for balance studies with pigs. Dent et al., (1970) concluded that female stock were more

efficient convertors of lysine and energy into live weight and lean meat gain. Therefore there is likely to be a reduction in the sensitivity of the experiment through the use of barrows (Bayley and Summers, 1968) and any sex by treatment interaction may alter the ranking of treatments in NR criteria. Studies demonstrating interactions involving sex for various criteria have been discussed (Section 2.3.8), and therefore caution must be exercised in relating the results of balance studies to a mixed population (Carr and Dunkin, 1969). Balance studies are usually restricted to pigs of more than 20 kg live weight where separate collection of dung and urine is desired, as smaller animals present difficulties of accurate separation.

For collection of excreta, experimental animals need to be confined individually in some form of metabolism cage and this necessarily provides an environmental change from the normal commercial systems of housing pigs in groups. With the possibility of stress factors being associated with this change, or some other reaction to being housed individually, there arises the problem that there may be a different or physiologically abnormal response to dietary treatment (ARC, 1967). However the evidence for differential responses due to the change in environment is conflicting. One study, using a restricted feeding scale for pigs of undefined sex kept individually in metabolism cages or in penned groups, suggested that the former grew significantly slower and had fatter carcasses (Livingston *et al.*, 1969). Cole *et al.* (1966) using *ad libitum* feeding and castrated males, also found caged animals had slower growth rates due to a reduced voluntary feed intake. They calculated that only half of this effect could be explained by a reduced energy requirement, although there would be difficulties involved in an accurate assessment of the influence of reduced exercise and any change in energy requirements associated with the absence of a possible influence of other members' body heat upon the individual's

microclimate. (For a discussion of heat losses from individual and groups of pigs, see Holmes, 1968). These workers suggested the results indicated a danger in applying results from work with metabolism crates to general husbandry conditions. Other workers have obtained differential growth rates but contradictory to those discussed above (Woodman and Evans, 1951; Oslage and Fliegel, 1964; Batterham and Manson, 1970). Woodman and Evans attributed the difference to decreased muscular activity in the cages. The pigs in the experiment of Oslage and Fliegel were at a near maximal feed intake and the increased growth rate in the cages was small (2%). Batterham and Manson (1970) noted a trend for superior growth rates and feed conversion efficiencies in cages but indicated that a direct comparison was not possible since the different housing conditions were not balanced for litter or sex. With the possibility of stress influencing N metabolism through a change in endocrine activity, Pearlman and Cassidy (1953) present evidence that hormonal changes can influence NR and the discussions of Munro (1964c) and Leatham (1964) contain reviews of the effect of hormones upon protein metabolism.

There appears to have been no systematic work conducted on how long the animal should be on the experimental treatment before balance studies are conducted. Both the quantity and the quality of dietary protein can have a significant impact upon the intra-cellular composition of tissues, particularly the liver (Allison, 1964). In reviewing this aspect of protein metabolism, Allison (1964) presents evidence for the protein/DNA ratio and the quantity of RNA in liver and muscle being influenced by N intake and protein quality. For the animal to be in a steady physiological state in relation to the imposed dietary treatment, there will need to be sufficient time for various mechanisms of the body, particularly the enzyme system, to adjust to any changes in nutrient intake. It is common for this

preliminary period to be of 10-14 days duration (Lassiter et al., 1956; McConnell et al., 1971), although some have been less (Cromwell et al., 1969).

In an attempt to minimise any influence of stress and also to regularise the gut contents of the animal before collection takes place, an acclimitisation period, where the animal is placed in the metabolism cage is usually incorporated into the experimental programme at the end of the preliminary period. The rate of passage of feed through the gut of 23 to 86 kg pigs has been estimated at 50 hours (Castle and Castle, 1957; Kidder et al., 1961). Therefore the acclimitisation period should be approximately three days to allow the excreta collected at the commencement of the balance to be related to the conditions for the remainder of the period.

Examining the length of the collection period, Lassiter et al. (1956) concluded that a seven day collection period offered little advantage following a preliminary period of sufficient length (10 days in the collection crates) over a five day collection period. In determining the length of the collection period, there must be a compromise between the need to collect sufficient data for statistical reliability and the influence of the increasing age or weight of the animal (Section 2.3.6). Periods of five to seven days are commonly decided upon (Robinson et al., 1964a; Ostrowski, 1969; Cromwell et al., 1969; McConnell et al., 1971).

Although more thorough work with smaller laboratory animals is common, usually only N leaving the body in urine and faeces is accounted for in studies with pigs. This necessarily assumes that other losses such as in skin and hair, are comparatively insignificant and constant between dietary changes in protein and amino acid nutrition. Some workers have postulated other important avenues of N loss from the body, such as in respired air (Costa, 1960), to explain NR with no change in weight. However Lewis and

Evans (1971) concluded that this is most unlikely in rats and chicks, and that carefully executed studies show no consistent discrepancy between balance and carcass analysis methods for estimating NR. The errors involved in estimating N output have been divided by Blaxter (1966) into random errors associated with sampling and systematic errors involving principally incomplete collection of excreta or deterioration of samples before analysis. Sampling and often sub-sampling is necessary in balance work with pigs, and the random errors can only be minimised by careful mixing and sampling with analyses done in duplicate or triplicate. Incomplete collection of excreta or losses of feed offered are largely overcome by the design and proper adjustment of the metabolism cages. However the method of collecting excreta can influence the degree of N loss before analysis. To prevent the loss of N as ammonia from urine, it may be collected under toluence (Carr and Dunkin, 1969) or mineral acid added to lower the pH (Martin, 1966; Carr, 1971). The results of Martin (1966) and work at the Massey University Animal Physiology Unit (unpublished) has shown that little N loss occurs from faeces or acidified urine when only collected once daily at normal ambient temperatures. Faeces are usually stored in a freezer until analysis, while urine can be satisfactorily kept in a chiller. In analysing faeces samples, some workers have found unacceptably high losses upon drying (Raymond and Harris, 1954; Colovos et al., 1957), although others found smaller losses with careful drying (Saben and Bowland, 1971). To avoid possible drying losses, some workers have developed techniques using wet faeces (Raymond and Harris, 1954; Carr and Dunkin, 1969).

Goldsmith et al. (1952) have listed other variables which they consider require careful evaluation in relation to interpreting the results of N balance experiments. These are related principally to the effect of energy on protein metabolism and are as follows: total daily calorie intake, distribution of non-protein calories in the diet, the relation of total dietary protein to the caloric value

of the ration, the time factor in feeding, the distribution of animal protein over the day's meal, general physiologic state of the animal and the status of body protein stores.

The previous discussion has highlighted the major difficulties associated with N balance studies for growing pigs. Although some of these are significant in certain situations, the N balance technique is still regarded as a useful experimental tool often used in conjunction with other criteria (Lassiter et al., 1956; Braude et al., 1968; Carr and Dunkin, 1969; Ostrowski, 1969; Jones et al., 1962). Several workers have found that NR as estimated by the balance technique gives a good correlation to the amount of edible meat in the carcass (Jones et al., 1962; Jung and Piatkowski, 1967; McConnell et al., 1972).

CHAPTER 4: MATERIALS AND METHODS

4.1 EXPERIMENT 1: THE GROWTH TRIAL

4.1.1 Introduction

In relation to the objectives referred to previously (Chapter 1), growth rates and feed conversion ratios for growing pigs fed the experimental diets described below were examined over the weight range 23-50 kg. No measurements of carcass composition were undertaken.

4.1.2. Composition of the Experimental Diets

4.1.2.1 Maize and meat and bone meal

Several studies have shown that meat and bone meal is an inferior protein source compared to many other feedstuffs of high protein content (Section 3.2). However it was used as the supplement in these experiments because of its large scale use in commercial pig production in New Zealand.

The protein level of experimental diets designed to investigate the order of limiting essential amino acids is important. In a discussion of amino acid supplementation of cereal grain diets for children, Bressani (1969) concludes: "The results of the extensive animal experimentation indicate that to be able to show the order of deficiency in a protein, it is necessary to decrease its level of intake." The diets used in this experiment were low in crude protein content and in the supply of certain essential amino acids relative to stated requirements for pigs of the weight range used (ARC, 1967). This was to allow for a more sensitive background so that responses to amino acid supplementation would be more evident (Harper, 1964; Rerat, 1971). Formulations giving a commercially acceptable level of performance were not the aim of this experiment.

Table 4.1.2.1.1 gives a partial proximate analysis of the three principal dietary ingredients.

TABLE 4.1.2.1.1 A partial Proximate Analysis of the maize and meat and bone meal used in Experiments I and II (per cent of air dry sample).

Fraction	Normal Maize	Opaque-2 Maize	Meat and Bone meal
Moisture	10.0	10.0	6.0
Ether Extract	4.3	5.0	11.8
Ash	1.2	1.3	28.6
Crude Protein	8.0	9.0	44.3

The higher ether extract, ash and crude protein values for opaque-2 maize as compared to normal maize is in agreement with published comparisons (Cromwell et al., 1967, 1968; Klein et al., 1971; Gipp and Cline, 1972). However the references quoted also usually showed a higher dry matter content in opaque-2 maize.

4.1.2.2 Level of inclusion of meat and bone meal

A preliminary experiment (preliminary Trial B) on a small scale was conducted to estimate the level of meat and bone meal which would be most suitable for the planned experiment.

The composition of the diets and the results pertaining to preliminary Trial B are summarised in Table A4.2. This indicates that with the diets used, there was no advantage in terms of live weight gain from increasing the level of meat and bone meal above 10% of the diet. On the basis of these results, a 10% inclusion rate for meat and bone meal was used.

4.1.2.3 Supplementation with synthetic amino acids

The discussion in Sections 3.1 and 3.2 indicates that, in dietary combinations of maize and meat and bone meal, lysine, methionine, tryptophan, isoleucine and threonine may be critical amino acids limiting the growth of the young animal.

A preliminary Trial A (composition of diet and results summarised in Tables A4.1a and A 4.1b) indicated that neither lysine nor methionine were likely to be first limiting to growth on all-maize diets (with vitamin/mineral supplementation).

Tryptophan, lysine and methionine in synthetic forms were added to the experiment according to a design to be described.

In view of limited facilities, only one level of supplementation with each amino acid was possible. For the purpose of these experiments, the biochemical data from the analysis of normal hybrid maize and meat and bone meal samples were used to calculate the amounts of L-lysine. HCl and DL-methionine hydroxyanalogue required to satisfy estimated requirements. (Tryptophan values were estimated from the literature for the initial calculations, but an analytical method was subsequently used and the result included in later calculations.) In adopting this approach, there was the possibility of levels of the supplemented amino acids becoming imbalanced with remaining essential amino acids (Waddell, 1958), but the higher levels were used in an effort to obtain definable responses. With the inconsistent published values for the availability of amino acids in maize and meat and bone meal (Section 3.3.2.3.2) there was little justification in using any other values than those resulting from chemical analysis.

The levels of supplementation with L-lysine. HCl, DL-methionine hydroxyanalogue and L-tryptophan, purity as stated by the manufacturer in brackets, were as follows; expressed on an air dry basis:

L-lysine. HCl	0.37 (98%)
DL-methionine hydroxyanalogue	0.30 (92%)
L-tryptophan	0.10 (90%)

4.1.2.4 Mineral and vitamin additives

In formulating the experimental diets, the aim was to supply adequate amounts of all known nutrients necessary for optimum performance in the growing pig apart from the amino acids. Table A4.3 gives the composition of a proprietory mineral/vitamin premix as supplied by the manufacturer, together with the recommendations of the ARC(1967). At the stated level of inclusion (0.22%), the supplement provided adequate levels of all the constituents for which requirements have been estimated apart from choline and thiamine. However, published figures suggest that predominantly maize diets are not likely to be limiting in these two components (ARC, 1967; Crampton and Harris, 1969).

Estimated requirements for Sodium and Chlorine are 0.1 and 0.15% of the dry matter of diets for growing pigs respectively. (ARC, 1967). Pure finely ground salt was added at a level of 0.25% (air dry basis) to satisfy this requirement apart from any contribution from the two main ingredients.

Recent research has indicated that the availability estimates for calcium and phosphorus in pig rations as previously used (ARC, 1967) are likely to be in error (Whittemore *et al.*, 1972). Therefore estimates of minimum levels will probably be adjusted from those which were used in the formulation of the experimental rations. Although the high ash content of meat and bone meal (Table 4.1.2.1.1)

contains significant quantities of calcium and phosphorus, the availability is uncertain and to ensure sufficient supplies of these two elements, steamed boneflour was included at a level of 2% (air dry diet).

The level of nicotinic acid in the diet deserves particular attention because of the well established ability of the growing pig to convert tryptophan into nicotinic acid when the latter is being consumed at inadequate levels. (ARC, 1967). Furthermore, the ARC review evidence that the nicotinic acid present in maize is almost completely unavailable to the pig. To reduce the possibility of dietary tryptophan being metabolised to provide niacin, an additional amount (2 g/100 kg) of the pure vitamin was added, further to that supplied in the vitamin/mineral premix.

The total amino acid analyses of the samples of the maizes and of the meat and bone meal used in the experiments are given in Table A4.5. The compositions of the complete mixed diets are tabulated in Table 4.1.2.4.1 and their calculated amino acid contents for 6 of the essential amino acids are presented in Tables 4.1.2.4.2 and 4.1.2.4.3 as per cent of dry matter and per mcal ADE (apparent digestible energy) respectively.

4.1.3 The Experimental Design

The four variables under consideration: maize type and the levels of dietary lysine, methionine and tryptophan, suggested a 2^4 factorial arrangement with 16 treatments. With research facilities permitting individually feeding 64 pigs, 4 replications per treatment were possible. It is generally accepted that with non-factorial designs, 8 replications per treatment are required to detect significance differences of a meaningful magnitude in growth studies with pigs. However, the efficiency of the factorial design, which allows for the use of every treatment result in the analysis of the mean effects (Snedecor and Cochran, 1967),

TABLE 4.1.2.4.1 Gross composition of the diets used in Experiments I and II

	GROUP I								GROUP II							
Treatment No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Code (6)	(1)	lt	ml	mt	pl	pt	pm	pmlt	l	t	m	mlt	p	plt	pml	pmt
Opaque-2 maize (kg)					87.25	87.5	87.25	86.75					8.75	87.0	86.75	87.0
Normal maize (kg)	87.5	87.0	86.75	87.0					87.25	87.5	87.25	86.75				
Meat & bone meal (kg)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Salt (kg)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Min. & vit. suppl (2) (kg)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Bone flour (kg)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Nicotinic acid (g)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
L-lysine HCl (3) (g)		370	370		370			370	370			370		370	370	
DL-methionine hydroxy-analogue (4) (g)			300	300			300	300			300	300			300	300
L-tryptophan (5) (g)		100	100	100		100		100		100	100	100		100		100

(1) Mean dry matter content 89.0%

(2) Refer Table A4.3.

(3) 92% activity.

(4) 98% activity.

(5) 90% activity

(6) p, m, l and t indicate diets containing opaque-2 maize, methionine, lysine and tryptophan respectively.

TABLE 4.1.2.4.2 Calculated amino acid contents of the experimental diets (% dry matter)

Diet	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Lysine	0.50	0.91	0.91	0.49	0.98	0.57	0.57	0.98	0.91	0 50	0.49	0.91	0.57	0.98	0.98	0.57
Methionine	0.16	0.16	0.50	0.50	0.16	0.16	0.50	0.50	0.16	0.16	0.50	0.50	0.16	0.16	0.50	0.50
½ cystine	0.14	0.14	0.14	0.14	0.16	0.16	0.16	0.16	0.14	0.14	0.14	0.14	0.16	0.16	0.16	0.16
Meth.+ Cys.	0.30	0.30	0.64	0.64	0.32	0.32	0.66	0.66	0.30	0.30	0.64	0.64	0.32	0.32	0.66	0.66
Tryptophan	0.08	0.20	0.08	0.20	0.13	0.24	0.13	0.24	0.08	0.20	0.08	0.20	0.13	0.24	0.13	0.24
Isoleucine	0.39	0.39	0.39	0.39	0.41	0.41	0.41	0.41	0.39	0.39	0.39	0.39	0.41	0.41	0.41	0.41
Leucine*	1.30	1.30	1.29	1.30	1.13	1.13	1.13	1.13	1.30	1.30	1.30	1.29	1.13	1.13	1.13	1.13
Threonine	0.42	0.42	0.42	0.42	0.50	0.50	0.50	0.50	0.42	0.42	0.42	0.42	0.50	0.50	0.50	0.50

* Ratio of leucine to isoleucine: normal maize 1 : 3.3, opaque-2 maize 1 : 2.7

TABLE 4.1.2.4.3 Calculated amino acid contents of the experimental diets (g/mcal apparent digestible energy)

Diet	1	2	3	4	5	6	7.	8	9	10	11	12	13	14	15	16
Lysine	1.51	2.76	2.76	1.48	2.97	1.72	1.72	2.97	2.76	1.51	1.48	2.76	1.72	2.97	2.97	1.72
Methionine	0.48	0.48	1.51	1.51	0.48	0.48	1.51	1.51	0.48	0.48	1.51	1.51	0.48	0.48	1.51	1.51
½ cystine	0.42	0.42	0.42	0.42	0.48	0.48	0.48	0.48	0.42	0.42	0.42	0.42	0.48	0.48	0.48	0.48
Meth.+ Cys.	0.90	0.90	1.93	1.93	0.96	0.96	1.99	1.99	0.90	0.90	1.93	1.93	0.96	0.96	1.99	1.99
Tryptophan	0.24	0.60	0.24	0.60	0.39	0.72	0.39	0.72	0.24	0.60	0.24	0.60	0.39	0.72	0.39	0.72
Isoleucine	1.18	1.18	1.18	1.18	1.24	1.24	1.24	1.24	1.18	1.18	1.18	1.18	1.24	1.24	1.24	1.24
Leucine	3.94	3.94	3.91	3.94	3.42	3.42	3.42	3.42	3.94	3.94	3.94	3.91	3.42	3.42	3.42	3.42
Threonine	1.27	1.27	1.27	1.27	1.51	1.51	1.51	1.51	1.27	1.27	1.27	1.27	1.51	1.51	1.51	1.51

suggests that the loss in precision may not be as great as the reduction in replication from 8 to 4 would suggest. The following table illustrates that the error degrees of freedom in the analysis of variance, an indication of the sensitivity of the experiment in detecting significant differences, only moves from 49 to 41 when the number of treatments is increased from 8 to 16 over the same number of animals. Therefore the 16 treatment design was adopted.

