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AN ASSESSMENT OF CURRENT DIETARY AMINO ACID
RECOMMENDATIONS FOR THE GROWING MEAT RABBIT
BASED ON WHOLE BODY AMINO ACID COMPOSITION.

A thesis presented in partial fulfilment
of the requirements for the degree of
Master of Agricultural Science at
Massey University

WENDY HELEN SCHULTZE

1986

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ABSTRACT

Reservations regarding the amino acid levels recommended by the National Research Council [NRC] (1977) and the Société de Chimie Organique et Biologique [AEC] (1978) for the growing meat rabbit, prompted the use of rabbit whole body amino acid composition values as a first approximation toward determining the ideal dietary amino acid balance, relative to lysine, for this species.

In the absence of whole body amino acid composition data for the growing rabbit, a technique was established for the processing and subsequent chemical analysis of the rabbit whole body. Using the established technique, twelve 53-day-old New Zealand White rabbits were prepared and representative whole body tissue samples were analysed to determine their amino acid contents.

The determined overall mean essential amino acid composition of rabbit whole body (g/kg dry matter) was, lysine 5.05; histidine 2.54; isoleucine 2.57; leucine 5.67; phenylalanine 3.66; tyrosine 2.82; threonine 3.24; valine 3.16; arginine 5.48; methionine 1.49; and cystine 2.32. A comparison of these determined rabbit whole body amino acid values, relative to lysine, compared with the recommendations of NRC (1977) and AEC

(1978), suggested, that the published requirements were overgenerous. In a subsequent study, aimed at determining whether the published dietary amino acid recommendations were indeed excessive, 81 five-week-old New Zealand White rabbits were fed one of a series of nine iso-caloric diets with progressively reduced amounts of crude protein (159 to 97 g/kg) but a fixed level of lysine (6.5 g/kg).

Over a 40-day period the growth performance of the rabbits was similar on the first six diets of the series, but thereafter with decreasing dietary crude protein content there was a linear decrease in growth rate and concomitant increase in the feed conversion ratio.

Urinary nitrogen and urinary urea excretion measured during the experimental period declined progressively from the first to the sixth diet of the series and then plateaued, findings which are in general agreement with the growth performance data. Urinary creatinine excretion showed a decline across diets, indicating, that the rabbits on the higher protein diets were leaner than their counterparts on the diets of lower crude protein.

As the gross amino acid composition of the first diet in the series equated with that of published recommendations, while that of diet six approximated rabbit whole body amino acid composition, it appears that the recommendations are overgenerous and that the dietary ideal amino acid balance may not be far removed from that of rabbit whole body composition. The need for further research to confirm these findings and define more exactly the dietary ideal amino acid balance for the growing rabbit is emphasized.

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INTRODUCTION.

Dietary protein is a major cost factor in intensive meat rabbit production. Consequently, it is important that the animals' requirements for amino acids are known so that they can be provided adequately and economically in the diet.

Although national recommendations of dietary amino acid requirements for the growing meat rabbit have been published, they cannot be accepted without reservation. This stems not solely from the limited nature of the data on which these recommendations are based, but also the approach which has been adopted in their determination. This approach being such as to cast doubt that the amino acid pattern identified adequately defines the dietary ideal amino acid balance.

Based on rabbit whole body amino acid composition as an index of the dietary amino acid pattern required for maximum growth performance, the present study was undertaken to clarify whether or not current published dietary amino acid recommendations for the growing meat rabbit adequately describe the dietary ideal amino acid balance.

CHAPTER 1

REVIEW OF LITERATURE.

1.1 Introduction.

The following review outlines and discusses energy and crude protein requirements of the young, growing rabbit post weaning, and raises the question of the importance of protein quality in the nutrition of the growing rabbit. Empirical estimates of amino acid requirements are reviewed and the deficiencies of these estimates discussed. The concept of ideal dietary amino acid balance is introduced and whole body amino acid composition is evaluated as a means of determining an estimate of the ideal dietary amino acid balance for the growing rabbit.

1.2 Anatomy and Physiology of Digestion in the Growing Rabbit.

The alimentary tract of the rabbit (Figure 1.1) is characteristic of monogastrics. The relative size of the rabbits' stomach is similar to the pig, but the rabbit differs from other monogastrics in that it has a relatively large caecum and small colon (refer Table 1.1). This size differentiation of the digestive organs peculiar to the rabbit is complete by the age of six weeks (Schlola, 1982).

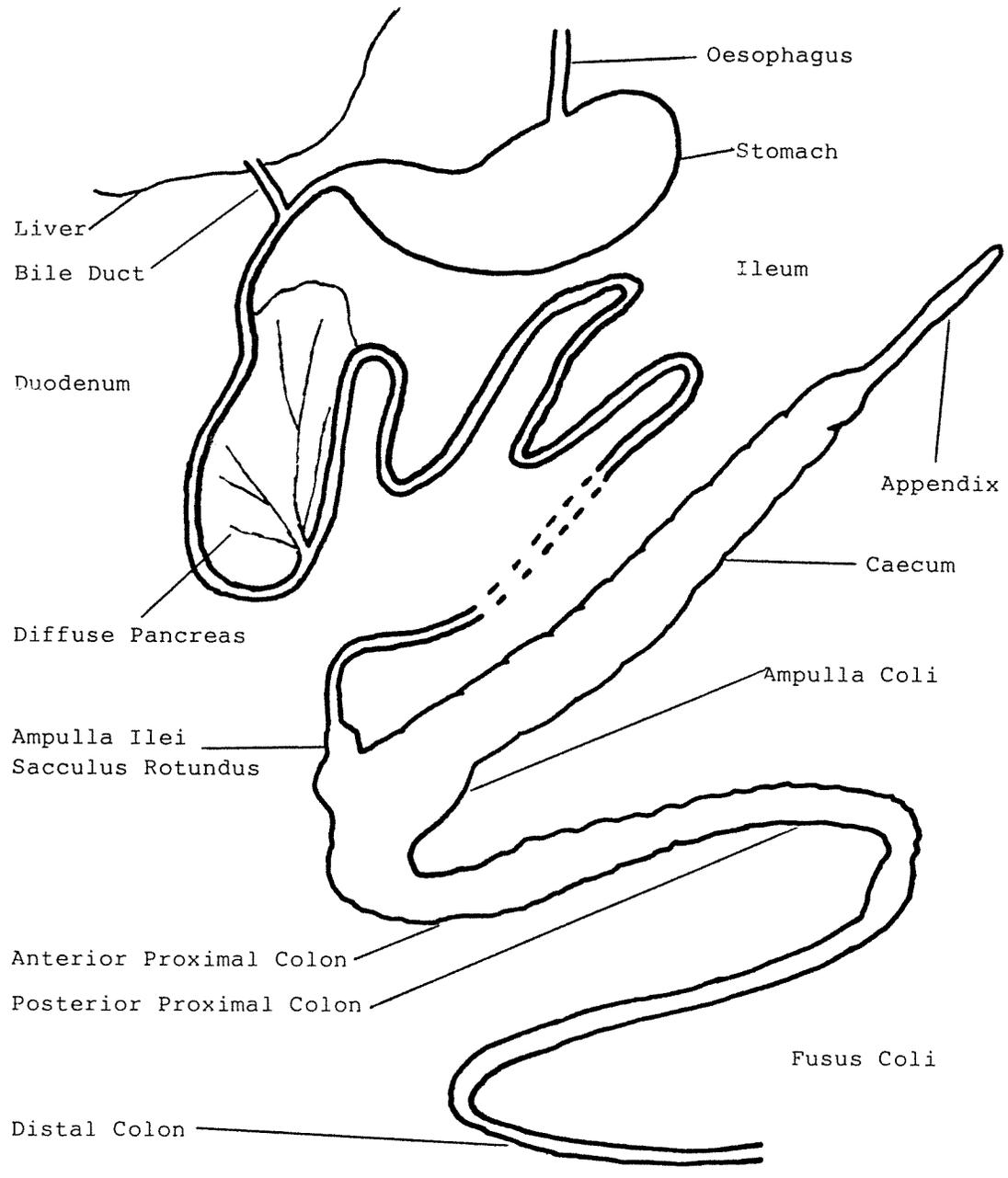


Figure 1.1 The digestive tract of the growing rabbit.
(adapted from Lang, 1981).

Table 1.1. Relative capacity of the digestive organs of
the rabbit and the pig (% of total).

(from Schlolaut, 1982)

| | Pig | Rabbit |
|-----------------|-----|--------|
| Stomach | 29 | 34 |
| Small intestine | 33 | 11 |
| Caecum | 6 | 49 |
| Colon | 32 | 6 |

A notable feature of the rabbits' digestive tract is, that the ileum terminates in an enlarged area called the sacculus rotundus or the ampulla ilei (refer Figure 1.1). Movement of digesta from the ileum is controlled by a valve. Once digesta has passed this point, the valve prevents any backflow (Pickard and Stevens, 1972). The ileo-caeco-colic junction is a complex part of the gut and has an important role in the physiology of the caecum and colon (to be discussed later in this section).

The main difference between the rabbit and other monogastric species is the practice of caecotrophy. Caecotrophy is the re-ingestion of a specially modified portion of the faecal material. This practice is more commonly called coprophagy. Coprophagy, however, is the re-ingestion of unmodified faeces, hence this term is less accurate when referring to the rabbit, which produces two types of faeces.

The practice of caecotrophy is made possible by the formation of two types of faeces, commonly called 'hard faeces' (those excreted) and 'soft faeces' (those re-ingested). This differentiation of digesta begins in the fifth week of life (Schlola, 1982) and appears to occur at the ileo-caeco-colic junction (Pickard and Stevens, 1972).

Food residues pass through the ileo-caecal valve, which prevents any backflow. Part may move into the caecum and part into the proximal colon. The proximal colon is subject to strong antiperistaltic waves which tend to push digesta into the caecum. The caecum is itself in constant movement and its contents are continually mixed by rapid contractions running from base to appendix and in the reverse direction. As well as antiperistaltic waves the proximal colon is subject to slow moving constrictions which pass from the anterior toward the posterior end. These movements cause a constant flux and reflux of material between the proximal colon and the caecum, the net result being that larger

particles and some fluid tend to move from the caecum into the colon and much of the fluid and small particles tend to move into the caecum. This retrograde movement of small particles is helped by secretion of water in the proximal colon and the absorption of water in the caecum. It has been suggested (Ruckebusch and Hörnicke, 1977), that because of the structure of the anterior proximal colon, the digestive movements cause large particles to be pressed to it's walls while an axial stream of fluid carries small particles back to the caecum. The result of this process is the selective retention of fluid and small particles (including micro-organisms) in the caecum while allowing the excretion of larger less digestible food particles (Pickard and Stevens, 1972).

`Hard' faeces are produced from the larger particles which are pushed along the circumference of the anterior proximal colon into the posterior proximal colon (refer Figure 1.1) where water is not absorbed, but mechanically squeezed out. Excretion occurs when the whole of the distal colon is evacuated in response to a single nervous stimulation. `Soft' faeces are produced after the caecal contents have been subjected to several hours of microbial action, usually in response to more digesta passing through the ileal valve. The complete elimination of hard faeces always precedes the single large contraction of caecum and proximal colon which initiates the rapid movement of caecal material through the distal colon and rectum, to produce `soft' faeces (Ruckebusch and Hörnicke, 1977).

A rapid rate of digesta passage in the rabbit (Pickard and Stevens, 1972; Laplace and Lebas, 1975) and the selective retention of small particles, at the expense of large particles, allows the rabbit to cope with a high fibre diet, but also leads to the low digestibility of fibre (usually the large particles) by the rabbit (Fonnesbeck, Harris, and Kearl, 1974). The rabbit is, however, efficient at digesting the protein portion of the diet (Schurg, Frei, Cheeke, Holton, 1977). Robinson, Cheeke, and Patton (1985) suggested, that the rabbit is inefficient at digesting grains and protein supplements (e.g. soyabean meal) in the small intestine due to the rapid rate of passage, and that re-ingestion via caecotrophy is necessary for the full utilization of dietary nutrients. The importance of the caecum and caecotrophy in the normal digestive functioning of the rabbit has also been reported by Herndon and Hove (1955), Thacker and Brandt (1955), and Stevens (1977).

The rabbit is different from other monogastric species in many aspects of its digestive physiology and anatomy. There is, therefore, a need to determine specific nutrient requirements for this species.

1.3 Crude Protein and Energy Requirements of the Growing Rabbit.

The rabbit requires both dietary energy and protein. These two nutrients, however, cannot be considered independently. Energy is required for protein synthesis and amino acids themselves can be sources of energy (Payne, 1972; and Low, 1981).

Table 1.3.1 Recommendations for the digestible energy content (MJ/kg) of grower rabbit diets.

| | |
|-----------------------------------|------------|
| Davidson and Spreadbury(1975) | 12.8 |
| Spreadbury and Davidson(1978) | 8.7 - 14.1 |
| Dehalle(1981) | 9.7 - 11.5 |
| Lebas,Laplace, and Droumenq(1982) | 9.9 - 12.3 |

Table 1.3.1 summarizes the recommended digestible energy contents of grower rabbit diets. The range of values reported appears to be due to the ability of the rabbit to increase voluntary feed intake on diets of low digestible energy content. Spreadbury and Davidson (1978) found, that over the range of 8.7 to 14.1 MJ digestible energy per kg diet, rabbits fed ad libitum adjusted their feed intake according to energy concentration to consume 1.34 MJ digestible energy per day. Lebas (1975), Dehalle (1981), and Buther, Bryant, Owen, Leach and Machin (1983) also reported, that rabbits on diets of varying digestible energy

concentration adjusted their intakes to maintain a constant daily energy intake, when fed ad libitum. Thus, over the normal range of digestible energy in commercial rabbit diets, the growing rabbit will adjust its intake to compensate to a constant energy intake. In this respect there is no single energy requirement for the diet of the growing rabbit, as long as they are fed ad libitum. This also means that crude protein requirements would be better expressed on a per unit of digestible energy basis, rather than per kg of diet. Despite this, however, most workers have expressed protein requirements per kg of diet which undoubtedly introduces variation.

A review of recommendations for optimum crude protein supply in grower rabbit diets is shown in Table 1.3.2. There is reasonably good agreement between workers. The variation in crude protein recommendations between workers may be explained by variations in factors such as sex, age, strain of rabbit, measure of response, feed intake, source of protein, level of protein, and level of energy.

Knowledge of the crude protein content of the diet, however, does not give any information on amino acid requirements, which may be important in the nutrition of the growing rabbit.

Table 1.3.2 Recommendations for optimum crude protein supply
in grower rabbit diets.

| Author | Levels of Protein Examined (g/kg diet) | Growth Rates Achieved (g/day) | Author(s) Recommendation (g crude protein /kg diet) |
|--------------------------------------|---|-------------------------------------|--|
| Davidson and Spreadbury(1973) | 110 - 260 | 40+ | 150 |
| Lebas(1973) | 110 - 290 | 20 - 38 | 180 - 200 |
| Colin(1974) | 130,170,210 | 18 - 39 | 170 |
| Omole(1977) | 140,180,220 | 18 - 21 | 180 |
| Omole(1982) | 100,140, 180,220,260 | 13 - 25 | 180 |
| Ouhayoun and Cheriet(1983) | 104,138,172 | 32 - 36 | 138 |
| Sanchez, Cheeke, and Patton(1985) | 175,190,205 | 38 | 175 |

1.4 Significance of Protein Quality in the Nutrition of the Growing Rabbit.

1.4.1 The Concept of Protein Quality.

Protein quality is sometimes regarded as synonymous with protein utilization during growth (Payne, 1972), whereas other workers (Cole 1979 and 1980; Fuller, Livingstone, Baird, and Atkinson, 1979; Low, 1981; and Fuller and Chamberlain, 1982) regard protein quality as simply reflecting the required pattern of amino acids for the growing animal. The latter approach has been adopted in this thesis.

Amino acids vary widely in their digestibility and also in availability; where digestibility is defined as disappearance from the digestive tract, and availability is defined as digestibility plus subsequent utilization. The lower the digestibility or availability the more that must be supplied in the diet to meet the requirement. There are three commonly used methods used to determine amino acid digestibility;

i) Faecal Analysis - digestibility is calculated by correcting faecal amino acid output for amino acid output on a protein free diet, and expressing this as a proportion of amino acid intake. The accuracy of this method has been questioned. Firstly, in respect of the determination of digestibility of dietary amino acids, important discrepancies may arise resultant from microbial

fermentation in the hindgut (Moughan and Smith,1982). This discrepancy may not be as important in the rabbit as in other monogastric species, as microbial protein could be utilized via caecotrophy. Secondly, the use of protein-free diets to estimate endogenous protein is prone to errors, as the level and source of dietary fibre influences the composition and quantity of endogenous protein (Buraczewska,1980; Moughan and Smith,1982).

ii) Ileal Analysis - measurement of amino acid digestibility at the terminal ileum, rather than faecal analysis, is more accurate for the pig (Zebrowska,1978; Moughan and Smith,1982). Whether this is true for the rabbit has not been determined. However, this method does not overcome the problem of inaccuracies due to endogenous protein.

It must be remembered that neither faecal nor ileal analyses are measures of actual availability, but measures of disappearance from the digestive tract. For example, some lysine may have been absorbed before the terminal ileum, but in an unavailable form (Zebrowska,1978).

iii) In vitro - McNab(1979) discusses a number of in vitro methods of determining amino acid availability, however, these methods are limited to some specific amino acids and lack a reliable in vivo comparison.

In summary, when determining protein quality it would be desirable to base the score on amino acids available to the animal rather than present in the feed, however, there is some difficulty in accurately and inexpensively determining amino acid availability.

To score proteins for quality based on amino acid composition it is necessary to first establish the ideal balance of amino acids required for the growing animal. The ideal balance of amino acids for growth is the one that leads to complete utilization of dietary protein by the growing animal, with neither additions nor removal of amino acids improving protein utilization (Cole, 1979). To maximise protein quality the dietary amino acids should be supplied to meet the ideal balance.

The balance of dietary amino acids can deviate from the ideal in a number of ways:

- 1) Deficiency - there may be one or more amino acids deficient in the diet which will limit protein synthesis. If one amino acid is limiting there will be other amino acid surplus to requirements.
- 2) Large imbalances - the balance of amino acids leads to a poorer production than might be expected on the basis of the levels of limiting amino acids (Harper, Benevenga, and Wohlhueter, 1970).
- 3) Antagonism - the excess of one amino acid causes an increased requirement for a structurally or chemically related amino acid (Harper, Benevenga, and Wohlhueter, 1970). Hence, an excess of

one amino acid can render another amino acid less useful or limiting (eg. excess lysine antagonises arginine).

4) Toxicity - detrimental effects of an excessive level of an individual amino acid which are not prevented by the addition of another individual or small groups of amino acids to the diet (Harper, Benevenga, Wohlhueter, 1970).

Deviations from the ideal balance of amino acids causes wastage of amino acids when one amino acid limits protein synthesis or an amino acid is rendered less useful and hence limits protein synthesis.

1.4.2 Protein Quality in the Nutrition of the Growing Rabbit.

Whereas dietary protein quality is generally considered to be important in monogastric nutrition, the question to be addressed here is whether amino acid supply and the balance of amino acids in the diet are important in the nutrition of the growing rabbit.

It has been suggested, that as a hindgut fermenter, the rabbit could utilize the amino acids synthesised by bacteria in the large intestine via caecotrophy (Davidson and Spreadbury, 1975; Hörnicke, 1981). Differences in chemical analysis between the coprophagic and normal faecal pellets support the view that rabbits derive some nutritional benefit from the ingestion of soft

faeces (refer Table 1.4.1).

Table 1.4.1 Composition of rabbit faeces (as a % of dry matter).
(from Portsmouth, 1976).

| | Normal Pellets | Caecotrophic Pellets |
|-----------------------|----------------|----------------------|
| Crude Protein | 11.0 | 35.0 |
| Ether Extract | 4.0 | 3.5 |
| Crude Fibre | 35.5 | 14.0 |
| Nitrogen-free Extract | 41.5 | 36.5 |
| Ash | 8.0 | 11.0 |

It can be seen (Table 1.4.1) that the protein level is markedly higher in the caecotrophic pellets. Also, compared to hard faeces there is not one amino acid which is lower in the caecotrophic pellets (refer Table 1.4.2).

Spreadbury (1978), by collaring rabbits, estimated that caecotrophy contributes 2g of crude protein per day to the growing rabbits total protein intake (approximately 10% of the total crude protein intake).

Table 1.4.2 Amino acid composition of caecotrophic and normal faeces (g per 100 g DM).

| Amino Acid | Normal Faeces | Caecotrophic Pellets |
|---------------|---------------|----------------------|
| Aspartic acid | 0.97 | 3.06 |
| Threonine | 0.54 | 1.79 |
| Serine | 0.45 | 1.34 |
| Glutamic acid | 1.01 | 3.30 |
| Proline | 0.54 | 1.28 |
| Glycine | 0.62 | 1.59 |
| Alanine | 0.58 | 1.80 |
| Valine | 0.63 | 1.69 |
| Methionine | 0.13 | 0.47 |
| Isoleucine | 0.53 | 1.28 |
| Tyrosine | 0.24 | 0.93 |
| Phenylalanine | 0.54 | 1.10 |
| Lysine | 0.60 | 1.57 |
| Histidine | 0.25 | 0.44 |
| Arginine | 0.35 | 0.91 |
| Leucine | 0.89 | 1.88 |

(from Ferrando, Wolter, Vitat, and Megard, 1970)

Caecotrophy, however, does not supply all amino acids required by the growing rabbit in sufficient amounts to support growth (Adamson and Fisher, 1971). Adamson and Fisher (1971) established that there were eleven dietary essential amino acids for the growing rabbit; histidine, arginine, glycine, isoleucine, leucine, lysine, threonine, tryptophan, valine, phenylalanine, and methionine. Omission of glycine resulted in weight gain approximately half that obtained with the complete mixture of amino acids, indicating that glycine can be synthesised by the rabbit, but is a dietary requirement for rapid growth. Omission of arginine and of isoleucine resulted in neither weight loss nor weight gain, and omission of the other amino acids found to be essential caused a sharp loss in body weight.

Davidson and Spreadbury (1975) hypothesised, that if the amino acid balance is important in the nutrition of the growing rabbit, then diets containing poor quality protein supplements (as judged by diets for non-ruminants) such as gelatin should not support such good growth as diets containing high quality protein supplements such as fishmeal. Diets of equal crude protein levels with 'poor' and 'high' quality protein supplements were fed to growing rabbits and the growth performance compared. Rabbits on 'poor' quality protein supplement diets grew, on average, ten grams per day (20%) slower than those on 'high' quality protein supplement diets. This work and that of Cheeke (1971), Cheeke and Amberg (1972), Adamson and Fisher (1973), Kennedy and Hershberger

(1974), Spreadbury (1974 and 1978) confirmed that amino acid balance is important in the nutrition of the growing rabbit.

It has also been shown, that like other monogastrics the growing rabbit is unable to utilize dietary non-protein nitrogen via bacterial synthesis in the caecum followed by caecotrophy (Olcese and Pearson, 1948; King, 1971; Cheeke, 1972; Lebas and Colin, 1973; and Hoover and Heitmann, 1975)

In theory it appears to be possible for the growing rabbit to utilize bacterial protein via caecotrophy, hence, amino acid supply would not be critical in the nutrition of the growing rabbit. In the practical experiments reviewed above, however, it has been shown that there are dietary essential amino acids and the balance of these acids in the diet is important in the nutrition of the growing rabbit, indicating that caecotrophy, although it may be important, does not supply enough protein for maximal growth.

Because the rabbit requires a supply of dietary amino acids, not crude protein or non-protein nitrogen, it becomes necessary to substitute protein requirement by the more specific one of amino acid requirement.

1.5 Best Estimates of Amino Acid Requirements.

The most comprehensive work on the amino acid requirements of the growing rabbit has been done by Adamson and Fisher(1973) and Davidson and Spreadbury (1973 and 1975).

Adamson and Fisher (1973) investigated the amino acid requirements of the six to seven-week-old female rabbit, using a chemically defined synthetic diet whose amino acid pattern was based on methionine supplemented isolated soya protein. Each of the 10 essential amino acids, except glycine, were studied in alphabetical order. The amino acid under study was isonitrogenously replaced with L-glutamic acid. Four or more levels of each amino acid were tested. The level of an amino acid estimated as the requirement, based on growth rate and feed intake, was incorporated into the starting diets of all future studies. The requirements estimated are given in Table 1.5.1.

Davidson and Spreadbury (1973) estimated the amino acid requirements for the growing rabbit from 0.75 - 2.5 kg liveweight. Their estimates were based on the analysis of diets that supported optimum growth (40g per day) and from a review of the literature. The requirements estimated are also given in Table 1.5.1.

Davidson and Spreadbury's 1975 paper appears to report the same experiment as the 1973 paper, however, the concluding recommendations were slightly different (refer Table 1.5.1).

Table 1.5.1 Estimates of the amino acid requirements (% of the diet) of the growing rabbit.

| | Adamson and Fisher(1973) | Davidson and Spreadbury(1973) | Davidson and Spreadbury(1975) |
|-----------------------------|-----------------------------|----------------------------------|----------------------------------|
| Arginine | 1.00 | 0.70 | 0.60 |
| Glycine | required | 0.50 | 0.50 |
| Histidine | 0.45 | 0.30 | 0.28 |
| Isoleucine | 0.70 | 0.60 | 0.56 |
| Leucine | 0.90 | 1.10 | 1.04 |
| Lysine | 0.70 | 0.90 | 0.90 |
| Methionine + Cystine | 0.60 | 0.55 | 0.55 |
| Phenylalanine + Tyrosine | 0.60 | 1.10 | 1.06 |
| Threonine | 0.50 | 0.60 | 0.58 |
| Tryptophan | 0.15 | 0.20 | 0.17 |
| Valine | 0.70 | 0.70 | 0.71 |

A number of studies have investigated the growing rabbit's requirements for lysine, methionine plus cystine, and arginine.

Lysine

Adamson and Fisher's (1973) recommended level of lysine for the growing rabbit is considerably lower than that estimated by Davidson and Spreadbury (1973). Colin (1974) and Colin and Allain (1978) using sesame oil based diets supplemented with synthetic L-lysine suggested a requirement for lysine of 0.65% and 0.60-0.75% of the diet, respectively, based on growth performance and nitrogen balance data. Both of these recommendations agree with Adamson and Fisher's recommendation of 0.70% of the diet. On the other hand Cheeke (1971), using lucerne/wheat based diets supplemented with synthetic L-lysine, and growth performance as a criterion of response, suggested a requirement of 0.93% of the diet and Spreadbury (1978), with inclusion level of 5.0 to 9.4 percent of the diet, obtained a response to lysine up to 0.94% of the diet, on ground-nut meal based diets using growth performance as the criterion of response, supporting Davidson and Spreadbury's recommendation of 0.90% of the diet (Table 1.5.2).

