# ILEAL ENDOGENOUS RESPONSE IN NITROGEN AND LYSINE TO DIETARY PROTEIN AND CELLULOSE IN THE CHICKEN

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**Dedicated to**

***Edina Cahyaningsih***

**ABSTRACT**

Four experiments were undertaken. The first examined the swelling properties of faeces and the storage modulus (G') or elastic properties of ileal digesta to determine whether they were suitable indices for describing feeds on the basis of digestive response. One set of feeds were, maize, barley, meat and bone meal and peas. A second set consisted of feeds of set 1 but in each case involved sorghum addition in the ratio of 1:1. The third set involved cellulose and a medicinal bulking agent, Granocol, introduced in increasing proportion. The second and fourth experiments were undertaken to quantify endogenous nitrogen (N) response (experiment 2) and endogenous lysine response (experiment 4) at the terminal ileum to increasing dietary intake of cellulose (experiment 2) and guanidinated gelatin (experiment 4). Experiment 3 explored procedures and response to the feeding of wet diets of the form that were employed in experiment 4.

In experiment 1, swelling index varied over a narrow range between 4 to 8 cc excreta/g excreta (DM). Barley and meat and bone meal differed significantly, but the addition of sorghum to each caused little change in swelling index. The addition to cellulose of Granocol over the range of ratios 5:0 to 3.5:1.5 altered swelling index but not significantly or linearly. The narrow range within which swelling index varied suggests its application is limited.

Storage modulus estimations yielded a wide range between 2.7 and 76 K Pa. Meat and bone meal plus sorghum and maize plus sorghum differed significantly from all other dietary treatments except the maize diet. The addition of Granocol to cellulose and of sorghum to the

cereals, meat and bone meal and peas produced responses that were inconsistent in both direction and magnitute. The lack of control over colloidal consistency of the liquid and solid phases of the ileal digesta suggests this form of measurement is of limited use.

In experiment 2, the concentrations of N and chromium (Cr) in the ileal digesta were consistent with the digestibility properties of the dietary components and the ratio of the components, cellulose and cornstarch comprising the test diets. The endogenous N excretion response to increasing dietary intake of cellulose was 1.15 mg N for each g intake of cellulose ($P<0.01$).

In experiment 3, the concentration of N in the ileal digesta increased with increasing concentration of gelatin in the test diets. For each gram intake of gelatin, 34 mg of ileal N was passaged ($P<0.01$). The apparent N digestibility of gelatin was estimated as 54 percent.

In experiment 4, guanidination of gelatin resulted in an 86% conversion of lysine to homoarginine. The concentration of chromium in the ileal digesta was low and diminished with increasing concentration of dietary gelatin. The estimate of the ileal excretion of N in response to increasing intake of guanidinated gelatin was extreme and untenable. The low ileal digesta cr concentrations were considered to be giving extreme and exaggerated values of digesta flow (g digesta DM/g food fed). Ileal lysine response to increasing levels of guanidinated gelatin was estimated as 34 mg lysine per g intake of guanidinated gelatin.

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# CHAPTER 1
# INTRODUCTION

The assessment of the quality of feedstuffs and diets fed to poultry is based on bird assays that estimate the quantity of nutrient and food energy extracted by the digestive process. When the amount extracted is related back to the amount fed, a coeficient of digestibility or metabolizability is obtained. Estimates of the nitrogen (N) and amino acid (AA) digestibility and the metabolizable energy (ME) of the feedstuffs are published and used by the food compounding industry in the provision of commercial diets. The assay methods involved take various forms, and the procedures for estimating amino acid and N digestibility are reviewed in chapter 3, but all attempt directly or indirectly to minimise the contribution of endogenous excretions in the response. The application of an endogenous correction is an attempt to account for and negate the endogenous influence. The methods involved in establishing the size of the correction vary and their magnitude appear to be influenced by the methods employed.

Central to the issue of endogenous corrections in N and AA digestibility studies is the determination of the composition and quantity of endogenous excretions. Endogenous AA and N output is a function of the components of excretion, their proportions in the excretion, their AA and N composition and the magnitute of the endogenous excretion. The components of endogenous excretions are desquamated cells, mucus, digestive enzymes, bile, and microflora together with unabsorbed hydrolysis by-products of their partial or complete digestion.

There is likely to be an accumulation of endogenous secretions in the lower ileum. It has been suggested by Gitler (1964) and Boorman (1976) that endogenous proteins released distally to the stomach will escape the denaturing and partial digestive processes of the stomach and that the further down the tract they are secreted the less exposure they will have to denaturation. Studies by Ochoa-Solano and Gitler (1968) using $^{35}$S-Methionine in rats to label endogenous proteins have shown their accumulation in the ileum and mucin, the glycoprotein of mucus has been shown by Hashimoto, *et al.* (1963) and Hoskins (1978) to be resistant to enzymatic digestion.

The mediators determining the size of endogenous excretions are under normal conditions dietary. Fat and carbohydrate dietary increases raised amylase and lipase activity of pancreatic juice (Hulan and Bird, 1972). When dietary protein was raised from 16 to 28% in diets, chymotrypsin activity was increased in the duodenum and jejunum and the feeding of unheated soybean meal decreased amylase, lipase and chymotrypsin activity (Dal Borgo, *et al.*, 1968). Poullain, *et al.* (1989) showed dietary whole protein produced more rapid mucosal growth in rats than hydrolysed dietary protein or free amino acids. Raising dietary levels of fibre in the rat and pig resulted in increased pancreatic secretions (Zebrowska, *et al.*, 1983 ; Zebrowska, 1985) and increased the activity or levels of secreted enzymes (Schneeman, *et al.*, 1982 ; Langlois, *et al.*, 1987). Dietary fibre has also been associated with stimulating mucosal growth (Vahouny, *et al.*, 1985 ; Johnson, 1988 ; Jacobs, 1983; 1986) and increasing microbial mass in faeces (Larsen, 1991).

It is clear that dietary change may influence secretions into the gut but the effect this may have on

amino acid composition or total amino acid and N endogenous excretion is uncertain. Snook and Meyer (1964b) found that approximately 90% of endogenous proteins of gut secretions and sloughed cells were digested and absorbed leaving 10% for excretion with feed residues. Boorman (1976) refers to the homeostasis of amino acid composition of endogenous excretions and a number of reports have suggested that AA composition may be independent of dietary supply (Nasset and Ju, 1961 ; Nasset, 1968 ; 1972). Parsons (1981) partitioned excreta by differential centrifugation or physical separation techniques into fractions comprising microbial sediment, insoluble matter which equated with feed residues and a soluble fraction comprising endogenous material emanating from the gut and urine, the three fractions showed remarkable similarity in AA composition. Others, on the other hand have noted an effect on endogenous AA patterns of different dietary protein sources (Holmes, *et al.*, 1974).

On balance it seems that dietary changes may influence endogenous secretions into the gut, but the processes of digestion and absorption mitigate against variation and result in terminal excretions that remain relatively constant in composition. Under this premise endogenous output of N and AAs would be a function of the magnitute of excretion and as such may be responsive to the components of diets that influence excreta volume. The major dietary components are protein, fibre and starch.

The primary purpose of the work described in the experimental section of this thesis was to quantify endogenous nitrogen and lysine response to changing intakes of the major dietary components, fibre in the form of cellulose and protein in the form of gelatin and guanidinated gelatin. A secondary exercise was undertaken

to characterise feedstuffs on the basis of their excreta and ileal digesta characteristics.

The experimental section is prefaced by a review of protein digestion and absorption in the fowl (chapter 2) and a study of the methods and issues associated with digestibility assays (chapter 3). The experimental section describes four experiments. The first explores two methods for characterising feedstuffs on the basis of their excreta or ileal digesta characteristics. Experiments 2 and 4 involve studies to quantify endogenous N response (experiment 2) and endogenous lysine response (experiment 4) at the terminal ileum to increasing dietary intake of cellulose (experiment 2) and a transformed protein, guanidinated gelatin (trial 4). Experiment 3 explored procedures and response to the feeding of wet diets of the form that were employed in experiment 4.

# CHAPTER 2
# PROTEIN DIGESTION AND ABSORPTION IN THE FOWL

Components of gut secretions, nitrogen, protein, amino acids and energy influence values obtained in digestibility studies of feed nitrogen and amino acids, and metabolisable energy determinations of feedstuffs. Various design procedures and corrections are adopted to lessen the effect of endogenous sources of bias and to improve the absolute nature and additivity of feedstuff coefficients. The success of these procedures is difficult to gauge. The purpose of this section is to review processes of digestion and absorption associated particularly with dietary protein that affect excretion of protein and its hydrolysis products from the gastrointestinal tract.

## 2.1. Mouth, Pharynx, Oesophagus and Crop

**2.1.1. Morphology and Function:** In birds the mouth and pharynx are not sharply delimited and in most species including the fowl there is no soft palate and the hard palate communicates with the nasal cavities by a median connection, the choanal slit (Hill, 1971 ; Duke, 1977). Teeth are absent and their function is accomplished by a horny beak, a heavily cornified tongue anterior to the tongue fold and the grinding action of the gizzard (Hill, 1976 ; Sturkie, 1976 ; Duke, 1977).

The cavity of the mouth is lined with stratified squamous epithelium. Taste buds are few in number (about twelve in the young chick) and are situated on the base of the tongue and on the floor of the pharynx (Hill, 1976). Anderson and Nafstad (1968) cited by Hill (1971) described other sensory organs and free nerve endings (possibly

pressure sensitive) located on the hard palate and beak. Tubular mucous secreting salivary glands are well developed in chickens (Duke, 1986). They are widely scattered through the mouth and pharynx and are simple branched or compound in form. Hill (1971) refers to maxillary glands (roof of mouth), palatine glands (adjacent to the choanal slit), spheno-pterygoid glands (roof of pharynx), anterior and posterior submandibular glands, lingual glands on the tongue, crico-arytenoid glands near to the opening of the larynx and small gland at the angle of the mouth. Whilst the glands of the sparrow contain appreciable amounts of amylase, those of the chicken and turkey do not (Duke, 1986).

Mastication does not occur and food is swallowed quickly. Deglutition is accomplished by the tongue and hyobranchio-lingual muscles and larynx with raising of the head playing a secondary role. The food is moistened by secretions of the mouth and there is reflex closure of the choanal slit (Hill, 1971 ; Duke, 1977). Estimates of the volume of saliva produced employing oesophageal fistula techniques range from 7 to 30 ml/24 hours (Leasure and Link, 1940 ; Belman, 1962 cited by Hill, 1971) although Belman and Kare (1961) suggest the volume may be greater.

The oesophagus is a distensible tube lined by stratified squamous epithelium which is divided into upper and lower sections by a diverticulum, the crop, at its entry into thorax. Food is conveyed by peristaltic contractions assisted by lubricating secretions from an abundance of mucous glands present in the upper and lower sections but not in the crop (Hill, 1971) though this seems contentious (Duke, 1977). When the gizzard is empty food by-passes the crop and moves directly into the proventriculus. When the gizzard contains food or is

contracting the oesophageal-ingluvial fissura controlling entry into the crop relaxes and boli are diverted into the crop (Sturkie, 1976). Many factors may control the rate of crop evacuation including wetness of the food, fineness of the food, excitement, fear, struggling, hunger and length of fasting (Hill, 1971 ; Sturkie, 1986).

## 2.2. Proventriculus and Gizzard

**2.2.1. Morphology and Function:** According to Hill (1971) the proventriculus or glandular stomach is lined with a glandular mucous membrane of simple columnar epithelium and lies between the lower oesophagus and the gizzard. The proventricular glands comprising tubular alveoli in the submucosa, drain by a series of ducts lined with mucous secreting neck cells, into the lumen via macroscopic papillae on the mucosal surface. The cells lining the alveoli are both acid (oxyntic) secreting and enzyme (pepsinogen) secreting and discharge zymogen for up to 3 hours following a single meal. The evidence of Chodnik (1947) cited by Hill (1971) suggests evacuation is phasic with only a proportion of cells discharging at any one time.

The gizzard, the second site of peptic proteolysis, is a heavily muscled grinding chamber lined internally with a thick abrasive-resistant coat, koilin, composed of hardened secretions of the gizzard glands. Beneath it is glandular, crypt forming, mucous membrane and a thin submucosa. Simple tubular glands open into the crypts of the microvilli and the lining cells of both glands and microvilli are mainly of one type, chief cells. The secretions of the gland cells (chief cells) are polysaccharide protein complexes and result in both hardened vertical koilin rods that form dentate processes

on the surface and thick horizontal bands of koilin (Hill, 1971).

The main functions of the proventriculus and gizzard are the production of gastric juice, proteolysis and the propulsion of juice and food into the duodenum. Sturkie (1986) suggests avian control of gastric secretion is similar to that of mammals and occurs in three phases. A cephalic phase in which the bird's sense of the presence of food or feeding activity initiates gastric secretion by the vagus nerve. A gastric phase initiated by the arrival of food in the stomach and activated by direct contact of ingested nutrients with the gastric mucosa and indirectly by autonomic nerve involvement and through the release of gastric hormones. An intestinal phase stimulated by arrival of food in the small intestine and invoked by the autonomic nerve supply and the production of intestinal hormones.

Cells producing the hormone gastrin have been found in the glandular and muscular stomach of birds (Polack, *et al.*, 1974 ; Larsson, *et al.*, 1974b, 1986) and in mammals are referred to as G cells (Butts, 1993). Gastrin probably stimulates secretions of HCl and pepsin by causing release of acetylcholine from releasing cells in the submucosa of the proventriculus which then acts directly on the chief cells. Vagal stimulation of gastric secretions in birds occurs directly without involvement of acetylcholine (Kokue and Hayama, 1975). There is no evidence in birds of structures producing the gastric secreting inhibitory hormone, enterogastrin, synthesised in mammals (Hill, 1971).

The hormone avian pancreatic polypeptide discovered in chickens (Kemmel, *et al.*, 1968 ; Larsson, *et al.*, 1974a) appears to be involved in the gastric phase of secretion

and is released from the pancreas in response to the presence of amino acids and HCl in the upper portion of the gut following a meal (Duke, *et al.*, 1982 ; Johnson and Hazelwood, 1982). According to Hazelwood, *et al.* (1973) it acts independently of the vagus nerve to increase secretion of pepsinogen and HCl.

Cholecystokinin and secretin are hormones produced in the upper small intestine of birds and mammals and are regulators of the intestinal phase of gastric secretion (Sturkie, 1986). Cholecystokinin stimulates $H^+$ secretion in chickens but unlike gastrin does not appear to affect pepsin secretion (Burhol, 1974 ; 1982). Secretin stimulates both $H^+$ and pepsin secretion in birds (Burhol, 1974 ; 1982) which differs from its function in mammals where it has an inhibitory action on $H^+$ production. Sturkie (1986) postulates that the difference in functions may be related to supporting the important mechanical role of the gizzard in digestion and to the mixing of gastric and intestinal contents by regularly recurring intestinal refluxes.

The volume of gastric juice produced by the proventriculus varies from 6-21 ml/hr during starvation and up to 38.8 ml/hr after histamine stimulation (Hill, 1971). According to Sturkie (1965 ; 1986) feeding increases gastric secretions, fasting decreases them and food affects them in proportion to its content of protein. Gastric juice is composed principally of water with some hydrochloric acid, pepsin, mucin and certain salts. Sturkie (1976) reviewed its composition and secretion rate under various conditions.

The proventriculus secrets the inactive precursor pepsinogen (zymogen) which is autocatalytically converted into its active form, pepsin, as the pH of the digesta changes in the proventriculus and gizzard. There are at least 5 chicken pepsinogens (Sturkie, 1976 ; 1986). They appear to have differing pH optima and in some cases specificities (Boorman, 1976). As the pH of gastric contents change the enzymes become maximally active in sequence and create an enzymatically active mixture over a wide pH range. According to Taylor (1968) the more susceptible peptide bonds to pepsin hydrolysis are those involving the aromatic amino acids and those involving leucine and valine.

## 2.3. The Small Intestine

**2.3.1. Morphology and Function** : The histology of the avian small intestine is comparable to that of mammals although there are some differences (Toner, 1965 ; Hill, 1971 ; Boorman, 1976). The mucous membrane forms numerous villi (and crypts of lieberkuhn) that vary in form and length according to species. In carnivorous birds they are fingerlike and well developed but in herbivorous birds they are flattered and leaflike (Ziswiler and Farner, 1972). The villi are formed by projecting cores of lamina propria covered by simple columnar mucosal epithelium containing many goblet cells. Brunners glands are absent in the chicken duodenum but in some bird species tubular glands that are homologous with brunner glands of mammals may be present (Calhoun, 1954 ; Ziswiler and Farner, 1972). Argentaffin cells are in high concentration and lie deep in the epithelial glands in the upper duodenum of birds (Hill, 1971).

### 2.3.1.1. Pancreatic Secretion

There are two components to pancreatic secretion, the electrolytic or aqueous phase containing cations $Na^+$, $K^+$, and $Ca^{2+}$ at concentrations in humans close to that of plasma (Wills, 1985) and bicarbonate ions, and the enzymatic phase comprising enzymes for the degradation of proteins, carbohydrates (amylases) and fats (lipases) (Sturkie, 1986), and ribonuclease and deoxyribonuclease (Hill, 1971). Pancreatic juice is pale yellow in colour, has a pH of 6.4 to 6.8 (chickens) and 7.4-7.8 (turkeys) and cannulation of the main pancreatic duct of 14-30 week old white leghorn chickens resulted in a secretion of 15-20 ml/day (Hulan, *et al.*, 1972 ; Sturkie, 1976). Bird (1971) measured the distribution of trypsin and amylase in different segments of the duodenum of 14-16 week old male chickens and found that most of the enzyme activity (55% and 75% respectively) took place in the last quarter near the entry of the pancreatic duct into the duodenum. In geese the concentration in pancreatic tissue of chymotrypsin was ten times that of trypsin (Nitzan, *et al.*, 1973) and Kokue and Hayama (1972) showed that the rate of secretion of pancreatic juice per unit of body weight was greater for the chicken than in mammals (Sturkie, 1986).

### 2.3.1.2. Proteolytic Enzymes of the Pancreas

Keller (1968) provides a clear account of the broad enzymatic processes involved in intraluminal protein digestion. The proteolytic enzymes of pancreatic juice comprise endopeptidases, the trypsins, the chymotrypsins and the elastases, and the exopeptidases, carboxypeptidase A and carboxypeptidase B. They are secreted into the intestine as zymogens and are activated by the protease,

enterokinase, secreted by the intestinal wall which converts the trypsinogens to trypsins. The process once started is autocatalytic and the trypsins formed hydrolyse bonds in other zymogens to form active enzymes which are themselves autocatalytic (Silk, *et al.*, 1985). The trypsins are thus central to proteolysis and their inhibition has consequences that exceed a simple diminution of tryptic hydrolysis of dietary protein (Boorman, 1976).

As described for pepsin, each endopeptidase is probably a mixture of enzyme species. Trypsins catalyse the hydrolysis of bonds involving basic amino acids such as lysine or arginine (Wills, 1985 ; Boorman, 1976). Chymotrypsin hydrolysis bonds involving aromatic amino acid residues whereas the elastases are relatively non specific. The exopeptidases split off terminal amino acids possessing free carboxyl groups. Type A carboxypeptidase has a specificity similar to pepsin splitting peptide bonds adjacent to aromatic amino acids whilst type B carboxypeptidases attack bonds involving basic amino acids (Wills, 1985). The activities of carboxypeptidases are inhibited in sequential hydrolysis by the proximity of proline and in the case of the type A, charged side chains whereas the B requires close proximity of a cationic side chain for maximum activity (Boorman, 1976).

The pancreatic enzymes are considered to be extrinsic enzymes which act in the lumen and are adsorbed onto the epithelial absorptive surface where they continue to catalyse fragmentation of the polypeptide chain. In contrast peptidases produced by the surface epithelial glands and cells of the intestine are intrinsic. They continue the proteolytic function both on the brush border and in the lumen where they occur as a result of

desquamation rather than secretion. Rhodes (1968) has reviewed and classified these enzymes.

### 2.3.1.3. Regulation of Pancreatic Proteolytic Enzymes

The sequence of events in mammals leading to the flow of pancreatic juice has been summarised by Sturkie (1986). In mammals the eating of a meal results initially in vagal stimulation which causes the pancreas to secrete low volumes of the enzymic component of pancreatic juice. If the meal is prevented from reaching the duodenum as in the case of sham feeding the secretion stops, but if the food reaches the duodenum, the food and gastric HCl stimulates release of secretin from the intestine. Secretin causes an initial secretion of the aqueous component from the pancreas. Dietary amino acids or peptides and fat on reaching the duodenum stimulate the production of intestinal cholecystokinin. Cholecystokinin produces a prolonged flow of both aqueous and enzymatic components of pancreatic juice.

Sturkie (1986) cites a number of authors whose work collectively suggests regulation of pancreatic flow in birds is similar to that in mammals. Pancreatic secretion begins immediately when fasted chickens are fed, but if the chickens are first vagotomised there is no initial response (Kokue and Hayama, 1972). Pancreatic secretion may increase from 0.4-0.8 ml/hr to 3 ml/hr immediately following feeding (Ivanov and Gotev, 1962 cited by Sturkie, 1986). Secretin has been found in the intestinal mucosa of turkeys (Dockray, 1972) and intravenous injections produced an increase in the secretion of the pancreatic aqueous component (Heatley, *et al.*, 1965). Vasoactive intestinal peptide has been isolated from the chicken intestine

(Nilsson, 1974) and has a similar function to secretin but is more potent (Vaillant, *et al.*, 1980). Administration of cholecystokinin to pigeons has been shown to increase pancreatic proteolytic enzyme activity (Webster and Tyor, 1966) and increase pancreatic secretions (Sabha, *et al.*, 1970) although in this work the synthesis of proteins by the pancreas was not increased. Porcine cholecystokinin was shown by Dockray (1975) to increase the rate of flow of pancreatic secretions and pancreatic protein in turkeys.

There is evidence that dietary composition affects the composition of pancreatic juice. Increased intake of dietary carbohydrates and fat increase amylase and lipase activity of pancreatic juice (Hulan and Bird, 1972). Dal Borgo, *et al.* (1968) reported an increase in chymotrypsin activity in the duodenum and jejunum when dietary protein increase from 16 to 25% percent.

### 2.1.3.4. Bile

The biliary system comprises the cystic duct bearing the gall bladder and draining bile mainly from the right lobe of the liver, and the hepatic duct which drains the left lobe. Both ducts anastomose on a common papilla with the pancreatic duct at the caudal end of the ascending limb of the duodenum (Hill, 1971). Bile production is stimulated by the presence of bile salts in the blood, by eating and cholecystokinin is probably involved in postprandial secretion (Sturkie, 1986). Formation of bile by the liver is a continuous process and secretory rates of about 1.0 ml/hr have been observed in concious 14 week old cockerels and anaesthetised 4 to 6 month-old birds (Hill, 1971).

In mammals bile contains water, protein, bile pigments, bile acids, cholesterol, neutral fats, urea and inorganic ions. Bacteria in the lower intestine deconjugate bile acids and alter them chemically by the reduction of hydroxyl groups. Deconjugated and unaltered bile acids are reabsorbed in the lower small intestine into the portal blood and taken up by the liver where they are reconstituted (Butts, 1993). The concentration of protein in bile is low, many are glycoproteins and the most abundant is albumin (Butts, 1993).

### 2.3.1.5. Intestinal Secretions

Evidence for the nature of secretions produced by the small intestine have been reviewed by Hill (1971), Duke (1976), Sturkie (1976 ; 1986). The small intestine is the primary site of chemical digestion. Intestinal pH ranges from about 5.6 to 7.2 in those species that have been tested (Herpol and Van Grembergen, 1967). The pH increases from the oral to aboral end and the pH of each portion of the tract is regulated by secretory activity within that portion (Hurwitz and Bar, 1968). Vagal stimulation increases the inherent motility of the tract (Duke, 1976 ; Hill, 1971 ; Sturkie, 1986). Intestinal secretion may be increased by duodenal distention (which probably directly stimulates release of intestinal hormones) by vagal stimulation (Hill, 1971) and by secretin (Kokas, *et al.*, 1967). Vagal stimulation has more an effect on mucous secretion than on secretion of digestive enzymes (Sturkie, 1986) and recent work suggests that whilst secretin stimulates duodenal secretions there are other hormones present in mucosal cell extract that are also involved (Sturkie, 1986).

Intestinal enzyme secretions include amylase, saccharidases, peptidases and lipase. Lactase and trehalase which acts on the common plant carbohydrate trehalose are not secreted but maltase, isomaltase and sucrase are present (Sturkie, 1986). Greatest disaccharidase activity is found in the upper ileum, with the duodenum having less and the lower ileum almost none (Sturkie, 1986). Hormones known to be produced by the chicken intestine are enterokinase, secretin, cholecystokinin and vasoactive intestinal peptide.