TABLE 4.1.3.1 Analysis of variance table for 8 and 16 treatments with 64 observations

Source	d.f.(8 treatments)	d.f.(16 treatments)
Mean	1	1
Blocks	7	7
Treatments	7	15
Error	49	41
Total	64	64

With 16 treatments and 8 blocks, it became necessary to confound one factorial effect with the differences between blocks. Cochran and Cox (1957) present a design which confounds the third order interaction with blocks by combining all the treatments which have a positive coefficient in the assessment of this interaction in one group, with the treatments carrying the negative coefficient in the other.

The arrangement of the treatments, together with their composition, is shown in Table 4.1.2.4.1.

4.1.4 Experimental Housing and Facilities

The test house was a modern totally enclosed facility, with the internal fittings set for this experiment to provide 8 pens, each with 8 individual feeding bails. Figure 4.1.4.1 gives a view down the central race, showing the individual feeding troughs, rope pulls for operating the locking gates,

FIG. 4.1.4.1 Internal view of the testing facility used for Experiment I.

and the sheet metal gate partitions for the dunging area behind the feeding bails. Entrance into the adjacent sleeping area was by a doorway approximately 250 cm wide. By means of a movable partition, the sleeping area could be varied in relation to the number and size of the pigs in each pen. Each sleeping area had an opening approximately 40 by 12 cm high up on the external wall, which by means of a sliding panel, could be varied in area. Two extractor fans were situated in the roof near the mid-line of the building with manual individual 3-speed control. Heat lamps were used in each sleeping area for 2-3 weeks after the animals were moved in. However, following this initial adjustment period, the extractor fans and inlet duets over the sleeping areas were used to try and keep the internal environment as constant as possible. Drinking water was available ad libitum from automatic nozzles situated in the dunging area.

4.1.5 The Experimental Animals

4.1.5.1 Selection

A total of 64 pigs comprising equal numbers of castrated males and females, were selected on the basis of age and weight when they weighed approximately 10 kg. They were from 8 crossbred litters: 6 Large White x Landrace; 1 Large White x Berkshire; 1 (Large White x Landrace) x Landrace.

Barrows and gilts were ranked separately according to weight, and then each divided into 4 groups each comprising 8 individuals of similar weight.

4.1.5.2 Pre-experimental management

Pigs were weaned at 3-4 weeks of age and fed ad libitum a "starter" ration based on barley and buttermilk powder. They were transferred in groups of 8 to the test house when the average weight was approximately 10 kg and fed 820 g/pig/day of the starter ration. Groups were assigned to pens

on a weight and sex basis, so that on each side of the central race, groups of gilts and barrows of a similar weight were allotted opposite pens.

It was observed in the two preliminary trials that animals often reacted to an abrupt change from a barley-based to a maize-based ration by a drop in appetite for several days. Where the nutrient balance of the diet was favourable, individuals soon adjusted to the new diets, but where this was not the case, refusals persisted. For the above reasons, a pre-experimental diet based on maize was formulated calculated to have a suitable balance of nutrients for pigs in the weight range 10-25 kg (ARC, 1967). Both opaque-2 and normal maize were included in equal proportions to allow adaptation to any differences in palatability between the two maizes. The content of the pre-experimental diet, together with estimated levels for the amino acids most likely to be critical in cereal - based diets (Section 3.1) is shown in Table A4.7.

Changeover from the starter to the pre-experimental mix occurred at approximately 16 kg live weight and the feeding level was adjusted to 680 g/pig/day, in a single feed until 20 kg when the level was increased to 820 g (Table 4.1.5.2.1). The feed was given once daily in a gruel form. No difficulties were experienced in changing from the starter to the pre-experimental diet.

4.1.5.3 Allocation of treatments

Each group of treatments (Table 4.1.2.4.1) was allocated at random to the pairs of pens facing each other across the central race (one of barrows and the other gilts), and then the 8 treatments in each group distributed at random to the individuals in each pen. Figure 4.1.5.3.1 gives a diagrammatic outline of the final layout.

TABLE 4.1.5.2.1 Daily Feed allowance (kg) according to live weight (kg) for the pre-experimental and experimental periods for Experiments I and II.

Live Weight	Pre-Experimental	Experimental[+]
16.0	0.68	
18.0	0.68	
20.5	0.82	
23.0		0.92
25.5		1.02
28.0		1.13
30.5		1.24
33.0		1.34
35.5		1.44
38.0		1.52
40.5		1.60
43.0		1.67
45.5		1.75
48.0		1.83
50.5		1.88
53.0		1.96
55.5		2.04

[+] Mean apparent digestible energy content of diets 3.88 mcal/kg dry matter (Table A5.6).

Pen	1	2	3	4
Treatment Group	II	I	I	II
Sex	B	G	B	G
	Central Raceway			
Pen	8	7	6	5
Treatment Group	II	I	I	II
Sex	G	B	G	B

FIG. 4.1.5.3.1 Final layout of the Test House for Experiment I (B = barrows, G = gilts).

4.1.6 Procedure during the Experimental Period

Each animal was weighed weekly, and 2 days before the start of the experimental period (23 kg), the level of feeding was increased to 920 g/pig/day. The scale of feeding during the experimental period is detailed in Table 4.1.5.2.1. This scale was adopted in view of the persistent and substantial refusals which were recorded in each of the preliminary trials for which the higher scale of feeding shown in Table A4.4 was followed. The experimental scale of feeding represented 95% on a digestible energy basis of that described by the ARC (1967). Pigs were fed individually once daily, with water added to the meal to form a gruel, but the ratio of meal to water was not rigidly specified. The maximum period animals were allowed access to the feed was 4 hours. After this time, any feed which had not been consumed was collected. Daily refusals for each pig were bulked for a 7 day period (sometimes varied 1 or 2 days either way). After thorough mixing and weighing (nearest 5 g), a sample of approximately 400 g was accurately weighed and the dry matter content determined by drying (71°C for 48 hours). Using a common dry matter value for the original experimental diets

(89% estimated by the same drying procedure), the air dry weight for the bulked refusals was calculated.

Shortly after transfer into the test house, all animals were treated for internal and external parasites.(1)

These treatments were repeated during the experimental period.

Troughs, feeding bails and dunging passages were cleaned once daily.

At the termination of the experimental period (50 kg), average daily gains and feed conversion ratios for each pig were calculated.

4.2 EXPERIMENT II: THE NITROGEN BALANCE TRIAL

4.2.1 The Aims of Experiment II.

4.2.1.1 As for Experiment I but using nitrogen balance as the means of assessment.

4.2.1.2 To determine the apparent digestible energy of the diets used in Experiment I.

4.2.2 Composition of the Experimental Diets

Due to limited facilities only one half of the treatments used in Experiment I could be included in Experiment II; these were those designated group II (Table 4.1.2.4.1).

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(1) "Atgard" Dichlorvos lig Anthilmentic T.V.L. Laboratory Ltd. "I.C.I. Mange and Lice Dressing" for farm animals. I.C.I. (N.Z.) Ltd.

4.2.3 The Experimental Design

A total of 30-35 individual balance determinations was considered to be a reasonable number for the purposes of this investigation. To examine any possible effect of age or weight upon N metabolism in response to dietary treatment, it was considered desirable to conduct balances at two weights. This suggested 15-17 balances at each of the weights. Since there were 16 treatments in the growth trial, investigation of all these diets by nitrogen balance would have allowed only one observation per treatment at each weight. As indicated above, 8 treatments from those used in Experiment I were chosen, and this permitted two replications within balances and treatments. Although it was considered unlikely that significant differences would be detected under these circumstances, any trends could provide useful information.

Therefore the treatments used in Experiment II corresponded to a partial factorial design.

4.2.4 Experimental Housing and Facilities

The experimental facilities provided 12 individual holding pens in one room and 8 metabolism cages in another. Both rooms were fully enclosed with the ambient temperature controlled by a heating/cooling unit. The thermostat was set at 21°C for the duration of the experiment.

Figure 4.2.4.1 shows 3 of the metabolism cages with cage 2 ready for use. Both the length and width of the crates was adjustable.

4.2.5 The Experimental Animals

4.2.5.1 Selection

Sixteen barrows were selected at approximately 15 kg on the basis of age. As there were 8 metabolism cages,

FIG. 4.2.4.1 A view of some of the metabolism cages used in Experiment II. (Note: The metabolism cage numbered 2 is fitted out ready for an experimental run: with a tray for catching spilled feed, polythene urine collecting funnel leading into a glass wool filter laying on wire gauze with a smaller funnel set in the lid of the plastic bucket, and a plastic sheet laying on the faeces collecting tray at the rear of the cage).

the 16 animals were selected as two groups of 8 pigs, spaced in time. Within groups, animals were taken from no more than 4 litters.

4.2.5.2 Pre-experimental management

Following selection, pigs were transferred individually to holding pens and fed and treated as for Experiment I animals.

4.2.5.3 Allocation of treatments

Within each group of 8 pigs, treatment diets were allocated at random. Each remained on the same diet for the duration of the experiment.

4.2.6 Procedure during the Experimental Period

Feeding and management during the experimental period was similar to that for Experiment I. However, as animals reached 30 kg and later 43 kg, they were transferred from holding pens individually to metabolism cages for periods of 7 days (3 days pre-collection period and 4 days collection period), for the purpose of estimating nitrogen balance, dietary ADE and ME (metabolisable energy). The procedure followed for the collection of faeces and urine and for the sampling and chemical analysis of excreta and feed were the same as those described by Carr and Dunkin (1971) except that urinary energy was also determined. For this purpose, duplicate 50 ml samples of urine were freeze dried (approximately 3 days) in petri dishes lined with light plastic of a known weight and determined energy content. The folded plastic containing the dried urine was then ignited in an adiabatic bomb calorimeter (40-50% of the total energy being represented by the plastic).

The two balances were designated A (30 kg) and B (43 kg).

Since there were no separate feeding bails in the holding pens, feed refusals were collected 24 hours after each feed in Experiment II.

4.2.7 Statistical Analysis

The data of nitrogen and energy digestibility were subjected to analysis by the split-plot method (Snedecor and Cochran, 1967) with experimental diets (treatments) as the main plots and balances (A and B) as sub-plots. By this method it was hoped to detect any balance by treatment interactions as well as balance and treatment effects separately. To be valid, main plot and sub-plot treatments must be assigned at random. This condition did not hold for the present experiment, since the experimental diets (main plots) were distributed at random within the two groups of replicates for balance A (sub-plot), but the animals remained on the same experimental diet subsequent to balance A and during balance B. Therefore the error terms in the statistical analysis will be biased, but this was not considered to be a serious limitation for the purposes of this analysis. The split-plot design allows more precise information to be gained on the sub-plot treatments (Snedecor and Cochran, 1967). However, there was little difference in the present experiment, 7 and 8 d.f. for the main plot and sub-plot errors respectively, and the loss of 1 d.f. in the sub-plot error due to missing data for some parameters meant this error mean square was often the larger of the two.

In addition to the analysis of variance with partitioning of any significant ($p < 0.05$) treatment sums of squares, the Duncan Multiple Range Test was used for comparisons between treatment means.

The treatments used corresponded to a partial factorial arrangement which resulted in confounding of factorial effects in the partitioned treatment sums of squares. The main effects P, M, L and T were confounded with the second order interactions (-MLT, -PLT, -PMT and - PML respectively), but since interactions of this order are only likely to assume significance with very large responses to the main factors (Snedecor and Cochran, 1967), this was not considered a serious limitation in the present

study. The first order interactions PM, PL and PT were confounded with the negative coefficients of the other interactions of this order (- LT, - MT and - LM respectively).

Calculations in the analysis of variance were taken to 6 decimal places but are rounded to 3 decimal places for presentation.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 EXPERIMENT I: THE GROWTH TRIAL

5.1.1 Health

Prior to transfer and for some time subsequent to the move to the test house, tail biting occurred in some of the pens. In most cases the incidence stopped after 2-3 weeks and the affected ones healed satisfactorily. However, from one pen two individuals (numbers 33 and 49) had to be isolated for 24 days and treated with antibiotics to facilitate healing. This occurred while the animals were on treatment, but as it was possible to return them to the pen and growth rate was not significantly affected, the data from these animals was included in the analysis.

Four animals were withdrawn from the experiment before the 50 kg final weight was reached. This decision was made when no gains had been recorded over the preceding 2 week period and/or serious weakness in the hind legs was evident. The cause of the leg weakness syndrome was not clear, although a veterinarian diagnosed one case as infectious arthritis. It is possible that the general malnutrition and protein depletion which occurred with these animals (refer Section 5.1.2) predisposed them to infection. However, alternative explanations include some specific interaction with the diets being ingested, and the longer periods these animals remained in the feeding bails (a maximum of 4 hours), although the latter cause seems unlikely. It was considered that sufficient observations had been recorded on these animals for regression of live weight on time to give growth rate information for inclusion in the statistical analysis. The terminating weights used were: 44.0, 38.8, 41.3 and 43.1 kg for pig numbers 9, 10, 34 and 35 respectively. Batterham (1970) records that one animal developed paralysis in the hind legs on a maize/meat and

bone meat diet supplemented with lysine and a MVA (mineral, vitamin and antibiotic) supplement.

5.1.2 Feed Refusals

Although a study of feed refusals was not one of the original aims of this experiment, they did provide information of some interest. Significant feed refusals and low growth rates were recorded for some individuals, in agreement with earlier observations using similar diets (Table A4.1b). The total feed refusals recorded are summarised in Table 5.1.2.1.

There appeared to be a strong relationship between the frequency and extent of refusals and treatment. A Chi test was conducted on the totals, and assuming the expected refusal total for each treatment was zero, the treatment effect was highly significant ($p < 0.005$).

However there were large differences between individual pigs receiving the same diet. This was particularly noticeable with treatment 9 (code 1), where one barrow had no refusals recorded. It is interesting to note that this pig, No. 33, was withdrawn and treated for tail biting after being on experiment for 15 days. From Table A5.2 which lists the refusals on a weekly basis, it can be seen that the two pigs showing the greatest total refusals had started refusing within 2 weeks of being introduced to the diet. Although pig 34 did not begin refusing until the fifth week, it seems unlikely that the lack of refusals for pig 33 was due to the preferential treatment.

Table 5.1.2.1 suggests that supplementation of normal maize/meat and bone meal diets with lysine and methionine, alone or in combination, produced a significant drop in appetite. Similar supplementation of opaque-2 maize had the same effect, but much less severe. Whereas adding both methionine and lysine gave the highest total weight of refusals with normal maize, the lysine supplemented diet gave the highest total with opaque-2 maize.

TABLE 5.1.2.1 Individual and treatment refusal totals (kg) for Experiment I (air dry weight).

Pig No.	Sex[2]	Tr. No.	Code[1]	Refusal Total	Tr. Total
1	B	1	(1)	0.8	
2	B	1	(1)	5.3	
4	G	1	(1)	0.6	6.7
9	B	3	ml	17.9	
10	B	3	ml	7.9	
11	G	3	ml	29.4	
12	G	3	ml	32.2	87.4
17	B	5	pl	0.8	
18	B	5	pl	2.0	
19	G	5	pl	4.2	
20	G	5	pl	2.5	9.5
25	B	7	pm	3.1	3.1
31	G	8	pmlt	2.0	
32	G	8	pmlt	0.8	2.8
34	B	9	l	10.5	
35	G	9	l	30.0	
36	G	9	l	18.9	59.4
41	B	11	m	0.4	
42	B	11	m	18.2	
43	G	11	m	15.9	
44	G	11	m	3.4	37.9
46	B	12	mlt	0.3	0.3
50	B	13	p	0.8	
51	G	13	p	0.2	
52	G	13	p	1.3	2.3
54	B	14	plt	0.2	
56	G	14	plt	1.4	1.6
57	B	15	pml	0.8	
58	B	15	pml	0.3	
60	G	15	pml	5.3	6.4
61	B	16	pmt	1.2	1.2

(1) p = opaque-2 (1) = normal maize
m = methionine l = lysine
t = tryptophan

(2) B = barrow G = gilt

Diets 3, 9 and 11 (and to a lesser extent 5 and 15), appeared to produce the classical symptom of amino acid imbalance viz, reduced voluntary intake (Harper, 1970). According to the theory developed from extensive studies, principally by Harper and co-workers with rats (reviewed in Harper et al., 1970), one way in which an imbalance can be induced is by supplementation of the diet with an amino acid other than the one first limiting in the dietary protein. Those closest to the critical amino acid in terms of deficiency are likely to have the greatest effect, for example the second limiting amino acid (Harper et al., 1964; Baker et al., 1968). However the comments of Harper (1964) may be noted: "The amino acid balance of corn is such that several amino acids are limiting for the rat fed a diet of corn and imbalances among these may be produced readily by amino acid supplementation. It is therefore difficult to distinguish among deficiencies, imbalances and antagonisms in corn diets supplemented with amino acids."

The addition of tryptophan to any diet supplemented with either lysine and/or methionine, eliminated refusals almost entirely. It can be inferred therefore that tryptophan was first limiting, certainly in diets containing normal maize. Since greater refusals occurred with lysine rather than methionine supplemented normal-maize diets, it is also concluded that lysine was second limiting. Although the differences are less definite, similar conclusions can be drawn for the opaque-2 maize diets.