Table 1.5.2 Estimates of dietary lysine requirement (% of the diet) of the growing rabbit.

| | |
|-------------------------------|-------------|
| Cheeke(1971) | 0.93 |
| Adamson and Fisher(1973) | 0.70 |
| Davidson and Spreadbury(1973) | 0.90 |
| Colin(1974) | 0.65 |
| Colin and Allain(1978) | 0.60 - 0.75 |
| Spreadbury(1978) | 0.94 |

Methionine plus Cystine

The requirements for the sulphur-containing amino acids are normally considered together, as a deficiency of cystine can result in it's synthesis from methionine. Thus, an adequate level of cystine will have a sparing effect on the methionine requirement.

There is some variability in the estimates of methionine plus cystine requirements of the growing rabbit (refer Table 1.5.3).

Table 1.5.3 Estimates of dietary methionine plus cystine requirement (% of diet) of the growing rabbit.

| | |
|----------------------------------|-------------|
| Cheeke(1971) | 0.45 |
| Adamson and Fisher(1973) | 0.60 |
| Colin, Arkhurst, and Lebas(1973) | 0.65 - 0.85 |
| Davidson and Spreadbury(1973) | 0.55 |
| Colin(1975a) | 0.60 - 0.65 |
| Prud'hon, Colin and Lebas(1977) | 0.65 |
| Colin(1978) | 0.57 |
| Spreadbury(1978) | 0.62 |

Colin, Arkhurst, and Lebas's early work (1973), using soyabean oil-meal based diets supplemented with various levels of DL-methionine, suggested the methionine plus cystine requirement of the growing rabbit, based on growth performance data, may be as high as 0.85% of the diet. However, Colin's more recent work (1975a and 1978) with lucerne/soyabean meal based diets supplemented with DL-methionine and L-cystine, suggests a lower requirement, based on growth performance data, of between 0.57 and 0.65% of the diet. The work of Adamson and Fisher (1973), Davidson and Spreadbury (1973), Prud'hon, Colin, and Lebas (1977) and Spreadbury (1978), based on various diets supplemented with DL-methionine, using growth performance as the criterion of

response, also report methionine plus cystine requirements to be in the range of 0.55 - 0.65% of the diet. Cheeke (1971) suggested a lower requirement for methionine plus cystine of 0.45% of the diet, based on the growth performance of rabbits on lucerne/wheat based diets supplemented with DL-methionine.

Arginine

Several estimates of arginine requirement have been made and most indicate a high requirement by the growing rabbit, more akin to avian than mammalian species. Table 1.5.4 shows the estimates from the literature.

Table 1.5.4 Estimates of dietary arginine requirements (% of diet) of the growing rabbit.

| | |
|--------------------------------------|-------------|
| McWard, Nicholson, and Poulton(1967) | 0.98 |
| Cheeke(1971) | 0.88 |
| Adamson and Fisher(1973) | 1.00 |
| Davidson and Spreadbury(1973) | 0.70 |
| Colin(1975b) | 0.90 - 1.00 |
| Davidson and Spreadbury(1975) | 0.60 |
| Adamson and Fisher(1976) | 0.60 |
| Spreadbury and Davidson(1978) | 0.56 |

The high dietary requirement is not the result of a lack of urea cycle enzymes (Cheeke and Amberg,1972). The possibility of a lysine-arginine antagonism has been investigated and discarded (Cheeke and Amberg,1972; Colin,1975b). High levels of arginine, up to 2.0%, have no adverse effects, suggesting that the rabbit has a high tolerance for it (Cheeke and Amberg,1972; Adamson and Fisher,1973; Colin,1975b). The more recent studies (Davidson and Spreadbury,1975; Adamson and Fisher,1976; and Spreadbury and Davidson,1978), which were based on diets providing other amino acids at established requirement levels, suggest a lower requirement than earlier work by Adamson and Fisher (1973)and Davidson and Spreadbury (1973), when the requirements for other

amino acids were not established.

Current Recommendations.

Although based on limited data (7 references), the Societe de Chime Organique et Biologique [AEC] (France) have published suggested amino acid requirements for the growing rabbit:Table 1.5.5. The National Research Council [NRC] (U.S.A.) have also published suggested amino acid requirements based on a more extensive review (24 references). Except for lysine and arginine the two committees agree on the recommendations for all amino acids.

Table 1.5.5 National recommendations of the amino acid requirements of the growing rabbit (% of the Diet).

| Amino Acid | NRC(1977) | AEC(1978) |
|-----------------------------|-----------|-----------|
| Lysine | 0.65 | 0.70 |
| Methionine + Cystine | 0.60 | 0.60 |
| Arginine | 0.60 | 0.90 |
| Histidine | 0.30 | 0.30 |
| Leucine | 1.10 | 1.10 |
| Isoleucine | 0.60 | 0.60 |
| Phenylalanine + Tyrosine | 1.10 | 1.10 |
| Threonine | 0.60 | 0.60 |
| Tryptophan | 0.20 | 0.20 |
| Valine | 0.70 | 0.70 |
| Glycine | Required | - |

It should be noted, that in some studies reviewed earlier, higher values have been reported for lysine(0.94%), methionine plus cystine(0.65%), arginine(1.0%), histidine(0.45%), and isoleucine(0.7%) requirements.

The preceding review of published amino acid requirements for the growing rabbit shows that there is variation in the estimates of requirements. Differences in experimental factors such as age, sex, strain of rabbit, feeding level, dietary composition, and methods used are undoubtedly responsible for a great deal of this variation.

1.6 Critical Evaluation of the Methodology Used to Determine Amino Acid Requirements.

Davidson and Spreadbury (1973 and 1975) estimated dietary essential amino acid requirements based on the analysis of diets which supported high levels of growth (40g per day). However, diets giving growth rates which are satisfactory may differ from those on which rabbits will achieve maximal growth rate and feed conversion efficiency. Also, comparisons between diets are complicated by differences in voluntary food intake and in the composition of the body weight gain. Clearly, such an approach is not the most critical and cannot be expected to give precise estimates of amino acid needs (Fuller, 1978).

All other estimates of amino acid requirements reviewed have been based on empirically derived dose-response relationships. In this method animals are given an amino acid in a series of increasing quantities in a diet which theoretically supplies

adequate quantities of all other nutrients, including the other essential amino acids, together with a total nitrogen supply sufficient to allow the synthesis of non-essential amino acids and other nitrogenous substances. The requirement is estimated as the input of amino acid for which maximum response is attained. Some investigators prefer to estimate the minimum input of amino acid which gives a response not significantly less than the maximum. The latter approach gives lower estimates of requirements than those based on the maximum response. The difference can be considerable in those instances where large deviations of amino acid input from the optimum produce only small alterations in response.

Secondly, there is no generally accepted single measurement of response. The determined amino acid requirement varies as to the type of response measured: growth rate, feed conversion ratio, or nitrogen retention.

A third difficulty inherent with the dose-response assay is, that of ensuring the amino acid under study is the only nutrient not supplied in the required amounts. This clearly requires prior knowledge of the requirements for all other nutrients, including all other essential amino acids. Many workers have attempted to overcome this problem by supplying all other amino acids in generous excess. This practice, however, may lead to imbalance effects. The feeding of imbalanced mixtures of amino acids is

likely to affect the growth rate of animals, via feed intake, and/or utilization of the amino acid under investigation (Harper, Benevenga, and Wohlhueter, 1970; Austic, 1978).

For growing rabbits fed ad libitum the energy content of the diet will determine the feed intake (refer section 1.3). Thus, amino acid requirements expressed as a percentage of the diet are imprecise. This problem can be overcome by expressing requirements in terms of grams of amino acid per MJ of energy. Colin and Allain (1978) reported variations in response to lysine with variations in energy content of the diet. They concluded that the rabbit required 2.4g lysine per 1000 kcal (4.2 MJ) of digestible energy. No other authors have expressed amino acid requirements per unit of energy.

A further problem inherent in the dose-response assay is the use of synthetic amino acids supplementary with normal acids from protein. The synthetic amino acids may be absorbed at a faster rate than the amino acids derived from digestion of the intact protein, thereby reducing the efficiency of protein synthesis. Batterham (1980) reviewed the use of free amino acids in mixed diets and concluded, that supplements of free lysine are inefficiently utilized by growing pigs fed once daily and that other synthetic amino acids would be similarly affected. An inefficient use of synthetic amino acids used in grading diets for dose-response assays may lead to over estimation of requirements.

Batterham (1980) also pointed out that the efficiency of use is improved by more frequent feeding (at least twice daily).

In view of the problems associated with determining amino acid requirements by analysis of diets which support optimum growth or dose response assays, it seems desirable to develop a method for estimating amino acid requirements which minimises the difficulties inherent in these approaches. Recently, in the nutrition of the growing pig (Cole 1979 and 1980; Fuller, Livingstone, Baird, and Atkinson, 1979), much attention has been given to the importance of considering the balance between dietary amino acids in determining amino acid requirements.

1.7 The Concept of Ideal Amino Acid Balance.

Cole (1979, 1980) has defined an ideal protein for growth in animals as one which is 'perfectly' balanced in terms of its amino acid content and its supply of non-essential nitrogen for growth. Additions or subtractions of amino acids would not improve its quality. Having determined the ideal balance of amino acids the protein requirements for the growing animal for various levels of production are viewed as requirements for amounts of ideal protein.

Determination of the composition of the ideal protein requires that a reference amino acid be chosen. The optimum levels of all other amino acids are then identified relative to this reference amino acid.

Williams, Curtin, Abraham, Loosli, and Maynard (1954) working with the rat, the chick, and the pig, chose lysine as a reference amino acid for several reasons. Firstly, it is the only amino acid which seems to be required as such in the diet. The keto or hydroxy analogues of the others appear to substitute satisfactorily. Secondly, the only known function of lysine is as a constituent of protein. Thirdly, the lysine requirement for growth appears to have been as extensively studied by nutritional experiments and the absolute requirement for lysine is as firmly established as for any other amino acid.

Having established the ideal amino acid balance, the absolute level of the reference amino acid required may be adjusted in relation to dietary energy content, feed intake and the potential performance standard of the animal.

1.7.1 Approaches to the Estimation of Ideal Amino Acid Balance.

Two approaches have been adopted to estimate the dietary ideal amino acid balance for growth, namely iterative experimental procedures and whole body amino acid composition. The first is reliant on empirically derived information and is based upon the determination of maximum utilization of dietary protein. Fuller, Livingstone, Baird, and Atkinson (1979) used this method to determine the ideal amino acid balance for growing pigs. A barley-based diet was supplemented with synthetic amino acids singularly and then various combinations of synthetic amino acids. Minimum urinary nitrogen was used as the criterion to determine the amino acid balance which led to the maximum protein utilization.

This iterative empirically derived information eliminates some of the problems discussed in section 1.6, as all essential amino acids are considered in relation to each other. It does not, however, necessarily avoid the problems of determining when the maximum response is attained, the possible inefficient use of synthetic amino acids, or the problem of different measurements of

response leading to different conclusions as to the optimum point of response. However, based on Moughan and Smith's work (1984), iterative empirically derived estimates of ideal amino acid balances for the growing pig are reasonably accurate.

In the nutrition of the growing rabbit there are no empirical estimates of ideal amino acid balance determined by iterative experimental techniques. To obtain this information would be costly in terms of both time and resources. Thus, it was considered desirable to evaluate a less costly alternative approach which attempts to estimate the ideal dietary amino acid balance via determination of the whole body amino acid composition.

1.8 The Use of Whole Body Tissue Analysis to Estimate the Ideal Amino Acid Balance for Growth

Based on his own classical studies and from an evaluation of the literature, Mitchell (1950) proposed the theory that an effective method of determining the requirements of a growing animal for dietary essential amino acids may be to firstly determine the requirement in grams per day of a single amino acid, such as lysine, and then to estimate the requirements of the others from the proportion existing between the essential amino acids and lysine in the body of the animal, these proportions to

be determined by amino acid assays of the entire carcass, or, approximately, even by amino acid assays of a dominant tissue such as muscle.

1.8.1 Basis for the use of Body Composition Data.

The function of amino acids in the animal body relates in the main to the replacement of essential tissue constituents that have been degraded in catabolic reactions, and the synthesis of proteins which are continually lost from the body (e.g. hair, intestinal epithelium, enzymes) as well as the formation of new protein in growth.

Mitchell (1950) proposed, that in the rapidly growing animal, the amino acids required for growth would dominate the animals total requirement for amino acids, hence, the composition of the tissues being formed during growth could be used to estimate amino acid requirements. In the mature animal, however, the requirements for maintenance would dominate amino acid requirements. Said and Hegsted (1970) confirmed, that for the rat, the amino acid requirements for maintenance and growth are not the same.

As the amino acid requirements for growth and maintenance differ, the success of Mitchell's proposal depends on the extent to which the formation of new tissue dominates the amino acid requirements of the growing animal. Boorman (1980) presents evidence, that the quantitative effects of maintenance on the pattern of amino acids required by the fast growing animal are small in comparison with deposition in new tissues.

1.8.2 Experimental Evidence.

Willams, Curtin, Abraham, Loosli, and Maynard (1954) investigated the validity of Mitchell's proposal, by comparing estimates of amino acid requirements calculated from carcass analysis with recommendations in the literature based on nutritional experiments.

The rat, the chick, and the pig were selected as animals for study. The carcass amino acid pattern was found to be comparable at different growth periods within each species. Aumaitre and Duee (1974) also reported the pattern of amino acids to be similar from birth to eight weeks of age in the pig and Duee, Calmes, and Desmoulin (1980) reported, that the amino acid composition of muscle crude protein was also unaffected by genotype. For the rat, the two estimates were remarkably similar with the exception of arginine, methionine, and tryptophan. The arginine difference

was not unexpected as the rat can synthesize this particular amino acid in appreciable amounts. If the methionine and cystine requirements were considered together, as they are for nutritional experiments as cystine has a sparing effect on methionine requirements, then the difference between the two methods disappeared. Willams, Curtin, Abraham, Loosli, and Maynard (1954) suggested, that the difference between the two methods in the estimation of tryptophan was due to an underestimation of requirement in the nutritional experiment due to a deficiency of niacin in the experimental diets. No reason was given for the calculated value of histidine being lower than the nutritional determination.

The agreement between the calculated and the nutritionally determined values was not as close for the chick as for the rat. Nevertheless, the correlation was high. Willams, Curtin, Abraham, Loosli, and Maynard (1954) pointed out the tentative nature and variability of the nutritionally determined values of the amino acid requirements of the chick, as a possible cause for the poorer agreement.

Willams, Curtin, Abraham, Loosli, and Maynard (1954) considered the agreement between the calculated and nutritionally determined values of amino acid requirements of the pig to be closer than expected, considering the limited data available from nutritional studies.

Fisher and Scott (1954) used Price, Taylor, and Russell's (1953) data on the amino acid composition of the chick carcass to re-evaluate the chicks' requirements for essential amino acids. They found good agreement between the carcass analysis values and the 1950 National Research Council's recommendations, except for, methionine, lysine, and phenylalanine. Phenylalanine was left out of the comparison due to a lack of nutritional data. When methionine plus cystine requirements were compared, rather than methionine, the agreement was improved. Fisher and Scott (1954) presented evidence, that the lack of agreement for lysine requirements was due to the nutritional estimate being too low.

Fuller, Livingstone, Baird, and Atkinson (1979) investigated the optimum amino acid supplementation of barley for growing pigs. They noted, that barley along with the optimum supplement of lysine, threonine, and histidine suggested a pattern of amino acids which was similar to the whole body protein pattern of pigs reported by Aumaitre and Duee (1974).

Based on work done with other monogastrics (rat, chicken, pig), whole body tissue analysis appears to be a promising technique to determine the ideal amino acid balance for the growing rabbit.

1.9 Summary and Conclusions.

The rabbit's digestive anatomy and physiology is characteristic of monogastrics. There are, however, certain aspects of the rabbit's digestive physiology and anatomy (particularly those related to caecotrophy), which are different from other monogastric species, indicating a need to determine specific nutrient requirements for the growing rabbit.

In theory, it appears that it is possible for the growing rabbit to utilize microbial protein synthesised in the caecum via caecotrophy, suggesting that dietary protein quality may be less important for this species than for other monogastric species. The review of practical experiments, however, showed there was actually nine dietary essential amino acids for rapid growth, and the balance of these amino acids (protein quality) influenced rabbit growth performance.

The review of estimates of amino acid requirements for growth in the rabbit showed variation between workers. A critical review of the methods used to estimate these requirements, indicated a need to develop a method which would minimise the difficulties inherent in the methods used. It was concluded that determining the ideal balance of amino acid required would greatly improve the accuracy of estimates.

In the absence of any iterative empirical estimates of ideal amino acid balance for the growing rabbit, the use of whole body amino acid composition was considered as a first approximation toward determining the ideal dietary amino acid balance. From work done with other monogastric species it was concluded, that whole body tissue analysis appeared to be a reliable technique to determine a first approximation of the dietary ideal amino acid balance for growth in the rabbit.

CHAPTER 2

THE WHOLE BODY AMINO ACID COMPOSITION OF THE GROWING
COMMERCIAL RABBIT2.1 Introduction

From the foregoing review of literature it appears from research undertaken with the pig, the chicken, and the rat that whole body amino acid composition can serve as a useful first approximation towards determining the ideal dietary amino acid balance for these species. Unfortunately as far as the growing rabbit is concerned, not only was there no comparable whole body amino acid composition data available, but no procedure has been established for the processing and chemical analysis of this species for the determination of whole body amino acid composition.

The objective of this study was to determine the amino acid composition of the empty whole body of the growing rabbit. Before doing so, however, it was necessary to establish a method for reducing the empty whole body of the rabbit to representative samples for amino acid analysis. Consequently the study was conducted in two parts.

2.2 Preliminary Study. Establishment of a method for obtaining a representative sample of ground rabbit whole body tissue.

2.2.1 Introduction

A number of workers have described methods of determining the chemical composition of the empty whole body of domestic animals [(Spreadbury, 1978(Rabbit); Aumaitre and Duée, 1974(Pig); Duee, Calmes and Desmoulin, 1980(Pig); Price, Taylor and Russell, 1953(Chicken); Morris and Moir, 1964(Sheep, Cattle, Chicken)]. The methods described involved freezing the empty whole body, then grinding while partially thawed, the whole body, chopped body, or individual joints. Samples were then taken and further prepared for analysis by freeze-drying, extracting the lipid, and fine grinding. On the basis of their reports a method for analysing the empty whole body of the growing rabbit was established.

It was considered desirable, however, to evaluate this method before subjecting it to routine use. In this respect the homogeneity of rabbit empty whole body tissue samples was tested by examining their nitrogen and lipid contents. Lipid and nitrogen determinations were used to ascertain the homogeneity of the samples because these assays are routine and provide reasonably accurate results.

2.2.2 Materials and Methods.

(i) Animals and Treatment.

One 49-day-old New Zealand White rabbit was obtained from a local producer. The rabbit was killed by dislocation of the cervical vertebrae and the digestive tract taken out and cleaned of contents by flushing with water. After removal of excess water the tract was replaced in the body cavity. Care was taken during this procedure to ensure that there was no loss of blood from the body. The empty body was then weighed, sealed in a plastic bag, and placed in deep freeze (-20°C), until required for mincing and chemical analysis of tissue samples.

(ii) Mincing of the Empty Body.

After removal from deep freeze the rabbit empty body was partially thawed by keeping it in a chiller overnight. Subsequently the chilled empty body was weighed to determine if any loss had occurred during the storage period. The whole body was then sawn, by hand, into eight segments. Fur and sawdust produced were collected and added to the sawn pieces of partially frozen empty body.

Each segment of whole body was fed into an industrial grinder (Hobart MFG Co Ltd, London, England) with a 10mm aperture plate. The auger and aperture plate of the grinder were cleaned and the resulting minced tissue was then reground twice, through the same aperture plate, with mixing by hand in between mincings but not cleaning out the grinder. The auger and 10mm aperture plate were then cleaned and the latter was replaced by a 6mm aperture plate. The minced empty body was ground a further four times, with mixing of the mass by hand between each mincing. The auger and aperture plate were cleaned before and after the final grinding. Care was taken to ensure that all segments of the rabbit empty body were thoroughly ground and mixed and that the loss of moisture was kept to a minimum.

(iii) Sampling of Minced Tissues

The minced material obtained from the rabbit empty body was spread out and marked into a grid, comprising six squares of equal dimensions. A grab sample of minced empty whole body was taken from the centre of each of the six squares of the grid, and these were combined to provide one composite sample of approximately 120g. This procedure was repeated a further three times to give four 120g samples of minced empty body in total for independent chemical analysis.

Each 120g sample of minced empty whole body was freeze-dried for 96 hours, weighed, returned to the freeze drier and reweighed at four hour intervals, until the weight remained constant. The freeze-dried samples of minced empty body were then crumbled using a domestic cheese grater, and stored in a desiccator.

(iv) Lipid Extraction and Nitrogen Analysis

The lipid content of each of the four freeze-dried whole body samples was determined six times on 4g sub-samples and a mean lipid content for each sample was then obtained. Lipid extraction was with petroleum ether (boiling point 40-60°C) for seven hours in a Soxhlet apparatus using a standard procedure (Association of Official Analytical Chemists [A.O.A.C.], 1970). Lipid contents were expressed on a freeze-dried basis.

The freeze-dried lipid extracted tissue, pertaining to each initial 120g sample of minced whole body tissue, was then put through a Retsch grinder (Retsch GmbH, 5657 HAAN, West Germany) with a 0.5mm sieve, which produced a fine fluffy powder for nitrogen analysis.

The nitrogen contents of the four freeze-dried, lipid-free samples of rabbit empty whole body were determined in triplicate by the Kjeldahl method (A.O.A.C., 1970).

Residual dry-matter determinations were done on the freeze-dried, lipid-free, whole body tissue so that nitrogen determinations could be expressed on an oven dry-matter basis. For this determination approximately 1g of the freeze-dried, lipid-free finely ground whole body minced tissue was oven dried at 80°C for three hours.

2.2.3 Results and Discussion.

There was no apparent weight loss during the deep freeze storage of the empty body.

The mean lipid content for each of the four freeze-dried empty body tissue samples is given in Table 2.2.1. There was good agreement between the four sample means and the coefficients of variation were low.

Table 2.2.1: The mean (± S.E.) lipid content (g/100g freeze-dried minced tissue) of each of the four rabbit whole body tissue samples.

| Sample No | Lipid Content | Coefficient of Variation(%) |
|-----------|------------------|-----------------------------|
| 1 | 28.29 \pm 0.33 | 2.89 |
| 2 | 28.54 \pm 0.18 | 1.58 |
| 3 | 28.99 \pm 0.11 | 0.94 |
| 4 | 28.84 \pm 0.12 | 1.04 |
| Overall | 28.67 \pm 0.11 | 1.91 |

The mean nitrogen content for each of the four freeze dried samples of empty whole body mince is given in Table 2.2.2. There was good agreement between the four sample means and the coefficients of variation were low. The overall coefficient of variation of the nitrogen content was similar to that for the lipid content (refer Table 2.2.1).

Table 2.2.2: The mean (\pm S.E.) nitrogen content (g/100g lipid-free, dry matter) of each of the four rabbit minced whole body tissue samples.

| Sample No | Nitrogen Content | Coefficient of Variation(%) |
|-----------|------------------|-----------------------------|
| 1 | 12.63 \pm 0.04 | 0.50 |
| 2 | 13.10 \pm 0.06 | 0.77 |
| 3 | 13.05 \pm 0.04 | 0.58 |
| 4 | 12.69 \pm 0.19 | 2.64 |
| Overall | 12.87 \pm 0.08 | 2.09 |

2.2.4 Conclusions

There was low variability in the lipid and nitrogen results obtained from the analysis of minced rabbit whole body tissue samples. On this basis it is considered that the method of whole body preparation described can provide a composite sample of minced tissue which is representative of the whole body of the rabbit.

2.3 Main Study. Determination of the Amino Acid Composition of Rabbit Whole Body Tissue

2.3.1 Introduction

From the previous study (refer section 2.2) it would appear that the mincing and sampling procedure adopted, resulted in representative samples of the empty whole body of the rabbit for chemical analysis.

The second part of this study involved the preparation and analysis of 12 rabbits to determine the whole body amino acid composition of the growing rabbit.

2.3.2 Materials and Methods

(i) Animals

Twelve 53-day-old New Zealand White rabbits (six male and six female) were obtained from the Massey University Small Animal House. The average liveweight was 1528g and the values ranged from 1365 to 1648g. The rabbits had been weaned at 35 days of age and after weaning they were reared in a group and fed a pelleted rabbit grower diet, hay, and water ad libitum.

(ii) Procedure

The twelve rabbits were slaughtered immediately (53 days of age) and their empty whole bodies were minced according to the procedure outlined previously (refer section 2.2.2(iii)).

One sample of minced empty whole body tissue was obtained from each rabbit and this sample was analysed six times for lipid content. The sampling and analytical procedures were the same as those described in section 2.2.2(iv).

The lipid-extracted material pertaining to the one sample of minced whole body tissue of each rabbit was ground for nitrogen and dry matter analyses as described previously (refer section 2.2.2(v)). Nitrogen analyses were conducted in duplicate and multiplied by the factor of 6.25 to give crude protein content.

The amino acid composition of the freeze-dried, lipid-free whole body tissue samples was determined, using a Waters high pressure liquid chromatograph (HPLC) amino acid analyser (Waters, U.S.A.). Duplicate sub-samples of material (120mg) were hydrolysed in 6N HCl for 24 hours at $110^{\circ} \pm 1^{\circ}\text{C}$. Tryptophan being destroyed during acid hydrolysis, was not determined.

The extraction of methionine and cystine from acid hydrolysis is unreliable, as these amino acids are partially destroyed during acid hydrolysis. Accordingly, performic acid treatment was used to oxidise methionine to methionine sulphone and cystine to cysteic acid. Duplicate samples of material (10mg) were oxidised with performic acid (packed in ice) for 16 hours. The performic acid was then evaporated off and the samples were hydrolysed in 6N HCl for 20 hours at $110^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

Nitrogen and amino acid values were expressed on an oven-dry basis.