Silk, *et al.* (1985) cite recent studies which indicate the presence of solubilised intestinal brush-border and cytoplasmic intestinal mucosal amino oligopeptidases in intestinal contents of humans. They function as exopeptidases and remove amino acid residues from the amino end of peptide chains. Duke (1977) reports both amino peptidases and carboxypeptidases have been found in the duodenal mucosa of chickens citing DeRycke (1962). Amino peptidases include di, tri and tetra peptidases and are formed in the small intestinal mucosa. They hydrolyse peptides to amino acids both at the surface of the mucosa before absorption and within the epithelial cells before amino acids enter the blood circulation (Larsen, 1991). These enzymes enter the intestinal lumen through the desquamation of the mucosal cells and assume a functionally significant role in the terminal stages of protein digestion in the ileum (Silk, *et al.*, 1985). In the ileum the activity of luminal peptidases is greater than in the jejunum and their importance relative to proteolytic activities on the surface of mucosal cells in the ileum may be increased. The products of luminal proteolysis are free amino acids and small peptides having a chain length of two to six amino acid residues (Silk, *et al.*, 1985).

## 2.4. Caeca, Colon and Cloaca

**2.4.1. Morphology and function:** The caeca are a pair of blind ended tubes that arise at the junction of the small and large intestine, extend forward for about half their length and then double back on themselves. They are lined with columnar epithelium which in the base region (blind-end) is relatively smooth but in the body and neck regions is formed into villus-like projections which suggests an absorptive function. Lymphoid tissue is scattered throughout the submucosa and occasional goblet cells are present in the lining epithelium (Hill, 1971).

The colon is short and narrow and extends from the ileo-caeco-colic junction to the cloaca. The mucosal epithelium is formed into short broad, villus-like projections that are lined with columnar cells and numerous goblet cells (Hill, 1971).

The cloaca is a spheroidal chamber into which the digestive and uro-genital tracts converge. It is separated from the colon by a muscular constriction and opens to the exterior at the vent. It is lined with columnar epithelium which forms short villus-like structures anteriorly and which are more flattered and leaf like towards the vent where it merges on the inner aspect of the upper and lower lips into stratified squamous epithelium (Hill, 1971).

The digestive functions of the avian caeca and colon have been reviewed by Hill (1971), Sturkie (1965 ; 1976 ; 1986) and Duke (1976). There is little evidence of digestion in the large intestine of birds other than in the caeca although there is evidence of water resorption in the colon and urine and fluid refluxing into the caeca (Hill, 1971 ; Sturkie, 1976). Only fluid portions enter the caeca (Clements, *et al.*, 1975) and the digesta that enters is

retained there up to four times longer than material that by-passes it (Sturkie, 1986) with the ratio of caecal to normal droppings varying depending on dietary composition from 1 to 7 for barley diets to 1 to 11 for wheat diets (Hill, 1971).

The caeca appears to be important in water absorption (Thronburn and Willcox, 1965) and may play a role in non protein metabolism and protein utilisation. In *Willow ptarmigans* urine refluxing into the caeca was degraded into ammonia and incorporated into amino acids by bacterial action, but only bacteria utilised the amino acids and none was absorbed by the host (Mortensen and Tindall, 1981). However, whereas in geese urea excretion was 5.7% greater following caecectomy similar procedures in chickens failed to demonstrate an increase in uric acid secretion (Kese and March, 1975). The feeding of protein sources that are less digestible such as unheated soybeans may result in significant portions of dietary protein escaping intestinal proteolysis and becoming available to degradation by caecal flora. Degradation by caecal bacteria appears to be substantial. Kessler, *et al.* (1981) showed caecectomised chickens excreted 15-30 percent more amino acids than intact birds and the studies of Low (1985) support these findings.

The caeca appear to have an important role in bacterial fermentation of dietary fibre and their effect responds to dietary preconditioning. Sturkie (1976) reviews early studies that indicated that the crude fibre of corn, oats and wheat were digested more poorly by caecectomised than by intact birds. The coefficient of digestibility change for corn fibre from 17.1 and 19.7 before caecectomy to 0.0 following it. Sturkie (1986) cites a number of studies which show that the caecal bacteria of domestic

fowls digest little or no cellulose. On the other hand, Duke, *et al.*(1984) demonstrated in turkeys that preconditioning with high fibre or high cellulose diets improved cellulose degradation from 2.8 percent in birds not preconditioned to 10.4 percent in birds preconditioned. The glucose freed by cellulose breakdown was absorbed by the caeca and used by the host birds. Bedbury and Duke (1983) conclude that preconditioning produces a caecal flora more capable of fibre digestion and cellulolysis.

Sturkie (1986) cites a number of studies that indicate wild galliforms may extract considerable energy from fermentation by caecal bacteria and there is evidence that indicates caecal size responds to increasing concentrations of dietary fibre (Gasaway, 1976) although dietary bulk rather than quality may be causing the effect (Sturkie, 1986).

## 2.5. Microbial Effects

The nutritional significance of populations of microorganisms in the digestive tract have been reviewed by Salter (1973) for poultry and Savage (1986) for mammals. In the fowl bacterial activity occurs throughout the gastrointestinal tract and is illustrated in Table 2.1. adapted from Jayne-Williams and Coates (1969). The dominant bacteria in the crop appears to be lactobacilli and weakly acid or weakly alkaline environments (pH 6.5 to 7.5), increasingly anaerobic conditions, the composition of digesta, the nutritional status of the host and the quality of the protein fed all appear to influence the size and nature of the bacterial populations (Jayne-Williams and Fuller, 1971). Although bacteria are more concentrated in the caeca there may be a greater turnover of bacterial

cells and a greater effect of bacterial activity on digestion in other parts of the tract as a result of the different evacuation rates of the caeca, small intestine and colon (MacNab, 1973).

Table 2.1. Microflora population in different sites of the digestive tract of poultry (Adapted from Jayne-Williams and Coates, 1969).

| Organisms | Organisms/g in contents of Crop | Duodenum | Ileum | Caecum |
|---|---|---|---|---|
| **Streptococci**<br>*Strep. faecalis*<br>*Strep. liquefaecalis*<br>*Strep. zymogenes*<br>*Strep. faecium* | $0 - 10^9$ | $10^2 - 10^6$ | $10^3 - 10^7$ | $10^5 - 10^{10}$ |
| **Lactobacilli**<br>*L. lactis*<br>*L. acidophilus*<br>*L. salivarius* | $10^7 - 10^{10}$ | $10^4 - 10^8$ | $10^5 - 10^9$ | $10^8 - 10^{10}$ |
| **Coliforms**<br>*E. coli*<br>*A. aerogenes* | $10^5 - 10^7$ | $10^2 - 10^6$ | $10^3 - 10^6$ | $10^7 - 10^{10}$ |
| **Clostridia**<br>*Cl. welchii* | not determined | $0 - 10^5$ | $0 - 10^7$ | $0 - 10^9$ |
| **Bacteroides** | 0 | 0 | 0 | $0 - 10^9$ |

Other organisms : *Cl. sporogenes, Cl. paraputrificum, Cl. tertium, Eubacterium sp.*, aerobic sporeformers, coryneforms, anaerobic cocci, micrococci, moulds and yeasts.

Mason (1984), Draser and Hill (1974) and Wrong, *et al.* (1981) provide evidence of the digestive activity of mammalian gut flora. Among the nitrogenous compounds metabolised are amino acids, maillard compounds (amino acid-sugar complexes), mucosal residues, mucoglycoproteins, uric acid and amines. The most significant reactions involve the formation of short chain fatty acids through the reduction of amino acids with the liberation of

ammonia. Other reactions involve the oxidative deamination of amino acids with the production of aldehydes and formation of succinate, fumarate, indole propionate, indole pyruvate as well as other phenolic acids. The acids lower the pH of the contents of the large intestine and lowers the activity of the deaminase-producing bacteria which are favoured by alkaline pH conditions.

Gut bacteria may also metabolize amino acids by decarboxylation and fission reactions. Fission reactions result in the formation of other amino acids, α-keto acids, cyclic compounds and amines and decarboxylases are known to be produced by some bacteria.

The intestinal microflora may use the breakdown products of their activities to synthesise their own proteins. This mainly involves the assimilation of ammonia but the utilisation of amino acids has also been reported by Payne (1975) as cited by Larsen (1991). Boorman (1976) does not discount the possibility that amino acids freed by bacterial activity may also be absorbed. The consistency of faecal amino acid composition over a variety of diet types noted by Parsons (1981) ; Boorman (1976) and Larsen (1991) may reflect the microbial composition (Mason, 1980) and consistency of terminal endogenous secretions of the gut (Boorman, 1976).

## 2.6. Endogenous protein secretions

Proteinaceous gastrointestinal secretions have four primary sources: the enzymes secreted along the tract and those arising from the pancreas and bile, desquamated epithelial cells, plasma protein secretions, and mucous secretions. The nature and function of the enzymes

secretions have been covered in earlier sections.

Table 2.2. drawn from Hill (1971) demonstrates that as digesta passes from the gizzard into the duodenum there is an approximate 3 fold dilution of exogenous nitrogen by endogenous sources. Somewhat similar relationships (four fold) have been reported by Boorman (1976) of studies of the dog and the rat and suggest that considerable digestion and absorption of endogenous protein must take place in more distal segments of the gut.

Table 2.2. Distribution of nitrogen in the digestive tract of 6 week old chickens.

| Food or intestinal segment | % N | $\%Cr_2O_3$ | $N/Cr_2O_3$ |
|---|---|---|---|
| Food | 3.55 | 0.458 | 7.75 |
| Crop | 3.55 | 0.352 | 10.03 |
| Proventriculus | 3.13 | 0.192 | 16.30 |
| Gizzard | 2.74 | 0.189 | 14.50 |
| Upper duodenum | 8.33 | 0.230 | 36.25 |
| Lower duodenum | 8.55 | 0.282 | 30.37 |
| Upper jejunum | 6.00 | 0.700 | 8.58 |
| Lower jejunum | 5.23 | 1.437 | 3.64 |
| Upper ileum | 4.54 | 1.274 | 3.65 |
| Lower ileum | 4.30 | 0.811 | 5.30 |

The secretion of plasma protein into the gastro-intestinal tract has been reviewed by Freeman (1964) and Rothschild, *et al.* (1970) and their role in mammalian digestive secretions summarised by Butts (1993). Estimates of the amount of plasma protein loss into the digestive tract range between 10 percent for plasma albumin (Freeman, 1964 ; Jeffries and Sleisenger, 1968) and up to 20% (Cuthbertson and Tilstone, 1972) although earlier estimates implied catabolism by way of the gut might be even greater (Boorman, 1976). With the exception of immunoglobulin A

which is secreted by the mucosa and adheres to and protects the mucosal surface, there is no evidence that plasma proteins are actively transported across the epithelial surface. A carrier mechanism proposed is that lymph arising from capillaries in the lamina propria is carried into the lumen along with desquamating epithelial cells (Butts, 1993).

Cellular exfoliation from the mucosal surface of the gastro-intestinal tract involves cells originating in the crypts migrating up the epithelial surface of the villi where they are shed at the apical surface (Boorman, 1976). The size and shape of the villus is governed by the rate of cell loss and replacement and to retain size and shape a control must exist that coordinates rates of shedding with rates of synthesis in the crypts (Creamer, 1974). Each cell is replaced in approximately 48 hours in the fowl (Imondi and Bird, 1966) and is similar to estimates for replacement time of the total gut cell population in dogs and humans of 4-6 days and in rats of 3-7 days (Butts, 1993).

Butts (1993) notes a number of factors that influence the growth of the mucosa in mammals. Dietary fibre stimulates mucosal growth and intestinal cell turnover (Jacobs and Lupton, 1984). Forms of dietary amino acid supply, whether whole protein, hydrolysed protein or free amino acids have influenced mucosal growth in the rat (Poullain, *et al.* 1989). The composition of the gut microflora and physical and chemical trauma may increase cell loss (Badawy, *et al.*, 1957) and the presence of normal bacterial flora shorters the average life of a cell to half that under bacterial free conditions (Strombeck and Guilford, 1990).

The composition and function of mucus secreted in the gasto-intestinal tract has been reviewed by (Allen, 1981 ; 1984) and Mantle and Allen (1989) and its role in mammalian digestive physiology summarised by Butts (1993). Mucus is a water and mucus glycoprotein (mucin) mixture that occurs in two forms, as a water insoluble gel adhering to the mucosal surface of the entire gut and as a soluble fraction that mixes with luminal juices and overlies the gel. Mucus is continuously released under resting conditions by simple exocytosis from mucus secreting columnar epithelial cells found throughout the gut and through cell exfoliation. It, together with the digestive enzymes and desquamated epithelial cells represent the major source of endogenous protein in the secretions of the gut. Specialised structures involved in mucus secretion include the salivary and oesophageal glands, mucus neck cells of the proventriculus, brunner's glands of the duodenum, goblet cells and the crypts of lieberkuhn of the intestine. It functions to protect the underlying mucosal epithelium from trauma of a mechanical, chemical or pathological nature and acts as a barrier separating gut flora and pathogens from contact with the lining epithelium. It is permeable to low molecular weight solutes (<1000 DA) but not to large molecules such as proteins and within its layers it may contain dietary proteins, peptides and amino acids, enzymes, plasma proteins, bile, microorganisms, sloughed cells, immunoglobulin A and digesta fragments and solutes and ions.

*In vitro* studies have shown that purified mucins of mammalian origin are susceptible to proteolysis by digestive enzymes with the release of degraded glycoprotein subunits (Mantle and Allen, 1989). Loss or degradation of mucus may also arise from the abrasive action of the passage of digesta and from the motile forces of digestion.

Cassidy, *et al.* (1981) concluded that cellulose and wheat bran supplemented diets increased secretion within the large intestine. It is apparent that a dynamic equilibrium must exist that maintains the integrity of the mucus barrier against continuous erosion of the mucus layers.

## 2.7. Digestion and Absorption of Proteins

Reviews on the physiology of digestion in poultry have been published by Sturkie (1965, 1976, 1986), Hill (1971), Boorman and Freeman (1976), Duke (1977) and in mammals extensive coverage by Snook (1973), Rerat, *et al.* (1976), Davenport (1982), Wills (1985), Strombeck and Guilford (1990), Larsen (1991), Rerat and Corring (1991), Butts (1993) and for pigs Low and Zebrowska (1989). Digestion is a luminal process in its early stages that involves the hydrolysis of macromolecules into shorter sub-units. This is followed by a rapid degradation into lesser units (by a process operating on the brush border surface of the intestine) small enough to enter the microscopic spaces between the villi and be absorbed into epithelial cells. This final stage or membrane digestion is brought about by enzymes native to (intrinsic) or adsorbed onto (extrinsic) the mucosal surface. The pancreatic enzymes are extrinsic factors that act both in the lumen and at adsorption sites on the digestive-absorptive surface. The intrinsic enzymes are the peptidases of the small intestine which act on the brush border of the cell and in the lumen when they arrive through the process of cellular exfoliation. Further digestion and absorption of residual digesta may result from bacterial proteolysis which in the fowl is most active in the caeca (Boorman, 1976). Protein digestion occurs predominantly in the stomach and proximal small intestine and the main site of absorption is the small intestine.

Protein molecules are denatured initially by acidic enzymatic hydrolysis in the proventriculus and gizzard. In mammals absence of this stage results in a reduction of amino acid absorption and a reduction in protein digestibility (Butts, 1993). Acidic chyme passes into the duodenum where it is subject to alkaline pancreatic secretions that raise luminal contents to near alkaline pH to provide optimal conditions for degradative activities of pancreatic and brush border proteolytic enzymes. The brush border contains peptidase activity against peptides 3-6 residues in the length while the dipeptidases appear to be present predominantly in the cytosol of the epithelial cells but with some present in the brush border. Some of the peptidases from the brush border and cytoplasm of the cells are solubilised in the lumen, in part from the breakdown of exfoliated epithelial cells, and assume a luminal hydrolytic activity (Butts, 1993).

Absorption of amino acids and peptides takes place throughout the small intestine but with predominant activity occuring in the jejunum. Transport of amino acids by intestinal enterocytes occurs by simple and facilitated diffusion and active transport. Amino acid transport systems are classified on the basis of substrate preference. Sturkie (1986) reviews in some detail avian absorptive process. Absorption occurs by attachment to a specific site on the mucosal epithelial membrane. Learner (1971) cited by Hill (1971) concluded that in the chicken there were at least three separate absorptive pathways for neutral amino acids: a system for methionine and related aliphatic compounds, one for glycine and one for proline and related amino acids. He also recognised a distinct pathway for basic amino acids. Among members of a group there is competition in the absorptive process although the absorption sites exhibit preference rather than

exclusiveness. L-lysine absorption is inhibited by L-arginine, L-phenylalanine or L-histidine and L-leucine absorption is inhibited by L-valine, L-isoleucine, or L-methionine, with little or no competition observed for glycine site (Gous, *et al.*, 1977).

Endogenous protein is not digested as rapidly as exogenous protein. The more distal segments of the intestinal tract may play a more important role in the absorption of amino acids of endogenous protein (Crampton and Nesheim, 1969). Boorman (1976) suggests in explanation that endogenous proteins are not subjected to the denaturing and partial digestive processes of the stomach. However, Nasset (1972) has pointed out that the ratio of endogenous to exogenous protein decreases along the small intestine, a phenomenon that is inconsistent with endogenous protein being resistant to digestion and absorption.

Intestinal mucosal uptake of peptides is mediated by a specific carrier system and is restricted to the uptake of dipeptides and tripeptides. The uptake of peptides is generally faster than the absorption of free amino acids and such uptake confers enhanced efficiency to the digestive process by enabling intracellular hydrolysis of dipeptides and tripeptides (Butts, 1993). Some peptides and proteins are absorbed intact into the blood plasma and are probably transported to and hydrolysed in tissue cells (Webb, 1990 ; Butts, 1993).

# CHAPTER 3

# NITROGEN AND AMINO ACID DIGESTIBILITY

## 3. DIGESTIBILITY

Digestibility is a measure of the amount that has been extracted from a food through the process of digestion and absorption. It may be estimated by measuring the amount excreted and substracting it from the amount fed. Excreted material may contain waste of endogenous origin, cell debris, mucus, digestive enzymes, bile and microflora and of metabolic origin, urine, as well as food residues. If a correction is applied to negate the influence of endogenous and metabolic sources the estimates derived are "true" rather than "apparent". The most commonly undertaken measurements are those on nitrogen (N) and amino acids (AA).

Papadopoulus (1985) defined apparent amino acid digestibility as "the difference between the amounts of amino acid intake in the diet and in the excreta or ileal digesta" as a proportion of the amount consumed in the food. Expressions describing apparent and true AA digestibilities as adapted from Bolton (1969) are given below

$$\text{Apparent digestibility} = \frac{\text{Dietary AA} - \text{Excreta AA}}{\text{Dietary AA}}$$

$$\text{True digestibility} = \frac{\text{Dietary AA} - (\text{Excreta AA} - (\text{Endogenous} + \text{Metabolic AA}))}{\text{Dietary AA}}$$

There are two common procedures for estimating digestibility in poultry. They are the total collection and ileal techniques. Measurements labelled "ileal" are indicative of the use of procedures which attempt to overcome distortions caused by urine contamination of faeces and distortions arising from hind gut microbial synthesis of protein and amino acids.

Digestibilities are estimates of the proportion of supplied nutrient that is removed from the gut by absorption into the circulatory system. In constrast, they are not measures of availability although they can be used as estimates of such. Availability refers to the proportion of supplied nutrients that are in a form, following digestion and absorption, capable of being used for a particular metabolic need. In feed formulation digestibilities may permit an improved estimate of the amount of nutrient extracted from the diet and may enable more efficient used of feed ingredients with ultimately a cost benefit.

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

## 3.1. DIGESTIBILITY *IN VITRO*

Digestibility *in vitro* involves determination by enzymatic assay 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 insoluble residue that remains after the digestion process and following filtration and washing (Low, 1982).

The techniques attempt to reproduce digestive conditions in the stomach and in the small intestine but they differ in two important respects. The secretion of intestinal juice is not constant in composition and the technique does not take into account digestive phenomena in the end section of the digestive tract (Larbier, 1985). Accordingly, the extent to which they may simulate *in vivo* digestion in any given situation is uncertain (Low, 1982).

Results from enzymatic or *in vitro* assays have been compared with those of ileal and faecal (total collection) and chemical digestibility studies. Rayner and Fox (1976) compared the amino acid digestibility of rapeseed meals using either pronase or hydrolyses 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 biologycal assays than those obtained using acid hydrolysis.

It has become apparent that the type of enzyme or enzyme complex and the enzyme-to-substrate ratio influence the *in vitro* result. Buchmann (1979) showed that for cereals multi-enzyme digestion equates more closely to *in vivo* methods. On the other hand, Johnston and Coon (1979) were more interested in exploring the effect of graded levels of pepsin on the digestibility of various animal protein source. They found that decreased levels of pepsin

increased the sensitivity of the assay and the increased the range of difference between amino acids between diets.

*In vitro* techniques are relatively quick, inexpensive and convenient methods of estimating digestibility. However, the factors that influence results include enzyme types, quantities and combinations, time of incubation, pH, particle size and temperature and are a source of variability between samples and between protein sources Sibbald (1987). Clandinin and Robblee (1952) attributed to enzymatic assays a possible quality control function much as in identifying the optimum duration of heat treatment to which protein sources such as soybean meal should be exposed.

Standardised enzymatic procedures may provide assays capable of measuring differences in protein digestibility for samples of the same product grown or manufactured under different conditions. However, they have value only to the extent that they reflect *in vivo* values (Sibbald, 1987).

## 3.2. DIGESTIBILITY *IN VIVO*

These are digestibility assays performed using birds as the target species. They include the methods of total collection, ileal assays and surgical techniques. These latter have been used in research to estimate the extent of bias associated with urine and microbial contamination. They include cannulation, caecectomy, exterioration of the colon (colostomy) and the use of germ-free birds.

### 3.2.1. TOTAL COLLECTION METHODS

These assays involve collecting all the excreta resulting from the feeding of a test diet. The assays take a number of forms and give rise to two types of values, apparent digestibility (AD) and true digestibility (TD) according to whether endogenous corrections are applied. Total excreta collection is a frequently used procedure because it is relatively simple to perform and in the modern assay the period of excreta collection is short. The assays provide digestibilities for the set of amino acids of a feedstuff in a single assay.

The methodology was 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.

The total collection method involves feeding birds, with a known quantity of the test food and recovering the excreta produced over a collection period. The difference in the quality of AA or N in the test food and the excreta as a proportion of the total consumed provides a measure of apparent amino acid digestibility (AAAD).
The formula may be derived as follows:

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

Expanding and converting to a proportion:

$$\frac{\text{Balance AA (mg)}}{\text{total AA intake (mg)}} = \frac{\text{(mg AA/g food intake x g intake) - (mg AA/g excreta x g excreta)}}{\text{(mg AA/g food intake x g food intake)}}$$

Simplifying and converting to a percentage:

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

A variant that results in true amino acid digestibility (TAAD) uses a correction to remove the bias caused by non-food residues in the excreta. These fractions have been referred to by Austic (1983) and are the endogenous and metabolic losses that comprise AAs of unabsorbed digestive juices, mucus, bile, desquamated epithelial debris, microbial debris and AAs of urinary origin (Sibbald, 1987).

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

Assays are mostly conducted on egg type chickens, meat chickens or adult egg type cockerels.

### 3.2.1.1. FREE-ACCESS METHODS

In this approach animals are accustomed to the test diet over a period of time, ranging from 2 days (Rostagno, 1973) to 7 days (Muztar and Slinger, 1980a). The purpose is to clear residues of the previous diet from the digestive tract and to establish a uniform rate of passage of digesta. The single ingredient assay is not recommended because many feedstuffs are unpalatable and unbalanced when fed alone (Sibbald, 1987) and many have adverse effects on body functions if fed over a number of days. Consequently, the assay commonly involves determining the digestibility of a basal diet and a test diet that contains the test ingredient and basal in known proportions. These assays

include the classical approach (Hill, *et al.*, 1966) and the "rapid assay" (see later) is a variant.

The free-access approach is exposed to the errors and distortions that result from selective and variable food intake (Sibbald, 1987) and which in the case of the latter are due to the relative size of the endogenous fraction (Sibbald, 1975).

### 3.2.1.2. INTUBATION

This method was developed by Sibbald (1976) for the determination of true metabolizable energy (TME) of feedstuffs for poultry. The procedure has been claimed to be rapid, simple, accurate and inexpensive and was applied by Likuski and Dorrell (1978) to determine the AA digestibility of feedstuffs. It involves fasting the experimental birds for 24 to 48 hours to clear the digestive tract of residues followed by the delivery of a known quantity of feed into the crop and subsequent quantitative collection of excreta over a 24 to 48 hour period. The collected excreta 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 obtain true N or AA digestibility.