Acker et al. (1959) found a significant reduction in feed consumption of pigs fed a 14% CP corn/soybean ration supplemented with lysine at the higher levels (0.1 and 0.15%) of those they used and attributed this to a palatability effect. They had recognised the depressing effects of excess amino acids in other species and were careful to add only to the extent of the calculated deficiency of each amino acid. However, this implies that they added the synthetic amino acid to raise the total level in the diet up to the animal's requirement with no reference to the

concentration of the second limiting amino acid; (Waddell, 1958); therefore an imbalance could have been produced. Baker et al. (1969) found that a marked reduction in intake and growth rate with added lysine to normal maize diets could be completely rectified by the further addition of tryptophan. These authors reviewed earlier experiments which had shown that reduced growth rate from the addition of lysine was due to a lowered voluntary feed intake, and concluded that the young pig is very sensitive to lysine imbalance. In contrast to the present results, the addition of methionine to the diet (where the only source of protein was maize grain) did not produce the normal signs of an amino acid imbalance.

The extent of the reductions in voluntary feed intake seen for some treatments may have been due to the relatively low total protein level of the diets. Amino acids imbalance are most readily created with low protein diets (Harper, 1969), and the 12.5% CP in the diets used here is low relative to published recommendations (ARC, 1967).

Batterham (1970) mentions the existence of refusals on maize/meat meal or maize/meat and bone meal diets which were supplemented with lysine and a MVA supplement alone or in combination. Only slight intermittent rejections were recorded for those diets supplemented with tryptophan. Although the level of protein supplement was higher than in the present experiment, the feeding level and the level of supplementation with lysine and tryptophan were very similar. Cromwell et al. (1967) also recorded a drop in voluntary feed intake when lysine was added to a normal maize diet unsupplemented with additional protein.

5.1.3 Average Daily Gain (ADG)

Weekly weights, recorded to the nearest 0.5 lb were converted to metric using a conversion factor of 0.4536 and are shown in Table A5.1 to the nearest 100 gm. Only those weights subsequent to the starting weight (23 kg) and less than the terminating weight (50 kg) are included. Also

shown are ADG's calculated either from the first and last weights for each individual or from the linear regressions of weekly weights on time. Linear regression, although reducing the variance associated with using only the first and last weights, assumes ADG remains constant with time. It is however, well established that the growth curve of young animals in terms of weight is of a sigmoid nature (Crampton and Harris, 1969). Also, in fitting regression lines, observations are assumed to be independent, which does not hold when the weights of a single animal are plotted. Fitting a quadratic expression did in one case produce a significant reduction in the deviations sums of squares compared to the linear model. Although the error term is necessarily biased, this indicated that a quadratic expression would be a better expression of the data (Beames and Sewell, 1969). In contrast, Lodge et al., (1972) considered that the relationship between 23 and 59 kg live weight was essentially linear. For the purpose of this analysis, a simple linear regression model was considered satisfactory and the regression coefficients used in the analysis of variance.

The data was analysed according to standard methods for partially confounded factorial designs and the full analysis of variance (AOV), with partitioning of the Treatment sums of squares (TSS) is shown in Table A5.8. The block sums of squares was not partitioned if the F value was not significant ($p < 0.05$). The significant mean squares resulting from the partitioned TSS are shown in Table 5.1.3.1.

The mean ADG over all treatments was 415 g/day with a coefficient of variation of 8.9%. Two of the main effects, P and T, were highly significant, and this resulted in two first order (PT and LT) and two second order (PMT and PLT) interactions reaching significance at the 1% and 5% levels respectively.

TABLE 5.1.3.1 Significant mean squares from the partitioned treatment sums of squares for average daily gain (g/day).

Factorial Effect (1)	Factorial Total	Mean Square
P	2517	98 988.891**
T	2831	125 227.516**
PT	- 1611	40 551.891**
LT	1419	31 461.891**
PMT	- 621	6 025.641*
PLT	- 647	6 540.766*

(1) In this table and all following tables,

P = Factorial effect due to opaque-2 maize

M = Factorial effect due to methionine

L = Factorial effect due to lysine

T = Factorial effect due to tryptophan

* $p < 0.05$, ** $p < 0.01$.

To examine these effects and interactions, two and three way tables are presented (Tables 5.1.3.2, 5.1.3.4, 5.1.3.5, and 5.1.3.6).

TABLE 5.1.3.2 Significant interaction ($p < 0.01$) of maize type by tryptophan on average daily gain (g/day).

	Basal[1] Tryptophan	Added Tryptophan	Mean	S.E.[2]
Normal	307	446	376	
Opaque-2	436	474	455	± 9
Mean	371	460		
S.E.		±9		

Standard error (S.E.) of the difference between means in the body of the table ± 13.

1 The term "basal" is used to indicate those diets where no supplementation with the partcular amino acid took place.

2 S.E. of the difference between means.

The significant effect of substituting opaque-2 maize for normal maize over all treatments can be clearly seen in Table 5.1.3.2: an increase in ADG from 376 to 455 g/day, or 21%. Also, the addition of tryptophan increased ADG from 371 to 460 g/day averaged over all treatments, an increase of 24%. The interaction resulted from a much greater response to the addition of tryptophan in the diets containing normal maize. While ADG at basal tryptophan levels with normal maize represented only 70% of that for opaque-2 (307 vs 436), it represented 94% in the comparison when tryptophan was added to each maize and averaged over all other effects (446 vs 474). Normal maize supplemented with tryptophan produced an average mean effect slightly above the unsupplemented opaque-2 maize diets. This suggests that the superior performance of the opaque-2 maize in the unsupplemented diets was due to the higher levels of tryptophan in this type (Table A4.5).

Cromwell et al. (1967) compared normal and opaque-2 maize in terms of ADG with and without protein supplementation. When opaque-2 was substituted for normal maize both on a weight and isonitrogenous basis (with no protein supplement), ADG was significantly increased. Opaque-2 also remained superior at several levels of soybean meal. There was no amino acid supplementation of opaque-2 maize, but the addition of tryptophan to normal maize (0.062%) produced a non-significant increase in performance. In a later study (Cromwell et al., 1969a), opaque-2 significantly improved growth rates during the finishing period (45-92 kg) only at a low protein level (11.3% CP), although the trend was in favour of the opaque-2 maize during a growing phase (21-45 kg) at 16 and 14% CP.

Klein et al. (1968) compared opaque-2 and normal maize on an isonitrogenous basis with no protein supplementation, and found the former to be superior. These results, indicating the superiority of opaque-2 maize both in the presence and absence of additional protein, are supported by other published work (Conrad et al., 1969; Sihombing et al., 1969; Gallo et al., 1968b, 1968c, 1969, 1970b; Henry and Boudon, 1971, Klein et al., 1971; Maner et al., 1971; Gipp and Cline, 1972; Marroquin et al., 1970; Gallo, Jimenez and Maner, 1968). However with pigs weighing initially 5 kg, Drews et al. (1969) reported a significant interaction between maize type and level of protein supplementation, so that while opaque-2 gave superior weight gains at lower levels of soybean meal, the reverse was the case at higher levels. No explanation could be given for the depression in growth which occurred on opaque-2 maize diets as the supplement was increased. Other workers have also found growth rate to be non-significantly different or the order of superiority reversed at higher levels of supplementation (Gallo, Jimenez and Maner, 1968; Bellamy, 1969; Cromwell et al., 1969; Sihombing et al., 1969; Henry Boudon, 1971).

The significant response in growth rate to the addition of tryptophan found in this experiment is in support of several published experiments where meat and bone meal was used as a supplement to normal maize (Bloss et al., 1953; Terrill, 1954; Meade and Teter, 1957; Meade, 1969; Batterham, 1970; Stockland et al., 1971). A constant but non-significant response to added tryptophan was reported by Luce et al. (1964), where meat and bone meal partially replaced soybean meal. However, the levels of supplementation were relatively low at 0.025 to 0.041%. Henson et al. (1954) found an increase in performance when a combination of lysine, methionine and tryptophan was added to a maize/meat and bone meal diet.

Where the supply of nicotinic acid is inadequate, tryptophan is irreversibly transformed to the vitamin (ARC, 1967), thus aggrevating any tryptophan deficiency which may be present. Batterham (1970) concluded from his results that nicotinic acid is first limiting to tryptophan in maize/meat and bone meal diets. In view of the additional niacin added to the experimental diets (Table 4.1.2.4.1) it seems likely that the response to tryptophan reported here was due to increased protein utilisation rather than alleviating a niacin deficiency.

The absence of a significant effect for lysine or methionine, alone or in combination, in the analysis of variance for ADG is in agreement with other work involving maize/meat and bone meal diets (Meade and Teter, 1957; Luce et al., 1964; Meade, 1969; Oestemer et al., 1970; Batterham, 1970; Stockland et al., 1971). The lack of a positive growth response to added lysine contrasts with the conclusions from many pig nutrition experiments with cereal diets, as reviewed by Ostrowski (1969) and Braude et al. (1972), and also with work involving unsupplemented normal and opaque-2 maize diets for rats and pigs (Gallo, Jimenez and Maner, 1968; Gallo et al., 1969; Bressani and Marenco, 1963). However other workers have concluded

TABLE 5.1.3.3. Growth response of rats and pigs to lysine and tryptophan supplementation of maize diets containing no other protein source (1).

Animal	Maize Type(2)	Growth Response lysine	tryp.	lys.+ tryp.(4)	Reference
Rats	NM	+			Mitchell and Smuts, 1953.
	NM	+			Sure et al., 1953
	NM	+			Bressani and Marenco,1963.
	NM	+		+	Rosenberg et al. 1960.
	NM	+		+	Howe et al.,1965.
	OP-2	+		+	Mertz, 1966.
	OP-2			+	Nelson,1966.
	NM		+		Hogan et al.,1955.
	NM	+	+	+	Bressani et al., 1968.
Pigs	OP-2	+		+	Gallo, Jimenez and Maner,1968.
	NM			+	
	OP-2	+			Gallo et al.,1969.
	NM			+	
	OP-2	+			Jensen et al.,1969.
	NM	-(3)	+	+	Cromwell et al., 1967.
	NM	-		+	Baker et al.,1969.
	NM		+	+	Ilori and Conrad 1970.
	NM			+	Gallo et al.,1968b.
	NM			+	Gallo and Pond,1968.
	OP-2			+	Nordstrom et al., 1970.
	NM			+	
	OP-2	-			Pick and Meade,1968.

(1) The absence of a + does not indicate a negative response. Either the experiment did not examine the treatment or there was no response.

(2) NM = normal maize, Op-2 = opaque-2.

(3) Indicates an imbalance with reduced feed intake and growth rate.

(4) Denotes an additional response of the two amino acids together over that to a single amino acid.

from amino acid supplementation studies of maize diets with no other added protein that the initial response is to tryptophan. Table 5.1.3.3. summarises some of the results from the literature for rats and pigs.

Bressani et al. (1972) discuss the controversy relating to the first limiting amino acid in maize protein. They conclude there may be sufficient variation in the essential amino acid content of maize grain particularly lysine, together with the effects of different types of processing, to account for these differences. The data collected by Harman et al. (1969) and FAO (1970) would support this view, particularly if lysine and tryptophan are nearly equally first limiting.

Where protein supplements have been used in work with maize, lysine has been demonstrated to be first limiting with all supplements apart from meat and bone meal (Clawson et al., 1963; Gipp and Cline, 1972; Thomas and Kornegay, 1972). Bayley and Summer (1968) concluded lysine and methionine were nearly equally limiting in maize/soybean meal diets.

The relatively low levels of tryptophan in maize grain and meat and bone meal compared to their substitutes has already been briefly discussed (Section 3.1, and 3.2). The present results indicate that tryptophan is first limiting in the maize/meat and bone meal combination, in agreement with those from some other studies using maize protein alone (Table 5.1.3.3). This table also shows that in many cases, the response to a combination of tryptophan and lysine was often larger than those to either amino acid alone.

A significant interaction of lysine by tryptophan occurred in the present experiment as shown in Table 5.1.3.4.

TABLE 5.1.3.4. Significant interaction ($p < 0.01$) of lysine by tryptophan on average daily gain (g/day).

	Basal[1] Tryptophan	Added Tryptophan	Mean	S.E.[2]
Basal lysine	393	437	415	± 9
Added lysine	350	482	416	
Mean	371	460		
S.E.	± 9			

Standard error (S.E.) of the difference between mean in the body of the table ± 13.

1 The term "basal" is used to indicate those diets where no supplementation with the particular amino acid took place.

2 S.E. of the difference between means.

Thus, in the absence of supplemental tryptophan, the addition of lysine over all treatments depressed growth by 11% while there was an average improvement of 10% in the presence of added tryptophan. In other words, the response to added tryptophan was much greater in the presence of extra lysine (from 393 to 437 vs from 350 to 482).

In studies reported by Baker *et al.* (1969), it was found that the marked reduction in growth rate from adding lysine to maize diets could be completely removed by the further addition of tryptophan so that growth on the lysine and tryptophan supplemented maize was greater than the unsupplemented control. Table 5.1.3.4 shows that in the experiment reported here, the main effect of lysine and tryptophan together was greater than for either amino acid

alone. Also, over all levels of dietary tryptophan, there was no effect from the addition of lysine alone. The three way table for the significant maize type, lysine by tryptophan interaction on ADG is shown below (Table 5.1.3.5.)

TABLE 5.1.3.5 Significant interaction ($p < 0.05$) of maize type, lysine by tryptophan on average daily gain (g/day).

Normal Maize

	Basal[1] Tryptophan	Added Tryptophan	Mean	S.E.[2]
Basal Lysine	343	417	380	±13
Added Lysine	271	474	373	
Mean	307	446		
S.E.	±13			

Opaque-2 Maize

	Basal Tryptophan	Added Tryptophan	Mean	S.E.
Basal Lysine	443	457	450	
Added Lysine	429	491	460	±13
Mean	436	474		
S.E.	±13			

Standard error (S.E.) of the difference between means in the body of the table ± 18.

1 The term "basal" is used to indicate those diets where the particular amino acid took place.

2 S.E. of the difference between means.

It can be seen that the depression in growth rate from adding lysine alone was much greater with normal maize than opaque-2 maize diets. However, because of this more pronounced effect the recovery in performance from adding tryptophan to the imbalanced normal maize diets was more spectacular, so that the two types of maize diets supplemented

with both lysine and tryptophan supported comparable growth (although the opaque-2 diets remained superior in this respect).

These results conflict to some degree with those of Meade (1969), who reported no improvement in the growth supported by normal maize/meat and bone meal diets from adding lysine in the presence of supplemental tryptophan. Gallo et al. (1969) reported a significant improvement in normal maize diets supplemented with lysine and tryptophan, but, contrary to the present results, this failed to produce performance equal to that obtained with unsupplemented opaque-2 maize. However in earlier work (Gallo et al., 1968b), lysine and tryptophan added to a normal maize diet did produce an ADG equivalent to that for an opaque-2 maize diet. In normal maize diets containing meat and bone meal Batterham (1970) and Stockland et al. (1971b) both reported a large additional response to the addition of lysine to tryptophan supplemented diets. In one analysis of variance (Batterham, 1970), the lysine by tryptophan interaction reached significance for meat meal but not meat and bone meal.

Acker et al. (1959) describe a crude protein level by lysine interaction in normal maize/soybean diets: at 12% CP growth rate and feed conversion efficiency were increased with added lysine but there was no consistent effect at 14% CP. A significant reduction in growth rate resulted from increasing levels of supplemental lysine at 14% CP, but the trend was not significant at 12% CP. Although the depression was greater at the higher CP levels, this appeared to be due to an imbalance, despite the fact that lysine was added only to a maximum of the calculated deficiency (Section 3.3.4). In a 10% CP maize/fish meal diet, Devilat and Skoknic (1971) induced an imbalance effect with reduced feed intake and ADG by adding 0.3% lysine. The second experiment reported by Pick and Meade (1968) suggested an imbalance effect on growth rate occurred when 0.1% lysine was added to a basal opaque-2 diet.

The remaining interaction which reached significance is shown in Table 5.1.3.6 for maize type, methionine by tryptophan on ADG.

TABLE 5.1.3.6 Significant interaction ($p < 0.05$) of maize type, methionine by tryptophan on average daily gain (g/day).

	Normal Maize			
	Basal[1] Tryptophan	Added Tryptophan	Mean	S.E.[2]
Basal Methionine	334	443	388	± 13
Added Methionine	280	449	364	
Mean	307	446		
S.E.	± 13			

	Opaque-2 Maize		
	Basal Tryptophan	Added Tryptophan	Mean
Basal Methionine	426	473	449
Added Methionine	446	475	460
Mean	436	474	
S.E.	± 13		

Standard error (S.E.) of the difference between means in the body of the table ± 18.

1 The term "basal" is used to indicate those diets where no supplementation with the amino acid took place.

2 S.E. of the difference between means.

This table again shows that the pigs on the normal maize diets were more sensitive to the addition of synthetic amino acids than were those fed the opaque-2 diets. The addition of tryptophan, as already discussed, produced a greater positive growth response in normal maize diets,

while the addition of methionine in the absence of added tryptophan depressed growth. In the presence of added tryptophan, methionine produced no response in either maize.

In maize/meat and bone meal diets Meade (1969) found that the addition of methionine to a tryptophan supplemented diet significantly reduced ADG. There was no evidence for such an effect in the present experiment (Table 5.1.3.6), although a non-significant depression was apparent when individual treatment means were compared for opaque-2 maize diets (pt vs pmt; Table 5.1.3.7). Meade (1969) and Meade and Teter (1957) reported that methionine supplementation alone was without effect in maize/meat and bone rations on the growth of young growing pigs. Stockland *et al*. (1971b) found a non-significant depression in ADG when methionine was added to a tryptophan supplemented maize/meat and bone meal diet, but the addition of methionine alone had no effect upon ADG, in contrast to the results of the present experiment.

Individual treatment means for ADG in Experiment I are shown in ranked form in Table 5.1.3.7.

TABLE 5.1.3.7 Ranked individual treatment means for average daily gain (ADG; g/day) in Experiment I.