2.3.3 Results and Discussion

The mean(±SE) lipid content of minced empty whole body for the 12 rabbits was 23.52 ± 0.95 g/100g freeze-dried matter, and the coefficient of variation was 13.97 percent. There was no significant difference ($p > 0.05$) in mean lipid content between the two sexes (Appendix I).

The mean crude protein and amino acid levels in the rabbit empty whole body are given in Table 2.3.1. Generally the standard errors are low, except for valine and glutamic acid. There was no apparent reason for the higher standard errors for these two amino acids. There is reasonably good agreement between the protein content determined by nitrogen analysis (crude protein), and by summing the individual amino acids (79.58 and 72.55 g/100g DM respectively). It was expected, that the total estimated by summing the individual amino acids would be lower than that estimated by crude protein determination, as tryptophan and proline levels were not determined, and therefore not included in the sum of individual amino acids.

The sexes did not differ significantly ($p > 0.05$) in levels of empty whole body crude protein, but there were some small sex differences in amino acid levels of the empty whole body (Appendix II).

Table 2.3.1: The mean (\pm S.E.) crude protein and amino acid levels (g/100g DM) in the rabbit whole body minced tissue (n = 12).

| | |
|----------------------------------|--------------------|
| Crude Protein | 79.58 \pm 0.40 |
| <u>Essential Amino Acids</u> | |
| Lysine | 5.047 \pm 0.098 |
| Histidine | 2.544 \pm 0.019 |
| Isoleucine | 2.570 \pm 0.023 |
| Leucine | 5.667 \pm 0.061 |
| Phenylalanine | 3.655 \pm 0.059 |
| Tyrosine | 2.818 \pm 0.023 |
| Threonine | 3.235 \pm 0.032 |
| Valine | 3.155 \pm 0.211 |
| Arginine | 5.479 \pm 0.073 |
| Methionine | 1.494 \pm 0.063 |
| Cystine | 2.321 \pm 0.073 |
| <u>Non-essential Amino Acids</u> | |
| Aspartic Acid | 6.869 \pm 0.079 |
| Serine | 4.169 \pm 0.042 |
| Glutamic Acid | 11.582 \pm 0.523 |
| Glycine | 6.850 \pm 0.108 |
| Alanine | 5.098 \pm 0.057 |

2.3.4 Conclusion.

There was no comparable data in the literature with which to compare the estimate of rabbit whole body amino acid composition given in Table 2.3.1. The low level of variability, however, does indicate the data given is a reasonable estimate of the rabbit whole body amino acid composition.

CHAPTER 3

COMPARISON OF EMPIRICAL AND THEORETICAL ESTIMATES OF
AMINO ACID REQUIREMENTS FOR THE GROWING RABBIT

3.1 Introduction

Currently, estimates of amino acid requirements of the growing rabbit are based on dose-response assays and analysis of diets on which satisfactory growth was obtained. These estimates, however, are not only variable but suspect as regards their being a measure of amino acid requirements, since the methodology on which they are based, as has been discussed, is far from sound.

As an alternative to the determination of individual amino acid requirements, the concept of an "ideal protein" has been proposed. This approach is currently being adopted in pig nutrition albeit based on limited data from a few iterative-type experiments. As yet in the nutrition of the growing rabbit the approach considering amino acid requirements against the background of an ideal protein has not received attention and consequently there are no iterative empirical estimates of the ideal dietary amino acid balance. As already discussed, however, it is possible that a knowledge of the amino acid composition of

the empty whole body may serve as a first approximation of the ideal dietary amino acid balance for the growing rabbit.

Accepting that the latter may be so, it is of interest to compare the current estimates of empty whole body amino acid composition with the recommendations of NRC(1977) and AEC(1978). Table 3.1.1 shows, that on this basis the NRC(1977) and AEC(1978) recommendations for most of the essential amino acids in rabbit grower diets may be overgenerous.

Table 3.1.1 The balance of essential amino acids, relative to lysine, obtained from rabbit whole body tissue analysis compared with the balance obtained from NRC(1977) and AEC(1978) recommendations (relative to lysine=100).

| | Whole Body Tissue Analysis | NRC Recommendations | AEC Recommendations |
|---------------|-------------------------------|------------------------|------------------------|
| Lysine | 100 | 100 | 100 |
| Histidine | 50 | 46 | 43 |
| Isoleucine | 51 | 92 | 86 |
| Leucine | 112 | 169 | 157 |
| Phenylalanine | | | |
| + Tyrosine | 115 | 169 | 157 |
| Threonine | 64 | 92 | 86 |
| Valine | 63 | 108 | 100 |
| Arginine | 109 | 92 | 129 |
| Methionine | | | |
| + Cystine | 62 | 92 | 86 |
| Tryptophan | - | 31 | 29 |

The present study was undertaken with the prime objective of determining if NRC(1977) and AEC(1978) recommendations of essential amino acids relative to lysine are overgenerous for the growing rabbit, as indicated by the currently obtained estimate of whole body amino acid composition. It was also an objective of this investigation to ascertain how far an estimate of whole body amino acid composition can serve, at least as a first approximation towards the determination of the dietary ideal amino acid balance for rabbit growth. The study aimed to investigate the effect on rabbit growth performance of a reduction in the levels of dietary essential amino acids from NRC(1977), AEC(1978) towards and subsequently below amino acid levels of body composition. In this respect it was planned to systematically reduce amino acid levels relative to a fixed lysine content in a rabbit grower diet prepared from conventional feedstuffs through a progressive reduction in crude protein content and to measure growth performance. The essential amino acid levels in the initial diet were the higher of the NRC(1977), AEC(1978) recommendations.

3.2 Materials and Methods

3.2.1 Animals and Housing.

The experiment was repeated in time to give three replicates. For each replicate 27 five-week-old New Zealand White rabbits were obtained from a local commercial cooperative. The rabbits were weaned at the time of purchase (five-weeks-old) and weighed on average 1027 ± 161 g.

The rabbits were caged individually in an insulated building equipped with forced ventilation. The cages (38cm x 67cm x 36cm high) were constructed entirely from wire and ensured total and separate collection of faeces and urine. Faeces fell through the wire base of the cage onto a perforated plastic collection screen below, which completely surrounded the base of the cage. Urine passed through the faeces collection screen, and was guided by a plastic funnel into a collection vessel (Figure 3.2.1). Each cage was equipped with an ad libitum feed hopper designed to minimise feed wastage and to allow the removal and collection of feed 'fines'. Fresh water was available ad libitum from a water bottle via a nipple drinker.

Water bottle with nipple drinker

Cage

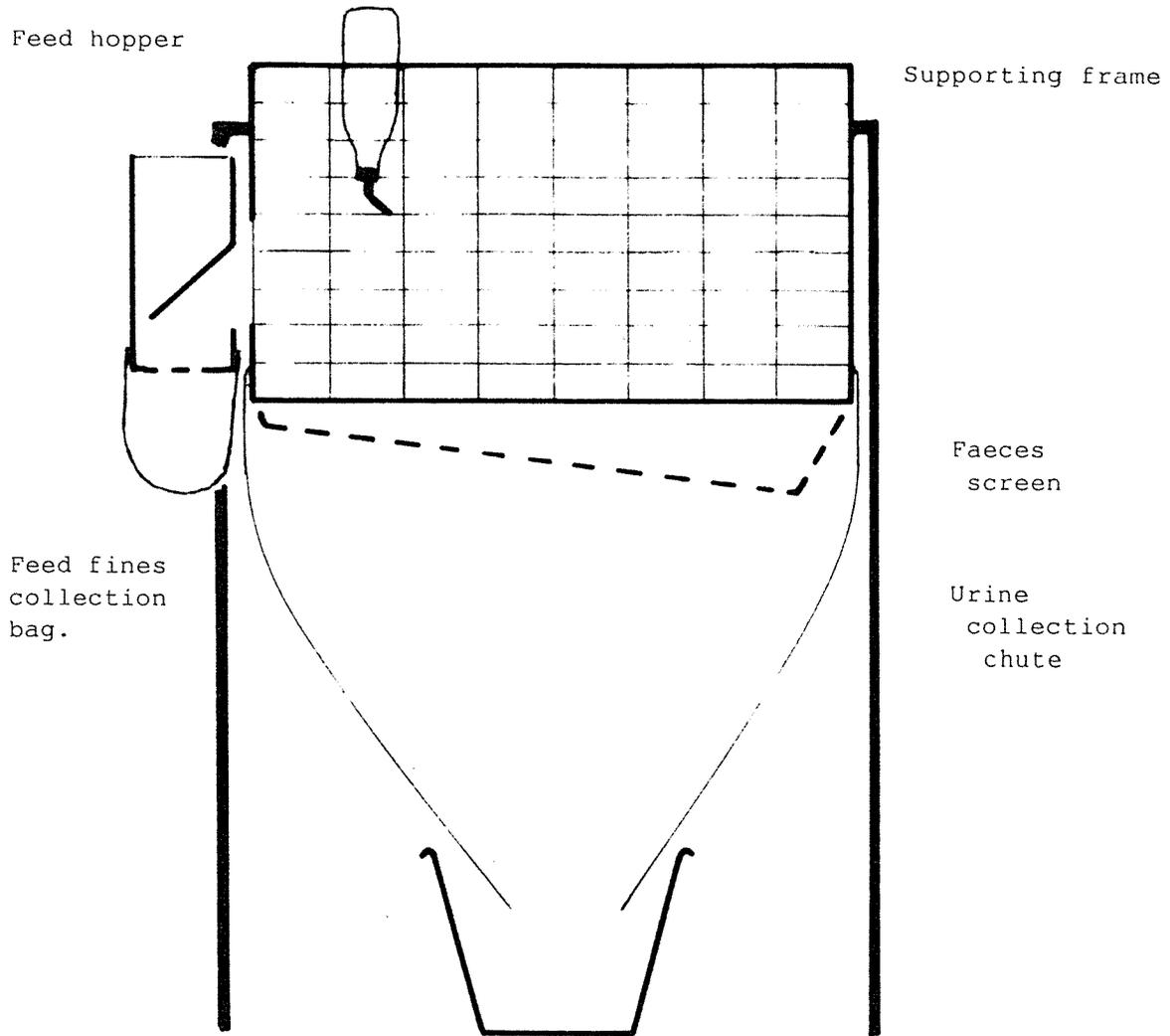


Figure 3.2.1 Diagram of a metabolism cage.

3.2.2 Treatments.

There were nine experimental treatments which comprised iso-caloric and iso-fibrous diets with progressively lower levels of crude protein, but fixed and equal gross levels of lysine, histidine, and tryptophan. Lysine was used here as the base amino acid to specify the ratio of the other dietary essential amino acids.

Gross amino acid levels in the first diet of the series equated with NRC(1977), AEC(1978) recommendations. As the recommendations were in general agreement but not identical, the higher recommendation of the two (except for lysine, as explained later) was taken for each amino acid. Gross amino acid levels in the subsequent eight diets, except lysine, histidine, and tryptophan, progressively diminished such that the gross amino acid levels in the ninth diet of the series were below even the gross levels of amino acids in the rabbit whole body rabbit tissue. Tryptophan was maintained at a constant level across all nine diets, as the tryptophan level in rabbit whole body tissue was not determined, hence, it was not possible to compare empirical and theoretical estimates. In formulating the first diet of the series a minimum value for tryptophan of 2.0g/kg diet was set, being the level recommended by NRC(1977) and AEC(1978). The actual level attained on diet one (2.1g/kg diet) was maintained across all diets. Histidine was also maintained at a constant

level across diets as it was the only amino acid higher in whole body rabbit tissue compared with the recommendations of NRC(1977), and AEC(1978) and was, therefore, considered not to be oversupplied in the latter empirical estimates. The dietary histidine level chosen was 3.3g/kg diet (50 percent of the gross lysine level), which was the value obtained for rabbit whole body tissue, this being higher than NRC(1977) or AEC(1978) recommendations.

The diets were prepared using the same ingredients to contain 159, 146, 140, 130, 128, 123, 111, 106, and 97 g of crude protein per kg of air-dry diet, by gradually reducing the levels of extracted decorticated soya-bean meal, blood meal, and wheat and raising the levels of maize starch and tallow (Table 3.2.1)

Purified cellulose (Avicel, Steetler NZ Ltd, Auckland.) was used to maintain a crude fibre level of 100g per kg air-dry diet throughout the series. Synthetic L-lysine monohydrochloride was added to each diet to maintain a level of 6.5g total lysine per kg air-dry diet throughout the range of diets. This lysine level of 6.5g (NRC,1977) was chosen, rather than the slightly higher AEC (1978) recommendation of 7.0g, to ensure that the balanced protein for maintenance and growth was below the upper limit to body protein retention of the growing rabbit. Synthetic L-histidine was used to maintain a level of 3.6g total histidine per kg of each air-dry diet while synthetic L-tryptophan addition to each

diet ensured that a level of 2.1g of tryptophan per kg air-dry diet was maintained across the nine diets (Table 3.2.1). A proprietary vitamin/mineral supplement (Tasman Vaccines Ltd, Auckland.) was added to each diet. Chromium sesquioxide was also added to each diet at a level of 2.5g per kg of air-dry diet, as it was planned in a separate study to determine the ileal digestibility of crude protein in the series of diets. An adequate quantity of each diet to meet the needs of the trial was mixed and pelleted, and then stored in deep freeze (-10°C) until required.

Table 3.2.2 gives the determined dry matter, crude protein, and gross energy contents of the experimental diets along with the calculated crude fibre levels and apparent digestible energy contents. The determined amino acid composition of the nine experimental diets is shown in Table 3.2.3.

Table 3.2.1. Ingredient composition of the nine experimental diets (g/kg air-dry weight).

| Ingredient | Diet | | | | | | | | |
|-------------------------------------|----------------|-------|-------|-------|-------|-------|-------|-------|----------------|
| | 1 ¹ | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 ² |
| Dried lucerne meal | 167.0 | 167.0 | 167.0 | 167.0 | 167.0 | 167.0 | 167.0 | 167.0 | 167.0 |
| Ground oats | 77.3 | 77.3 | 77.3 | 77.3 | 77.3 | 77.3 | 77.3 | 77.3 | 77.3 |
| Maize meal | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 | 33.3 |
| Wheat meal | 522.5 | 512.5 | 502.5 | 492.4 | 482.5 | 472.5 | 462.5 | 452.5 | 442.5 |
| Maize starch | 0.0 | 20.9 | 41.7 | 62.6 | 83.5 | 104.4 | 125.2 | 146.1 | 167.0 |
| Ex dec soya-bean meal | 120.0 | 105.0 | 90.0 | 75.0 | 60.0 | 45.0 | 30.0 | 15.0 | 0.0 |
| Blood meal | 3.6 | 3.2 | 2.7 | 2.2 | 1.8 | 1.3 | 0.9 | 0.4 | 0.0 |
| Tallow | 20.0 | 22.9 | 25.9 | 28.8 | 31.7 | 34.6 | 37.6 | 40.5 | 43.4 |
| Purified cellulose | 50.0 | 50.7 | 51.4 | 52.8 | 53.5 | 54.2 | 54.2 | 54.9 | 55.6 |
| L-lysine monohydrochloride | 0.0 | 0.6 | 1.3 | 1.9 | 2.5 | 3.2 | 3.8 | 4.5 | 5.1 |
| DL-methionine | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 |
| L-histidine | 0.0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | 1.6 |
| L-tryptophan | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 |
| Dicalcium phosphate | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Salt | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Chromic oxide | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamin/mineral premix ³ | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |

¹ Equates with higher of NRC (1977), AEC (1978) recommendations

² All essential amino acid levels below whole body composition

³ Tasmix Vitamin/Mineral Premix containing: Vitamin A (440 IU); Vitamin D (660 IU); Vitamin E (19 mg); Vitamin B₁ (1.4 mg); Vitamin B₂ (3.5 mg); Vitamin B₆ (4.0 mg); Pantothenic acid (12 mg); Niacin (14 mg); Choline (220 mg); Vitamin B₁₂ (11 mg); Iron (70 mg); Cobalt (0.4 mg); Copper (180 mg); Zinc (50 mg); Selenium (0.15 mg); Manganese (45 mg).

Table 3.2.2. Proximate chemical analysis of the nine experimental diets (g/kg air-dry weight).

| Component | Diets | | | | | | | | |
|--------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Dry Matter | 882.3 | 884.5 | 871.3 | 878.7 | 880.6 | 881.0 | 887.3 | 888.8 | 892.4 |
| Crude Fibre ¹ | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Crude Protein (N x 6.25) | 158.9 | 145.8 | 140.4 | 129.6 | 127.6 | 122.5 | 111.3 | 105.9 | 97.1 |
| Gross Energy (MJ/kg) | 17.36 | 17.43 | 17.32 | 17.34 | 17.53 | 17.46 | 17.47 | 17.52 | 17.67 |

¹ Tabulated values - Poultry Research Centre, Massey University

Table 3.2.3. Determined amino acid composition of the nine experimental diets (g/kg air-dry weight)

| Amino Acid | Diet | | | | | | | | |
|-------------------------|------|------|------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| <u>Essential</u> | | | | | | | | | |
| Lysine | 6.6 | 6.5 | 6.6 | 6.5 | 6.7 | 6.2 | 6.3 | 6.2 | 6.2 |
| Histidine | 3.5 | 3.5 | 3.5 | 3.6 | 3.6 | 3.3 | 3.3 | 3.1 | 3.6 |
| Isoleucine | 5.0 | 4.5 | 4.5 | 3.9 | 3.7 | 3.6 | 2.9 | 2.5 | 2.6 |
| Leucine | 10.7 | 9.8 | 9.5 | 8.4 | 8.2 | 7.7 | 6.8 | 6.1 | 5.7 |
| Phenylalanine | 6.9 | 6.3 | 6.1 | 5.5 | 5.4 | 5.1 | 4.4 | 4.0 | 3.6 |
| Tyrosine | 4.5 | 4.2 | 4.1 | 3.7 | 3.5 | 3.3 | 2.9 | 2.7 | 2.4 |
| Threonine | 5.4 | 5.0 | 4.8 | 4.2 | 4.1 | 3.9 | 3.4 | 3.1 | 2.8 |
| Valine | 6.4 | 6.0 | 5.7 | 5.1 | 4.8 | 4.6 | 3.7 | 3.3 | 3.4 |
| Arginine | 8.9 | 8.0 | 7.7 | 6.8 | 6.4 | 6.1 | 5.2 | 4.6 | 4.1 |
| Methionine | 3.1 | 3.1 | 2.9 | 2.6 | 2.6 | 2.4 | 2.4 | 2.1 | 2.1 |
| Cystine | 2.7 | 2.6 | 2.6 | 2.5 | 2.4 | 2.2 | 2.0 | 2.1 | 1.8 |
| Tryptophan ¹ | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 |
| <u>Non-essential</u> | | | | | | | | | |
| Aspartic acid | 12.9 | 12.0 | 11.6 | 9.9 | 9.7 | 9.2 | 7.9 | 7.0 | 6.1 |
| Serine | 7.4 | 6.8 | 6.6 | 5.8 | 5.7 | 5.4 | 4.9 | 4.5 | 3.9 |
| Glutamic acid | 28.2 | 26.0 | 25.8 | 23.3 | 23.0 | 21.9 | 19.5 | 18.4 | 16.2 |
| Glycine | 6.6 | 6.0 | 5.5 | 4.9 | 4.8 | 4.5 | 4.1 | 3.7 | 3.4 |
| Alanine | 6.4 | 5.8 | 5.5 | 4.9 | 4.8 | 4.5 | 4.1 | 3.7 | 3.4 |

¹ Tabulated values - Poultry Research Centre, Massey University

3.2.3 Experimental Procedure.

(i) Growth Trial.

During the first eight days the diet was gradually changed from commercial pellets and hay to the allocated experimental diet. Within each replicate the rabbits were allocated to the diets at random. Following the eight-day preliminary period the rabbits were weighed (15.00h) and placed on trial. Each replicate of the trial was of 40 days duration, this being divided into five periods each of eight days.

The scale of feed intake was restricted to 5.00 percent of body weight. The rabbits were weighed at the end of each eight-day period at 15.00h and the feeding level was adjusted for the following eight-day period. The rabbits were fed approximately half their daily ration at 09.00h and the remainder at 19.00h. All spillages, feed refusals (feed remaining in hopper prior to the 09.00h feeding of a new collection period), and 'fines' were collected and weighed at the end of each eight-day period.

(ii) Metabolism Trial

The procedure was as described above except that during the third eight-day period of each replicate urinary and faecal outputs were collected from each rabbit. Faeces were collected daily on days six, seven, and eight and urine daily on days seven and eight. Any spillages, feed refusals, and 'fines' on days five, six, and seven were collected and weighed separately from those over the whole eight day period. Urine was collected over acid (10 M sulphuric acid x 2.5 percent urine volume), weighed and stored in deep freeze (-10°C) until required for chemical analysis. Total faecal outputs were weighed at the end of the three-day collection period and frozen (-10°C) until required for chemical analysis.

During the fifth eight-day period of each replicate faecal outputs were collected from each rabbit. Faeces were collected daily on days six, seven, and eight. Any spillages, feed refusals, and 'fines' on days five, six, and seven were collected and weighed separately from those over the whole eight day period. Total faecal outputs were weighed at the end of the three-day collection period and frozen (-10°C) until required for chemical analysis.

Representative samples of the air-dry diets collected during the first metabolism period were ground in a hammermill with a 1.0mm sieve (Glen Creston, Stanmore, England.) On removal from deep freeze the total faeces output of each rabbit collected during the first metabolism period was well mixed and a sub-sample of approximately 35g taken. Any rabbit hair adhering to the surface of the faecal pellets was removed. Approximately 10g of the sub-sample was freeze-dried for 96 hours and ground in the hammer mill with a 1.0mm sieve, to await analysis of its nitrogen content. The remainder of the sample was crushed and mixed in a mortar and then sampled for the determination of dry matter, organic matter, and gross energy contents. The faecal outputs collected during the second metabolism period were mixed and sub-sampled as for the first period, but only nitrogen contents were determined.

It was critical for this study that, the daily amount of dietary balanced protein available for growth and maintenance in the rabbit be lower than the genetic limit to rabbit whole body protein retention. In this respect it can be shown, assuming an average rabbit body weight during the present trial of 1700g, and a daily food intake of 5.00 percent of body weight (85g), an average apparent faecal nitrogen digestibility of 0.80 (NRC,1977) and a chemical score of 0.93 (0.65 g/kg lysine supplied in diet/0.70 recommended by NRC,1977), that a rabbit of average body weight on the diet with the highest nitrogen level (diet 1, 2.54

percent nitrogen) would have received around 1.6g of nitrogen daily for maintenance and growth. The latter figure is lower than literature estimates for nitrogen retention in growing New Zealand White rabbits of 1.75g nitrogen per day (Colin,1975b) and 1.92g (Colin and Allain,1978).

3.2.4 Analytical Methods.

(i) Dry Matter.

Duplicate determinations were performed on samples of feed(1.5g), and faeces(0.5g). The samples were placed in pre-weighed Pyrex beakers and weighed to the nearest 0.1mg. The samples were oven dried at 80°C for three hours. After cooling in a dessicator, the beakers and their contents were weighed. Dry matter content was expressed as a proportion of the weight of the original sample.

(ii) Gross Energy.

Duplicate determinations were performed on feed and faeces. Analyses were performed on an adiabatic bomb calorimeter (A.Gallenkamp and Co.Ltd.,Christopher St.,London.). The air-dry feed samples were pelleted before combustion whereas fresh faeces were wrapped in cellophane to enhance combustion. Gross energy

values were expressed in megajoules(MJ) per kg.

(iii) Total Nitrogen.

Duplicate determinations were performed on feed, faeces, and urine. Total nitrogen content was determined using a Kjelec Auto 1030 Analyzer (supplied by Tecator, Sweden). The method is based on the Kjeldahl method (Hiller, Plazin, and Van Slyke, 1948). The material was digested in hot concentrated sulphuric acid in the presence of potassium sulphate and selenium as a catalyst. This was followed by automated distillation of ammonia into boric acid and subsequent titration against standardised 0.2N sulphuric acid. Total nitrogen values were expressed on a dry matter basis for feed and faeces samples and as a percentage of fresh weight for urine samples.

(iv) Urea.

The concentration of urea in urine samples was measured colorimetrically on an autoanalyser (Technicon Instruments Corp., Tarrytown, New York, U.S.A.). The method is based on the reaction of urea and diacetyl monoxime in the presence of thiosemicarbazide under acid conditions. For a detailed description, refer Marsh, Fingerhut, and Miller (1965). A standard curve was derived by analysing a series of urea solutions of known concentrations. The concentration of urea in each sample was determined with reference

to this standard curve. Initial studies with the autoanalyser demonstrated that the repeatability between samples was such as to eliminate the need for duplication.

(v) Creatinine.

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

(vi) Amino Acids.

Amino acid compositions were determined on duplicate samples of feed. The method of analysis was the same as that described in chapter 2 (refer section 2.3.2(ii)).

3.2.5 Analysis of Data.

(i) Measurements.

Daily growth rates of the rabbits (g/day) over the period of the trial were obtained following the subtraction of initial from final bodyweights and division of this figure by 40, the length of each replicate of the trial.

Feed conversion ratios were calculated by dividing the weight of pellets consumed (g) over the trial period by the liveweight gain over that period and expressing the outcome as g feed consumed per g of liveweight gain.

The faecal collection pertaining to period three of replicates one and three was used to determine the dietary apparent dry matter, nitrogen, and energy digestibilities of the experimental diets. The faecal collection pertaining to period five of replicates one and three was used to determine the apparent nitrogen digestibilities only. Total urine output in period three of replicates one and three followed by the analysis of its nitrogen and urea content, permitted the calculation of daily urinary nitrogen (mg/kg liveweight/g nitrogen intake/day) and daily urea excretions (mg/kg liveweight/g nitrogen intake). Urine and faecal nitrogen outputs pertaining to period four of

replicates one and three were used to calculate nitrogen retention values.

$$NR = \frac{(N_I - [N_u + N_f])}{N_I}$$

where:

NR is nitrogen retention (g/day)

N_I is nitrogen intake (g/day)

N_u is urinary nitrogen (g/day)

N_f is faecal nitrogen (g/day)

Urinary creatinine excretions (mg/kg liveweight/day) were also determined for period three of replicates one and three of the trial respectively.

(ii) Statistical.

All the measured data described in the previous section were subjected to analysis of variance for a randomised block design layout (Snedecor and Cochran, 1980). The linear model was of the form:

$$y_{ij} = \mu + d_i + b_j + (db)_{ij} + e_{ij}$$

where:

y_{ij} is the observation on the i^{th} rabbit in the j^{th} block

μ is the general mean;

d_i is the effect of the i^{th} diet;

b_j is the effect of the j^{th} block;

$(db)_{ij}$ is the interaction between the i^{th} diet and the j^{th} block; and

e_{ij} is the residual effect which is assumed to be normally distributed with mean zero and variance σ^2 .