Sibbald (1987) claims that the method delivers a known amount of feed and eliminates selective feeding. It avoids feed spillage, and overcomes feed intake variation. The optimum meal size depends upon the size of the bird and the nature of the feed. Sibbald (1987) recognizes that a large meal size may reduce the effect of experimental error although Sibbald (1979) found that TAADs of plant

feedstuffs were not affected by meal size in assays on adult cockerels. Sibbald (1977) reports that feed inputs of greater than 40 g may result in an increase in the incidence of regurgitation in adult cockerels.

### 3.2.1.3. RAPID ASSAYS

Farrell (1978) described a rapid assay for the determination of apparent metabolizable energy (AME). It involved the used of adult cockerels trained to consumed 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 1 hour period. Excreta is then collected for a recommended 24-36 hours. 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 formula that applies is as follows:

$$\% \text{ AAAD} = \frac{\text{D AA/g T} - \text{D AA /g B} \times \text{P}}{(1-\text{P})\ \text{AA/g I}} \times 100$$

where
D = Digestibility
T = test diet
I = test ingredient
B = basal diet
P = the proportion of the basal in the test diet
g = grams
AAAD = Apparent Amino Acid Digestibility

and where in general

$$\text{D AA/g F} = \text{AA/g F} - \frac{\text{AA/g } E_f \times \text{g } E_f}{\text{g F}}$$

where
F = food
$E_f$ = excreta arising from the food (After Yap, 1991).

### 3.2.2. ILEAL COLLECTION METHODS

These methods are advocated on the basis that the effects of fermentation by bacteria of the hind gut on undigested residues and hence on digestibility are avoided (Achinewhu and Hewitt, 1979 ; Raharjo and Farrell, 1984). In addition the procedure removes the urinary influence on digestibility (Austic, 1983 ; Sibbald, 1987).

The procedure (adapted from Payne, *et al.*, 1968) involves a N free basal diet, for example cornstarch, sugar, corn oil, cellulose, salt and a vitamin and mineral supplement, to which is added in known proportion the test ingredient and an indicator, commonly chromic oxide, to form the test diet. The test diet is fed to young chick which subsequently are slaughtered and samples of digesta removed from their terminal ileums.
Amino acid digestibility may be calculated using the expression

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

The method requires that the concentrations of chromic oxide and amino acids in the feed and the digesta be known.

Apparent digestibilities may be corrected to "true" estimates by feeding separately the nitrogen free basal containing an indicator. The formula for obtaining "true" digestibilities is given below (After Yap, 1991) and is based on an assumption that the test food and basal will influence the weight and composition of endogenous excreta equally for each gram of either fed.

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

The sensitivity of ileal assays has been investigated by a number of workers. Raharjo and Farrell (1984) compared the digestibility of animal and plant proteins using the whole ileum, the final 10 cm 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 obtained using terminal ileum samples as compared to excreta samples 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 procedures, but both procedures were equally effective in demonstrating the influence of heat damage on digestibility in chicks. On the other hand, Varnish and Carpenter (1971 ; 1975) reported that the digestibility of amino acids in the 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.

### 3.2.3. SURGICAL METHODS

These have been adopted by researchers for the most part in an endeavour to isolate and quantify the influence on digestibility values of microorganisms inhabiting the hind gut and caeca and urine voided jointly with faeces at the cloaca. They include cannulation, caecectomy and exterioration of the cloaca (colostomy).

### 3.2.3.1. ILEAL CANNULATION

Ileal cannulation was used by Raharjo and Farrell (1981), as an alternative to the ileal slaughter method. They used a glass T-piece cannula which was inserted in the terminal ileum of adult cockerels (2-3 kg) about 2.5 cm anterior to the ileocaecal junction. When digesta was required the screw cap and plug were replaced by a small plastic vial which screwed onto the cannula stem. The collection period of about 30 minutes atarted about 5 hours following the feeding of a single meal (100 g) fed over the period of one hour.

The procedure serves the dual purpose of providing a means of sampling ileal contents and preserving birds for reuse. Johns, *et al.* (1986b) used the technique and reported 60% of the cannulated cockerels were still healthy 9 months after surgery. Problems to do with the method have been cited by Sibbald (1987). These include 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 and the influence of feeding frequency on marker recovery.

### 3.2.3.2. COLOSTOMISED BIRDS

Colostomy involves exteriorisation of the colon. It enables a separation of urine from faeces in birds. The technique was explored by Rothchild (1947) and refined by Tao, *et al.* (1969). The latter authors found the technique to be satisfactory and were able to collect urine and faeces over a 2-4 month period. O'Dell, *et al.* (1960) found that the amino acid content contributed 3% of the nitrogen in chicken urine. They concluded that this was unlikely to have 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/day which equates with 38 mg of amino acids per day.

### 3.2.3.3. CAECECTOMISED BIRDS

Most microbial fermentation in the large intestine of chickens is thought to occur in the caeca. A review has been made by MacNab (1973) on the functions of the avian caeca. These include: (1) water absorption (Thornburn and Wilcox, 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.

Caecectomy is usually applied to adult cockerels. The ceaca are removed at the ileo-caecal-colon junction and the birds given six or more weeks to recover before trial procedures are commenced. The results of a number of studies suggest that caeca are involved with a net removal of amino acids from the digesta leading to an increase in digestibility (Payne, *et al.*, 1971 ; Nesheim and Carperter, 1967 ; Johns, *et al.* (1986a) and Parsons, 1984 ; 1986). In their studies caecectomy lowered digestibility indicating the caeca of intact birds had a functional role in AA extraction.

## 3.3. THE CONTRIBUTION OF ENDOGENOUS SOURCES TO AMINO ACIDS AND NITROGEN EXCRETION.

There is considerable interest in the effect of dietary components on the composition of endogenous excretion and on its magnitute. Various methods are employed to quantify endogenous AA and N output and the values derived are used to correct digestibility to "true" estimates, but the "true" estimates are only as valid or reliable as the endogenous estimates are representative. Many studies on dietary influences on endogenous excretion have been conducted. The results are variable.

Dietary fibre has been shown to influence endogenous nitrogen excretion from rats (Meyer, 1956) and pigs (Whiting and Bezeau, 1957). The effect of dietary fibre on endogenous amino acid excretion from birds fed once, by crop intubation, is unclear. Muztar and Slinger (1980b) reported that excretion of endogenous amino acids was greater when cockerels were precision fed 20 g, rather than 10 g, of purified wood cellulose. However, Sibbald (1980, 1981) found no response to endogenous nitrogen or amino acid excretion when adult birds were precision fed graded concentrations of cellulose in diets. Green (1988) found that increments in the dietary concentration of the sources of fibre (in this case cellulose up to 120 g/kg diet) had no influence on the quantities of endogenous amino acids or nitrogen excreted. Parsons *et al.* (1983) reported that the excretion of endogenous amino acids of fasted adult cockerels and those fed a protein-free low-fibre or high-fibre diet were 148.5, 299.5 and 392 mg/bird/day respectively.

Green and Kiener (1989) found that there was a similarity between caecectomised and intact birds in

endogenous output of nitrogen and all the amino acids except threonine. These results suggest that endogenous amino acid excretion is only very minimally influenced by bacterial activity or retention in the ceca. In other work, germ-free chicks excreted more endogenous amino acids than did conventional chicks. This suggested there was a substantial microbial influence (Salter, *et al.*, 1974 ; Parsons, *et al.* 1982b).

### 3.3.1. MEASUREMENTS OF ENDOGENOUS EXCRETION

Various estimates of the endogenous N and amino acid excretion in chickens have been reported. Green and Kiener (1989) found that the quantity of endogenous nitrogen secreted in caecectomised and intact birds fed a protein-free diet was 82.5 mg and 81.5 mg per bird per day respectively. Muztar and Slinger (1980b) using fasted birds obtained greater levels of 525.6 mg N per day. For total endogenous amino acids the results of Green and Kiener (1989) were 492.5 mg and 458.5 mg per bird per day for caecectomised and intact birds respectively, as opposed to 373.8 mg and 331.1 mg reported by Green, *et. al.* (1987a,b). Perez, *et al.* (1993) determined endogenous amino acids at the terminal ileum and recorded 312.2 mg per bird per day. The endogenous amino acid secretion in chickens as reported by various workers is summarised in Table 3.1.

Various methods have been used to measure endogenous N and amino acids.

Table 3.1. Endogenous excretion (mg/day) of amino acids in chickens as determined by different methods

| Amino acid | Lower Ileum | | Intact | | Caecectomised | | | Fasted |
|---|---|---|---|---|---|---|---|---|
| Bird | (1) | (2) | (3) | (4) | (2) | (3) | (4) | (5) |
| Essential Amino Acid (EAA) | | | | | | | | |
| Histidine | 23.4 | 8.8 | 9.5 | 29 | 11.9 | 12.0 | 37 | 24.8 |
| Arginine | 20.1 | 18.7 | 25.0 | 31 | 20.1 | 24.5 | 29 | 22.7 |
| Threonine | 13.1 | 19.8 | 28.5 | 28 | 31.5 | 39.0 | 33 | 15.9 |
| Valine | 19.3 | 18.5 | 28.5 | 26 | 21.7 | 32.5 | 26 | 14.8 |
| Methionine | 7.8 | 5.1 | 6.0 | 8 | 4.7 | 5.0 | 8 | 5.9 |
| Isoleucine | 17.2 | 14.1 | 19.0 | 19 | 15.0 | 20.0 | 20 | 11.0 |
| Leucine | 22.1 | 19.5 | 29.5 | 33 | 21.1 | 30.0 | 33 | 17.0 |
| Phenylalanine | 11.8 | 12.1 | 18.5 | 27 | 12.7 | 19.5 | 22 | 10.0 |
| Lysine | 15.4 | 22.4 | 27.5 | 33 | 20.8 | 26.5 | 26 | 19.6 |
| Non-Essential Amino Acid (NEAA) | | | | | | | | |
| Aspartic Acid | 22.9 | 29.5 | 40.5 | 42 | 34.1 | 43.0 | 44 | 25.1 |
| Glutamic Acid | 36.6 | 45.6 | 62.5 | 63 | 48.8 | 65.0 | 67 | 43.7 |
| Serine | 24.4 | 22.3 | 32.0 | 35 | 28.7 | 37.5 | 35 | 16.5 |
| Glycine | 18.1 | 30.4 | 36.0 | - | 30.3 | 36.5 | - | 162.8 |
| Alanine | 18.6 | 19.2 | 25.5 | - | 19.7 | 26.0 | - | 14.0 |
| Proline | 14.6 | 21.1 | 33.0 | - | 26.6 | 36.5 | - | 21.4 |
| Tyrosine | 18.2 | 12.4 | 19.0 | 29 | 13.0 | 19.5 | 19 | 7.4 |
| Cystine | 8.7 | 11.6 | 17.5 | - | 13.1 | 19.0 | - | 5.4 |

1) Perez, *et al.* (1993)
2) Green, *et al.* (1987)
3) Green and Kiener (1989)
4) Johns, *et al.* (1986)
5) Muztar and Slinger (1980b)

### 3.3.1. PROTEIN-FREE METHOD

This method involves using a nitrogen-free diet to obtain an estimate of endogenous Nitrogen or 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 hours collection period in adult cockerels. 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 acids than unfed birds.

The protein-free method has been criticised on the basis of being unphysiologycal. Despite this the method is used widely, for example Janssen (1979), Bielorai, *et al.* (1987), Green and Kiener (1989), Zuprizal, *et al.* (1992) and Perez, *et al.* (1993). There is evidence that the amount of protein secreted into the intestine is reduced by the feeding of protein-free diets (Snook and Meyer, 1964a ; Fauconneau and Michel, 1970). The reasons that have been given are:

1. The absence of dietary protein causes a reduction in the rate of cell replication and cell protein turnover in the intestine (Munro and Golberg, 1964 ; Millward, *et al.*, 1976 ; Muramatsu, 1990 ; Moughan and Rutherfurd, 1990).
2. The absence of dietary protein may increase the breakdown and re-absorption of secreted enzymes (Munro and Golberg, 1964 ; Fauconneau and Michel, 1970 ; Moughan and Rutherfurd, 1990).
3. The absence of dietary protein causes a lower secretion of digestive enzymes and mucoprotein (Moughan and Rutherfurd, 1990).

On the other hand, protein-free diets consist largely of starch fibre, and endogenous secretion is mainly

affected by the type and level of dietary fibre used (Meyer, 1956 ; Parsons, 1983 and Raharjo and Farrell, 1984). These effects are likely to be related to the observation that an increase in the intake of fibre causes an increase in digestive secretions (Schneeman, *et al.* 1982) and an increase in intestinal cell turnover rate (Vahouny and Cassidy, 1986). Contrasting results have been reported by Muztar and Slinger (1980b), Sibbald (1980) and Green (1988).

### 3.3.1.2. FASTING-BIRD METHOD

This involves 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 birds as its own control.

### 3.3.1.3. REGRESSION METHOD

This method involves 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.

### 3.3.1.4. HOMOARGININE METHOD

This method provides an estimate of a single endogenous amino acid, lysine. It involves transforming the lysine in a protein source such as gelatin to homoarginine

by a process known as guanidination. Homoarginine is not normally present in the intestine. In chickens, the method has been used by Siriwan, *et al*. (1987 ; 1989). The method depends on the assumptions that guanidinated protein is digested and absorbed at a similar rate and in the same manner as other dietary proteins, that homoarginine does not cause altered protein metabolism in the animal, and that there is no significant arginase activity in the digestive tract that would convert homoarginine back to lysine. The procedure also relies on high conversion rates of lysine into homoarginine. The highest conversion achieved by Rutherfurd and Moughan (1990) was 95%. In rats fed guanidinated gelatin endogenous excretion was greater than in rats fed a protein-free diet (Moughan and Rutherfurd, 1990). The effect was attributed to dietary protein. The method has particular application in species and circumstances in which lysine is a first limiting amino acid.

# Chapter 4
# EXPERIMENTAL

In recent years increasing interest has been expressed in the effect of non starch polysaccharides on digestibility and metabolizable energy of foods fed to poultry. Conventional *in vivo* methods assess quality on the basis of nitrogen and amino acid digestibility and metabolizable energy. These procedures vary in the directness of their measures. Some involve intubation of test ingredients, others, incur a variable feed intake, and involve free access to diets and contain the test ingredient at some proportion in the food fed. Corrections may be applied to account for endogenous secretions and in ileal assays indicators are used to quantify feed residues. The purpose of experiment 1 was to investigate the swelling properties of faeces and the storage modulus (G') or elastic properties of ileal digesta to determined whether they provide indices suitably variable and sensitive to characterise feeds on the basis of digestive response. Such measures are relatively direct and may be more suitable for certain feedstuffs, in particular, the cereals and legumes, whose fibre contents may show considerable water holding capacity and viscous properties.

Experiment 2 and 4 were undertaken to quantify endogenous N and (in the experiment 4) endogenous lysine response to increasing intake of cellulose (Avicel) and protein (guanidinated gelatin) respectively. The guanidination of gelatin involves a transformation of protein lysine to homoarginine. Lysine appearing in terminal gut secretions is reasonably assumed to be endogenous. Digestibility and metabolizable energy assays undertaken to assess food quality are designed to

trivialise the impact of endogenous secretions or the outcomes are corrected to minimise endogenous bias. There is evidence (Parsons, 1981 ; Nasset, 1968 ; 1972; Boorman, 1976) that terminal gut secretions vary little in composition and are independent of dietary provision, but that dietary components (fibre, protein and starch) may influence the volume of secretions (Sturkie, 1986 ; Larsen, 1991 ; Butts, 1993). Experiments 2 and 4 were undertaken to define endogenous response to dietary cellulose and dietary protein.

Experiment 2 involved the precision feeding of meat chickens with artificial diets involving cellulose and cornstarch. Difficulty was experienced in the intubation procedure with delivering some of the quantities of food involved. Experiment 3 was undertaken to explore procedures involved and the response to feeding by intubating wet diets of the form that would be employed in experiment 4. In addition, since the response estimates rely in large measure on the reliability of the chromium concentrations of the ileal digesta the trial would produce additional estimates of chromium concentrations and assist in gauging the reliability of outcomes of experiment 4.

**EXPERIMENT 1.**

# FAECAL SWELLING AND ILEAL DIGESTA DEFORMATION AS INDEXES OF WATER HOLDING CAPACITY AND VISCOSITY OF FEEDS

## OBJECTIVES

a. To investigate the faecal residues of feeds for their capacity to retain water.
b. To investigate the ileal digesta of feeds for their resistance to deformation.

In recent years there has been an upsurge in interest in the physio-chemical properties of non starch polysaccharides (NSP) particularly in respect to their solubility and viscosity characteristics and the impact of these on feed digestibility and utilisation (Annison, 1991 ; 1993).

The hydrophillic properties of non starch polysaccharides are due to the presence of hydroxyl groups and are considerably enhanced by sugar residues with free polar groups. Soluble non starch polysaccharides such as the glucans, arabinoxylans, pectin, mucilages and to a varying extent the hemicelluloses form intramolecular associations and networks which bind water molecules and impart a degree of viscosity that is a function of several factors including molecular size and degree of branching, the presence of charged groups and the concentration of NSP's in the medium (Larsen, 1991).

Insoluble fibre sources such as cellulose and the xylans are in contrast non viscous and entrap water molecules mechanically, much like a sponge, but according

to Van Soest (1984) and Robertson and Eastwood (1981) to a lesser degree than the soluble fibres. Viscosity has been linked with anti-nutritive effects (Larsen, 1991), whereas according to Smits and Annison (1994) there is no evidence that the bulking properties of non viscous NSP's impair digestibility, indeed it may well decrease anti-nutritive bacterial effects in the gut by diminishing the time available for bacterial fermentation.

This trial was carried out firstly to investigate procedures involved in obtaining an index of water holding capacity of the faecal material of feedstuffs and sensitivity of the measure between and within feed types. The index employed was swelling capacity and was defined as the volume of faecal material voided in cc per g of faecal dry matter.

The second objective was again exploratory. It was reasoned that if the viscosity of digesta was of sufficient persistance and magnitute to interfere with digestive physiology in the distal part of the ileum it might impart degrees of elasticity to digesta collected in colloidal form that would be measureable by deformation rheology.

## MATERIALS AND METHODS

Sixty 23 day old Ross female meat chickens were received on 12 of March 1993 and raised in a single pen in the Environmental Physiology shed until 42 days of age when 48 were randomly selected, weighed and placed in individual bird cages. Over the next seven days, the period over which they had free access to food, was steadily reduced from 24 hours on day 42 to one hour on day 48. Thereafter until 54 days of age they had access to feed for one hour a day. The

birds were then denied food for at least 36 hours and intubation commenced at 9 am on day 56. Excreta was collected in one set of transparent calibrated plastic cups at 12 hours after intubation and in a second set at 21 hours. Immediately following completion of the second collection birds were intubated again with the same test diets they had previously received. Four hours later they were sacrificed by a 1 ml intracardial injection of sodium pentobarbitone (Pentobarb 300). The final 15 cm of ileum was clamped and removed and the contents squeezed into small plastic bags and sealed.

Excreta samples were stored in a refrigerator in sealed cups until the following week when volume measurements were made. Ileal digesta samples were stored overnight in a refrigerator and the next day measured for their elastic properties. Throughout, the birds received 16 hours of continous light per day and had free access to water at all times. Except for the treatment diets, they received a broiler ration in pelleted form described in appendix 6.

There were 12 dietary treatments randomly assigned over 48 birds to give 4 birds per treatment. Treatments were intubated 25 g quantities of the following diets.

| Code | Treatment diet |
|---|---|
| A1 | Maize |
| A2 | Barley |
| A3 | Meat and Bone Meal |
| A4 | Peas |
| A5 | Maize + Sorghum (1 : 1) |
| A6 | Barley + Sorghum (1 : 1) |
| A7 | Meat and Bone Meal + Sorghum (1 : 1) |
| A8 | Peas + Sorghum (1 : 1) |
| A9 | Cellulose + Granocol (5.0 : 0.0) |
| A10 | Cellulose + Granocol (4.5 : 0.5) |
| A11 | Cellulose + Granocol (4.0 : 1.0) |
| A12 | Cellulose + Granocol (3.5 : 1.5) |

The cellulose used in this and subsequent trials was purchased as Avicel RC 591 (from Commercial Minerals, Auckland). Granocol, a medicinal bulking agent, was obtained from Schering (NZ) Ltd Auckland and had a documented swelling capacity of not less than 20 ml/g. The reported composition of 100 g granocol (granules) was 47.3 g Bassorit, 8.276 g Cortex frangulae, 33.068 Wheat starch, 5.911 Talc, 0.709 g Sodium bicarbonate, 0.749 g Shellac and 1.622 g Caramel colour. For the determination of swelling index (faeces (cc) per g dry matter) faecal material was categorised as either paste, solid, two-phase or suspension. The two-phase category had both solid and aquaeous components. Excreta classified as suspensions were predominantly liquid with suspended solid material that settled out on standing. Liquid phases were decanted or siphoned off and to all cups a measured volume of water was added to convenient volume marks which previously had been tested for volume accuracy. The excreta was then freeze dried. Swelling index (SI) was calculated as follows:

$$\text{Swelling index (SI)} = \frac{\text{Final volume (cc) - Added water (cc)}}{\text{Weight (DM) of excreta residues}}$$

The elastic property in ileal samples was measured by storage modulus g' in pascalls using a Bohlin VOR Rheometer (Bohlin Rheology, Muhlacker, Germany). Approximately 1 g of digesta was used between two vertically opposed serrated Bohlin oscillation plates set to a gap of 0.5 mm and operated with a torsion element of 4.62 cm at room temperature. Storage modulus is defined as that part of the shear stress that is in phase with the shear strain divided by the strain under sinusoidal conditions (Howard, 1989). It is a ratio measuring the tangential force per unit of area giving rise to unit deformation.

Test diets were analysed for Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) using the procedure of James and Theander (1981) (Appendix 1). Nitrogen analyses were undertaken according to the methods of AOAC (1975) on a Kjeldahl 1030 auto analyser (Tecator, Sweden) (Appendix 2).

Correlation and analysis of variance procedures were conducted using the General Linear Model (GLM) procedure of Statistical Analytical System (SAS, 1985). Duncan's Multiple Range Test was used in the determination of significant differences.

## RESULTS

Appendix 7 provides details of the measurements obtained in the determination of swelling index and storage modulus. Appendix 11 details components of analyses of variance conducted for swelling index for both 12 and 9 hour collections, for storage modulus and for dietary NDF and ADF.

Two way analysis of variance comparing differences between swelling index of the 12 and 9 hour collection periods and differences due to diet indicated that the mean swelling index for the 12 hour collection of 6.167 cc/g was not significantly different to that for the 9 hour collection of 7.151 cc/g (Table 4.1.1. and Appendix 8). The swelling indices associated with meat and bone meal, meat and bone meal + sorghum, cellulose and cellulose + granocol (4.5:0.5) were significantly smaller than those of peas + sorghum ($P<0.05$).

Table 4.1.1. The effect of diet and period of excreta collection on swelling index (cc/g).

| Feedstuff | Period of excreta collection | | Mean |
|---|---|---|---|
| | 1st 12 hour | 2nd 9 hour | |
| A1 | 6.970 | 6.661 | 6.730[ab] |
| A2 | 8.186 | 7.478 | 7.832[ab] |
| A3 | 4.135 | 4.102 | 4.118[b] |
| A4 | 7.223 | 6.366 | 6.856[ab] |
| A5 | 5.295 | 6.965 | 6.130[ab] |
| A6 | 7.470 | 9.626 | 8.548[ab] |
| A7 | 4.112 | 5.958 | 5.035[b] |
| A8 | 7.889 | 12.420 | 10.155[a] |
| A9 | 4.771 | 5.362 | 5.066[b] |
| A10 | 5.392 | 5.260 | 5.326[b] |
| A11 | 6.389 | 7.556 | 6.937[ab] |
| A12 | 6.346 | 7.859 | 7.130[ab] |
| Mean | 6.167[a] | 7.151[a] | |

mean with different subscripts are significantly different (P<0.01)

The swelling indices of diets associated with the 12 hour collections (SI.1) and those associated with the 9 hour collections (SI.2) were separately analysed for differences (Table 4.1.2.). Swelling indices were not significantly different for the 9 hour collection. For the 12 hour collection barley (8.16 cc/g), barley + sorghum (7.47 cc/g) and peas + sorghum (7.78 cc/g) had significantly greater ($P<0.05$) swelling indices than meat and bone (4.14 cc/g) and meat and bone meal + sorghum (4.11 cc/g) and the swelling index of barley was greater than that of cellulose ($P<0.05$).