Treatment No.	Code	ADG	Subscript(1)
8	pmlt	498	a
14	plt	483	ab
2	lt	474	abc
12	mlt	474	abc
6	pt	462	abc
16	pmt	451	abc
15	pml	447	abc
7	pm	445	abc
13	p	441	abc
4	mt	423	bcd
10	t	411	cd
5	pl	410	cd
1	(1)	376	d
11	m	310	e
9	l	291	ef
3	ml	250	f

(1) Treatment means which do not share a common subscript are significantly different ($p < 0.05$).

The addition of methinonine to lysine plus tryptophan supplemented normal or opaque-2 maize diets did not significantly influence ADG (Table 5.1.3.7). Published work has shown similar results (Gallo et al., 1968a; Baker et al., 1969; Meade, 1969; Ilori, 1971; Stockland et al., 1971b).

It may be concluded from the above discussion that tryptophan and lysine are the first two limiting amino acids in that order for normal and opaque-2 maize/meat and bone meal diets, but that an amino acid other than methionine is third limiting. Since Baker et al. (1969) came to the same conclusion, using only maize grain protein as a source of amino acids for the growing pig, it appears that the addition of meat and bone meal at a level of 10% does not influence the first two limiting amino acids or their order. Rosenberg et al. (1960) concluded from work with rats that any one of four amino acids may be third limiting in maize grain protein: methionine, isoleucine, threonine and valine.

Although the main effects for lysine and methionine were not significant in the analysis of variance, Table 5.1.3.7 shows that for individual treatments the addition of lysine and/or methionine to normal maize diets significantly reduced performance from the unsupplemented treatment. This reflected the drop in appetite previously discussed (Section 5.1.2). The addition of methionine or methionine plus lysine to opaque-2 maize diets slightly increased performance, while the addition of lysine suppressed ADG, although none of these differences was significant. Gallo, Jimenez and Maner (1968) reported a significant reduction in the live weight gain of pigs fed methionine supplemented opaque-2 maize diets compared with those fed diets containing extra lysine. The trend was in the opposite direction in the present experiment, suggesting a significant role of the meat and bone meal supplement in altering the importance of these imbalance effects for individual amino acids. Baker et al. (1969) found that methionine added to lysine plus tryptophan supplemented low protein maize diet did not

significantly increase performance. A marked reduction in growth rate occurred with lysine supplementation, but methionine plus lysine had no effect.

The small increase in ADG from the further addition of methionine to lysine plus tryptophan supplemented opaque-2 maize would suggest that this amino acid may be third limiting in diets containing this mutant and supplemented with meat and bone meal. Only a small improvement may have been possible due to another amino acid becoming limiting with a slight increase in the level of methionine. The complete lack of a response to methionine in normal maize diets containing added lysine and tryptophan suggests that some other amino acid is third limiting in diets containing this maize type. However the large imbalance effect with this amino acid alone would indicate, apart from some specific effect due to methionine in the growing pig, that it is close to being third limiting.

5.1.4 Feed Conversion Ratio (FCR)

Table A5.3 lists the total feed offered, total refusals, net feed intake and FCR for individual animals in Experiment I. The approximate weekly distribution of feed refusals is shown in Table A5.2.

The FCR data were subjected to the same analysis of variance as outlined for ADG, and Table A5.10 shows the mean squares together with the partitioning of the treatment sums of squares. The significant mean squares are reproduced in Table 5.1.4.1.

TABLE 5.1.4.1 Significant mean squares ($p < 0.01$) resulting from the partitioning of the treatment sums of squares for feed conversion ratio (kg feed/kg grain).

Factorial Effect	Factorial Total	Mean Square
P	-0.293	5.500**
T	-0.318	0.318**
PT	-0.209	0.209**
LT	-0.135	0.135**

** $p < 0.01$.

The mean FCR over all treatments was 3.161 ± 0.18 kg meal/kg live weight gain, with a coefficient of variation of 11%.

The significant factorial effects are presented in Tables 5.1.4.2 and 5.1.4.3.

TABLE 5.1.4.2 Significant interaction ($p < 0.01$) of maize type by tryptophan on feed conversion ratio (kg meal/kg live weight gain).

	Basal Tryptophan[2]	Added Tryptophan	Mean	S.E.[1]
Normal	3.98	2.93	3.45	± 0.09
Opaque-2	2.98	2.76	2.87	
Mean	3.48	2.85		
S.E.	± 0.09			

(1) Standard error (S.E.) of the difference between means.

S.E. of the difference between means in the body of the table ± 0.12.

(2) The term "basal" is used to indicate those diets where no supplementation with the particular amino acid took place.

TABLE 5.1.4.3 Significant interaction ($p < 0.01$) of lysine by tryptophan on feed conversion ratio (kg meal/kg live weight gain).

	Basal Tryptophan[2]	Added Tryptophan	Mean	S.E.[1]
Basal Lysine	3.36	3.00	3.18	± 0.09
Added Lysine	3.60	2.69	3.15	
Mean	3.48	2.85		
S.E.	± 0.09			

(1) Standard error (S.E.) of the difference between means.

S.E. of the difference between means in the body of the table ± 0.12.

(2) The term "basal" is used to indicate those diets where no supplementation with the particular amino acid took place.

The ranked treatment means are shown in Table 5.1.4.4, together with subscripts from a Duncan Multiple Range Test. The order is seen to be almost identical to that for ADG in Table 5.1.3.7. Therefore it may be concluded that the significant reductions in voluntary intake which occurred on some of the treatment gave a corresponding reduction in growth rate, so that the ranking of treatment by either criterion gave similar results.

Because of the higher variability in the FCR data compared with ADG, only 2 of the main effects and 2 first order interactions were significant. Also, in Table 5.1.4.4, a lower number of treatments were significantly different from each other. For example, whereas the addition of lysine to a normal maize diet produced a significant reduction in ADG (23% when comparing individual treatments), the deterioration in FCR was not significant (14%). Again, supplementing a normal maize diet with lysine and methionine

TABLE 5.1.4.4 Ranked treatment means for feed conversion ratio (FCR; kg feed/kg live weight gain) in Experiment I.

Treatment No.	Code	FCR	Subscript (1)
8	pmlt	2.65	a
14	plt	2.66	a
2	lt	2.73	a
12	mlt	2.73	a
6	pt	2.83	a
15	pml	2.88	a
16	pmt	2.90	a
7	pm	2.93	a
13	p	2.98	a
4	mt	3.09	ab
5	pl	3.11	ab
10	t	3.16	ab
1	(1)	3.57	bc
11	m	3.96	cd
9	l	4.07	cd
3	ml	4.33	d

(1) Treatment means which do not share a common subscript are significantly different ($p < 0.05$).

produced a 33.5% decrease in growth rate, while feed efficiency decreased by only 21%. Batterham (1970), using maize/meat meal and meat and bone meal diets supplemented with amino acids, also found that percentage changes in FCR were less than those for ADG.

Under ad libitum feeding conditions, Meade (1969) found the addition of lysine or methionine alone to normal maize/meat and bone meal diets did not affect growth rate, while FCR significantly improved. However Stockland et al. (1971b), also with ad libitum feeding conditions, found the significant differences for ADG and FCR were the same in treatment comparisons. They recorded a much greater relative increase in maize/meat and bone meal diets from the addition of tryptophan in terms of ADG than in Experiment I. The improved utilisation of dietary protein may have increased appetite on these diets so that the total response was greater.

The close relationship which is known to exist between growth rate and feed conversion efficiency under restricted feeding conditions also holds in the present experiment, where amino acids imbalances produced large reductions in feed intake and ADG. For this reason, detailed consideration of the results pertaining to FCR in addition to those relating to ADG is not considered necessary. A comparison of the two sets of data shows that any increase or decrease in ADG was accompanied by a corresponding decrease or increase in FCR.

5.2 EXPERIMENT II : THE NITROGEN BALANCE TRIAL

5.2.1 Health

The health of animals on experiment was good in contrast to some of the individuals in the growth trial (Experiment I) on the same treatments. Possible reasons for this difference are discussed below.

5.2.2 Feed Refusals

The barrows in Experiment II received group II of the experimental diets used in the growth trial (Table 4.1.2.4.1). Table 5.1.1.2.1 shows that pigs fed diets 9 and 11 refused appreciable quantities of feed in Experiment I. However it was noticeable that both the fraction of pigs recording refusals and the total weekly refusals were less in the balance experiment (Table A5.7). (Only refusals for treatments 9 and 11 are shown since these diets were the only ones refused in the N balance trial). Refusals which occurred while the animals were in the metabolism cages are included in the totals in Table A5.7. Although the duration of the growth period was less in Experiment II, and refusals became more pronounced at the heavier weights in Experiment I (Table A5.2), these two factors are inadequate to account for the differences between the experiments in feed refusals. Variability between animals also makes a true comparison difficult, but it is seen that no severe refusals occurred in Experiment II.

The environment and management of the animals in the two experiments were different in some aspects and this probably affected the consumption of the test diets. In the growth trial, there was a maximum of 4 hours access to feed for a single period each day. In the N balance trial, animals were allowed free access to their daily allocation of feed given once daily between, and twice daily during, the two balance periods. Feed spilt from the troughs in the holding pens (between balances) and which

was not collected could have introduced a source of error, but the associated underestimation of refusals is unlikely to explain the large difference seen.

The other major differences between the two experiments were that in Experiment II a constant ambient temperature (21°C) was maintained and pigs were penned individually. Harper (1970) reviews work with rats which shows that if voluntary intake is stimulated with certain imbalanced diets by cold stress or insulin injections, growth rates can increase to be comparable to those on control diets and the animals appear healthy. However this should be differentiated from forcibly increasing the intake on a diet with a severe amino acid deficiency, where the effects of the deficiency are magnified and death may result (Harper and Benevenga, 1969). Therefore, some imbalanced diets will support body weight gains if injected in sufficient quantities, there is some physiological mechanism which depresses appetite and animals show lower or negative weight gains. In Experiment II, some factor (or combination of factors) was operating to increase voluntary intake on those diets which had induced significant refusals in Experiment I. Perhaps the 24 hour access to the daily allocation of feed in Experiment II (versus a 4 hour maximum in Experiment I) was sufficient to compensate for the reduced appetite and slower rate of eating observed on the imbalanced diets in Experiment I, so that total weight of refusals for these diets was less in Experiment II. However it was not possible to clearly differentiate those factors which were important in producing the difference in voluntary intake for the two experiments. This remains a possible field for future study.

5.2.3 Apparent Digestible Dry Matter (ADDM), Apparent Digestible Energy (ADE) and Metabolizable Energy (ME)

The data collected relating to dietary energy and dry matter digestibility are summarised in Table A5.4 A summary

of the mean squares from the analysis of variance for live weight, gross energy intake, apparent digestible dry matter, ADE and ME is shown in Table A5.9. There were no significant difference between either treatments or balances for ADDM(%), ADE(%) or ME (%). The balance and overall means are shown in Table 5.2.3.1.

The ADDM contents of the experimental diets are comparable with values reported in the literature for maize-based diets. Cromwell et al. (1969) found dry matter (DM) digestibilities of 83.7, 86.9 and 86.8, 86.9 for opaque-2 and normal maize respectively, with the first comparison for insonitrogenous diets with respect to opaque-2 and the second with respect to normal maize. The diets contained no protein supplements. Higher values of 87.0 - 89.4 were reported by McConnell et al., (1971) in normal maize/soybean diets. Sihombing et al. (1969) found no consistent difference between normal and opaque-2 maize in DM digestibility over several levels of soybean meal.

TABLE 5.2.3.1 Balance and general mean values for live weight, gross energy intake (GEI), apparent digestibility of dry matter (ADDM) and energy (ADE), and metabolisable energy (ME) for Experiment II.

	Balance				Experimental
	A	B	± S.E.(1)	Sig.	Mean
Live weight (kg)	31.4	43.8	0.18	***	
GEI (mcal/4 days)	18.0	24.8	0.30	***	
ADDM (%)	85.0	84.0	0.49	N.S.	85.0
ADE (%)	85.0	85.0	0.53	N.S.	85.0
ME (%)	83.0	83.0	0.65	N.S.	83.0
ME/DE (2)					98.0

(1) Standard error (S.E.) of a mean

(2) Ratio of metabolisable energy to apparent digestible energy.

*** $p < 0.005$; N.S.= non significant ($p > 0.05$).

Henry and Boudon (1971) report ADE coefficients of 82.0 and 81.7% for normal and opaque-2 maize diets respectively. The values in the present experiment were higher for pigs of a similar weight and fed similar quantities of feed. Their gross energy values of 4366 and 4395 kcal/kg DM were comparable to the overall value in the present experiment of 4360 kcal/kg DM in diets containing meat and bone meal. Jordan (1971) reports ADE and ME values for normal maize as 88.4 and 85.6% of gross energy, giving an ME/DE ratio of 0.97, similar to that found in the present experiment. May and Bell (1971) conclude that the ratio ME/DE in pig rations containing normal levels of protein is 0.98. McConnell et al. (1972) give DE values of approximately 86% for 40 kg pigs on maize/soybean diets. There was a trend for DM and DE to improve in trials at 70 and 95 kg.

5.2.4 Nitrogen Balance

The data collected relating to N metabolism are presented in Table A5.4. Mean squares resulting from the analysis of variance for nitrogen intake, apparent digestible N, and N retention are shown in Table A5.9.

In Table 5.2.4.1 are summarised the balance means for the 5 indices associated with the N balance. The large and highly significant difference in N intake was expected due to the increased intake of feed for Balance B.

The balance period effect for apparent digestible N(%) failed to reach significance and the mean values shown in Table 5.2.4.1 indicate that the overall value was 84%, with a low SE for each balance mean of $\pm$ 0.6.

TABLE 5.2.4.1 Mean values within balances for nitrogen balance information in Experiment II.

	Balance A	Balance B	±S.E.(1)	Sig.	Experimental Mean
NI (g/4days)(2)	97	134	1.6	***	
ADN (%)	84	84	0.6	N.S.	84
NR (g/4 days)(4)	44	53	2.0	*	49
NR (%ADN)	53	46	1.9	+	50
NR (g/kg/4 days)	1.38	1.21	0.05	+	1.30

(1) Standard error of mean
(2) NI = nitrogen intake
(3) ADN = apparent digestible nitrogen
(4) NR = nitrogen retention

*** $p < 0.005$, * $p < 0.05$, + $p < 0.10$, N.S. $p > 0.10$.

Some results have indicated that the opaque-2 mutation results in the grain protein being more digestible for the pig (Cromwell et al., 1969; Sihombing et al., 1969; Marroquin et al., 1970; Shimanda and Martinez, 1970; Henry and Boudon, 1971). Cromwell et al. (1969) suggest this may be due to the lower zein content of the mutant (Section 3.1.1), which has been shown to be poorly digested in the pig. In maize diets containing no protein supplementation, they report absorption coefficients for normal and opaque-2 maize or 77.07 and 82.31 with 31 - 55 kg barrow pigs. When the %ADN results are averaged separately irrespective of balance period for diets 9-12 (normal maize) and 13-16 (opaque-2 maize), the results are 83 and 85% respectively, suggesting a similar difference to those indicated above. Marroquin et al. (1970) reported apparent digestible protein values for normal and opaque-2 maize/soybean diets of 78.5 and 82.3% respectively. Similar values of 62.3-75.4 and 68.9-76.9 have been reported for diets of the same basic composition (Sihombing et al., 1969). Therefore it appears that the

present experimental diets tended to give higher values for digestible N than those reported in the literature for maize-baised diets. This may have been due to a combination of the presence of meat and bone meal as the protein supplement, the relatively low level of total protein in the diet and also the restricted feed intake.

The balance mean square from NR (g/4 days) was significant ($p < 0.05$) in the analysis of variance, and Table 5.2.4.1 shows that this criteria increased from 44 to 53 g/4 days from balance A to B over all treatments. In Section 2.3.6, several reports on the N deposition of the growing pig as related to increases in live weight were reviewed. Although the evidence is conflicting, it was concluded that when allowed maximum expression, the absolute levels of N deposition probably remain approximately constant over much of the growing period for modern strains of pigs. The results of recent studies on N retention would suggest that the potential of the growing pig to retain N is approximately 20 g/day (Poppe and Wiesemuller, 1968; McConnell *et al.*, 1971), for the period 40-90 kg. Average NR values of 11 and 13.3 g/day for balances A and B suggest that the treatments were grossly deficient in the supply of essential amino acids for maximum protein synthesis at the feeding level used, The low retention values are unlikely to have resulted from a low genetic capability for protein deposition (Henning and Kleeman, 1967; McConnell *et al.*, 1971). because of the breeding of the pigs used in these experiments. The increased feed intake during the second balance period, with the increased supply of the limiting amino acid, allowed a greater absolute NR. This occurred even though it may be assumed that a greater fraction of the absorbed amino acid N would be used in the repair and maintenance of the larger total muscle mass.

In low protein diets, McConnell *et al.* (1971) presented results which indicated that absolute NR increased in the body weight range 40-70 kg. A similar trend is indicated from the results of Robinson *et al.*, (1964a) with

14% CP and low energy 20% CP diets.

However it should also be pointed out that the potential for over-estimating apparent NR is increased with an increasing volume of material handled in the balance experiment. Duncan (1966) considers the greater accuracy with smaller animals is probably due to apparatus allowing more complete collection of excreta and the samples for analysis represent a greater proportion of the total. There is also the possibility of an increased loss of N from the excreta before sampling because of the greater quantity of N involved. Since the errors involved in estimating NR are compounded by the method of calculation (Allison and Bird, 1964; Waterlow et al., 1960), the greater amounts of feed offered to larger animals and the increased possibility of losses from excreta will tend to magnify errors.

Although the absolute amount of N deposited with increasing live weight significantly increased, the efficiency with which absorbed N was utilised (% NR) tended to decrease, as shown in Table 5.2.4.1. The mean square for the balance effect failed to reach significance at the 5% level ($p < 0.10$, Table A5.9), but the results support the well established fact that the growing pig shows a reduced efficiency in utilising dietary protein with increasing body weight (Rerat, 1972). An increasing proportion of the absorbed N is utilised for purposes other than protein anabolism and ultimately excreted in the urine.