Where a significant effect of treatment was found in the digestibility data, the method of least significant difference (Little and Hills, 1978) was used to test which differences between means were statistically significant.

Repeated measures analysis (Morrison, 1976) for a randomised block design was used to test whether or not there were any differences in apparent faecal digestibility of crude protein between treatments, replicates, and/or time periods (periods three and five).

Intersecting regression lines were fitted to the growth rate, feed conversion ratio, urinary nitrogen, and urea excretion data, by the method of least squares, to satisfy either of two theoretical responses. The common features of the two responses were a period of linear response and a plateau. Depending on the

response parameter, the linear response was either a positive or a negative relationship, and a constraint was imposed in making the second regression line horizontal to form a plateau (Morris,1983).

Several non-linear curves were also fitted to the growth rate, feed conversion ratio, urinary nitrogen, urea nitrogen, and creatinine excretion data. The curve of best fit (residual sums of squares), was chosen. Routine E4FDF NAG FORTRAN LIBRARY ROUTINE was used to fit the curves (Numerical Algorithms Group, 1983). The curves were differentiated so that slopes at given points could be calculated.

3.3 Results.

3.3.1 Growth Performance.

(i) Rabbit Health.

One rabbit on diet seven died during the experiment, the cause was unknown. A further three rabbits were removed from trial. Two of these rabbits, from diets six and nine respectively, consistently had low feed intakes. As they appeared healthy it is probable that they simply did not settle to individual cages and/or the experimental diets. The third rabbit removed from the trial was from diet seven. It failed to grow and was an excessive fur chewer. It was slaughtered and sent for post mortem but no cause was found for the failure to gain weight. The other 77 rabbits involved in the experiment settled into their individual cages, accepted the experimental diets and remained in good health for the duration of the trial. Fur chewing did occur to varying degrees among one third of the rabbits, but it was not confined to any particular treatment.

(ii) Daily Feed Intake.

Over the 40-day trial period there were no significant differences ($p < 0.05$) in daily feed intake between treatments (Table 3.3.1). There was a significant difference ($p < 0.01$)

between replicates, but no diet by replicate interaction (refer Appendix III). The overall mean(\pm SE) daily feed intake was 4.70 ± 0.02 percent of liveweight, indicating low wastage and/or refusals when compared to the daily amount of feed offered (5.00 % of liveweight).

Table 3.3.1. Least squares means (\pm SE) for daily feed intake of the rabbits over the 40-day trial period (as % of liveweight).

| Diet | No. of Rabbits | Daily Feed Intake |
|----------------------|----------------|-------------------|
| 1 | 9 | 4.73 ± 0.04 |
| 2 | 9 | 4.78 ± 0.04 |
| 3 | 9 | 4.71 ± 0.04 |
| 4 | 9 | 4.76 ± 0.04 |
| 5 | 9 | 4.68 ± 0.04 |
| 6 | 8 | 4.70 ± 0.04 |
| 7 | 7 | 4.74 ± 0.05 |
| 8 | 9 | 4.61 ± 0.04 |
| 9 | 8 | 4.63 ± 0.04 |
| Significance Level | | N.S. |
| (between treatments) | | |

In this and subsequent tables N.S. = Not significant.

(iii) Growth Rate and Feed Conversion Ratio.

At the commencement of the trial the mean(+SE) overall liveweight of the rabbits was $1278 \pm 129\text{g}$. There were no significant differences between treatments or replicates in initial liveweight of the rabbits (refer Appendix IV).

The growth rates and feed conversion ratios of the rabbits over the 40-day period of the trial are given in Table 3.3.2. Over the period of the trial growth rates and feed conversion ratios were significantly ($p < 0.05$, $p < 0.01$ respectively) influenced (refer Appendix V) by dietary treatment (Table 3.3.2).

When an intersecting regression line model was fitted to the growth and feed conversion ratio data, predicted points of intersection between the horizontal plateau and a linear response were found for the two variables. Figure 3.3.1 shows that as the crude protein level of the diet was reduced from 159 to 114g per kg, which was accompanied by a widening of the ratio between lysine, and the other amino acids (except histidine and tryptophan which were held constant), there was no decline in growth rate. Below 114g of crude protein per kg of diet, i.e. between diets six and seven, growth rate declined linearly from 27.4 g/day to 22.1 g/day on diet nine which contained 97g crude protein per kg of diet. The regression equation (Figure 3.3.1, Table 3.3.3) shows a decrease of 0.3 g/day in growth rate for every one percent

reduction in dietary crude protein content below the predicted break point at 114g crude protein per kg of diet. As shown in Figure 3.3.1, the break point for the feed conversion ratio data occurred at a dietary crude protein level of 117 g/kg i.e.between diets six and seven. The mean feed conversion ratios were inversely proportional to the mean liveweight gains.

Table 3.3.2 Least squares means (\pm SE) for growth rate (g/d) and feed conversion ratio (g feed/g liveweight gain) for the rabbits over the 40-day trial period.

| Diet | No of Rabbits | Growth Rate | Feed Conversion Ratio |
|------|---------------|----------------|-----------------------|
| 1 | 9 | 27.2 \pm 1.3 | 2.83 \pm 0.08 |
| 2 | 9 | 27.6 \pm 1.3 | 2.89 \pm 0.08 |
| 3 | 9 | 28.0 \pm 1.3 | 2.91 \pm 0.08 |
| 4 | 9 | 27.3 \pm 1.3 | 2.90 \pm 0.08 |
| 5 | 9 | 26.1 \pm 1.3 | 2.95 \pm 0.08 |
| 6 | 8 | 27.7 \pm 1.4 | 2.95 \pm 0.09 |
| 7 | 7 | 26.5 \pm 1.6 | 2.92 \pm 0.11 |
| 8 | 9 | 24.3 \pm 1.3 | 3.20 \pm 0.08 |
| 9 | 8 | 22.0 \pm 1.4 | 3.26 \pm 0.09 |

| | | |
|----------------------|--------|--------|
| Significance Level | p<0.05 | p<0.01 |
| (between treatments) | | |

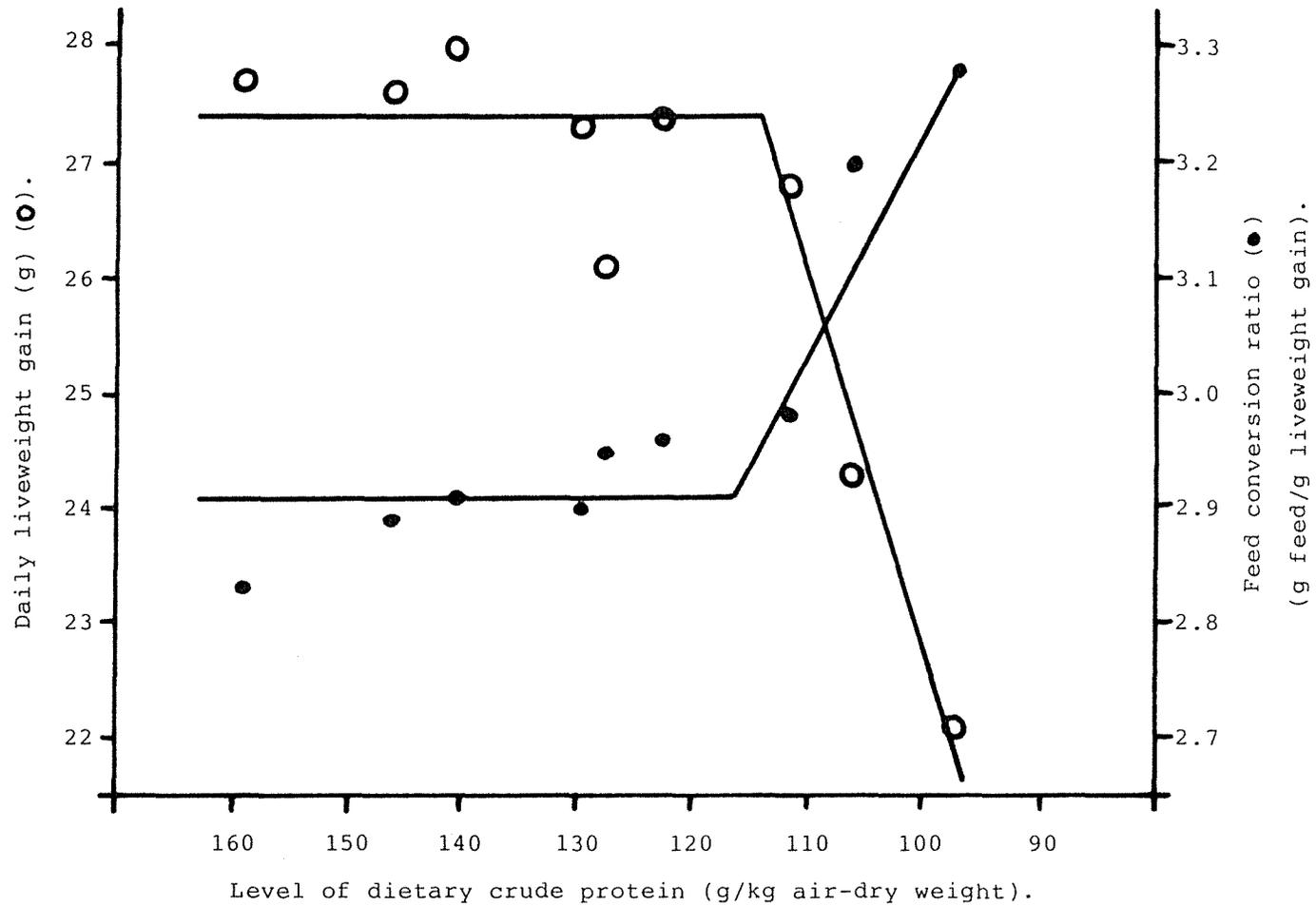


Figure 3.3.1 Application of an intersecting regression line model to dietary treatment means for growth rate and feed conversion ratio over the 40-day trial period.

Table 3.3.3. Application of an intersecting regression line model to dietary treatment means for growth rate and feed conversion ratio over the 40-day trial period.

| | | Variation explained (%) | Residual sum of squares | Predicted break point (g crude protein/kg air-dry diet) |
|--|-------------------------------------|-------------------------|-------------------------|---|
| Daily liveweight gain (g) | $y = -9.453 + 0.323x$ $y = 27.4$ | 91.77 | 2.522 | 114.1 |
| Feed conversion ratio (g feed/g liveweight gain) | $y = 5.244 - 0.020x$ $y = 2.91$ | 90.06 | 0.018 | 116.7 |

y = parameter of response, and x = crude protein level in the diet

The growth and feed conversion ratio data are shown plotted in Figure 3.3.2, where curves giving the best fit to the data were applied as outlined previously (section 3.2.5(ii)). The curves and their differentials are shown in Table 3.3.4. From the differential equations slopes were calculated at 10 g crude protein per kg of diet intervals (refer Appendix VI). These indicated that the slope began to change rapidly below the dietary crude protein level of 120g per kg diet i.e.between diets six and seven for the growth rate data. For the feed conversion ratio data the change in slope occurred in the same region(between diets six and seven), but it was less pronounced than for the growth rate data (refer Figure 3.3.2).

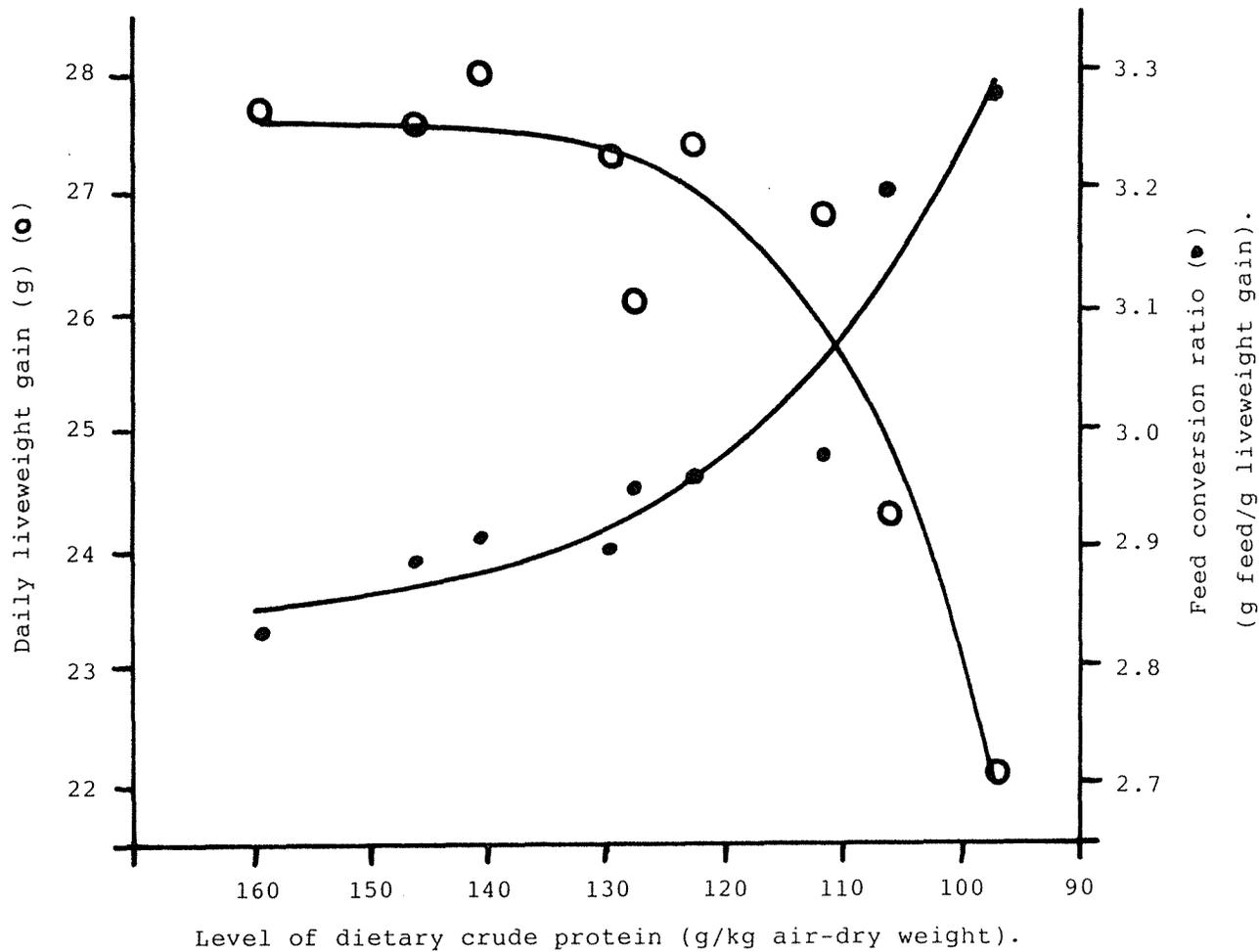


Figure 3.3.2 Application of curves to dietary treatment means for growth rate and feed conversion ratio of the rabbits over the 40-day trial period.

Table 3.3.4. Application of curves to dietary treatment means for growth rate and feed conversion ratio of the rabbits over the 40-day trial period.

| | Curve | Residual sum of squares | Variation explained (%) | Differential |
|---|---|-------------------------------|-------------------------------|--|
| Daily liveweight gain (g) | $y = \frac{1}{1 + be^{-cx}}$ $a = 27.6472$ $b = 2490.4474$ $c = 0.0946$ | 3.04 | 90.08 | $\frac{dy}{dx} = \frac{abce^{-cx}}{(1 + be^{-cx})^2}$ $a = 27.6742$ $b = 2490.4474$ $c = 0.0946$ |
| Feed conversion ratio (g feed/g liveweight gain) | $y = a + be^{-c(x - 150)}$ $a = 2.8343$ $b = 0.0309$ $c = 0.0510$ | 0.014 | 92.27 | $\frac{dy}{dx} = -bce^{-c(x - 150)}$ $a = 2.8343$ $b = 0.0309$ $c = 0.0510$ |

3.3.2 Metabolism Study.

All rabbits remained healthy during the metabolism trials. As shown in Table 3.3.5 mean daily feed intakes were below the daily level of feed offered (5.00 % of liveweight), but were similar across the dietary treatments (refer Appendix VII).

Table 3.3.5 Least squares means(+SE) for daily feed intake of the rabbits (as % liveweight) during the period of the metabolism trials (three days).

| Diet | No. of Rabbits | Daily Feed Intake |
|----------------------|----------------|-------------------|
| 1 | 6 | 4.26 ± 0.05 |
| 2 | 6 | 4.29 ± 0.05 |
| 3 | 6 | 4.28 ± 0.05 |
| 4 | 6 | 4.28 ± 0.05 |
| 5 | 6 | 4.21 ± 0.05 |
| 6 | 6 | 4.19 ± 0.05 |
| 7 | 6 | 4.28 ± 0.05 |
| 8 | 6 | 4.11 ± 0.05 |
| 9 | 6 | 4.27 ± 0.05 |
| Significance Level | | N.S. |
| (Between treatments) | | |

Mean values for daily urinary nitrogen, urea, and creatinine excretions for rabbits on the nine diets are shown in Table 3.3.6. As the rabbits were on diets differing in nitrogen concentration, dietary nitrogen intakes were not constant for all the rabbits. Accordingly, the urinary nitrogen and urea data were expressed in terms of mg urinary metabolite excretion per kg of liveweight per g nitrogen intake. Statistical analysis of the data (refer Appendix VIII) showed that, urinary nitrogen, urea, and creatinine excretions were significantly ($p < 0.01$, $p < 0.01$, and $p < 0.01$ respectively) influenced by dietary treatment.

Table 3.3.6 Least squares means (\pm SE) for daily urinary nitrogen, urea (mg/kg liveweight/g nitrogen intake) and creatinine (mg/kg liveweight) excretion for the rabbits on the nine diets.

| Diet | Nitrogen | Urea | Creatinine |
|--|----------|--------|------------|
| 1 | 240 | 413 | 45.05 |
| 2 | 211 | 314 | 40.45 |
| 3 | 168 | 268 | 39.09 |
| 4 | 170 | 265 | 34.58 |
| 5 | 154 | 243 | 34.98 |
| 6 | 167 | 264 | 36.40 |
| 7 | 139 | 196 | 31.35 |
| 8 | 146 | 195 | 33.50 |
| 9 | 109 | 176 | 29.44 |
| S.E. | 14 | 28 | 2.53 |
| Significance Level (between treatments) | p<0.01 | p<0.01 | p<0.01 |

When an intersecting regression line model was fitted to the urinary nitrogen and urea data, predicted points of intersection between the linear response and the horizontal plateau were found for the two variables. Figure 3.3.3 shows that, as the dietary crude protein level was reduced from 159 to 113g per kg diet, which was accompanied by a widening of the ratio between lysine and the other amino acids (except histidine and tryptophan which were held constant), urinary nitrogen excretion declined linearly. Below a dietary crude protein level of 113 g per kg diet, urinary nitrogen excretion was constant. The regression equation (Figure 3.3.3, Table 3.3.7) indicates an increase of 2.2 mg/kg liveweight/g nitrogen intake in daily urinary nitrogen for every one percent increase in crude protein content of the diet above the predicted break point at 113g crude protein per kg diet (i.e.between diets six and seven). Urea excretion (Figure 3.3.4) showed a very similar trend to the urinary nitrogen with a break point at 112g of crude protein per kg diet (i.e.between diets six and seven). The regression equation (Figure 3.3.4, Table 3.3.7) indicated an increase of 4.1 mg/kg liveweight/g nitrogen intake in daily urea excretion for every one percent increase in crude protein content of the diet above the predicted break point at 112g crude protein per kg diet.

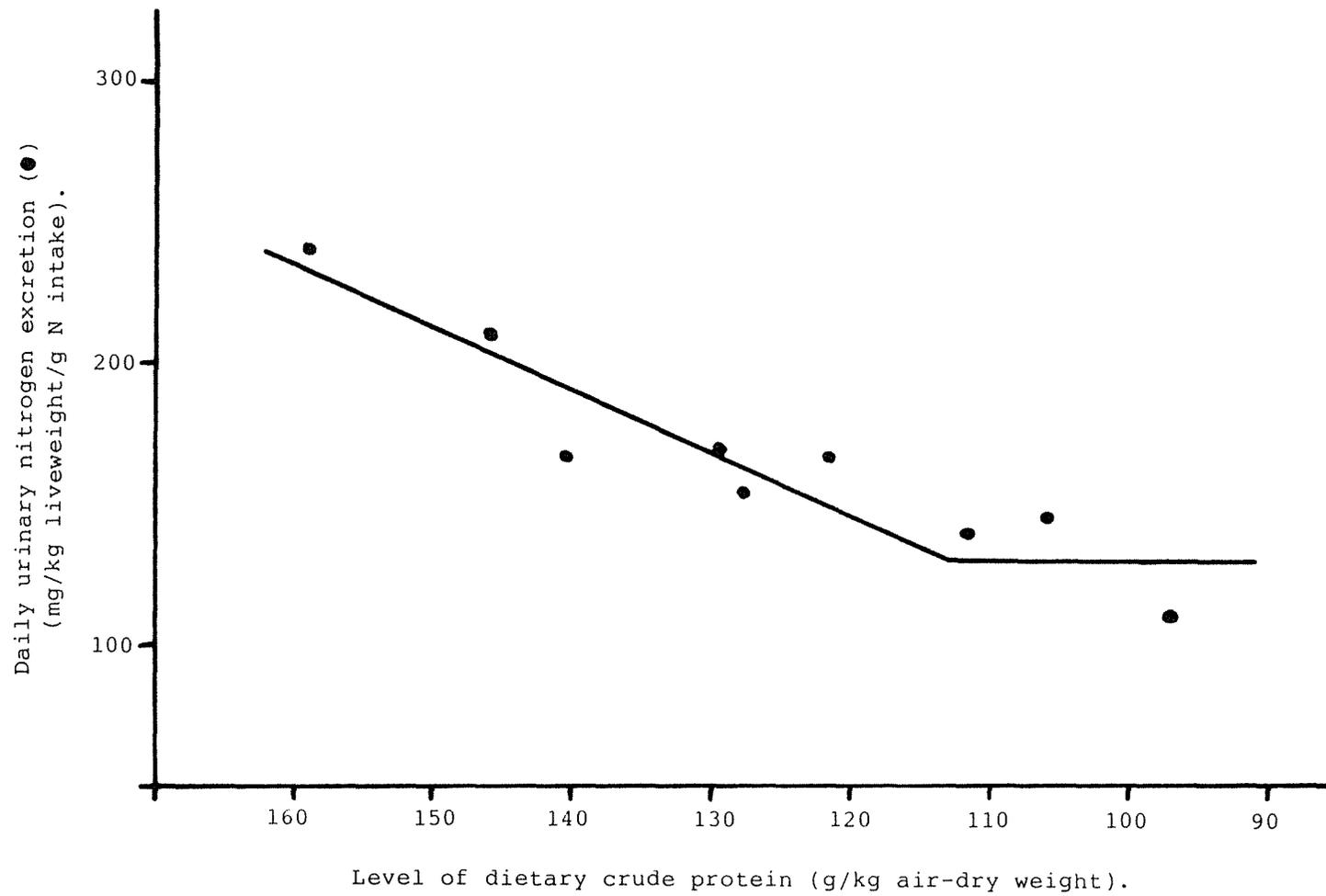


Figure 3.3.3 Application of an intersecting regression line model to dietary treatment means for daily urinary nitrogen excretion.

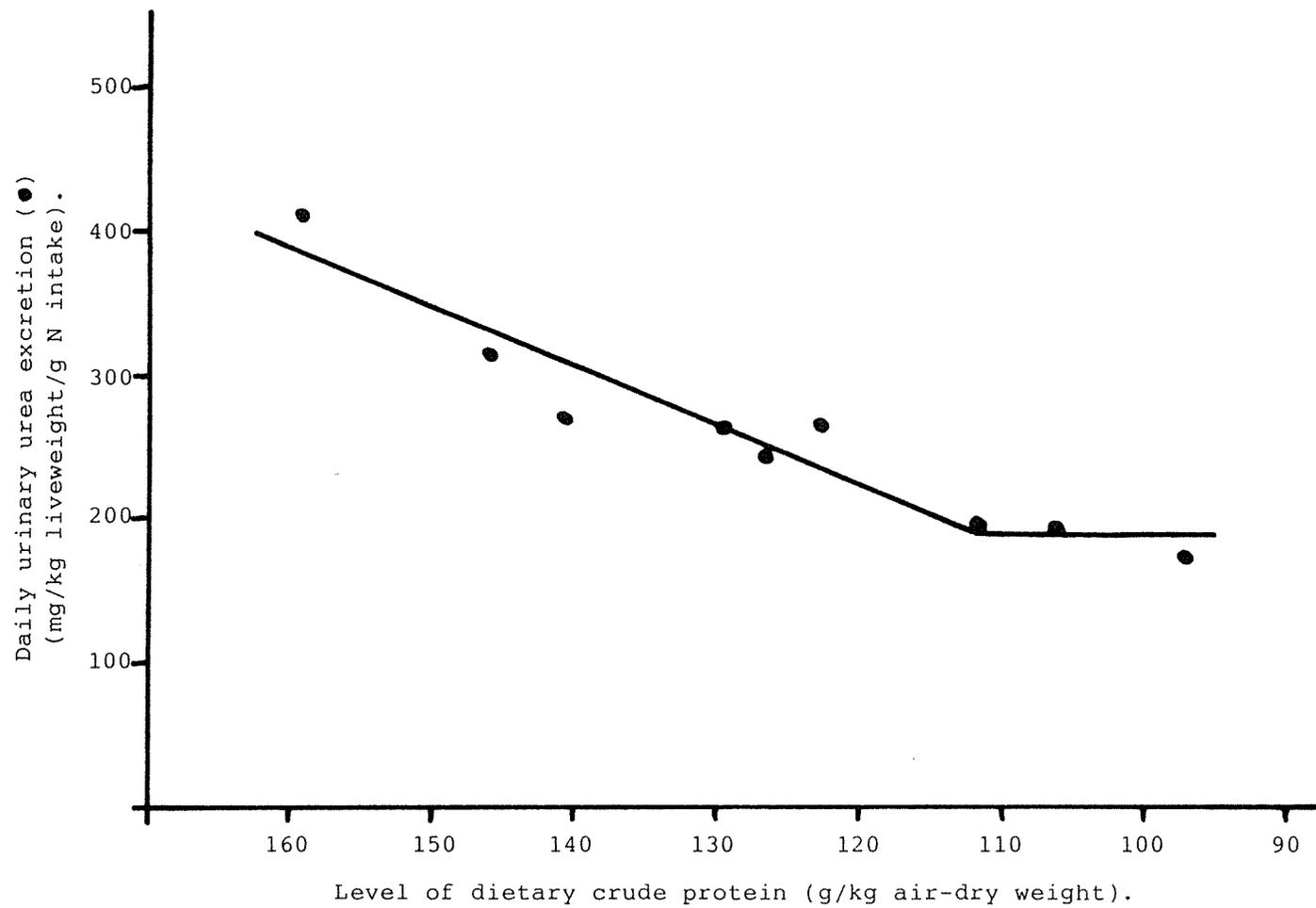


Figure 3.3.4 Application of an intersecting regression line model to dietary treatment means for daily urinary urea excretion.

