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DEVELOPMENT OF AN ASSAY FOR THE DETERMINATION OF DIETARY APPARENT ILEAL NITROGEN AND AMINO ACID DIGESTIBILITIES IN THE MEAT CHICKEN

A THESIS PRESENTED IN PARTIAL FULFILMENT OF
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ABSTRACT

Biological procedures which quantify protein value are expected to beneficially influence efficiency of nutrient provision in dietary formulations for the livestock industry. This thesis provides a review of two widely employed quantitative assessments, "Digestibility" and "Availability" and in the experimental section describes a series of experiments undertaken on meat chicken to refine assay procedures involved in the determination of protein and amino acid apparent ileal digestibility values.

Five experiments were undertaken.

(1a) A comparison of two feeding procedures with three diets differing in particle size with a view to evaluating the effect of feeding behaviour on the final composition of the test diet in the crop.

(1b) Determination of crop residue composition associated with time following feeding with two feeding procedures and two diets differing in particle size.

(2) A comparison of the effects of two slaughter procedures and two flushing solutions on ileal N digestibility of meat and bone meal.

(3) A comparison of the effect of time of slaughter following feeding on ileal N values of two diets.

(4) Determination of the effect of length of ileum on ileal N digestibility values of two diets.

(5) Determination of the effect of age on ileal N and AA digestibilities of two diets.

In 1a there was clear evidence that bird eating behaviour and particle size of the test diet influenced crop content proportions of a number of criteria, more notably chromium and nitrogen. For coarse particle diets an intubation feeding procedure produced a closer match of material in the crop with that of the untouched diet than a free access provision of food procedure.

In 1b the results of the study were inconclusive and no satisfactory cause for inconsistencies that developed between treatments could be found.

In 2, two slaughter procedures, euthanasia by sodium pentobarbitone and asphyxiation by carbon dioxide, resulted in significantly different ($P<0.05$) apparent ileal nitrogen (N) digestibility. Differences between flushing solutions, distilled water and physiological saline were small and not significant ($P<0.05$).

In 3, for two diet types, N and dry matter (DM) digestibilities were relatively constant over sampling times of 2 to 5 hours following the start of feeding. Ileal digesta sampled quantities tended to be greatest at the 4 hour sampling interval.

In 4, differences in N and DM digestibilities of digesta samples drawn from sections of the ileum up to 30 cm in length as measured from the ileo-caecal junction were generally small and non significant.

In 5, bird age had no significant effect on N and DM digestibilities.

The main conclusions drawn were that intubation better retained the integrity of food reaching the crop. Sodium pentobarbitone was a preferable method of slaughter. Ileal length sampled needed to be kept as short as consistant with providing adequate sample material and largest sample sizes were obtainable around 4 hours following the start of feeding.

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LIST OF ABBREVIATIONS

AA(s)	Amino acid(s)
AAAD	Apparent amino acid digestibility
ADF	Acid detergent fibre
AME	Apparent metabolizable energy
BV	Biological value
CO_2	Carbon dioxide
Cr_2O_3	Chromic oxide
DM	Dry matter
FA	Free access
FDNB	1-fluoro-2,4-dinitrobenzene
I	Intubation
LSD	Least significant difference
MBM	Meat and bone meal
N	Nitrogen
NDF	Neutral detergent fibre
NPR	Net protein retention
NPU	Net protein utilization
PER	Protein efficiency ratio
PFAA	Plasma free amino acid
PRC	Poultry Research Centre
TAAD	True amino acid digestibility
TME	True metabolizable energy

REVIEW OF LITERATURE

CHAPTER 1

INTRODUCTION

Diets fed to commercial poultry typically contain ingredients in proportions that meet gross nutrient requirements and which result in minimum dietary cost. Such diets are formulated with the aid of least cost linear programmes and feed mills have centralized computing operations to benefit from this technology. The diets produced are based on ingredient metabolizable energy, gross amino acids, vitamins and minerals, fatty acids and cost considerations and are constrained by local availability of feedstuffs and preferences for certain inclusions or levels of ingredients. These contraints are additionally influenced by field experience, personal leanings to a particular nutritional doctrine and rankings of quality. Quality in this context refers to digestibility or availability particularly of amino acids, antinutritional and tainting factors, growth factors and those affecting feeding behaviour (rather that metabolizable energy and protein content, amino acid balance and mineral and vitamin levels).

Dietary requirements or nutrient specifications are based on literature values or calculated directly using factorial methods from local data. Nutritional recommendations put out by authoratitive bodies such as the National Research Council (NRC) (1984) and the Agricultural Research Council (ARC) (1975) represent a concensus of scientific opinion based on a large body of research data and experience. Recommendations are provided as gross nutrient values either in units per kilogram of diet, units per bird per day or as a ratio involving metabolizable energy.

Since the advent of complete diets, made possible by the advance in knowledge of vitamin and mineral requirements and manufacture in the first half of this century, it has become apparent that the use of gross nutrient values, particularly with respect to amino acids (AAs),

inadequately describe the net quantity available to the birds. The quantity of nutrient absorbed is conditional on factors that enhance and impede digestion or absorption, that cause complexing, or which result in direct loss of nutritional substances in the faeces.

Some early assay procedures attempted to place a qualitative value on feedstuffs particularly in respect to protein but they are today of limited usefulness. The most notable ones have been Biological Value (BV), Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) and are described briefly below.

Biological Value (BV) is the percentage of nitrogen retained that is absorbed from the feed. The method was first employed by Thomas (1909) and later developed by Mitchell (1924). Protein concentration supplied by the test ingredient is set in the test diet at about 10 percent. The formula for deriving BV illustrates the procedures involved,

$$BV = \frac{\text{N intake} - (\text{faecal N} - \text{MFN}) - (\text{Urinary N} - \text{EUN})}{\text{N intake} - (\text{faecal N} - \text{MFN})} \times 100$$

where MFN = metabolic faecal nitrogen

EUN = endogenous urinary nitrogen

In poultry the denominator cannot be measured unless surgical techniques are undertaken to separate the excreta into urinary and faecal components. Hence for poultry BV may be redefined as the percentage of N retained that is consumed.

BV reflects the content of the limiting AA in the protein. The values tend to be lower when food protein either contains a deficiency or an excess of any particular AA. Among the protein sources, egg protein is considered to have a BV of about 100; meat protein, 72-79; cereal protein; 50-65 and gelatin 12-16. Allision and Anderson (1945) and Allision (1955) and Forkes et al., (1956) outline potential problems associated with the procedures.

Protein Efficiency Ratio (PER) is the ratio of the weight gain per unit of test protein consumed. The test material is the sole source of protein in the test diet and is included to supply about 10 percent protein. The method was first employed by Osborne et al. (1919) using the following formula.

$$PER = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

The result may be compared to that of a standard protein source such as casein by way of proportion and related to a constant standard value of 2.5 (Chapman et al., 1959).

$$PER = \frac{\dfrac{\text{Weight gain (g)}}{\text{Protein (test) consumed}}}{\dfrac{\text{Weight gain (g)}}{\text{Protein (standard) consumed}}} \times 2.5$$

Problems associated with the assay have been raised by Bender and Doell (1957a) and Harper (1981). Bender and Doell (1957b) proposed a modified PER method, in which the weight gain of the experimental group was compared with a group on a protein-free diet, to give net protein retention (NPR) calculated as follows:

$$NPR = \frac{\text{Weight gain of TPG - weight loss of NPG}}{\text{Weight of protein consumed}}$$

where TPG = group fed on test protein
NPG = non-protein group

The modified method allows for the maintenance and growth of the experimental animal (Harper, 1981) and enables evaluation of low quality feedstuffs which tend to result in little growth.

Net protein efficiency (NPU) measures efficiency of N utilization by comparing body N resulting from feeding a test protein with that resulting from feeding a comparable group of animals a protein-free diet for the same period (Miller and Bender, 1955). The following formula describes the procedures involved.

$$NPU = \frac{\text{Body N with test protein - Body N with protein-free diet}}{\text{Total N intake}}$$

Summers and Fisher (1961) reported that the water content of the carcass is closely related to the carcass N content. Using this relationship the N content of the carcass may be estimated by determining only the carcass moisture content to reduce the analytical procedures.

Stucki and Harper (1962) have questioned the constant ratio of carcass moisture against N content with age in the animals. Summers et al. (1964) and Fisher and Griminger (1969) raise additional problems associated with the procedures.

These assays assess quality of a protein source in terms of efficiencies of N retention or as implied by weight gain. Body weight gain and N retention however reflect the extent to which the protein source provides the balance of AAs necessary for the growth function. The assays are of limited value for response is fundamentally influenced by the extent levels and balance of AAs match metabolic needs for protein accretion. The assessments cover the feeding value of a protein source in isolation and do not address the compound diet situation in which imbalances and deficiencies in AAs of dietary ingredient components are balanced by other protein sources in the diet.

An assay form that circumvents this problem and which in recent times has become a standard procedure for evaluating diets and protein sources is that of the digestibility assay (McNab, 1976). It is designed to provide an estimate of the proportion of N or AA retained from the quantity fed. The assay involves 3 issues.

(1) The concentration of N and or AAs in the feed.
(2) The fraction of it which is digested and absorbed (digestibility).
(3) The fraction of it which is absorbed and in a form suitable for utilization (its availability). This latter statement receives further attention in chapter 3.

In digestibility measurements, levels and imbalances of AAs in a protein source are not expected to affect digestion or the digestibility result other than for the effect the protein source may have on excretion of endogenous fractions and the degree to which such endogenous fractions can be accurately allowed for by correction.

Investigation of digestibility has increased since the advent of Sibbald's (1976) rapid bioassay for the determination of true metabolizable energy (TME) of feedstuffs for poultry. His TME procedure involves fasting birds for 24-48 hours following the force-feeding of a known quantity of test material into the crop and subsequent collection of the total excreta for 24-48 hours. Recently, the assay has been extended to determine the AA digestibility of feed ingredients (Likuski and Dorrell, 1978; Parsons, 1981). Farrell (1978) has also described a rapid procedure for the determination of apparent metabolizable energy (AME) for poultry. This procedure involves training adult cockerels to consume their daily feed in one hour and collecting excreta for 24-36 hours. This procedure has also been adopted for and used in N and AA digestibility determinations (Raharjo and Farrell, 1984; Wallis and Balnave, 1984).

Both the Sibbald and Farrell rapid procedures to determine the amino acid digestibility of feedstuffs employ total excreta collection techniques. This procedure has been widely critized on the basis of the

high density of microbial bacteria in the hind gut (caeca and large intestine) and the effect it may have on AA digestibility (Mason et al., 1976; Zebrowska and Buraczewski, 1977; Parsons, 1981). On the other hand these effects have been considered to be of little or no significance in poultry since the passage of feed through the large intestine is relatively fast and the area is relatively small as compared to other monogastric species.

This controversy has resulted in further examination of ileal digestibility techniques. The use of ileal amino acid digestibility was first suggested by Payne et al. (1968) to eliminate the possible effect of intestinal microflora in the hind gut. This procedure has been used increasingly and more researchers have tended towards the use of ileal AA techniques in preference to faecal AA digestibility assays (Soares et al., 1971; Raharjo and Farrell, 1984).

An alternative approach to assessing protein quality is by way of availability assays. These are of interest on a number of counts. The scientific literature has not always clearly distinguished between digestibility and availability or clearly stated what availability purports to show. As a measure it suffers from problems concerning the effect of AA imbalances and dietary levels between test and control diets on the criteria of measurement, protein accretion and body weight gain. In general assays are restricted to producing AA availability information on one amino acid at a time, a circumstance that restricts their usefulness for commercial application.

In contrast digestibility assays are relatively fast and inexpensive (Austic, 1983) and provide the digestibility of several or all AAs in a feedstuff in a single assay.

This study has been undertaken with a view to outlining the issues associated with current digestibility and availability assays and refining techniques for obtaining digestibility values using ileal slaughter methods on meat chickens. The experimental procedures involved the following studies.

(1a) A comparison of two feeding procedures with three diets differing in particle size with a view to evaluating the effect of feeding behaviour on the final composition of the test diet in the crop.

(1b) Determination of crop residue composition associated with time following feeding with two feeding procedures and two diets differing in particle size.

(2) A comparison of the effects of two slaughter procedures and two flushing solutions on ileal N digestibility of meat and bone meal.

(3) A comparison of the effect of time of slaughter following feeding on ileal N values of two diets.

(4) Determination of the effect of length of ileum on ileal N digestibility values of two diets.

(5) Determination of the effect of age on ileal N and AA digestibilities of two diets.

The feeding procedures involved free access and intubation and ileal assays involved slaughter and the removal of a section of the gut from which digesta was sampled. Accordingly a brief description of the anatomy and physiology of digestion follows as an introduction to a more rigorous review in chapters 2 and 3 of digestibility and availability. The anatomical and physiological description is based on and taken primarily from Duke (1977, 1986).

1.1 Anatomy of the Digestive Tract

The anatomy of the fowl's digestive system is most notably different from that of mammals in the oral area, the presence of a crop in the oesophagus and a muscularly lined stomach or gizzard. In fowls there is no soft palate and hence the mouth and pharynx are not sharply delimited. The function of teeth is accomplished by a horny beak and gizzard.

The crop takes the form of a simple enlargement or pouch off the oesophagus about 20 cm below the pharynx in adult fowls. The glandular stomach or proventriculous is situated immediately proximal to the gizzard and has primarily a secretory role. The gizzard has 2 pairs of muscles called the musculari intermedii and musculi lateralis.

The small intestine has a duodenum, 20 cm long which forms a complete loop, but beyond that there are no delimited areas. Meckel's diverticulum, the vestige of the yolk sac, has been used loosely in this thesis to define the start of the second half of the small intestine and for purposes of decriptive convenience has been used as the junction of the jejunum and ileum. The small intestine is about 120 cm long in adult birds and contains villi that are generally more numerous and slender and taller than the villi of mammals. Brunners glands are absent in chickens (Calhoun, 1954), although Duke (1977) reports that in some species tubular glands homologous to Brunners glands in mammals, are present. The villi are served with a well defined network of blood capillaries but no lacteals (Graney, 1967).

Paired caeca (17.5 cm long in adults) extend laterally and distally from the junction of the small and large intestine. The large intestine is short (about 11.3 cm in adults) and enters into the cloacal chamber.

The left hepatic duct (of the liver) communicates with the duodenum whereas the right duct sends a branch to the gall blander. The bile duct empties into the duodenum near the distal loop. The pancreas lies within the duodenum loop. It consists of three lobes and its secretions reach the duodenum via three ducts.

1.2 Secretions of the Digestive Tract

Generally the salivary glands of birds have only mucus secreting cells but amylase has been found in the saliva of poultry (Duke, 1977). Food passes quickly through the oesophagus which lubricates its passage by the secretion of mucus. Mucus is also secreted by the crop of the fowl and amylase has been found in the crop although its origins are unclear. Work by Pritchard (1972) suggests non bacterial digestion of carbohydrates can occur in the crop.

The proventriculus contains predominantly 2 gland types - simple mucosal glands secreting mucous and compound glands, homologous to the chief and parietal cells of the mammal stomach, secreting mucous, HCl and pepsinogen.

Mechanical digestion and preliminary acid proteolysis occurs mostly in the muscular stomach. The pH of gastric juice for fowls ranges up to 2.5. Long (1967) indicates the chick secretes 8.8 ml per kilogram body weight per hour of gastric juice which is considerably greater than for man, the dog, the rat and the monkey. Similarly the acid concentration is greater, but the pepsin content per unit volume is lower although it is greater in terms of pepsin units per kilo gram body weight per hour (Duke, 1977).

The intestinal mucosa has been shown to possess proteolytic activity in chickens (Kokas et al., 1967) and DeRycke (1962) as cited by Duke (1977) reported finding aminopeptidases and carboxypeptidases in the duodenal mucosa. Additionally intestinal amylase (Duke, 1977) has been found in chickens and intestinal maltase and sucrase in a number of bird species (Zoppi and Schmerling, 1969). The pH of the tract increases from the oral to the aboral end and Hurwitz and Bar (1968) report the pH of each portion of the tract is regulated by secretory activity within that portion. PH ranges have been reported between 5.6. and 7.2 (Herpol and van Grembergen, 1967, as cited by Duke,1977).

The pancreas secretes digestive enzymes and an acqueous solution containing buffering compounds which act to neutralize the acid chyme and provide pH conditions of between 6 and 8. The pancreas is the major source of amylase and pancreatic lipase has been demonstrated in chickens (Polyakov, 1958, as cited by Duke, 1977). Pancreatic proteolytic activity has been demonstrated (Duke, 1977). Chicken chymotrypsin and trypsin have been reported (Duke, 1977) and DeRycke (1962) as cited by Duke (1977) has found dipeptidase, aminopeptidase and carboxypeptidase activities.

The pancreatic secretory rate is relatively greater in the fowl than in dogs, rats and sheep (Kokue and Hayama, 1972) and Duke (1977) reports it is less affected by fasting in the fowl than in these mammals.

The secretion of bile into the duodenum aids in the neutralization of chyme and the emulsification of fats and bile salts are reabsorbed in the lower ileum.

Several important factors are believed to occur in the caeca (McNab, 1973). The most notable perhaps is the microbial digestion of cellulose. However Duke (1977) suggested there was no conclusive evidence that the fowl derived any nutritional benefit from the breakdown of cellulose or from other nutrients released upon breakdown of the plant cell walls. Urine may pass from the cloaca along the colon to the caeca. Thorburn and Willcox (1965) reported excreta moisture content was increased by 1-2 % following caecectomy. Microbial synthesis of vitamins in particular B-vitamins has been reported by Coates et al., (1968) in experiments using a conventional and germ-free environment. However according to these studies vitamins are apparently not absorbed by the caeca.

1.3 Absorption from the Digestive Tract

The first one fourth of the ileum has been shown to be the most important site for absorption of fats, carbohydrate, and amono acids. Bile salts are absorbed largely in the lower ileum, amino acids coming from exogenous proteins are mostly absorbed in the upper half of the ileum, and the breakdown products from endogenous proteins are absorbed primarily in the lower half of the ileum (Crompton and Nesheim, 1969) as cited by Duke (1977).

Chickens possess a sodium-dependant mobile-carrier system for active transport of sugars similar to that of mammals (Alvarado and Monreal, 1967).

The absorption of D-glucose, D-galactose, D-xylose, 3-methyl glucose, α-methyl glucoside, and possibly D-fructose is active. Seven other monosaccharides are apparently passively transported (Bogner, 1960; Hudson and Levin, 1966; Fearson and Bird, 1968) as cited by Duke (1977).

According to Duke (1977) the in-vivo absorption of 18 L-amino acids into isolated segments of the intestine of chickens was studied by Tasaki and Takahashi (1966), who observed that the absorption rate was not dependent on molecular weight. Instead they observed that those amino acids with large nonpolar side chains (eg. methionine, valine, leucine) were absorbed more readily than those with polar side chains. Duke (1977) observed that most amino acids are actively absorbed, but not all have separate transport mechanisms. He cites an example drawn from Paine et al., (1959) in which L-methionine and L-histidine are both actively absorbed more rapidly than their D-isomers. He reports Tasaki and Takahashi (1966) as finding that the absorption of leucine or phenylalanine was inhibited by methionine.

Moreto and Planas (1989) report that the proximal caeca, close to the ileorectal junction, has well-developed villi and microvilli and is able to transport sugars and amino acids against a concentration gradient, by

mechanisms virtually identical to those in the small intestine. However contribution to the overall nutrient absorption is limited because the absorbing epithelium is exposed to the intestinal contents only during the filling and emptying of the caecal segments. In addition Low and Zebrowska (1989) have reported the colon in chickens may have an amino acid absorptive function.

In mammals, fats are absorbed into the lymph lacteals of the villi, whereas in birds, fat is absorbed directly into the blood (Noyan et al., 1964). Duke (1977) reports Noyan et al., (1964), Carew et al., (1972) and Hurwitz et al., (1973) as finding that approximately 80-95 % of the fatty acids present in the intestine of adult chickens are absorbed. In newly hatched chicks less is absorbed (Carew et al., 1972) as cited by Duke (1977).

CHAPTER 2

DIGESTIBILITY

Digestibility is defined as the difference between the amount of an amino acid in the diet and that in excreta or ileal digesta (Low, 1982; Papadopoulos, 1985; Sauer and Ozimek, 1986) as a proportion of the quantity fed. Measurement in poultry is usually accomplished by total excreta collection methods or by use of an indicator.

In obtaining digestibility values using poultry there are 3 perceived difficulties. They involve distortions of digestibility values consequent on:

(1) urine being a component of excreta;
(2) endogenous matter of other than food origin viz digestive secretions, microbial debris, epithelial cell debris, and bile contributing to feed residues; and
(3) hind gut microbial synthesis of protein and amino acids from digesta.

There are essentially two practical procedures for estimating digestibility in poultry. They are the total collection and ileal techniques. Alternative names for the "total collection" procedure are "total excreta", "excreta" or "faecal". Of these the latter is a loosely applied term and not a literal representation of the waste material collected. Measurements labelled "true" as opposed to "apparent" represent those for which an attempt has been made to correct for endogenous secretions. Measurements labelled "ileal" are indicative of the use of procedures which attempt to overcome post absorptive distortions caused by urine contamination of faeces and distortions arising from hind gut microbial synthesis of protein and amino acids from digesta residues.

Research procedures have been developed to estimate the extent of the bias associate with the abovementioned problem areas. They include cannulation, caecectomy, exterioration of the colon (colostomy) and the use of germ-free birds.

Digestibilities are measurements that provided an estimate of the proportion of supplied nutrient that is removed from the gut by absorption into the circulatory system. In contrast the measurements are not estimates of the proportion of supplied nutrients that are in a form suitable for a particular metabolic need. It is expected however that they will provide an estimate of the extent to which supplied nutrients are available to the animal in the form that was intended. As such in feed formulation they may permit a higher degree of precision in the supply of absorbable dietary nutrients and make more effective and efficient use of feed ingredients with ultimately a cost benefit.

Assays performed on target species are termed direct. Indirect approaches involve enzymatic in vitro studies.

2.1 Direct Procedures

2.2 Methods Involving Total Collection or Excreta Sampling

Under these procedures two digestibility values may be obtained. They are apparent excreta or faecal digestibility (AED or AFD), also termed apparent digestibility (AD) and true excreta or faecal digestibility (TED or TFD), commonly termed true digestibility (TD). Corresponding terminology for amino acid (AA) and nitrogen (N) digestibility are defined below.

apparent amino acid digestibility ---------- AAAD
apparent nitrogen digestibility ---------- AND
true amino acid digestibility ---------- TAAD
true nitrogen digestibility ---------- TND

Many assays employ total excreta collection techniques because they are relatively straight forward and the assay period is short. The assays provide digestibility values for the required amino acids of a feedstuff

in a single assay. The methodology was first used by Kuiken and Lyman (1948) to determine amino acid digestibility in rats. Bragg et al. (1969) proposed the excreta collection method for determining digestibility in poultry.

In its simplest form the total collection method involves feeding assay birds, commonly adult cockerels or growing chicks, with a known quantity of the test food and collecting the excreta produced over a representative period. The difference in quantity of the AA or N in the test food and excreta as a proportion of the total consumed provides a measure of AAAD.