Table 4.1.2. The effect of diet on faecal swelling index within period of collection, 12 hours (SI.1) and 9 hours (SI.2) in cc/g.

| Feedstuff | SI.1* | SI.2$^{ns}$ |
|---|---|---|
| A1 | 6.789$^{abc}$ | 6.661 |
| A2 | 8.186$^{a}$ | 7.478 |
| A3 | 4.135$^{c}$ | 4.102 |
| A4 | 7.223$^{abc}$ | 6.366 |
| A5 | 5.295$^{abc}$ | 6.965 |
| A6 | 7.470$^{ab}$ | 9.626 |
| A7 | 4.112$^{c}$ | 5.958 |
| A8 | 7.889$^{ab}$ | 12.420 |
| A9 | 4.771$^{bc}$ | 5.361 |
| A10 | 5.392$^{abc}$ | 5.260 |
| A11 | 6.389$^{abc}$ | 7.556 |
| A12 | 6.346$^{abc}$ | 7.859 |

*) significant (P<0.05)
ns) non significant

Table 4.1.3. details the effect of diet on the storage modulus of ileal digesta. The storage modulus of the ileal digesta of maize (55.4 K Pa), meat and bone meal + sorghum (76.2 K Pa) and maize + sorghum (66.8 K Pa ) were significantly greater (P<0.01) than that of barley (10.9 K Pa) and that of the digesta of each of the cellulose containing diets whose values ranged from 17.8 (cellulose:granocol, 4.5:0.5) to 2.8 K Pa (cellulose: granocol, 3.5:1.5). The storage modulus for the digesta of meat and bone meal plus sorghum was also significantly greater (P<0.01) than that of peas plus sorghum (4.02 K Pa).

Table 4.1.3. The effect of diet on the storage modulus (G') of ileal digesta (K Pa).

| Feedstuff | Storage modulus** |
|---|---|
| A1 | 55.375$^{abc}$ |
| A2 | 10.890$^{de}$ |
| A3 | 37.418$^{cd}$ |
| A4 | 24.450$^{de}$ |
| A5 | 66.825$^{ab}$ |
| A6 | 13.695$^{de}$ |
| A7 | 76.150$^{a}$ |
| A8 | 40.175$^{bcd}$ |
| A9 | 11.380$^{de}$ |
| A10 | 17.835$^{de}$ |
| A11 | 3.490$^{de}$ |
| A12 | 2.758$^{de}$ |

**) significant (P<0.01)

Neutral detergent fibre and ADF measurements of the treatment diets are given in Table 4.1.4. Diets of meat and bone meal (35.6%), barley (23.6%) and meat and bone meal plus sorghum (27.2%) had significantly greater (P<0.01) NDF levels than maize (18.4%), peas (19.3%), maize plus sorghum (16.6%), barley plus sorghum (19.5%), peas plus sorghum (27.2%) and the cellulose containing diets whose values ranged between 16.3 and 18%. Similar dietary relationships existed with respect to ADF measurements. Diets of meat and bone meal (31.2%), barley (19.3%) and meat and bone meal plus sorghum (23.1%) had significantly greater (P<0.01) ADF levels than maize (14.4%), peas plus sorghum (11.1%) and the cellulose containing diets whose values ranged between 9.8 and 12.5%. For both NDF and ADF, the meat and bone meal measurements were greater (P<0.01) than all diets.

Table 4.1.4. Dietary treatment NDF and ADF measurements in percent (DM).

| Feedstuff | NDF | Mean** | ADF | Mean** |
|---|---|---|---|---|
| A1 | 17.281 | $18.379^{d}$ | 13.613 | $14.380^{cd}$ |
| A1 | 19.478 | | 15.148 | |
| A2 | 24.510 | $23.581^{bc}$ | 20.072 | $19.289^{bc}$ |
| A2 | 22.653 | | 18.506 | |
| A3 | 38.730 | $35.623^{a}$ | 34.371 | $31.233^{a}$ |
| A3 | 32.517 | | 28.096 | |
| A4 | 18.094 | $19.286^{cd}$ | 9.236 | $10.382^{d}$ |
| A4 | 20.476 | | 11.529 | |
| A5 | 16.570 | $16.599^{d}$ | 13.340 | $13.340^{d}$ |
| A5 | 16.627 | | - | |
| A6 | 18.967 | $19.477^{cd}$ | 15.356 | $15.544^{cd}$ |
| A6 | 19.986 | | 15.732 | |
| A7 | 26.147 | $27.246^{b}$ | 22.299 | $23.128^{b}$ |
| A7 | 28.150 | | 23.957 | |
| A8 | 17.763 | $17.246^{d}$ | 11.600 | $11.090^{d}$ |
| A8 | 16.729 | | 10.581 | |
| A9 | 16.171 | $16.371^{d}$ | 12.192 | $12.452^{d}$ |
| A9 | 16.572 | | 12.712 | |
| A10 | 16.158 | $16.540^{d}$ | 11.308 | $11.589^{d}$ |
| A10 | 16.922 | | 11.870 | |
| A11 | 17.657 | $17.464^{d}$ | 10.429 | $10.730^{d}$ |
| A11 | 17.271 | | 11.032 | |
| A12 | 18.395 | $18.007^{d}$ | 9.553 | $9.875^{d}$ |
| A12 | 17.619 | | 10.198 | |

Mean with different subsripts are significant ($P<0.01$)

Correlations were obtained between SI.1 and NDF, SI.1 and ADF, SI.2 and NDF, SI.2 and ADF, Storage Modulus and NDF, Storage Modulus and ADF, and Swelling Index.1 and Storage Modulus. The set of coefficients of correlation are given below. All correlation coefficients were insignificant. Figures 1 to 7 respectively describe the relationships.

| | Coeficients of correlation | | | |
|---|---|---|---|---|
| | Swelling Index 1 n=12 | Index 2 n=12 | Storage Modulus (G') n=12 | Swelling Index 1 n=12 |
| NDF | 0.1861 | 0.1774 | 0.2341 | - |
| ADF | 0.1251 | 0.1598 | 0.3971 | - |
| Storage Modulus (G') | - | - | - | 0.2183 |

Figure 4.1.1. Correlation between swelling index of the 1st 12 hours excreta collection (SI.1) and the percentage of dietary NDF.

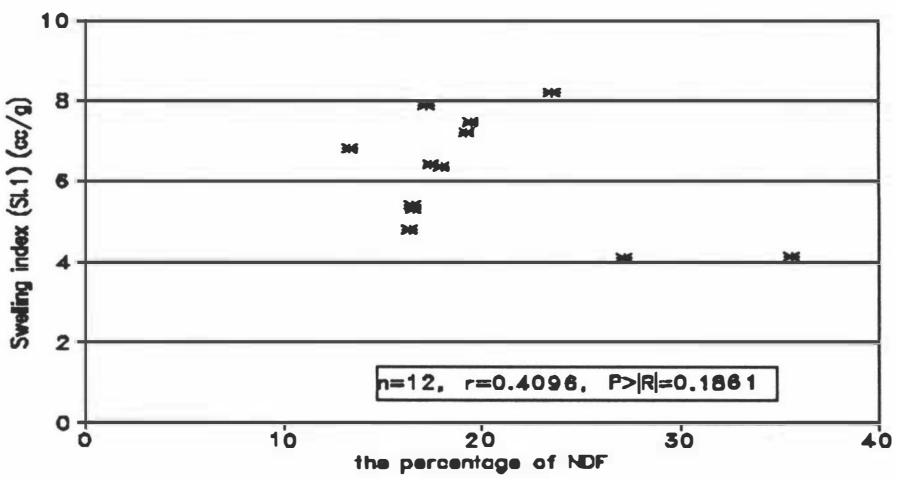


Figure 4.1.2. Correlation between swelling index of the 1st 12 hours excreta collection (SI.1) and the percentage of dietary ADF.

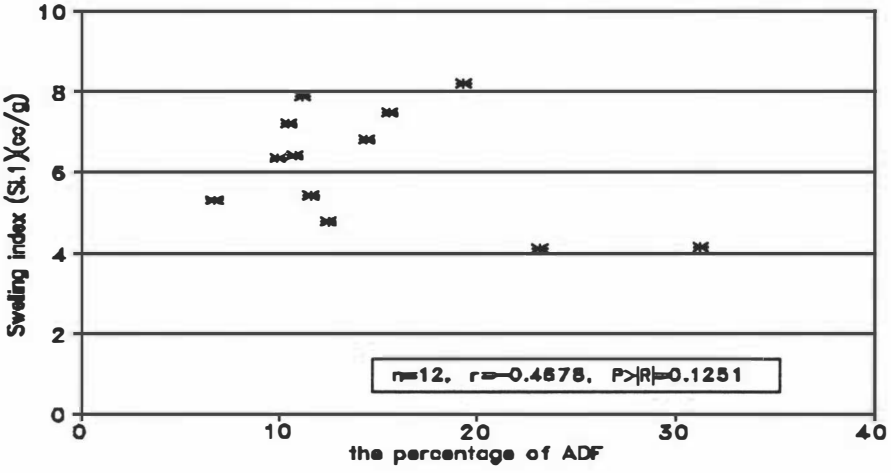



Figure 4.1.3. Correlation between swelling index of the 2nd (9 hours) excreta collection (SI.2) and the percentage of dietary NDF.

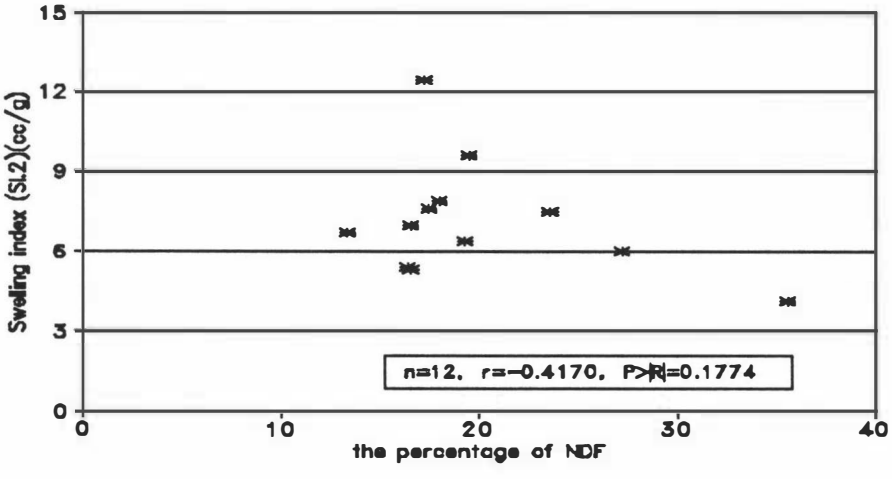


Figure 4.1.4. Correlation between swelling index of the 2nd (9 hours) excreta collection (SI.2) and the percentage of dietary ADF.

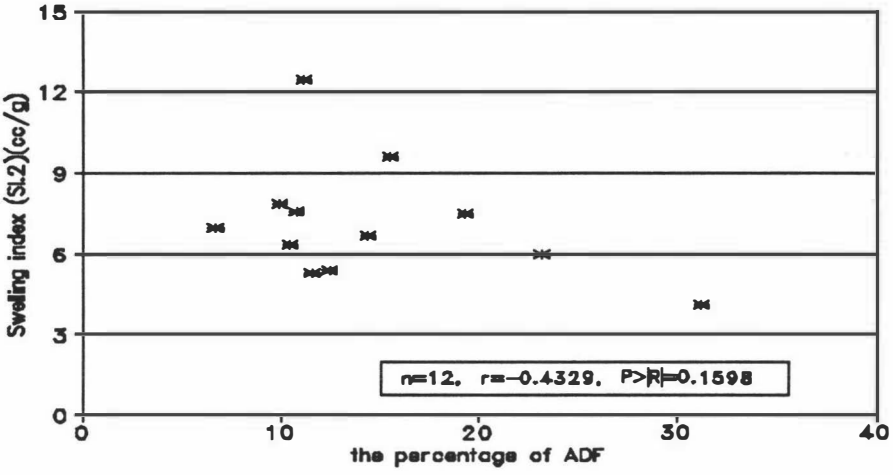



Figure 4.1.5. Correlation between storage modulus (G') and the percentage of dietary NDF.

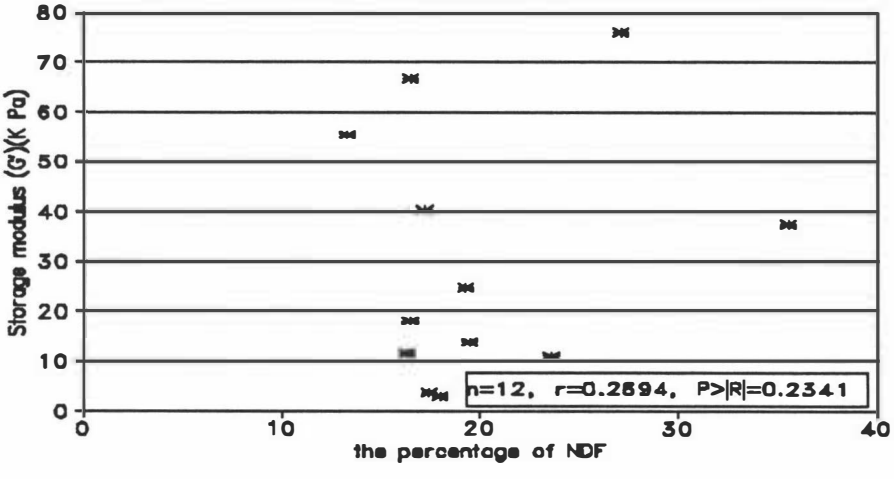


Figure 4.1.6. Correlation between storage modulus (G') and the percentage of dietary ADF.

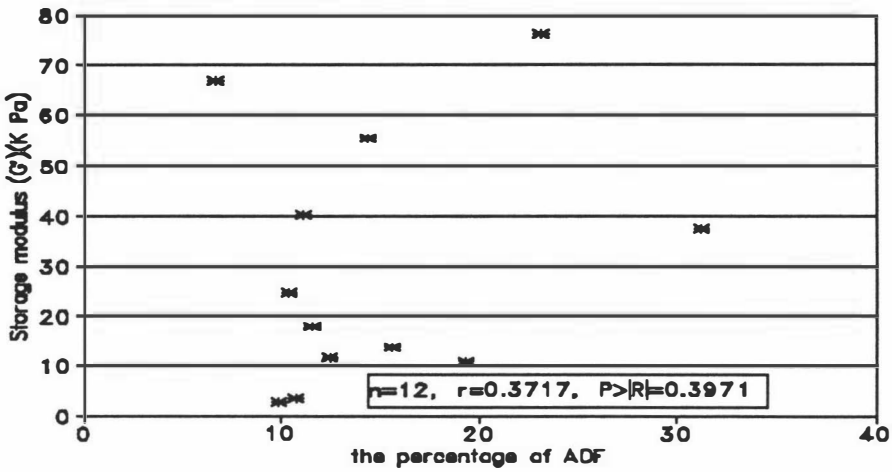
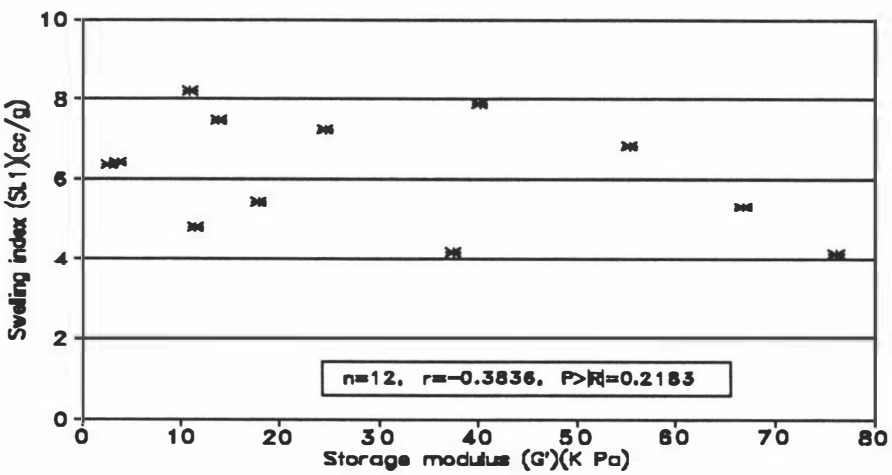



Figure 4.1.7. Correlation between swelling index (SI.1) of the 1st 12 hours excreta collection and storage modulus (G').



## DISCUSSION

The more effective measures of swelling index were obtained from collections of excreta voided within 12 hours of intubation. There were significant ($P<0.05$) swelling index differences between diets of the 12 hour collections, but no differences were detected for diets of the second or 9 hour collection. The time of collection effect was attributed to excreta of 12 hour collections contributing greater proportions to final volumes with correspondingly less variation and improved accuracy.

Distinguishing test diets on the basis of faecal swelling was successful in but few of the diets. The faecal swelling index of barley was different to that of meat and bone meal, and that of meat and bone meal + sorghum was different to those of barley + sorghum and peas + sorghum. However, the swelling index of the digesta of barley was not influenced by the addition of sorghum and nor was that of meat and bone meal. Similarly the swelling indices of the faeces associated with the feeding of maize and peas were not affected by the addition of sorghum to diets. In summary the dietary addition of sorghum made no different to swelling index when included at 50 percent of the diet even though the swelling index of the digesta of the diets to which it was added differed significantly. The addition of granocol seemed to add marginally to bulk but treatment differences over the range of indices obtained 4.77 cc/g to 6.35 cc/g were not significant.

The narrow range over which swelling index varied for the set of ingredients tested suggests there may be insufficient variation in swelling index for it to have useful application. No significant relationship could be

established between swelling index and NDF or ADF of the test diets.

Examination of ileal digesta for storage modulus suggested that meat and bone meal + sorghum and maize + sorghum gave rise to different digesta characteristics than barley + sorghum, peas, meat and bone meal and barley. There was no serial change in storage modulus in the cellulose containing treatments (A9 - A12) in response to increasing levels of granocol, although the addition of sorghum to test diets A1 to A4 raised the storage modulus of diets A5 to A8 but not by uniform amounts and not significantly except in the case of meat and bone meal plus sorghum. With the addition of sorghum the storage modulus of A1 increased from 55 to 67 K Pa, A2 increased from 11 to 14 K Pa whereas A3 and A4 increase from 37 to 76 and from 25 to 40 K Pa respectively. The range of values recorded, 2.76 for A12 to 76.15 for A7 suggests a wide degree of variation may exist between ingredients but as an index its value is offset by the considerable variation within ingredients (refer Appendix 7). No significant correlations were established between storage modulus and NDF and ADF of the test diets or between swelling index and storage modulus.

## EXPERIMENT 2.

## THE EFFECT OF CELLULOSE, DIETARY RATIO OF CELLULOSE AND CORNSTARCH AND FEEDING LEVEL ON ILEAL NITROGEN EXCRETION

### OBJECTIVE

To determined the effects of:

a. Dietary cellulose on nitrogen output in the ileal digesta
b. Dietary ratios of cellulose and cornstarch on ileal nitrogen concentration
c. Intubation levels of feeding on ileal nitrogen excretion

The purpose of the trial was to obtain a regression relationship between the feeding of cellulose and terminal endogenous nitrogen as determined from sampling digesta at the ileal caecal junction. The procedure involved feeding a series of cellulose and cornstarch test diets of differing ratios, ascertaining the quantity of digesta (DM) produced for each unit quantity of test diet fed using the expression

$$\text{digesta (DM) g/g diet fed} = \frac{\text{mg Cr/g test diet}}{\text{mg Cr/g digesta}}$$

and from analysis of percent nitrogen in the ileal digesta calculating the quantity of ileal nitrogen associated with dietary quantities supplying serial increments in cellulose whilst maintaining levels of cornstarch constant. Concomitantly the effect of different cellulose to cornstarch ratios on ileal nitrogen excretion was evaluated.

The assumption underlying the use of indicators evenly mixed in test diets is that the indicator will passage uniformly and be completely eliminated with the digesta. A number of studies, refer Vohra and Kratzer (1967) and Vohra (1972) have suggested this may not be so. At the completion of 24 hours following feeding some 12 percent of chromic oxide as indicator was not accounted for (Vohra and Kratzer, 1967) and the studies of Yap (1991) showed that chromic oxide concentration in the digesta varied over time of sampling following feeding.

An approach adopted to minimise variations arising from departures from uniform indicator flow is to sample at times when digesta passage through the terminal ileum is relatively large, i.e. at times between when the digesta begins to passage and when its flow diminishes at the end. An extension of the rationale is that uniformity of chromic oxide passage or its concentration in ileal samples may be affected by quantities of diet fed. The purpose of objective c was to evaluate whether levels of feeding over the range 15-25 g was influencing results.

## MATERIALS AND METHODS

Sixty 25 day old female Ross Meat chickens were received on 3rd of July 1993, and housed in a single pen under 23 hours light per day in the Environmental Physiology shed until 36 day of age. Fourty eight of them were then individually weighted and placed randomly in single bird cages and acclimatised as describe below under 16 hours light per day. Up until dietary treatment procedures commenced (46 DOA) the birds received a finisher type broiler diet as pellets (Appendix 6.). The birds had free access to water at all times.

From 36 DOA the birds were fed *ad libitum* until 8.00 pm on day 43 when feeders were removed. On day 44, the birds had access to food between 8.00 am and 8.00 pm. On the following day feed was supplied between 12 midday and 8.00 pm and on day 46 they had feed before them only between 4.00 and 8.00 pm. The birds were then denied food for at least 36 hours (8.30 am on day 48) when intubation of test diets began.

The treatments consisted of 4 diets, labelled 1 through to 4, containing cellulose and cornstarch in the ratio of 1:1, 2:1, 3:1 and 4:1, fed over 3 intubation levels 15, 20 and 25 g randomised over 48 birds. There were thus 12 treatments each replicated over 4 birds. Dietary treatments were mixed on the day prior to intubation. Diets were intubated in their air dry state.

| Feeding level (g) | Diet* 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 15 | TR 1 | TR 2 | TR 3 | TR 4 |
| 20 | TR 5 | TR 6 | TR 7 | TR 8 |
| 25 | TR 9 | TR 10 | TR 11 | TR 12 |

*) Diet 1 contained cellulose and cornstarch with ratio 1:1
Diet 2 contained cellulose and cornstarch with ratio 2:1
Diet 3 contained cellulose and cornstarch with ratio 3:1
Diet 4 contained cellulose and cornstarch with ratio 4:1

Intubation took place over a 5 hour period and ileal sampling took place some 3 to 7 hours after intubation in accordance with the first appearance of droppings showing evidence of chromic oxide content. Intubation 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. The birds were sacrified by a 1 ml intracardial injection of sodium pentobarbitone (Pentobarb 300). Ileal digesta samples were obtained by clamping and removing the final 15 cm of ileum and flushing the digesta into small plastic bags with 10 ml of water. The bags were sealed, stored overnight in a freezer and then unsealed and placed in a freeze dryer on the following day.

Ileal samples were analysed for nitrogen (N) and chromium (Cr). Nitrogen was determined by the micro-kjeldahl technique using 100 mg samples on a Kjeldahl 1030 auto analyser (Tecator, Sweden) (Appendix 2). Chromium was analysed according to the procedure of Costigan and Ellis (1987)(Appendix 3.).

Analyses were conducted on N and Cr data using the two-way analysis of variance procedure of Statistical Analytical System (SAS, 1985). The difference in mean N and Cr concentration between and across feeding levels and dietary types, were tested using Duncan's Multiple Range Test. Similar analyses were also conducted for total N. Regression analyses were undertaken for the relationship between changing dietary cellulose and total digesta N both by feeding level and over all feeding levels.

## RESULTS

Appendix 8. gives chromium and nitrogen concentrations obtained from laboratory determinations of sampled ileal digesta and dietary treatments.

The concentration of N in the digesta did not differ significantly among feeding levels (Table 4.2.1., Figure

4.2.2.) and ranged between 0.104% (20 g) and 0.139% (25 g), but dietary ratios influenced response with the ratio 2:1 (cellulose to cornstarch) resulting in significantly greater percent N ($P<0.05$) at 0.155% than ratios 3:1 (0.105%) and 4:1 (0.096%)(Figure 4.2.1. refers).

Table 4.2.1. The effect of feeding level and dietary type (cellulose: cornstarch) on percentage N in the digesta (DM basis).

| Feeding level (g) | The type of diets 1 Ratio 1:1 | 2 2:1 | 3 3:1 | 4 4:1 | Mean |
|---|---|---|---|---|---|
| 15 | 0.157 | 0.107 | 0.080 | 0.127 | 0.114[a] |
| 20 | 0.082 | 0.123 | 0.117 | 0.099 | 0.104[a] |
| 25 | 0.136 | 0.227 | 0.119 | 0.071 | 0.139[a] |
| Mean | 0.121[ab] | 0.155[a] | 0.105[b] | 0.096[b] | |

Means with different superscripts are significantly different ($P<0.05$)

Figure 4.2.1. Relationship between %N in the digesta and diet type.