When NR was expressed on a unit live weight basis (g/kg/4 days), the balance mean square failed to reach significance at the 5% level ($p < 0.10$). However Table 5.2.4.1 shows that the trend was for a decreased retention of N on a unit weight basis with increasing live weight. This indicates that as the animal increases in live weight, the proportion of the weight gain which constitutes protein is decreasing. Rerat (1972) reviewed published work which used several criteria to indicate that the ratio of muscle to fat decreases rapidly during the growing-finishing phase of the pig's development.

On examining the analyses of variance with reference to the mean squares due to treatment, the significant treatment mean square for N intake was unexpected. The partitioning of the treatment sums of squares showed that this was due principally to the higher N content of the opaque-2 maize (Table 4.1.2.1.1), although lysine supplementation also appeared to have an effect. There is now a considerable body of evidence to show that N intake influences the results of biological assays for protein quality (Section 2.3.10). Although the influence of the level of intake is more pronounced with lower quality proteins, it was assumed that the increased N content of the opaque-2 maize diets (Table A5.5), was unlikely to be confounding factor to the effects of amino acid balance in the present experiment.

The results of % NR are presented in more detail in Table 5.2.4.2, because of the relevance of the measure as an indicator of protein quality.

The code for the ranked treatment means indicates a trend for the combination of lysine and tryptophan to produce the highest proportion of N retained. This criteria can be viewed as representing apparent biological value (Crampton and Harris, 1969). Ideally the diets should all have had the same crude protein content since this has an important effect upon biological value (Henry, 1965). A closer control of protein intake could have been possible in the present experiments, with the treatments mean square for N intake significant (Table A5.9). The range of N intakes was 91-106 and 104-149 g/4 days for balances A and B respectively.

The balance means over all treatments of 52.9 and 46.8 compare favourably with other published results for the BV of maize protein for the growing pig of 49-61 (Armstrong and Mitchell, 1955) and using the rat 55-62.8 (FAO, 1970).

TABLE 5.2.4.2 Treatment and balance means for nitrogen retention (%ADN) with standard errors for a split-plot experiment with missing data (Cochran and Cox, 1950). (1)

Treatment No.	Code	Balance		Treatment Mean
		A	B	
12	mlt	58.5	56.0	57.3
14	plt	56.5	54.5	55.5
11	m	56.5	47.5	52.0
15	pml	59.0	42.5	50.8
16	pmt	53.5	45.5	49.5
9	l	48.0	45.0	46.5
13	p	46.5	45.5	46.0
10	t	44.5	37.5	41.0
Balance Mean		52.9	46.8	
Overall Treatment Mean				49.8

In normal maize diets containing 15% soybean meal, McConnell et al. (1971) report apparent BV figures of 54.8 and 50.9 for pigs approximately 40 kg, being influenced by the strain of pig used. With pigs averaging approximately 31 kg and using opaque-2 and normal maize with no protein supplementation, Cromwell et al. (1969) indicated % NR values of 49.8 and 35.3 respectively when an equivalent amount of protein was supplied by each maize. Henru and Boudon (1971), in

(1) Standard error (S.E.) of the difference between two treatment means ± 4.04 (± 5.51 if the comparison includes treatment 16).
S.E. of the difference between the two balance means ± 2.82.
S.E. of the difference between two treatment means in the same balance period ± 6.67 (± 10.04 if the comparison involves treatment 16).
S.E. of the difference between two balance means with the same treatment ± 7.51 (± 7.96 if the comparison involves treatment 16).

digestibility trials with male castrates (initial weight 30.1 kg), report values of 53.3 and 59.5% NR for diets containing normal and opaque-2 maize respectively. Under the conditions of the present experiment, the BV's were very similar for the two maizes, although the design did not allow an accurate or sensitive test for the maize effect alone. The values were 51.9, 53.8.and 46.5, 47.0 for normal and opaque-2 maize in balance periods A and B respectively. There was a trend for the opaque-2 to be superior and a slight indication that the difference was greater at the lighter weight. As indicated previously for NR (g/4 days), there was no significant reduction in % NR with the diets producing severe imbalance effects in Experiment I. However, working with children and dogs, Bressani (1962, 1969) has been able to demonstrate decreases in NR through amino acid supplementation producing imbalances.

The treatment mean squares for NR (g/4 days) was significant ($p < 0.05$) and Table A5.9 shows the partitioned TSS. The two main effects P and L were significant ($p < 0.05$), and the second order interaction PM was significant at the 10% level. The significant P (presence of opaque-2 maize) effect, (confounded with -MLT), suggested that diets containing opaque-2 maize supported greater absolute NR, and this is in agreement with the significant maize effect in the treatment sums of squares for ADG and FCR seen in Experiment I. Table 5.2.4.3 gives the ranked treatment means for NR (g/4 days) together with lines expressing the results of a Duncan Multiple Range Test.

TABLE 5.2.4.3 Ranked treatment means for nitrogen retention (g/4 days) over both balance periods.

Treatment No.:	14	12	15	16	13	11	9	10
Code :	plt	mlt	pml	pmt	p	m	l	t
Mean (1) :	59.5	55.8	53.3	49.5	47.3	43.5	40.5	37.3

(1) Those means not underscored by the same line differ significantly ($p < 0.05$).

Relating to the positioning of the treatments, it may be noted that within treatments for NR (g/4 days) there was often great variability which was not always related to gross NI. The removal of one value and recalculation of the mean with three observations often changed the position of a treatment by one or more placings. This illustrates the susceptibility of a low number of replications to individual variability, as is common in N balance work. However several treatments were significantly different in the multiple comparison. The combination of lysine and tryptophan with methionine in normal maize diets produced a significant increase in NR above the addition of lysine or tryptophan alone. This is in support of the significant lysine by tryptophan interaction reported for ADG and FCR in Experiment I, suggesting the response in growth was due to an increased utilisation of dietary protein in the presence of additional lysine and tryptophan. The addition of these two amino acids to opaque-2 maize gave the highest absolute values recorded, but this was not significantly different from the unsupllemented diet of this maize type. The significant maize effect in the partitioned treatment sums of squares is seen in 4 out of the top 5 placings being diets containing the mutant. This is in support of published work showing that the substitution of opaque-2 for normal maize produced a rise in absolute NR (Henry and Boudon, 1972; Cromwell *et al.*, 1969).

Vermorel and Keller (1967) suggest the leucine/isoleucine ratio in maize grain protein could depress NR, particularly where the proportion of dispensable amino acids and the general N content of the diet is low; the latter applying in the experimental diets used here. Extensive work has been conducted with rats in examining a possible leucine/isoleucine antagonism in maize protein, with some positive evidence (De Muelenaere et al., 1967b; Spolter and Harper, 1961), while other results have been inconclusive (Rosenberg et al., 1960). The ratio of leucine/isoleucine in the present experiments was 1:3.3 and 1:2.7 for diets containing normal and opaque-2 maize respectively (Table 4.1.2.4.2). There remains the possibility that some of the superiority of the mutant, and differences to response to some combinations of synthetic amino acids, may have been due to the change in this ratio.

Higher mean NR values for normal maize supplemented with lysine and methionine alone compared with tryptophan alone was unexpected on the basis of results from Experiment I, although the differences were non-significant. A much lower rate of refusals for treatments 9 and 11 in Experiment II and the possible reasons for this have already been discussed (Section 5.2.2). These results for absolute NR would suggest that if levels of feed intake are similar, the animal is able to utilise the amino acids in these imbalanced diets for protein synthesis to a similar extent as when fed a more balanced spectrum of amino acids. However, only trends are indicated here and experiments allowing a more sensitive evaluation of the effects of amino acid imbalance and feed intake upon N metabolism would be required to collect more definitive information.

When NR was expressed on a unit live weight basis (g/kg/4 days), there was a significant ($p < 0.05$) effect due to treatment. The partitioned treatment sums of squares for NR (g/kg/4 days) is shown in Table A5.9, with the P and L main effects reaching significance ($p < 0.05$) and the

first - order interaction PM significant at the 10% level. The ranked treatment means are shown in Table 5.2.4.4, together with the results of the Duncan Multiple Range Test.

TABLE 5.2.4.4 Ranked treatment means for nitrogen retention (g/kg/4 days).

Treatment No.:	14	12	15	16	13	11	9	10
Code :	plt	mlt	pml	pmt	p	m	l	t
Mean (1) :	1.56	1.50	1.45	1.33	1.25	1.16	1.10	1.02

(1) Those means not underscored by the same line are significantly different ($p < 0.05$).

The order of superiority with respect to NR on a unit live weight basis is the same as for absolute NR (Table 5.2.4.2). The multiple treatment comparison also gave similar conclusions for significant differences between treatments.

There was no suggestion of a treatment by balance interaction for any of the criteria studied apart from live weight (which was due to the expected very large mean square to balance). However the experimental design did not allow for a sensitive test of the treatment by balance interaction.

5.3 SOME COMPARISONS BETWEEN THE RESULTS OF EXPERIMENTS I AND II

A considerable difference in the extent of feed refusals has been discussed in Section 5.2.2. Average daily gains (ADG) and nitrogen retention values for animals from Experiment II were subsequently compared with the growth data from Experiment I. However, in drawing these comparisons, the possibility of error due to some factors not being common to the two experiments was appreciated. Those which may be important have been discussed in Section 5.2.2.

5.3.1 A Comparison of Live Weight Gains

Animals in Experiment II were introduced to the experimental diets at the same live weight as in Experiment I and were fed to the same scale. Table A5.10 includes the live weights according to treatment and ADG calculated by linear regression for pigs in Experiment II. For the same diets, average daily gains with barrows and gilts plus barrows from Experiment I are also shown and compared with the growth rates recorded in Experiment II.

From Table A5.10 it can be seen that the growth performances on the respective treatments were very similar for the two experiments. Correlation coefficients of the mean ADG for each treatment in Experiment II with the mean for the Barrows only ($r = 0.95$) and gilts plus barrows ($r = 0.90$) in Experiment I were both highly significant ($p < 0.01$). However there was a trend for animals in Experiment II to show superior growth rates. The conflicting published information on this aspect of balance studies has been reviewed in Section 3.4. With little apparent advantage from considering barrows only, the treatment means for the 4 observations in Experiment I were used in subsequent calculations. However there were some interesting observations for treatments 9 and 11. Excluding the results for the gilts on treatment 9 substantially improved this treatment mean in Experiment I and reduced the difference in growth rate between the two experiments from 25 to 11%. This was probably the principal cause for the superior correlation coefficient using barrows only in the comparison. The mean for treatment 11 was slightly improved by excluding the gilts, but this increased the difference between the experiments for this treatment. The increase in mean ADG from excluding the gilts for treatments 9 and 11 would suggest that perhaps the gilts reacted more strongly to the dietary imbalance of amino acids. The 4 highest refusal totals recorded in Experiment I were for gilts, as shown in Table 5.1.2.1 (treatments 3 and 9). However, no clear trend for a differential response between the sexes

was seen for the remaining treatments, and the design of the experiment did not allow a sensitive test for the effects of sex.

From a review of the literature, it was concluded in Section 2.3.8 that gilts are more sensitive to changes in dietary protein quality and quantity. Bayley and Summer (1968) consider that this limits the usefulness of using only barrows in NR studies. There appears to be no published work conducted looking specifically at the influence of sex on the response to dietary amino acid balance in the growing pig and this remains a possible field for future study.

5.3.2 A Comparison of Growth Rates from Experiment I for the Group II diets (treatments 9-16) with Nitrogen Retention Information from Experiment II

To examine a possible relationship between growth rate and absolute nitrogen retention (NR), the growth period for Experiment I was divided into two sub-periods, with the mean live weights corresponding approximately to those for balances A and B in Experiment II. For sub-period A the weekly live weights greater than 23 kg but less than 36.5 kg were used and for sub-period B, live weights greater than 36.5 kg. The resulting regression coefficients, together with the NR data, are listed in Table A5.11. In only 6 cases was the residual mean square not significantly reduced by calculating a two coefficient model ($p < 0.05$). Two individuals on treatment 9 and one on treatment 11 showed a lower ADG during the second sub-period, although in two cases the difference in the residual mean square from a single regression coefficient was no significant. However this indicates that for these animals, the feed refusals for sub-period B were a greater fraction of the total feed offered, so that feed intake relative to body weight declined.

A regression was calculated of the mean ADG for treatments 9-11 for each sub-period in Experiment I on the

corresponding response as NR (g/day) in Experiment II. When all the treatments were considered, the regression coefficients were not significant ($p > 0.05$). However, when the values for treatments 9 and 11 were excluded, the regression coefficients achieved significance at the 5% level (Table A5.11). The two regression lines for mean live weights of approximately 31 (Balance A, sub-period A) and 44 kg (Balance B, sub-period B) are shown in Figure 5.3.2.1. The respective equations are as follows:

Balance A (31.4 kg) $Y = 268.40 + 9.45X$ ($S.E._b = \pm 2.41$)

Balance B (43.8 kg) $Y = 378.51 + 12.97X$ ($S.E._b = \pm 3.25$)

where, Y = ADG (g/day)

X = NR (g/day)

$S.E._b$ = Standard error of the regression coefficient.

The two regression coefficients were not significantly different from each other ($p > 0.05$) in terms of residual variances and slope, the pooled regression coefficient being 11.86 with S.E. $\pm$ 1.24

These results are evidence of a significant positive relationship between the ability of the animal to retain dietary N and the observed growth rate expressed as ADG using these experimental diets. The position of the values for treatments 9 and 11 are included in Figure 5.3.2.1 with treatment 9 giving the lower ADG in each case. In relation to growth, a much higher rate of NR occurred on these "imbalanced" diets than could be anticipated from the significant relationship with the other treatments. In comparing the growth rate data from the two experiments (Section 5.3.1), it was apparent that much higher weight gains resulted for treatment 9 and for one animal on treatment 11 in Experiment II, despite a significant correlation between the two sets of results. This was due to a higher voluntary feed intake (Section 5.2.2). Also NR (g/4 days)

on these two diets was not significantly different from that on three other treatments (Table 5.2.4.3).

The good relationship between growth and N retention in the growing pig in these experiments, apart from the imbalanced diets, supports the conclusions of several studies using growing pigs with these two criteria (Ostrowski, 1969; Henry and Boudon, 1971; Cromwell *et al.*, 1969; Jones *et al.*, 1962). No carcass quality information was collected in the present experiments, but the results of other comparative studies have shown a good relationship between estimated NR and the lean/fat ratio in the carcass (Cunningham *et al.*, 1962b; Jones *et al.*, 1962; Cunningham and Friend, 1966; Cooke et al., 1966; Jung and Piatkowski, 1967; McConnell *et al.*, 1971, 1972). However, Braude *et al.* (1968) found evidence for an increase in carcass lean which was not reflected in a change in total NR. Also, the results of Blair *et al.* (1968a) and Madsen and Matensen (1964) indicate that supplementation with lysine, although significantly increasing live weight gain and feed conversion efficiency, had no significant effect on the rate and efficiency of lean meat gain. However the trend was for both these criteria to improve with higher lysine levels and the use of only two replications per treatment at each slaughter weight would demand wide differences to reach significance. Their observations suggest however that it may be more difficult to observe changes in NR or lean meat gain due to smaller effects from changes in protein quality than for growth rate. This, coupled with the lower replication usually unavoidable in the balance technique, would make significant differences by this method difficult to detect.

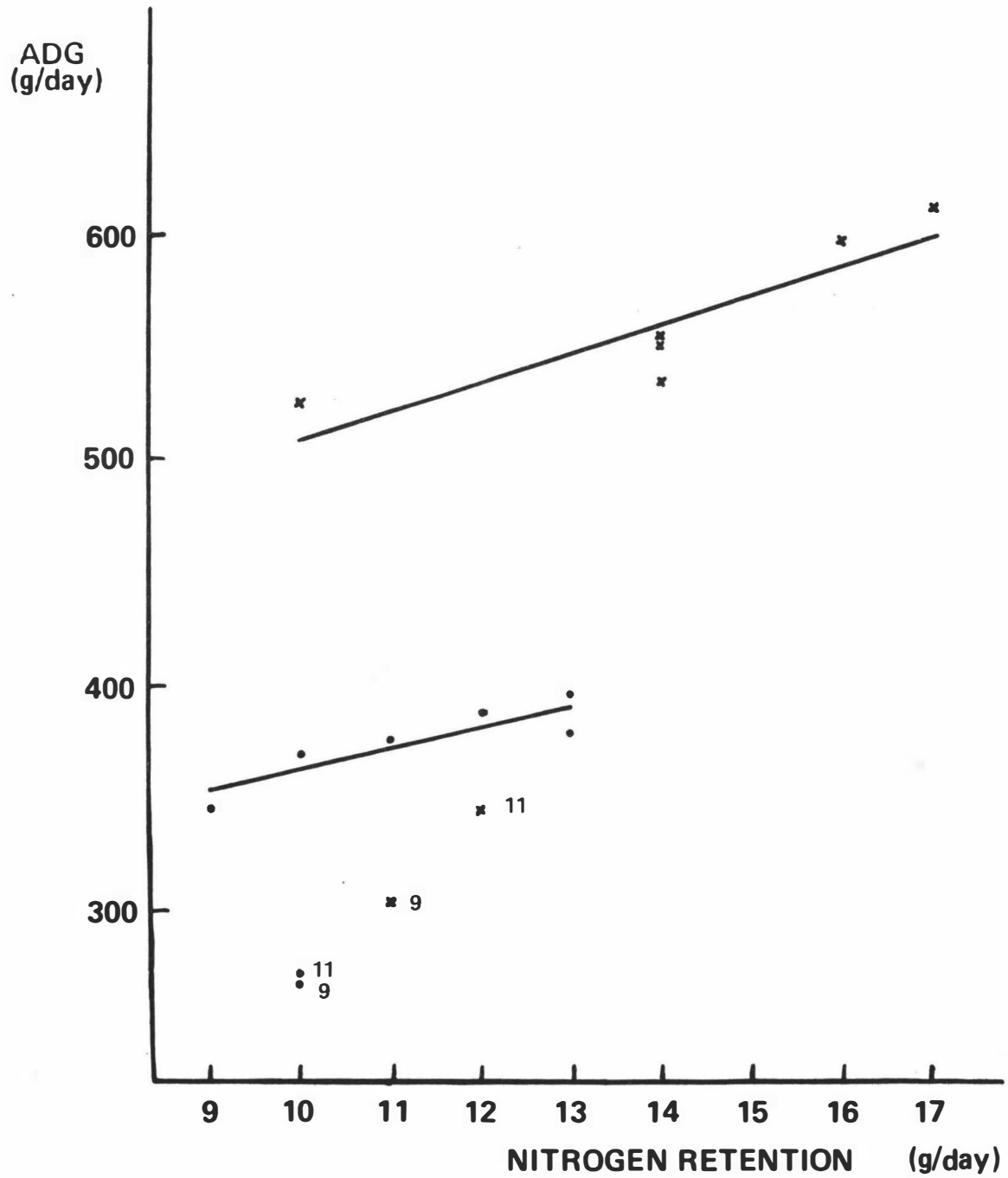

FIG. 5.3.2.1 Regression of ADG (Experiment I) on Nitrogen Retention (Experiment II) for Balance A(·) and Balance B (x), with treatments 9 and 11 excluded.