A working formula may be derived as follow:

Balance of AA (mg) = total AA intake (mg) - total excreta AA (mg)

Expanding and converting to a proportion:

$$\frac{\text{balance of AA}}{\text{total AA intake (mg)}} = \frac{(\text{mg AA / g food intake} \times \text{g intake}) - (\text{mg AA / g excreta} \times \text{g excreta})}{(\text{mg AA /g food intake} \times \text{g food intake}}$$

Simplifying and converting to a percentage:

$$\% \text{ AAAD} = 1 - \frac{(\text{mg AA / g excreta} \times \text{g excreta})}{(\text{mg AA / g food intake} \times \text{g food intake})} \times 100$$

A variant which results in TAAD introduces a correction to remove sources of bias associated with non-food residues in the excreta. These sources are referred to by Austic (1983) as endogenous losses and comprise AAs resulting from unabsorbed digestive juices, mucus, bile, desquamated epithelial debris, microbial debris and AAs of urinary origin (Sibbald, 1987).

Austic (1983), Papadopoulos (1985) and Sibbald (1987) outline a number of methods that have been used to measure endogenous losses. They included excreta collected from fasted birds, excreta collection from birds fed a nitrogen-free diet and regression techniques involving extrapolation to obtain an intercept value at zero intake. These are outlined in more detail in section 2.1.3.

The correction term for endogenous amino acids,
(mg AA /g endogenous excreta) x g endogenous excreta, when applied to the working formula gives TAAD values.

$$\% \text{ TAAD} = \frac{1 - [(\text{mg AA/g excreta} \times \text{g excreta}) - (\text{mg AA/g endogenous excreta} \times \text{g endogenous excreta})]}{\text{mg AA/ g food intake} \times \text{g food intake}} \times 100$$

2.2.1 Total Collection Assay Procedures

Assays are usually conducted on egg type chicks, broilers or adult egg type cockerels. Three common assay forms are presented to illustrate several of the more prominent features and issues. They are the methods of free-access, intubation and the rapid assay approach.

2.2.1a Free-access Methods

In this approach stock are accustomed to the test diet or feedstuff over a period that has ranged from 2 days (Rostagno et al., 1973) to 7 days (Muztar and Slinger, 1980a). The purpose is to allow clearance of all residues of the previous diet from the digestive tract and to establish a uniform rate of passage of the test feed and feed residues (Schneider and Flatt, 1975). The single ingredient assay is not recommended because many feedstuffs are unpalatable and unbalanced when fed alone

(Sibbald, 1987) and may have adverse effects on body functions if fed over a number of days. Some feedstuffs contain toxic substances that may affect digestive tract functioning during the accustomization period (Sibbald, 1987). The free-access approach is exposed to the errors that result from selective feed intake (Sibbald, 1987) and the distortions affecting uncorrected assay values that may arise from variable food intake (Sibbald, 1987) and low food intake (Sibbald, 1975) and which are due to the relative size of the endogenous fraction.

2.2.1b Intubation

Sibbald (1976) developed a simplied procedure for the rapid determination of true metabolizable energy (TME) of feedstuffs for poultry. This procedure has been claimed to be rapid, simple, accurate and inexpensive. The procedure was applied by Likuski and Dorrell (1978) to the determination of AA digestibility in feedstuffs. It involves fasting the experimental birds for 24 to 48 hrs to clear the digestive tract of previous residues followed by the placing of a known quantity of feedstuff directly into the crop and subsequent quantitative collection of excreta for 24 to 48 hrs. The excreta collected is frozen, freeze-dried, weighed, ground and cleared of contaminants and then analysed for N or AA content. In addition, the excreta of fasted control birds is collected, the N or AA content determined and used as a measure of the endogenous N or AA output. A correction is made to the excreta term to abtain true N or AA digestibility.

Sibbald (1987) reports that the intubation procedure ensures full intake of a precise amount of a feedstuff or feed at a known time. The technique prevents the birds from selecting preferred components. It avoids feed spillage, feed wastage, and overcomes feed intake variation. The optimum food provision depends upon the size of the bird and the form and the nature of the feed. Sibbald (1987) recognizes that increasing the level of feed fed reduces the effect of experimental

error. However, feed inputs greater than 40 g have resulted in an increased incidence of regurgitation (Sibbald, 1977). For adult White Leghorn cockerels the optimum input recommended is 30-40 g of pelleted or 25-30 g of ground feed. The input should be about 1-2 % of body weight (Sibbald, 1987). Sibbald (1979c) found that the level of feed input had no significant effect on true amino acid digestibility values in plant feedstuffs in assays on adult cockerels.

2.2.1c Rapid Assay

Farrell (1978) described a rapid assay for the determination of apparent metabolizable energy (AME). It involved the use of adult cockerels trained to consume their daily feed allowance (80-110 g) in 1 hour. The assay has been employed to obtain N and AA digestibility values in both adult cockerels and growing meat chicken stock. The assay involves presenting to groups of birds either a pelleted basal diet or a pelleted mixture of the basal diet and the test material (50:50 w/w) for a 1 hour period. Excreta is then collected for a recommended 24-36 hrs. In the assay endogenous losses are not corrected for but they are expected to be relatively small and inconsequential if food intake is large.

The working formula for obtaining AAAD values under the above approach is derived in Appendix 1.

2.2.2 Excreta Sampling Procedures --- Indicators and Markers

Indicators have been used in digestibility work because they obviate the need to measure the amount of test food used and the quantity of waste material produced. They involve measuring the weight of indicator per unit weight of test diet and per unit of waste material. A widely used indicator is chromic oxide (Sibbald, 1987).

Working formulas for the determination of AAAD using indicators are derived as follows and illustrate the principles involved and the measurements required.

waste = intestinal residues

indicator fed = indicator excreted

indicator / g food eaten = waste x indicator / g waste

$$\text{waste} = \frac{\text{indicator/ g food} \times \text{g food eaten}}{\text{indicator/ g waste}}$$

AA absorbed per 1 g food eaten = AA/g food eaten - waste x AA/ g waste

$$= \text{AA/ g food eaten} - \frac{\text{indicator/ g food} \times \text{AA/g waste}}{\text{indicator/ g waste}}$$

Converting to a proportion

$$\frac{\text{AA absorbed/g food eaten}}{\text{AA/g food eaten}} = \frac{\text{AA/g food eaten} - \frac{\text{indicator/g food} \times \text{AA/g waste}}{\text{indicator/g waste}}}{\text{AA/g food eaten}}$$

$$\text{AAAD} = 1 - \frac{\text{indicator/ g food}}{\text{indicator/ g waste}} \times \frac{\text{AA/ g waste}}{\text{AA/ g food eaten}}$$

Converting to a percentage

$$\% \text{ AAAD} = 100 - \frac{100 \times \text{indicator/ g food}}{\text{indicator/ g waste}} \times \frac{\text{AA/ g waste}}{\text{AA/ g food eaten}}$$

The procedures require that the indicator mixes uniformly with the test food and waste material representative of the test diet fed is sampled. Further information on the use of indicators is given in section 2.2.1b.

An allied procedure imposed to delineate test food excreta residues from that associated with the pre and post assay feed is the use of markers such as ferric oxide. Food containing the marker is given during the acclimatization period and again following the provision of the test diet (Sauer et al., 1974). The unmarked excreta material produced between stages of marked material is considered to be the excreta corresponding to the test food. In work with chickens, Bragg et al. (1969) inserted a 10 hours fast period between the holding diet and the test diet. Sibbald (1987) suggests problems may arise in the separation of marked and unmarked material and the general properties of indicators referred to in section 2.3.1b should apply.

2.2.3 Endogenous Correction

Amino acids lost through endogenous sources, ie sources originating in the body and not of direct food origin may be measured and substracted from those of the excreta related to the test food to give ultimately true digestibility measurements.

Endogenous secretions in poultry comprise digestive juices, mucous, bile, epithelial cell debris, microbial debris and urine (Sibbald, 1987). The purpose of correcting for endogenous excretion is to remove the impact of their magnitude on digestibility values. The accuracy of true digestibility remains, however, uncertain. This is because there are

four ways of measuring endogenous secretion and comparisons have not always proved compareable (Parsons, 1981). In addition ample evidence exists to suggest that the type and quantity of food fed impacts on endogenous secretions in an unpredictable manner Meyer (1956), Whiting and Bezeau (1957), Rostagno et al. (1973) and Krawielitzki et al. (1977) as cited by Sibbald and Price (1980).

Parsons (1981) provided an estimate of the proportion of amino acids in components of excreta. Working with adult cockerels and using differential centrifugation or physical separation techniques, he partitioned excreta amino acids into fractions arising from microbial sediment, insoluble matter which equated with feed residues and a soluble fraction comprising endogenous material emanating from the gut and urine. He found approximately 31, 26 and 43 % of the amino acid content was present in the microbial, insoluble and soluble fractions, respectively. In additional work he reported the AA urinary contribution to the total amino acid content of excreta produced over a 48 hour period by cockerels surgically modified and force-fed two 30 g amounts of a nitrogen-free diet over a 6 hr period to be approximately 33 %. By difference the soluble endogenous secretion from the gut could be expected to be around 10 % (43%-33%).

The work of Snook and Meyer (1964b) supports this conclusion. They found that approximately 90 % of endogenous proteins consisting of gut secretions and sloughed cells were digested and absorbed leaving 10 % for secretion with feed residues.

Several approaches have been employed to measure endogenous amino acid losses. They are as follows.

(1) Measuring the amino acids of excreta produced by unfed birds and using these values as a measure of the endogenous losses of fed birds. Likuski and Dorrell (1978) used this method to determine the true amino acid digestibility of feedstuffs. Some amino acids were more than 100 % digestible which indicated that the measure for endogenous production overestimated that in fed birds. Subsequently Muztar and Slinger (1981) suggested use of the fed bird as its own control.

(2) Using a nitrogen-free diet to obtain an estimate of endogenous amino acid excretion. Parsons (1981) compared this approach with that using starved birds. Endogenous amino acid secretion was twice as high using a low fibre N-free diet for a 48 hrs collection period in adult cockerels. Low (1980) suggested that the absence of protein in the feed can change the metabolism of the animal away from what ould be considered to be a normal response. The results obtained by Muztar and Slinger (1980b) were consistent with those of Parsons et al. (1983). They found roosters fed on a N-free diet excreted more amino acid than unfed birds.

(3) Feeding birds with several levels of a single protein source. The intercept of the regression line provides an estimate of endogenous amino acid output (Bielorai et al., 1985). Siriwan and Bryden (1986) reported this procedure gave greater estimates of endogenous amino acid excretion than the N-free diet method.

(4) A lesser used method is to feed birds with a protein diet which is completely digested and absorbed.

Various feed characteristics are reported to influence endogenous amino acid output. Nasset (1964) suggests a homeostatic condition operates in the secretion of endogenous nitrogen into the digestive tract to prevent wide fluctuations in the amino acid mixture. Hence endogenous N secretion could be expected to vary with the quality and quantity of dietary protein. McNab and Shannon (1972) fed fish meal, soyabean meal and field bean meal to birds. The N values of the duodenal contents increased 2, 5 and 15 fold over that of the feed N respectively. Bolton (1961) and Imondi and Bird (1965) and Bird (1968) found that most of the N leaving the duodenum was absorbed in the jejunum but that absorption in the ileum was not significant. Imondi and Bird (1966) suggested this phenomena was linked with the length of the villi which decreased in size gradually from the duodenum to the ileum.

Parsons et al. (1983) found dietary carbohydrate has a substantial effect on the secretion of endogenous N in chickens. High fibre diets

resulted in a greater amino acid secretion in N-free diets than low fibre though the difference was not significant. Similar findings have been reported for rats (Meyer, 1956; Whiting and Bezeau, 1957). Beames and Eggum (1981) consider the response may result from high fibre diets being passaged more rapidly with a corresponding increase in the sloughing off of cells. Hallsworth and Coates (1962) have suggested such diets may increase the secretion of mucous.

On the other hand, Sibbald (1980) and Sibbald (1981) found the feeding of cellulose, sand and sawdust did not influence endogenous N secretion.

Antinutritive factors such as trypsin inhibitors and tannins have been reported by Green et al. (1973) and Rostagno et al. (1973) to increase the secretion of endogenous nitrogen. Other factors influencing endogenous N have been reported. They include period of starvation time, age and body weight of birds (Sibbald, 1981), levels of protein (Snook and Meyer, 1964a), levels of feed (Shires et al., 1980), type of feed (Isshiki et al., 1988) and dry matter of feed (Meyer, 1956).

2.3 Ileal Collection Methods

Many researchers have suggested that amino acid digestibility can be accurately determined from the analysis of ileal digesta for by using this material the effects of fermentation by bacteria of the hind gut on undigested residue and hence on digestibility can be avoided (Achinewhu and Hewitt, 1979; Raharjo and Farrell, 1984). In addition such procedures remove the influence of metabolic activity reflected in the urinary component of excreta on digestibility (Austic, 1983; Sibbald, 1987). In this subsection methods of measurement of ileal digesta are outlined first. This is followed by a review of evidence relating to the magnitude of the hind gut bacterial effect and that of urinary mixing on digestibility values. The chapter ends with a brief survey of environmental, bird and feedstuff properties that may affect digestibility measurements.

2.3.1 Ileal Slaughter Assays

Payne et al. (1968) first made use of the ileal digesta of chicks to measure amino acid digestibility. The procedure involves a N free basal diet consisting of perhaps cornstarch, sugar, corn oil, cellulose, salt and a vitamin and mineral supplement to which is added in known proportion the test material and an indicator, commonly chromic oxide, to form the test diet. The test diet is fed to young chicks and at some defined period subsequently the chicks are slaughtered and samples of digesta removed from their terminal ileums.

Amino acid digestibility may be calculated using the following equation:

$$\% \text{ AA digestibility} = 100 - \left(100 \times \frac{\% Cr_2O_3 \text{ in feed} \times \% \text{ AA in digesta}}{\% Cr_2O_3 \text{ in digesta} \times \% \text{ AA in feed}}\right)$$

The method requires that the proportions of chromic oxide and amino acids being investigated be known or obtained for the feed and the digesta.

The digetibility so obtained may be corrected to give true values by feeding separately a nitrogen free basal containing an indicator and correcting the AA per unit of digesta arising from the test food by the correction factor so derived.

A formula for true ileal AA digestibility is as follows.

$$TAAD\ \% = 100 - \left(\frac{100 \times \%Cr_2O_3 \text{ test}}{\%Cr_2O_3 \text{ digesta}_{test}} \times \frac{\%AA \text{ digesta}_{test}}{\%AA \text{ test}} - \frac{100 \times \%Cr_2O_3 \text{ basal}}{\%Cr_2O_3 \text{ digesta}_{basal}} \times \frac{\%AA \text{ digesta}_{basal}}{\%AA \text{ test}}\right)$$

The correction assumes that the test food and basal influence the weight and composition of endogenous excreta equally for each gram of either fed.

A number of studies have been carried out to ascertain the relative sensitivity of ileal assays. Varnish and Carpenter (1971, 1975) reported that the digestibility of amino acids in heat-damaged muscle protein or propionylated lactalbumin was 5 to 12 % lower when measured from the ileal digesta as compared to the faecal excreta of chicks. No differences were observed for good-quality muscle protein. Elwell and Soares (1975) found similar amino acid digestibilities by partial faecal collection and ileal collection procedures for fish meal and soybean meal in chicks. Raharjo and Farrell (1984) compared the digestibility of several animal and plant proteins as obtained using the whole ileum, the 10 cm portion of the terminal ileum, the post caeca region and the excreta. Average apparent N digestibilities were 62, 74, 74 and 57 %, respectively. They considered the greater digestibility associated with sampling from the terminal ileum as compared to use of the terminal excreta was the result of urinary nitrogen. Low (1985) found the amino acid digestibilities of meat and bone meal subjected to different heat treatments were greater for those based on ileal digesta than for excreta collection precedures, but both procedures were equally effective in demonstrating the influence of heat damage on digestibility in chicks.

The inconsistency in the relative magnitude of digestibility between ileal slaughter and faecel collection procedures and in their capacity to demonstrate effects arising from heat damage may be attributed to a number of factors that are confounded in the preceding reports. They include the influence of urine on digestibility values, the microbial contribution of the hind gut to excreta amino acid profiles, level of feeding and characteristics of the diet fed.

Aspects of ileal methodology have received considerable attention in recent years and these are reviewed under four categories.

2.3.1a The Effect of Passage Rate

The digestive tract of poultry differs in a number of respects from that of monogastric animals. The length of various parts of the digestive tract of the chicken are shown in Table 1. The rate of passage of digesta through the digestive tract can be expressed in several ways. The time required for the first appearance of faeces after feeding (Hillerman et al., 1953; Tuckey et al., 1958). The time required to completely clear the digestive tract (Cherry and Siegel, 1978) and the time required to empty the crop (Keith et al., 1927; Heuser, 1945).

Sturkie (1976) described methods by which the rate of passage of feed in poultry may be measured. These are summarised as followed:

(1) Birds slaughtered at different times following feeding to observe the location of feed in the intestinal tract.
(2) The food may be stained with certain dyes so that it may be recognised in the faeces.
(3) Certain types of feed, such as oats, may be recognised in the faeces without marking (Browne, 1922).
(4) The passage of food may be observed with X-rays.
(5) The food may be collected by placing cannulas into the different portions of the tract.

In chickens, Jensen et al. (1962) observed that although the feed marker made its first appearance in the excreta 1 hour following feeding the peak of excreta production took place 4-6 hours following feed intake. They found that pellets had a faster passage rate than ground pellets and mash when fed to chicks. Kaminska and Summers (1988) studied the passage rate in White Leghorn and broiler birds of the same age. The marker first appeared about 1-1.5 hours following feeding and maximum excreta voiding took place about 4 hours post feeding. The rate of passage when expressed as excreta produced per unit of time was not different between the two types of birds.

The rate of passage is influenced by consistency, hardness and water content of the feed (Sturkie, 1976), the type of feed and the level of intake (Sibbald, 1979a) and the physiological state of the birds (Tuckey et al., 1958). The first feed entering the crop is passaged rapidly through the proventriculus and into the gizzard in starved birds (Halnan, 1949). Heuser (1945) found that rate of passage depended on the type of feed and was related to the time require for grinding in the gizzard.

High environmental temperature increased rate of passage in White Pekin ducks (Wilson et al., 1980). Mateos et al. (1982) observed that when the level of supplemental fat was increased from 0 to 30 %, the average first appearance time of the feed marker was increased from 3.0 to 4.5 hours. The addition of antibiotics in the feed may slow down the passage time in chickens and turkeys (Hillerman et al., 1953). Old birds may passage food more slowly than young birds (Thornton et al., 1956). Disease may also slow passage rate (Aylott et al., 1968; Duke et al., 1969).

Jones and Sibbald (1979) and Sibbald (1979b, 1979d) found that feeds pass through the digestive tract within 24 hours, but some feedstuffs such as rapeseed products, dehydrated alfalfa meals, low density oats, meat meals and fish meals have a longer passage time.

Table 1. Absolute and relative lengths of various parts of the digestive tract of the chicken at two ages.

Part	Age, 20 days		Age, 1.5 years	
	cm	% of total	cm	% of total
Upper esophagus	7.5	8.3	20.0	9.8
Lower esophagus	4.0	4.4	15.0	7.4
Duodenum	12.0	13.3	20.0	9.8
Ileum	49.0	54.1	120.0	58.9
Caeca	5.0	5.5	17.5	8.6
Colon + cloaca	4.0	4.4	11.3	5.5
Total	90.5	100.00	203.8	100.00

From Calhoun, Microscopic Anatomy of the Digestive System of the Chicken, Iowa State U. press, Ames. 1954.

2.3.1b The Effect of Indicators

The derivation of digestibility formulas involving indicators are given in section 2.1.2.

The digestibility of amino acids can be determined by the used of indicators in the food. This obviates the need for measuring the quantity of food consumed and that of residues produced. They are employed when samples are collected by way of an ileal cannula or in slaughter techniques. Markers need to be indigestible, unabsorbable and inert. Ideally they should have properties that enable them to mix uniformly with the feed, pass through the digestive tract at a rate that permits them to maintain a constant ratio with that of the feed residues over time and be free of pharmacological or physiological effects on the digestive tract (Sibbald, 1979e).

For solid digesta the most widely used indicator is chromic oxide (McNab and Shannon, 1972; Achinewhue and Hewitt, 1979). Alternative indicators have included ferric oxide (Bragg et al., 1969), cellulose (Bolton, 1964) and acid insoluble ash (Wallis and Balnave, 1984).

Fluid digesta can be determined using soluble markers such as phenol red (Uden et al., 1980), cobalt ethylenediamine tetracetic acid (Gonalons et al., 1982), and polyethylene glycerol (Roudybush et al., 1974). Lipid digesta can be measured by the addition of tridodecyl ether (Carlson and Bayley, 1970) to the fed material.

Vohra and Kratzer (1967) reported that chromium can be absorbed by the chickens and in their work recovery was only 88%. Dansky and Hill (1952) found most of the chromium was recoverable within 24 hrs in chicks. Jensen et al. (1962) reported that some markers may pass through the digestive tract faster than the feed. Vohra (1972) suggested that although there are some problems with chromium analysis, they can be overcome by the used of the atomic absorption spectrophotometry.

2.3.1c The Effect of Different Slaughter Methods

In the ileal slaughter technique, birds have been killed by cervical dislocation (Bolton, 1961; Soares and Kifer, 1971; Skurry and Cumming, 1975; Raharjo and Farrell, 1984) and killed by injection of sodium pentobarbitone (Bolton, 1965; McNab and Shannon, 1972; Achinewhu and Hewitt, 1979; Wallis and Balnave, 1984; Bielorei et al., 1985) or by way of carbon dioxide inhalation (May et al., 1988).

The method of slaughter appears to be important. The problem of contamination of digesta with mucosal shedding at death has been studied. Summer and Robblee (1985) reported that the apparent digestibility of amino acids between birds sacrificed by anaesthesia (halothane) and cervical dislocation were not significantly different. The averall means were 83.9 and 82.9%, respectively. Bolton (1964) sacrificed adult chickens by cervical dislocation. The studies showed agonal spasms caused movement of the digesta up and down the gut and raised the possibility that this effect may influence digestibility results. Bolton (1962) found that the consequences of struggling during death can caused a movement of Coliform organism from the crop to the gizzard and could result in nonsterile eggs. When birds were killed under anaesthesia by an overdose of sodium pentobarbitone, struggling was absent and the oviduct was completely sterile.

In studies with sheep. Badawy (1964) suggested that when the animals were sacrificed, the intestinal contents might be contaminated with the shedding of epithelium from the lining of the intestine. Badawy et al. (1957) and Badawy et al. (1958) stated that when sheep were shot and bled, the intestines underwent peristalsis and shedding cells increased the nitrogen content of the digesta. On the other hand when sodium pentobarbitone was used, peristaltic movements were reduced, and the mucosal lining remained intact. Badawy et al. (1958) and Fell et al. (1961) reported that cell shedding occurs within about 10 minutes of the cessation of respiration in rats and sheep, and is accelerated by bleeding and increases progressively with time after death. Cell shedding may also result from prolong vascular engorgement or spasm (Fell, 1969).

The above studies indicate that the use of sodium pentobarbitone may avoid cell shedding and the movement of digesta during death and may constitute a preferred method of sacrifice.

2.3.1d The Effect of Digesta Removal

In chickens, the ileum is generally considered to be that portion of the gut extending from Meckel's diverticulum to the ileocaecal junction. Methods of digesta removal usually involve gently flushing with distilled water using a syringe or gentle digital extrusion into containers. McNab and Shannon (1972) concluded that the collection of digesta by digital extrusion probably caused mucosal shedding into the sample. Payne et al. (1968) and Soares and Kifer (1971) used a 5% trichloroacetic acid solution to wash digesta from the ileum subsequent to analysis of the digesta for amino acids.