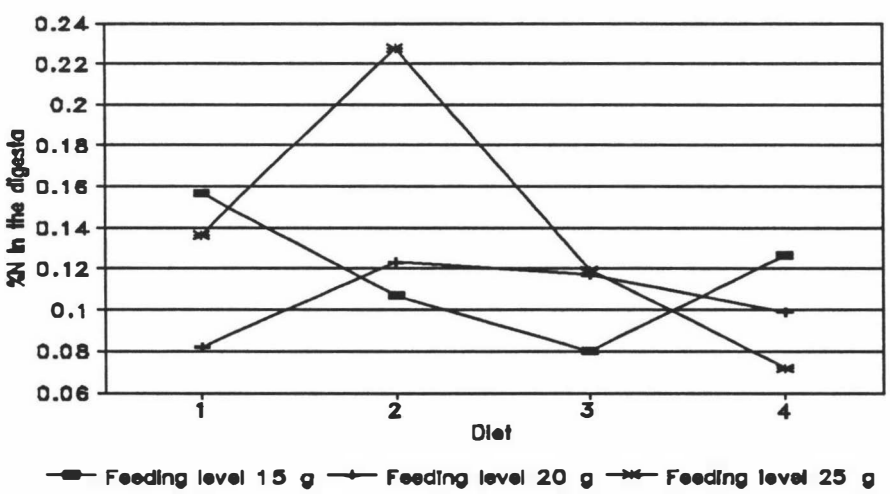
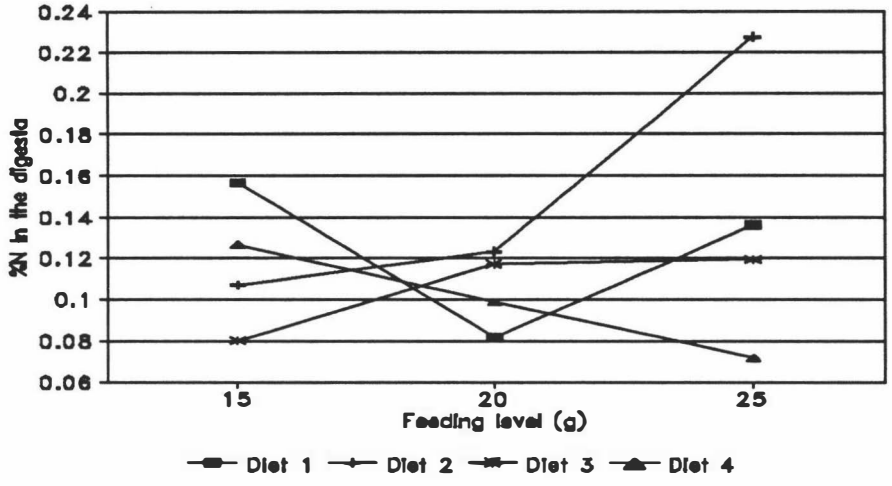


Figure 4.2.2. Relationship between %N in the digesta and feeding level.




The concentration of chromium in the ileal digesta was not significantly influenced by feeding levels (Table 4.2.2., Figure 4.2.4) for which means of 0.235, 0.227 and 0.224 percent were obtained for feeding levels of 15, 20 and 25 g respectively, but dietary type had a significant effect ($P<0.05$), refer Table 4.2.2, Figure 4.2.3. The ratio 1:1 (cellulose to cornstarch) resulted in a greater chromium concentration (0.281%) than diets of ratios 2:1 (0.231%), 3:1 (0.203%) and 4:1 (0.206%).

Table 4.2.2. The effect of feeding level and dietary type on percentage Cr in digesta (DM basis).

| Feeding level (g) | The type of diets 1 Ratio 1:1 | 2 2:1 | 3 3:1 | 4 4:1 | Mean |
|---|---|---|---|---|---|
| 15 | 0.274 | 0.235 | 0.221 | 0.215 | 0.235[a] |
| 20 | 0.277 | 0.219 | 0.203 | 0.205 | 0.227[a] |
| 25 | 0.293 | 0.237 | 0.184 | 0.199 | 0.224[a] |
| Mean | 0.281[a] | 0.231[b] | 0.203[b] | 0.206[b] | |

Means with different superscripts are significantly different ($P<0.05$)

Figure 4.2.3. Relationship between % Cr in the digesta and diet type.

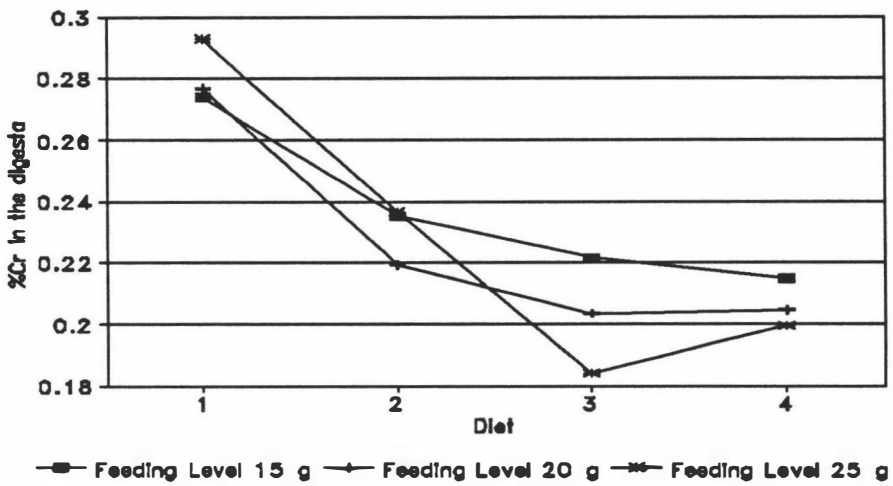
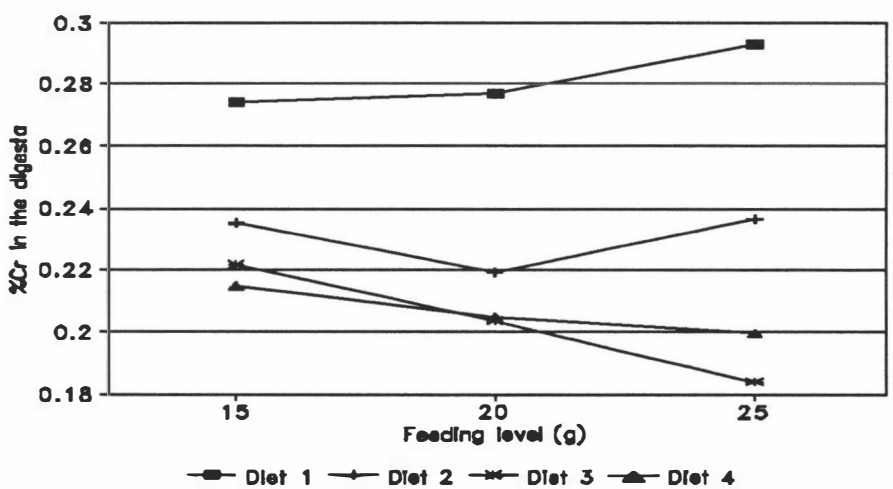



Figure 4.2.4. Relationship between % Cr in the digesta and feeding level.



Dietary ratios were formulated and feeding levels defined such that application of the expression

$$\frac{\text{mg Cr/g test diet}}{\text{mg Cr/g digesta}} \times \text{mg N/g digesta} \times \text{feeding level (g)} \times \text{multiplier}$$

to each treatment would give a series of endogenous N values equivalent to cellulose: cornstarch intakes of 5:5, 10:5, 15:5 and 20:5 gram in each feeding level set of treatments. The relationship between multiplier, dietary ratios and cellulose and cornstarch equivalent intake at each feeding level is as follows:

| | Dietary type | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | ratio 1:1 | 2:1 | 3:1 | 4:1 |
| Feeding level 15 g | | | | |
| multiplier | 10/15 | 1 | 20/15 | 25/15 |
| cellulose | 5 | 10 | 15 | 20 |
| cornstarch | 5 | 5 | 5 | 5 |
| Feeding level 20 g | | | | |
| multiplier | | 15/20 | 1 | 25/20 |
| cellulose | 5 | 10 | 15 | 20 |
| cornstarch | 5 | 5 | 5 | 5 |
| Feeding level 25 g | | | | |
| multiplier | 10/25 | 15/25 | 20/25 | 1 |
| cellulose | 5 | 10 | 15 | 20 |
| cornstarch | 5 | 5 | 5 | 5 |

Multiplier adjusted estimates of endogenous N excretion equivalent to incremental intakes of cellulose of 5, 10, 15 and 20 g at constant cornstarch intakes of 5 g in mg was as follows:

| Feeding level (g) | Diet type | | | | Overall |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| cornstarch (g) | 5 | 5 | 5 | 5 | mean |
| cellulose (g) | 5 | 10 | 15 | 20 | |
| 15 | 19.016 | 20.434 | 22.148 | 33.238 | 23.364 |
| 20 | 11.234 | 26.671 | 33.798 | 36.848 | 27.169 |
| 25 | 14.727 | 35.749 | 39.417 | 27.131 | 29.830 |
| Overall mean | 14.617[b] | 26.900[a] | 31.787[a] | 32.330[a] | |

Means with different superscripts are significantly different (P<0.01)

There was a highly significant difference in ileal N excretion between different levels of cellulose intake (dietary types)(P<0.01), but feeding levels had no effect. Greater intakes of cellulose resulted in greater ileal excretion of N although only the cellulose intake of 5 g at 14.617 mg excreted was significantly (P<0.01) different to the other outputs of 26.9, 31.787 and 32.330 mg N recorded for intakes of 10, 15 and 20 g cellulose respectively.

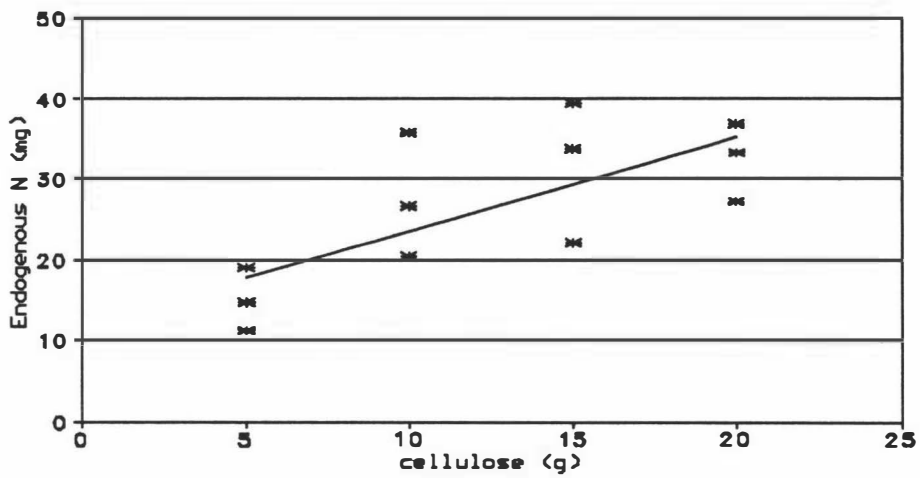
Endogenous N was regressed against cellulose intake (Figure 4.2.5 refers) using pairs of values over all feeding levels. The regression model (linear) and the slope were highly significant at P<0.01, and the intercept was also significant at P<0.05. The regression equation

$$Y = 12.041 + 1.154\ X$$

where Y = endogenous N (mg)
and X = quantity of cellulose (g)

suggests that endogenous N increased by 1.154 mg for each gram increment of cellulose fed and that 5 g of cornstarch fed alone would result in 12.041 mg of endogenous N at the terminal ileum.

Figure 4.2.5. Relationship between endogenous N and increasing intake of dietary cellulose.

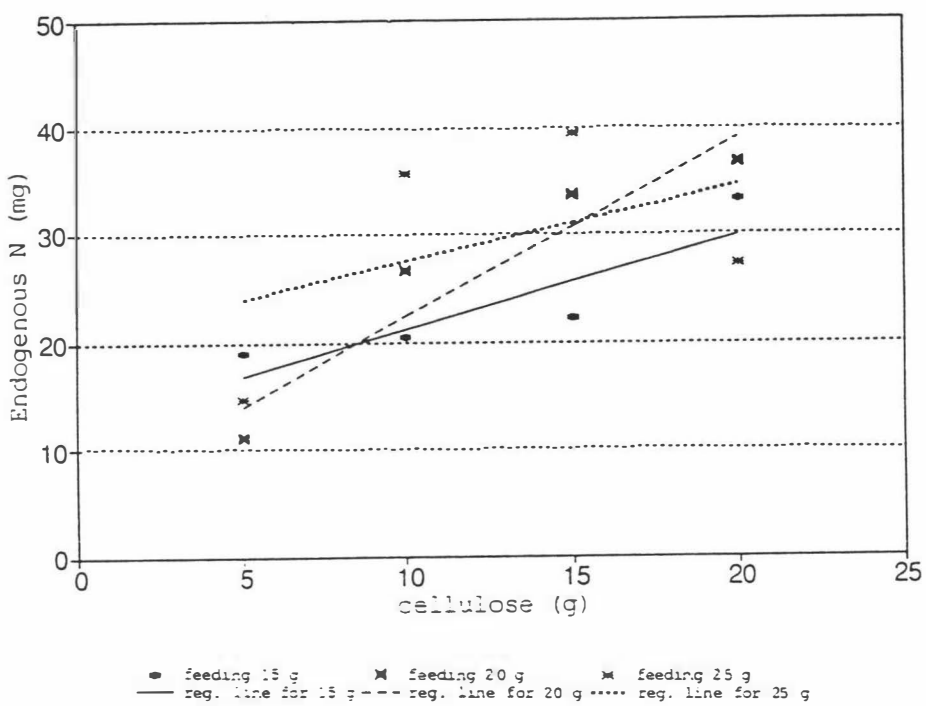



Regression of endogenous N against cellulose intake was analysed by pairs of values within feeding levels (Figure 4.2.6.). The regression analyses together with significant components are presented below. Both the model (linear) and slope at feeding levels of 15 and 20 g were highly significant ($P<0.01$), but for the 25 g feeding level, the intercept and the slope were not significantly different to zero. The intercept was significant ($P<0.01$) only in the case of the 15 g regression line.

| Intubation level | Regression equation | Level of significant model | intercept | slope |
|---|---|---|---|---|
| 15 | Y = 12.489 + 0.870 X | *[1] | * | * |
| 20 | Y = 5.642 + 1.670 X | * | ns[2] | * |
| 25 | Y = 20.301 + 0.721 X | ns | ns | ns |

1) Significant at P<0.05
2) Non significant

Figure 4.2.6. The regression relationship between endogenous nitrogen and increasing intake of dietary cellulose by feeding level.




## DISCUSSION

Difficulty was experienced in the delivery of test diets into the crop and resulted in extended feeding times of up to 13 minutes in the feeding of 25 g quantities. This was attributed to impaction of feed in the crop for delivery of 15 g amounts seldom took longer than 3-4 minutes.

Difficulty was also experienced in predicting the optimal time to slaughter following feeding. The work of Yap (1991)

involving standard diets suggested adequate digesta volumes could be expected at 4-5 hours following feeding, but in this trial many birds had not defecated chromium containing material at 4 hours and palpation of bird crops indicated sizable amounts of feed were still present. Consequently sampling of digesta was undertaken for the most part between half and one hour after Cr containing faeces appeared and this resulted in birds being sacrified between 4 and 9 hours following feeding. In 4 cases insufficient sample material was obtained and in a number of other cases disappointingly small volumes of digesta were collected.

Ileal N concentration was numerically greater in the 1:1 and 2:1 ratio diets and in the latter case significantly greater than the two higher ratio diet types. Chromium digesta concentration showed a similar trend with the 1:1 and 2:1 ratio diets containing numerically greater concentrations and significantly so in the 1:1 ratio class than the 3:1 and 4:1 diet types. The chromium results suggest smaller quantities of digesta were being passaged per unit of diet fed in the 1:1 and 2:1 ratio diets, which is consistant with expectations if cornstarch were wholely absorbed and cellulose completely eliminated and perhaps supports or provides an explanation for the significant differences in endogenous N response to cellulose feeding levels of between 5 and 20 g.

No significant differences were detected in either N or Cr concentrations when diets were fed at 15, 20 or 25 grams and this suggests that for cellulose-cornstarch diets within the ratios operating, 15 g of feed was a satisfactory feeding amount.

It was demonstrated that endogenous N excretion was significantly different between cellulose intakes of 5 and

those of 10 to 20 g. The regression equation generated, ($Y = 12.041 + 1.154 X$) indicated that the feeding of the cornstarch component in diets at levels of 5 g was associated with a terminal ileal N excretion of approximately 12 mg of N ($P<0.05$) and that each gram of cellulose fed was resulting in an output of 1.154 mg of N ($P<0.01$). This response implies that cellular sloughing as an effect of cellulose passage or the capacity of cellulose to retain endogenous secretions or both is as a whole a significant component of N ileal excretion.

## EXPERIMENT 3.

# THE EFFECT OF WET FEEDING OF GELATIN CELLULOSE-CORNSTARCH DIETARY RATIOS AND FEEDING LEVELS ON ILEAL N AND Cr EXCRETION

## OBJECTIVE

To explore the effects of feeding level and dietary ratios of gelatin cellulose-cornstarch mixtures intubated in wet form, on N and Cr ileal digesta concentration and ileal N excretion.

In experiment 2 serious difficulties were experienced in intubating cellulose-cornstarch mixtures in dry form into the crop. This trial was undertaken to explore the practicalities and effects of feeding wet diets comprising gelatin and a cellulose-cornstarch base on ileal N and Cr excretion, as a prelude to investigations involving guanidinated gelatin.

## MATERIALS AND METHODS

Sixty ,28 day old Ross meat chickens were received at the Poultry Research Unit on 13 July 1993. They were housed in a single pen in the Environmental Physiology shed until 38 days of age (DOA) when 48 were randomly selected, individually weighed and placed in single bird cages numbered from 1 to 48 in the order in which they were weighed. Whilst floor housed they received 23 hours of light each day, but in their period in cages they were given 16 hours light each day. They had free access to water at all times and up until intubation procedures

commenced the food fed was a pelleted broiler finisher diet described in Appendix 6.

The birds were given free access to diets until 43 DOA when feed was withdrawn at 4 pm. It was resupplied for 5 hours between 11 am and 4 pm on day 44, and again but for 4 hours between 1 and 5 pm of day 45 and finally between 5 pm and 8 pm on day 46. They were then fasted for at least 36 hours when treatments were begun at 8.30 am on day 48.

Treatments involved intubation of air dry amounts of 15, 20 and 25 g of mixtures of different ratios of gelatin and a cellulose to cornstarch base fed in wet form by application of 5 parts
water by volume (cc) to 1 part test diet by weight (g). The base dietary component had a ratio of 3 parts cellulose to 1 part cornstarch. There were 4 dietary gelatin to base ratios, 0.5:1, 1:1, 1.5:1 and 2:1 labelled respectively dietary ratios 1, 2, 3 and 4, each fed over 3 air dry feeding levels of 15, 20 and 25 g to give 12 treatments in all labelled treatments 1 through 12 as illustrated below.

| Feeding Level (g) | Diet ratios* 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 15 | TR1 | TR2 | TR3 | TR4 |
| 20 | TR5 | TR6 | TR7 | TR8 |
| 25 | TR9 | TR10 | TR11 | TR12 |

*) diet 1 contained gelatin and base with ratio 0.5:1.0
diet 2 contained gelatin and base with ratio 1.0:1.0
diet 3 contained gelatin and base with ratio 1.5:1.0
diet 4 contained gelatin and base with ratio 2.0:1.0

The treatments were randomised over the 48 birds to give 4 birds per treatment. There were 2 exceptions to the above format. The first bird treated, TR 3, received a diet of 30 ml water:15 g diet and bird 2, TR 11, received 50 ml

water:25 g diet. Diets were mixed the day before treatment diets were applied and water was added immediately before intubation using calibrated 35 ml plastic disposable syringes.

Intubation was completed over a 3 hour period using equipment and procedures described in experiment 2. Ileal sampling took place between 4 and 9 hours after intubation, in general between and 1 hour following the first appearance of Cr in the droppings. The birds were sacrificed by injection of 1 ml of sodium pentobarbitone (Pentobarb 300) into the heart. Ileal digesta samples were obtained by clamping and removing the final 15 cm of ileum and flushing the digesta into small plastic bags with 10 ml of water. The bags were sealed, stored overnight in a freezer and then unsealed and placed in a freeze dryer on the following day.

Ileal samples obtained were analysed for nitrogen (N) and chromium (Cr) following the same methods as described under experiment 2. All digesta samples were analysed in duplicate for N and Cr determinations, whenever possible.

Analyses were conducted on N and Cr data using the two-way analysis of variance procedure of Statistical Analytical System (SAS, 1985). The difference in mean N and Cr concentration between and across feeding levels and dietary types were tested using Duncan's Multiple Range Test. Similar analyses were conducted for total N. Regression analyses were undertaken for the relationship between changing dietary gelatin and total digesta N both by feeding level and over all feeding levels.

## RESULTS

Appendix 9. gives chromium and nitrogen data obtained from laboratory analysis of sampled ileal digesta and dietary treatments. The concentration of N in the ileal digesta was unaffected by feeding level (Table 4.3.1., Figure 4.3.1) with percent N varying between 2.637% for the 15 g feeding level and 3.658% for the level of 20 g. Dietary ratios on the other hand had a significant effect at $P<0.05$. The percentage of N in the ileal digesta was significantly greater in the 1:1 and 1.5:1 and 2:1 ratio diets than the 0.5:1 diet (Table 4.3.1., Figure 4.3.2.) with values of 3.471, 3,580 and 4,609 percent respectively versus 1.904%.

Table 4.3.1. The effect of feeding levels and the type of diets on the percentage of N in the ileal digesta (DM basis).

| Feeding level (g) | Diet 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|
| 15 | 1.310 | 2.790 | 2.238 | 4.787 | 2.637[a] |
| 20 | 2.100 | 3.456 | 4.497 | 4.888 | 3.658[a] |
| 25 | 2.303 | 3.998 | 4.006 | 4.153 | 3.579[a] |
| Mean | 1.904[b] | 3.471[a] | 3.580[a] | 4.609[a] | |

Means with different superscripts are significantly different ($P<0.05$).

Figure 4.3.1. Relationship between % N in the digesta and feeding levels by dietary type.

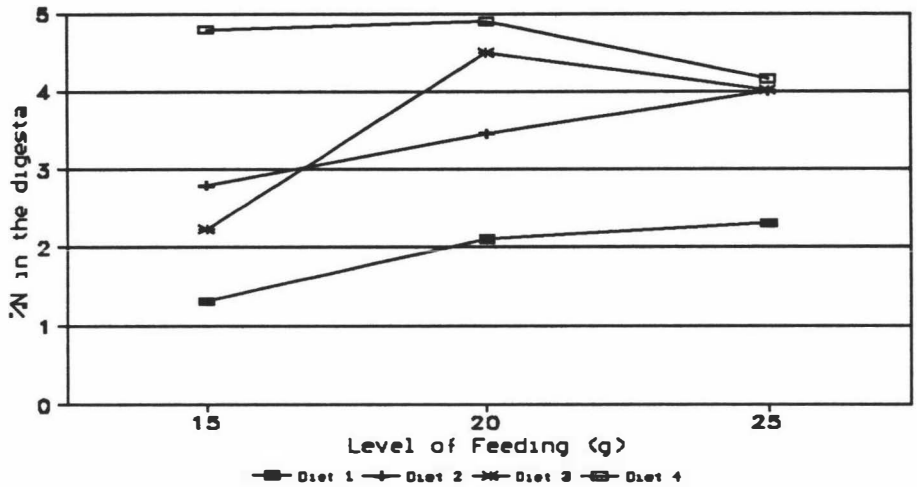
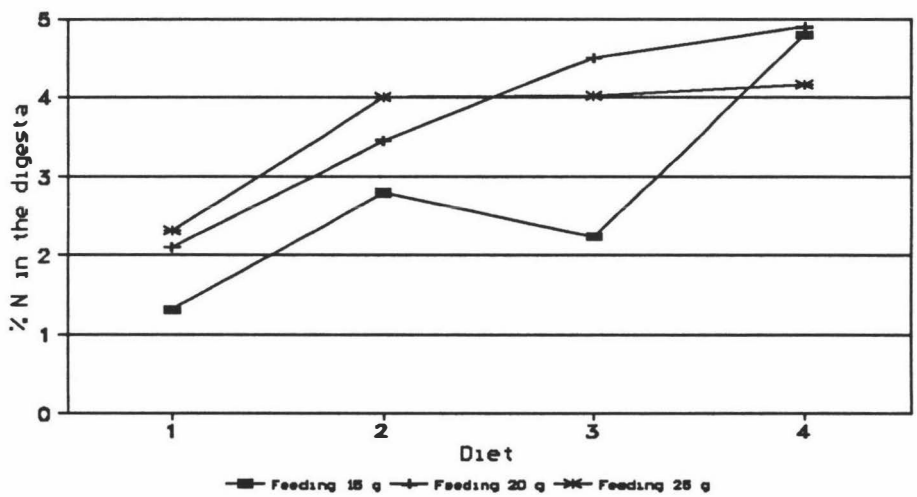



Figure 4.3.2. Relationship between % N in the digesta and dietary type by feeding level.