CHAPTER 6 : SUMMARY AND CONCLUSIONS

6.1 GENERAL PERFORMANCE

The feeding scale used in Experiments I and II was based upon a table presented by ARC (1967). Adjusted to 85% of that scale on a weight basis, the daily intake of ADE was 95% of the figures quoted, due to the higher ADE value of the diets used in these experiments (3.3 mcal DE/kg). The ARC reviewers present expected daily live weight gains and over the 25-50 kg period, 95% of the ADG was calculated as 662 g/day. The general mean for ADG in Experiment I was 415 g/day, suggesting that the basal diet of 90% maize/10% meat and bone meal was inadequate for supporting near-optimum growth rates for growing pigs.

Average absolute NR values of 11 and 13.3 g/day also indicated that the supply of essential amino acids was only allowing approximately 50% of the potential rate of N deposition in pigs of the weight range used in these experiments. These conclusions are supported by the calculated levels of amino acids both on a dry matter basis (Table 4.1.2.4.2) and per mcal of ADE (Table 4.1.2.4.3), where the limiting amino acid in any dietary combination is supplying only approximately 50-60% of the estimated requirement (ARC, 1967; Rerat and Lougnon, 1968).

6.2 OPAQUE-2 MAIZE

The substitution of opaque-2 maize for normal maize produced superior ADG and FCR irrespective of the type of amino acid supplementation. This produced a significant main effect due to opaque-2 maize (P) in the partitioned TSS for these criteria. Treatment means for the two maizes were significantly different ($p < 0.05$) with no amino acid supplementation, lysine alone, methionine alone and lysine plus methionine. Although only one feeding level and

protein supplement was used, and the diets were relatively low in total crude protein, these results also suggest that lower protein and/or amino acid supplementation would be required for equivalent performance to be obtained in opaque-2 as opposed to normal maize diets.

The N balance studies supported observations made in Experiment I: in the analyses of variance, the maize effect was significant for NR (g/4 days, g/kg/4 days).

6.3 AMINO ACID IMBALANCE

An imbalance effect, characterised by a reduction in voluntary feed intake and growth rate, was induced in normal maize/meat and bone meal diets by the addition of lysine (0.37%) and methionine (030%), either alone or in combination in Experiment I. Feed conversion efficiency was only significantly depressed with the lysine plus methionine conbinations. Lysine supplementation of opaque-2 maize diets non-significantly depressed growth and feed conversion efficiency, while the addition of methionine or methionine plus lysine had no effect. It was concluded that diets containing opaque-2 maize are less likely to induce imbalance effects with amino acid supplementation, probably due to the higher levels of the limiting amino acids in the grain protein of the mutant.

Although not examined statistically, there was the suggestion from a consideration of individual feed refusal totals and growth rate data that gilts may be more sensitive to dietary amino acid imbalance than castrates.

6.4 LIMITING AMINO ACIDS

The main effect for tryptophan in the analyses of variance for ADG and FCR was highly significant ($p < 0.01$). Also highly significant for these two criteria was the lysine by tryptophan interaction: the response to these two amino acids together being greater than to either alone

for the two maizes. The addition of lysine plus tryptophan to normal maize increased ADG and FCR so that performance was superior but not significantly different from an unsupplemented opaque-2 maize diet. It was therefore concluded that higher levels of these two amino acids in the mutant maize grain was responsible for its superiority in maize/meat and bone meal diets.

The addition of methionine to diets containing supplemental lysine and tryptophan was without effect for normal maize but produced a non-significant increase in ADG and FCR for diets containing opaque-2 maize. The four highest treatment means for ADG and FCR were the normal and opaque-2 maizes supplemented with the lysine plus tryptophan or the lysine plus tryptophan plus methionine combinations.

It was concluded that tryptophan was the first limiting amino, followed by lysine, for normal and opaque-2/meat and bone meal diets. The results suggested that methionine may be third limiting in the opaque-2 maize diets, but that some other amino acid is third limiting in normal maize/meat and bone meal diets.

These conclusions were supported in part by the N balance studies: the two treatments of normal maize plus lysine, methionine and tryptophan and opaque-2 maize plus lysine and tryptophan producing the highest values for absolute NR (g/4 days), % NR, and NR g/kg/4 days.

Since many studies with rats and pigs have shown that tryptophan and lysine are the two essential amino acids limiting the utilisation of maize grain protein, it was concluded that the addition of meat and bone meal at the level used in these experiments did not alter this situation for the two protein sources combined. However although the evidence for the first limiting amino acid in maize protein is conflicting, the relatively low level

of tryptophan in the meat and bone meal supplement results in this amino acid being clearly limiting in dietary combinations of these two feedstuffs.

6.5 NUTRIENT DIGESTIBILITY

In Experiment II, where half the dietary treatments used in Experiment I were examined, there was no significant effect ($p < 0.05$) of treatment diet or balance period upon ADDM (85%), ADE (85%), ME (83%), or ADN (84%).

6.6 NITROGEN METABOLISM

Treatment and balance effects were significant ($p < 0.05$) for absolute NR (g/4 days) and NR (g/kg/4 days), but only at 10% for the balance effect of the latter. In the partitioned TSS, the maize and lysine main effects were significant ($p < 0.05$) and the maize by methionine interaction significant at the 10% level for these two criteria. However, confounded effects do not allow accurate interpretation of these results. Absolute NR (g/4 days) significantly ($p < 0.05$) increased, but NR as related to live weight (g/kg/4 days) non-significantly ($p < 0.10$) decreased, from Balance A to B.

The treatment and balance mean squares for %NR were significant at the 10% level, with a trend for this criteria (apparent biological value) to decrease at the second balance and to improve with the use of opaque-2 maize and/or the addition of lysine plus tryptophan.

6.7 MANAGEMENT AND THE EFFECTS OF AMINO ACID IMBALANCE

In contrast to Experiment I, there were no significant feed refusals recorded for individuals in Experiment II. It was concluded that differences in management between the two experiments, and particularly with regard to the time animals were allowed access to feed was responsible.

The higher intakes of the "imbalanced" diets in Experiment II appeared to produce no ill effects and supported NR values similar to several of the remaining treatments examined in Experiment II.

6.8 GROWTH RATE FOR GROWING PIGS PENNED AS INDIVIDUALS AND IN GROUPS

There was a highly significant correlation between the average daily gains for Experiments I and II, both when barrows only and gilts plus barrows from Experiment I were used in the comparison. However there was a trend for growth rates to be superior in Experiment II by 1 to 25%, the largest improvement being for treatment 9 which induced severe imbalance effects in Experiment I.

6.9 THE RELATIONSHIP BETWEEN LIVE WEIGHT GAIN AND NITROGEN RETENTION

A regression of ADG (g/day) for Experiment I on NR (g/day) from Experiment II for treatments common to both was significant ($p < 0.05$) only when the data for treatments 9 and 11 were excluded. It was concluded that the positive response in terms of ADG to amino acid supplementation in Experiment I was due to an improved utilisation of dietary protein, leading to a greater deposition of body protein.

TABLE A1.1 Maize grain production in New Zealand (taken from the Monthly Abstract of Statistics, Sept. 1972; Dept. of Statistics Wellington, N.Z.).

Date[1]	Acres (,000)	Yield (,000 bushells)
1959 - 60	8.3	703
1960 - 61	6.6	404
1961 - 62	7.3	544
1962 - 63	8.0	609
1963 - 64	9.6	744
1964 - 65	9.7	927
1965 - 66	8.1	762
1966 - 67	7.6	728
1967 - 68	14.5	1413
1968 - 69	17.6	2014
1969 - 70	20.0	2308
1970 - 71 (2)	23.2	

(1) Relates to holdings of 10 acres and over.

(2) Estimated.

TABLE A3.1a Published values for the crude protein and essential amino acid contents (per cent air dry weight) of normal hybrid maize, along with respective data for the sample used in Experiments I and II.

Crude Protein	Tryptophan	Lysine	Methionine	Cystine(a)	Meth. + Cys.	Threonine	Isoleuc.	Leucine	Phenylal.	Tyrosine(a)	Phen. + Tyr.	Arginine	Valine	Histidine	Reference
9.6	0.11	0.27	0.20	0.19	0.39	0.48	0.38	1.10	0.45	0.39	0.84	0.57	0.73	0.22	McDonald et al., 1966
8.4	(b)	0.22	0.14			0.29	0.19	0.94	0.36	0.28	0.64	0.34	0.28	0.19	Crampton & Harris, 1969
9.0	0.09	0.21			0.34										Massey University, unpub.
9.0	0.06	0.25	0.17	0.12	0.29	0.31	0.28	0.98	0.40	0.35	0.75	0.40	0.40	0.22	Harmon et al., 1969
9.0[c]	0.05	0.26	0.17	0.12	0.29	0.36	0.41	1.17	0.32	0.55	0.87	0.32	0.46	0.23	Orr & Watt, 1957
9.1	0.09	0.24	0.15	0.14	0.29	0.32	0.31	1.10	0.45	0.39	0.84	0.46	0.42	0.27	Cromwell et al., 1967
8.6		0.30	0.15	0.13	0.28	0.33	0.27	1.10	0.43	0.40	0.83	0.46	0.43	0.27	Cromwell et al., 1968
8.9	0.10	0.38	0.14	0.14	0.28	0.29	0.34	1.02	0.41	0.27	0.68	0.52	0.47	0.34	Drews et al., 1969
9.5	0.07	0.25	0.18	0.15	0.33	0.34	0.35	1.19	0.46	0.36	0.82	0.40	0.46	0.26	FAO, 1970
9.6	0.07	0.28	0.12	0.16	0.28	0.40	0.40	1.41	0.56	0.50	1.06	0.47	0.55	0.25	Gipp & Cline, 1972
8.5	0.01[d]	0.28	0.07			0.24	0.25	0.84	0.48			0.45	0.33	0.26	)
8.6	0.01	0.31	0.12			0.39	0.31	0.90	0.42			0.50	0.52	0.26	) Hesby et al., 1972
9.0	0.06	0.23	0.15			0.29	0.25	0.95	0.37			0.38	0.35	0.21	Jensen et al., 1969
8.9	0.09	0.27	0.17	0.11	0.28	0.34	0.27	1.06	0.44			0.45	0.45	0.27	)
8.2	0.09	0.25	0.15	0.14	0.29	0.33	0.25	0.74				0.45	0.39	0.25	) Klein et al., 1971(f)
9.6	0.07	0.28	0.12	0.16	0.28	0.40	0.40	1.41	0.56			0.47	0.55	0.25	Klein et al., 1971
9.3	0.08	0.29	0.21			0.34	0.36	1.12	0.41			0.45	0.50	0.24	Lyman et al., 1956
10.0	0.06	0.28	0.30	0.19	0.49	0.35	0.50	1.61	0.54	0.49	1.03	0.46	0.72	0.29	Maner et al., 1971
10.5		0.29	0.21	0.13	0.34	0.39	0.42	1.46	0.56	0.42	0.98	0.50	0.53	0.32	Mertz et al., 1965
8.0	0.06[e]	0.23	0.12	0.12	0.24	0.28	0.26	1.02	0.34	0.26	0.60	0.30	0.32	0.19	Sample of consignment used in Experiments I and II

(a) Cystine and tyrosine are not essential for the pig, but are included here because the level of intake of these two amino acids influences the dietary requirement for methionine and phenylalanine respectively.
(b) A blank space denotes value not given.
(c) Assumed for purpose of calculation.
(d) Analytical method not given.
(e) Analyzed by the method of Spies, 1968.
(f) Microbiological assay.

TABLE A3.1b Published values for the crude protein and essential amino acid contents (per cent air dry weight) of Opaque-2 maize, along with respective data for the sample used in Experiments I and II.

Crude Protein	Tryptophan	Lysine	Methionine	Cystine[a]	Meth. + Cystine	Threonine	Isoleuc.	Leucine	Phenylal.	Tyrosine[a]	Phen. + Tyr.	Arginine	Valine	Histidine	Reference
11.6	0.15	0.49	0.16	0.20	0.36	0.38	0.37	0.97	0.51	0.45	0.96	0.79	0.57	0.40	Cromwell et al., 1967
11.5	(b)	0.54	0.14	0.17	0.28	0.47	0.38	1.00	0.51	0.46	0.97	0.76	0.58	0.36	Cromwell et al., 1968
10.4	0.10	0.51	0.13	0.20	0.33	0.33	0.36	0.94	0.42	0.29	0.71	0.73	0.56	0.46	Drews et al., 1969
10.0	0.01[c]	0.50	0.03			0.34	0.30	0.71	0.38			0.73	0.34	0.35	)
10.1	0.01	0.43	0.09			0.48	0.34	0.90	0.47			0.73	0.50	0.32	) Hesby et al., 1972
9.5	0.12	0.35	0.18			0.37	0.32	0.85	0.38			0.56	0.51	0.27	Jensen et al., 1969
11.7	0.15	0.57	0.11	0.18	0.29	0.45	0.34	0.97	0.61			0.77	0.56	0.36	)
11.7	0.15	0.40	0.15	0.18	0.33	0.41	0.33	0.91				0.66	0.48	0.32	) Klein et al., 1971[e]
11.8	0.17	0.50	0.20	0.09	0.29	0.48	0.43	1.12	0.55			0.71	0.63	0.37	)
11.0	0.15	0.53	0.23	0.09	0.32	0.48	0.44	1.11	0.55			0.81	0.65	0.40	)
10.3	0.10	0.41	0.46	0.19	0.65	0.34	0.37	0.82	0.39	0.36	0.75	0.64	0.58	0.28	Maner et al., 1971
10.6		0.50	0.20	0.15	0.35	0.41	0.40	1.04	0.51	0.38	0.89	0.69	0.58	0.32	Mertz et al., 1965
10.1	0.11	0.52	0.14	0.14	0.28	0.40	0.38	0.93	0.46	0.26		0.65	0.60	0.32	)
10.4	0.10	0.47	0.13	0.15	0.28	0.38	0.35	0.91	0.43	0.22		0.56	0.57	0.32	) Oestemer et al., 1970
10.1	0.10	0.46	0.09	0.14	0.23	0.36	0.34	0.83	0.39	0.24	0.63	0.60	0.54	0.32	)
9.0	0.11[d]	0.30	0.12	0.14	0.26	0.36	0.28	0.85	0.43	0.32	0.75	0.39	0.45	0.18	Analysis of sample from consignment used in Experiments I and II.

(a) Cystine and tyrosine are not essential for the pig, but are included here because the level of intake of these two amino acids influences the dietary requirement for methionine and phenylalanine respectively.
(b) A blank space denotes value not given.
(c) Analytical method not given.
(d) Analyzed by the method of Spies, 1968.
(e) Microbiological assay.

TABLE A3.2 Published values for the crude protein and essential amino acid content (per cent air dry weight) of meat and bone meal, along with respective data for the sample used in Experiment I and II.

Crude Protein	Tryptophan	Lysine	Methionine	Cystine[a]	Meth. + Cystine	Threonine	Isoleucine	Leucine	Phenylal.	Tyrosine[a]	Phenyl. + Tyrosine	Arginine	Valine	Histidine	Reference
50.0	0.35	2.81	0.68	(b)	0.68	1.70	1.74	3.22	1.75	1.22	2.97	3.74	2.45	0.90	McDonald et al., 1971
50.6	0.20	3.50	0.70	0.60	1.30	1.80	1.70	3.10	1.80			4.00	2.40	0.90	Crampton & Harris,1969
44.4	0.19	2.10		1.10											)
54.0		3.00	0.70												)Massey University,
53.7		2.60	0.80												) unpublished.
	0.55	3.28	0.89	0.49	1.38	1.93	1.79	3.76	2.13	1.40	3.53	4.33	2.78	1.12	FAO, 1970
45.0	0.18	2.20	0.53	0.26	0.79	1.80	1.70		1.80			2.70	2.40	1.50	)
50.0	0.26	2.60	0.67	0.33	1.00	1.63	1.28		1.70			3.35	2.25	0.96	)Allen, 1972
45.2	0.27	2.81	0.53	0.51	1.04	1.51	1.05	2.50	1.35	0.86	2.21	3.34	1.61	1.04	Payne et al., 1972
44.3	0.24	2.42	0.41	0.28	0.69	1.37	1.22	2.67	1.49	1.04	2.53	2.85	1.80	0.75	Analysis of samples from the consignment used in Experiments I and II

(a) Cystine and tyrosine are not essential for the pig, but are included here because the level of intake of these two amino acids influences the dietary requirement for methionine and phenylalanine respectively.

(b) A blank space denotes values not given.

TABLE A 4.1a Gross composition (per cent of air dry diet) in preliminary Trial A.