2.3.2 Ileal Cannulation

This is a procedure involving the insertion of a cannula usually into the terminal ileum. It has been employed in adult stock and serves the dual purpose of providing a means of sampling ileal contents and of preserving the test birds for reuse.

Ileal cannulation in chickens has been used by Raharjo and Farrell (1984), Summer et al. (1982), Thomas and Crissey (1983) and Johns et al. (1986b). Thomas and Crissey (1983) found some types of cannulas caused sampling problems and only half of the cannulated birds could provide samples. Johns et al. (1986b) reported only 60% cannulated cockerels were still healthy 9 months after surgery. Other problems have been cited (Sibbald, 1987). These involve the effect of cannulation on

digestibility, factors associated with the free flow of digesta through the cannula, effects consequent on particle size of the feed, the influence of feeding frequency, marker recovery and collection procedure.

2.4 Studies with Colostomized Birds

In poultry, faeces and urine are mixed and excreted together. A purpose of colostomizing birds in studies related to digestibility is to enable separate collection of urine and faeces to ascertain the impact of each on excreta values. Sibbald (1987) suggests that debiting the amino acid or nitrogen of urine to the faecal concentration and using the combined value to estimate the amount removed from the quantity fed is a departure from the definition of digestibility and results in measurements he terms metabolizable amino acids. As metabolizable amino acids are a measure of both digestibility and subsequent metabolizable and physiological utilization (Austic, 1983), they incorporate a metabolic effect and are potentially subject to the influence of additional factors affecting variation.

Bragg et al. (1969) found amino acid content of gut residues or excreta was higher in colostomized chicks than intact chicks. Sibbald (1987) cites Gruhn (1974) as finding in a comparative study that intact birds gave lower estimates of amino acid absorption than did colostomised birds.

The urinary amino acid contribution to excreta has been reported to be small and as such would have a negligible effect on digestibility (Sykes, 1971; Terpstra, 1979). Tasaki (1987) was unable to detect amino acids in the urine and concluded that separation of faeces and urine was unnecessary in amino acid digestibility studies. On the other hand O'Dell et al. (1960) showed that the amino acid content amounted to 3% of the nitrogen in chicken urine. They also concluded that this was

unlikely to have a significant effect on amino acid digestibility. Parsons (1981) reported Teekell et al. (1968) as obtaining an amino urinary nitrogen excretion in hens of 6 mg/d which equates with 38 mg of amino acids per day. In Parsons (1981) own work he obtained an essential amino acid excretion level over 48 hours in colostomized cockerels fed a nitrogen free low fibre diet of 176 mg which equated with 100 mg/day total amino acids, an amount approximately 2.5 times as great as the findings of Teekell et al. (1968). He observed that surgical procedures may have increased urinary excretion and cites the work of Low and Zebrowska (1977) in support of that observation.

His work provides a useful insight into the proportional contribution of amino acids in excreta. He found that the contribution of amino acids in the excreta of adult poultry fed a stock layer ration was 31% attributable to a microbial sediment component, 26% attributable to insoluble residues (feed residues) and 43% attributable to a soluble fraction comprising urine and endogenous secretions of the gut. In a different experiment on colostomized cockerels referred to in the preceding paragraph the urinary essential amino acid fraction of the total excreta (faeces and urine) was 33%. The quantity in the urine (176 mg/48 hrs) equates to a urinary nitrogen content of 28 mg/48 hrs or 14 mg/24 hrs. If it is assumed that 3kg body weight cockerels have a maintenance nitrogen excretion of about 445 mg/d (Sim, 1986) then the 3% urinary nitrogen contributed by amino acids referred to previously under O'Dell et al. (1960) is equivalent to a 13.4 mg/day output of urinary amino acids, a quantity in keeping with the findings of Parsons (1981).

Further, if the soluble fraction of the excretion partition of Parsons (1981), contributes 43% of the total amino acid excretion under stock layer feed conditions of feeding and the urine contributes 33% under nitrogen free diet feeding conditions, it is clear that the urinary impact on amino acid digestibility may be substantial.

2.5 The Effect of the Intestinal Microflora upon Amino Acid Excretion

Determination of nitrogen and amino acid digestibility using faecal collection procedures have been widely criticized on the grounds that the intestinal microflora especially of the caeca and large intestine may have a substantial effect on the amount of nitrogen and amino acids excreted. Substantial effects have been observed in pigs (Holmes et al., 1974; Zebrowska, 1978) and rats (Mason and Palmer, 1973).

Zebroswka (1978) found evidence of considerable degradation in pigs. In 2 studies he found the equivalent of 80-90% of nitrogen of an enzymatic hydrolysate of casein infused into the large intestine using an ileocaecal cannula was excreted as urea nitrogen. According to studies by Binder (1970) as cited by Austic (1983) and those of Zebroswka (1978), the pig hind gut does not appear to play a significant role in the absorption of amino acids for protein formation in the host animal. Instead evidence in the form of digestibility studies reported by Holmes et al. (1974) and Sauer et al. (1980) as cited by Austic (1983) indicates hind gut fermentations may result in a net synthesis of some amino acids which contribute to the protein composition of the bacterial population. A table presented by Austic (1983) in which amino acid digestibility obtained by ileal and faecal procedures are compared for a variety of feedstuffs, demonstrates consistantly lower ileal digestibility and supports the concept that hind gut fermentation results in a net loss of amino acids from intestinal residues.

The studies in swine provide a useful guideline to influences different regions of the gut in chickens may have on digestibility values. In contrast to the pig, chickens have a relatively short large intestine of relatively reduced capacity (Austic, 1983; Papadopoulos,1985). In addition the rate of passage of digesta in fowls is relatively rapid (Papadopoulos, 1985). Perhaps partly in consequence, in general, the effect of the intestinal microflora on the digestibility of nitrogen or individual amino acids in poultry have been considered to be less important or of little significance. However clear evidence exists of bacterial activity.

Austic (1983) reports Mason et al. (1976) as finding that 50% or more of the faecal protein (on a dry matter basis) was contributed of microorganisms in pigs whereas in poultry, Parsons (1981) found the microbial protein contributed 31% of the essential amino acids of the excreta. Bryden and Bluett (1986) conducted an experiment using a physical separation technique to separate the microbial fraction from the ileal digesta of chicks. Approximately 12% of the digesta was microbial. Just (1980) reporting on pigs found that the bacterial activity in the large intestine may have a 3-5% influence on nitrogen digestibility. Skurray and Cumming (1975) concluded that for chicks the bacterial activity was greater in the posterior than anterior segment of the small intestine, and that the caeca contained the highest population of microflora. Barnes (1972) found bacterial counts were considerably higher in the caeca contents and numbered approximately 10^8/g (wet weight) of the intestinal contents for poultry. Anaerobic bacteria capable of decomposing uric acid were found at high levels by Barnes and Impey (1974) and these researchers suggested that the microflora of the
caeca may play a substantial role in reutilization of uric acid.

Terpstra (1977) summarised factors which can influence fermentation in the digestive tract. They were as follows: (1) The time required for feed to pass through the digestive tract. (2) The amount of nitrogen which reaches the lower intestine. (3) The amount of energy reaching the lower intestine.

2.5.1 Studies with Caecectomised Birds

These studies where they involve a comparison of intact versus caecectomised birds in digestibility assays and employ total excreta collection techniques, remove the potential issue of urinary constituents confounding interpretation (as urine is common to both). They provide an unbiassed comparison of the effect of caecal activity on digestibility under conditions of urinary exposure.

Most microbial fermentation in the large intestine area of chickens is thought to occur in the caeca. A review has been made by McNab (1973) on the functions of the avian caeca. These include: (1) water absorption (Thornburn and Willcox, 1965); (2) carbohydrate digestion or microbial decomposition of cellulose; (3) protein digestion or non-protein nitrogen absorption; (4) microbial synthesis of vitamins and their absorption: (5) immunization (Mayhew, 1934). Lev and Briggs (1956) found the microflora of the caeca in two-day old chicks reached a concentration that was very similar to that found at 10, 16 and 30 days after feeding. It has been suggested that the microflora may be able to synthesize amino acids (Austic, 1983) or utilize undigested amino acids without nutritional value to the host.

Several studies have been conducted comparing amino acid digestibility as determined by assays conducted on intact or caecectomised birds. Nitsan and Alumot (1963) found that caecectomised chicks fed raw soybean meal had poorer performance than intact chicks. Payne et al. (1971) examined the apparent protein digestibility of fish meal in birds. Digestibility in intact birds was slighly greater than that from caecectomised chickens. Studies by Nesheim and Carpenter (1967) showed that the apparent digestibility of heat-damaged cod flour was 68 % for caecectomised chicks, but for intact chicks, it was 77 %. For good quality cod muscle, there was no difference. Johns et al. (1986a) reported that the apparent amino acid digestibility of heat treated meat and bone meal measured using caecectomised cockerels was lower than that obtained using intact cockerels. Parsons (1984, 1986) force-fed 30 g of meat meal to caecectomised and intact cockerels. The true digestibility of all amino acids were lower for caecectomised than for intact cockerels. For plant feeds Picards (1983) found that the true amino acid digestibility was not significantly different between caecectomized and intact birds. Green et al. (1987) tested several cereals and a protein-free diet using cockerels. The endogenous amino acid output between intact and caecectomized birds was not significantly different when the protein-free diet was used. The apparent digestibilities of nitrogen and amino acids in cereals were inconsistent between intact and caecectomised birds and this was considered to be due to the variability

in endogenous amino acid excretion. Kessler et al. (1981) found that fasted caecectomised roosters excreted more amino acids than fasted intact roosters over a 24 hr collection period.

These results suggest ths caeca are involved with a net removal of amino acids from the digesta leading to an increase in digestibility. The extent of the effect may depend on the protein source.

2.5.2 Studies with Germ-free Birds

These studies compare measurements obtained using germ-free and conventional birds. Their general purpose is to show the effect of bacterial contamination on measurements. In the context of these studies, germ-free means free of contamination by bacteria, yeasts, moulds, fungi, protozoa, parasites; in general, free of all other life.

Several studies have been conducted comparing the utilization of nitrogen and amino acids between germ-free and conventional chicks. Miller (1967) reported no differences between net protein utilization values obtained with germ-free and conventional chicks fed a casein-gelatin diet. Salter and Coates (1971) fed germ-free and conventional chicks with ^{14}C-labelled freeze-dried and heat-damaged egg albumin and found the ^{14}C:nitrogen ratio was higher in the large intestine of the conventional as compared to the germ-free chicks. They and Salter (1973) reported that the ammonia concentration of caecal residues was five times higher for conventional as compared to germ-free chicks. The studies concluded that bacterial activity in the lower gut of chicks did not improve the availability of amino acids but such activity may influence amino acid digestibility involving the total collection procedure. Salter and Fulford (1974) found the true digestibilities of amino acids in the freeze-dried and heat-damaged egg albumin were no different for germ-free and conventional chicks. Salter et al. (1974) found that germ-free chicks excreted more endogenous amino acids than

conventional chicks when a nitrogen-free diet was provided. In addition, the nutritional values in several poor quality protein feedstuffs were not substantially different in both types of chicks. However, Soares et al. (1971) found that monocontaminated germ-free chicks fed fish meal consistently excreted smaller amounts of amino acids than conventional chicks fed fish meal. They concluded that the conventional chicks may underestimate the digestibility of amino acids. Coates (1976) concluded that the effects of intestinal microbial life on protein digestibility seemed to be small, but that it may degrade endogenous proteins.

2.6 The Influence of Miscellaneous Variables on Digestibility

2.6.1 Level of Protein

Yokota (1978) used faecal collection assays to investigate the effect of levels of protein on N digestibility in colostomised cockerels. They found no significant difference in N digestibility between test diets containing 6% and 71% test protein. Feed intake was the same in a protein-free and the low protein test diet and was twice that of the high protein treatment. Keulder (1978) compared the effect of two levels of protein on amino acid digestibility in chicks based on excreta collection procedures using Brown Fishmeal as the protein source. The amino acid digestibility of the 13% and 29% test protein diets were not significantly different. Brown and Squance (1967) employing faecal collection studied graded levels of mixtures of animal and cereal proteins in feeds. Results indicated that 2.5 to 25% protein inclusion had no effect on true N digestibility.

2.6.2 Age and Temperature

McNab and Shannon (1972) studied protein digestibility in the ileum of 13, 17 and 24-week old pullets and found there were no effects of age on N digestibility. Equivalent results were observed by Hvidsten and Bjϕrnstad (1978) in a comparative study involving adult colostomised hens and chicks. Good agreement was recorded for N digestibility across all feedstuffs tested except that of meat and bone meal. Similar results were obtained by Keulder (1978) who worked with chicks ranging in age between 21 and 62 days. There were no significant differences in faecal amino acid digestibility. The author suggested that 42 day old chicks were easy to handle and should provide sufficient ileal sample. However, Fonolla et al. (1981) using total excreta collection procedures and male broilers found that protein digestibility decreased with age. Wallis and Balnave (1984) reported that the ileal digestibility of all amino acids were significantly higher at 50 than 30 days of age in broilers. Håkansson and Erissson (1974) concluded that the digestibility of N decreased and that of fat increased with the age in poultry. Wilson et al. (1980) found that high environmental temperatures had an effect on amino acid digestibility in female but not in male broilers.

2.7 Indirect Procedures

2.7.1 Enzymatic Assays

These involve the determination of the quantity of amino acids released from a protein source material on its incubation with proteolytic enzymes. The simplest assays involve incubation of the feedstuff with one enzyme at a particular pH, eg. pepsin at pH 2. Some involve a second enzyme or enzyme complex incubation step eg. the former step followed by pancreatic proteases at pH 7-8. Digestibility is obtained by a comparison of the amino acid or nitrogen content of the test

material with that of the final insoluble residue remaining (Low, 1981) following digestion, filtration and washing.

Simple enzyme assays have been described by Sneffner et el. (1956) and Rayner and Fox (1976) and multi-enzyme assays by Akeson and Stahmann (1964) and Saunders and Kohler (1972). Some assays have been developed to rely on the release of a specific amino acid such as tryptophan (Lombard and de Lange, 1965).

Resuls from enzymatic assays have been compared with those of ileal and faecal and chemical digestibility studies. Rayner and Fox (1976) compared the amino acid digestibility of rapeseed meals using either pronase or hydrolysis with 6M HCl. More amino acids were released under the acid hydrolysis procedure than by the action of pronase. On the other hand Datta (1978) found the lysine digestibility of poultry diets as estimated by pepsin:pancreatin digestion equated more closely with results obtained from biological assays than to those obtained using acid hydrolysis. Saunder and Kohler (1972) used pronase B and chick pancreatic material to digest wheat millfeeds and found the amino acid profiles of undigested residues to be identical to those resulting from in-vivo procedures. Bielorai et al. (1983) reported similar findings. The amino acid compositions in the residues of feather meal digested by pepsin and pancreatin and by in-vivo procedures were alike. Clunies and Leeson (1984) reported a correlation coefficient, r, of 0.93 for the N digestibility of finely ground (capable of passing through a 0.40 mm screen) poultry diets as obtained using enzymatic techniques and in-vivo ileal methods in rats.

It has become apparent that the type of enzyme or enzyme complex and the enzyme-to-substrate ratio influence the in-vitro result. In general, for cereals, multi-enzyme digestion equates more closely to in-vivo methods (Buchmann, 1979). Johnston and Coon (1979) explored the effect of graded levels of pepsin on the digestibility of various animal protein sources. They found that decreased levels of pepsin increased the sensitivity of the assay and increased the range of difference between amino acids between diets. Carpenter (1958) suggests single

enzyme digestion of a mixed diet may give erroreous results because some proteins are more susceptible to attack by one particular enzyme than by another.

The conditions under which enzymes interact with substrates can have profound affects on the rate and end products of digestion. Sibbald (1987) cites the work of Kratzer and Porter (1962) who studied the effect of pH on the digestion of milk and soyabean proteins by pepsin. Raising the pH to 4 decreased the rate of digestion of soyabean protein more than milk protein and caused major changes in peptide formation.

The accumulation of the end products of enzymatic digestion may affect continuing enzyme activity (Sibbald, 1987). The enzymatic digestion system of Ford and Salter (1966) takes place in a column that permits the products of digestion to be eluted. In the system described by (Gauthier et al., 1982) two stages of digestion are employed involving pepsin then pancreatin. The latter stage takes place under continuous dialysis.

In-vitro techniques are relatively quick, inexpensive and convenient methods of estimating digestibility. However the factors that influence results which include enzyme types, quantities and combinations, time of incubation, pH, particle size and temperature are a source of variability in in-vitro digestibilities both between samples and between protein sources and these may confound rankings between ingredients. Sibbald (1987) reports Clandinin and Robblee (1952) as attributing to enzymatic assays a possible quality control function. In the reported case in respect to identifying the optimum duration of heat treatment to which protein sources such as soyabean meal should be exposed.

Enzymatic enzymes clearly defined and controlled for a protein source material may provide a suitable form of assay, predicting differences in protein digestibility for samples of the same product grown under or subjected to different field or manufacturing conditions. However they have value only to the extent that they reflect in-vivo values. Since the digestive environment provided by in-vitro techniques cannot

reproduce the complex and dynamic conditions of the gut (Low, 1982) the degree to which they simulate in-vivo digestion in any given situation must remain uncertain.

CHAPTER 3

AVAILABILITY

Sibbald (1987) describes availability as "an abstract concept which can be defined but not measured". He suggests "a nutrient is bioavailable if on entering a living tissue it can be used for normal metabolic function". It may be argued that the way in which the body disposes, utilizes or otherwise handles ingested material constitutes a metabolic response and all such responses are normal for the state of the animal.

He suggests "utilization of an absorbed molecule is proof of bioavailability, but excretion is not evidence of a lack of bioavailability" and illustrates his views by stating "minerals absorbed in excess of storage capacity are usually excreted but may be bioavailable because they have potential utility". The remarks highlight four issues. They are:

(1) Utilization is a prerequisite for availability.
(2) The animal decides the fate of the absorbed compound.
(3) The metabolic pathway deployed is governed by the physiological state of the animal.
(4) Absorbed compounds and elements have a potential for utilization. By implication this potential is a function of the nutritional role of the material and the discrete structural form in which it is present.

Zebroswska (1978) recognizes that availability incorporates the processes of digestion and absorption before utilization can proceed, but does not qualify the form of utilization.

De Muelenaere et al. (1967b) defined amino acid availability as "that portion" (proportion) "of an amino acid present in a protein which is used for growth , development and maintenance of an animal in so far as it is dependent on the digestibility of the protein, enzyme inhibiting substances, and rate of release of the amino acid in the intestinal tract". All metabolic activity may be considered to contribute directly

or indirectly to growth development and maintenance. The definition does not address the question of structural form or complex of which the amino acid forms a part or whole and the impact of compound structure on subsequent utility following absorption.

Sauer and Ozimek (1985) defined availability as "the proportion of the AA in the diet that is absorbed in a form suitable for utilization and as such is measured by the slope-ratio method eg. Batterham et al. (1979)". This definition like that of de Muelenaere et al. (1967b) makes no distinction as to the utilization that may be imposed as a consequence of structural form. By way of example, an AA in one form may be excreted directly it is absorbed. In another form it may undergo accretion into protein. Both forms are available for metabolic purposes and both forms are utilized. However only one form is retained and contributes in a direct sense to weight gain or nitrogen retention.

Papadopoulos (1985) defined availability as "the degree to which AAs are present in a form suitable for digestion, absorption and metabolic processes". The definition of Low (1982) is "that proportion of an AA in a diet which is not combined with compounds which interfere with its digestion, absorption and metabolism". By omission both imply that the form of utilization is inconsequential.

However specificity of utilization is important. In addressing this issue Austic (1983), referring to growth as a measure of AA availability, states that its use is based on the "assumption that body or carcass composition is constant". His comments imply that body weight is used because under the foregoing assumption it is a valid index of protein accretion. Again by inference Sibbald (1987) sees the role of protein accretion as being important to the concept of AA availability by stating in comments relating to growth assays, initially, N retention is preferable because AAs at suboptimal intakes, should affect tissue protein synthesis.

In modern poultry diet formulation AAs are supplied to provide for the birds need for AAs for net protein accretion. Whilst utilization of AAs may provide an incidental source of energy, heat and substrate elements and compounds, the dietary supply is intended to provide AA adequacy for protein formation to support the end processes of growth production and replacement. Measures of availability are intended to be an index of the extent to which the AAs supplied have been available to the bird for use for these ends.

Accordingly the definition of Sauer and Ozimek (1985) is modified by qualifying "utilization" and is presented as a preferred statement of availability as follow:

> The proportion of the AA in the diet that is absorbed in a form with the same potential for utility as its pure or unmodified form.

Availability as measured is an index. It is commonly obtained by way of a ratio in which a test is related to a standard or reference result. It may in some assay forms be obtained by difference. The reference diet is usually composed of a highly available source material such as crystalline AAs or lactalbumin. For purposes of standardization it is convenient to attribute to the reference diet an availability of 100 %.

Assay procedures employed may be in-vivo and as such are usually direct ie. applied to the target species. Examples are the growth and plasma AA assays. They may also be in-vitro or indirect and take the form of microbial or chemical techniques.

The values obtained are frequently described as relative and quantitative measures. In the former case the measures are suitable for ranking as opposed to the latter case in which they are considered suitable for direct adoption.

3.1 Direct Procedures

3.1.1 Growth Assays

The most direct approach to determining availability of an amino acid is by way of the growth assay (Hill et al., 1966; Robel and Frobish, 1977). It constitutes a preferred approach in so far as it may be conducted on target species, constituents of the basal diet may be of a type used commercially and the response criteria, usually body weight gain is a measure used commercially to evaluate performance.

The assay is commonly designed in accordance with the slope-ratio model of Finney (1951). A test diet comprising a basal deficient in the AA under test is supplemented at a series of levels with the test AA source and response is compared to that achieved with a reference diet consisting of the basal and incremental levels of a synthetic form of the AA under test or a reference protein source such as lactalbumin. The ratio of the slope of the regression lines provides the measure of availability. For the assay to qualify as valid the slopes generated should be linear and have a common intercept (Carpenter et al., 1972; Sibbald, 1987). Commonly body weight gain is made the dependent variable and is related to either dietary concentration of the test AA (Ousterbout et al., 1959) or total test AA consumed (Campbell, 1966) as the independent variable.

The synthetic or reference protein AA is assumed to be 100% available. Sibbald and Wolynetz (1985) in trials using adult cockerels, however found L-lysine HCl to be only 92% available.

For poultry, assays are normally conducted on chicks and should run for as long as it takes to achieve a representative response. In the experiments of Netke and Scott (1970), Smith and Scott (1965a) and Combs et al. (1968), the test period was 5 days. Robel and Frobish (1977) compared the availability of AAs of soyabean meal as obtained using 4, 6 and 8 day assays. The 8 day assay gave the highest AA availability. Hill et al. (1966) reported a 10% reduction in lysine availability using a 4 day test period as compared to a 5 day period.

The reliability of the growth assay has been questioned on a number of counts. The creation of diets deficient in one AA and the necessary use of a protein source in the test diet to provide incremental levels of the AA under test, introduces the possibility that dietary imbalances in AAs may distort the normal response to graded increases in the test AA (Fisher and Shapiro, 1961; Carpenter and Woodham, 1974; Harper et al., 1970).

The difference in protein content and AA balance within the test and reference diet may affect the growth response and bias resultant availability values (Gupta and Elvehjem, 1957; Uwaegbute and Lewis, 1966; de Muelenaere et al., 1967a; Guo et al., 1971; Baker, 1978; King, 1982).

The work of de Muelenaere et al. (1967b) suggests the energy to protein ratio of the supplemental diets may influence availability outcome and de Muelenaere et al. (1967a) as cited by Austic (1983) found starch and fibre influenced the availability of lysine in rat assays.