The concentration of Cr in the ileal digesta was affected ($P<0.05$) by feeding level, refer Table 4.3.2., Figure 4.3.3., with greater concentrations of Cr occurring at the 25 g level, 0.525%, than at the 15 g level, 0.398%. The concentration of Cr in the 2:1 ratio diet at 0.614% was

significantly greater (P<0.05) than all other dietary types (Table 4.3.2., Figure 4.3.4.). Percent Cr in the ileal digesta of the 1.5:1 diet type at 0.478 was numerically greater than that of diet type 1:1 (0.422%) and was significantly greater (P<0.05) than the value recorded for diet type 0.5:1 of 0.325.

Table 4.3.2. The effect of feeding levels and the type of diets on the percentage of Cr in the ileal digesta (DM basis).

| Feeding level (g) | Diet 1 | 2 | 3 | 4 | Mean |
|---|---|---|---|---|---|
| 15 | 0.334 | 0.334 | 0.494 | 0.418 | $0.398^{b}$ |
| 20 | 0.310 | 0.434 | 0.380 | 0.616 | $0.423^{ab}$ |
| 25 | 0.331 | 0.476 | 0.558 | 0.807 | $0.525^{a}$ |
| Mean | $0.325^{c}$ | $0.422^{bc}$ | $0.478^{b}$ | $0.614^{a}$ | |

Means with different superscripts are significantly different (P<0.05).

Figure 4.3.3. Relationship between % Cr in the digesta and feeding levels by dietary type.

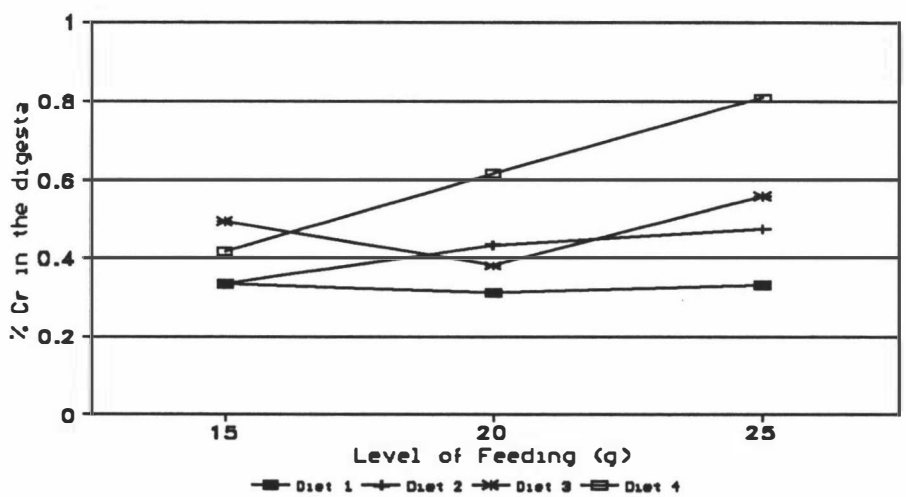
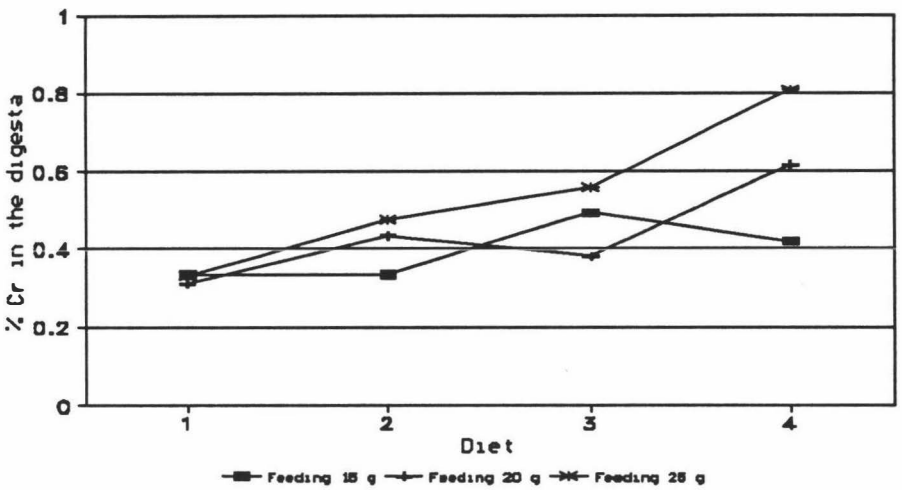


Figure 4.3.4. Relationship between % Cr in the digesta and dietary types by feeding level.




Dietary ratios were formulated and feeding levels defined such that application of the expression

$$\frac{\text{mg Cr/g test diet}}{\text{mg Cr/g digesta}} \times \text{mg N/g digesta} \times \text{feeding level} \times \text{multiplier}$$

to each treatment would give a series of digesta N values equivalent to gelatin: base intakes of 5:10, 10:10, 15:10 and 20:10 grams in each feeding level set of treatments. The relationship between multiplier, dietary ratios and gelatin and base equivalent intakes at each feeding level is as follows:

| | Dietary type | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | ratio 0.5:1.0 | 1.0:1.0 | 1.5:1.0 | 2.0:1.0 |
| Feeding level 15 g | | | | |
| multiplier | 1 | 20/15 | 25/15 | 30/15 |
| gelatin | 5 | 10 | 15 | 20 |
| base | 10 | 10 | 10 | 10 |
| Feeding level 20 g | | | | |
| multiplier | 15/20 | 1 | 25/20 | 30/20 |
| gelatin | 5 | 10 | 15 | 20 |
| base | 10 | 10 | 10 | 10 |
| Feeding level 25 g | | | | |
| multiplier | 15/25 | 20/25 | 25/25 | 30/25 |
| gelatin | 5 | 10 | 15 | 20 |
| base | 10 | 10 | 10 | 10 |

Multiplier adjusted estimates of ileal N excretion, equivalent to incremental intakes of gelatin of 5, 10, 15 and 20 g at a constant base intake of 10 g in mg was as follows:

| Feeding level (g) | Diet type 1 | 2 | 3 | 4 | Overall mean |
|---|---|---|---|---|---|
| base (g) | 10 | 10 | 10 | 10 | |
| gelatin (g) | 5 | 10 | 15 | 20 | |
| 15 | 178.642 | 562.776 | 407.711 | 1021.330 | 506.981[a] |
| 20 | 294.980 | 495.879 | 882.373 | 861.818 | 618.559[a] |
| 25 | 304.954 | 549.569 | 673.388 | 461.126 | 499.668[a] |
| Overall Mean | 259.525[b] | 533.647[ab] | 654.491[a] | 781.425[a] | |

Means with different superscripts are significantly different (P<0.01).

There were no significant differences in ileal excretion between different feeding levels, but increasing gelatin caused significant elevation in ileal N of the 15 and 20

g intakes (654 and 781 mg respectively) over the 5 g intake (260 mg ) at $P<0.01$.

Endogenous plus dietary N was regressed against gelatin intake using pairs of values over all feeding levels (Figure 4.3.5). The model(linear) and the slope were highly significant ($P<0.01$). The regression equation

$$Y = 128.789 + 34.348 X$$

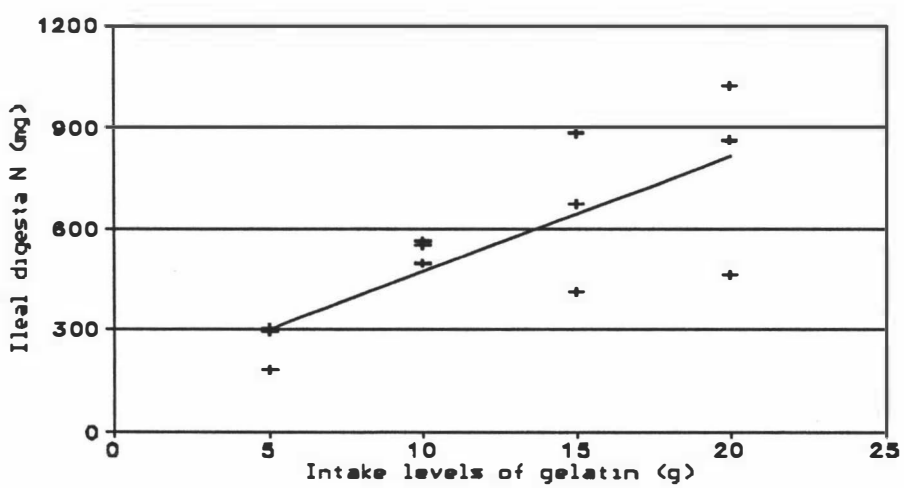
in which

Y = the total N in the digesta (mg)

X = levels of protein fed per intubation (g)

suggests that ileal endogenous plus dietary N increased by approximately 34 mg for each gram increment of gelatin fed and that 10 g of the cellulose-cornstarch base if fed alone would result in approximately 129 mg N at the terminal ileum.

Figure 5.3.5. Relationship between N at the terminal ileum and increases in dietary gelatin.

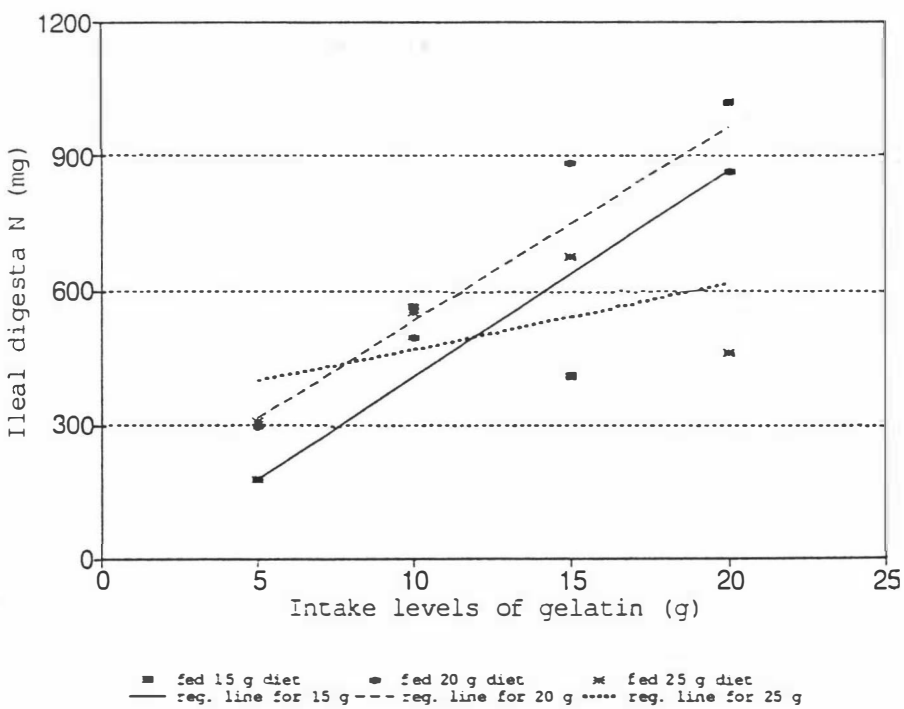


Regression of ileal N on gelatin intake was analysed by pairs of values within feeding levels. The regression analyses together with significance components are presented below (Figure 4.3.6.). The model(linear) and slope were significant in the 15 and 20 g feeding level regressions.

| Intubation level | Regression equation | Level of significant model | intercept | slope |
|---|---|---|---|---|
| 15 | Y = -50.727 + 45.929 X | *[1] | ns[2] | * |
| 20 | Y = 99.133 + 43.285 X | * | ns | **[3] |
| 25 | Y = 330.238 + 14.119 X | ns | ns | ns |

1) significant (P<0.05)
2) non significant
3) highly significant (P<0.01)

Figure 4.3.6. The regression relationship between ileal N and increasing intake of gelatin by feeding level.



## DISCUSSION

The wet method of feeding overcame problems attributed to impaction experienced in experiment 2. Delivery of food was easier and took place more rapidly. Regurgitation was experienced in the feeding of 25 g (air dry basis) in some cases and in a number of birds the feeding of 25 g in wet form exceeded the capacity of the birds. In these cases a little less than the total volume of food in wet form was delivered.

The concentration of N in the ileal digesta was unaffected by feeding levels, but was least in the 0.5:1 dietary ratio ($P<0.05$) and greatest (nonsignificant) in the 2:1 dietary ratio. The response suggests that increasing protein concentration in the diet resulted in increasing N concentration in the digesta of the terminal ileum.

The concentration of Cr in the ileal digesta varied significantly over feeding levels with greater Cr concentration in the digesta of the 25 g feeding level (DM) than the 15 g level (DM). This implies their was less digesta produced per g of food fed in the greater feeding level than in the smaller. This may be related to overfeeding of birds receiving the 25 g (DM) feeding level.

The Cr concentration in the digesta of birds receiving diets with the 2 larger ratios of gelatin to base were significantly or numerically greater than the concentration recorded for the lower 2 dietary ratios. This implies that diets of higher gelatin concentration produced lesser digesta.

Increases in ileal N produced for incremental increases of gelatin intake from 5 through to 20 g under conditions of

a constant 10 g intake of base were significant ($P<0.01$) and regression analysis indicated that ileal N was increasing at approximately 34 mg for each g increase in gelatin consumed. The response suggests that increasing gelatin concentration in diets of gelatin and a mixture of cellulose to cornstarch in the ratio of 3:1 has a marked and significant impact on N excretion at the terminal ileum. Assuming the N content of gelatin is 6.25 mg per 100 mg then the net efficiency of N utilisation of gelatin at the terminal ileum was 34/62.5=54 percent. Some fraction of the N may have been endogenous and hence actual gelatin utilisation may have exceeded 54 percent.

The intercept of the regression line of approximately 128 mg was not significant and the quantitative effect of feeding 10 g of the base alone remains uncertain.

**EXPERIMENT 4.**

## THE ESTIMATION OF ENDOGENOUS LYSINE USING GUANIDINATED GELATIN

### OBJECTIVE

To measure the effect of dietary guanidinated gelatin on endogenous lysine at the terminal ileum.

In the guanidination of gelatin, lysine is converted to homoarginine. If transformation is complete it follows that lysine appearing in the digesta is endogenous in origin. This trial involved feeding diets of guanidinated gelatin and a cellulose-cornstarch base in different ratios to 68 day old meat chickens and establishing the lysine response as determined from terminal ileal digesta samples.

### MATERIALS AND METHODS

Ross female meat chickens were received when 32 days of age on 11 of October 1993 and housed in a single pen in the Environmental Physiology shed at the Poultry Research Unit until 50 days of age when 32 were randomly selected, individually weighed and placed in single bird cages. For the next 5 days they were allowed free access to food up until 4 pm on day 55 when the food was withdrawn. The birds were allowed access to food between 11 am and 6 pm on the following day and on day 57 they were fed between 1 and 8.30 pm. They were then fasted for 36 hours until 8.30 am on day 59 in readiness for intubation. However, the guanidination of gelatin had not been completed and the birds were returned to *ad libitum* feeding conditions and

the events of the previous 9 days repeated. On Tuesday, 16 November at 8.30 am when the birds were 68 days of age intubation commenced. The birds were run under 23 hours of light each day during the period of pen housing, and 18 hours of light during the period in cages. They had free access to water at all times and other than for the intubated diets they received a broiler finisher pelleted diet described in appendix 6.

The treatments were 4 diets, labelled 1 through to 4, containing guanidinated gelatin (GG) and a base in the ratios of 0.5:1, 1:1, 1.5:1 and 2:1 respectively. The base was the same as that used in experiment 3 and comprised a cellulose to cornstarch mixture in the ratio of 3:1. Birds were fed 20 g (air dry) of each diet mixed with water in the ratio of 1 g diet to 5 cc of water. The water was added immediately prior to feeding using calibrated 35 ml disposable syringes. The treatments were allocated randomly over the 32 birds to give 4 treatment each of 8 birds. Intubation equipment and procedures were those outlined in experiment 3. Intubation was completed within 2 hours and birds were sacrificed some 3 to 7 hours following intubation on the basis of degree of crop emptiness and on the appearance of Cr in the droppings. The birds were sacrificed and digesta samples obtained using the same procedures as those described in experiment 3. Digesta samples were collected in small plastic bags and stored overnight in a freezer before they were transferred to the freeze dryer.

For amino acid analyses the digesta of birds of the same treatment were subsampled and pooled. The remainder was then used in the analysis by bird of Cr and N. Gelatin was obtained from the Sigma Chemical Company (PO BOX 14508, St Louis, Missouri, 63178 USA) and was specified as type A,

from porcine skin and approximately 60 bloom. It was guanidinated according to Rutherfurd and Moughan (1990) with some modifications, refer appendix 4. Digesta nitrogen and Cr concentrations were determined by the methods described previously (experiment 2 and 3). Amino acid content was determined on duplicate 5 mg ileal samples using a Waters ion-exchange HPLC system, utilising post O-phthalaldehyde derivation and fluorescence detection. Samples were first hydrolysed in 6M glass distilled HCl containing 0.1% phenol for 24 hours at 110$\pm$ 2° C in an evacuated sealed tube. Amino acid analysis of guanidinated gelatin utilised the same technique.

Analyses were conducted on N and Cr and lysine data using the one-way analysis of variance and regression procedures of Statistical Analytical System (SAS, 1985). Duncan's Multiple Range Test was used to assess the significance of differences between treatment means.

## RESULTS

Amino acid analysis of the guanidinated gelatin (Table 4.4.1.) gave the composition of homoarginine as 1.281% and that of lysine as 0.213%. The percentage of transformation of lysine was therefore 86%.

Appendix 10 gives chromium and nitrogen data obtained for experiment 4. The concentration of Cr and N in the ileal digesta is shown in Table 4.4.2.

Table 4.4.1. Mean of amino acid contents (duplicates) of ileal digesta (%) and guanidinated gelatin.

| Amino acids (%) | Ileal digesta TR 1 | TR 2 | TR 3 | TR 4 | Guanidinated Gelatin |
|---|---|---|---|---|---|
| Aspartic acid | 1.272 | 1.941 | 1.891 | 2.687 | 4.236 |
| Threonine | 0.573 | 0.797 | 0.774 | 1.376 | 1.331 |
| Serine | 0.645 | 0.994 | 0.915 | 1.499 | 3.082 |
| Glutamic acid | 1.304 | 2.079 | 2.039 | 3.063 | 7.778 |
| Proline | 0.999 | 1.983 | 1.931 | 2.144 | - |
| Glycine | 1.314 | 2.281 | 2.174 | 2.698 | 25.975 |
| Alanine | 0.575 | 1.206 | 1.188 | 1.573 | 5.488 |
| Valine | 0.539 | 0.815 | 0.883 | 1.296 | 1.151 |
| Methionine | 0.174 | 0.374 | 0.319 | 0.614 | 0.140 |
| Isoleucine | 0.383 | 0.516 | 0.508 | 1.023 | 0.662 |
| Leucine | 0.655 | 0.993 | 0.916 | 1.736 | 1.518 |
| Tyrosine | 0.336 | 0.415 | 0.430 | 0.912 | 0.429 |
| Phenylalanine | 0.433 | 0.604 | 0.605 | 1.304 | 0.946 |
| Histidine | 0.201 | 0.381 | 0.342 | 0.630 | 0.743 |
| Lysine | 0.450 | 0.635 | 0.654 | 1.238 | 0.213 |
| Arginine | 0.627 | 1.222 | 1.151 | 1.725 | 3.416 |
| Homoarginine | - | - | - | - | 1.281 |

Table 4.4.2. The effect of changing dietary ratios of guanidinated gelatin and base on ileal digesta Cr and N concentration (DM basis).

| | Type of Diet | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Mean |
| Ratio | 0.5:1 | 1:1 | 1.5:1 | 2:1 | |
| Cr (%) | 0.117 | 0.137 | 0.071 | 0.019 | 0.094$^{ns}$ |
| Cr (mg/g digesta) | 1.171 | 1.367 | 0.709 | 0.189 | 0.939$^{ns}$ |
| N (%) | 4.171$^{b}$ | 7.155$^{a}$ | 7.661$^{a}$ | 8.201$^{a}$ | 6.882* |
| N (mg/g digesta) | 41.706$^{b}$ | 71.548$^{a}$ | 76.614$^{a}$ | 82.005$^{a}$ | 68.815* |

ns) non significant
*) significant at P<0.05

The concentration of N in the ileal digesta was significantly less (P<0.05) in the 0.5:1 ratio treatment at 4.171% than in the 1:1 (7.155%), 1.5:1 (7.661%) and 2:1 (8.201%) ratio treatments. The concentration of Cr was not significantly different between treatments and ranged between 0.137% for the 1:1 ratio treatment and 0.019% for the 2:1 treatment.

The concentration of lysine in the ileal digesta increased from 4.50 mg/g digesta for the 0.5:1 ratio diet to 12.38 in the 2:1 ratio diet. Differences were significant at P<0.01 (Table 4.4.3. refers). The concentration of lysine was significantly greater in the digesta of diet 2:1 than in that of diets 1.5:1 and 1:1 and that of all three were significantly greater than the lysine concentration in the digesta of diet 0.5:1.

Table 4.4.3. The effect of increasing dietary concentration of guanidinated gelatin on ileal digesta lysine concentration.

| | Diet | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Mean |
| Ratio | 0.5:1 | 1:1 | 1.5:1 | 2:1 | |
| Lysine (mg/g digesta) | 4.50[c] | 6.35[b] | 6.54[b] | 12.38[a] | 7.143** |

**) significant (P<0.01)

Digesta lysine concentration (DM basis) was regressed on the concentration of guanidinated gelatin in test diets (Figure 4.4.1). The regression model and the slope were significant (P<0.05), and the intercept was not significant. The regression equation fitted

$$Y = -2.731 + 0.194\ X$$

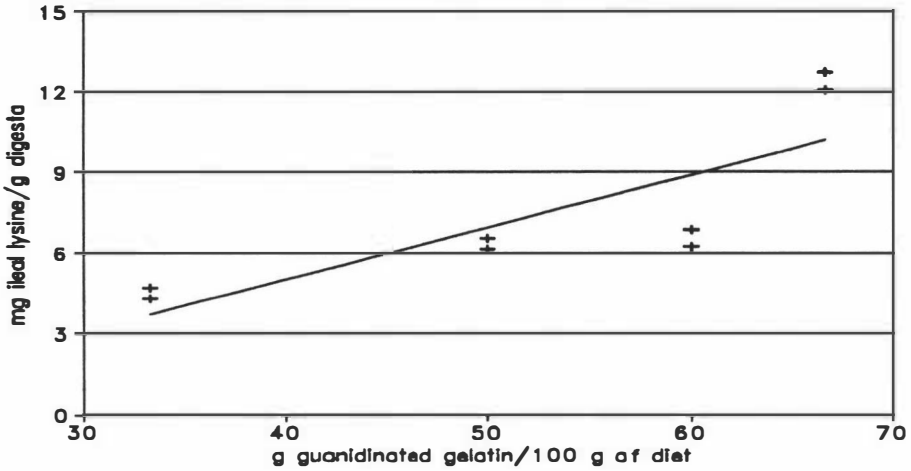
in which

Y = lysine ileal excretion (mg/g digesta)

X = g guanidinated gelatin/100 g of diet

suggests that the feeding of the cellulose-cornstarch mixture alone at a level of 10 g would result in negative 2.731 mg of ileal lysine per g digesta (DM)(non significant) and that each gram of increase of guanidinated gelatin per 100 g diet would raise digesta lysine concentration by 0.194 mg/g digesta (DM)(P<0.05).

Figure 4.4.1. Relationship between lysine concentration in ileal digesta and dietary concentration of guanidinated gelatin.




Dietary ratios were formulated such that for an actual feeding of 20 g application of the expression

$$\frac{\text{mg Cr/g test diet}}{\text{mg Cr/ g digesta}} \times \text{mg N*/g digesta} \times \text{feeding level} \times \text{multiplier}$$

*) mg lysine/g digesta (for determination of endogenous lysine excretion)

to each treatment would give a series of digesta N or lysine values equivalent to guanidinated gelatin:base intakes of 5:10, 10:10, 15:10 and 20:10 g.

The relationship between multiplier, dietary ratios and guanidinated gelatin and base for equivalent guanidinated gelatin intakes of 5 through to 20 g are shown in Table 4.4.4.