Table 3.3.7. Application of an intersecting regression line model to dietary treatment means for daily urinary nitrogen and daily urinary urea excretions.

| | | Variation Explained (%) | Residual sum of squares | Predicted break point (g crude protein/kg air-dry diet) |
|--|--|-------------------------------|-------------------------------|--|
| Daily urinary nitrogen excretion (mg/kg liveweight/g N intake) | $y = - 117.02 + 2.20x$ $y = 131.67$ | 87.16 | 15.30 | 113.0 |
| Daily urinary urea excretion (mg/kg liveweight/g N intake) | $y = - 173.66 + 4.13x$ $y = 189.00$ | 90.42 | 40.35 | 112.0 |

y = parameter of response and x = crude protein level in the diet

The urinary nitrogen and urea excretion data are shown plotted in Figures 3.3.5 and 3.3.6 where curves giving the best fit to the data were applied as outlined previously (section 3.2.5(iii)). The curves and their differentials are shown in Table 3.3.8. From the differential equations slopes were calculated at dietary crude protein intervals of 10g/kg (refer Appendix IX). Although the slope decreased from 159 to 97g crude protein per kg diet, there was no obvious point at which the slope changed for the urinary nitrogen or the urea data (refer Appendix IX).

A linear model was fitted to the daily urinary creatinine excretion data (Figure 3.3.7). Over the dietary crude protein range of 97 to 159 g crude protein per kg diet, urinary creatinine excretion decreased by 2.3mg per kg liveweight for every one percent decrease in dietary crude protein.

The urinary creatinine data are also shown plotted in Figure 3.3.8, where the curve giving the best fit to the data was applied. The curve and the differential are shown in Table 3.3.8. From the differential equation slopes were calculated. The slope of the curve decreased very little over the nine diets (159 to 97g crude protein per kg air-dry diet) (refer Appendix IX). On comparing the percentage variation explained by fitting a curve(92%) and a linear model(91%), the two models were found to fit the data equally well.

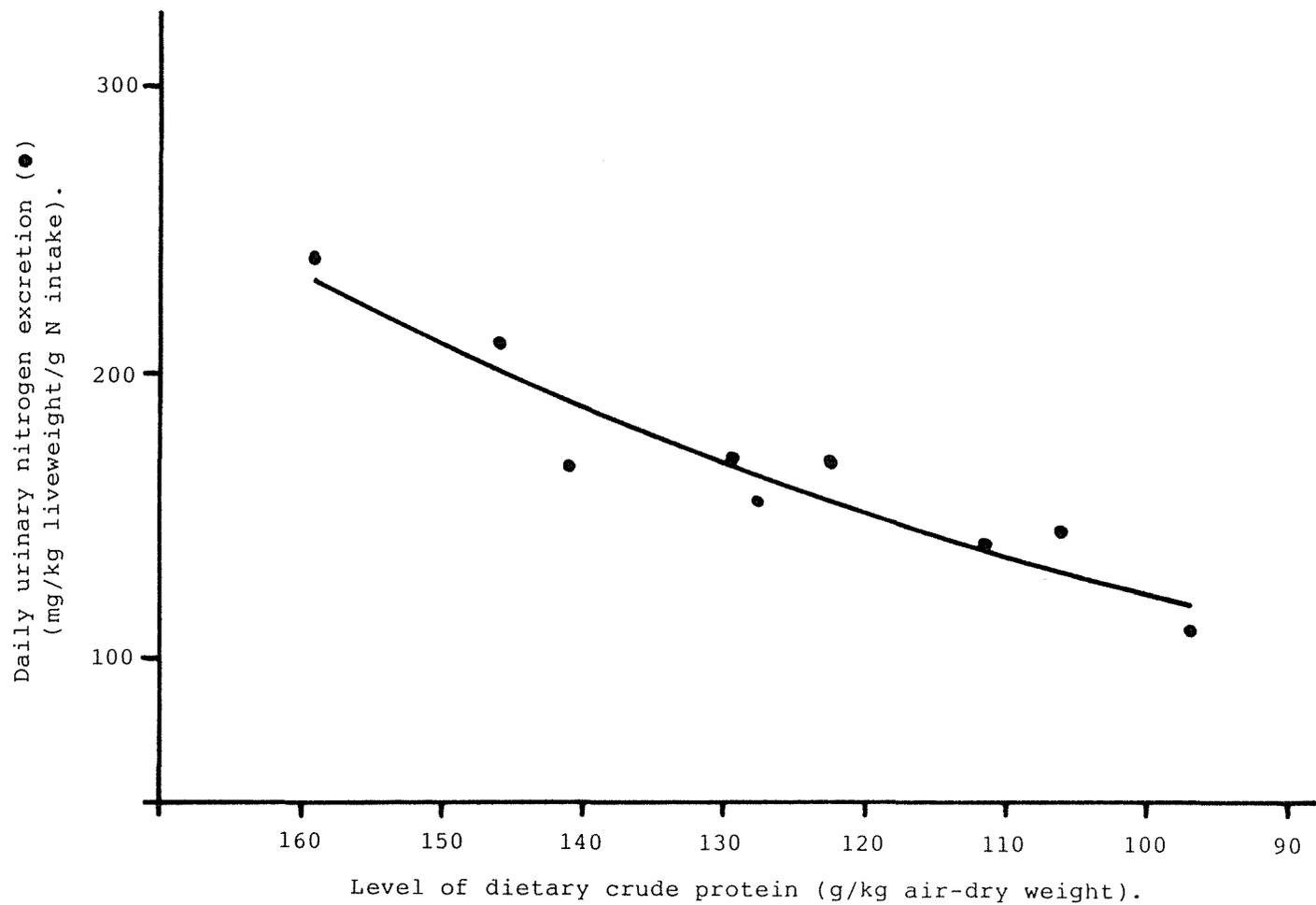


Figure 3.3.5 Application of a curve to dietary treatment means for daily urinary nitrogen excretion.

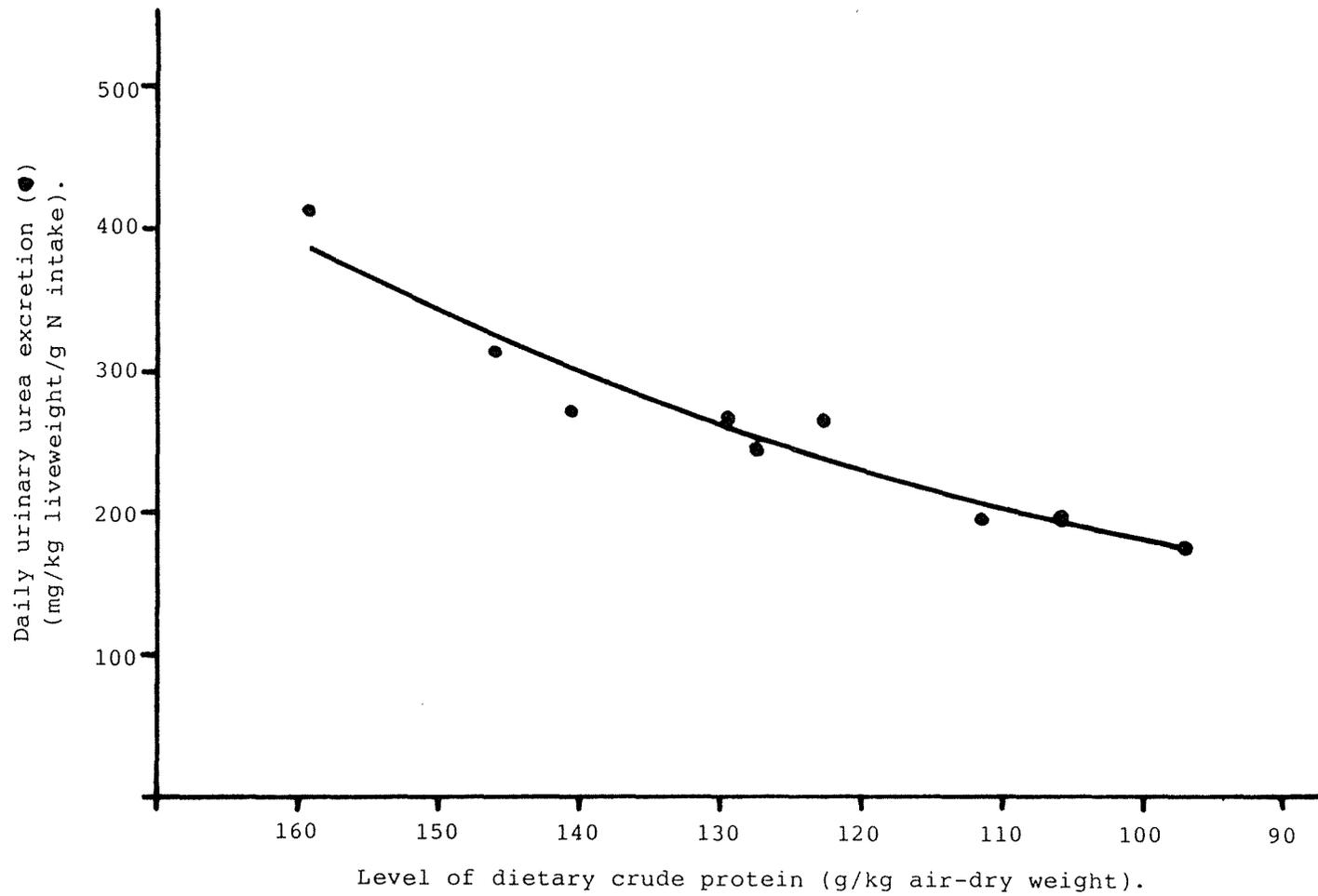


Figure 3.3.6 Application of a curve to dietary treatment means for daily urinary urea excretion.

Table 3.3.8. Application of curves to dietary treatment means for daily urinary nitrogen, urea, and creatinine excretions.

| | Curve | Residual sum of squares | Variation Explained (%) | Differential |
|--|---|-------------------------------|-------------------------------|---|
| Daily urinary nitrogen excretion (mg/kg liveweight/g N intake) | $y = ae^{bx}$ $a = 41.6791$ $b = 0.0108$ | 1220 | 89.76 | $\frac{dy}{dx} = abe^{bx}$ $a = 41.6791$ $b = 0.0108$ |
| Daily urinary urea excretion (mg/kg liveweight/g N intake) | $y = ae^{bx}$ $a = 49.4024$ $b = 0.0129$ | 3049 | 92.76 | $\frac{dy}{dx} = abe^{bx}$ $a = 49.4024$ $b = 0.0129$ |
| Daily urinary creatinine excretion (mg/kg liveweight) | $y = ae^{bx}$ $a = 16.1190$ $b = 0.00631$ | 14.07 | 92.41 | $\frac{dy}{dx} = abe^{bx}$ $a = 49.4024$ $b = 0.0129$ |

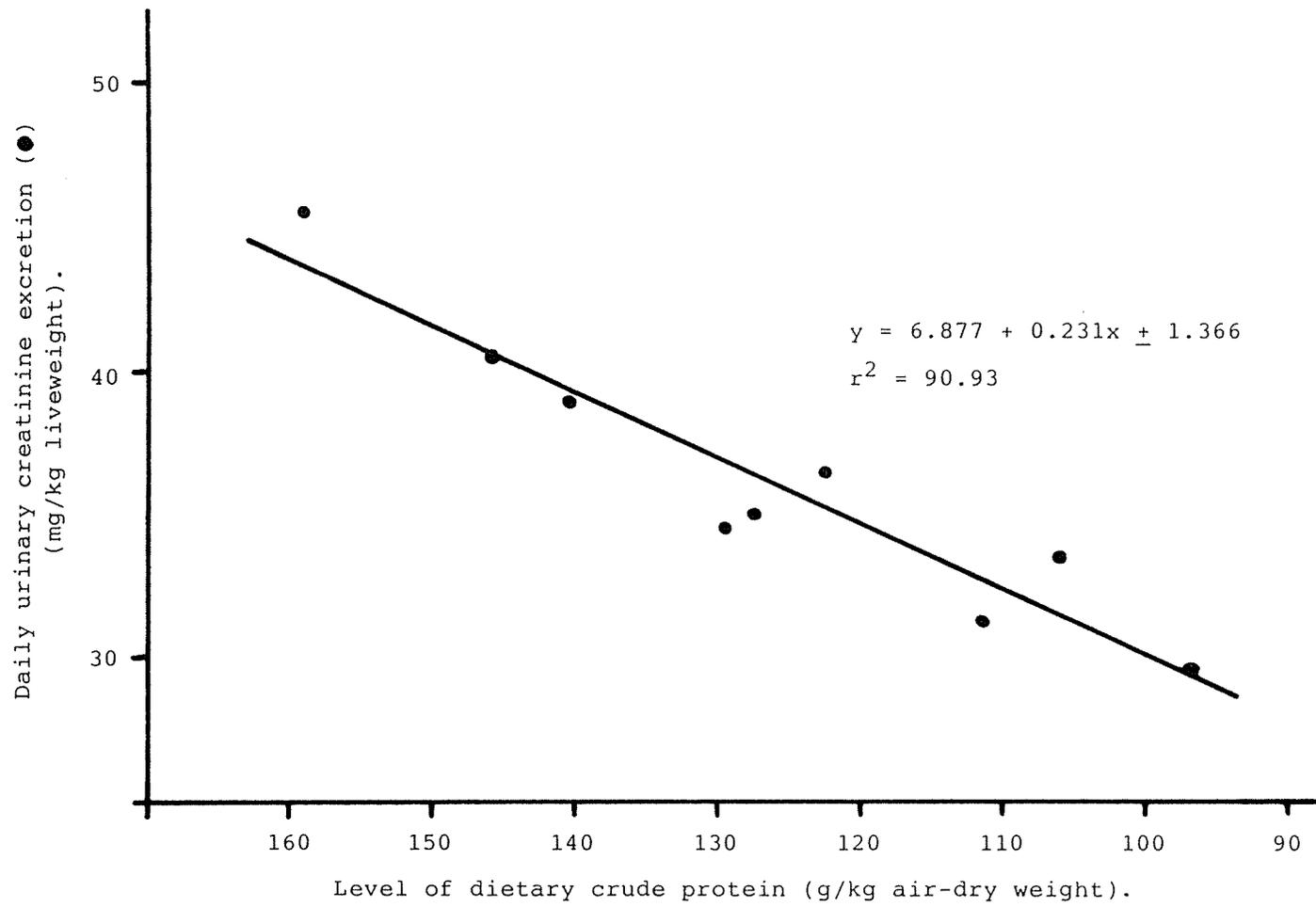


Figure 3.3.7 Application of a regression line model to dietary treatment means for daily urinary creatinine excretion.

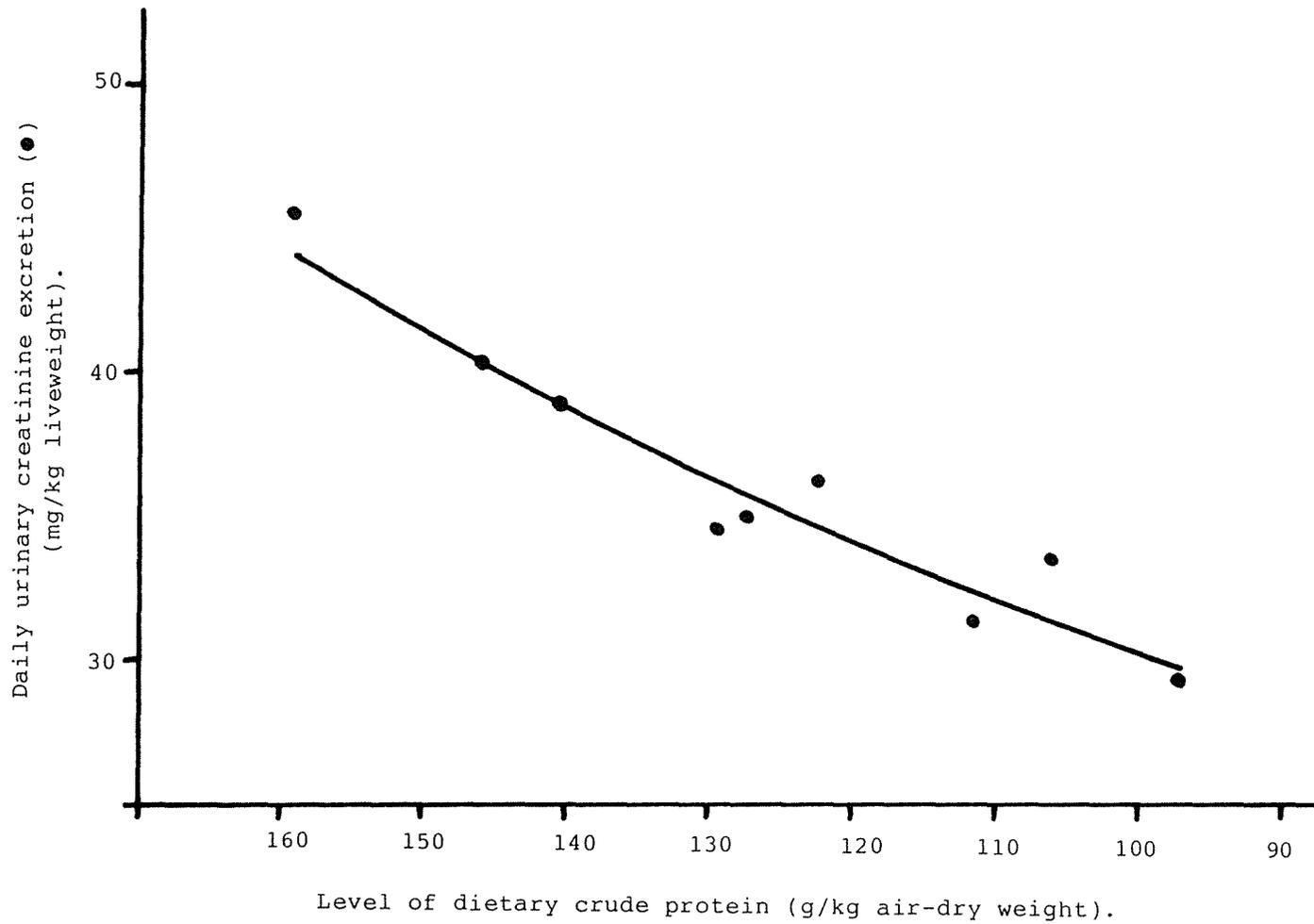


Figure 3.3.8 Application of a curve to dietary treatment means for daily urinary creatinine excretion.

During the metabolism study individual rabbit faecal outputs were collected daily as described (section 3.2.3(ii)) This permitted the calculation of dietary apparent digestible energy contents and the apparent faecal digestibility of the crude protein fraction of the diet.

The apparent crude protein digestibility data (refer Appendix X) proved to be highly variable between rabbits within diets, between diets, and between collection periods. Overall the mean(\pm SE) dietary crude protein digestibility was 79.73 ± 0.51 % [C.V.=4.26%] (n=108). The high variability may be the result of the relatively short collection period (three days) adopted. Of prime importance in this study was that dietary crude protein digestibility did not show a linear trend across the nine dietary treatments (refer Appendix X).

Nitrogen retention data relating to the nine diets were affected by the high variability of the apparent crude protein digestibility and were therefore also highly variable (refer Appendix XI). Overall the mean(\pm SE) nitrogen retention was 0.774 ± 0.021 g per day [C.V.=20.4%] (n=54).

The apparent faecal energy digestibility data were also variable. The overall mean(\pm SE) dietary apparent digestible energy value was 13.14 ± 0.76 MJ per kg diet [C.V.=7.30%] (n=54). Although, some significant differences between the diets were

detected (refer Appendix XII) there was no linear trend between diets one to nine of the series.

3.4 Discussion.

This study was undertaken with the objective of identifying the applicability of current NRC(1977) and AEC(1978) estimates of amino acid requirements for growth in the rabbit. The measurement of rabbit whole body amino acid composition introduced the concept of ideal amino acid balance and also served to indicate that current estimates of amino acid requirement may be overgenerous. The study is discussed firstly in terms of the experimental design adopted and secondly with reference to the results obtained.

3.4.1 Experimental Design.

It was considered important that the diets were formulated to be iso-caloric, so that dietary energy level was not influencing treatment effects. Each diet was formulated to contain 12.80 MJ DE per kg diet, based on tabulated pig data for New Zealand feed ingredients (Poultry Research Centre, Massey University), in the absence of comparable information for the growing rabbit. A reasonably high dietary energy level was chosen, based on a review of recommendations, such as to minimize the deamination of amino acids for the supply of energy.

The actual overall mean apparent faecal digestible energy level measured during the metabolism trial was 13.14 MJ per kg diet, which was close to the formulated value of 12.80 MJ DE per kg of diet. It was significant, however, that the determined digestible energy values of the diets were not constant across treatments. This may have been due to the procedure adopted in formulating the diets (reduction in diet protein level by gradually reducing the levels of soya bean meal, blood meal, and wheat and increasing the levels of maize starch, and tallow), leading to a linear trend in dietary digestible energy content across treatments. In this respect, although the differences in digestible energy content of the diets were statistically significant, they were nevertheless small (the greatest difference between any two successive diets was 0.5 MJ DE per kg of diet) and there was no linear trend in digestible energy across the nine dietary treatments.

There was no apparent reason for these erratic differences in digestible energy content between diets prepared from similar ingredients. Thorbek and Chwalibog (1981), however, reported a marked variation in measuring diet digestibility in the growing rabbit. They suggested that the observed high individual variation may be associated with the practice of caecotrophy. Lang (1981) in a review article also noted that there was wide variation in the digestibility coefficients of some commonly used feedstuffs in rabbit diets.

These reports of difficulties in the measurement of diet digestibility in the growing rabbit, combined with the fact that the faeces collection period in the present study was short (three days), led to the conclusion that the erratic differences in nutrient digestibility observed between the diets were probably due to experimental variation rather than real differences.

It was critical in this study, that the daily balanced dietary protein available for growth and maintenance in the rabbit over the 40-day trial period be provided at a level below the rabbits upper genetic limit to daily body protein deposition. Hence, a growth response to an amino acid becoming limiting would be immediate and complete.

Prior to the conduct of the trial it was estimated, that a rabbit on the diet with the highest protein level would be receiving around 1.6g of nitrogen daily for maintenance and growth. This level was below those reported in the literature for nitrogen retention in growing New Zealand White rabbits of 1.75g nitrogen per day (Colin, 1975b) and 1.92g (Colin and Allain, 1978). Nitrogen retention values measured during the metabolism period were variable and subject to the inaccuracies discussed in the previous section. The overall mean nitrogen retention was 0.774g per day, with the highest record nitrogen retention being 1.139g per day. Although the individual nitrogen retention values were variable they do indicate, that even the highest nitrogen

retention was well below the potential of the growing rabbit reported in the literature (Colin, 1975b; Colin and Allain, 1978).

Of further importance in the design of the present experiment, was that the levels of dietary tryptophan and histidine relative to lysine be held constant across the nine diets. The histidine level given by NRC(1977) and AEC(1978) was lower than that found in the rabbit whole body tissue, and on this basis may have been limiting for growth. Accordingly, the histidine level of the diets was held constant and based on the rabbit whole body tissue level. In the absence of a rabbit whole body tissue value for tryptophan, the dietary level of this amino acid was also held constant across diets and was provided according to the highest of the NRC(1977), AEC(1978) recommendations. The latter recommendation for tryptophan requirement of the growing rabbit was based on the analysis of diets which supported high levels of growth (Davidson and Spreadbury, 1973). Hence, if anything this requirement is likely to be overgenerous, not limiting. As mentioned earlier the supplementation of diet protein with synthetic amino acids may lead to an inefficient use of the free synthetic acids, due to differences in rate of absorption in comparison with those occurring naturally in the protein. Batterham (1984) estimated, that only 50 percent of synthetic lysine is utilized when fed in combination with acids from natural proteins once daily to pigs. Batterham (1980) suggested, that frequent feeding, at least twice

daily, would increase the efficiency of use of free synthetic amino acids. Partridge, Low, and Keal (1985) confirmed that twice daily feeding improved the efficiency of dietary nitrogen utilization in the presence of free lysine when compared to once a day feeding. Batterham (1984) estimated, that twice daily feeding increases the utilization of free lysine to at least 80 percent. It was also noted, that although little work has been done on the utilization of other free synthetic amino acids they are likely to be affected by the feeding regime in a similar manner (Batterham, 1984).

The rabbits on trial were fed their diets, which contained synthetic amino acids, twice daily and it was also noted, that they consumed each meal over a period of several hours. This feeding regime combined with periodic consumption of the diet should have minimized the effect of synthetic amino acids being inefficiently used in combination with naturally occurring amino acids in the dietary protein.

The analysis of the rabbit growth response and metabolism data also poses problems with respect to the most appropriate statistical technique to use. Growth performance and urinary nitrogen and urea excretion data were analysed using an intersecting regression line technique. It was assumed there was a linear response until a threshold value, at which the response abruptly ceases. Although this model may apply to individuals in

a population, the response of those individuals will vary. The integration of a set of intersecting regression line responses necessarily passes through a curve (Morris, 1983). Whenever the response of a number of similar animals is being examined a model incorporating this curvilinear response would ideally be applied.

According to Morris (1983), the consequence of fitting an intersecting regression line model to a set of experimental data is almost always to underestimate the optimum dose. In the present study, however, rather than trying to determine an exact response point, the area of response was of greater consequence. It was assumed here, that an amino acid became limiting somewhere around the predicted break point, but, that further investigation would be required to identify the exact point where the break occurred.