Several authors have recognised the value of using more direct measures to estimate availability. De Muelenaere et al. (1967a) reported a difference in lysine availability determined by rat assay when measures were based on lysine accretion as opposed to body weight. Austic (1983) advocates accretion of nitrogen in the carcass as a more meaningful response criteria than body weight gain. Sibbald (1987) points out however that Uwaegbute and Lewis (1966) found N retention to be in close agreement with weight gain but recognises that tissue protein synthesis is but one component of body weight gain.

A disadvantage of the growth assay that has inhibited its general application is that it estimates the availability of only one AA at a time. In addition unless the basal is made up, at least in part of synthetic amino acids, the range of AAs that can be made satisfactorily limiting by practical ingredients is limited. Sibbald (1987) claims in addition that the assays are slow, costly and laborious, and though conceding that they provide useful comparative data, suggests that the current need is for a simple and rapid assay approach.

3.1.2 Plasma Amino Acid Assays

Plasma free amino acid (PFAA) assays have been used to measure changes or differences in the concentration of free amino acids in blood plasma or serum subsequent to ingestion of food (Sibbald, 1987). A comparison of the concentration of the AA level achieved between a test diet and a reference source provides a measure of the amino acids availability to the animal. Corrections have involved PFAA values obtained on the feeding of a protein free diet (Hill and Olsen, 1963). Crystalline AA diets have been used as reference diets (Smith and Scott, 1965a; Dean and Scott, 1966).

Zimmerman and Scott (1967) observed that protein-free fed chicks gave rise to lower plasma AA levels, than starved birds. The results of Denton et al. (1953) suggest that the PFAA values of portal blood are generally greater than those of systematic blood for some time after feeding and Bielorai et al. (1972) as cited by Sibbald (1987) observed that monitoring portal blood gave an improved estimate of intestinal free AAs than the use of heart blood.

Smith and Scott (1965a) observed that the plasma AA technique can be used to compare the relative availability of AAs in proteins subjected to different processing treatments. They found (Smith and Scott, 1965b) that the test AA was more available in properly heated soyabean meal than raw or heat damaged material. This finding is supported by Hill and Olsen (1967) who found a decrease in the plasma lysine of chicks fed autoclaved soyabean meal. However it was also found (Smith and Scott, 1965b) that supplementation of the test diet with the first limiting AA reduced all AA levels in the plasma.

Jones (1964) reports interaction effects may influence availability measures. A deficiency of arginine in the diet caused a sharp accumulation of lysine in the plasma.

Dietary concentration of the first limiting AA may influence plasma levels. Zimmerman and Scott (1965) concluded that plasma levels of the

first limiting AA remained low until its concentration in the diet exceeded 10% of that required for maximum growth. Thereafter the plasma AA level increased linearly with a concurrent reduction in growth.

Various factors have been reported to influence plasma AA results. They include feed intake and sampling time (Hewitt and Lewis, 1972), age (Askelson and Balloun, 1963), frequency of feeding (Knipfir et al., 1972), environmental temperature (McNaughton et al., 1978), composition of feed protein, intake of AA, pattern of AA release during digestion and rate of absorption (Lewis, 1967).

McNab (1979) suggests measurements obtained using the plasma AA assay have not been widely correlated with other biological procedures and the results only represent the balance between supply to and removal from the blood when sampling.

Sibbald's (1987) assessment of the method is that the PFAA assay is not suitable for making quantitative estimates of AA availability in feedstuffs. He suggests its most promising application is in estimating relative efficiencies of amino acid isomers and analogues.

3.2 Indirect Procedures

3.2.1 Microbiological Assays

These assays rely on the following principles and procedures:

(1) Microbiological growth is a function of the release of amino acids and peptides from an innoculum containing the protein source under test.
(2) Growth is used for obtaining a measure of the availability of amino acids in the protein source.
(3) The measure of availability obtained corresponds to that for target animals.

The assays involve reference back to the growth achieved with an innoculum containing a standard source of the amino acids under test. The assays involve use of only microorganisms whose requirements for an amino acid or group of amino acids are known.

Several strains of microorganisms have been used to estimate the availability of amino acids. They include Streptomyces faecalis (Bunyan and Price, 1960), Streptomyces durens and L. arabinosus (Ford, 1964) and E. coli (Rayne et al., 1977). Two that have received particular prominence, Streptomyces zymogenes (Ford, 1960) and Tetrahymena pyriformis (Dunn and Rockland, 1947; Pilcher and Williams, 1954) are considered here.

3.2.1a Streptomyces zymogenes

Ford (1962, 1964) reported that the bacterium S. zymogenes required methionine, tryptophan, arginine, histinine, leucine, isoleucine, valine and glutamic acid for optimal growth. Ford (1964) used this assay to measure the availability of methionine in feedstuffs. The procedure involved partial enzymic hydrolysis followed by incubation with the proteolytic S. zymogenes. Growth was measured turbidimetrically or from acid production (Ford, 1962). Waterworth (1964) compared availability obtained using the microbiological assay with that of a growth chick assay using animal protein as test materials. There was good agreement between the two assays. Miller et al. (1965) also found this organism gave good agreement with the chick assay in the measurement of methionine availability. For meat, fish and whale meals the correlation coefficient was 0.93.

Ford (1964) and Miller et al. (1965) found that finely ground samples and the concentration of enzyme used during the predigestion period may increase the value of available methionine. Miller (1967) concluded that availability obtained using carbohydrate containing materials should be checked by comparison with those resulting from bioassay procedures.

3.2.1b Tetrahymena pyriformis

The protozoan tetrahymena pyriformis has been used to assess a wide range of sources and requires the same amino acids for growth as the rat (Sibbald, 1987). It has been used to estimate the availability of lysine, methionine, arginine and histidine in feedstuffs (Scott and Smith, 1966). The test food is provided at graded levels to the organism and the growth compared with that obtained for graded doses of the test amino acid. Growth has been determined by counting the number of organisms per millilitre of culture with a hemacytometer. It has often proved difficult to distinguish between cells and food particles.

The method has been modified and found to give close correlations with the FDNB procedure (Shorock, 1976) and the chick growth assay (Shepherd et al., 1977).

With respect to microbial assays generally, Sibbald (1987) has summarised a number of factors that may affect growth response. These include the composition of the growth medium with respect to carbohydrate, amino nitrogen, D-isomers of amino acids and vitamins and feed preparation procedures including grinding and choice and concentration of enzymes used in the predigestion of innoculum. He also questions whether peptides used by microorganisms are available to mammals and birds to the same extent.

3.2.2 Chemical Assays

3.2.2a FDNB

Of chemical assay methods employed to define protein quality the 1-fluro-2,4-dinitrobenzene (FDNB) procedure of Carpenter and his associates (Carpenter and Ellinger 1955a,b; Carpenter et al., 1957) and Carpenter (1960) has received most attention. It measures the quantity

of reactive lysine in a protein source. This is purported to be a measure of available lysine.

Excessive heat treatment and prolonged storage may damage proteins by the Maillard or Browning reaction. In this reaction, aldehyde groups of reducing sugars react with free amino groups, particularly the epsilon amino group of lysine, and amino sugar complexes are formed that are resistant to enzymatic attack during digestion. Chemical analysis of protein sources subjected to severe heat treatment have showed lysine content to be largely unchanged but growth assays suggest a large fraction of the lysine has been rendered unavailable.

The principle of the assay relies on the FDNB reacting with unbound (free) epsilon amino groups of peptide chains to form dinitrophenyl (DNP) compounds which on refluxing in acid (acid hydrolysis) are released. Following filtration the absorbance of the filtrate is measured colorimetrically and dinitrophenyllysine (DNPL) determined by comparison with a lysine containing standard. The availability of lysine is obtained by direct reference to the quantity of DNPL obtained or from the difference in lysine of the protein source before and after treatment with FDNB. The latter forms the basis of the approach of Roach et al. (1967) and is known as the Silcock available lysine method.

An implicit assumption is that lysine residues whose epsilon amino groups are not free are biologically unavailable (Sibbald, 1987). Venkatesan and Rege (1968) have raised the possibility that the position of lysine within the protein molecule may render it free from reaction with FDNB. Further assumptions on which the validity of the assay rests have been raised by Matheson (1968). They included possible measuring error due to colour interference by compounds released by protein sources of a vegetable origin. Partial destruction of DNPL on acid hydrolysis and the potential formation of coloured histidine derivitives confounding adsorption measurements.

However Sibbald (1987) reports Booth (1971) as concluding that this latter approach was free of most of the disadvantages of

"Nitrophenylation methods". Boyne et al. (1961) reports that for animal proteins the methods give a good correlation with chick growth tests and reflect available lysine content. Laksesvela (1958) found close agreement between lysine availability of heated herring meal as determined from the FDNB assay and that obtained using a chick growth assay. Major and Batterham (1981) reported close agreement between results obtained using the slope-ratio growth assay on chicks and the FDNB approach. McBee and Marshall (1978) compared amino acid availability by the FDNB assay and a microbiological assay. The overall mean was approximately 11 % higher for the FDNB procedure. Low (1982) reports Batterham et al. (1979) as finding a rather weak relationship between the in-vitro approach to determinig lysine availability using the methods of Carpenter (1960) and Roach et al. (1967) and that obtained by pig or rat assay for three protein sources, cottonseed, fishmeal and soyabean meal.

Sibbald (1987) concludes that the FDNB asssay provides useful quality control information particularly in respect to identifying heat damage. He suggests the data produced are more useful for ranking samples than for providing absolute values. He warns that the correlation between a protein quality index and an estimate of available lysine can only be strong if lysine is the first limiting amino acid.

3.2.2b D-methylisourea

Several other techniques have been developed in response to problems recognized with the FDNB assay. Carpenter and March (1961) and Butterworth and Fox (1963) reported that with tests using vegetable protein concentrates, acid hydrolysis can cause losses of 20-30 % to added dinitrophenyl-lysine. To overcome such losses in carbohydrate rich protein sources, Mouron and Bujard (1963) proposed a method to measure lysine and free ε-amino groups by reaction with D-methylisourea. Maga (1981) found the addition of various grades of carbohydrate source in this assay did not affect levels of available lysine. A disadvantageous feature was the long incubation period necessary to

allow for the transformation of lysine residues to homoarginine, a fundamental step as homoarginine is stable under acid hydrolysis (Nair et al., 1978). It has also been found that other factors such as pH and concentration of the reagent may affect the results.

3.2.2c Dye-binding Procedure

Dye-binding procedures rely on the properties of sulfonated dyes such as acid Orange 12 to bind to the functional amino groups of basic amino acids lysine, histidine and arginine if they are free (uncomplexed). The technique is easier and faster than the FDNB method yet correlates well with FDNB available lysine values (Sibbald, 1987). A procedure aimed at improving specificity involves mixing a sample of the protein source with a solution of the dye and measuring the degree of adsorption. By blocking the reactive lysine in a second sample by propionylation and then subjecting the sample to dye binding, a measure of the amount of reactive or available lysine is provided by the difference in dye binding capacity of the unblocked and blocked lysine samples. Goh et al. (1979) and Walker (1979) reported high correlations between the binding of acid Orange and FDNB lysine. Others as reported in Sibbald (1987) confirmed the usefulness of this procedure.

Lakin (1973) suggests that for the food industry the dye-binding procedure would be a useful means of monitoring the extent of heat damage to protein in processed food. Sibbald (1987) concurs stating dye-binding seemed best suited to a quality control role in its present form of developmeant.

CHAPTER 4

EXPERIMENTAL

4.1 EXPERIMENT 1

4.1.1 OBJECTIVES

(1a) To determine whether the feeding behaviour of meat chickens results in particle selection when the birds are given free access to diets designed to differ in particle size.

(1b) To determine whether there is stratification of the crop contents of meat chickens with time when the birds are given feeds designed to be fine (meat and bone meal based) or coarse (wheat based) under free access and intubation conditions of feeding.

The purpose of objective (1a) was to obtain evidence on the significance of particle selection in digestibility assays involving free access and intubation methods of supplying test foods. Whilst it is recognised that simplicity of assay procedure is an important feature of routine digestibility assays and feeding by free access offers considerable advantages in labour, effort and time, particle size separation and/or selection of test diets may give rise to a design bias and lower the sensitivity and reliability of assay techniques. In project (1a) the free access method was tested against an alternative feeding procedure, intubation, which might remove the bird and textural components of any potential intake bias. Three assays were conducted, one on each of 3 mash feeds, wheat based (WD) representatve of a mix of coarse and fine particles, meat and bone meal based (MBM) considered to be a relatively fine particle mix and a standard commercial meat chicken diet (CD), introduced to provide information relevant to commercial type whole diet mixes. Following feeding treatments and euthanasia, samples of crop

contents were analysed for nitrogen (N), chromium (Cr), neutral detergent fibre (NDF) and acid detergent fibre (ADF) and these latter used to assess the effect of feeding methods.

The purpose of objective (1b) was to obtain information on the composition of the crop contents with time as a measure of the degree to which components of test diets might be selectively passaged. Selective passaging from the crop is a potential source of variation in the composition of ileal digesta over time and consequently ileal digestibility values. Two assays were undertaken. One involved free access and one intubation methods of feeding. Meat and bone meal based, representing relatively fine and ground wheat based, representing coarse type treatment diets, were tested under each feeding method. At regular intervals following feeding, treatment birds were killed, crop contents sampled and subsequent laboratory determined N, Cr, NDF, and ADF values used to access the effect of time following feeding within test diets for each feeding method.

4.1.2 MATERIALS AND METHODS

Ninety male and ninety female newly hatched Ross strain meat chickens were received from Golden Coast Hatcheries Ltd. of Levin and raised in separate pens on wood shavings under 23 hrs of light per day. Approximately 72 of each sex were transferred at 23 days of age to a bank of 8 suspended grower cages set up as two parrellel rows in the PRC brooder shed. Each cage was partitioned into three 62 x 62 x 37 (cm) compartments and to each compartment 3 males and 3 females were randomly allocated to give 24 groups of birds. Under caging conditions they received 14 hours of daily light and were allowed 6, 7, or 8 days acclimatization before assay procedures were started at 29, 30, or 31 days of age. Up until assays commenced they had unlimited access to water and to a pelleted broiler starter feed (refer Table 2) least cost formulated according to NRC (1984) nutrient requirements.

At 29 days of age birds were individually weighed and those of extreme weight and culls were discarded and group sizes made up so as to provide in all 22 compartments each containing 3 birds of each sex.

Six compartments were allocated to experiment (1a) and 8 to each of experiments (1b) meat and bone meal based [(1b)MBM] and [(1b)WD] treatments.

On days 29, 30, and 31 birds of experiment 1(a), 1(b)MBM and 1(b)WD respectively were placed for 4 days on their treatment diets supplied ad libitum. Then following a fasting period of 24 hours the experimental treatments were applied.

TREATMENTS

Experiment 1(a). There were 3 diet types each fed under 2 methods of feeding to give 6 treatments coded as followed.

Diet Type	Feeding method	
	Free access (FA)	Intubation (I)
Commercial diet	FA CD	I CD
MBM based	FA MBM	I MBM
Wheat based	FA WD	I WD

The ingredient composition of the test diets is given in Table 3. Diets were provided in mash form. They were blended in a Hobart mixer and chromic oxide (Cr_2O_3) was added as an indicator. Free access treatment birds were given unlimited access to their test diets for a 1 hr period by compartment following which they were removed to the laboratory and asphyxiated using CO_2 gas. Intubation birds were individually force-fed 25 grams (air dry basis) of test diet. Force feeding was accomplished using a stainless steel funnel with a 17.2 cm long stem and a 17.7 cm long plunging rod. The external and internal diameter measurements of

the stem were respectively 0.9 cm and 0.75 cm. The end of the stem was inserted into the crop and following feeding the birds were returned to their cages. Half an hour after feeding they were removed to the laboratory and killed with CO_2 gas.

Table 2. Ingredient and nutrient composition (g/kg air dry) of the least-cost starter broiler diet

Ingredients	Diet
Maize	430.00
Barley	189.50
Meat and bone meal	103.00
Soybean meal	100.00
Blood meal	43.00
Peas	50.00
Skim milk powder	15.90
Corn oil	10.00
DL-methionine	1.60
Sodium chloride	2.00
Vitamin and mineral premix[1]	4.50
Coccidosis	0.50
Total	1000.00
Nutrients	
Crude protein, %	230.00
Metabolizable energy (Kcal/kg)	3240.00
Arginine	17.77
Lysine	15.25
Histidine	8.86
Isoleucine	7.50
Leucine	21.31
Methionine	4.96
Threonine	9.81
Tryptophan	2.20
Valine	12.02
Calcium	12.00
Available phosphate	8.35

1. Technik product's broiler starter vitamin and mineral premix, supplied by Technik Product, Massey, Auckland, N.Z.. At a recommended inclusion level of 4.5 kg/tonne of feed the premix contributed per kilogram the following nutrients: Vit A, 10,300 IU; Vit D_3, 2,500 IU; Vit E, 40.00 mg; Vit K, 3.75 mg; Vit B, 1.00 mg; Vit B_2, 6.50 mg; Vit B_6, 6.00 mg; Vit B_{12}, 0.01 mg; Calcium pantothenate, 18.00 mg; Niacin, 30.00 mg; Folic acid, 2.00 mg; Biotin, 0.06 mg; Flovomycin, 50.00 mg; Ethoxyquin, 125.00 mg; Choline, 650.00 mg; Molydenum, 2.00 mg; Manganese, 120.00 mg; Iron, 7.00 mg Cobalt, 1.00 mg; Zinc, 90.00 mg; Iodine, 1.50 mg; Selenium, 0.15 mg.

Table 3. Ingredient composition (g/kg air dry) of the experimental diets.

Ingredients	Diets		
	Commercial diet	Meat and bone meal diet	Wheat diet
Maize	450.00	---	---
Barley	205.00	---	---
Soybean meal	212.30	---	---
Meat and bone meal	100.00	500.00	---
Wheat	---	---	908.50
Maize starch	---	317.00	---
Sucrose	---	80.00	---
Maize oil	12.00	50.00	50.00
Purified cellulose	---	40.00	---
Potassium carbonate	---	3.00	---
Sodium chloride	2.50	2.50	4.00
Dicalcium phosphate	---	---	20.00
Calcium carbonate	10.00	---	10.00
DL-methionine	0.70	---	---
Vitamin and mineral premix[1]	4.50	4.50	4.50
Chromic oxide	3.00	3.00	3.00
Total	1000.00	1000.00	1000.00

1. Technik product's broiler starter vitamin and mineral premix (refer Table 2).

Experiment 1(b). There were 2 test diets, MBM and WD both as described for experiment 1a (Table 3 refers) applied over 2 methods of feeding, free access and intubation. For each diet / feeding method class there were 4 slaughter periods. In all there were 16 treatments each of 6 birds. The treatment were coded as follows:

Time of slaughter from start of feeding (hr).	Meat and Bone Meal based diet		Wheat based diet	
	Free access	Intubation	Free access	Intubation
½		I MBM (½)		I WD (½)
1	FA MBM (1)		FA WD (1)	
2	FA MBM (2)	I MBM (2)	FA WD (2)	I WD (2)
3	FA MBM (3)	I MBM (3)	FA WD (3)	I WD (3)
4	FA MBM (4)	I MBM (4)	FA WD (4)	I WD (4)

4.1.3 SAMPLING AND ANALYTICAL PROCEDURES

Following slaughter the contents of the crops of each individual bird were collected in labelled plastic cups. Crops were flushed several times with distilled water. Crop samples were immediately placed in a freezer and held at -20°C and later freeze dried. All samples of crop contents and test feeds were finely ground by passing them through a 1 mm sieve before chemical analysis.

Duplicate determinations of N, Cr, NDF and ADF were made on each sample of crop contents and duplicate determinations were made on each of 6 subsamples of each test diet.

Nitrogen was analysed by the macro-Kjeldahl technique using 300 mg samples (Association of Official Agriculture Chemists, AOAC, 1975) on a

Kjeldahl 1030 auto analyser (Tecator, Sweden) (Appendix 11). Chromium determinations using 150 mg of test feed and 50 mg of crop sample were made by atomic absorption spectrophotometry (AA\AE spectrohpotometer 451, Instrumentation Laboratory Inc. USA.) after the procedure of Costigan and Ellis (1987) (Appendix 12). Neutral and acid detergent fibre were analysed following the procedure of the James and Theander (1981) (Appendix 13).

4.1.4 STATISTICAL ANALYSIS

For Exp 1a within each test diet for each criterion tested data was analysed by one way analysis of variance (Snedecor and Cochran, 1972). Where the Fisher test showed significance all combinations of treatments were tested by Minitab's Two Sample t test for two populations without necessarily equal variances. All statements of significance refer to the 5 percent level of probability ($P<0.05$). For Exp 1b within each feeding procedure and for each criterion examined data was analysed by one way analysis of variance (Snedecor and Cochran, 1972). Where the Fisher test showed significance all combinations of slaughter times were tested by Minitab's Two Sample t test for two populations without necessarily equal variances. All statement of significance refer to the 5 percent level of probability ($P<0.05$).

4.1.5 RESULTS 1a.

Experiment (1a). The percentages of Cr, N, NDF and ADF of crop feed samples on a dry matter (DM) basis and related N, NDF, and ADF to Cr ratios across the two methods of feeding and as obtained directly from the test diets are given in Table 4 (commercial diet), Table 5 (meat and bone meal based diet) and Table 6 (wheat based diet).

For the commercial diet (CD) there were significant differences in the crop feed content of Cr between free access (FA) and intubation (I) methods of feeding and between these and the test diet. Percent Cr increased from 0.246 % (FA) to 0.278 % (I) and 0.287 % (CD). The N content of the FA treatment was at 3.542 %, significantly lower than that of the intubation (3.739 %) and the test food (3.732 %). As a consequence the N : Cr ratios were significantly different for the free access and intubation treatments and the commercial diet. Additionally the free access treatment had significantly greater NDF and ADF : Cr ratios (52.67 and 11.99 respectively) than the intubation treatment (44.91 and 10.67 respectively) and the commercial diet (44.43 and 10.55) respectively.

For the MBM diet the percent of ADF in the FA treatment was significantly greater (1.579 %) than the intubation treatment (1.457 %) and the MBM test diet (1.385 %). The ADF to Cr ratios differed significantly in a corresponding fashion. The percentages of Cr, N, and NDF were not significantly different across the treatments.

For the wheat diet, intubation treatment results were in close agreement with analyses conducted on the wheat based diet. The free access treatment Cr content was significantly lower at 0.259 % than both the intubation treatment (0.288 %) and the wheat diet (0.286 %). Its N content was significantly greater 2.069 % (FA) versus 1.991 % (I) and 1.982 % (WD) and its NDF and ADF contents were significantly greater at 10.865 % versus 10.237 % (I) and 10.220 % (WD) for NDF and 3.331 % versus 3.005 % (I) and 3.034 % (WD) for ADF.

Table 7 shows the amount of feed in the crop as a percentage of the feed received for the 3 diets across the 2 feeding methods at time of slaughter. The percentage of food in the crop remaining ranged from 84.82 % (CD) to 82.43 % (WD) under free access methods of feeding and was between 91.77 % (CD) and 89.39 % (WD) for intubation practices.

Table 4. The percentage of Cr, N, NDF, and ADF of crop samples (dry matter basis) and related N, NDF and ADF to Cr ratios of treatments receiving the commercial diet by feeding method and corresponding values for the test diet.