Table 4.4.4. Multipliers used to obtain ileal excretion of nitrogen and endogenous lysine for specified intakes of dietary components.

| | Diet | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| GG (g) | 5 | 10 | 15 | 20 |
| Base (g) | 10 | 10 | 10 | 10 |
| Feeding level 20 g | | | | |
| Multiplier | 15/20 | 1 | 25/20 | 30/20 |

The ileal N excreted for intakes of 5:10 and 10:10 g were significantly less ($P<0.01$) at 2631 mg, and 10357 mg respectively than that of 20:10 g (GG:base) of 65403 mg (Table 4.4.5. refers). Intake of 15:10 (24864 mg) was not significantly different to those of the 5:10, 10:10 and 20:10 g (GG:base).

Table 4.4.5. Total ileal N excretion (mg) associated with different guanidinated gelatin intakes at constant base intakes.

| | Diet | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| GG (g) | 5 | 10 | 15 | 20 |
| Base (g) | 10 | 10 | 10 | 10 |
| Total N (mg)** | 2631[b] | 10357[b] | 24864[ab] | 65403[a] |

**) significant ($P<0.01$)

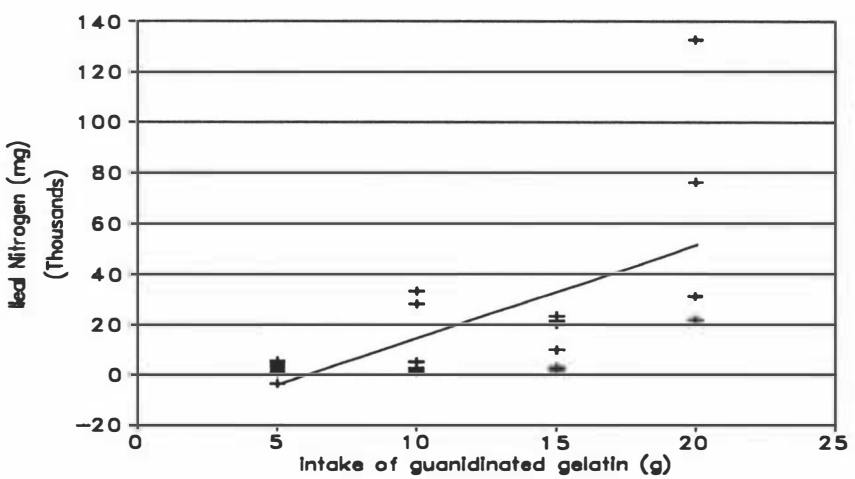
Total ileal N was regressed against guanidinated gelatin intake (Figure 4.4.2). The regression model(linear) and slope were highly significant ($P<0.01$), but the intercept was not significant. The regression equation fitted

$$Y = -22085.630 + 3719.710\ X$$

in which Y = the total N ileal excretion (mg)
and X = intake of guanidinated gelatin (g)

indicated that the feeding of the cellulose-cornstarch mixture alone at a level of 10 g would result in negative -22085.630 mg of ileal N excretion (non significant) and that each gram of dietary guanidinated gelatin would increase excretion by 3719.710 mg ($P<0.01$).

Figure 4.4.2. Relationship between total ileal N and increasing intakes of guanidinated gelatin at a constant base intake.




The total ileal lysine excreted differed significantly ($P<0.01$) for an intake of 20:10 (GG:base) at 5895.2 mg than those of 5:10, 10:10 and 15:10 (GG:base) of respectively 173.1 mg, 278.5 mg and 691.3 mg (Table 4.4.6).

Table 4.4.6. Total ileal lysine excretion (mg) associated with different guanidinated gelatin intakes at a constant base intake.

| | Diet | | | | Mean |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| GG (g) | 5 | 10 | 15 | 20 | |
| Base (g) | 10 | 10 | 10 | 10 | |
| Feeding level 20 g | | | | | |
| Ileal lysine(mg) | 172.93[b] | 278.71[b] | 691.82[b] | 5895.24[a] | 1759.54** |

*) Significant (P<0.05)

Total ileal lysine was regressed against guanidinated gelatin intake (Figure 4.4.3.). The regression model(linear) and slope were significant (P<0.05), but the intercept was not significant. The regression equation fitted

$$Y = -2635.251 + 351.583\ X$$

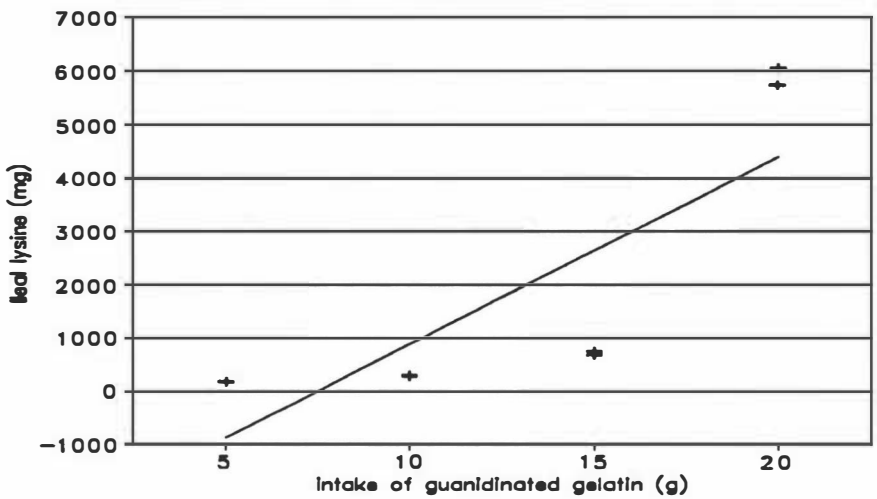
in which

Y = total ileal lysine excretion (mg)

and X = intake of guanidinated gelatin (g)

indicated that the feeding of the cellulose-cornstarch mixture alone at a level of 10 g would result in negative 2635 mg of ileal lysine excretion (non significant) and that each gram of dietary guanidinated gelatin would increase excretion of lysine by 351.583 mg (P<0.05).

Figure 4.4.3. Relationship between total ileal lysine and increasing intakes of guanidinated gelatin at a constant base intake.




The estimate of ileal N and lysine excretion associated with increasing guanidinated gelatin intakes at a constant base intake of 10 g was reanalysed this time excluding the extreme response data of treatment 4. The response for total ileal N was not significantly different over diets 1 to 3, but for total ileal lysine that of diet 3 (691.82 mg) was significantly greater ($P<0.05$) than than of diet 2 (278.71 mg) and diet 1 (172.93 mg). Respective regression equations were for total ileal N

$$Y = -9615.022 + 2223.237\ X$$

and for total ileal lysine

$$Y = -137.210 + 51.817\ X$$

where Y = mg of N or lysine

and X = intake of guanidinated gelatin in grams

Figure 4.4.4. Relationship between total ileal N and increasing intakes of guanidinated gelatin at a constant base intake.

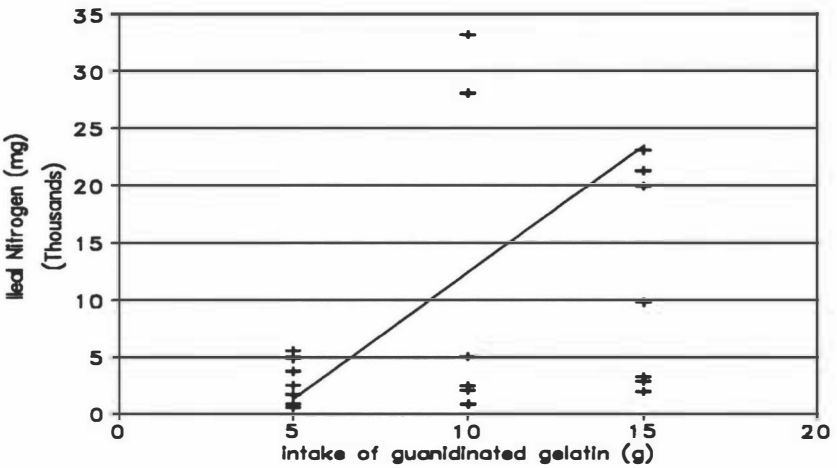
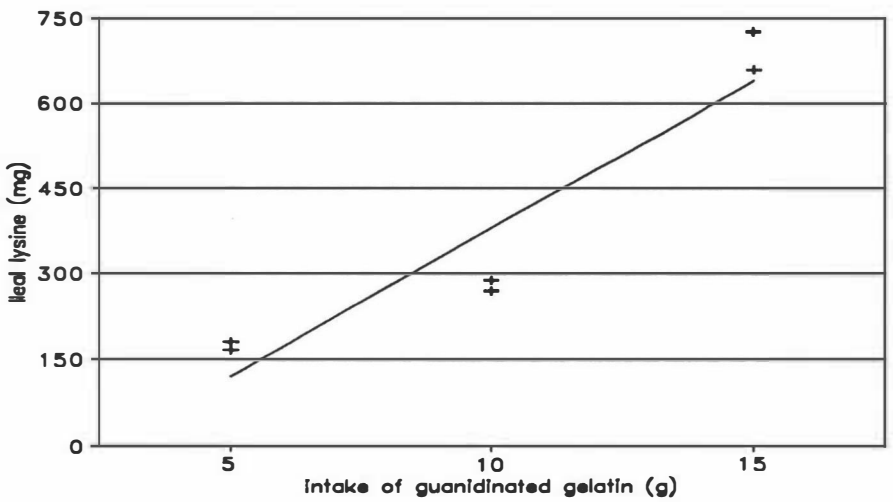



Figure 4.4.5. Relationship between total ileal lysine and increasing intakes of guanidinated gelatin at a constant base intake.




Both the regression model(linear) and slope were significant at $P<0.05$ for ileal N and $P<0.01$ for ileal lysine, but in both cases the intercepts were not

significantly different to zero (Figure 4.4.4. for ileal N and 4.4.5. for ileal lysine refer).

## DISCUSSION

Difficulty was experienced in reliably predicting digesta flow through the terminal ileum. In some cases the digesta volume collected was disappointingly small and in some cases the colouration of digesta suggested chromium content was less than usually experienced.

The concentration of N in the digesta increased with increasing concentration of guanidinated gelatin in the diet. The direction of this change was consistent with a diminishing N utilisation efficiency in the gut with increasing dietary concentrations of protein. In this trial the concentration in the diet of guanidinated gelatin increased from 33 2/3 to 66 2/3%.

The concentration of Cr in the digesta diminished (non significant) from 0.117% in the 0.5:1 (GG:base) diet to 0.019% in the 2:1 ratio diet. Such a trend implies more digesta is being produced at higher dietary ratios than lower and suggests that feed residues are greater with increasing concentration of guanidinated gelatin in the diet or conversely that feed residues are increasing as dietary cellulose concentration diminishes. This is inconsistent with the digestion characteristics of protein and cellulose and casts doubt on the validity of extrapolations involving the chromium measurements, particularly of diet 4.

The concentration of ileal lysine increased significantly from 4.5 mg/g digesta in diet 0.5:1 to 12.38 in diet 2:1. This is consistent with endogenous lysine excretion increasing with increasing concentration of protein in the diet, and is in agreement with Siriwan, *et al*. (1989). The regression equation fitted was significant for the model (linear) and the slope.

The use of multipliers to estimate ileal N and lysine excretion with increasing intakes of guanidinated gelatin at constant intakes of base involved Cr determinations by way of the expression

$$\frac{\text{mg Cr/g test diet}}{\text{mg Cr/g digesta}}$$

that calculates the quantity of digesta produced for each unit quantity of food fed. The ileal N excretions associated with 5, 10, 15 and 20 g intakes of guanidinated gelatin at constant base intakes of 10 g were excessive and invalid in the case of N and lysine. Some intakes were resulting in more N excreted than protein fed. It is concluded that the digesta Cr concentrations obtained, incorrectly characterised the quantity of digesta passaged and resulted in extreme and corrupt values of N and lysine. The fundamental cause of this problem seemed to lie with an inability to obtain satisfactory volumes of digesta and an inability to identity satisfactory slaughter times.

The % digesta Cr in diet 3 (0.071) was about half that of diet 2 (0.137) and the value of diet 4 (0.019) was about one seventh that of diet 2. Diet 4 data was excluded in an endeavour to obtain regression slopes less influenced by strong directional change in digesta Cr concentrations. The slope for N indicated 2223 mg of N was passaging in the

ileal digesta for each gram of guanidinated gelatin fed and suggests the feeding of gelatin was causing negative N balance. This was not considered sensible and consequently both N and lysine regression slopes involving dietary treatments 1 to 3 only are not considered representative of a physiological response.

# CHAPTER 5
# SUMMARY AND CONCLUSIONS

In experiment 1, swelling index and storage modulus (G') were employed to assess the water swelling and elasticity characteristics of the faeces and ileal digesta of four commonly used feedstuffs, two cereals, maize and barley, meat and bone meal and the legume, peas. Further treatments included mixing in a 1:1 ratio the aforementioned foodstuffs with sorghum. A final four dietary treatments involved assessing cellulose and cellulose mixed in increasing ratio with a medicinal bulking agent, Granocol.

Swelling index was measured on excreta collected after 12 hours and on that collected between 12 and 21 hours following feeding. The second collection was reduced in volume and showed swelling index variation within ingredients sufficiently large to deny the stage of collection of useful application. The first collection resulted in satisfactory volumes of excreta, but the range of swelling indices, 4.112 to 8.186 and the insensitivity of the measure to the addition of sorghum indicated that the method was unlikely to yield a measuring index that would differentiate between different varieties of cereal or legumes. The index did differentiate between barley and meat and bone meal but not between maize and meat and bone meal and the response to added sorghum was small and non significant in all cases. There was a marginal movement in swelling index to the addition of granocol to cellulose, but it was not significant and irregular.

Storage modulus estimates resulted in a large range of moduli, between 2.7 and 76 K Pa, but the addition of granocol to cellulose and of sorghum to the cereals, peas and meat and bone meal produced responses that were inconsistant in both direction and magnitute. Thus addition to cellulose of granocol in the ration of 4.5:0.5 raised storage modulus from 11.3 to 17.8 K Pa, but additional granocol resulted in values of 3.5 to 2.8 K Pa. Addition of sorghum to meat and bone meal raised storage modulus from 37.4 to 76.2 K Pa, but addition of sorghum to barley increased G' from 10.9 to 13.7 K Pa, responses that are difficult to reconcile.

Current physico-chemical evaluations of fibre or the digesta of fibrous foods commonly measure viscosity and involve solubilization, centrifugation and assessment of the supernatant (Larsen, 1991 ; Smits and Annison, 1994). In these procedures the phase on which viscosity is measured is liquid. In assessing the elastic properties of ileal digesta the substrate is coloidal with both solid and acqueous phases. The proportional contribution of the phases cannot be controlled and their proportions will alter the coloidal properties of the digesta.

Neither measure, swelling index or storage modulus were significantly correlated to dietary levels of neutral detergent fibre and acid detergent fibre or to each other. It is concluded that both measures are unlikely to prove useful as indices for measuring the physico-chemical or digestibility characteristics of feedstuffs.

Endeavours to quantify relationships between endogenous N and amino acid excretion at the terminal ileum to the dietary components, protein, starch and fibre may have considerable application if the extent to which

terminal gut excretions respond to changes in diet composition change little in composition but vary in volume. Experiment 2 to 4 were undertaken to elicit information on the degree of response to dietary cellulose and protein at the terminal ileum.

In experiment 2, the feeding of diets containing increasing ratios of cellulose to cornstarch significantly reduced the concentration of N in the ileal digesta. If it can be assumed that cellulose is inert and that cornstarch is wholy digested and absorbed and the food residues passaging at the terminal ileum are cellulose, then the outcome suggests that the gut secretory response was greater for diets with larger proportions of cornstarch or lesser for increasing proportions of cellulose and that the influence of digesta bulk on the excretion of exfoliated cells and mucus was relatively small compared to the response and elimination from the tract of gut enzymes.

Chromium concentration in the ileal digesta was relatively constant for diets 2:1, 3:1 and 4:1 (cellulose: cornstarch) at between 0.203 and 0.231 percent, but that of diet 1:1 was raised significantly (0.281%). These concentrations are consistant with increasing digesta cellulosic material arising from increasing ratios of the dietary cellulose component and compare favourably with concentrations obtained for chromium in experiment 3 in which the dietary proportion of cellulose and cornstarch was very much reduced by replacement with gelatin. In experiment 3, chromium concentrations were 0.325 percent for diet 0.5:1 (gelatin:cellulose+cornstarch base) and increased to 0.614 percent for diet 2:1. It is to be expected that as the proportion of digestible component is increased (gelatin) the quantity of ileal digesta produced will be diminished and the concentration of digesta

chromium will rise. This representation is reflected by the direction of response in experiment 3. Thus the chromium concentrations in the ileal digesta have a logical interpretation and changes were in directions that were in accordance with the expected digestibilities of dietary components.

In consequence the measure, response of endogenous N to changing levels of cellulose of 1.154 mg N for each g intake of dietary cellulose appears to be sound.

The concentration of N in the ileal digesta in experiment 3 increased with increasing concentration of gelatin in the test diets. Regression analysis indicated that 34 mg of ileal N was being passaged for each gram of gelatin fed and calculations suggested this amounted to an apparent N digestibility of 54 percent with a true digestibility somewhat greater.

The wet method of feeding facilitated delivery of the artificial diets into the crop and although difficulty was experienced in predicting slaughter times that would yield large digesta volumes, the chromium and N concentrations of the digesta suggested that representative responses were obtained.

In experiment 4, guanidination of the protein gelatin resulted in an 86 percent transformation of lysine into homoarginine. This potentially was a source of confounding and in future activities the guanidination procedure should be repeated a sufficient number of times to ensure greater levels of transformation are obtained. The ileal digesta N response to increasing levels of dietary gelatin was clearly corrupt. The regression slope indicated greater N was being excreted than gelatin fed. The response was

attributed to very low digesta chromium concentration (values of 0.117 to 0.019% were obtained) and ultimately to an inability to predict optimal slaughter times and obtain satisfactory volumes of ileal digesta. N concentrations in the digesta were approximately two times those obtained in experiment 3. The explanation for this remains obscure. With the chromium concentration of the sample of digesta being small it suggests a significant proportion of the N was other than dietary. The unreconcilable chromium and N concentrations indicated that the lysine response to increasing dietary levels of guanidinated gelatin were faulted and probably misrepresentative.

The use of multipliers to obtain response in N or lysine endogenous excretion to changing levels of cellulose and guanidinated gelatin is a technique that eliminates confounding in respect to which of two changing components in a two component diet is causing the response. There seems no valid reason why in future studies the base fraction of a two component mixture should not be made more compatible with standard feeds and reduce the artificiality of the test diet. Thus, in experiment 2 where the test diet consisted of a base, cornstarch, to which the test fraction cellulose was added in changing proportion, the test diet could have contained a base fraction composed perhaps of a conventional chick diet to which the cellulose could have been added. Likewise, in studies on the effect of starch and guanidinated gelatin intake on endogenous AA and N excretion the base in each case could be made more "normal" in composition without incurring confounding. The clarification of issues giving rise to unconfounded responses when dealing with two component mixtures is the most significant contribution of this work to the determination of endogenous AA and N excretion.

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## APPENDIX 1.

## DETERMINATION OF NEUTRAL DETERGENT FIBRE (NDF) AND ACID DETERGENT FIBRE (ADF)

**Reagents**

Neutral detergent solution

α-amylase solution

Acid detergent solution

Na Lauryl sulphate or Na dodecyl sulphate

$Na_2$ Ethylenediaminetetra acetic acid (EDTA)

Na Tetraborate decahydrate

$Na_2$ Hydrogen phosphate anhydrous ($Na_2HPO_4$)

NaOH

HCl

Cetyl trimethylammonium bromide (CTAB)

$H_2SO_4$

**Preparations**

1. Neutral detergent (ND) solution

   60.00 g Na lauryl sulphate (or Na dodecyl sulphate)

   37.22 g $Na_2$ EDTA

   13,62 g Na tetraborate decahydrate

   9.12 g $Na_2$ hydrogen phosphate anhydrous ($Na_2HPO_4$)

   Weight 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 of 6.9 - 7.1 and adjust with NaOH or HCl if necessary.

2. 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.

3. Acid detergent (AD) solution

Put 2 litre conical flask containing about 1 litre of distilled water in sink. Wearing goggles, carefully add 56 ml concentrated sulphuric acid ($H_2SO_4$). Make up to 2 litre with distilled water = 5% w/w $H_2SO_4$. Weight 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 on bench overnight to allow the mixture completely dissolve.

**Analytical procedure:**

DAY 1.

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

(2) Accurately weigh about 1 g of sample into 400 ml beakers. Add boiling beads and assign "W" to the weight of each sample.

(3) 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).

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

(5) Boil some distilled water in the jug for rinsing the beakers and washing the samples through the crucible.

(6) While the mixture is still hot, filter samples through correspondingly labelled crucibles. Use the vacuum system to assist and rinse all sample off the watchglasses and beaker sides free using a washbottle containing hot distilled water.

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

DAY 2.

(8) Remove crucibles out of the furnace and cool in a desiccator. Weigh the crucibles with sample residue as CNDF = crucible + neutral detergent residue (NDF).

(9) Put crucible sideways into 600 ml beakers and cover with AD solution. Use watchglass to cover beakers. Boil gently (as previously) on a hot plate for 1 hour.

(10) Boil distilled water and take beakers to the vacuum system. Using hot water, wash crucible sides and bottom to remove all sample from the crucibles into the beakers.

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

DAY 3.

(12) Remove crucibles containing sample from the oven, cool in a desiccator and then weigh as CADF = crucible + AD residue (ADF).

(13) Calculation:

a. $\% \text{ NDF} = \frac{\text{CNDF} - \text{C}}{\text{W}} \times 100$

b. $\% \text{ ADF} = \frac{\text{CADF} - \text{C}}{\text{W}} \times 100$

## APPENDIX 2.

## DETERMINATION OF KJELDAHL NITROGEN CONTENT WITH A KJELTEC 1030 AUTO ANALYSER SYSTEM

**Reagents**

Sulphuric acid, concentrated analytical grade N-free.
Kjeltab (Se) (macro or micro)
Sodium Hydroxide analytical grade 35-40%
Receiver solution
Hydrochloric Acid (0.01 M, 0.1 M, 0.2 M or 0.5 M)

**Preparations**

1. Sodium hydroxide

   Weigh 2 kg Sodium Hydroxide disolve in 5 litres distilled water. Make it gradually, not the whole lot.

2. Receiver Solution

   Disolve 100 g Boric Acid in 10 litres distilled or deionised water. Add 100 ml Bromocresol Green solution (0.1 g in 100 ml methanol). Add 70 ml Methyl Red solution (0.07 g in 70 ml methanol). Add 1 ml 1 M (4%) Sodium Hydroxide (2 mg in 5 ml) until greeny-black colour.

**Analytical procedure:**

(1) Dry and well-mix samples are finely ground to pass through 1 mm mesh.
(2) Weigh the samples (less than 0.1 g for micro and 0.8 g for macro) using weighing boats and quantitatively transfer into 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 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 preheat digester (420 $^{0}$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 samples solution to hand temperature and dilute with distilled water (10 ml for micro and 30 ml for macro) and mixed.

(11) Start up the Kjeltec Auto 1030 Analyser following the procedure witten in the manual.

(12) Then, total N or crude protein can be calculated 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 N

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 0.5 M HCl. For semi-micro analysis use 0.1M HCl, and for micro analysis use 0.01M HCl.

## APPENDIX 3.

## CHROMIUM ANALYSIS USING ATOMIC ABSORPTION SPECTROMETRY (AAS)

**Reagents**

Phosphoric acid/manganese sulphate solution

Potassium bromate

Standard chromium solution for calibration

Stock (equivalent to 1000 g $Cr_2O_3$/ml)

Working standard

**Preparations**

1. Phosphoric acid/manganese sulphate solution
   a. 10% w/v $MnSO_4.4H_2O$
   b. 85% w/w Orthophosphoric acid
   c. mix a. and b. with the proportion of 3 : 97 (v/v)
2. 4.5 % w/v aqueous solution Potassium bromate
3. Standard chromium solution for calibration
   Blank (chromium-free) : prepared by ashing and digesting chromium-free sample using the same relative amounts of reagents as in each sample determination.
4. Stock (equivalent to 1000 g $Cr_2O_3$/ml) : dissolve 1.9355 g potassium dichromate in distilled water and make up to 1 litre.
5. Working standards : Standards in the range equivalent to 0 - 25 g Cr2O3/ml made up from stock solution diluted to volume with the blank.

**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 and weight them thereafter.

(3) Weight the samples (15-40 mg for ileal sample and 100 g for diet) in the the beakers and dry at 105°C overnight or until a constant weight. Cool in the desiccator and reweight to obtain dry matter.

(4) Ash at 500°C furnace overnight to ensure a complete combustion.