Diet	1	2	3	4	5
Opaque-2 maize	96.00				
Normal maize		96.00	96.00	96.00	96.00
Boneflour	4.00	4.00	4.00	4.00	4.00
Min. & Vit.(1)	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
L-lysine.HCL				0.70	0.70
DL-methionine			0.30		0.30

(1) For the composition of the mineral and vitamin supplement, see Table A4.3

TABLE A4.1b Summary of results for preliminary Trial A.(1)

Group	A	B	C	D
Period I				
Diet	1	2	3	4
Days on test	22	24	22	27
Growth rate (g/day)	191	145	150	68
Feed intake (kg)	989	989	953	653
FCR (kg feed/kg gain)	5.18	6.82	6.35	9.60
Live weight (kg)	19.3	19.0	19.4	16.8
Period II(2)				
Diet	4	6(3)	5	1
Days on test	21	21	21	21
Growth rate (g/day)	109	535	132	295
Feed intake (kg)	943	1352	871	1116
FCR (kg feed/kg gain)	8.65	2.53	6.60	3.78
Live weight (kg)	24.8	29.5	24.2	22.9

(1) Means of 5 observations, 5 pigs/group which were fed as a group.
(2) Consecutive to Period I.
(3) A ration based on barley, fish meal and meat and bone meal.

FCR = Feed Conversion Ratio.

TABLE A4.2 Gross dietary composition (per cent air dry diet) and summary of results for preliminary Trial B.

Diet	1	2	3	4	5	6
Opaque-2 maize	90.00		85.00		80.00	
Normal maize		90.00		85.00		80.00
Meat and bone meal	10.00	10.00	15.00	15.00	20.00	20.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Min. & vit. (1)	+	+	+	+	+	+
Calc. crude protein	13.1	12.8	15.0	14.7	16.8	16.6
Calc. lysine	0.58	0.44	0.69	0.55	0.80	0.67
Summary of Results (2)						
Days on treatment	55.2	55.0	59.0	53.4	63.4	52.0
Growth rate (g/day) (3)	376	213	345	150	313	177
Feed intake (kg/day)	1.27	1.09	1.23	0.91	1.13	0.91
FCR (kg feed/kg gain)	3.40	6.31	3.52	8.32	3.77	5.55

(1) Supplying Mn, Zn, Fe, Cu, Nicotinic acid, Pantothenic acid, vitamins A, D_3 and E to satisfy respective published requirements (ARC, 1967).

(2) Means of 5 individually fed animals per treatment.

(3) Average initial weight 20.5 kg.

FCR = Feed Conversion Ratio.

TABLE A4.3 Stated contribution of minerals and vitamins to the diets used in Experiments I and II by the proprietory supplement[1] incorporated, along with respective recommendations (ARC, 1967).

	Contribution (per kg air/ dry weight)	Recommendation (per kg air/ dry weight)
Vitamin A (iu's)	4410	1610
Vitamin D_3 (iu's)	662	221
Vitamin E (iu's)	20	1.5
Vit. K (Menedione)(mg)	-	-
Vit. B_1 (Thiamine)(mg)	1.48	1.5
Vit. B_2 (Riboflavin)(mg)	3.5	2.4
Vit. B_6 (Pyridoxine)(mg)	4.0	2.4
Vit. B_{12} (ug)	11.0	10.1
Pantothenic acid (mg)	12.0[2]	10.1
Biotin	-	?
Nicotinic acid (mg)	15.0	12.1
Folic acid	-	-
Choline (mg)	221[3]	803
Iron (ppm)	70	27 (Toxic level 5000)
Zinc (ppm)	150	23 (Toxic level 2000)
Manganese (ppm)	45	? (Toxic level 1000)
Copper (ppm)	180	1.8 (Toxic level 500)
Iodine (ppm)	2.1	?
Cobalt	0.33	?
Selenium (ppm)	0.15	? (Toxic level 7)
3 Nitro (mg)	27.6	-
Oxytetracycline	-	-
Semolina carrier	453	-

(1) Tasman Vaccine Laboratories (N.Z.) Ltd.

(2) Conversion factor: 1 g Pantothenic acid = 1.115 g Ca-D-pantothenate.

(3) Conversion factor: 1 g choline = 1.153 g choline chloride.

TABLE A4.4 Daily feed allowances (kg) according to live weight (kg) for preliminary Trials A and B.

Live Weight	Preliminary Trials A and B
<18.0	0.91
18.0	0.97
20.5	1.06
23.0	1.14
25.5	1.24
28.0	1.35
30.5	1.44
33.0	1.49
35.5	1.56
38.0	1.65
40.5	1.72
43.0	1.81
45.5	1.87
48.0	1.95
50.5	2.01
53.0	2.07
55.5	2.14
58.0	2.20

TABLE A4.5 Total amino acid analysis for the maize and meat and bone meal samples used in Experiments I and II (per cent air dry weight).

Amino Acid	Opaque-2 Maize	Normal Maize	Meat and Bone Meal
Hydroxy-lysine			0.20
Tryptophan(1)	0.11	0.06	0.24
Lysine	0.30	0.23	2.42
Histidine	0.18	0.19	0.75
Ammonia	0.16	0.15	0.56
Arginine	0.39	0,30	2.85
Hyrdoxy-proline			2.42
Aspartic acid	0.96	0.48	3.06
Threonine	0.36	0.28	1.37
Serine	0.45	0.38	1.63
Glutamic acid	1.67	1.54	4.93
Proline	0.83	1.11	3.16
Glycine	0.45	0.31	5.62
Alanine	0.59	0.55	2.65
Half cystine	0.14	0.12	0.28
Valine	0.45	0.32	1.80
Methionine	0.12	0.12	0.41
Isoleucine	0.28	0.26	1.22
Leucine	0.85	1.02	2.67
Tyrosine	0.32	0.26	1.04
Phenylalanine	0.43	0.34	1.49
	8.94	7.96	40.53

(1) Analyzed by the method of Spies (1968). The procedures and results are summarised in Table A4.6.

TABLE A4.6 Derivation of standard curves and results of tryptophan analyses of the dietary ingredients used in Experiments I and II.

Derivation of Standard Curves(1)

ug Tryptophan	% Transmittance (600 mu)		Log Transmittance	
Exp. I & II	Exp. I	Exp. II	Exp. I	Exp. II
12	98.5	100.0	1.994	2.000
30	90.0	90.0	1.955	1.954
48	89.0	-	1.950	-
60	84.0	75.0	1.925	1.875
90	68.5	55.5	1.837	1.744
120	46.5	39.0	1.668	1.591

Results of Tryptophan Analyses (2)(3)

Replicate		Opaque-2 Maize		Normal Maize		Meat and Bone Meal	
Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II	Exp. I	Exp.II
1	1	0.074	0.107	-	0.090	0.294	0.249
2	2	0.176	0.111	0.050	0.092	0.254	0.246
	3		0.114		0.073		0.262
	4		0.113		0.083		0.262

Means (Exp. I + II)			
Dry matter basis	0.12	0.07	0.26
Air dry basis	0.11	0.06	0.24

(1) Procedure G (Spies and Chambers, 1948).

(2) Pronase hydrolysis of corn without prior water extraction (Spies, 1968), and analysis of pronase solution and pronase hydrolysates (Spies and Chambers, 1949).

(3) Per cent dry matter.

TABLE A4.7 Gross composition (per cent) of the pre-experimental diet and the estimated content of selected nutrients (dry matter basis).

Opaque-2 maize	39.78
Normal maize	39.78
Meat and bone meal	10.00
Fish meal	10.00
Min. and Vit. suppl.(1)	0.22
Salt	0.22
ADE (kcal/kg)	3730
Crude protein (%)	19.7
Lysine (%)	1.06
Methionine plus cystine (%)	0.68
Tryptophan (%)	0.20

ADE = Apparent Digestible Energy.

(1) For the composition see Table A4.3

TABLE A5.1 Live weights (kg) according to week on treatment and average daily gains (ADG) for each pig in Experiment I.

Pig No.	Code	Sex[4]	Week on Treatment																ADG$_1$[1]	ADG$_2$[2]	±S$_b$[3]	Means ADG$_1$	Means ADG$_2$
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					
1	(1)	B	23.8	25.4	27.7	30.8	33.1	35.8	38.3	42.2	45.4	49.9							414	409	16		
2	(1)	B	24.5	26.5	27.7	29.0	30.6	32.2	34.9	37.6	39.5	42.2	45.1	47.6	49.9				302	306	11		
3	(1)	G	23.8	25.4	27.4	30.2	33.3	35.2	38.1	41.3	44.7	49.0							400	395	15		
4	(1)	G	24.3	26.5	27.9	30.8	33.1	35.8	38.6	42.0	45.4	49.2							396	392	15	378	376
5	lt	B	24.3	26.1	28.6	31.5	34.9	38.8	42.9	46.9									463	471	23		
6	lt	B	24.9	26.3	29.0	32.0	35.2	39.2	43.1	47.6									463	470	26		
7	lt	G	23.6	25.4	27.2	29.9	33.1	37.2	40.8	45.4	49.9								470	475	27		
8	lt	G	24.0	25.9	28.1	31.5	34.9	38.6	42.9	47.2									472	479	25	467	474
9	ml	B	24.5	25.4	26.1	27.4	28.1	29.0	30.8	32.2	33.6	34.9	36.1	38.6	41.5	42.2	44.0	+41.5	199	203	9		
10	ml	B	24.7	26.8	28.6	31.1	32.7	34.9	37.6	40.1	38.8								251	282	18		
11	ml	G	24.7	25.4	27.2	29.3	31.3	32.7	34.2	36.1	38.8	40.4	43.1	44.9	45.8	48.3	+48.3		259	268	5		
12	ml	G	24.3	25.2	27.0	29.0	29.7	32.0	33.3	34.7	36.3	38.6	40.4	42.0	44.7	46.3	48.5		248	247	5	239	250
13	mt	B	23.1	24.9	27.7	30.2	32.9	36.1	39.0	42.9	46.7								421	421	15		
14	mt	B	23.4	25.6	27.9	30.8	33.3	36.5	39.5	43.8	47.2								425	425	15		
15	mt	G	24.5	26.3	28.1	30.8	34.0	36.5	40.4	43.5	47.6								413	415	18		
16	mt	G	24.3	26.3	28.8	31.5	34.2	38.3	40.8	44.5	48.1								425	430	13	421	423
17	pl	B	24.7	25.9	28.4	31.3	35.4	39.0	43.1	46.3									440	464	25		
18	pl	B	24.5	25.9	28.1	31.1	34.0	37.4	40.6	44.5	48.3								425	434	19		
19	pl	G	24.7	25.6	27.9	31.1	33.8	35.8	38.8	40.6	44.2	48.1							371	372	13		
20	pl	G	23.1	24.7	26.8	29.0	31.5	33.6	36.3	39.2	43.1	46.7							374	370	16	403	410
21	pt	B	24.0	25.4	28.1	31.5	34.9	38.6	43.1	46.3									454	474	22		
22	pt	B	23.6	25.9	27.7	30.6	34.0	37.4	40.8	46.0	49.9								470	474	24		
23	pt	G	24.0	26.3	28.1	31.8	34.5	37.9	41.5	46.0	49.4								454	461	19		
24	pt	G	23.8	25.2	27.7	30.8	33.3	36.7	40.4	44.2	47.9								429	440	18	452	462
25	pm	B	24.0	25.9	28.1	30.8	34.2	37.6	42.4	43.3	47.2								413	429	17		
26	pm	B	24.5	27.4	28.8	31.8	35.4	39.2	42.9	47.2									463	461	24		
27	pm	G	24.9	26.8	28.6	31.3	34.2	37.2	40.6	44.5	48.8								425	424	21		
28	pm	G	23.4	24.9	27.2	29.9	33.3	37.0	40.8	44.5	49.0								458	465	22	440	445
29	pmlt	B	24.7	27.2	29.9	34.0	37.6	41.7	44.9	48.8									491	503	13		
30	pmlt	B	24.3	26.3	29.0	31.5	35.2	40.4	42.9	46.3									449	467	21		
31	pmlt	G	24.9	26.8	29.5	33.6	37.2	40.8	45.6	47.6									463	494	20		
32	pmlt	G	24.5	27.2	30.4	34.0	38.3	43.1	45.8										508	529	19	478	498
33	l	B	24.0	25.2	27.0	29.5	33.3	36.1	39.5	43.5	46.7								405	422	22		
34	l	B	23.8	24.7	25.9	27.7	30.4	32.4	33.6	36.1	38.3	39.9	41.3	+41.3					249	267	8		
35	l	G	23.4	25.9	28.1	28.8	30.4	32.7	32.4	34.9	36.7	38.8	39.7	41.3	40.1	41.7	43.1	+43.1	201	198	9		
36	l	G	24.0	24.7	26.3	28.1	29.7	31.8	33.1	35.8	37.4	39.0	42.0	44.7	46.9				273	276	9	282	291
37	t	B	23.4	25.4	27.2	28.6	31.5	35.4	37.6	40.4	44.2	47.9							389	389	17		
38	t	B	23.6	25.6	27.4	30.4	33.3	36.3	39.7	43.8	46.9								417	424	17		
39	t	G	23.6	25.4	27.9	30.2	32.9	35.8	38.8	42.4	46.5								409	405	17		
40	t	G	24.5	26.3	28.1	31.1	34.0	37.2	39.9	44.5	48.1								421	425	20	409	411

TABLE A5.1 continued

Pig No.	Code	Sex(4)	Week on Treatment 0	1	2	3	4	5	6	7	8	9	10	11	12	ADG_1(1)	ADG_2(2)	$\pm S_b$(3)	Means ADG_1	Means ADG_2
41	m	B	24.7	26.1	27.9	28.8	30.8	33.1	36.1	38.8	41.3	44.9	48.3			337	336	18		
42	m	B	24.0	26.5	28.4	29.0	30.6	32.4	34.7	36.3	39.9	41.7	44.5	45.6	48.8	294	289	10		
43	m	G	23.1	25.4	28.1	30.2	32.2	34.5	37.2	39.0	40.4	42.9	46.0	46.3	49.7	316	309	6		
44	m	G	24.7	26.5	28.1	29.3	31.1	33.6	36.1	38.8	41.3	43.1	45.6			298	304	10	311	310
45	mlt	B	24.0	26.1	28.1	30.6	34.2	37.9	41.5	46.5						458	454	27		
46	mlt	B	23.6	25.9	27.9	30.4	33.1	36.3	40.6	44.5	49.0					454	449	24		
47	mlt	G	23.8	26.8	30.2	33.1	37.0	41.3	45.6							518	516	18		
48	mlt	G	24.5	26.8	28.8	31.8	35.6	39.7	43.3	47.4						467	476	23	474	474
49	p	B	24.5	26.3	28.8	32.9	33.6	37.0	40.8	44.7	49.2					441	434	23		
50	p	B	24.0	27.2	29.9	33.8	34.9	38.6	42.9	46.9						467	452	20		
51	p	G	24.5	27.7	29.9	32.9	35.8	39.7	43.8	48.3						486	475	21		
52	p	G	24.0	26.3	28.1	30.4	33.1	36.5	41.1	42.2	46.0					393	401	17	447	441
53	plt	B	23.1	25.4	27.4	29.9	33.1	36.3	40.1	45.4	49.0					462	464	25		
54	plt	B	24.5	27.4	30.8	33.3	37.0	41.5	46.0							513	505	23		
55	plt	G	24.3	26.8	29.5	32.9	36.1	39.7	44.2	48.5						495	495	20		
56	plt	G	24.3	24.9	27.2	30.6	34.0	38.3	42.4	45.8						440	467	30	478	483
57	pml	B	24.7	27.9	29.9	32.7	35.6	39.5	42.9	46.7						449	443	16		
58	pml	B	24.0	26.3	29.5	32.0	36.1	38.6	42.6	47.2						472	467	18		
59	pml	G	23.8	25.9	29.0	32.0	34.7	38.1	42.6	46.7						467	466	20		
60	pml	G	24.5	27.2	28.8	30.6	33.8	37.2	41.5	44.0	46.9					401	410	18	447	447
61	pmt	B	24.9	27.4	30.2	32.4	35.8	39.7	44.0	46.7						444	454	18		
62	pmt	B	23.6	26.1	28.6	31.5	34.5	37.6	42.2	45.6	49.9					470	469	17		
63	pmt	G	23.4	25.9	28.4	31.1	34.2	37.6	41.1	45.6	49.7					470	468	18		
64	pmt	G	23.6	24.9	27.0	29.5	32.2	35.6	39.2	42.6	46.0					401	413	20	446	451

(1) ADG_1 = $\frac{\text{Final wgt. - Initial wgt.}}{\text{number of days}}$ (g/day).

(2) ADG_2 = Daily gain calculated by linear regression (g/day).

(3) S_b = Standard error for ADG_2.

(4) B = Barrow
G = Gilt.
Withdrawn from treatment before reaching 50 kg, final weight used in the calculations appears before the plus sign.

TABLE A5.2 Refusals of diets (g) by pigs in Experiment I. According to week (approximate) on treatment.

Pig No.	Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	(1)					768				/					
2	(1)		366	859	1269	165			1410	216	411	198	405/		
4	(1)					594	42								
9	ml	952	790	1137	1481	1620	1250	1232	725	2360	1140	1180	1130	774	2100+
10	ml				1100	1025	1012	1180	3580+						
11	ml			282	838	1939	2346	3300	2380	3180	3080	2540	3860	5680/	
12	ml		757	521	1695	2320	3402	3680	2140	3960	2460	2960	3200	2360	3376/
17	pl							820/							
18	pl		78				960	990	/						
19	pl	652	240	98	344	657	757	1270	189	/					
20	pl				562	832	252	376	143	322/					
25	pm							3094(1)	/						
31	pmlt							2009(1)	/						
32	pmlt						780/								
34	l					844	1531	1309	2109	2440	2297+				
35	l		92	1046	1932	1230	2120	1530	2220	1950	2340	4620	3240	4120	3566+
36	l	608	1037	645	1089	1788	2130	1970	2600	2540	2620	1210	694/		
41	m		115							314	/				
42	m	154	1096	2022	1930	1350	1260	2010	2480	1300	1650	1640	1276/		
43	m		252	609	1026	805	968	2140	2170	1700	2440	2260	1571/		
44	m					374		388	1254	545	797/				
46	mlt			322					/						
50	p				800			/							
51	p		167					/							
52	p						169	1087	/						
54	plt			215			/								
56	plt						1420	/							
57	pml						22	768/							
58	pml					324		/							
60	pml					195	849	1897	2360/						
61	pmt							1185(1)	/						

(1) These refusals associated with an outbreak of scouring.