	Feeding procedure		
	Free access	Intubation	Test diet
% Cr	0.246±0.018a	0.278±0.009b	0.287±0.006c
% N	3.542±0.119a	3.739±0.074b	3.732±0.084b
% N / % Cr	14.46±0.73a	13.47±0.35b	12.99±0.41c
% NDF	12.897±0.530a	12.460±0.631a	12.772±0.472a
% NDF / % Cr	52.67±3.33a	44.91±2.72b	44.43±1.51b
% ADF	2.937±0.152a	2.956±0.209a	3.031±0.092a
% ADF / % Cr	11.99±0.79a	10.67±0.99b	10.55±0.49b

± values are standard deviations.

Means in the same row without a letter in common are significantly different at $P<0.05$.

Table 5. The percentage of Cr, N, NDF, and ADF of crop samples (dry matter basis) and related N, NDF and ADF to Cr ratios of treatments receiving the meat and bone meal diet by feeding method and corresponding values for the test diet.

	Feeding procedure		
	Free access	Intubation	Test diet
% Cr	0.264±0.019a	0.254±0.010a	0.258±0.003a
% N	4.333±0.289a	4.191±0.117a	4.213±0.020a
% N / % Cr	16.45±2.09a	16.51±0.33a	16.36±0.19a
% NDF	14.788±2.162a	14.849±0.796a	15.337±0.602a
% NDF / % Cr	56.38±11.66a	58.58±4.65a	59.61±2.55a
% ADF	1.579±0.097a	1.457±0.146b	1.385±0.079b
% ADF / % Cr	6.07±0.54a	5.76±0.79b	5.38±0.37b

± values are standard deviations.

Means in the same row without a letter in common are significantly different at $P<0.05$.

Table 6. The percentage of Cr, N, NDF and ADF of crop samples (dry matter basis) and related N, NDF and ADF to Cr ratios of treatments receiving the wheat diet by feeding method and corresponding values for the test diet.

	Feeding procedure		
	Free access	Intubation	Test diet
% Cr	0.259±0.013a	0.288±0.007b	0.286±0.002b
% N	2.069±0.026a	1.991±0.018b	1.982±0.001b
% N / % Cr	8.00±0.45a	6.92±0.19b	6.93±0.06b
% NDF	10.865±0.116a	10.237±0.236b	10.220±0.317b
% NDF / % Cr	41.99±2.36a	35.55±0.54b	35.72±1.25b
% ADF	3.331±0.156a	3.005±0.044b	3.034±0.060b
% ADF / % Cr	12.86±0.72a	10.44±0.18b	10.60±0.19b

± values are standard deviations.

Means in the same row without a letter in common are significantly different at $P<0.05$.

Table 7. Crop feed as a percentage of the feed received (eaten) for 3 diets across 2 feeding methods at time of slaughter.

Diets	Feeding procedure	
	Free access@	Intubation
Commercial diet	84.82	91.77±3.08
Meat and bone meal	82.99	91.22±2.00
Wheat diet	82.43	89.39±4.57

± values are standard deviations.

@ Compartment feeding resulted in one feed consumption value per treatment.

4.1.6 DISCUSSION 1a.

For the commercial diet, the reduced indicator proportion in the crop feed associated with FA may have been caused by the birds preferentially selecting the large particle size fragments in the food. The larger particles consisted of maize, soybean meal and barley and it was visually evident that the finer matrix of food contained a greater proportion of indicator than the coarser. Under intubation it was noticeable that the crop food was drier than that imbibed under FA conditions. It also resulted in indicator adhering to the crop surface and it was difficult to clear this completely by the washing out procedure with distilled water. There was evidence of regurgitation over each of the feeding methods and this was ascribed to the distress caused by asphyxiation with CO_2.

For the MBM diet, both the free access and intubation procedures resulted in proportions of components of the feed being in close agreement with those of the test diet. The diet contained large proportions of maize starch (31.7 %), maize oil (5.0 %) and purified cellulose (4.0 %) and their fine particle size and the resultant texture of the diet may have prevented the birds from selecting from the diet. There was evidence of regurgitation over both methods of feeding and the intubation procedure was associated with drier crop surface and adherence of indicator to the crop surface.

For the wheat diet there were significant differences in Cr and N proportions in the crop feed between the free access and the intubation feeding approaches with the proportions in the intubation samples being not dissimilar to those of the test food. The difference may have been caused by the 91 % content of cracked wheat in the test diet. It seems likely that the birds preferentially selected the larger particle leaving the finer dietary constituents which appeared to contained a higher proportion of Cr. Other problems such as regurgitation and dry crop material and adherence of indicator to the crop surface in intubation treatments were also evident.

Overall the study indicated that the preferred method of feeding, with respect to maintaining proportion, for coarse diets such as the wheat based and commercial diet was intubation. For the uniformly fine diet (MBM based) the free access and intubation feeding approaches had little effect on the composition of food reaching the crop. Hence in subsequent experimental work the intubation procedure was used in assays involving the wheat based diet but free access was employed for treatments involving the MBM based diet. Operationally the free access feeding procedure was easier to implement.

4.1.7 RESULTS 1b.

Experiment (1b). The percentages of Cr, N, NDF and ADF in crop feeds of birds fed the MBM and wheat diets using 2 feeding procedures, free access and intubation as determined for different slaughter times following the start of feeding are given in Table 8 (MBM diet) and Table 10 (wheat diet) and corresponding treatment N, NDF and ADF to Cr ratios are given in Table 9 (MBM diet) and Table 11 (wheat diet).

For the MBM diet (Table 8) under intubation feeding, no significant differences were apparent in crop proportions with time following feeding for Cr, N, NDF and ADF, but for free access treatments the concentration of Cr was smaller (0.245 %) at the 4 hr slaughter time than at the 2 hr (0.264 %) and 1 hr (0.269 %) intervals. Nitrogen content was significantly lower at the 4 hr slaughter point than at the 2 hr mark (4.008 % versus 4.303 %). The proportion of NDF was significantly greater at the 3 hr (17.834 %) and 4 hr (18.699 %) times than at the 1 hr (13.055 %) and 2 hr (14.157%) slaughter intervals, whereas ADF content was significantly lower at 3 hr and 4 hr following feeding (1.081 % and 1.112 % respectively) than at 1 hr (1.421 %).

With respect to N, NDF, and ADF to Cr ratios in MBM crop contents (Table 9), no significant differences were observed over the 4 slaughter times

for intubation treatments, but NDF : Cr and ADF : Cr ratios were significantly different for the 3 and 4 hr slaughter points than the 1 and 2 hr times (NDF) and 1 hr point (ADF).

For the wheat diet (Table 10) under intubation, the percentage of Cr of the crop content was significantly smaller at the 3 hr (0.243 %) than the 2 hr (0.279 %) and ½ hr (0.280 %) times. The N concentration of the crop samples of birds slaughtered at 3 and 4 hr (1.879 and 1.913 %) was significantly smaller than that found for the ½ hr slaughter time (2.003 %). For ADF, the content at 2 hr following feeding (2.992 %) was significantly greater than at 3 hr (2.669 %).

In contrast for the free access, the percent of Cr and N in the crop samples did not change significantly with time. The NDF concentration was significantly greater at 2 hr (10.899 %) than at 1 hr (10.410 %) and ADF concentration showed a significantly decrease to 2.904 % at 3 hr from 3.111 % at 1 hr.

With respect to the N, NDF and ADF to Cr ratios associated with the wheat diet under intubation (Table 11), the N : Cr ratio at 3 hr (7.963) was significantly greater than the 2 hr (7.062) and 1 hr ratios (7.170). A similar response was achieved for NDF : Cr. The 3 hr slaughter value (41.952) was significantly greater than the 2 hr (36.377) and 1 hr (36.787) ratios. For ADF : Cr the ratio at 3 hr (11.163) was significantly greater than that at the ½ hr mark (9.947).

In contrast under conditions of free access (Table 11) the 4 hr ratio for N : Cr of 8.755 was significantly greater than that at 1 hr (7.980). For NDF : Cr the ratio was significantly greater when birds were slaughtered at 2 hr (45.865) than at 1 hr (41.000). On the other hand no significant differences were observed for ADF : Cr ratios.

In Table 12, data are provided showing the rate of removal of the MBM and wheat diet from the crop for the free access and intubation feeding procedures. For the MBM free access treatments, the proportion left decreased from 84.65 % at 1 hr to 10.60 % (3 hr) and 6.96 % at 4 hr.

For intubation feeding the proportion remaining after ½ hr was 89.14 % and this dropped to 30.05 % at 3 hr and 13.01 % at 4 hr.

For wheat treatments, under free access crop food decreased from 82.46 % (1 hr) to 28.52 % at 4 hr, whilst for the intubation treatments the proportion of crop food remaining was 89.97 % after ½ hr and 5.67 % after 4 hrs from the start of feeding.

Table 8. The percentage of Cr, N, NDF and ADF in the MBM based diet (dry matter basis) as sampled from bird crops according to feeding treatment and time to slaughter following start of feeding (n=6).

Time to slaughter following start of feeding (hr)	Feeding procedure		Test diet
	Free access	Intubation	
% Cr			0.258±0.003
1, # ½	0.269±0.015a	#0.252±0.013a	
2	0.264±0.012a	0.251±0.010a	
3	*0.264±0.019ab	0.252±0.018a	
4	*0.245±0.008b	*0.227±0.030a	
% N			4.213±0.020
1, #½	4.321±0.230ab	#4.219±0.080a	
2	4.303±0.136a	4.200±0.047a	
3	*4.193±0.123ab	4.125±0.086a	
4	*4.088±0.123b	*4.069±0.229a	
% NDF			16.704±0.221
1, #½	13.055±2.134a	#16.771±0.596a	
2	14.157±0.747a	16.337±1.201a	
3	*17.834±1.614b	15.882±0.912a	
4	*18.699±2.072b	*18.940±2.608a	
% ADF			1.385±0.079
1, #½	1.421±0.160a	#1.267±0.134a	
2	1.098±0.177b	1.383±0.158a	
3	*1.081±0.166b	1.483±0.249a	
4	*1.112±0.138b	*1.491±0.337a	

* Mean based on 5 values.

± values are standard deviations.

Means without a letter in common are significantly different at $P<0.05$.

Table 9. Ratio of N:Cr, NDF:Cr and ADF:Cr in the MBM based diet (dry matter basis) as sampled from bird crops according to feeding treatment and time to slaughter following start of feeding (n=6).

Time to slaughter following start of feeding (hr).	Feeding procedure		Test diet
	Free access	Intubation	
% N / % Cr			16.355±0.190
1, #½	16.135±1.711a	#16.778±0.921a	
2	16.295±0.665a	16.757±0.791a	
3	*15.934±1.181a	16.455±1.108a	
4	*16.704±0.221a	*18.076±1.862a	
% NDF / % Cr			59.610±2.546
1, #½	48.595±8.336a	#66.613±1.940a	
2	53.653±3.781a	65.193±6.117a	
3	*67.776±7.801b	63.415±6.137a	
4	*76.320±7.421b	*85.176±20.550a	
% ADF / % Cr			5.382±3.367
1, #½	5.287±0.586a	#5.047±0.677a	
2	4.167±0.755b	5.523±0.743a	
3	*4.072±0.411b	5.920±1.025a	
4	*4.534±0.418b	*6.608±1.462a	

* means based on 5 values.

± values are standard deviations.

Means without a letter in common are significantly different at $P<0.05$.

Table 10. The percentage of Cr, N, NDF and ADF in the wheat based diet (dry matter basis) as sampled from bird crops according to feeding treatment and time to slaughter following start of feeding (n=6).

Time to slaughter following start of feeding (hr)	Feeding procedure		Test diet
	Free access	Intubation	
% Cr			0.286±0.002
1, #½	0.254±0.009a	#0.280±0.010a	
2	0.239±0.017a	0.279±0.011a	
3	0.244±0.008a	0.243±0.028ab	
4	0.238±0.019a	0.254±0.030ab	
% N			1.982±0.010
1, #½	2.026±0.027a	#2.003±0.016a	
2	2.036±0.034a	1.971±0.034ab	
3	1.992±0.050a	1.913±0.076b	
4	2.039±0.058a	1.879±0.090b	
% NDF			10.220±0.317
1, #½	10.410±0.176a	#10.282±0.387a	
2	10.889±0.315b	10.143±0.303a	
3	10.223±0.745ab	10.110±0.798a	
4	10.635±0.355ab	10.029±0.310a	
% ADF			3.034±0.060
1, #½	3.111±0.076a	#2.779±0.026ab	
2	3.049±0.150ab	2.992±0.120b	
3	2.904±0.109b	2.669±0.219a	
4	3.048±0.072ab	2.735±0.285ab	

± values are standard deviations.

Means without a letter in common are significantly different at $P<0.05$.

Table 11. Ratio of N:Cr, NDF:Cr and ADF:Cr in the wheat based diet (dry matter basis) as sampled from bird crops according to feeding treatment and time to slaughter following start of feeding (n=6).

Time to slaughter following start of feeding (hr).	Feeding procedure		Test diet
	Free access	Intubation	
% N / % Cr			6.928±0.055
1, #½	7.980±0.375a	#7.170±0.304a	
2	8.563±0.721ab	7.062±0.219a	
3	8.360±0.405ab	7.963±0.670b	
4	8.755±0.676b	7.458±0.633ab	
% NDF / % Cr			35.718±1.245
1, #½	41.000±1.535a	#36.787±1.407a	
2	45.865±4.440b	36.377±2.213a	
3	41.915±2.437ab	41.952±4.108b	
4	44.865±4.308ab	39.893±4.080ab	
% ADF / % Cr			10.602±0.189
1, #½	12.353±0.563a	#9.947±0.378b	
2	12.832±1.379a	10.737±0.789ab	
3	11.920±0.561a	11.163±1.896a	
4	12.852±1.111a	10.790±0.314b	

± values are standard deviations.

Means without a letter in common are significantly different at $P<0.05$.

Table 12. Crop feed (DM) as a percentage of the feed received (DM) (eaten) for 2 diets across 2 feeding procedures at different slaughter intervals following initial feeding time.

Time of slaughter following initial feeding	Feeding procedure	
	Free access@	Intubation
MBM diet		
1 hr, #½ hr	84.65	#89.14±2.53a
2 hr	45.30	50.75±7.83b
3 hr	*10.60	30.05±10.17c
4 hr	*6.97	*13.01±9.28d
Wheat diet		
1 hr, #½ hr	82.46	#89.97±3.14a
2 hr	46.66	45.04±6.32b
3 hr	34.54	15.10±7.97c
4 hr	28.52	5.67±4.19d

* Mean based on 5 values.

± values are standard deviations.

@ Compartment feeding resulted in one feed consumption value per treatment.

Means without a letter in common are significantly different at $P<0.05$.

4.1.8 DISCUSSION 1b.

The results showed that for MBM diet / intubation the percentage dietary components assessed with time were not significantly different. For wheat diet / intubation there was evidence of changing composition towards lesser proportions with time but except in the case of % N this was not consistently significant. Thus in the case of N only the 3 and 4 hr slaughter proportions were significantly lower than the ½ hr values and in the case of Cr and ADF, significant differences as between the 2 and 3 hr slaughter intervals were not obtained when either was compared to the ½ and 4 hr points.

With MBM diet / free access, proportions did change significantly with time for all percentage criterion, but for the wheat diet, though there were significant proportional changes at the 2 and 3 hr marks for NDF and ADF, the 1 and 4 hr determinations were not significantly different.

The results indicate that for the MBM diet, intubation resulted in a flow of food components out of the crop over time in a way which maintained the proportional composition of the crop contents. This was not so for free access methods of feeding. The corresponding ratios of N : Cr, NDF : Cr and ADF : Cr suggest counter balancing of selective passaging by corresponding movement of the other component of the ratio did not take place to any marked extent.

For wheat diets under intubation feeding, there was some evidence of irregular selective movement out of the crop. With the exception of N, initial changes in proportional content with time were righted by the 4 hr slaughter time. The appropriate ratios suggest that counter balancing movements by components of the ratios were not large.

For wheat diet / free access, though some evidence developed in results relating to NDF and ADF proportions that suggested selective passaging at certain times, at the 4 hr point significant differences were not evident. With respect to the relevant ratios, the movement of Cr, N, NDF and ADF from the crop was such that differences between hrs for ADF

which proved significant when expressed as a proportion of dry matter, were not when assessed as units per unit of Cr. Expressing NDF as a ratio with Cr resulted in the same slaughter time effects as for NDF and the ratios of N : Cr indicated significant differences between the 1 hr and 4 hr slaughter times.

The results of the study were inconclusive. Though evidence surfaced in the free access / MBM and intubation / wheat diet treatments to show Cr and N proportions tended to get less with time and that in the case of the free access / MBM, intubation / wheat diet and free access / wheat diet treatments that ADF got lesser but NDF proportions increased with time, there were a number of exceptions. For intubation / MBM there were no significant differences with time in the proportions of Cr, N, NDF and ADF and in the case of the intubation / wheat diet treatments, changes that had occurred by the 3 hr sampling time were not reflected at the 4 hr sampling (eg. Cr and ADF). Similar inconsistencies were apparent in the free access / wheat diet treatments for NDF and ADF. In addition the proportions of Cr and N in the free access / wheat diet treatments did not alter significantly with time. No cause for the inconsistancies could be found.

On the other hand, the crop has a food storage function and it is also a region in which hydration of feed occurs. An explanation for the response exhibited by the free access / MBM and intubation / wheat diet treatments in which Cr, N and ADF proportions lessened with time may be that the less soluble fractions of the food such as NDF, remained in the crop for longer periods of time to allow water to penetrate. However results overall are not entirely supportive of this explanation notably those of intubation / MBM and free access / wheat diet.

4.2 EXPERIMENT 2

4.2.1 OBJECTIVE

To investigate the influence of two slaughter procedures, CO_2 asphyxiation and euthanasia by sodium pentobarbitone, and the effect of two flushing solutions, distilled water and physiological saline on apparent ileal N digestibility.

There is strong evidence from work completed on sheep (Badawy, 1958) that cause of death may influence the degree of peristallic intestinal movement, affect levels of epithelial cell shedding into the digestive tract and increase N content of the digesta. Studies by Bolton (1964) on adult cockerels sacrificed by cervical dislocation, indicated that agonal spasms may cause movement and contamination by digesta between regions of the tract. The purpose of comparing the two slaughter methods was to ascertain whether such effects under the proposed slaughter methods were of sufficient magnitude to influence ileal N digestibility and whether measurable changes may result from osmotic differences in two readily available flushing solutions, distilled water and physiological saline (containing 0.9 g NaCl / 100 ml distilled water, w/v).

The trial involved obtaining ileal samples from 4 treatment groups of 6 (3 male and 3 female) 35 day-old meat chicken fed the MBM test diet described in Exp. 1. In accordance with the finding of Exp 1a, the free access method was used to supply treatment birds with the test diet.

4.2.2 MATERIALS AND METHODS

Newly hatched meat chickens of the Ross strain, 18 males and 18 females in number, were received from Golden Coast Hatcheries Ltd. of Levin and grown in separate floor pens by sex under similar growing and feeding conditions to those described in Exp. 1. All the birds were transferred to suspended growing cages in the PRC brooder shed at 23 days of age. They were housed 3 males and 3 females per compartment. The birds were weighed and assigned to cage compartments in a way which minimised

differences in treatment (compartment) mean body weight. They were given a 6 days acclimatization period during which they received the pelleted starter feed (Table 2 refers). They then received the test diet for 4 days. The birds were then fasted for 24 hrs before receiving on day 35 a period of 1 hr free access to the test diet to enable ileal sampling to take place sequentially.

Treatments: There were 4 treatments each of 3 males and 3 females coded as follows.

Method of flushing	Method of slaughter:	
	Carbon dioxide	Sodium pentobarbitone
Distilled water	CO_2 D	Na D
Physiological saline	CO_2 P	Na P

Four hours following the supply of MBM test diet mash to the treatment compartment feed troughs, treatment birds were removed for ileal sampling. Asphyxiation with CO_2 took place in a wooden crate supplied with a hinged lid and modified to receive a supply of CO_2 by way of an air hose inserted through a side face a few centimetres above the base of the crate. Birds allocated to the sodium pentobarbitone treatments received a 1 ml injection of sodium pentobarbitone (300 mg / ml, Pento 300, South Island Chemical Ltd., Christchurch, N.Z.) directly into the heart. When the birds were immobilized they were bled by severing the blood vessels of the neck to avoid contamination of the samples with blood. The abdomen was opened and the ileum was exposed. Hemostats were used to clamp off the terminal 15 cm of ileum to prevent loss of material from the sampled region. The section on removal was dried with absorbent paper. Digesta was collected by flushing gently from the proximal end with distilled water or physiological saline using a plastic syringe into small plastic bags which were then sealed and stored in a deep freeze.

4.2.3 CHEMICAL ANALYSIS

The test feed was ground and the samples of ileal contents and test feed analysed for N and Cr following the same procedures as described under Exp 1 except that micro-Kjeldahl (100 mg) techniques were employed in the N determinations and approximately 35 mg ileal digesta samples used in Cr determinations. All samples were analysed in duplicate for N and Cr.

Apparent ileal N digestibility was calculated according to the following formula and expressed on a dry matter basis.

$$\% \text{ N digestibility} = 100 - \frac{\% \text{ Cr in feed} \times \% \text{ N in digesta}}{\% \text{ Cr in digesta} \times \% \text{ N in feed}} \times 100$$

4.2.4 STATISTICAL ANALYSIS

All treatments were tested against each other by one-way analysis of variance (Snedecor and Cochran, 1972). Main class effects were similarly tested. All significant results refer to the 5 % level of probability ($P<0.05$).

4.2.5 RESULTS

The means of N digestibility expressed as a percent for treatments and main classes are given in Table 13. A summary of related analysis of variance information is given in Appendix 2. The number of observations for treatment means was 6 and for main class effects, 12. All combinations of treatment interactions were insignificant. Consequently main class effects were tested. For methods of slaughter, sodium pentobarbitone resulted in significantly greater apparent ileal N digestibility (80.23 %) than slaughter by CO_2 asphyxiation (72.90 %). Flushing digesta using distilled water resulted in a mean N

digestibility (76.52 %) not significantly different from that obtained using physiological saline (76.61 %).

Table 13. The effect of different slaughter procedures and flushing solutions on apparent ileal N digestibility (%).

Flushing solution	Slaughter procedure		Main class: Flushing solution
	Carbon dioxide	Sodium pentobarbitone	
Distilled water	73.41±9.34	79.82±4.75	76.52±8.38a
Physiological saline	72.39±9.11	80.65±5.54	76.61±7.82a
Main class: Slaughter procedure	72.90±8.81a	80.23±4.94b	

± values are standard deviations.
Class means without a letter in common are significantly different at $P<0.05$.

4.2.6 DISCUSSION

The results of this study indicated that different slaughter procedures significantly affected apparent ileal N digestibility in the MBM based test diet. CO_2 inhalation treatment birds had lower N digestibility while higher N digestibility was observed in sodium pentobarbitone slaughter treatments.

CO_2 inhalation caused extreme struggling in birds before death ensued after 2-3 minutes. In some cases, defecation and regurgitation accompanied the struggling activity. These consequences may explain the lower digestibility associated with CO_2 inhalation treatments. It is postulated that struggling may have influenced the passage rate of digesta in the gut and interfered with N content. Such an effect has been reported by Bolton (1964) who used a cervical dislocation slaughter procedure which caused agonal spasms of the intestine with consequent digesta movement within the gut. Another reported factor that may influence digestibility accuracy is sloughing of epithelial cells in the gut lumen with its consequent effect on N content of the digesta. This is a time related phenomenon and in this work may have been minimised by the short intervals of 6 minutes that elapsed between death and sampling of ileal contents.