Curvilinear functions were also fitted to the growth, feed conversion, urinary nitrogen excretion, and urinary urea excretion data and a comparison made with the linear regression models. Although, fitting a curve to the data may be more correct, difficulties can arise in determining over what range of inputs there is a change in the response curve. The calculation of slopes of the tangents and visual assessment can be used to assess a region in which the slope of response changes. These methods, however, rely on individual interpretation of what is a significant change. Curves may also predict continued small

responses in a region where it is very doubtful whether any response is occurring, thus overestimating the optimum dose (Morris,1983).

In summary, although intersecting regression line models and curvilinear models are subject to limitations in determining a point of optimum input of a nutrient, both statistical models are considered to be useful in determining a range of dietary amino acids in which the dietary ideal amino acid balance for the growing rabbit lies. Further iterative empirical experiments will be required to determine the actual optimum balance.

3.4.2 Growth performance and metabolism data.

If the dietary essential amino acid levels, relative to lysine, recommended by NRC(1977) and AEC(1978) are overgenerous, as indicated by the whole body amino acid composition of the rabbit, then it is to be hypothesised, that these dietary levels may be reduced without affecting rabbit performance until such time as an amino acid other than lysine, (and in the case of this study histidine or tryptophan which were held constant) becomes limiting. Further decreases in the level of the first limiting amino acid are expected to lead to concomitant decreases in rabbit performance. Also, if rabbit whole body amino acid composition can serve, at least as a first approximation, towards the

determination of the dietary ideal amino acid balance for rabbit growth, then it is to be hypothesised that any decline in performance would occur when dietary amino acid levels, relative to lysine, fall below those of the whole body composition.

Although the first diet was formulated, using tabulated values, to have essential amino acid levels equal to or above those recommended by NRC(1977) or AEC(1978), an analysis of the diets showed, that only phenylalanine and tyrosine were above the levels recommended. All other essential amino acids (except for tryptophan for which there were no determined values) were below NRC(1977) and AEC(1978) recommendations in diet one of the series. Therefore, if the NRC(1977) and AEC(1978) recommendations were accurate, then it would be expected, that the growth performance of the rabbits would have declined over the range of the dietary treatments. There was, however, no linear decline observed in daily liveweight gain nor in the efficiency of conversion of feed to bodyweight over the range of diets.

Both the intersecting regression line and curvilinear analysis of the growth response data indicated, that an essential amino acid component or possibly the non-essential amino acid component, became limiting around a crude protein level of 115g per kg diet. This indicates, that NRC(1977) and AEC(1978) amino acid recommendations, relative to lysine, for the growing rabbit are overgenerous.

Of NRC's(1977) crude protein recommendation for the growing rabbit of 160g per kg diet, 40 percent of the protein was essential amino acids, compared to AEC's(1978) recommendation of 150g per kg diet of which 45 percent of the protein was essential amino acids. The remaining 60 and 55 percent of the crude protein requirement, as given by the NRC(1877) and AEC(1978) respectively, was therefore for non-essential amino acids or nitrogen for the synthesis of non-essential amino acids. The crude protein content of the nine dietary treatments in this study was composed of 42 percent essential amino acids, the remaining 58 percent being non-essential amino acids or nitrogen available for their synthesis. The supply of non-essential amino acids as a proportion of the total crude protein should not, therefore, have become limiting at the dietary crude protein level of around 115g per kg, when growth performance was first observed to decline. The response detected was most probably due to a dietary essential amino acid level limiting growth performance.

On comparing the two models of response, it is of note, that they predicted the same area of response and statistically both models fitted the data equally well. This supports the argument that either model may be used to determine an approximate area of response.

It is of significance to compare the dietary amino acid composition, whereupon the growth performance of the rabbits had begun to decline, with that of rabbit whole body tissue. Figure 3.4.1 relates the body amino acid composition values to the dietary crude protein scale employed in the experiment, and illustrates the dietary protein level at which each essential amino acid recommendation, as indicated by whole body amino acid composition was met.

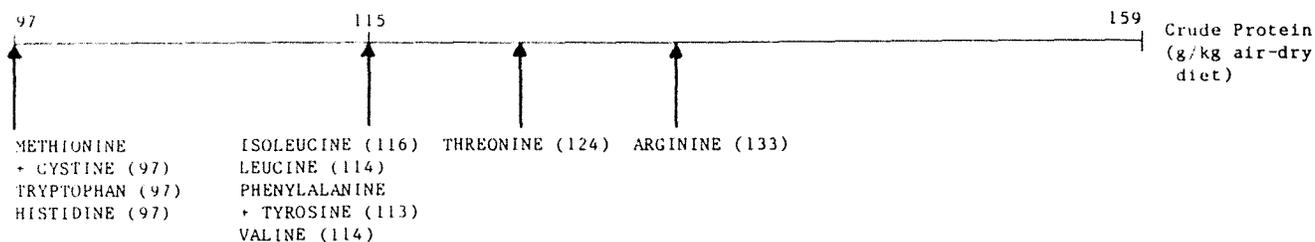


Figure 3.4.1 Relationship between the amino acid recommendation as determined from whole body tissue analysis and the crude protein levels of the experimental diets (values for individual amino acids indicate the level of dietary crude protein which would meet requirements for these amino acids as indicated by whole body tissue analysis).

Although the level of methionine plus cystine decreased as the dietary crude protein level declined, it did not fall below the level required, relative to lysine, as suggested by whole body amino acid levels (refer Figure 3.4.1). Tryptophan and histidine were maintained at a constant level, considered to be adequate, across the range of crude protein levels. All other essential

amino acid levels fell below those recommended by whole body tissue analysis at an approximate crude protein level of 115 g per kg air-dry diet, the area around which a decline in growth performance was detected, or in the case of threonine and arginine at a higher dietary crude protein level, namely 124g and 133g of crude protein per kg diet respectively. This indicates, that arginine or threonine could have been the amino acid which limited growth performance, around the dietary crude protein level of 115g per kg.

Whole body amino acid composition indicates, that arginine would have become limiting below the dietary crude protein level of 133g per kg. Growth performance, however, did not decline until well below this level, which would suggest that whole body amino acid composition overestimates the requirement for arginine in the growing rabbit. One possible explanation for body composition overestimating the arginine requirement, would be if the rabbit was able to synthesize arginine to supplement its dietary requirement. Whether the rabbit is able to synthesize arginine is not clear. Cheeke and Amberg (1972) reported that the rabbit does produce the urea cycle enzymes and can theoretically synthesize arginine. Adamson and Fisher (1971), however, consider that arginine is an essential amino acid, not semi-essential, for growth in the rabbit. The growth performance data from this trial indicated, that the higher AEC(1978) recommendation for arginine is overgenerous and that the actual requirement for arginine,

relative to lysine, lies below the level suggested by whole body amino acid composition. This supports the suggestion that the rabbit can synthesize arginine to supplement its dietary requirement.

Based on the intersecting regression line analysis it would appear, that the threonine requirement suggested by the whole body tissue analysis was overgenerous. As mentioned earlier, however, the so called 'break point' has been used to indicate a region rather than a point of response. The curves fitted to the growth performance data demonstrate, that this region of response covers a range of crude protein levels. Based on the growth performance curves and Figure 3.4.1, threonine may well have been the first amino acid to limit growth performance as the dietary crude protein level decreased across the range of experimental diets.

The growth performance data would suggest, that whole body amino acid composition may be used as a first approximation towards determining the ideal dietary amino acid balance for the growing rabbit. A comparison of the ratio of essential amino acids relative to lysine in the growing rabbit carcass and the growing pig carcass (refer Table 3.4.1) are not dissimilar suggesting, that protein quality is as important in the nutrition of the growing rabbit as it is in the growing pig.

Table 3.4.1 Amino acid composition of ideal protein for the growing rabbit and the growing pig, as indicated by whole body tissue analysis (lysine = 100).

| Amino Acid | Rabbit (present study) | Pig (Aumaitre and Duée, 1974) |
|---------------|---------------------------|----------------------------------|
| Lysine | 100 | 100 |
| Histidine | 50 | 38 |
| Isoleucine | 51 | 52 |
| Leucine | 112 | 101 |
| Phenylalanine | | |
| + Tyrosine | 115 | 96 |
| Threonine | 64 | 55 |
| Valine | 63 | 70 |
| Methionine | | |
| + Cystine | 62 | 43 |
| Arginine | 109 | - |

A further hypothesis in this study was, that as a dietary amino acid other than lysine, histidine, or tryptophan became limiting there would be an effect on urinary metabolite excretion. Therefore, urinary nitrogen, urea, and creatinine excretions were adopted as criteria for determining rabbit response to dietary amino acid level.

Assuming, that on the higher crude protein diets there was an excess supply of amino acids relative to lysine, it was expected, that the excretion of urinary, nitrogen and urea per unit of crude protein intake would have declined with a decreasing dietary crude protein content until such point as an amino acid relative to lysine became limiting for growth. Thereafter it was expected, that urinary, nitrogen and urea excretions per unit of crude protein intake would have increased.

The intersecting regression line analysis of urinary, nitrogen and urea excretion data indicated a decline in excretion until the dietary crude protein was reduced to a level of around 133g per kg of the diet indicating, that above this level amino acids relative to lysine were supplied in excess. There was, however, no indication of a subsequent increase in the metabolite excretions with a further decrease in dietary crude protein content. Because urinary metabolites arise from processes other than the deamination of imbalanced amino acids it may be, that these metabolite excretions are not particularly precise

determinants of response patterns. However, the present data do indicate a plateauing of response, suggesting that if diets of lower crude protein content had been included in the experiment, a further increase in metabolite excretion might have been detected.

Along with the intersecting regression line model, curves were also fitted to the data and demonstrated an equally good fit. Although the slope of the curves relating to urinary nitrogen and urea excretion declined across the nine diets, it was not possible to identify a distinct region of change in response or a plateauing effect.

In summary, although the urinary nitrogen, and urea excretion data generally support the hypothesis that NRC(1977) and AEC(1978) amino acid recommendations, relative to lysine, for the growing rabbit are overgenerous, they do not clearly indentify an area in which an amino acid becomes limiting.

With respect to urinary creatinine excretion it has been established (Borsook and Dubnoff, 1947), that this metabolite may relate to lean body mass. In consequence, urinary creatinine excretion expressed per unit of liveweight could be a potential indicator of body composition.

In the present study the urinary creatinine excretion data showed a linear decline from the diet with the highest crude protein level to that with the lowest content of crude protein, suggesting, that the rabbits on the high crude protein diets were leaner than their counterparts on the low crude protein diets.

The growth performance data, and urinary, nitrogen and urea excretion data suggest, that there was no effect of dietary amino acid level relative to lysine until the region of 115g crude protein per kg diet. The diets were iso-caloric (DE) and due to the nutrient compositions of the diets, those of higher crude protein may have supplied somewhat less metabolizable energy than the diets of lower crude protein content. On this basis it was expected, that the rabbits on the higher crude protein diets would have the highest lean content. As rabbits on diets seven to nine (below 115g crude protein per kg diet) grew more slowly and converted feed to body weight gain less efficiently than their counterparts on the higher crude protein levels, it was expected that the former rabbits would have been relatively fatter at a given age. Thus, the present creatinine data are consistent with the other findings of this study.

Taylor, Cole, and Lewis (1979) obtained carcass composition data in a trial with pigs, similar to the present study. Their carcass data, although more variable than the growth performance data, showed there to be no apparent difference in body

composition across the dietary crude protein range at which growth performance was constant. Below the dietary crude protein level which lead to a linear decline in growth performance, carcass composition showed a similar response, with pigs on the lower crude protein diets having a relatively higher fat content.

It seems likely that the effect of nutrition on urinary creatinine excretion was, at least in part, an indirect one due to differences in body composition of the rabbits fed the various diets, but, there may have also been a direct effect of dietary crude protein and amino acid composition on urinary creatinine excretion (Fisher, 1965). It would be useful in future studies to determine whether differences in body composition do occur across the range of dietary amino acid compositions investigated, and whether urinary creatinine is a useful indicator of body composition in the rabbit.

3.4.4 Conclusions.

Based on this study, it would appear that NRC(1977) and AEC(1978) recommendations for the amino acid requirements of the growing rabbit, relative to lysine, are overgenerous, and that whole body amino acid composition can serve, as a first approximation, towards the determination of the dietary ideal amino acid balance for the growing rabbit. It must be noted, however, that the design of the trial was such that it was only possible to determine an area in which the dietary ideal amino acid balance lies, not to determine the actual ideal amino acid balance. To determine the actual ideal amino acid balance for the growing rabbit, one must resort to more detailed and expensive iterative-type experiments involving dietary supplements and removal of amino acids in an appropriate manner.

Once the dietary ideal balance of amino acids for the growing rabbit, relative to lysine, has been determined, the absolute level of lysine required can be estimated, knowing that no other amino acid will be limiting or imbalanced in relation to lysine. The proportion of other essential and non-essential amino acids, relative to lysine, will not change.

It is appreciated that the digestible amino acid balance of the diets may deviate from the gross amino acid patterns. Ultimately the aim must be to express dietary amino acid levels and indeed formulate diets using digestible amino acid values. It has been noted (Moughan and Smith, 1982), that for the pig it would be ideal to express amino acid requirements in terms of ileal digestibilities not faecal. Whether this is so for the rabbit, which recycles digesta via caecotrophy after it has passed the terminal ileum, is not known. It may well be adequate to express digestibilities for the rabbit in terms of faecal values.

As with all nutritional studies the ultimate question is whether the biological optimum is also the economic optimum. As future studies are done to determine the actual ideal amino acid balance and the optimum absolute level of lysine in grower rabbit diets, it will be necessary to determine the economic effect of deviations from the biological optimum. Accepting less than maximum growth performance may result in economic savings which will ultimately determine the viability of a commercial rabbit meat producing unit.

Appendix I.

Lipid Content of Rabbit Empty Whole Body.

I.1 The mean (\pm S.E.) lipid content (g/100g freeze-dried, minced tissue) in the rabbit empty whole body(n=12).

| Rabbit No | Lipid Content | Coefficient of Variation(%) |
|-----------|------------------|-----------------------------|
| 1 | 22.22 \pm 0.27 | 3.01 |
| 2 | 23.35 \pm 0.28 | 2.89 |
| 3 | 21.43 \pm 0.33 | 3.74 |
| 4 | 22.46 \pm 0.17 | 1.88 |
| 5 | 24.07 \pm 0.33 | 3.35 |
| 6 | 21.50 \pm 0.21 | 2.40 |
| 7 | 20.00 \pm 0.22 | 2.74 |
| 8 | 29.67 \pm 0.35 | 2.92 |
| 9 | 25.03 \pm 0.42 | 4.16 |
| 10 | 28.63 \pm 0.35 | 3.01 |
| 11 | 25.28 \pm 0.39 | 3.81 |
| 12 | 18.50 \pm 0.17 | 2.22 |
| Overall | 23.52 \pm 0.95 | 13.97 |

I.2 The mean (\pm S.E.) lipid content (g/100g) of the six freeze-dried, whole body, minced tissue samples from six male and six female New Zealand White rabbits, and the 95% confidence limits for the mean difference.

| Sex of Rabbit | | 95 % |
|------------------|------------------|-------------------|
| | | Confidence Limits |
| Male | Female | |
| 22.05 \pm 0.42 | 24.53 \pm 1.84 | (-6.27 , 2.22) |

Appendix II

The mean crude protein and amino acid levels (g/100g DM)
in the male and female rabbits empty whole body minced tissue,
and the 95% confidence limits for the mean differences.

| | Sex | | 95 % C.I. for the difference between the sexes. |
|----------------------------------|----------------|----------------|---|
| | Male | Female | |
| Crude Protein | 79.37 ± 0.48 | 79.79 ± 0.67 | (-1.95, 1.12) |
| <u>Essential Amino Acids</u> | | | |
| Lysine | 5.214 ± 0.148 | 4.879 ± 0.095 | (.030, .066) |
| Histidine | 2.528 ± 0.025 | 2.560 ± 0.031 | (-.130, .066) |
| Isoleucine | 2.588 ± 0.042 | 2.552 ± 0.021 | (-.066, .138) |
| Leucine | 5.754 ± 0.087 | 5.579 ± 0.075 | (-.017, .367) |
| Phenylalanine | 3.713 ± 0.045 | 3.597 ± 0.111 | (-.142, .375) |
| Tyrosine | 2.826 ± 0.024 | 2.811 ± 0.042 | (-.097, .126) |
| Threonine | 3.255 ± 0.048 | 3.215 ± 0.045 | (-.112, .192) |
| Valine | 3.296 ± 0.265 | 3.013 ± .0342 | (-.413, .979) |
| Arginine | 5.631 ± 0.103 | 5.326 ± 0.060 | (.007, .602) |
| Methionine | 1.528 ± 0.084 | 1.461 ± 0.100 | (-.129, .264) |
| Cystine | 2.179 ± 0.058 | 2.462 ± 0.110 | (-.547, -.018) |
| <u>Non-essential Amino Acids</u> | | | |
| Aspartic Acid | 6.842 ± 0.116 | 6.896 ± 0.118 | (-.384, .276) |
| Serine | 4.213 ± 0.046 | 4.126 ± 0.070 | (-.069, .242) |
| Glutamic Acid | 11.713 ± 0.782 | 11.452 ± 0.766 | (-.257, .778) |
| Glycine | 7.095 ± 0.133 | 6.605 ± 0.097 | (.077, .904) |
| Alanine | 5.207 ± 0.064 | 4.989 ± 0.072 | (-1.69, -1.54) |

Appendix III

Analysis of variance of daily feed intake (as % bodyweight)

The experiment included nine dietary treatments and was conducted in three replicates.

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 2.015 | N.S. |
| Replicate | 2 | 10.026 | p<0.01 |
| Treatment x Replicate | 16 | 1.402 | N.S. |
| Error | 50 | | |
| Total | 76 | | |

Appendix IV

Analysis of variance of initial bodyweight of the rabbits.

The experiment included nine dietary treatments and was conducted in three replicates.

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 0.385 | N.S. |
| Replicate | 2 | 2.353 | N.S. |
| Treatment x Replicate | 16 | 0.677 | N.S. |
| Error | 50 | | |
| Total | 76 | | |

Appendix V

Analysis of variance of rabbit growth rate(g/d) over the 40-day trial period.

The experiment included nine dietary treatments and was conducted in three replicates.

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 2.184 | P<0.05 |
| Replicate | 2 | 0.190 | N.S. |
| Treatment x Replicate | 16 | 0.712 | N.S. |
| Error | 50 | | |
| Total | 76 | | |

Appendix V - continued

Analysis of variance of feed conversion ratio (g feed/g liveweight gain) over the 40-day trial period.

The experiment included nine dietary treatments and was conducted in three replicates.

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 3.110 | p<0.01 |
| Replicate | 2 | 5.406 | p<0.01 |
| Treatment x Replicate | 16 | 0.891 | N.S. |
| Error | 50 | | |
| Total | 76 | | |

Appendix VI

Slopes calculated from the differentials of the curves fitted to the growth rate and feed conversion ratio data.

| Level of dietary crude protein (g/kg air-dry weight) | Slope | |
|---|------------------------------|---|
| | Daily liveweight gain (g) | Feed conversion ratio (g feed/g liveweight gain) |
| 160 | 1.74×10^{-3} | 9.46×10^{-4} |
| 150 | 4.46×10^{-3} | 1.58×10^{-3} |
| 140 | 1.14×10^{-2} | 2.62×10^{-3} |
| 130 | 2.90×10^{-2} | 4.37×10^{-3} |
| 120 | 7.22×10^{-2} | 7.28×10^{-3} |
| 110 | 1.70×10^{-1} | 1.21×10^{-2} |
| 100 | 3.56×10^{-1} | 2.02×10^{-2} |

Appendix VII

Analysis of variance of daily feed intake during the
metabolism collection

The experiment included nine dietary treatments and was conducted in two replicates with three rabbits per replicate. All rabbits were offered the diets at a level of 5% of liveweight.

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 1.635 | N.S. |
| Replicate | 1 | 0.072 | N.S. |
| Treatment x Replicate | 8 | 1.628 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Appendix VIII

Analysis of variance of daily urinary nitrogen excretion
(mg/kg liveweight/g N intake).

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

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 7.490 | p<0.01 |
| Replicate | 1 | 0.031 | N.S. |
| Treatment x Replicate | 8 | 0.394 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Appendix VIII - continued

Analysis of variance of daily urinary urea excretion
(mg/kg liveweight/g N intake).

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

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 6.828 | p<0.01 |
| Replicate | 1 | 0.081 | N.S. |
| Treatment x Replicate | 8 | 0.545 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Appendix VIII - continued

Analysis of variance of daily urinary creatinine excretion
(mg/kg liveweight).

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

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 3.605 | p<0.01 |
| Replicate | 1 | 1.229 | N.S. |
| Treatment x Replicate | 8 | 0.686 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Appendix IX

Slopes calculated from the differentials of the curves fitted to the daily urinary nitrogen, urea, and creatinine excretions.

| Level of dietary crude protein (g/kg air-dry weight) | Slope | | |
|---|--|--|--|
| | Daily urinary nitrogen(mg/ kg liveweight/ g N intake) | Daily urinary urea (mg/kg liveweight/ g N intake) | Daily urinary creatinine (mg/kg liveweight) |
| 160 | 2.53 | 5.02 | 0.28 |
| 150 | 2.27 | 4.41 | 0.26 |
| 140 | 2.04 | 3.88 | 0.25 |
| 130 | 1.83 | 3.41 | 0.23 |
| 120 | 1.64 | 3.00 | 0.22 |
| 110 | 1.48 | 2.63 | 0.20 |
| 100 | 1.33 | 2.31 | 0.19 |

Appendix X

Least squares means for apparent faecal Crude Protein

Digestibilities(%) - Repeated Measures Analysis.

| Diet | Apparent Faecal Crude Protein Digestibility | |
|--|---|----------|
| | Period 1 | Period 2 |
| 1 | 83.01 | 81.85 |
| 2 | 80.29 | 76.05 |
| 3 | 83.20 | 76.03 |
| 4 | 79.43 | 80.53 |
| 5 | 83.52 | 85.19 |
| 6 | 85.87 | 84.91 |
| 7 | 74.50 | 83.20 |
| 8 | 76.11 | 74.95 |
| 9 | 78.53 | 69.93 |
| S.E. | 3.28 | |
| Significance Level (between treatments) | p<0.05 | |

Appendix X - continued

Repeated measures analysis of variance of apparent faecal
crude protein digestibility.

The experiment included nine dietary treatments, two collection periods (16 days apart), and was conducted in two replicates.

Treatments, replicates, and times were regarded as fixed variables.

| Source of variation | d.f. | Chi-square | Significance Level |
|-------------------------------|------|------------|--------------------|
| Times | 1 | 0.042 | N.S. |
| Replicate | 1 | 0.000 | N.S. |
| Treatment | 8 | 18.014 | P<0.05 |
| Times x Replicate | 1 | 1.712 | N.S. |
| Times x Treatment | 8 | 6.190 | N.S. |
| Replicate x Treatment | 8 | 4.180 | N.S. |
| Times x Replicate x Treatment | 8 | 10.446 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Appendix X - continued.

Least squares means(\pm SE) for apparent faecal crude protein digestibility(%) - Analysis of variance for each time period.

| Diet | Apparent Faecal Crude Protein Digestibility | |
|--|---|----------|
| | Period 1 | Period 2 |
| 1 | 82.43 bc | 82.17 |
| 2 | 78.17 ab | 79.76 |
| 3 | 79.62 abc | 81.07 |
| 4 | 79.98 abc | 80.55 |
| 5 | 84.36 bc | 82.05 |
| 6 | 85.39 c | 79.49 |
| 7 | 78.85 abc | 79.51 |
| 8 | 75.53 a | 75.22 |
| 9 | 74.23 a | 76.93 |
| S.E. | 2.32 | 2.23 |
| Significance Level (between treatments) | p<0.05 | N.S. |

Means followed by a common letter are not significantly different at the five percent level of significance.

Appendix X - continued

Analysis of variance of faecal crude protein digestibilityANOVA Table - Period 1.

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 2.603 | p<0.05 |
| Replicate | 1 | 0.721 | N.S. |
| Treatment x Replicate | 8 | 1.229 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Least significant difference = 6.73 (at the 5% level).

ANOVA Table - Period 2

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 1.069 | N.S. |
| Replicate | 1 | 0.751 | N.S. |
| Treatment x Replicate | 8 | 0.466 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Appendix XI

Analysis of variance of nitrogen retention(g/day).

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

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 3.060 | p<0.01 |
| Replicate | 1 | 0.561 | N.S. |
| Treatment x Replicate | 8 | 1.204 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Appendix XII

Least squares means (\pm SE) apparent faecal digestible energy
(MJ/kg air-dry diet) values of rabbits on the nine diets.

| Diet | Digestible energy |
|----------------------|-------------------|
| 1 | 13.00 abc |
| 2 | 12.51 a |
| 3 | 12.76 ab |
| 4 | 13.26 bcd |
| 5 | 13.60 d |
| 6 | 13.59 d |
| 7 | 13.30 cd |
| 8 | 13.02 abc |
| 9 | 13.26 bcd |
| S.E. | 0.18 |
| Significance level | p<0.01 |
| (between treatments) | |

Means followed by a common letter are not significantly different

Appendix XII - continued

Analysis of variance of digestible energy level of diets.

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

Treatments and replicates were regarded as fixed variables.

ANOVA Table

| Source of variation | d.f. | F-ratio | Significance Level |
|-----------------------|------|---------|--------------------|
| Treatment | 8 | 4.040 | p<0.01 |
| Replicate | 1 | 6.429 | p<0.05 |
| Treatment x Replicate | 8 | 1.282 | N.S. |
| Error | 36 | | |
| Total | 53 | | |

Least significant difference = 0.52 (at the 5% level of significance).

BIBLIOGRAPHY

- ADAMSON, I. and FISHER, H. (1971) - The amino acid requirement of the growing rabbit: Qualitative needs. Nutrition Reports International 4: 59-64.
- ADAMSON, I. and FISHER, H. (1973) - Amino acid requirement of the growing rabbit: Quantitative needs. Journal of Nutrition 103: 1306-1310.
- ADAMSON, I. and FISHER, H. (1976) - Further studies on the Arginine requirement of the rabbit. Journal of Nutrition 106: 717-723.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1970) - Official methods of analysis. 11th Edition Ed: Horwitz, W.
Pub: Association of Official Analytical Chemists.
- AUMAITRE, A. and DUÉE, P.H. (1974) - [Amino acid composition of piglet body proteins between birth and 8 weeks of age.] Annales de Zootechnie 23: 231-236.