\+ Pigs removed from experimental diets at weights less than 50 kg. / End of Experimental feeding.

TABLE A5.3 Feed allowances (kg), feed refusals (kg) and feed conversion ratios (FCR: kg feed/kg gain) for each pig in Experiment I.

Pig No.	Code	Sex(1)	Total feed Offered	Total Refusals	Net feed Intake	FCR	Mean
1	(1)	B	83.8	0.8	83.1	3.24	
2	(1)	B	115.1	5.3	109.8	4.27	
3	(1)	G	83.8		83.8	3.37	
4	(1)	G	84.5	0.6	83.8	3.40	3.57
5	lt	B	63.6		63.6	2.75	
6	lt	B	64.3		64.3	2.79	
7	lt	G	71.9		71.9	2.70	
8	lt	G	63.1		63.1	2.69	2.73
9	ml	B	123.4	17.9	105.5	5.31	
10	ml	B	70.9	7.9	63.0	3.99	
11	ml	G	124.9	29.4	95.5	3.92	
12	ml	G	131.6	32.2	99.4	4.11	4.33
13	mt	B	71.6		71.6	3.04	
14	mt	B	72.4		72.4	3.04	
15	mt	G	73.7		73.7	3.17	
16	mt	G	74.8		74.8	3.11	3.09
17	pl	B	63.5	0.8	62.7	2.76	
18	pl	B	73.0	2.0	71.0	2.92	
19	pl	G	84.6	4.2	80.4	3.43	
20	pl	G	79.9	2.5	77.4	3.32	3.11
21	pt	B	62.7		62.7	2.70	
22	pt	B	73.1		73.1	2.75	
23	pt	G	75.4		75.4	2.92	
24	pt	G	73.0		73.0	2.96	2.83
25	pm	B	74.2	3.1	71.1	2.96	
26	pm	B	64.8		64.8	2.87	
27	pm	G	74.3		74.3	3.13	
28	pm	G	72.2		72.2	2.77	2.93
29	pmlt	B	66.9		66.9	2.71	
30	pmlt	B	63.1		63.1	2.76	
31	pmlt	G	66.6	2.0	64.6	2.67	
32	pmlt	G	55.3	0.8	54.5	2.45	2.65

TABLE A5.3 continued

Pig No.	Code	Sex(1)	Total feed Offered	Total Refusals	Net feed Intake	FCR	Mean
33	1	B	71.8		71.8	3.04	
34	1	B	87.7	10.5	77.2	4.13	
35	1	G	131.3	30.0	101.3	5.22	
36	1	G	108.9	18.9	90.0	3.88	4.07
37	t	B	82.1		82.1	3.35	
38	t	B	72.8		72.8	3.07	
39	t	G	71.1		71.1	3.14	
40	t	G	73.8		73.8	3.10	3.16
41	m	B	93.0	0.4	92.6	3.94	
42	m	B	113.3	18.2	95.1	3.92	
43	m	G	116.8	15.9	100.8	3.89	
44	m	G	90.8	3.4	87.5	4.11	3.96
45	mlt	B	62.1		62.1	2.79	
46	mlt	B	73.1	0.3	72.8	2.89	
47	mlt	G	54.0		54.0	2.49	
48	mlt	G	63.8		63.8	2.74	2.73
49	p	B	74.0		74.0	3.04	
50	p	B	65.2	0.8	64.4	2.91	
51	p	G	65.2	0.2	65.1	2.80	
52	p	G	72.8	1.3	71.6	3.19	2.98
53	plt	B	72.7		72.7	2.80	
54	plt	B	54.0	0.2	53.8	2.54	
55	plt	G	64.9		64.9	2.68	
56	plt	G	61.3	1.4	59.8	2.62	2.66
57	pml	B	65.0	0.8	64.2	2.96	
58	pml	B	64.1	0.3	63.8	2.79	
59	pml	G	63.3		63.3	2.77	
60	pml	G	74.6	5.3	69.3	3.02	2.88
61	pmt	B	65.4	1.2	64.2	2.89	
62	pmt	B	74.2		74.2	2.82	
63	pmt	G	73.2		73.2	2.79	
64	pmt	G	71.4		71.4	3.09	2.90

(1) B = Barrow
G = Gilt

TABLE A5.4 Summary of Results for Experiment II.

Code	l				t				m				ltm			
Pig No.	3		15		1		10		8		16		5		12	
Balance Period	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Mean L. Wgt. (kg)	32.0	42.2	31.5	42.6	30.4	44.2	31.3	43.5	31.5	44.2	32.7	43.1	32.0	42.9	31.8	42.6
GNI[1] (g/4 days)	91	104	94	131	94	129	94	129	82	124	94	116	96	133	97	134
GEI[2] (mcal/4 days)	17.5	20.0	18.0	25.1	18.4	25.2	18.4	25.2	16.3	24.5	18.6	22.9	18.2	25.2	18.4	25.2
Digestible DM[3] (%)	88	83	87	87	88	84	83	86	87	84	86	84	86	85	86	87
ADN[4] (g/4 days)	78	83	79	108	81	104	76	107	66	99	76	99	82	112	83	114
ADN (%)	86	79	84	83	85	81	80	83	81	80	81	85	85	84	85	85
NR[5] (g/4 days)	39	31	36	56	36	35	34	44	41	38	39	56	51	64	45	63
NR (g/ day)	9.8	7.8	9.0	14.0	9.0	8.8	8.5	11.0	10.3	9.5	9.8	14.0	12.8	16.0	11.3	15.8
NR[6] (%)	50	38	46	52	45	34	44	41	62	38	51	57	62	57	55	55
NR (g/kg/4 days)	1.22	0.73	1.14	1.31	1.18	0.79	1.09	1.01	1.30	0.86	1.19	1.30	1.59	1.49	1.42	1.48
ADE[7] (%)	88	84	87	87	89	84	83	86	87	85	87	91	87	85	87	87
ME[8] (%)	86	81	85	85	86	81	81	84	86	82	84	89	85	83	85	85

TABLE A5.4 continued

Code	p				plt				pml				pmt					
Pig No.	6		9		2		14		4		11		7		13		Mean	
Balance Period	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Mean L. Wgt. (kg)	29.7	44.5	30.8	46.7	32.9	45.1	31.5	43.3	31.5	44.7	30.6	45.1	31.1	43.8	31.5	42.2	31.4	43.8
GNI[1] (g/4 days)	102	139	101	143	105	148	105	144	106	149	106	149	82	136	101	139	97	134
GEI[2] (mcal/4 days)	18.4	25.1	18.4	25.9	18.4	25.8	18.4	25.1	18.4	25.7	18.3	25.8	14.9	24.6	18.4	25.2	18.0	24.8
Digestible DM[3] (%)	85	86	80	84	83	84	81	83	86	83	83	83	80	83	85	84	84.5	84.5
ADN[4] (g/4 days)	87	121	81	122	90	128	88	121	91	124	90	127	-[9]	116	86	119	82	113
ADN (%)	85	87	79	86	86	87	84	85	86	83	85	85	-	85	84	86	84	84
NR[5] (g/4 days)	45	58	33	53	58	72	43	65	57	45	49	62	-	55	44	52	44	53
NR (g/day)	11.3	14.5	8.3	13.3	14.5	18.0	10.8	16.3	14.3	11.3	12.3	15.5	-	13.8	11.0	13.0	11.0	13.3
NR[6] (%)	52	48	41	43	64	56	49	53	63	36	55	49	-	48	51	43	53	47
NR (g/kg/4 days)	1.51	1.30	1.07	1.13	1.76	1.60	1.36	1.50	1.81	1.01	1.60	1.37	-	1.26	1.40	1.23	1.38	1.21
ADE[7] (%)	86	87	79	84	82	83	82	84	87	84	84	83	-	84	85	85	85	85
ME[8] (%)	84	85	77	81	80	82	79	82	85	82	81	80	-	81	83	83	83	83

(1) Gross nitrogen intake
(2) Gross energy intake
(3) Apparent digestible dry matter
(4) Apparent digestible nitrogen
(6) Nitrogen retention
(6) NR as a per cent of ADN
(7) Apparent digestible energy
(8) Metabolizable energy
(9) Results in error, values for analysis calculated by a method for missing plots of a split-plot design (Cochran and Cox, 1950).

TABLE A5.5 Nitrogen content (per cent air dry weight) of diets used in Experiment II (mean of two analyses).

Diet	Code	
9	1	2.0233
10	t	1.9902
11	m	1.9606
12	mlt	2.0549
13	p	2.1440
14	plt	2.2184
15	pml	2.2403
16	pmt	2.1408

TABLE A5.6 Gross energy content (kcal/kg air dry weight) of some of the diets used in Experiment I (mean of two analyses).

Diet	
1	3880
3	3884
4	3873
5	3885
7	3870
Mean	3878

TABLE A5.7 Total feed refusals (kg) recorded in Experiments I and II for pigs fed diets 9 and 11 (1 and m respectively), according to live weight (kg).

Experiment I				Experiment II			
Pig No.	Diet	Live Weight	Total Refusals	Pig No.	Diet	Live Weight	Total Refusals
33	9	24.0 - 46.7	-	3	9	24.5 - 43.5	5.8
34	9	23.8 - 41.3	10.5	15	9	23.6 - 44.2	0.1
35	9	23.4 - 43.1	30.0	8	11	25.6 - 46.3	0.8
36	9	24.0 - 46.9	18.9	16	11	23.4 - 41.3	4.3
41	11	24.7 - 48.3	0.4				
42	11	24.0 - 48.8	18.2				
43	11	23.1 - 49.7	15.9				
44	11	24.7 - 45.6	3.4				

TABLE A5.8 Experiment I: Summary of analysis of variance of average daily gains (ADG) and feed conversion ratios (FCR).

Source	d.f.	Mean Squares / F tests ADG (g/day)	FCR (kg feed/kg gain)
Block	7	571.61	0.018
Treatment(1)	14	22820.88***	1.207***
P	1	98988.89***	5.510***
M	1	618.77	0.033
L	1	19.14	0.019
T	1	125227.52***	6.452***
PM	1	4882.52*	0.160
PL	1	1198.89	0.047
PT	1	40551.89***	2.790***
ML	1	1305.02	0.024
MT	1	1691.27	0.042
LT	1	31461.89***	1.172***
PML	1	523.27	0.008
PMT	1	6025.64**	0.286
MLT	1	456.89	0.023
PLT	1	6540.77**	0.334
Error	42	1358.08	0.123

*** $p < 0.01$ ** $p < 0.05$ * $p < 0.10$; where no level of significance is shown: $p > 0.10$.

(1) The third order interaction PMLT was confounded with blocks and so excluded from the treatment sums of squares.

TABLE A5.9 Experiment II: Summary of analyses of variance.

Source	d.f.	Mean Squares / F tests LW (kg)	GEI (g/4 days)	ADDM (%)	ADE (%)	ME (%)
Main Plots:						
Block	1	0.113	3.578	0.578	0.000	1.125
Treatment	7	0.741	2.235*	7.191	8.768	9.982
Error	7	0.810	1.269	4.177	8.071	7.554
Sub-Plots:						
Balance	1	1223.888****	371.963****	0.300	0.500	0.125
Bal. by Tr.	7	2.571**	0.989	4.128	2.571	3.554
Error	8	0.492	1.437	3.904	4.500	6.714

Main Plots:		NI (g/4 days)	ADN (%)	NR (g/4 days)	NR (%)	NR (g/kg/4 days)
Block	1	101.531	0.281	0.125	18.000	0.002
Treatment(1)	7	306.746***	7.210	236.554**	112.339*	0.152**
P (-MLT)	1	1417.781****		528.125**		0.328**
M (-PLT)	1	2.531		153.125		0.130
L (-PMT)	1	236.531**		496.125**		0.349**
T (-PML)	1	38.281		153.125		0.095
PM (-LT)	1	26.281		325.125*		0.162*
PL (-MT)	1	81.281		0.125		0.000
PT (-ML)	1	344.531**		0.125		0.002
Error	7	36.531	3.853	61.696	32.643	0.037
Sub-plots:						
Balance	1	1137.781****	0.781	722.000**	300.125*	0.228*
Bal. by Tr.	7	44.496	6.496	40.571	26.625	0.026
Error	7	39.781	5.179	73.857	56.357	0.048

**** $p < 0.005$ *** $p < 0.01$ ** $p < 0.05$ * $p < 0.10$; where no level of significance is shown $p > 0.10$.

(1) The treatment sums of squares was only partitioned where it was significant at the 5% level.

LW = Live Weight

GEI = Gross Eenergy Intake

ADDM = Apparent Digestible Dry Matter

ADE = Apparent Digestible Energy

ME = Metabolizable Energy

NI = Nitrogen Intake

ADN = Apparent Digestible Nitrogen

NR = Nitrogen Retention

TABLE A5.10 Live weights (kg) according to week on treatment and average daily gains (ADG g/day) for each pig in Experiment II with corresponding data for pigs of Experiment I fed the same diets.

Pig No.	Code	Week on Treatment 0	1	2	3	4	5	6	7	8	9	10	11	ADG (1)	ADG (2)	ADG (3)	± S_b (4)[b]	ADG (5)	ADG (6)	ADG (7)	% (8)	Diff. (9)
3	1	24.5	26.3	28.1	30.6	33.3	35.8	38.6	40.8	43.5				389	389	347	0.008					
15	1	23.6	26.1	27.4	29.7	33.3	36.5	40.8	44.2					518	486	424	0.026	386	345	291	+12	+33
1	t	24.7	26.5	28.6	32.0	34.7	37.6	41.5	46.7					485	745	440	0.026					
10	t	24.7	27.2	29.9	32.7	35.2	37.9	41.1	45.8					389	680	413	0.017	427	407	411	+5	+4
8	m	25.6	27.2	31.5	31.5	33.8	36.3	38.6	42.2	46.3				-	583	348	0.022					
16	m	23.4	23.4	24.0	25.9	27.4	28.8	31.5	33.0[+]	34.5	36.7	39.5	41.3	195	518	246	0.012	297	313	310	-5	-4
5	mlt	24.7	26.8	30.4	33.3	36.3	40.6	44.9						421	616	480	0.021					
12	mlt	23.8	26.8	29.9	33.3	36.3	39.9	45.1						486	745	493	0.020	487	452	474	+8	+3
6	p	24.0	25.9	28.6	30.8	34.2	38.1	41.7	46.9					324	745	462	0.028					
9	p	24.5	26.5	29.3	32.4	35.4	39.0	43.5						454	583	450	0.022	456	443	441	+3	+3
2	plt	23.1	25.2	27.2	30.8	34.7	37.9	42.6	47.4					551	680	498	0.028					
14	plt	24.9	27.0	29.7	33.3	36.7	40.6	46.0						518	777	498	0.030	498	485	483	+3	+3
4	ml	25.6	27.9	29.9	33.1	36.3	39.2	43.1	46.3					454	454	428	0.015					
11	pml	23.8	26.5	28.8	32.4	35.6	39.0	43.3	46.9					518	518	476	0.016	452	455	447	-1	+1
7	pmt	27.0	29.5	32.4	34.2	37.6	41.7	45.8						421	583	440	0.025					
13	pmt	24.3	26.5	29.5	33.3	36.5	40.1	44.2						551	583	480	0.016	460	462	451	-0.4	+2

[+] Estimated

(1) ADG on balance A (preliminary and balance period).

(2) ADG on balance B (preliminary and balance period).

(3) ADG over the whole growth period by linear regression.

(4) Standard error of the regression coefficient.

(5) Mean ADG for ADG (3).

(6) Mean ADG for barrows on Group II diets in Exp. I.

(7) As for (6) with gilts included.

(8) Difference of (5) from (6) as a % of (6).

(9) Difference of (5) from (7) as a % of (7).

TABLE A5.11 Average daily gains (ADG g/day) from Experiment I and nitrogen retentions (NR g/day) from Experiment II for those animals fed group II (diets 9-16) of the experimental diets, with regression equations including these results.

Diet	Raw Data Sub-Period/Balance(1)	X(2)	Y(3)
9	A	10	269
	B	11	303
10	A	9	346
	B	10	523
11	A	10	271
	B	12	345
12	A	12	388
	B	16	596
13	A	10	369
	B	14	556
14	A	13	396
	B	17	612
15	A	13	379
	B	14	551
16	A	11(5)	375
	B	14	535

Regression Analysis

No.(4)	n	ΣY	ΣXY	Σx^2	ΣY^2	ΣX	S_b(6)	b
1	8	2793	31060	16	993305	88	10.753	21.063*
2	8	4021	55631	40	2113085	108	13.940	33.688*
3	6	2253	25660	13.3	847503	68	2.414	9.45**
4	6	3373	48158	28.8	1902251	85	3.247	12.965**

Regression Equations

No.(4)	
3	$Y = 268.40 + 9.45X$ ($S_b = \pm 2.41$)
4	$Y = 378.51 + 12.97X$ ($S_b = \pm 3.25$)

** $p < 0.05$ * $p < 0.10$

(1) Sub-periods for Experiment I: A=23 - 37 kg; B=37 -50 kg. Mean Live weights for balances in Experiment II: A = 31.4 kg; B = 43.8 kg.
(2) Mean NR for Experiment II.
(3) Mean ADG for Experiment I.
(4) 1 = Sub-period A/Balance A with all diets; 2 = Sub-period B/Balance B with all diets; 3 = as for 1 but excluding diets 9 and 11; 4 = as for 2 but excluding diets 9 and 11.
(5) Includes a value calculated for a missing plot.
(6) Standard error of the regression coefficient (b).

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