In contrast, sodium pentobarbitone slaughter procedure birds had higher N digestibility. This procedure caused immobilization within about 2 seconds and the birds lapsed into unconsciousness quietly. The greater N digestibility resulting from these treatments may have been caused by an absence or reduction in involuntary intestinal contractions. In addition sampling under this procedure could be implemented more rapidly and on average was completed within 3 minutes of death. Slaughter procedures also have implications in terms of animal welfare considerations and in this respect euthanasia is a method of choice.

No significant difference was observed on N digestibility by the use of two different flushing solutions. Average N digestibility was 76.5 %. The results suggested either solution could be used to flush the ileal digesta. However distilled water is to be preferred because it is more readily obtained and as such reduces the complexity of the assay.

4.3 EXPERIMENT 3

4.3.1 OBJECTIVE

To investigate the effect of time of slaughter after feeding on apparent ileal N digestibility.

Under the two feeding procedures being employed, intubation and free access the amount that can be safely lodged in the crop or which is consumed is relatively small amounting to 20-25 g in the case of intubation for 5 week old meat chickens and about 40 g for the same stock when given free access to the MBM diet for 1 hr (Exp 1 and 2). Potential problems arise in respect to obtaining sufficient sample quantities for subsequent analytical work and in obtaining samples that are free from digesta component distortions (resulting from differential flow rates of N and Cr) and which give digestibility values that reflect the real values.

In the literature there is considerable variation in elapse time between feeding of the test diet and ileal digesta sampling. Varnish and Carpenter (1971) studied the ileal amino acid digestibility of chicken muscle and lactalbumin by fasting birds overnight and slaughtering at 2.75 or 3.75 hrs after the start of feeding. Raharjo and Farrell (1984) studied ileal amino acid digestibility of plant and animal proteins by fasting birds for two days, then giving them 1 hr access to test feed and slaughtering them 5 hr after the cessation of feeding.

These trials were undertaken to examine the effects of sampling time on apparent ileal N digestibility over the two diets MBM and wheat described in Exp 1 (Table 3). Five groups of 6 birds (3 males and 3 females) were allocated to each feeding treatment and serially slaughtered 2, 3, 4, 5 and 6 hrs after the start of feeding.

4.3.2 MATERIALS AND METHODS

Forty male and the same number of female newly hatched Ross strain meat chickens were received and grown under the same procedures as decribed in Exp 2. After individual weighings and distribution into treatment groups in suspended cages there were 10 groups of 6 birds (3 males and 3 females) per compartment. At 29 days of age 5 groups were given free access to the MBM test diet for 4 days and at 30 days of age the remaining 5 groups were presented with the wheat diet ad libitum for a similar period of time. Following a fasting period of 24 hrs the MBM treatments were allowed free access to the test diet for a 1 hr period after which the groups were sacrificed using sodium pentobarbitone (refer Exp 2) according to the treatment times. At 36 days of age the wheat diet treatment birds were intubated and at sequential times thereafter, according to treatment, sacrificed with sodium pentobarbitone by the method described in Exp 2.

For each dietary type there were 5 euthanasia times, 2, 3, 4, 5 and 6 hrs following the start of feeding. Procedures used in obtaining digesta samples were those described in Exp 2. Distilled water was used to flush digesta from the terminal 15 cm of ileum.

Laboratory procedures were those described under Exp 2. One-way analysis of variance procedures (Snedecor and Cochran, 1972) in conjunction with Least Significant Difference measurements were used to test treatment differences. All significant results refer to the 5 % level of probability ($P<0.05$).

4.3.3 RESULTS

Tables 14 and 15 summarise sampling means applicable to MBM treatments taken between 2 and 6 hr after the start of feeding for % N digestibility, % dry matter digestibility and digesta weight (g) (Table 14) and N and Cr as a percent of dry matter (Table 15). Appendix 3 details the relevant analysis of variance data. Tables 16 and 17 detail results for the same factors as obtained for the wheat based diet. Appendix 4 details the relevant analysis of variance data.

For MBM dietary treatments, N digestibility at the 2 hr interval (88.65 %) was significantly greater than that at the 6 hr point (56.00 %). Between times digestibility remained relatively constant ranging between 81.78 % (4 hr) and 78.75 % (5 hr). The N and Cr data (Table 15) (Figure 1) suggests this effect was caused primarily by disproportionate levels of Cr over hours. At the 2 hr mark the Cr proportion of 1.526 % was significantly greater than the 3 hr (0.992 %), 4 hr (0.943 %) and 5 hr (0.824 %) proportions and these were significantly greater than the 6 hr level of 0.417 %. On the other hand N as a proportion of the dry matter varied non significantly over time.

For the wheat dietary treatments, N digestibility at the 2, 3, 4 and 5 hr marks were significantly greater ranging from 85.74 % (2 hr) to 90.80 % (3 hr) than at the 6 hr interval (77.76 %). The related Cr data (Table 17) (Figure 2) shows marked but non significant variation in the proportion of Cr in the digesta dry matter with time, values ranging from 0.921 % at 2 hr to 1.297 % at 5 hrs. For N as a percentage of the digesta (DM) the 2 hr through 5 hr results were significantly smaller than the 6 hr result of 1.294 % and this suggests that disproportionate movement in N flow caused the significantly lower N digestibility recorded for the 6 hr interval.

More digesta (DM) was collected at the 4 hr points (708 mg) compared to the 2 hr (667 mg) and 5 hr (569 mg) for MBM diet (Table 14). Similar trends were also observed for the wheat diet in which greatest digesta quantities were obtained at 4 hr (461 mg), following by 5 hr (360 mg) and 2 hr (357 mg) slaughter times (Table 16). In addition, the digesta collected was one-third less for wheat than for MBM treatments.

Table 14. The effect of the time of slaughter following the start of feeding on N and DM digestibilities and digesta weigh (DM) for 35 days old meat chickens fed the MBM based test diet.

Time of initial feeding (hr)	% N digestibility	% dry matter digestibility	Digesta weigh (DM)(g)
2	88.65±3.19a	84.11±2.57a	0.667±0.198a
3	80.65±4.57b	73.60±4.25b	0.490±0.191a
4	81.78±3.26ab	74.19±4.43b	0.708±0.186a
5	78.75±4.85b	70.81±3.20b	0.569±0.263a
6	56.00±11.27c	48.39±10.86c	0.300±0.346a

Means without a letter in common are significantly different at P<0.05.
± values are standard deviations.

Table 15. The effect of the time of slaughter following the start of feeding on percentage of N and Cr in digesta (dry matter basis) for MBM diet.

Time of slaughter after start of feeding (hr)	% N / g	% Cr / g
2	2.852±0.256a	1.526±0.206a
3	3.083±0.250a	0.922±0.150b
4	3.010±0.395a	0.943±0.150b
5	3.060±0.402a	0.824±0.095b
6	3.062±0.835a	0.417±0.178c

Means without a letter in common are significantly different at P<0.05.
± values are standard deviations.

Table 16. The effect of the time of slaughter following the start of feeding on N and DM digestibility and digesta weigh for 36 days old meat chickens fed the wheat based test diet.

Time of initial feeding (hr)	% N digestibility	% dry matter digestibility	Digesta weigh (DM) (g)
2	85.74±3.53a	72.00±4.03ab	0.357±0.162a
3	90.80±0.95a	76.28±4.34ab	0.287±0.119a
4	89.21±2.85a	77.55±5.49a	0.461±0.146a
5	89.40±2.94a	77.09±6.73a	0.360±0.036a
6	77.76±10.96b	68.83±11.17b	0.339±0.172a

Means without a letter in common are significantly different at P<0.05.
± values are standard deviations.

Table 17. The effect of the time of slaughter following the start of feeding on percentage of N and Cr in digesta (dry matter basis) for wheat diet.

Time of slaughter after initial of feeding (hr)	% N / g	% Cr / g
2	0.917±0.324a	0.921±0.213a
3	0.699±0.169a	1.137±0.297a
4	0.840±0.254a	1.193±0.422a
5	0.888±0.216a	1.297±0.403a
6	1.294±0.316b	0.952±0.408a

Means without a letter in common are significantly different at P<0.05.
± values are standard deviations.

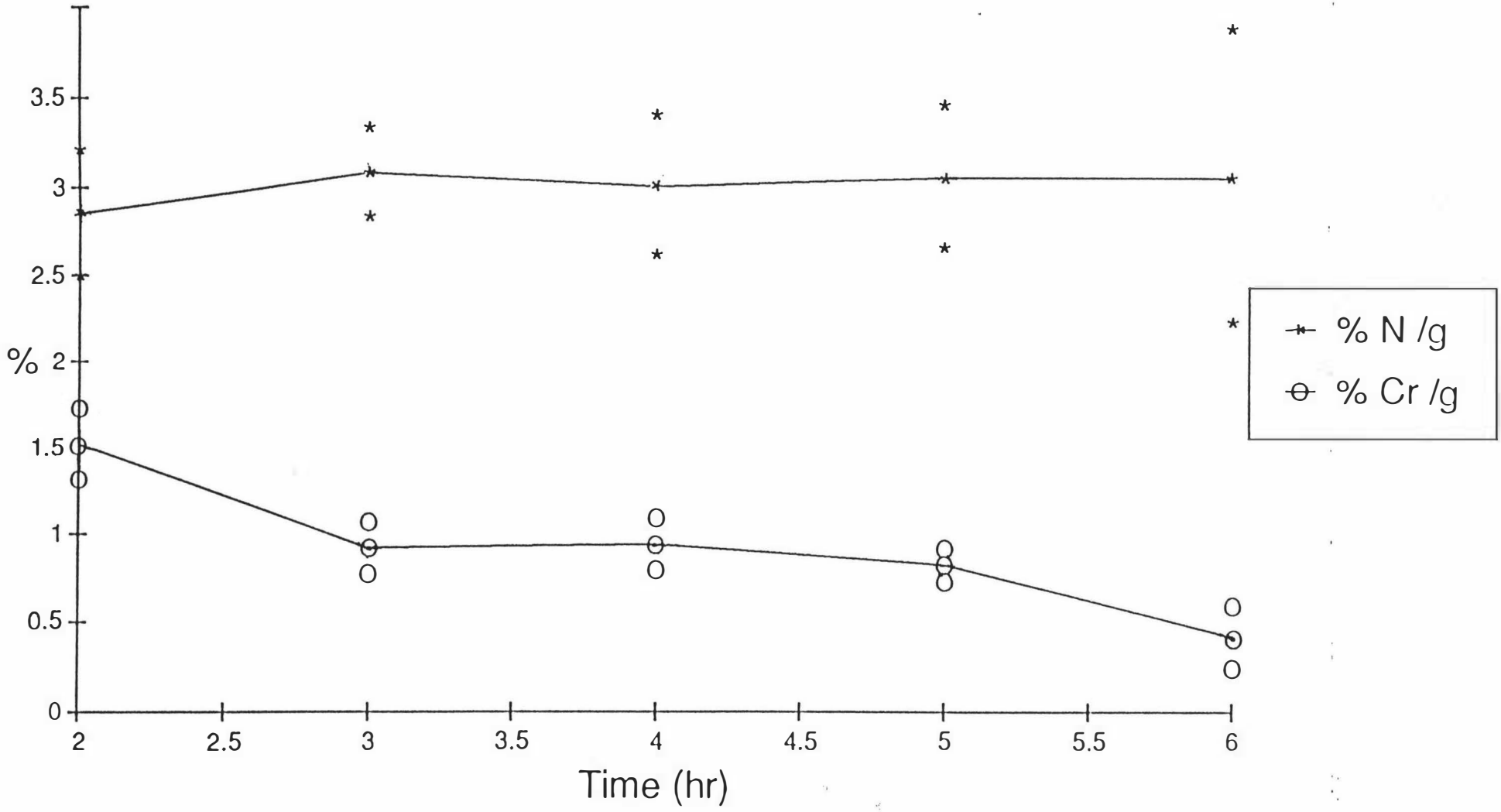

Figure 1. The effect of the time of slaughter following the start of feeding on percentage of N and Cr in digesta (dry matter basis) for MBM diet.

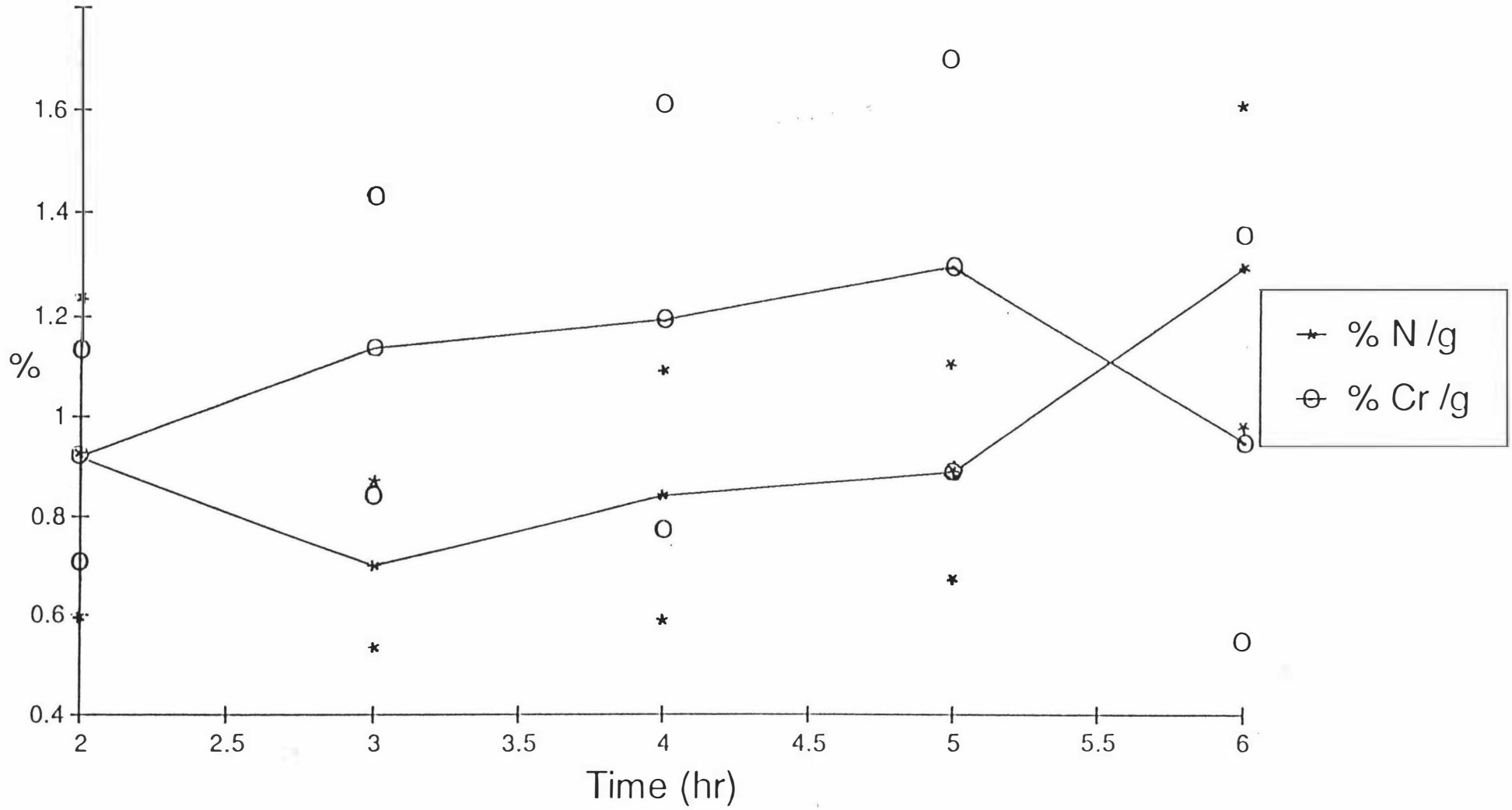

Figure 2. The effect of the time of slaughter following the start of feeding on percentage of N and Cr in digesta (dry matter basis) for wheat diet.

4.3.4 DISCUSSION

For the MBM treatments although the weight of digesta collected was not significant different over the 2 to 6 hr slaughter times, numerically more digesta material was collected at 4 hr than at 6 hr and the difference in quantity was quite marked. The significantly lower N and DM digestibility obtained by sampling at 6 hrs may have been caused by coarse and indigestible particles such as bone and hair being retained in the gizzard for a longer time. Visual inspection showed an increasing fraction of this material appearing in the gizzard with time following feeding and a decreasing amount of indicator. This observation suggests the fraction of finer particles including Cr passed through the intestine more rapidly. This is consistent with finding highest digestibility at 2 hrs and lowest at 6 hrs following feeding. Further, coarse particles may increase the destruction of mucosal cells and the secretion of mucus so adding to this effect.

A similar trend was apparent in the wheat treatments. Nitrogen and DM digestibilities were relatively constant over sampling times of 2 to 5 hrs. However, a significantly lower value was obtained at the 6 hr slaughter time. The intubation / wheat treatments resulted in about one-third less ileal digesta than obtained for the MBM dietary treatments due in part to the lesser food intake associated with the intubation technique. Again however, greatest ileal digesta (460 mg) was collected at the 4 hr slaughter time. The significantly smaller N and DM digestibilities obtained at the 6 hr sampling time may have been caused by factors described previously for the MBM treatments. Observations of the gizzard showed an increasing coarse and indigestible particle build up with increasing slaughter time and an apparent reduction in the fraction or Cr at the 6 hr sampling time.

The study indicated that ileal N digestibility remained relatively constant over the 3 to 5 hr period following the start of feeding. Ileal digesta quantities were numerically greatest at the 4 hr sampling interval for both the MBM based and wheat based test diets and hence this time interval for reasons of sample size represents a preferred time of slaughter.

4.4. EXPERIMENT 4

4.4.1 OBJECTIVE

To investigate the effect of length of ileum used to obtain digesta samples on ileal N digestibility.

The length of the ileum sampled is governed primarily by the need to obtain sample material on which digestion is complete and by factors relating to the need to obtain adequate replication and sample size to meet the requirements of statistical needs and laboratory analysis.

Raharjo and Farrell (1984) reported that N digestibility was higher in the terminal 10 cm of ileum and rectum than in the excreta and whole ileum (Meckel's diverticulum to ileo-caecal junction). Many experiments involving ileal digestibility have been based on samples collected from the whole ileum (Payne et al., 1968; Varnish and Carpenter, 1975; Achinewhu and Hewitt, 1979). This trial was undertaken to examine how critical and sensitive length of ileum sampled, as measured from the caecal junction, was to N digestibility determination. Assessment was made using two diets MBM based and wheat based (Table 3 refers).

4.4.2 MATERIALS AND METHODS

Day-old Ross strain meat chickens comprising both males and females in equal numbers were received from Golden Coast Hatcheries Ltd. of Levin and grown in floor pens under similar circumstances and conditions as described for Exp 2. They were transferred to cage conditions at 23 days of age, acclimatized and 5 days before slaughter (35 days), randomly assigned to 8 treatment groups each of 3 males and 3 females and individually weighed. Cull birds and birds of extreme weight were replaced and treatment birds then provided for 4 days with the MBM or wheat test diets provided ad libitum. Following a fasting period of 24

hrs the MBM treatment birds were given free access for 1 hr to the test diet and the wheat dietary treatment birds intubated a 25 g (air dry basis) amount.

All feeding took place sequentially at time intervals that permitted digesta sampling to take place 4 hrs following the start of feeding. Birds were sacrificed by a 1 ml intra-cardial injection of sodium pentobarbitone, bled and the abdomen was opened. Digesta was flushed gently using distilled water into plastic bags, the bags sealed and contents subsequently frozen.

Treatments according to length and diet were as followed:

Treatment diet	Treatment length (cm) of ileum as measured from the ileo-caecal junction			
MBM diet	10	15	30	45
Wheat diet	10	15	30	45

Laboratory analysis conducted on N and Cr were evaluated using methods described under Exp 2. One-way analysis of variance (Snedecor and Cochran, 1972) in conjunction with the Least Significant Difference test were used to assess results for significance. All results of significance refer to the 5 % level of probability ($P<0.05$).

4.4.3 RESULTS

Table 18 and 20 summarise results for the MBM and wheat test diets respectively for the criteria, N digestibility (%), dry matter digestibility (%) and digesta weight (g). Table 19 and 21 detail corresponding data for the MBM and wheat based diets for proportions of N
and Cr in the digesta. Appendix 5 and 6 details the relevant analysis of variance data for MBM and wheat treatments respectively.

For the MBM treatments, length of ileum sampled had no significant effects on N digestibility and proportions of N and Cr in the digesta dry matter. N digestibility values ranged between 83.84 % and 83.44 % for sampled lengths between 10 and 30 cm and was 79.39 % for the digesta of the 45 cm ileal length. Nitrogen and Cr proportions ranged between 2.670 % (15 cm length) to 3.473 % (45 cm length) for % N and from 1.103 % (15 cm) to 0.907 % (45 cm) for % Cr (Figure 3). A trend similar to that for N digestibility was observed in dry matter digestibilities. A substantial increase in the length of ileum sampled resulted in a non significant decrease in the dry matter digestibility.

For the wheat based treatments length of ileum sampled had no significant effect on N digestibility and proportions of N in the digesta. N digestibility decreased sequentially from 88.95 % in the 10 cm ileal sample to 84.11 % in the 45 cm sample. N as a proportion of the digesta varied between 1.037 % for the 10 cm ileal sample and 0.810 % for the 15 cm sample (Figure 4). Chromium as a proportion of the digesta (DM) was significantly greater (1.804 %) in the 10 cm ileal sample than in ileal portions of greater length (Figure 4). The Cr proportions for the 15 cm, 30 cm and 45 cm portions were 1.067, 1.246 and 1.155 % respectively.

Table 18. The effect of sampling length of ileum on N and DM digestibility and digesta weight (DM) for 35 days old meat chickens fed a MBM diet.

Terminal length of ileum (cm)	% N digestibility	% dry matter digestibility	Digesta weigh (DM) (g)
0-10	83.69±4.07a	76.90±4.26a	0.356±0.100a
0-15	83.44±5.11a	76.91±4.83a	0.660±0.210a
0-30	83.84±3.98a	75.75±4.09a	1.089±0.142b
0-45	79.39±6.35a	73.47±3.50a	1.906±0.542c

Means without a letter in common are significantly different at $P<0.05$.
± values are standard deviations.

Table 19. The effect of sampling length of ileum on N and Cr proportions in the digesta for 35 days old meat chickens fed a MBM diet.

Terminal length of ileum (cm)	% N / g (DM)	% Cr / g (DM)
0-10	2.670±0.693a	1.088±0.178a
0-15	2.783±0.877a	1.103±0.240a
0-30	2.712±0.680a	1.053±0.196a
0-45	3.473±1.066a	0.907±0.184a

Means without a letter in common are significantly different at $P<0.05$.
± values are standard deviations.

Table 20. The effect of sampling length of ileum on N and DM digestibility and digesta weight for 35 days old meat chickens fed a wheat diet.

Terminal length of ileum (cm)	% N digestibility	% Dry matter digestibility	Digesta weigh (DM) (g)
0-10	88.95±4.26a	78.91±2.85a	0.242±0.098a
0-15	86.13±3.50a	74.20±4.13a	0.409±0.099ab
0-30	85.46±6.69a	74.82±7.25a	0.767±0.177b
0-45	84.11±2.44a	73.78±4.55a	1.341±0.713c

Means without a letter in common are significantly different at P<0.05.
± values are standard deviations.

Table 21. The effect of sampling length of ileum on N and Cr proportion in the digesta for 35 days old meat chicken fed a wheat test diet.