- AUSTIC,R.E. (1978) - Nutritional interactions of amino acids.
Feedstuffs July 17th:24-26.
- BATTERHAM,E.S. (1980) - Amino acid availability in pig diets
with special reference to natural proteins and synthetic
amino acids. In:Recent Advances in Animal Nutrition,
1979 Proceedings of the 13th Nutrition Conference for
Feed Manufacturers. Ed:Haresign,W. and Lewis,D.
Pub: Butterworths, London.
- BATTERHAM,E.S. (1984) - Utilization of free lysine by pigs.
Pig News and Information 5: 85-88.
- BOORMAN,K.N. (1980) - Dietary constraints on nitrogen retention.
In:Protein Deposition in Animals. Proceedings of the 29th
Easter School in Agricultural Sciences, University of
Nottingham. Eds:Buttery,P.J. and Lindsay,D.B.
Pub: Butterworths, London.
- BORSOOK,H. and DUBNOFF,J.W. (1947) - The hydrolysis of
phosphocreatine and the origin of creatinine.
Journal of Biological Chemistry 168: 493-510.

BURACZEWSKA, L. (1980) - Secretion of nitrogenous compounds into the small intestine of pigs (short note).

In: Current Concepts of Digestion and Absorption in Pigs. Eds: Low, A.G. and Partridge, I.G.

Pub: National Institute for Research in Dairying.

Reading, England.

BUTHER, C.; BRYANT, M.J.; OWEN, E.; LEACH, I.; and MACHIN, D.H.

(1983) - The effect of dietary metabolizable energy concentration upon the pre- and post-weaning performance of growing rabbits.

Animal Production. 36: 229-236.

CHEEKE, P.R. (1971) - Arginine, lysine and methionine needs of the growing rabbit. Nutrition Reports International 3: 123-128.

CHEEKE, P.R. (1972) - Nutrient requirements of the rabbit Feedstuffs 44(48): 28.

CHEEKE, P.R. and AMBERG, J.W. (1972) - Protein nutrition of the rabbit. Nutrition Reports International 5: 259-266.

- COLE,D.J.A. (1979) - Amino acid nutrition in the pig. In:Recent Advances in Animal Nutrition Proceedings of the 12th Nutrition Conference for Feed Manufacturers, University of Nottingham. Eds:Haresign,W. and Lewis,D. Pub:Butterworths, London.
- COLE,D.J.A. (1980) - The amino acid requirements of pigs - the concept of an ideal protein. In:Pig News and Information. 1:201-205.
- COLIN,M. (1974) - [Lysine supplementation of sesame oil-meal diets in the rabbit. Effects on growth performances and nitrogen balance estimated by means of two methods.] Annales de Zootechnie 23: 119-132.
- COLIN,M. (1975a) - [Effects on the growth of the rabbit of L-lysine and DL-methionine supplementation of simplified plant diets.] Annales de Zootechnie 24: 465-474.
- COLIN,M. (1975b) - [Effect of the dietary arginine content on growth and nitrogen balance in the rabbit: relationship with the lysine level.] Annales de Zootechnie 24: 629-638.

- COLIN,M. (1978) - [Effects of methionine or cystine supplementation of sulphur amino acid deficient diets on growth performance of rabbits.] Annales de Zootechnie 27: 9-16.
- COLIN,M. and ALLAIN,D. (1978) - [Lysine requirement of the growing rabbit as related to the energy concentration of the diet.] Annales de Zootechnie 27: 17-31.
- COLIN,M.; ARKHURST,G. and LEBAS,F. (1973) - [Effects of methionine addition to the diet on growth performance in the rabbit.] Annales de Zootechnie 22: 485-491.
- DAVIDSON,J. and SPREADBURY,D. (1973) - [Research at the Rowett Research Institute on the nutrition of meat rabbits.] Proceedings of International Convention on Rabbit Production ERBA (Italy) 230-240.
- DAVIDSON,J. and SPREADBURY,D. (1975) - Nutrition of the New Zealand White rabbit. Proceedings of the Nutrition Society 34: 75-83.
- DEHALLE,C. (1981) - [Balance between protein and energy supplies in growing rabbit feeding.] Annales de Zootechnie 30: 197-208.

- DUEE, P.H.; CALMES, R. and DESMOULIN, B. (1980) - [Amino acid composition of muscle crude protein according to the pig breed.] Annales de Zootechnie 29: 31-37.
- FERRANDO, R.; WOLTER, R.; VITAT, J.C. and MEGARD, J.P. (1970) - Teneur en acides amines des deux categories de feces du lapin: coecotrophes et feces dures. Academie des Sciences, Comptes Rendus, Serie D, Sciences Naturelles 270: 2202-2204.
- FISHER, H. (1965) - Variations in the urinary creatinine excretion of rats fed diets with different protein and amino acid content. Journal of Nutrition 85: 181-186.
- FISHER, H. and SCOTT, H.M. (1954) - The essential amino acid requirements of chicks as related to their proportional occurrence in the fat-free carcass. Archives of Biochemistry and Biophysics. 51: 517-519.
- FONNESBECK, P.V.; HARRIS, L.E. and KEARL, L.C. (1974) - Digestion of plant cell walls by animals. Journal of Animal Science 39: 182.

FULLER, M.F. (1978) - Amino acids in the nutrition of the pig.

Rowett Research Institute Report of Studies in Animal Nutrition and Allied Sciences 34: 116-128.

FULLER, M.F. and CHAMBERLAIN, A.G. (1982) - Protein requirements

of pigs. In: Recent Advances in Animal Nutrition. Studies in Animal Nutrition. Ed: Haresign, W. Pub: Butterworths, London.

FULLER, M.F.; LIVINGSTONE, R.M.; BAIRD, B.A. and ATKINSON, T. (1979) -

The optimum amino acid supplementation of barley for the growing pig. 1. Response of nitrogen metabolism to progressive supplementation. British Journal of Nutrition 41: 321-331.

HARPER, A.E.; BENEVENGA, N.J. and WOHLHUETER, R.M. (1970) - Amino

acid imbalances. Physiological Reviews 50: 428-558.

HERNDON, J.F. and HOVE, E.L. (1955) - Surgical removal of the

cecum and its effect on digestion and growth in rabbits. Journal of Nutrition. 57: 261-270.

HILLER, A.; PLAZIN, J. and VAN SLYKE, D.D. (1948) - A study of

conditions for Kjeldahl determinations of nitrogen in proteins. Journal of Biological Chemistry 176: 1401-1420.

- HOOVER,W.H. and HEITMANN,R.N. (1975) - Cecal nitrogen metabolism and amino acid absorption in the rabbit. Journal of Nutrition 105: 245-252.
- HÖRNICKE,H. (1981) - Utilization of caecal digesta by caecotrophy (soft faeces ingestion) in the rabbit. Livestock Production Science 8: 361-366.
- KENNEDY,L.G. and HERSHBERGER,T.V. (1974) - Protein quality for the non-ruminant herbivore. Journal of Animal Science 39: 506-511.
- KING,J.O.L. (1971) - Urea as a protein supplement for growing rabbits. British Veterinary Journal 127: 523-528.
- LANG,J. (1981) - The nutrition of the commercial rabbit. Part I: Physiology, digestibility and nutrient requirements. Nutrition Abstracts and Reviews 51: 197-225.
- LAPLACE,J.P. and LEBAS,F. (1975) - [The digestive transit in the rabbit III. Influence of the hour and mode of administration of the faecal excretion of cerium-141 in ad libitum fed rabbits. Annales de Zootechnie 24: 255-265.

LEBAS,F. (1973) - [Growth performances of the rabbit as affected by the protein level of soyabean or sesame diets.] Annales de Zootechnie 22: 83-92.

LEBAS,F. (1975) - [Effect of energy level in the food on growth performance in the rabbit.] Annales de Zootechnie 24: 281-288.

LEBAS,F. and COLIN,M. (1973) - [Effect of adding urea to a poor protein diet in growing rabbits.] Annales de Zootechnie 22: 111-113.

LEBAS,F; LAPLACE,J.P.; and DROUMENQ,P. (1982) - [Effect of the dietary energy content in rabbit.] Annales de Zootechnie 31: 233-256.

LITTLE,T.M. and HILLS,F.J. (1978) - AGRICULTURAL EXPERIMENTATION DESIGN AND ANALYSIS. Pub: John Wiley and Sons, New York.

LOW,A.G. (1981) - Amino acid use by growing pigs. In:Recent Advances in Animal Nutrition. Proceedings of the 14th Nutrition Conference for Feed Manufacturers, University of Nottingham. Ed:Haresign,W. Pub:Butterworths, London.

- MARSH,W.H.; FINGERHUT,B. and MILLER,H. (1965) - Automated and manual direct methods for the determination of blood urea. Clinical Chemistry 11: 624-627.
- McNAB,J.M. (1979) - The concept of amino acid availability in farm animals. In: Recent Advances in Animal Nutrition Proceedings of the 13th Nutrition Conference for Feed Manufacturers, University of Nottingham.
Ed: Haresign,W. and Lewis,D. Pub: Butterworths, London.
- McWARD,G.W.; NICHOLSON,L.B. and POULTON,B.R. (1967) - Arginine requirement of the young rabbit. Journal of Nutrition 92: 118-120.
- MITCHELL,H.H. (1950) - Some species and age differences in amino acid requirements. In: Protein and Amino Acid Requirements of Mammals. Ed:Albanese,A.A. Pub:Academic Press Inc, New York.
- MORRIS,J.G. and MOIR,K.W. (1964) - Methods of determining the chemical composition of dead animals. In: Carcass Composition and Appraisal of Meat Animals: Selected Papers. Ed:Tribe,D.E. Pub:Commonwealth Scientific and Industrial Research Organisation, Australia.

- MORRIS, T.R. (1983) - The interpretation of response data from animal feeding trials. In: Recent Advances in Animal Nutrition. Ed: Haresign, W. Pub: Butterworths, London.
- MORRISON, D.F. (1976) - Multivariate Statistical Methods. Second Edition. Pub: McGraw-Hill, Inc, New York.
- MOUGHAN, P.J. and SMITH, W.C. (1982) - Aspects of protein digestion and absorption in monogastrics with particular reference to dietary formulation. Proceedings of the Nutrition Society of New Zealand. 7: 55-65.
- MOUGHAN, P.J. and SMITH, W.C. (1984) - Assessment of a balance of dietary amino acids required to maximise protein utilisation in the growing pig (20-80 kg liveweight). New Zealand Journal of Agricultural Research. 27: 341-347.
- NATIONAL RESEARCH COUNCIL (1977) - Nutrient Requirements of Rabbits. Pub: National Academy of Sciences - National Research Council, Washington, Publication 331.
- NUMERICAL ALGORITHMS GROUP (1983) - NAG FORTAN LIBRARY MANUAL, MARK 10.

- OLCESE, O. and PEARSON, P.B. (1948) - Value of urea in the diet of rabbits. Proceedings of the Society of Experimental and Biological Medicine. 69: 377-379.
- OMOLE, T.A. (1977) - Influence of level of dietary protein and supplementary copper on the performance of growing rabbits. British Veterinary Journal. 133: 593-599.
- OMOLE, T.A. (1982) - The effect of level of dietary protein on growth and reproductive performance in rabbits. Journal of Applied Rabbit Research. 5(3): 83-88.
- OUHAYOUN, J. and CHERIET, S. (1983) - [Comparative utilization of diets with different crude protein levels in rabbits selected on growth rate and in farm rabbits.] Annales de Zootechnie 32: 257-276.
- PARTRIDGE, I.G.; LOW, A.G. and KEAL, H.D. (1985) - A note on the effect of feeding frequency on nitrogen use in growing boars given diets with varying levels of free lysine. Animal Production. 40: 375-377.
- PAYNE, P.R. (1972) - Protein quality of diets, chemical scores and amino acid imbalances. In: Protein and Amino Acid Functions. The International Encyclopaedia of Food and Nutrition, Vol. II. Ed: Bigwood, E.J.

Pub:Pergamon Press, Oxford.

PICKARD,D.W. and STEVENS,C.E. (1972) - Digesta flow through the rabbit large intestine. American Journal of Physiology. 222: 1161-1166.

PORTSMOUTH,J. (1976) - The nutrition of rabbits. In:Proceedings of the 10th Nutrition Conference for Feed Manufacturers
Pub: Butterworths, London.

PRICE,W.A.(JR); TAYLOR,M.W. and RUSSELL,W.C. (1953) - The retention of essential amino acids by the growing chick. Journal of Nutrition. 51: 413-422.

PRUD'HON,M.; COLIN,M. and LEBAS,F. (1977) - [Effects of methionine supplementation on feeding behaviour characteristics of growing rabbits.] Annales de Zootechnie 26: 421-428.

ROBINSON,K.L.; CHEEKE,P.R. and PATTON,N.M. (1985) - Effect of prevention of coprophagy on the digestibility of high-forage and high-concentrate diets by rabbits. Journal of Applied Rabbit Research 8: 57-59.

- RUCKEBUSCH, Y. and HÖRNICKE, H. (1977) - Motility of the rabbit colon and caecotrophy. Physiology and Behaviour. 18: 871-878.
- SAID, A.K. and HEGSTED, D.M. (1970) - Response of rats to low dietary levels of essential amino acids. Journal of Nutrition. 100: 1363-1375.
- SANCHEZ, W.K.; CHEEKE, P.R. and PATTON, N.M. (1985) - Effect of dietary crude protein level on the reproductive performance and growth of New Zealand White rabbits. Journal of Animal Science. 60: 1029-1039.
- SCHLOLAUT, W. (1982) - The nutrition of the rabbit.
Pub: Roche Information Service, Animal Nutrition Dept.
- SCHURG, W.A.; FREI, D.L.; CHEEKE, P.R. and HOLTAN, D.W. (1977) - Utilization of whole corn plant pellets by horses and rabbits. Journal of Animal Science. 45: 1317-1321.
- SNEDECOR, G.W. and COCHRAN, W.G. (1980) - Statistical Methods Seventh Edition. Pub: Iowa State Univ Press.

SOCIÉTÉ DE CHIMIE ORGANIQUE ET BIOLOGIQUE [AEC] (1978) - Amino acid recommendations; Rabbits. From: Animal Feeding AEC France - energy, amino acids, vitamins, minerals. Document No 4.

SPREADBURY, D. (1974) - Protein and amino acid requirements of the growing meat rabbit. Proceedings of the Nutrition Society 33: 56A.

SPREADBURY, D. (1978) - A study of the protein and amino acid requirements of the growing New Zealand White rabbit with emphasis on lysine and the sulphur-containing amino acids. British Journal of Nutrition. 39: 601-631.

SPREADBURY, D. and DAVIDSON, J. (1978) - Some observations on the arginine requirement of the growing New Zealand White rabbit. Journal of the Science Food and Agriculture 29: 1017-1022

STEVENS, A.G. (1977) - Digestibility and coprophagy in the growing rabbit. Proceedings of the Nutrition Society 36: 4A.

TAYLOR, A.J.: COLE, D.J.A. and LEWIS, D. (1979) - Amino acid requirements of growing pigs. 1. Effects of reducing protein level in diets containing high levels of lysine. Animal Production. 29:327-338.

THACKER, E.J. and BRANDT, C.S. (1955) - Coprophagy in the rabbit.

Journal of Nutrition. 55: 375-385.

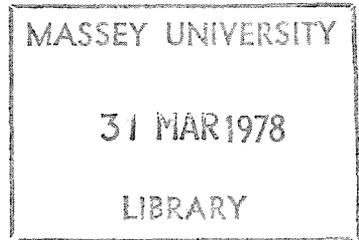
THORBEC, G. and CHWALIBOG, A. (1981) - [Growth, digestibility, nitrogen and energy metabolism in growing rabbits fed different feed compounds.] Beretning fra Statens Husdyrbrugs forsog. 510.

WILLIAMS, H.H.; CURTIN, L.V.; ABRAHAM, J.; LOOSLI, J.K. and MAYNARD, L.A. (1954) - Estimation of growth requirements for amino acids by assay of the carcass. Journal of Biological Chemistry. 208: 277-286.

ZEBROWSKA, T. (1978) - Determination of available amino acids in feedstuffs for monogastrics. Feedstuffs. Dec 25th: 15-17, 43

Leadership and Organizational Functioning: Organizational Regression

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INTRODUCTION: ISSUES ERRONEOUSLY PERCEIVED AS PERSONALITY PROBLEMS OF THE LEADER

THIS PAPER IS A CONTINUATION of earlier efforts to apply psychoanalytic object-relations theory, a psychoanalytic theory of group processes, and an open-systems theory of social organizations to the study of psychiatric institutions and of therapeutic methods carried out in such institutions (Kernberg, 1975a, 1975b). In this paper I focus on the relationships among the administrator's personality, the organizational structure, group processes occurring in the organization, and organizational tasks.

Sometimes, the carrying out of all tasks in the areas of treatment, research, and education seems to be limited by the leader's personality problems. Very often the staff see the leader as arbitrary and authoritarian, as though he were misusing his power to impose courses of action detrimental to commonly shared goals. But this perception may be a misperception. There is often a shared conception—or fantasy—among staff that depicts the leader as not understanding, as arrogant and revengeful, but careful analysis of the total situation by outside consultants, particularly when modern approaches of organizational diagnosis are utilized, may reveal various, and at times quite complex, situations.

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The effectiveness of leadership of the organization does not depend exclusively or predominantly on the leader's personality. The first requirement for effective functioning of an organization—including its leadership—is the adequate relationship between the organization's overall task and its administrative structure; the task has to be meaningful rather than trivial and, given the available resources, feasible rather than overwhelming. Psychiatric institutions operate within various environments, and their effectiveness in carrying out therapeutic, educational, and research tasks depends upon the adequacy of their human and material resources, as well as upon the nature of their interaction with the environment. When any of these conditions are not met, that is, when resources are insufficient for the tasks to be carried out, or when the normal flow of resources and "products" across the boundaries of the institution breaks down, or when contradictory goals or failure to clarify priorities interfere with the functional relationship between task and administrative structure, the task-group structures in the organization deteriorate, morale breaks down, and the group processes within the organization regress; this regression, in turn, powerfully affects the quality and effectiveness of leadership.

Bion (1959) has described the regression that takes place in group processes in terms of basic emotional assumptions ("basic assumptions group"): the "fight-flight" assumption, the "dependency" assumption, and the "pairing" assumption. These assumptions constitute the basis for group reactions that potentially always exist but are particularly activated when task structure breaks down.

The "dependency" group perceives the leader as omnipotent and omniscient, while considering themselves inadequate, immature, and incompetent. This idealization of the leader is matched by desperate efforts to extract knowledge, power, and goodness from him in a greedy and forever-dissatisfied way. The failure of the leader to live up to such an ideal of perfection is at first met with denial, and then with a rapid, complete devaluation of him and a search for substitute leadership. Thus, primitive idealization, projected omnipotence, denial, envy, and greed, together with defenses against these, characterize the dependency group, and its members feel united by a common sense of needfulness, helplessness, and fear of an outside world vaguely experienced as empty or frustrating.

The "fight-flight" group is united against vaguely perceived external enemies and expects the leader to direct the fight against such enemies, as well as to protect the group from any in-fighting. Any opposition to the "ideology" shared by the majority of the group, however, cannot be tolerated, and the group easily splits into subgroups which fight each other. Frequently, one subgroup becomes subservient to the idealized leader, while another subgroup attacks the first one or is in flight from it. The group's tendency to forcefully control the leader or to experience itself forcefully controlled by him, to experience "closeness" in a shared denial of intragroup hostility, and to project aggression onto an outgroup, all are prevalent. In short, splitting, projection of aggression, and "projective identification" are predominant, and the search for nurture and dependency characteristic of the dependency group is here replaced by conflicts around aggressive control, with suspiciousness, fight, and dread of annihilation prevailing.

The "pairing" assumption leads the group to focus on two of its members—a couple (frequently, but not necessarily, heterosexual) to symbolize the group's hopeful expectation that the selected pair will "reproduce itself," thus preserving the group's threatened identity and survival. The fantasies experienced about this selected pair express the group's hope that, by means of a magical "sexual" union, the group will be saved from the conflicts related to both the dependent and fight-flight assumptions. The pairing group, in short, experiences generalized intimacy and sexual developments as a potential protection against the dangerous conflicts around dependency and aggression (which, we may add, have a pregenital character, in contrast to the genital one of the pairing group).

The development of group fight-flight or dependency assumptions moves the leadership of originally task-oriented groups into stands complementary to the emotional needs of their members or staff; that is, a staff who expects a primitive kind of leadership from an omnipotent, giving figure (in the dependent group) or a powerful or dangerous, controlling authority (in the fight-flight group) tempts or provokes the task leadership to regress.

The general implication is that a breakdown in the effectiveness of work created by various internal factors and relationships be-

tween the organization and the environment induces regressive group processes first, and regression in the functioning of the leadership later. If these group processes remain undiagnosed, only their end-product may be visible, in the form of what appears to be primitive, inadequate leadership and, more specifically, negative effects of the leader's personality on the organization.

The group processes occurring in psychiatric institutions are, however, influenced not only by the degree of task orientation and task appropriateness on the part of administrative and therapeutic structures. The very nature of the task carried out in psychiatric institutions, particularly in settings where severely regressed patients are treated, also exerts a powerful influence on these group processes. I am referring here to the replication of the pathological internal world of object relations induced by borderline and psychotic patients in the group processes involving staff and these patients on each service, ward, or section. At certain times, severely regressed patients may induce basic assumption group processes in both formal and informal patient and staff groups on a service, and this negatively affects group leadership and, finally, perhaps the administrative structure of the entire service. In this regard, one might say that the nature of the "product" handled by psychiatric institutions, namely, primitive and deep human conflict, deeply influences the functioning of such institutions.

Again, in this case, only the end-product of a chain process may be visible, and the administrator of the service or of the hospital may appear as arbitrary, threatening, and irrational. Only a careful organizational analysis may bring to the surface the relationship between problems of patients at the grass-roots level, on the one hand, and the problems among leadership at the top, on the other.

It is, of course, possible that serious psychopathology in the leader is, indeed, responsible for the problems of morale, of breakdown of task groups, and the development of regressive group processes. The problem, then, is to differentiate the symptomatic activation of emotional regression in the leader, reflecting problems in the institution, from the deterioration of organizational functioning reflecting psychopathology in the leader.

The traditional analysis of institutional management has focused on the leader's personality, particularly his "inborn" characteristics

(such as "charisma") and his "authoritarian" qualities. Psychoanalytic thinking has focused on the distorted perception that staff may have of the administrator or leader as a function of the irrational relations with authority that stem from infantile conflicts, particularly from the oedipal situation. More recent sociological thinking has stressed the "role" aspects of leadership; that is, the activation of socially sanctioned and recognized functions in which leader and follower have mutually reinforcing perceptions and behavior. This sociological analysis focuses on the confusion that often develops in organizations between the leader's personality, his behavior in carrying out certain roles, and the perception of his behavior on the part of staff who cannot easily differentiate role from personality (particularly, of course, when perception of the leader is distorted by unconscious conflicts).

More recently still, the application of psychoanalytic methods to the study of small-group processes has revealed the activation in nonstructured and informal groups of primitive emotional contents and defensive operations that are ordinarily latent in all individuals and only become manifest in patients with severe regression, such as borderline conditions and the functional psychoses. These findings further complicate the study of the interaction involving the personality of the leader, his behavior, the perception of his behavior by the staff, and the mutual induction of regressive behavior of staff and leader under the influence of regressive group processes. It is at this point that a systems approach may be helpful in clarifying, not only the mutual influences of the leader's personality, group processes, organizational structure, and organizational tasks, but also in pointing to the major origin of the distortions affecting all of these. A systems approach to organizations considers the institution as an overall system dynamically and hierarchically integrating various subsystems (such as the personality of leadership, the nature of group processes, in addition to the ordinary task systems and administrative structures of the organization), and conceives of the environment of the organization as suprasystems affecting the institution in dynamically and hierarchically organized ways (Dolgoff, 1973; Levinson and Klerman, 1967; Rice, 1963, 1969).

A systems approach is not only a theoretically satisfying model

which permits such integration, it can have significant diagnostic and corrective impact on the work in psychiatric institutions. Such a systems approach is in contrast to linear and mechanical models, which attribute the sources of organizational disturbances to one or another of the sub- or suprasystems mentioned. Systems analysis is, for example, in contrast to a model that conceives the fluctuation of group processes (from task-oriented to regressive) as a major origin of all institutional conflicts; if the leader appears to be authoritarian or demagogic, it is "the group that is making him behave this way"; if patients become regressed, it is because "the group is putting its illness into them," etc. Or else—another example of a nonsystems approach—the leader is to blame for everything; staff feels powerless and paralyzed because of the nature of the irrational, authoritarian leadership. The attribution of blame to the leadership often not only reflects an effort of staff to deny serious conflicts among staff and patients and to project them onto the leader (the director of the unit, service, or the institution), but may also serve to protect staff from awareness of its own responsibility in protecting and perpetuating an authoritarian structure.

At times, it is the nature of the task that is overwhelming, as when an ideology which conceives of psychiatric treatment as so powerful that all patients should, ideally, be able to improve, is painfully confronted with the illusionary nature of this conviction (as demonstrated by treatment outcomes over an extended period of time). Then, too, there are institutions directed by leaders who are actually very poor. It is important to be able to differentiate this type of situation from the much more frequent one in which the problem of top leadership represents a symptom rather than the cause. It can happen that most of the energy of an institution seems to be spent in the direction of "curing" its leader; it may well be that the astonishing capacity of so many people in so many places to tolerate such a situation over an extended period of time indicates how gratifying it is to attribute the cause of all problems to the administrator, rather than focus upon the painful and complex interaction of the various systems involved in bringing about his behavior.

So far, I have not mentioned the political dimension of conflicting interests among groups in institutions which influence their

relation to the task as well as to the leader and management at large. If we conceive of political strivings as the conscious or unconscious efforts of individuals or groups to defend their interests and expand their influence over individuals and/or groups at their "boundaries," political action becomes a normal aspect of institutional interactions. Insofar as group interests stem from group members' identifications with values of a social, cultural, or professional nature, conflicts develop between belonging to "task" or to "nontask" determined groups—"sentience" groups (Miller and Rice, 1967). "Sentience" here refers to the emotional bonds influencing group formation and cohesiveness; such emotional bonds may derive from the task performance itself or from past or present, real or fantasied things in common that link individuals in groups.¹ Political strivings may reflect the efforts to bring about an optimal equilibrium between these conflicting identifications.