Terminal length of ileum (cm)	% N / g (DM)	% Cr / % g (DM)
0-10	1.037±0.201a	1.804±0.598a
0-15	0.810±0.240a	1.067±0.253b
0-30	0.921±0.219a	1.246±0.332b
0-45	1.018±0.098a	1.055±0.198b

Means without a letter in common are significantly different at P<0.05.
± values are standard deviations.

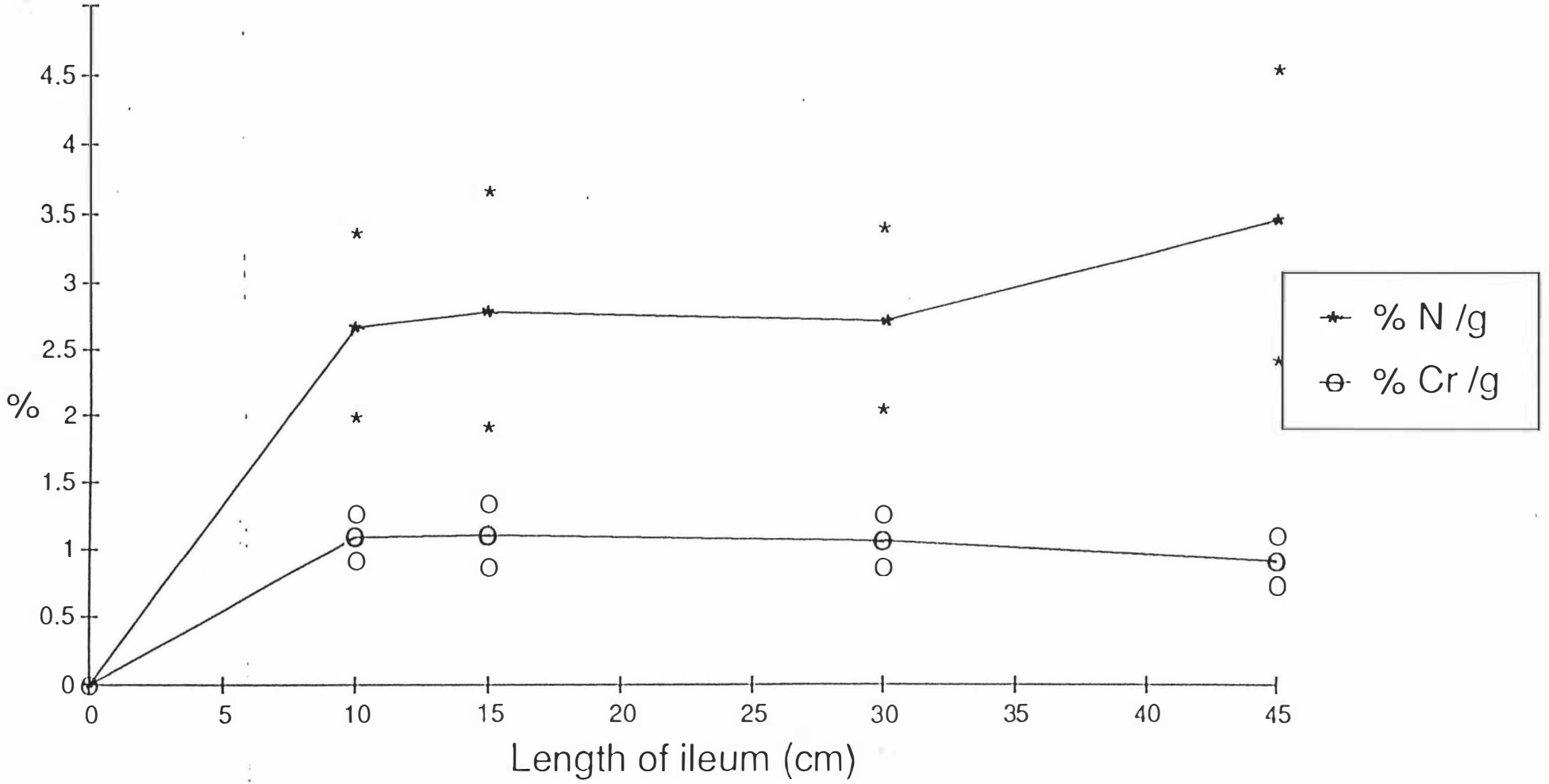

Figure 3. The effect of sampling length of ileum on nitrogen and chromium proportion in the digesta for 35 days old meat chicken fed a meat and bone meal test diet.

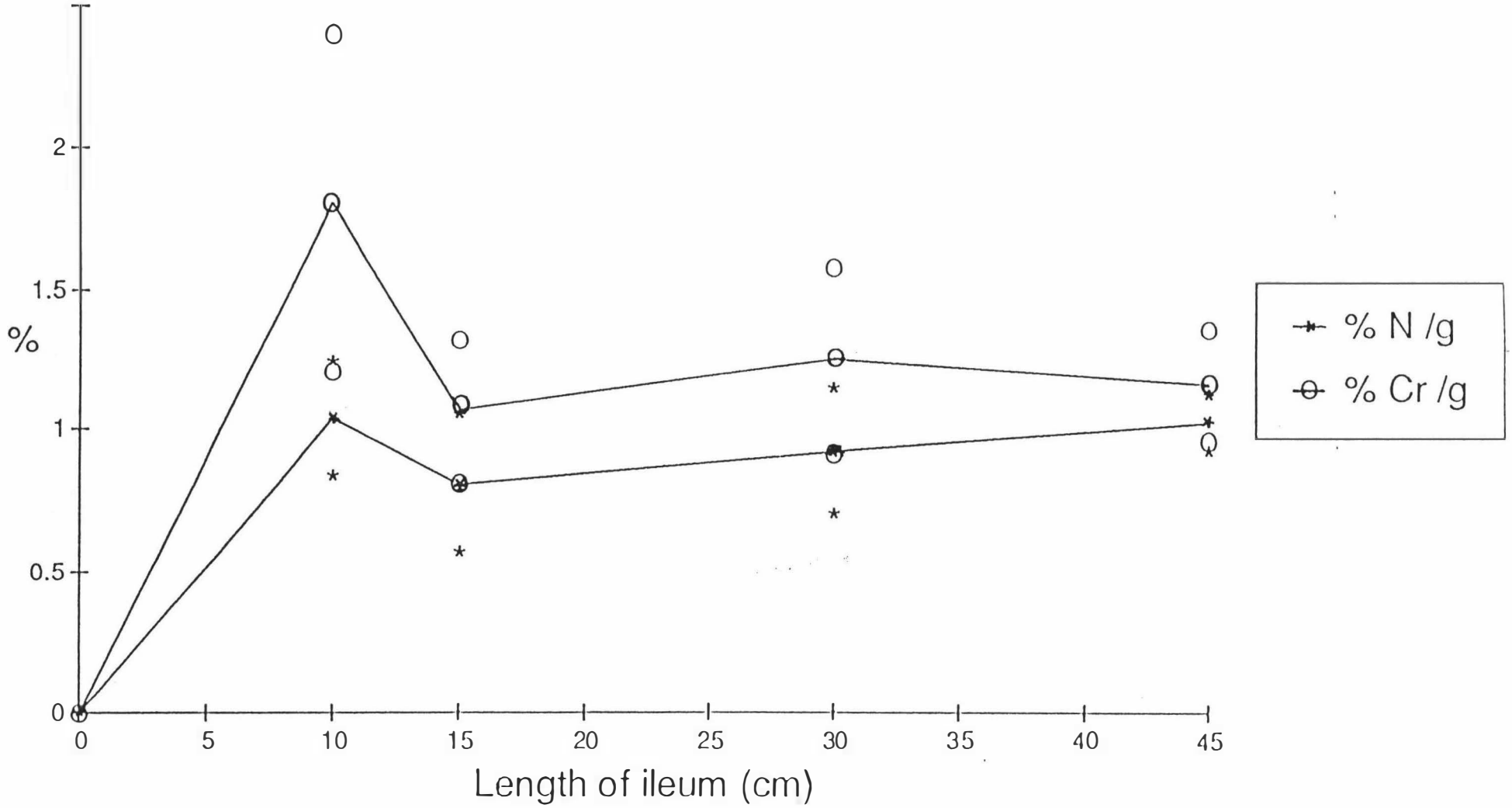

Figure 4. The effect of sampling length of ileum on nitrogen and chromium proportion in the digesta for 35 days old meat chicken fed a wheat test diet.

4.4.4 DISCUSSION

For the MBM diet, there was little numerical difference in N and DM digestibilities within 0-30 cm treatments (Table 18). This would suggest that N and DM had been largely absorbed before the digesta reached the terminal 30 cm of ileum. On the other hand, N digestibility was about 4 % lower (non significant) for the 0-45 cm compared to the 0-30 cm treatment.

The result reflects the fact that N absorption may be relatively substantial up to the terminal 30 cm of ileum and suggests results based on samples drawn from the whole ileum may underestimate nutrient digestibility. A further consideration is the quantity of digesta collected. In the case of the 0-10 cm treatment 356 ± 100 mg of digesta was collected an amount that may be insufficient for chemical analysis. On the other hand for the 0-15 cm segment, an average of 660 mg was obtained. Though on this basis it may be argued that 0-30 cm would provide still more sample it should be appreciated that the length chosen represents a proportion of the total ileum which is a function of the age and size of the bird. Drawing from too large a segment in young stock may introduce incidental bias. As a precautionary measure segment size is perhaps best kept as small as consistant with the needs of the assay.

Similar trends were also observed for the wheat diet. N and DM digestibilities decreased gradually (non significant) in the terminal 0-10 cm to 0-45 cm treatments with differences of 4.84 and 5.13 % respectively. However, about one-third less digesta was collected for wheat treatments than for MBM fed birds for corresponding segments.

This may be explained by the small quantity (25 g air dry basis) of feed fed under intubation as compared to about 45 g (air dry basis) consumed for MBM free access treatments. Although more digesta may be collected from the terminal 0-30 cm or 0-45 cm of ileum, this may result in lower N digestibility. The 0-10 cm segment gave marginally sufficient sample for chemical analysis.

The results suggest that N and DM digestibilities may not be greatly affected by small changes in the length of ileum sampled and that this feature may be used to justify and increase in ileal sample length in circumstances of insufficient digesta content. In the terminal 15 cm of ileum about 660 mg and 410 mg of ileal digesta from MBM and wheat diets were collected respectively. This length provided sufficient sample size for chemical analysis and full replication.

4.5 EXPERIMENT 5

4.5.1 OBJECTIVE

(5a) To investigate the effect of age on ileal N digestibility in meat chickens.

(5b) To investigate the ileal amino acid digestibility of two test diets using meat chickens.

Conflicting results have been reported in the literature on the effect of age on N digestibility. McNab and Shannon (1972) reported no effect, but Fonolla et al. (1981) and Hakansson and Eriksson (1974) found protein digestibility decreased, whilst Wallis and Balnave (1984) found an increase of ileal amino acid digestibility with age. With ileal digestibility procedures it may not prove practical to standardize age of slaughter. Indeed, an age range for ileal sampling would achieve a desirable degree of flexibility.

The purpose of the trial was to investigate the effect on N digestibility of slaughter between the ages of 4 and 8 weeks in rapidly growing stock across the two test diets, MBM based and wheat based (Table 3 refers) using preferred assay procedures developed through Exp 1-4. In addition the results of assays conducted at one age have been used to analyse and compare the ileal amino acid digestibilities within the two test diets.

4.5.2 MATERIALS AND METHODS

Sufficient meat chicken day old stock were obtained from Golden Coast Hatcheries Ltd. of Levin to provide 2 groups of 6 birds (3 males and 3 females) at each of 3 slaughter times, 4, 6 and 8 weeks of age. The stock were grown by sex in pens under conditions as described in Exp 2. Groups of birds assigned to 6 and 8 week slaughter treatments were fed a

broiler grower mash diet from 25 days of age until provision of the test diets. Table 22 outlines the ingredients and nutrient composition of the broiler grower diet. Twelve days before slaughter, groups of birds were removed to suspended cages fitted with heaters and given a 6 day acclimatization period before being individually weighed and assigned, 3 males and 3 females, to each of 2 cage compartments. They were given ad libitum access to test diets for 4 days, then fasted for 24 hrs and then given their test diets either by free access for a 1 hr period in the case of the MBM based diet or by intubation in the case of the wheat based diet. Intubated birds received 15, 30 and 40 g (air dry basis) of wheat based diet according to whether they were assigned to 4, 6 or 8 week slaughter treatments.

Four hrs following the start of feeding, treatment birds were sacrificed by a 1 ml intra-cardial injection of sodium pentobarbitone (300 mg / ml) in the case of 4 and 6 week old and a 1.5 ml injection in the case of 8 week old birds. The digesta of the terminal 15 cm of ileum were collected in sealable plastic bags by flushing the ileal gently with distilled water and stored in a deep freeze until laboratory analyses were undertaken. Nitrogen and Cr were analysed by methods described previously (Exp 2). Amino acids were analysed by ion-exchange chromatograph using a Waters High Pressure Liquid Chromatograph (HPLC) (James and Treloar, 1984) (Appendix 14). Preparation involved acid hydrolysis of feed and ileal digesta samples (approximately 40 mg) in 6M HCl in evacuated sealed tubes at 110° C for 24 hrs. A separate preparation procedure for the determination of methionine was not carried out and tryptophan and cystine were not determined.

All the samples were analysed in duplicate for N, Cr and amino acids. One-way analysis of variance (Snedecor and Cochran, 1972) in conjunction with Least Significant Difference testing was used to assess significance at the 5 % level of probability ($P<0.05$) between treatment means of different ages for each criterion examined within a test diet. In the AA digestibility analyses for each diet 6 birds were used to obtain six samples. Duplicate AA analyses were undertaken on each sample.

4.5.3 RESULTS

Table 23 provides a matrix of treatment means obtained over 3 ages for birds fed the MBM based diet for the measurements, N digestibility (%), DM digestibility (%), length of ileum as measured from Meckel's diverticulum to the ileo-caecal junction (cm), body weigh (g) and feed consumption (g). Table 24 provided corresponding treatment means for birds intubated with the wheat based diet. Analysis of variance tables for each comparison of means are provided in Appendix 7 for MBM based treatments and Appendix 8 for wheat based treatments.

For the MBM based dietary treatments apparent ileal N digestibilities across ages were not significantly different and were 76.12 % (4 weeks), 78.12 % (6 weeks) and 79.33 % when slaughtered at 8 weeks of age. For the wheat based dietary treatments N digestibilities were 89.15 % (4 weeks), 90.37 % (6 weeks) and 87.92 % (8 weeks). Treatment means were not significantly different.

The standard deviations of the means for N digestibility for the MBM treatments were large ranging between 8.47 % (4 weeks) and 5.30 % (6 weeks) and suggests that many more birds would be needed at each treatment level to improve the estimates of the treatment means. On the other hand for the wheat based diet treatment standard deviations of the means were considerably smaller (2.35 % for 4 weeks to 3.34 % for 8 weeks). No significant age effect on N digestibility was apparent. Similar trends were also observed for DM digestibility for both diets. The results suggest that the age effect is unlikely to contribute greatly to N and DM digestibilities values for meat chickens assayed between 4 and 8 weeks of age.

Tables 25 and 26 outline the amino acid composition on a percent of dry matter basis and apparent ileal amino acid digestibilities of the MBM test diet (Table 25) and the wheat based diet (Table 26). Appendix 9 and 10 details the relevant analysis of variance data for MBM and wheat treatments respectively.

For the MBM based diet the digestibilities of amino acids were divisible into 3 categories. Those at the highest level which were significantly greater than those at the lowest level and an intermediate category which were not significantly different from each other but of which some were significantly different from amino acids in either the high or low categories. One amino acid Methionine (87.89 %) qualified for the high level and one amino acid Glycine (74.51 %) qualified for low level category. Thirteen amino acids qualified for the intermediate level. They were Aspartic acid (78.66 %), Threonine (81.81 %), Serine (76.33 %), Glutamic acid (82.00 %), Alanine (78.38 %), Valine (84.99 %), Isoleucine (87.28 %), Leucine (86.54 %), Tyrosine (84.44 %), Phenylalanine (82.28 %), Histidine (77.83 %), Lysine (81.19 %) and Arginine (79.98 %).

Digestibilities of amino acids in the wheat diet were generally greater than those of the MBM test diet. Mean amino acid digestibility was 88.28 % versus mean N digestibility for the wheat treatments of 90.37 %. Amino acids that were most digestible and whose digestibilities were not significantly different were Glutamic acid (95.22 %), Methionine (91.66 %), Leucine (91.50 %) and Tyrosine (91.10 %). Amino acid that were least digestible and whose digestibilities were not significantly different were Histidine (80.41 %), Threonine (84.07 %) and Phenylalanine (84.53 %). The digestibilities of other amino acids lay within the boundaries defined by these two extreme groups. They were Isoleucine (90.86 %), Serine (89.94 %), Valine (89.02 %), Arginine (88.89 %), Alanine (87.90 %), Glycine (87.55 %), Aspartic acid (86.63 %) and Lysine (84.72 %).

Table 22. Ingredient and nutrient composition (g/kg) of the broiler grower diet.

Ingredients	Broiler grower diet
Maize	452.00
Barley	205.00
Meat and bone meal	100.00
Soybean meal	212.80
Corn oil	12.00
DL-methionine	0.70
Sodium chloride	2.50
Dicalcium phosphate	10.00
Vitamin and mineral premix[1]	4.50
Coccidiosis	0.50
Total	1000.00
Nutrients	
Crude protein (% N x 6.25)	200.00
Metabolizable energy (Kcal/kg)	3200.00
Arginine	18.63
Histidine	7.61
Isoleucine	7.10
Leucine	16.70
Lysine	11.28
Methionine	3.80
Threonine	7.62
Tryptophan	1.88
Valine	8.67
Calcium	11.00
Available phosphate	5.66

1 Technik product's broiler starter vitamin and mineral premix (refer Table 2).

Table 23. The treatment N digestibility (%), DM digestibility (%), digesta weigh (g), length of ileum between Meckel's diverticulum to the ileo-caecal junction (cm) and body weight (g) in three difference ages of birds for the MBM based diet.

	Age		
	4 week old	6 week old	8 week old
% N digestibility	76.12±8.47a	78.12±5.30a	79.33±7.02a
% DM digestibility	68.85±5.69a	71.23±5.25	73.60±5.64a
Digesta weigh (DM)(g)	0.477±0.207a	0.614±0.126a	0.718±0.713a
Length of ileum (cm)	54.8±7.3a	63.0±5.8b	66.3±5.1b
Body weigh (g)	918±39a	1822±137b	2818±299c
Feed consumption* (g/b) (air dry basis)	27.46	40.74	52.64

* Means of the individual bird based on group feeding.

Means in the same row without a letter in common are significantly different at $P<0.05$.

± values are standard deviations.

Table 24. The treatment N digestibility (%) , DM digestibility (%), digesta weigh (g), length of ileum between Meckel's diverticulum to the ileo-caecal junction (cm) and body weight (g) in three difference ages for the wheat based diet.

	Age		
	4 week old	6 week old	8 week old
% N digestibility	89.15±2.35a	90.37±3.02a	87.92±3.34a
% DM digestibility	81.85±2.71a	82.08±5.29a	81.01±3.68a
Digesta weigh (DM)(g)	0.318±0.092a	0.461±0.080b	0.477±0.124b
Length of ileum (cm)	53.0±4.3a	59.8±4.3b	66.8±3.1b
Body weigh (g)	893±43a	1790±147b	2812±195c
Feed consumption* (g/b) (air dry basis)	15.0	30.0	40.0

* Force feeding procedure were used.

Means in the same row without a letter in common are significantly different at $P<0.05$.

± values are standard deviations.

Table 25. Amino acid composition (DM) and apparent ileal amino acid digestibility (DM) of the MBM based diet as measured in 42 days old meat chickens.

Amino acid	% composition	% digestibility
Aspartic acid	1.9038	78.66±5.34ab
Threonine	0.8789	81.81±4.09bc
Serine	1.0336	76.33±8.31ab
Glutamic acid	3.1793	82.00±5.29bc
Glycine	3.4377	74.51±5.05a
Alanine	1.8030	78.38±6.05ab
Valine	1.0063	84.99±4.07bc
Methionine	0.4200	87.89±3.44c
Isoleucine	0.7668	87.28±3.59bc
Leucine	1.5714	86.54±3.57bc
Tyrosine	0.6098	84.44±5.55bc
Phenylalanine	0.8570	82.28±5.38bc
Histidine	0.5256	77.83±5.10ab
Lysine	1.4466	81.19±5.60b
Arginine	1.5703	79.98±9.31ab
Mean	---	81.63
Nitrogen	3.9570	78.12±5.30

± values are standard deviations.

Means without a letter in common are significantly different at $P<0.05$.

Table 26. Amino acid composition (DM) and apparent ileal amino acid digestibility (DM) of the wheat based diet as measured in 42 days old meat chickens.

Amino acid	% composition	% digestibility
Aspartic acid	0.6507	86.83±3.93bc
Threonine	0.3558	84.07±3.68ab
Serine	0.6289	89.94±2.70c
Glutamic acid	3.6384	95.22±3.11d
Glycine	0.5468	87.55±4.13bc
Alanine	0.4344	87.90±3.48bc
Valine	0.4006	89.02±3.07c
Methionine	0.1892	91.66±2.61cd
Isoleucine	0.3162	90.86±2.53c
Leucine	0.7549	91.50±1.76cd
Tyrosine	0.4004	91.10±1.90cd
Phenylalanine	0.5304	84.53±6.00ab
Histidine	0.3491	80.41±5.67a
Lysine	0.3261	84.72±4.60b
Arginine	0.6238	88.89±2.79bc
Mean	---	88.28
Nitrogen	1.8150	90.37±3.02

Means without a letter in common are significantly different at $P<0.05$.
± values are standard deviations.

4.5.4 DISCUSSION

Age of birds had no significant effect on N and DM digestibility for MBM based treatments (Table 23). However the N and DM digestibilities were slightly greater (non significant) for older birds and the length of the ileum sampled in relation to bird size may have contributed to this effect. With age, the length of ileum increased from an average of 55 cm for 4 week old to 66 cm for 8 week old birds. In this case, the 15 cm sampling segments represented 27.3 %, 23.8 % and 22.7 % of the whole ileum for 4, 6 and 8 week old birds respectively. It has been noted under Exp 4 that greater digestibility was associated with treatments in which the ileum sampled represented a relatively small fraction of the total ileum. In addition, with age the diameter of the ileum increased and this resulted in a larger sample size for the same length of ileum drawn.

In general, the apparent ileal amino acid digestibilities were in agreement with those reported by Johns et al. (1986b) who examined MBM using 3 week old chicks. In their study, they reported greater amino acid digestibilities for ileal determinations in chicks than for those obtained using cannulated cockerels. The apparent ileal amino acid digestibilities of this study are also greater than apparent faecal amino acid digestibilities reported by Johns et al. (1986a) and Jenssen et al. (1979) for MBM using intact cockerels.

A similar trend was observed for the wheat based treatments. Overall N and DM digestibilities were greater for wheat than for MBM treatments. For this test diet, birds were force-fed 15 g, 30 g and 40 g (air dry basis) at 4, 6 and 8 week old of age respectively. These amounts represent 1.68 %, 1.68 % and 1.42 % of the treatment bird body weight. The amount of digesta sampled increased with age due to the greater diameter of the tract of older birds. However at 8 weeks of age, bird average body weight was 2.8 kg. At this size they were less easy to handle and work with than 6 week old stock which achieved an average body weight of 1.8 kg.

From the point of view of intubation and ease of handling 6 week old rather than 8 week old birds were preferred. In terms of digesta sample size intubation may give rise to marginal samples in 4 week old stock.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The review part of this project has examined current assay procedures involved in assessing quality of protein sources and the project work has focussed on assay techniques designed to minimise bias associated with hind gut influences with a view to further refining methodology. Whilst the evidence for a quantitative and marked effect of the caecum and colon on N and AA utilization is clear for pigs and rats these influences have less explicitly been demonstrated for the fowl. With the latter species Parsons (1981) among others has demonstrated sizable AA excretion in the urine and Dukes (1977) reports a water extraction and AA, protein synthesis/degradation function of the caeca on urine and digesta. Although AAs from these sources may be utilized by the microbial population of the hind gut, the absorption of them from this area in the fowl, if it occurs at all, appears to be of little utility. Although the effect of caecal function on protein accretion in the host is doubtful there are a number of studies illustrating differences in digestibility values as between assays excluding or including caecal function. Given the evidence it seems likely a bias of undefined magnitude exists involving digestibility assays which incorporate caecal influences and consequently in this project, experiments were undertaken on methodolody concerned with ileal in preference to total excreta collection procedures.

In experiment 1a there was clear evidence that bird eating behaviour and particle size of the test diet influenced crop content proportions of Cr and N. Thus the relatively coarse grained diets, the commmercial diet and the wheat diet resulted in significantly different Cr and N crop content proportions between free access and intubation procedures and the undisturbed test diets with the intubation treatment results being nearer the test diet proportion values than those of the free access. With the relatively finely ground test diet, meat and bone meal, this feature was not evident. Visual inspection indicated less uniformity of particle distribution in the coarse diets than in the meat and bone

based diet. The results suggested that in feeds in which texture interfered with the uniform distribution of particles, the intubation technique was likely to provide a closer match of material in the crop with that of the test diet than intake under free access conditions.

The results of the experiment 1b were inconclusive. Though evidence surfaced in the free access MBM and intubation wheat diet treatments to show Cr and N proportions tended to get less with time and that in the case of the free access MBM, intubation wheat diet and free access wheat diet treatments that ADF got lesser but NDF proportions increased with time, there were a number of exceptions. For intubation MBM there were no significant differences with time in the proportion of Cr, N, NDF and ADF and in the case of the intubation wheat treatments, changes that had occurred by the 3 hour sampling time were not reflected at the 4 hour sampling (eg. Cr and ADF). Similar inconsistencies were apparent in the free access wheat treatments for NDF and ADF. In addition the proportions of Cr and N in the free access wheat diet treatments did not alter significantly with time. No cause for these inconsistencies could be found.

The results of experiment 2 conducted on a MBM based diet indicated that different slaughter procedures significantly affected apparent ileal N digestibility. The CO_2 inhalation treatment birds had lower N digestibility than counterparts sacrificed by sodium pentobarbitone. Evidence presented in the discussion suggested that struggling during CO_2 asphyxiation may have influenced digestibility. Sodium pentobarbitone as the preferred procedure was also associated with a reduction in the time taken to collect digesta material. No significant difference in digestibilities were observed in the use of two different flushing solutions. However distilled water was considered a preferred solution on the grounds of assay convenience.

Experiment 3 was designed to establish the optimum time of sampling from the ileum following the start of feeding. In both the MBM and wheat treatments N and dry matter digestibility were relatively constant over sampling times of 2 to 5 hours with greater values at the 1 hour and lesser values at the 6 hour marks. Observations of the gizzard contents revealed an increasing coarse and indigestible particle build up with increasing time to slaughter and an apparent reduction in the fraction of Cr at the 6 hour sampling time. Ileal digesta quantities were numerically greatest at the 4 hour sampling interval for both the wheat based and MBM based diets and for this reason and on balance represented a preferred sampling time.

For both MBM and wheat treatments of experiment 4 differences in N and dry matter digestibility of samples of digesta drawn from section of ileum up to 30 cm in length as measured from the ileo-caecal junction were generally small and non significant. It was apparent that the length of the ileum sampled as a proportion of ileal length varied with age, a feature which suggested that drawing from too large a segment in young stock for the purposes of meeting sample size requirements may give rise to an incidental source of bias. On this basis and as a precautionary measure the results led to the conclusion that length of the lower ileum selected should be kept as small as consistent with obtaining sufficient digesta sample.

In experiment 5 age of birds had no significant effect on N and dry matter digestibilities for MBM and wheat based treatments. The procedures highlighted two procedural issues. Difficulty may be experienced in handling during intubation of 8-week old meat chickens and in terms of digesta sample size requirements, intubation may give rise to marginal samples in 4-week old stock. The small range of digestibility across 3 ages within both the MBM (3.2%) and wheat (2.5%) test diets was a measure of the precision of the assay in its developed form. Operationally and on balance the six week slaughter age proved most satisfactory and in future work this age is recommended.

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APPENDIX

APPENDIX 1

Derivation of an equation for determining Apparent Amino Acid digestibility values (AAAD) involving test and basal diets.

Additivity is assumed.

T = test diet

B = basal diet

I = test ingredient

P = the proportion of the basal in the test diet

Et, Eb, Ei = excreta arising from the test diet, the basal diet and the test foodstuff, respectively

AA = amino acid under investigation

D = digestible

g = gram(s)

AAAD = apparent amino acid digestibility of the test ingredient as a fraction

$$AA\ T = AA\ B\ (P) + AA\ I\ (1-P) \quad (1)$$

Expanding

$$AA/g\ T \times g\ T = AA/g\ B \times g\ T \times P + AA/g\ I \times g\ T \times (1-P) \quad (2)$$

$$AA/g\ T = AA/g\ B \times P + AA/g\ I \times (1-P) \quad (3)$$

Now

$$AA/g\ T = \frac{D\ AA/g\ T + AA/g\ E_t \times g\ E_t}{g\ T} \quad (4)$$

and

$$AA/g\ B = \frac{D\ AA/g\ B + AA/g\ E_b \times g\ E_b}{g\ B} \quad (5)$$

and

$$AA/g\ I = \frac{D\ AA/g\ I + AA/g\ E_i \times g\ E_i}{g\ I} \quad (6)$$

Substituting equations 4, 5 and 6 into 3

$$\frac{D\ AA/g\ T + AA/g\ E_t \times g\ E_t}{g\ T} = \frac{[D\ AA/g\ B + AA/g\ E_b \times g\ E_b]\ P}{g\ B} + \frac{[D\ AA/g\ I + AA/g\ E_i \times g\ E_i](1-P)}{g\ I} \quad (7)$$

Reshaping

$$D\ AA/g\ I = \frac{[D\ AA/g\ T + AA/g\ E_t \times g\ E_t]}{g\ T} - \frac{[D\ AA/g\ B + AA/g\ E_b \times g\ E_b]\ P}{g\ B} - \frac{[AA/g\ E_i \times g\ E_i]\ (1-P)}{g\ I} \quad (8)$$

But

$$\frac{AA/g\ E_t \times g\ E_t}{g\ T} - \frac{(AA/g\ E_b \times g\ E_b)P}{g\ B} - \frac{(AA/g\ E_i \times g\ E_i)(1-P)}{g\ I} = 0 \quad (9)$$

Hence

$$D\ AA/g\ I = \frac{D\ AA/g\ T - D\ AA/g\ B \times P}{1-P} \quad (10)$$

Converting (to the proportion of AA in 1 g of I)

$$\frac{D\ AA/g\ I}{AA/g\ I} = AAAD = \frac{D\ AA/g\ T - D\ AA/g\ B \times P}{(1-P)\ AA/g\ I} \quad (11)$$

Alternatively

$$D\ AA/g\ T - D\ AA/g\ B \times P = D\ AA/g\ I\ (1-P) \quad (12)$$

$$\frac{D\ AA/g\ T - D\ AA/g\ B \times P}{(1-P)} = D\ AA/g\ I \quad (13)$$

As a proportion

$$\frac{D\ AA/g\ T - D\ AA/g\ B \times P}{(1-P)AA/g\ I} = \frac{D\ AA/g\ I}{AA/g\ I} \quad (14)$$

$$\frac{D\ AA/g\ T - D\ AA/g\ B \times P}{(1-P)AA/g\ I} = AAAD \quad (15)$$

The final form of the formula is given below:

$$AAAD = \frac{D\ AA/g\ T - D\ AA/g\ B \times P}{(1-P)\ AA/g\ I}$$

APPENDIX 2

Summary of one way analysis of variance tables of paired comparisons between all combinations of treatments and for main classes for Exp 2.

Treatment x Treatment	Error D.F.	Treatment M.S	Error M.S	F-ratio	P<0.05
CO_2:distilled water x CO_2:physiol. saline	10	3.08	85.12	0.04	NS
CO_2:distilled water x Na-pento.:distilled water	10	123.46	54.90	2.25	NS
CO_2:distilled water x Na-pento.:physio. saline	10	157.25	58.93	2.67	NS
Na-pento.:distilled water x CO_2:physio. saline	10	165.54	52.82	3.13	NS
Na-pento.:physio. saline x CO_2:physio. saline	10	204.35	56.85	3.59	NS
Na-pento.:physio. saline x Na-pento.:distilled water	10	2.04	26.63	0.08	NS
Main class effects					
Method of slaughter	22	322.74	51.03	6.32	Sig.
Flushing solution	22	0.05	65.70	0.00	NS

APPENDIX 3

Summary of one way analysis of variance tables of treatments for MBM (dry matter basis) in Exp 3.

Treatment x Treatment	Error D.F.	Treatment M.S.	Error M.S.	F-ratio	P<0.05
% N / g	25	0.05	0.24	0.22	NS
% Cr / g	25	0.94	0.03	36.80	Sig
N digestibility	25	924.01	38.46	24.04	Sig
DM digestibility	25	1045.49	34.53	30.28	Sig
Digesta weight	25	0.16	0.06	2.63	NS

APPENDIX 4

Summary of one way analysis of variance tables of treatments for wheat diet (dry matter basis) in Exp 3.

Treatment x Treatment	Error D.F.	Treatment M.S.	Error M.S.	F-ratio	P<0.05
% N / g	25	0.15	0.04	4.26	Sig
% Cr / g	25	0.16	0.13	1.21	NS
N digestibility	25	166.65	30.05	5.55	Sig
DM digestibility	25	86.15	47.04	1.83	NS
Digesta weight	25	0.02	0.02	1.29	NS

APPENDIX 5

Summary of one way analysis of variance tables of treatments for MBM (dry matter basis) in Exp 4.

Treatment x Treatment	Error D.F.	Treatment M.S.	Error M.S.	F-ratio	P<0.05
% N / g	20	0.05	0.07	0.65	NS
% Cr / g	20	0.04	0.04	1.07	NS
N digestibility	20	27.56	24.69	1.12	NS
DM digestibility	20	15.74	17.62	0.89	NS
Digesta weight	20	2.72	0.09	29.45	Sig

APPENDIX 6

Summmary of one way analysis of variance tables of treatments for wheat diet (dry matter basis) in Exp 4.

% N / g	20	0.05	0.04	1.27	NS
% Cr / g	20	0.54	0.13	4.24	Sig
N digestibility	20	24.95	20.26	1.23	NS
DM digestibility	20	33.38	24.63	1.36	NS
Digesta weight	20	1.42	0.14	10.15	Sig

APPENDIX 7

Summary of one way analysis of variance tables of treatments for MBM in Exp 5.

Treatment x Treatment	Error D.F	Treatment M.S	Error M.S	F-ratio	P<0.05
N digestibility	15	15.69	49.65	0.32	NS
DM digestibility	15	33.82	30.58	1.11	NS
Digesta weight	15	0.09	0.03	2.96	NS
Length of ileum	15	210.06	37.48	5.60	Sig

APPENDIX 8

Summary of one way analysis of variance tables combination of treatments for wheat diet in Exp 5.

Treatment x treatment	Error D.F	Treatment M.S	Error M.S	F-ratio	P<0.05
N digestibility	15	9.02	8.61	1.05	NS
DM digestibility	15	1.90	16.29	0.12	NS
Digesta weight	15	0.05	0.01	4.57	Sig
Length of ileum	15	287.06	15.44	18.59	Sig

APPENDIX 9

One way analysis of variance of amino acid digestibility for MBM in Exp 5.

	D.F.	Sum squares	Mean square	F-ratio
Treatment	14	1550.63	110.76	3.59
Error	75	2312.45	30.83	
Total	89	3863.08		

APPENDIX 10

One way analysis of variance of amino acid digestibility for wheat diet in Exp 5.

	D.F.	Sum squares	Mean square	F-ratio
Treatment	14	1184.54	84.61	6.30
Error	75	1007.20	13.43	
Total	89	2191.73		

APPENDIX 11

Determination of Kjeldahl Nitrogen Content with a Kjeldahl Auto System

Analytical procedure:

(1) Dry and grind the samples to pass through a 1 mm sieve.

(2) Weigh the samples (less than 0.1 g for micro; approximately 0.1 g for semi-micro and 0.8 g for macro) directly into the digestion tubes or into weighing boats and quantitatively transfer to the digestion tubes.

(3) Add a Kjeltab (micro or macro) to each digestion tube containing a sample to be analysed.

(4) Add concentrated sulphuric acid (10 ml for macro and 5 ml for semi-micro and micro) from a dispenser and mix carefully by gently swirling the tube by hand or using a test tube mixer.

(5) Place the digestion tubes and stand with the prepared samples beside the digester and fit the exhaust manifold to the digestion tubes. Turn on the vacuum source (water aspirator) to maximum flow.

(6) Place stand, tubes and exhaust manifold in the preheated digester (420° C).

(7) Digest for 3-5 minutes with maximum flow through the exhaust manifold. Then adjust the airflow until fumes are just contained.

(8) Continue digestion until the mixture is clear and colourless (usually 20-45 minutes).

(9) Remove the digestion tubes containing the exhaust manifold from the digestor into a stand and allow the entire assembly to cool.

(10) Cool sample solution to hand temperature and dilute with distilled water and mixed (30 ml for macro and 10 ml for semi-micro and micro).

(11) Start up the "Kjeltec Auto 1030 Analyser" as for the instructions (see manual).

(12) Calculate nitrogen or crude protein using the formula:

$$\% N = \frac{14.01 \times M \times f \times 100 \times (\text{ml titrant} - \text{ml blank})}{\text{mg sample}}$$

$$= \frac{1.401 \times M \times f \times (\text{ml titrant} - \text{ml blank})}{\text{mg sample}}$$

where 14.01 = the atomic weight of nitrogen

M = the molarity of titrant HCl (mole/litre)

f = standard Kjeldahl factor = 1.00 for % N.

For macro analysis the recommended titrant concentration is 0.2M or 0.5M HCl. For semi-micro analysis use 0.1 HCl, and for micro analysis use 0.01M HCl.

Reagents and preparation:

Sulphuric acid, concentrated analytical grade N-free

Kjeltab (Se) (macro or micro)

Sodium hydroxide analytical grade 35-40% : Dissolve 400 g of NaOH in 1 litre of distilled water or deionised water.

Receiver solution : 1% Boric Acid with bromocresol green/methyl red indicator solution.

Preparation:

a. Dissolve 100 g Boric Acid with 10 litres of distilled or deionised water (1% solution).
b. Add 100 ml bromocresol green solution (100 mg in 100 ml methanol).
c. Add 70 mg methyl red solution (70 mg in 70 ml methanol).
d. Add 1 ml 1M (4%) sodium hydroxide (2 ml in 5 ml) to obtain a greeny-black color.

Hydrochloric acid: Depending on the sample size and nitrogen contents, HCl ranging from 0.01 M-0.50 M may be used for titration.

APPENDIX 12

Chromium Analysis - Atomic Absorption Spectrometry (AAS)

Analytical procedure:

(1) Dry and grind the samples to pass through a 1 mm sieve.

(2) Dry beakers for 3 hrs at 105° C and cool in desiccator, weigh. Weigh the samples (25-50 mg for ileal digesta and 100 mg for diet) into the beakers and dry at 105° C overnight or until a constant weight. Cool in the desiccator and reweigh to obtain dry matter.

(3) Ash at 500° C furnace overnight (ensure complete combustion).

(4) Add 3 ml of manganese sulphate/phosphoric acid solution to each beaker and swirl. Cover the beakers with a glass plate and place in a 140° C heating block for 20 minutes.

(5) Remove the glass plate and place the beakers on an insulated surface and allow to cool to below 100° C. Add 4 ml of 4.5 % w/v potassium bromate to each beaker and place in a heat block and cover the beakers with a glass plate. Then raise the heat block to 220° C (approximately 45 minutes).

(6) Remove the glass plate and place the beakers on an insulated surface. Carefully add 15 ml of distilled water at 60° C to each beaker and allow to cool.

(7) Rinse into 50 ml volumetric flasks with distilled water and make up to volume. Stand to allow ash to settle out.

(8) Read on atomic absorption spectrophotometer at 357.9 nm with a nitrous oxide/acetylene flame.

Reagents and preparation:

Phosphoric acid/manganese sulphate solution

a. 10% w/v $MnSO_4.4H_2O$

b. 85% w/w orthophosphoric acid

mix a. and b. with the proportion of 3 : 97 (vol/vol).

Potassium bromate

4.5% aqeous solution.

Standard chromium solutions for calibration:

Blank (chromium-free) : Prepared by ashing and digesting chrimium-free sample, using the same relative amounts of reagents as in each sample determination.

Stock (equivalent to 1000ug Cr_2O_3/ml) : Dissolve 1.9355 g potassium dichromate in distilled water and make up to 1 litre.

Working standards : Standards in the range equvalent to 0-20 ug Cr_2O_3/ ml made up from stock solution diluted to volume with the blank.

APPENDIX 13

Determination of Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF)

Analytical procedure:

DAY 1

(1) Place the crucibles in furnace at 500° C for 2 hours. Cool in a desiccator and weigh and record weight as C = weight of crucible.

(2) Weigh samples of about 1 g into 400 ml beakers. Assign "W" to the weight of each sample. Add 50 ml of neutral detergent (ND) solution, cover with a watchglass and bring to boil on a hotplate (set to 400° C initially and later to 300° C prior to boiling).

(3) Add another 50 ml ND solution and 2 ml α-amylase solution. Bring back to the boil and simmer again for 30 minutes.

(4) While the mixture is still hot filter samples into correspondingly labelled crucibles. Use the vacuum system and wash the watch glasses and beaker sides free of all sample using a washbottle containing distilled water.

(5) Wash the residue several times until no detergent is present. Place the crucibles with sample in the oven at 105° C overnight.

DAY 2

(6) Remove crucibles out of the oven and cool in a desiccator. Weigh the crucibles with sample residue as CNDR = crucible + neutral detergent residue (NDR).

(7) Put crucibles sideways into 600 ml beakers and cover with acid neutral detergent (AD) solution. Use watchglass to cover beakers. Boil gently (as previously) on a hotplate for 1 hour.

(8) Remove beakers to the vacuum system. Using hot water, wash crucible sides and bottom to remove all sample from the crucibles into the beakers

(9) Place the crucibles in the vacuum system and using low suction, filter the samples into the crucibles, washing several times with hot distilled water to remove all the detergent. Dry in 105° C oven overnight.

DAY 3

(10) Remove crucibles containing sample from the oven, cool in a desiccator and then weigh as CADR = crucible + acid detergent residue (ADR).

(11) Calculation:

$$a.\ \%\ NDR = \frac{CNDR - C}{W} \times 100$$

$$b.\ \%\ ADR = \frac{CADR - C}{W} \times 100$$

Reagents and preparation:

Neutral detergent solution:

60.00 g Na lauryl sulphate (or Na dodecyl sulphate)

37.22 g Na_2 ethylenediaminetetra acetic acid (EDTA)

13.62 g Na tetraborate decahydrate

9.12 g Na_2 hydrogen phosphate anhydrous (Na_2HPO_4).

Weigh each into a 2 litre conical flask. Dissolve in 1.5 litre of distilled water. Then add 20 ml ethylene glycerol and make up to 2 litre with distilled water and stir. Check the pH is in the range 6.9-7.1 and adjust with NaOH or HCl if necessary.

α-amylase solution:

Dissolve 1 g α-amylase in 30 ml distilled water in a 50 ml volumetric flask. Add 10 ml ethoxyethanol and make up to 50 ml with distilled water. Store in a refrigerator and replace about every week.

Acid detergent solution:

To a 2 litre conical flask containing about 1 litre of distilled water and wearing goggles, carefully add 56 ml concentrated sulphuric acid. Make up to 2 litre with distilled water = 5 % w/w H_2SO_4. Weigh 40 g cetyl trimethylammonium bromide (CTAB) into a large beaker, dissolve in 5 % H_2SO_4 and make up to 2 litre with 5 % H_2SO_4. Leave overnight to allow the mixture to dissolve completely.

APPENDIX 14

Determination of Amino Acid - Acid Hydrolysis Procedure

Analytical procedure:

(1) Weigh out finely ground samples (approximately 50 mg) using weighing scoops with elongated ends.

(2) Transfer the weighed samples quantitatively into the hydrolysis tubes by first tilting the tubes to approximate 30°. Insert the elongated ends of the scoops into the tubes. Gradually return the tube to its vertical position, tapping the scoop gently. The sample will slide into the tube.

(3) Add 1 ml of 2.5 um Norleucine standard into the tubes.

(4) Add 20 ml of the 6N HCl to each tube by first pipetting the acid into a small beaker. Then using a dropper, wash the acid through the scoop into the tube.

(5) Pass oxygen-free nitrogen through each tube for 60 seconds before handtightening the stoppers.

(6) Leave to stand in a freezer for 30-60 minutes (optional).

(7) Deaerate the tubes using water aspirators. Unscrew the stoppers slowly.

(8) When finished degassing, screw up the stoppers to hand tightness plus 3/4 of a turn.

(9) Transfer the tubes to the Pierce Reacti-Therm heating block and hydrolyse at 110±1° C for 24 hours.

(10) Filter the hydrolysates into the 500 ml RB flask, using Whatman No. 6 filter paper. Rinse tubes 3 times with demineralised water. Rinse also the stoppers and funnels.

(11) Attach the flasks to the rotary evaporators and evaporate the hydrolysates to dryness. Wash 3 times with demineralised water evaporating to dryness each time.

(12) Transfer quantitively the dried hydrolysates into 25 ml volumetric flasks, using 5 x 5.0 ml aliquots of pH 2.2±0.03 sodium citrate loading buffer. Make up to exact volume.

(13) Filter each hydrolysate into a sample bottle using Whatman No. 3, 5 or 6 filter paper. Store samples in a freezer.

(14) Defrost the required samples either by subjecting them to cold running water or by standing them in the refrigerator overnight.

(15) Centrifuge the hydrolysates at 5° C at 17000 rpm for 20-30 minutes.

(16) Transfer the centrifuged hydrolysates (usually 40 ul, depending on the percentage N content) into vials. The vials are loaded into the Waters High Pressure Liquid Chromatograph (HPLC) to obtain the analysis.

(17) Decant the remaining centrifuged samples into the corresponding sample bottles and store them in the freezer.

Reagents and preparation:

6N HCl:

a. Filter 1.0 litre of demineralised water into a vacuum flask through a 0.45 cellulose nitrate millipore.

b. Degas the filtered water for 15-20 minutes on a magnetic stirrer/hot plate at 250° C.

c. Cool, then decant the degassed water into a 2 litre measuring cylinder.

d. Add 1.1 litre concentrated HCl to 0.9 litre degassed water. Filter again through a millipore filter.

e. Transfer the 6N HCl into a 2 litre volumetric flask and store in refrigerator.

2.5 um Norleucine

2.2 % Sodium citrate