However, when political strivings evolve into an ideological commitment to establish an optimal equilibrium between politically opposite groups regardless of task requirements, a new complication for organizational functioning has arisen. The purpose of psychiatric institutions is basically a professional and technical, rather than political, one, and serious distortions in the task and in group processes, administrative structures, and leadership may evolve when political objectives replace task-oriented or functional ones. For example, "democratization" of an administrative structure may be perceived as an ideal solution to organizational conflicts, with consequent deterioration of task groups, specialized skills, and individual functions and responsibilities. It is an illusion that authoritarianism in institutions can be successfully overcome by democratization of them, rather than by a functional analysis of task requirements and the functional administrative structures corresponding to them. There are dictatorships of groups as well as of individuals, and these can result as much from paralysis as from capriciousness at the top.

¹ "We shall therefore talk of *sentient system* and *sentient group* to refer to that system or group that demands and receives loyalty from its members; and we shall talk of *sentient boundary* to refer to the boundary around a sentient group or sentient system" (p. xiiin).

Let me offer a few clinical examples of the preceding theoretical formulations.

Case 1

In a department of psychiatry, a chronic problem of morale and chaotic functioning of the nursing service brought about the hiring of a supposedly very strong director of nursing with full authority for reorganization of that department. The assumption had been that the department had discouraged strong nursing in the past, and that to import this strong nurse represented a commitment to nursing. The new director of nursing did indeed establish a powerful personal leadership, increasing the power and prestige of the nursing service in the process. However, she developed a new organizational structure, "team nursing," without considering the recent development of interdisciplinary team work that was occurring throughout the department at the same time. This resulted in new conflicts between the nursing staff and other professions, and resentment among nursing staff of administrative interventions on the part of other disciplines affecting them.

The new director of nursing had difficulties in conveying to her staff the department's emphasis on interdisciplinary work, and in conveying to the department the new nursing plans and developments. In other words, she had trouble carrying out "boundary functions" as an essential part of her leadership. After a period of growing conflict between the department's administration and the nursing staff, in the course in which mutual distortions, accusations, and incomprehension escalated rapidly, she resigned and left. After which the myth developed among the nursing staff that she had been forced to leave because she was "too strong a leader." This myth disappeared only when a new director of nursing, who was, if anything, even stronger than her predecessor, was hired. The new director was able to diagnose and carry out successfully the basic tasks of the nursing service in consonance with the task definition of the entire department. Eventually, the department experienced the second director of nursing as highly effective and not at all as "coming on strong," and the nursing staff itself gradually recognized her as a much better leader. This case illustrates problems in task definition and boundary control. Technical fail-

ures in leadership induced regressive group processes across the boundaries between nursing staff and the rest of the department; personality problems of the individual leader were not the issue.

Case 2

The activities department of a psychiatric hospital perceived its leader as inefficient, weak, and wavering; an organizational analysis of the functions of the department revealed that important changes had occurred (derived from the "unitization" or compartmentalization of the hospital), resulting in a contradiction between independent hospital units, on the one hand, and an integrated organization of the activities department, on the other. This contradiction had brought about an unbearable complication in scheduling meetings, communications, and interdisciplinary work. A change in the administrative structure and functions of the activities department in consonance with the new developments of the hospital was achieved, with the result that the activities staffing and functions became flexibly integrated into the new units, while certain activities specialists were still available for the hospital at large. The consequent resolution of tensions within the hospital and the activities department itself brought about a fundamental change in how the activities department perceived its leadership. The director and his associates were now seen as strong and reliable. We see here how the perception of "weak leadership" was a symptom of problems in the organizational structure of the department rather than a reflection of the personalities involved.

Case 3

In one hospital service, an acute conflict erupted among the service director, several senior consultants, the psychiatric resident in charge of the treatment of a very difficult case, and various other staff members who took positions in one of two feuding fields. An "in-group," led by the service director, considered that the patient had been treated "too leniently," that insufficient hospital-milieu structure had been provided, and that acting out of rebelliousness against authority figures on the part of the resident had complicated the treatment situation: the patient was seen as acting out the resident's rebellion. An "out-group," consisting of various staff

members, the psychiatric resident in charge of the case, and his supervisor, all felt that the patient's ego weakness had been underestimated, that more time and patience was needed rather than consistent confrontation, and they experienced the service director as rather ruthless and domineering in his handling of the clinical conferences in which the case was discussed. Analysis of the situation showed that quite specific intrafamilial dynamics of the patient had been activated and projected onto the relationships among the staff, intensifying the potential conflicts around authority and power of all those involved in the patient's treatment. Once this was clarified and the split among the staff was healed, better understanding of the patient's dynamics could be utilized in his psychotherapeutic and hospital-milieu treatment (Kernberg, 1975a). This illustrates the regressive effects of the patient's pathology on the nature of the interactions among staff and on the perception of the leadership of the service as authoritarian.

Case 4

Conflicts developed in a psychiatric hospital between the departments of rehabilitation, occupational therapy, and recreational therapy. These conflicts were first perceived as personal conflicts around power on the part of the leaders of two of these three groups; it later emerged that the leader of one of the groups had indeed been given some authority over the other two, but without a clear mandate over who controlled the joint boundaries of all three departments. Insofar as the three departments continued to function autonomously and no clear coordination or integration of all their activities was possible, the leader who had been tentatively selected to direct the entire area projected an image of uncertainty and doubt.

The question arose: where is the problem? In the personality of the leader who was not able to assert his authority over the entire department? In the nature of the administrative structure of these three departments, which were confusingly intermingled and spread over the entire hospital? In the nature of the task, which had become unclear as changes in the hospital philosophy and utilization of occupational, recreational, and rehabilitative services clashed with the departments' traditional background of experience?

The last example illustrates the diagnostic process required to answer the question of where the source of the problem resides. In order to arrive at an answer, it is helpful to start by defining, first, the nature of the task and its constraints; second, the optimal administrative structure required for the task; third, the nature and amount of the authority functionally required by the leader; fourth, the leader's technical and conceptual skills and liabilities; and, finally, the leader's personality characteristics which might be involved in the problem.

For practical purposes, it is sometimes helpful to simply hire a new administrative leader, selecting a person with known and proven conceptual, technical, and personal skills (Katz, 1955) to diagnose the nature of the task and the required administrative structure for task performance, so that the selection of the leader precedes the adequate diagnosis of all other factors in the hope that a solution will become available before the problem is fully diagnosed. An alternative method also exists, namely, first diagnosing the nature of the task and the required administrative structure for that task, and only then searching for the "right person" for the leadership function of that administrative structure. This second method is slower and requires more input from the organization at large before a decision can be made regarding the required new leader, but it may be less risky than the first method. It is much easier to hire the right person when the nature of the task and its constraints have been clarified. However, time considerations or political organizational constraints may make the first method preferable. In any case, the analysis of task priorities and respective administrative requirements should provide a safety margin against later problems which may re-emerge—rightly or wrongly—under the mask of personality difficulties of the new leader. Hidden contradictions between the apparent, expressed goals and the real, underlying goals of organizations sometimes reveal themselves in the symptomatic act of selecting one incompetent or naive leader after another for an impossible task.

From the administrator's viewpoint, unresolved problems in his personality and unresolved problems within the nature of the specific tasks of the organization and its administrative structures are not the only sources of regressive pressures on his functioning. The exec-

utive administrator of a psychiatric institution occupies various boundaries. First, he occupies the boundary between the organization and its social environment, and contradictions in and pressures from the social environment, as well as those stemming from within the institution, may affect his psychological functioning. Second, he is at the boundary between his professional background or convictions (his "sentience") and the nature of the task required within the organization. Third, he is also at the boundary between his personal value systems and ethical commitments, on the one hand, and the task requirements of relating himself to a human, social organization, on the other. Conflicts of loyalty, regarding moral convictions and other alternatives requiring courage may at times become prominent and create regressive pressures on his functioning. In summary, from a practical standpoint, the major forces determining the effectiveness of leadership stem from: (a) the leader's personality characteristics, (b) the nature of his technical and conceptual skills, (c) the adequacy of task definitions, availability of human and material resources, and priority settings of the institution, and (d) the adequacy of the administrative structure to the task requirements.

A major instrument permitting the administrator to evaluate the optimal functioning of his system is the exploration of group processes within his unit, ward, service, hospital, department, or institution. The technical utilization of his knowledge of group processes will permit him to evaluate the degree to which task groups are "work groups" or are being influenced by basic group assumptions (Bion, 1959). The analysis of the content of any regressive group processes may reveal the nature of the "hidden agenda" of the institution and therefore provide a test of the adequacy of task performance and of the administrative structure. At the same time, and insofar as patients are treated as individuals and/or as groups within the institution, the analysis of such regressive group processes will permit the carrying out of very important diagnostic work regarding the conflicts within the internal world of object relations of patients. Both kinds of regressive pressures, that is, the organization's "hidden agendas" and the distortions of the social processes induced by regressed patients, will highlight the distorted way in which the administrator is viewed or the transferenceal

reactions to him as he carries out his professional and administrative roles. The analysis of regressive group processes, in short, may reveal the effects of organizational and/or patients' conflicts, and thus help to evaluate by elimination to what extent the administrator's personality factors are complicating the situation and creating stress and regression in the group processes in the organization.

FRUSTRATION OF BASIC HUMAN NEEDS IN THE LEADERSHIP FUNCTION

Various aspects of administration or management induce powerful regressive pressures on the administrator's psychological functioning. Among these, the very loneliness of his position, the loss of the spontaneous and unconstrained feedback from peers, the uncertainty that is part of significant decision-making, all may induce anxieties. Oedipal fears of failure or defeat, frustration of dependent needs, and general activation of conflicts around aggression in the administrator as a leader of and participant in various group processes all contribute to inducing such a regressive pull. Nor is this all. There is the general "invasive" nature of administrative concerns—the invasion of the privacy of his thinking, around the clock, by pressing organizational issues for which no immediate solution can be found; the invasion of his life space as his public image infiltrates areas of privacy and reduces his time and space for careless leisure and freedom; the threat to the freedom of his fantasy life as his internal relations to people and nature, to art and leisure, all become contaminated by stress related to responsibilities that always remain with him.

Aggressive Needs

In the realm of aggression, although creative administration may permit the expression of aggressive needs in sublimated form, there are also temptations for resolving such tensions by the sudden exertion of authority. Groups only too readily tempt their leaders into impulsive action, but the leader must resist these temptations: he is usually aware that loss of control over his angry impulses may have devastating effects far beyond those occurring in other,

ordinary situations. The role aspects of his functioning—the formal organizational authority he enacts—and the unavoidable transference distortion of the perception of his behavior on the part of staff may amplify his expression of aggression dangerously and may bring about paranoid distortions in the minds of his staff.

The activation of primitive aggressive needs in the administrator ordinarily depends more upon the regressive pull of group processes in the organization than upon his personality characteristics. There certainly are leaders with strong sadistic trends, and given the amplification of the leader's aggressive behavior by the staff's transference perceptions and reactions, even relatively minor outbursts become major issues in the organization, but the influence of group processes triggering off and amplifying such reactions in the leader cannot be underestimated. For example, when a regressive group process corresponding to Bion's fight-flight assumption occurs, the leader of this basic assumption in the group—often representing "the voice of the opposition"—may provoke the administrative leader into a personal fight.

Often the most extreme, paranoid, oppositional member of the staff takes over group leadership at such a juncture and seems to control the group as well as the administrative leader himself, a development that may induce paranoid, regressive processes in the administrator, who may now fear that the most vehement and irrational of his opponents has completely taken control of the group. The administrator may react with exaggerated fear, anger, and authoritarianism against the "challenger," and thereby miss the internal conflicts in the staff group, that is, the "silent support" for the challenge that exists in the group, and also miss the criticism of the violence among themselves on the part of other staff. The administrator's awareness of group processes and of his own reactions to them may be very helpful to him in transforming such a potentially dangerous situation into a creative one.

The leader of any group or organization is constantly faced with the expression of aggression of various sources from those under him. From the viewpoint of individual psychology, the aggression directed at the parental images and its expression and/or projection onto the leader is an important aspect of group life: disappointments and rage, rebellious hatred are the counterparts of idealization

of and submission to the leader stemming from oedipal and preoedipal relations to the parents. Bion (1959) suggests that the inordinate expectations of the dependent group bring about frustrations and hatred of the task leader who frustrates the needs for total gratification and the group's longings for unlimited dependency. The fight-flight group struggles with aggression against the task leader, who is perceived in distorted, paranoid ways as a vengeful, dangerous authority. In more global terms, the task leaders' consistently pointing to reality in terms of the task, and thus destroying the hopes and longings of basic assumptions, evokes frustration and aggression.

Because the leader or executive head of an organization is only human and does have limitations and makes mistakes, there are always grounds for the staff's feeling frustrated and angry with him, for rationalizing the deeper levels of irrational hatred of him in terms of his human limitations. Therefore, hatred of authority usually seems logical enough, and this compounds the actual distortions of leadership functions.

In any case, the various origins of hatred of leadership are usually condensed, and it is frequently hard to judge whether the leader is hated because the administrative structure is authoritarian, because he is incompetent, because he frustrates his followers' needs for idealization and unrealistic expectations, or because of individual psychopathology of all those involved, obviously including the leader himself. Ideally, the answer to the question of the origin of the hatred of the leader should come from an analysis of the primary task of the organization, the adequacy of the administrative structure and functional leadership to the task, etc.

Only after these questions have been answered in the negative, can one raise the question of whether the personality aspects of key leaders are injecting pathological degrees of aggression into the system, or whether pathological regressions within groups are temporarily activating basic group assumptions, and thus excessive aggression.

Only after studying general morale problems within the organization and the task orientedness of the relations among groups within it may it be possible to conclude that regressive group phenomena are not the primary factor, and that the psychopathology of

individuals, particularly the leader, is involved. The point is that distortions of organizations can be caused by individual psychopathology within crucial administrative points of the organizational structure, but that this diagnosis can only be made after eliminating all other possible causes of emotional regression within the organization. This viewpoint is in contrast to the analysis of organizational conflicts exclusively in terms of individual psychopathology, or of group processes, organizational structure, or political factors.

If the leader appears reasonably adequate to his task and shows no significant personality disturbance, and if there are no major organizational problems evident at that point—that is, the administrative structure is adequate to task performance and the external environment is relatively stable—the question of “inappropriate” aggression of staff toward the leader can often be resolved in terms of the need for the leader to tolerate a certain amount of aggression without undue concern over it. In practice, when the leader is loved without reserve, and nobody is ever angry with him, something must be wrong. Decisions, when they are meaningful decisions, always cause somebody pain. Naturally, those painfully affected blame the man on top, and the man on top must be able to tolerate this. Tolerance of aggressive outbursts of rebellious behavior of staff without overreacting is part of what is required of a good leader: this is one reason why severely narcissistic and paranoid personalities make poor task leaders. Often the administrator’s tolerance of temporary irrational staff outbursts may in itself decrease the fears that underlie the expression of such anger, and thus create an emotionally corrective experience for everybody concerned.

Sexual Needs

An increase of oedipal sexual temptations in the leader is the counterpart of the activation of oedipal aggressive rivalries around the issues of power and control within the hierarchy of the institution. While one genetic aspect of the drive for assuming positions of power is that of taking the place of father and becoming the domineering male of the social group, the staff’s unconscious perception of the male leader as being the owner of all the women in the institution, and the oedipal orientation of female staff toward

him as a complement to this shared myth, is an additional, potential source for sexual temptations in leader and staff for acting out such oedipal conflicts. The reverse situation develops when a woman is the leader of the organization. In either situation, the prevalent social conventions and taboos regulating public and private interactions between the sexes exert a strong influence on these dynamics. The sexual politics of institutions, that is, the political equilibrium reached in the power struggle and sexual tensions involving men and women as complementary or opposite sentience groups, is often played out at the top of the institution, as in the proverbial relation between the boss and his secretary, between the chief doctor and head nurse, etc.

Psychiatric institutions are mostly male dominated and reproduce the culturally dominant (apparent) control by "sadistic-controlling" men over "masochistic-subservient" women. Therefore, the political struggles between the sexes as expressed in regressive group phenomena often take the form of men (apparently) dominating the public decision-making process, and of women, in (apparent) admiration of and subservience to male-made decisions, carrying out orders while yet protesting in passive ways against such submission by means of inducing guilt in men over the mistreatment to which they subject women. The conflicts between physicians and nurses concerning who in effect make the final decisions on any psychiatric service is one illustration of this problem. The mutual "sexual teasing" among staff, with unconscious efforts to make the other sex the first to cross the forbidden boundary from professional to sexual relations in order to retaliate by inducing massive guilt in the offender, is another aspect of the same problem. Behind the temptations and fears of crossing sexual boundaries are those of crossing hierarchical boundaries with acting out of oedipal rebellion implicit in the process. Above all, because psychiatric institutions deal with patients who have not been able to satisfactorily resolve their oedipal problems outside the hospital, strong pressures deriving from the nature of the task of treating such patients may exacerbate all these potential conflicts among staff.

The danger that unresolved oedipal conflicts of the senior administrator may trigger off a sharp increase of oedipal conflicts throughout the entire institution is often present. This situation

becomes complicated by the frequent sexualization at the administrative level of conflicts actually related to the leader's frustrated dependency needs. Oedipal and preoedipal regressive pressures may combine to activate the administrator's sexualized dependent relationships, typically that of "the great man" who is "babied" by "mothering" women, who are often admiring and subservient and yet dominant, in his immediate "entourage."

In general, groups operating under the basic assumption of pairing experience intimacy and sexual developments as a potential protection against the dangers and conflicts around dependency and aggression. Sexual pairing may also represent a real or fantasied escape from the dangerous and/or controlling group pressures in the organization, and symbolize a condensation of oedipal rebelliousness against the "established order" with the defensive sexualization of more primitive conflicts around aggression and dependency.

Thus, there may be sexually excited and romanticized pressure around the administrator which fosters a sexualized bond between him and some leading administrative member of the other sex. Under optimal circumstances, this bond is expressed in a working relationship mildly infiltrated with sublimated eroticized trends. Actually, a certain eroticization of work relationships may be enhancing to the work group. But when regressive pressures lead to the crossing of sexual boundaries, a couple's sexual intimacy may bring about, not only an exaggerated condensation of the work group with sexualized sentience, and with the consequent distortions in ordinary work boundaries and relationships, but may also induce a freeing of the aggressive components related to oedipal conflicts in such sexualized relations, with a general breakdown of interpersonal relations in the system. In organizational terms, it may be said that the sexualization of relationships among staff increases their level of aspiration to such an extent that ordinary gratifications at work will (sooner or later) fall disastrously short of such increased expectations, and a general breakdown of morale will ensue.

For the administrator, there is an obvious need that his sexual gratifications occur outside the boundaries of his administrative functions. This might seem too trivial to mention were the regressive

pressures for sexualized relations within the administrative boundaries not so strong. At the same time, if and when a functional, mutually respectful, and open work relationship between the sexes develops in an organization, eroticized perhaps but still maintaining work boundaries, the exhilarating experience of men and women who work together as friends without necessarily having to become sexually engaged can be a most creative experience and indirectly foster a sexually mature and tolerant atmosphere, which, in psychiatric institutions, will help in the treatment of patients.

From a broader viewpoint, general conflicts among the sexes within the social, cultural, and economic environment, for example, socially fostered and ritualized sadomasochistic relationships between women and men, sexual exploitation, and teasing, are automatically expressed as part of the sexualized tension within organizations and threaten to distort task relationships. This is seen in the masochistic submission of nurses to doctors and in the manipulative exploitation of sexual seductiveness to reestablish a (real or fantasied) equilibrium between sadistically behaving men and masochistically behaving women. If political tensions among sentience groups and across task boundaries become sexualized, such sexual aggression, submission, and teasing acquire political significance and express sexual politics in an organization. Sexual politics, powerfully reinforced by general sexual sentience, may interfere with the task relationships and task structure.

One implication of what I have been saying is that, in order to bring sexual politics out into the open, the extent to which sexual sentience and task sentience are related must be diagnosed, not with the intention of stripping away the barriers of privacy but to avoid the misperceptions shared by groups and both sexes when sexual politics operate within the institution. For example, the shared rebelliousness of young female nurses and male physicians against their respective female and male leadership may require diagnosis in order to avoid general distortions in the relations among nursing staff and medical staff.

All of this is not to be construed to mean that satisfactory sexual relationships cannot develop among individual staff members of organizations; on the contrary, people often establish lasting sexual and marital relationships with those they meet at work. Usually,

when this happens, the work relationship between them shifts; one or both might even withdraw from work. Should a married couple or one with a stable relationship continue in a work relationship as well, there is a need to consolidate the sexual relationship in terms other than that of the work relationship proper; the couple that marries will have to establish a close relationship, in areas separate from those of the task. In turn, it will become particularly important that organizational leadership watch the maintenance of the task when a couple functions within a certain task system.

It is often not satisfactory when husband and wife work together as hierarchical leader and subordinate in the same task system, for this may have a deleterious effect on task relations with their peers. Couples frequently are perceived as powerful and even threatening alliances of power within organizations, so that even when task relationships are scrupulously observed, groups do not so perceive them and react to them with fear, aggression, resentment, and suspicion.

Even under ideal circumstances this may reduce the couple's effectiveness. Under less than optimal circumstances, the couple may be "sucked in" by the role-inducing, shared fantasies and behavior of groups. Furthermore, the couple may be tempted to act out its own conflicts by projecting aggression onto the environment, and by developing an idealized bond that tends to exploit their alliance to the disadvantage of others in the organization. At times, the powerful member of the couple controls the more subservient or masochistic one for his or her needs; when such a couple in fact occupies a position of leadership, distortion of exercise of authority may occur and typically authoritarian relationships develop, which may become quite destructive. There is wisdom in the administrative principles within universities that are intended to prevent just such an eventuality. At the other extreme is the danger of unwarranted discrimination against couples because of the shared fantasies about them within the institution: it is unfortunate when two creative persons are limited in their development and/or contributions by such an organizational bias.

If "to work and to love" are the principal tasks in life, creative developments within organizations should permit the placing of Eros at the service of work and the placing of work at the service of sublimated love. The main objective of an organization is not to

satisfy the human needs of its members but to carry out a task; one objective of intelligent leadership is to permit the gratification of human needs in carrying out that task.

Dependent Needs

The major regressive pressure on the leader usually derives from the frustration of his dependency needs. There are many reasons for such frustration. For one thing, the potential activation of Bion's basic assumption of dependency is, at least to some extent, always present. Then, too, the administrator carries the burden of responsibility for the entire institution, for processes that to some extent are outside his control and boundaries. He is also confronted with a staff whose freedom of expression of the dependency needs is greater than his own. The "carefree" attitude of those under him, and the comparatively greater availability for support, applause, and gratification they have, create additional pressures for the leader. Those subordinates who do well are often rewarded by the leader, but top administrators usually receive few direct human rewards when they are effective. Staff takes it for granted when things go well; whenever anything goes wrong, the leader is the first to be held responsible.

There are, of course, compensating aspects in the work as such. When the administrator has the realistic awareness that his job has been done well, that he has been able to introduce and carry out new ideas and programs, and that he has permitted and stimulated his staff to grow and become creative, important gratification of his needs may occur. In general, creativity in administrative work may simultaneously gratify dependency needs (by projection), narcissistic needs (by success and approval), and oedipal strivings (by administrative victories). The administrator's immediate group of co-workers, as part of their working relation with him, can provide him with gratification of his dependency needs. In this regard, mutual gratification of dependency needs on the part of the senior administrative staff is an important, realistic requirement of work situations, particularly in large institutions.

Another major compensating factor for the frustration of the administrator's dependency needs is the availability of friendship and support outside his own administrative boundaries. Realistic

gratification of the administrator's instinctual needs in his daily life outside the work situation becomes very important in the long run. An excessive search for the gratification of dependency needs from his subordinates may distort the administrative structure and burden the staff excessively. There is a delicate balance between the administrator's being so reserved and self-contained that he feeds staff's dehumanizing distorted perceptions of him, or of his relying so much on gratification and support from staff that he overwhelms them and decreases their concentration on actual work. This delicate balance also raises the issue of the extent to which the administrator should or should not share with his staff his concerns and difficulties across the external boundaries of the organization.

The leader's openness about himself may increase staff's understanding of his own constraints, clarify distortions derived from their perception of his role (that is, from confusing his role with his personality), and increase staff morale. However, for the leader to burden staff with his problems may induce in them not only anxiety about problems they cannot solve, but also a tremendous increase of the expectation that "with openness and humanness all problems will get solved," that is, an unreasonable expectation of the effectiveness of reasonableness, so to speak. In other words, paradoxically, the "ideal" leader in terms of his openness, warmth, and nondefensiveness may, by the same token, so increase expectations from him and from external reality outside the institution that disappointments become unavoidable. In short, there is a danger that the supposedly "perfect" administrator fosters primitive idealizations related to the dependency assumptions of staff, and such idealizations necessarily lead to disappointment reactions.

The regressive pull of needs around aggression, dependency, and sex may derive from the personality characteristics of the administrator, from aspects of the reality of his relations with staff, and, particularly, from the regressive group processes among staff. Whatever the origin of these pressures, they ordinarily are compensated for by a variety of factors. Major among these is the overcoming of oedipal conflicts in the normal capacity for achieving and experiencing success and to use success creatively. I believe it is an Arabian proverb that says that every man, in his life, should be able to plant a tree, write a book, and have a child. Translated into the

functions of an administrator, the planting of a tree may represent getting things done or building new things; the writing of a book, the development of new ideas and knowledge; and the having of a child, the creative development of the human resources of the institution and encouragement of the staff to grow and develop their capacity for good and gratifying human relationships in the process of carrying out significant work.

REFERENCES

- Bion, W.R. (1959), *Experiences in Groups*. New York: Basic Books.
- Dolgoff, T. (1973), Organizations as Sociotechnical Systems. *Bull. Menninger Clin.*, 37:232-257.
- Katz, E. (1955), Skills of an Effective Administrator. *Harvard Business Review*, Jan.-Feb., pp. 33-42.
- Kernberg, O. F. (1975a), Modern Hospital Milieu Treatment of Schizophrenia. In: *New Dimensions in Psychiatry: A World View*, ed. S. Arieti and G. Chrzanowski. New York: John Wiley & Sons, pp. 202-220.
- _____ (1975b), A Systems Approach to Priority Setting of Interventions in Groups. *This Journal*, 25:251-275.
- Levinson, D. J., and Klerman, G. L. (1967), The Clinical-Executive. Some Problematic Issues for the Psychiatrist in Mental Health Organizations. *Psychiatry*, 30:3-15.
- Miller, E. J., and Rice, A. K. (1967), *Systems of Organization*. London: Tavistock Publications.
- Rice, A. K. (1963), *The Enterprise and Its Environment*. London: Tavistock Publications.
- _____ (1969), Group and Intergroup Processes. *Human Relations*, 22:565-584.

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