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

(6) Remove the glass plate and add 4 ml of 4.5 % Potassium bromate to each beakers. Place back in a heat block and cover the beakers with a glass plate. Then raise the heat block to 220°C (approximately 45 minutes).

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

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

(9) Read using Atomic Absorption Spectrometry (AAS) at 357.9 nm with a nitrous oxide/acetylene flame.

## APPENDIX 4.

## GUANIDINATION OF DIETARY LYSINE (GELATIN)

**Reagents**

0.6M O-Methylisurea (MIU)

$Ba(OH)_2$

Gelatin

2M HCl

2M NaOH

**Analytical Procedure**

a. Prepare 38% (w/v) $Ba(OH)_2$ with boiling deionised water. Reheat the $Ba(OH)_2$ solution almost to boiling point until maximum dissolution has occured, then quickly add to sufficient MIU to make a 0.6M solution.

b. Stand for 30 minutes.

c. Remove the resulting $Ba(OH)_2$ by transferring solution into 250 ml tubes and centrifuging 6400g 10 min at room temperature using Sorval $RC_2$-B with GSA rotor.

d. Pool the supernatant.

e. Add the filtrate a minimum volume of distilled water and then recentrifuge again. Collect the supernatant and adjust its pH to 11.0 with 2M HCl.

f. Make solution up to 90% of volume with distilled water. Add 10% protein solution in 0.6M MIU. At the mean time adjust the pH of solution to 10.6 with either 2M HCl or 2M NaOH.

g. Incubate 20°C for 6 days and chech pH daily.

h. After 6 days remove unreacted MIU by ultrafilter. However, in this experiment ultrafiltration failed. While waiting for Dowex-500, the process of guanidination was repeated again.

i. After the second incubation finished, unreacted MIU was then removed using Dowex-500. The result was convertion rate of dietary lysine into homoarginine was 86%.

## APPENDIX 5.

## DETERMINATION OF AMINO ACID - ACID HYDROLYSIS PROCEDURE

**Reagents and preparations**

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 10-20 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 2 litre volumetric flask and store in refrigerator.

2.5. µm Norleucine

2.2 % Sodium citrate

**Analytical procedure:**

(1) Weigh out finely ground samples (approximately 50 mg) using weighing scoops with elongated ends.
(2) Transfer the weighed samples quantitatively into a hydrolysis tubes by first tilting the tubes to approximately 30°C. 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 µm Norleucine standard into the tubes.
(4) Add 20 ml of 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 hrs.

(10) Filter the hydrolysate 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 flask to the rotary evaporators and evaporate the hydrolisates to dryness. Wash 3 times with demineralised water evaporating to dryness each time.

(12) Transfer quantitatively the dried hydrolysates into 25 ml volumetric flasks, using 5x5.0 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 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 µl, depending on the percentage N content) into vials. The vials are loaded into the waters High Pressure Liquid Chromatograph (HPLC) for analysis.

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

## APPENDIX 6.

### INGREDIENT COMPOSITION
### OF PELLETED BROILER FINISHER DIET, EXPERIMENTS 1 TO 4

| Ingredient | percentage |
|---|---|
| Wheat | 10.00 |
| Barley | 11.35 |
| Maize | 45.00 |
| Broll | 5.00 |
| Soybean Meal | 18.80 |
| Meat and Bone Meal | 7.50 |
| Limestone | 0.90 |
| Salt | 0.08 |
| Sodium Bicarbonate | 0.28 |
| Lysine | 0.39 |
| DL-Methionine | 0.40 |
| Premix | 0.25 |

# APPENDIX 7.

## DATA FOR CALCULATIONS OF SWELLING INDEX AND STORAGE MODULUS

| 1st twelve hours | | | | | 2nd nine hours | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| code | note | A | B | C | code | note | A | B | C | D1 | E1 | SI.1 | D2 | E2 | SI.2 | Storage modulus |
| A.1.1 | suspension | 5.4 | 92 | 53.8 | A.1.1 | suspension | 0.4 | 30 | 26.3 | 38.2 | 5.2898 | 7.221 | 3.7 | 1.0368 | 3.569 | 59.7 |
| A.1.2 | suspension | 6 | 40 | 20 | A.1.2 | paste | 0 | 30 | 24.2 | 20 | 3.9938 | 5.008 | 5.8 | 1.4353 | 4.041 | 43.3 |
| A.1.3 | two-phase | 1.2 | 60 | 32 | A.1.3 | paste | 0 | 30 | 25 | 28 | 4.1487 | 6.749 | 5 | 0.9311 | 5.370 | 54.0 |
| A.1.4 | suspension | 32.4 | 80 | 53.3 | A.1.4 | two-phase | 4 | 40 | 26.7 | 26.7 | 3.2513 | 8.212 | 13.3 | 0.9732 | 13.666 | 64.5 |
| A.2.1 | solid | 11.2 | 80 | 43 | A.2.1 | two-phase | 4.85 | 30 | 18.7 | 37 | 4.7019 | 7.869 | 11.3 | 1.2895 | 8.763 | 13.5 |
| A.2.2 | two-phase | 5.4 | 50 | 17 | A.2.2 | two-phase | 0.05 | 50 | 38.3 | 33 | 4.408 | 7.486 | 11.7 | 1.7182 | 6.809 | 8.97 |
| A.2.3 | two-phase | 0 | 60 | 35.2 | A.2.3 | two-phase | 0.8 | 60 | 51 | 24.8 | 4.0985 | 6.051 | 9 | 1.4601 | 6.164 | 10.2 |
| A.2.4 | suspension | 23.7 | 60 | 25.5 | A.2.4 | two-phase | 4.4 | 40 | 27 | 34.5 | 3.0427 | 11.339 | 13 | 1.5901 | 8.176 | ** |
| A.3.1 | two-phase* | 14 | 70 | 42 | A.3.1 | two-phase | 4.1 | 30 | 18 | 28 | 7.5065 | 3.730 | 12 | 1.9622 | 6.116 | 76.3 |
| A.3.2 | two-phase | 0.6 | 40 | 20 | A.3.2 | paste | 0 | 40 | 35 | 20 | 7.2262 | 2.768 | 5 | 2.6412 | 1.893 | 14.7 |
| A.3.3 | two-phase | 4.8 | 50 | 28.5 | A.3.3 | solid | 0 | 60 | 46.4 | 21.5 | 4.7912 | 4.487 | 13.6 | 4.2714 | 3.184 | 2.97 |
| A.3.4 | two-phase | 8.4 | 50 | 16.8 | A.3.4 | two-phase | 5.7 | 40 | 24.2 | 33.2 | 5.9784 | 5.553 | 15.8 | 3.0298 | 5.215 | 55.7 |
| A.4.1. | solid | 0 | 50 | 13 | A.4.1. | solid | 0 | 30 | 18 | 37 | 9.3619 | 3.952 | 12 | 2.7863 | 4.307 | 27.3 |
| A.4.2 | suspension | 22 | 101 | 22 | A.4.2 | two-phase | 6.1 | 40 | 20 | 79 | 9.4803 | 8.333 | 20 | 1.9752 | 10.126 | 25.8 |
| A.4.3 | suspension | 19.5 | 124 | 45.2 | A.4.3 | solid | 0 | 40 | 29.5 | 78.8 | 8.9908 | 8.765 | 10.5 | 2.2501 | 4.666 | 25.6 |
| A.4.4 | two-phase | 6 | 50 | 28 | A.4.4 | nothing | 0 | 0 | 0 | 22 | 2.805 | 7.843 | 0 | 0 | 0 | 19.9 |
| A.5.1 | suspension | 9.8 | 50 | 28.5 | A.5.1 | two-phase | 0.95 | 30 | 23 | 21.5 | 4.1573 | 5.172 | 7 | 0.9263 | 7.557 | 75.4 |
| A.5.2 | two-phase | 3.8 | 50 | 19.5 | A.5.2 | two-phase | 0.25 | 30 | 23 | 30.5 | 4.3539 | 7.005 | 7 | 1.0517 | 6.656 | 69.1 |
| A.5.3 | two-phase | 0 | 70 | 41.8 | A.5.3 | two-phase | 2.4 | 40 | 29.8 | 28.2 | 5.2064 | 5.416 | 10.2 | 1.1710 | 8.711 | 53.5 |
| A.5.4 | solid | 0 | 50 | 39.2 | A.5.4 | paste | 0 | 40 | 33 | 10.8 | 3.012 | 3.586 | 7 | 1.4181 | 4.936 | 69.3 |
| A.6.1 | solid | 0 | 60 | 12.7 | A.6.1 | solid | 0 | 30 | 27 | 47.3 | 3.8034 | 12.436 | 3 | 0.7596 | 3.949 | 21.5 |
| A.6.2 | solid | 0 | 40 | 21 | A.6.2 | paste | 0 | 30 | 23 | 19 | 4.579 | 4.149 | 7 | 1.2026 | 5.821 | 15.0 |
| A.6.3 | two-phase | 4.4 | 60 | 38.2 | A.6.3 | two-phase | 0 | 60 | 27 | 21.8 | 3.6362 | 5.995 | 33 | 1.4730 | 22.403 | 11.1 |
| A.6.4 | two-phase | 2.2 | 50 | 25.6 | A.6.4 | two-phase | 0.2 | 30 | 23.6 | 24.4 | 3.3434 | 7.298 | 6.4 | 1.0110 | 6.330 | 7.18 |
| A.7.1 | two-phase | 0 | 50 | 30 | A.7.1 | two-phase | 1.6 | 30 | 21 | 20 | 6.0094 | 3.328 | 9 | 1.8482 | 4.870 | 105 |
| A.7.2 | two-phase | 1.4 | 40 | 16 | A.7.2 | two-phase | 0 | 30 | 22.6 | 24 | 6.3111 | 3.803 | 7.4 | 4.0911 | 1.809 | 57.8 |
| A.7.3 | two-phase | 17.4 | 60 | 20.3 | A.7.3 | two-phase | 1.6 | 50 | 37 | 39.7 | 7.4607 | 5.321 | 13 | 1.5659 | 8.302 | 66.1 |
| A.7.4 | two-phase | 1 | 50 | 30.8 | A.7.4 | solid | 0 | 50 | 37 | 19.2 | 4.8071 | 3.994 | 13 | 1.4685 | 8.853 | 75.7 |
| A.8.1 | paste | 19.9 | 113 | 46.5 | A.8.1 | suspension | 0.8 | 60 | 53 | 66.5 | 7.138 | 9.316 | 7 | 1.3634 | 5.134 | 45.0 |
| A.8.2 | solid | 0 | 70 | 42.5 | A.8.2 | two-phase | 4.2 | 30 | 18 | 27.5 | 6.6579 | 4.130 | 12 | 1.4560 | 8.242 | 30.0 |
| A.8.3 | two-phase | 2.2 | 70 | 25.2 | A.8.3 | two-phase | 6.2 | 40 | 20.6 | 44.8 | 6.1814 | 7.248 | 19.4 | 1.6298 | 11.903 | 59.2 |
| A.8.4 | suspension | 5.8 | 102 | 28 | A.8.4 | two-phase | 6.4 | 40 | 15.5 | 74 | 6.8119 | 10.863 | 24.5 | 1.0041 | 24.400 | 26.5 |
| A.9.1 | paste | 0 | 124 | 7 | A.9.1 | two-phase | 0.15 | 30 | 20.5 | 117 | 23.8642 | 4.903 | 9.5 | 1.9720 | 4.817 | 13.8 |
| A.9.2 | solid | 0 | 113 | 35 | A.9.2 | solid | 0 | 40 | 23.5 | 78 | 22.0098 | 3.544 | 16.5 | 4.1247 | 4.000 | 23.2 |
| A.9.3 | solid | 0 | 124 | 13 | A.9.3 | two-phase | 1.4 | 50 | 40 | 111 | 25.4973 | 4.353 | 10 | 2.4152 | 4.140 | 5.2 |
| A.9.4 | paste | 0 | 164 | 19.3 | A.9.4 | two-phase | 1.4 | 50 | 29 | 144.7 | 23.0216 | 6.285 | 21 | 2.4741 | 8.488 | 3.32 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A.10.1 | paste | 0 | 124 | 18 | A.10.1 | paste | 0 | 60 | 30 | 106 | 17.4185 | 6.085 | 30 | 5.1644 | 5.809 | 9.34 |
| A.10.2 | paste | 0 | 113 | 8.5 | A.10.2 | two-phase | 0 | 60 | 51 | 104.5 | 23.2141 | 4.502 | 9 | 2.9165 | 3.086 | 16.5 |
| A.10.3 | paste | 0 | 113 | 16 | A.10.3 | paste | 0 | 70 | 36.2 | 97 | 17.9706 | 5.398 | 33.8 | 5.3895 | 6.276 | 21.9 |
| A.10.4 | paste | 0 | 124 | 16.8 | A.10.4 | two-phase | 1 | 50 | 36 | 107.2 | 19.1952 | 5.585 | 14 | 2.3854 | 5.869 | 23.6 |
| A.11.1 | paste | 0 | 128 | 0 | A.11.1 | solid | 0 | 92 | 25 | 127.5 | 17.0818 | 7.464 | 67 | 7.8956 | 8.486 | 5.32 |
| A.11.2 | paste | 0 | 128 | 0 | A.11.2 | paste | 0 | 40 | 13 | 127.5 | 20.3069 | 6.279 | 27 | 3.8273 | 7.055 | 3.13 |
| A.11.3 | paste | 0 | 113 | 10.6 | A.11.3 | paste | 0 | 60 | 21 | 102.4 | 17.6709 | 5.795 | 39 | 5.3178 | 7.334 | 3.06 |
| A.11.4 | paste | 0 | 128 | 0 | A.11.4 | solid | 0.8 | 40 | 17.4 | 127.5 | 21.1845 | 6.019 | 22.6 | 3.0742 | 7.352 | 2.45 |
| A.12.1 | paste* | 0 | 194 | 0 | A.12.1 | two-phase | 6.2 | 60 | 45 | 194 | 21.9541 | 8.837 | 15 | 1.6025 | 9.360 | 2.41 |
| A.12.2 | two-phase* | 0 | 114 | 19.5 | A.12.2 | two-phase | 6.1 | 60 | 45.6 | 94.5 | 24.9747 | 3.784 | 14.4 | 1.7786 | 8.096 | 4.08 |
| A.12.3 | paste | 0 | 102 | 13.6 | A.12.3 | paste | 0 | 60 | 4.1 | 88.4 | 13.4668 | 6.564 | 55.9 | 7.4961 | 7.457 | 2.62 |
| A.12.4 | paste | 0 | 124 | 21.8 | A.12.4 | paste | 0 | 60 | 16.8 | 102.2 | 16.4814 | 6.201 | 43.2 | 6.6238 | 6.522 | 1.92 |

*=2 cups ** = not enough sample

Note :

A = Liquid removed (cc)
B = Final volume (cc)
C = Water added (cc)
D = Faecal volume (cc)
E = Faecal dry matter (g)

SI = Swelling index
1 = 1st 12 hour collection
2 = final 9 hour collection

## APPENDIX 8.

## DATA FROM Cr AND N COMPOSITION ARRANGED ACCORDING TO DIETS USED IN EXPERIMENT 2.

| Treatment | rep. | Cr Concentration in the digesta | | Cr Concentration in diet | N Concentration in the digesta | |
|---|---|---|---|---|---|---|
| | | % | mg/g | mg/g | % | mg/g |
| TR 1 | 1 | 0.2359 | 2.359 | 2.940 | 0.099 | 0.99 |
| | 3 | 0.3678 | 3.678 | 3.087 | 0.153 | 1.53 |
| | 4 | 0.2189 | 2.189 | 3.200 | 0.218 | 2.18 |
| TR 2 | 1 | 0.2463 | 2.463 | 2.973 | 0.088 | 0.88 |
| | 2 | 0.2755 | 2.755 | 3.047 | 0.166 | 1.66 |
| | 3 | 0.1836 | 1.836 | 2.980 | 0.088 | 0.88 |
| | 4 | 0.2344 | 2.344 | 3.060 | 0.086 | 0.86 |
| TR 3 | 1 | 0.2109 | 2.109 | 3.033 | 0.079 | 0.79 |
| | 2 | 0.2147 | 2.147 | 3.073 | 0.109 | 1.09 |
| | 3 | 0.2377 | 2.377 | 3.047 | 0.065 | 0.65 |
| | 4 | 0.2233 | 2.233 | 3.000 | 0.067 | 0.67 |
| TR 4 | 1 | 0.2496 | 2.496 | 3.093 | 0.112 | 1.12 |
| | 3 | 0.1969 | 1.969 | 3.047 | 0.187 | 1.87 |
| | 4 | 0.1976 | 1.976 | 3.060 | 0.081 | 0.81 |
| TR 5 | 1 | 0.3574 | 3.574 | 3.005 | 0.087 | 0.87 |
| | 2 | 0.2219 | 2.219 | 2.990 | 0.077 | 0.77 |
| | 3 | 0.2347 | 2.347 | 3.015 | 0.084 | 0.84 |
| | 4 | 0.2935 | 2.935 | 2.990 | 0.079 | 0.79 |
| TR 6 | 1 | 0.1987 | 1.987 | 2.995 | 0.211 | 2.11 |
| | 2 | 0.248 | 2.48 | 3.005 | 0.055 | 0.55 |
| | 3 | 0.2112 | 2.112 | 3.050 | 0.103 | 1.03 |
| TR 7 | 1 | 0.2664 | 2.664 | 3.005 | 0.143 | 1.43 |
| | 2 | 0.2525 | 2.525 | 3.005 | 0.115 | 1.15 |
| | 3 | 0.1898 | 1.898 | 3.060 | 0.176 | 1.76 |
| | 4 | 0.1051 | 1.051 | 2.995 | 0.033 | 0.33 |
| TR 8 | 1 | 0.1791 | 1.791 | 3.015 | 0.133 | 1.33 |
| | 2 | 0.2292 | 2.292 | 3.010 | 0.118 | 1.18 |
| | 3 | 0.1860 | 1.860 | 3.065 | 0.054 | 0.54 |
| | 4 | 0.2244 | 2.244 | 3.035 | 0.090 | 0.90 |
| TR 9 | 1 | 0.3263 | 3.263 | 3.028 | 0.093 | 0.93 |
| | 2 | 0.248 | 2.480 | 3.020 | 0.190 | 1.90 |
| | 4 | 0.3041 | 3.041 | 2.996 | 0.126 | 1.26 |
| TR 10 | 1 | 0.2178 | 2.178 | 3.016 | 0.222 | 2.22 |
| | 2 | 0.2483 | 2.483 | 3.020 | 0.240 | 2.40 |
| | 3 | 0.2061 | 2.061 | 3.032 | 0.341 | 3.41 |
| | 4 | 0.2738 | 2.738 | 3.016 | 0.105 | 1.05 |
| TR 11 | 1 | 0.2103 | 2.103 | 3.044 | 0.123 | 1.23 |
| | 2 | 0.2051 | 2.051 | 3.008 | 0.103 | 1.03 |
| | 3 | 0.1356 | 1.356 | 3.012 | 0.092 | 0.92 |
| | 4 | 0.1854 | 1.854 | 2.972 | 0.159 | 1.59 |
| TR 12 | 1 | 0.1882 | 1.882 | 2.984 | 0.104 | 1.04 |
| | 2 | 0.2008 | 2.008 | 3.008 | 0.077 | 0.77 |
| | 3 | 0.2060 | 2.060 | 3.036 | 0.072 | 0.72 |
| | 4 | 0.2024 | 2.024 | 3.020 | 0.032 | 0.32 |

## APPENDIX 9.

## DATA FROM Cr AND N COMPOSITION ARRANGED ACCORDING TO DIETS USED IN EXPERIMENT 3.

| Treatment | rep. | Cr Concentration in the digesta % | Cr Concentration in the digesta mg/g | Cr Concentration in diet mg/g | N Concentration in the digesta % | N Concentration in the digesta mg/g |
|---|---|---|---|---|---|---|
| TR 1 | one | 1 | 0.3083 | 3.083 | 0.906 | 9.06 |
| TR 1 | one | 2 | 0.2989 | 2.989 | 1.319 | 13.19 |
| TR 1 | one | 3 | 0.3171 | 3.171 | 1.183 | 11.83 |
| TR 1 | one | 4 | 0.4116 | 4.116 | 1.832 | 18.32 |
| TR 2 | two | 1 | 0.334 | 3.34 | 2.099 | 2 0.99 |
| TR 2 | two | 3 | 0.3973 | 3.973 | 1.468 | 14.68 |
| TR 2 | two | 4 | 0.2698 | 2.698 | 4.803 | 48.03 |
| TR 3 | tri | 1 | 0.4262 | 4.262 | 1.101 | 11.01 |
| TR 3 | tri | 2 | 0.3689 | 3.689 | 4.471 | 44.71 |
| TR 3 | tri | 3 | 0.4351 | 4.351 | 2.203 | 22.03 |
| TR 3 | tri | 4 | 0.747 | 7.47 | 1.177 | 11.77 |
| TR 4 | four | 2 | 0.3325 | 3.325 | 5.36 | 53.6 |
| TR 4 | four | 3 | 0.5293 | 5.293 | 5.327 | 53.27 |
| TR 4 | four | 4 | 0.3935 | 3.935 | 3.675 | 36.75 |
| TR 5 | one | 1 | 0.291 | 2.91 | 1.03 | 10.3 |
| TR 5 | one | 2 | 0.2766 | 2.766 | 1.572 | 15.72 |
| TR 5 | one | 3 | 0.3543 | 3.543 | 3.813 | 38.13 |
| TR 5 | one | 4 | 0.3192 | 3.192 | 1.984 | 19.84 |
| TR 6 | two | 1 | 0.3493 | 3.493 | 1.658 | 16.58 |
| TR 6 | two | 2 | 0.3869 | 3.869 | 4.869 | 48.69 |
| TR 6 | two | 3 | 0.5992 | 5.992 | 2.989 | 29.89 |
| TR 6 | two | 4 | 0.4015 | 4.015 | 4.309 | 43.09 |
| TR 7 | tri | 1 | 0.3652 | 3.652 | 1.881 | 18.81 |
| TR 7 | tri | 2 | 0.3757 | 3.757 | 5.251 | 52.51 |
| TR 7 | tri | 3 | 0.4258 | 4.258 | 6.359 | 63.59 |
| TR 7 | tri | 4 | 0.3544 | 3.544 | 4.497 | 44.97 |
| TR 8 | four | 2 | 0.7495 | 7.495 | 4.065 | 40.65 |
| TR 8 | four | 3 | 0.7718 | 7.718 | 5.359 | 53.59 |
| TR 8 | four | 4 | 0.3274 | 3.274 | 5.24 | 52.4 |
| TR 9 | one | 1 | 0.3088 | 3.088 | 1.41 | 14.1 |
| TR 9 | one | 2 | 0.3342 | 3.342 | 1.463 | 14.63 |
| TR 9 | one | 3 | 0.2898 | 2.898 | 2.066 | 20.66 |
| TR 9 | one | 4 | 0.3913 | 3.913 | 4.273 | 42.73 |
| TR 10 | two | 1 | 0.5056 | 5.056 | 3.712 | 37.12 |
| TR 10 | two | 2 | 0.3401 | 3.401 | 5.236 | 52.36 |
| TR 10 | two | 3 | 0.5059 | 5.059 | 5.242 | 52.42 |
| TR 10 | two | 4 | 0.5509 | 5.509 | 1.801 | 18.01 |
| TR 11 | tri | 1 | 0.2092 | 2.092 | 2.701 | 27.01 |
| TR 11 | tri | 2 | 0.4021 | 4.021 | 4.65 | 46.5 |
| TR 11 | tri | 3 | 1.049 | 10.49 | 4.915 | 49.15 |
| TR 11 | tri | 4 | 0.5715 | 5.715 | 3.758 | 37.58 |
| TR 12 | four | 1 | 0.8781 | 8.781 | 3.959 | 39.59 |
| TR 12 | four | 2 | 0.8208 | 8.208 | 5.62 | 56.2 |
| TR 12 | four | 4 | 0.721 | 7.21 | 2.88 | 28.8 |

## APPENDIX 10

## DATA FROM Cr AND N COMPOSITION ARRANGED ACCORDING TO DIETS USED IN EXPERIMENT 4.

| Treatment (Tr) | Replication | Chromium analysis in the digesta (%) | mg/g | N analysis in the digesta (%) | mg/g |
|---|---|---|---|---|---|
| 1 | 1 | 0.2597 | 2.597 | 5.488 | 54.88 |
| 1 | 2 | 0.092 | 0.92 | 3.635 | 36.35 |
| 1 | 3 | 0.0526 | 0.526 | 4.402 | 44.02 |
| 1 | 4 | 0.1557 | 1.557 | 2 | 20 |
| 1 | 5 | * | * | * | |
| * | | | | | |
| 1 | 6 | 0.0455 | 0.455 | 4.966 | 49.66 |
| 1 | 7 | 0.1717 | 1.717 | 3.456 | 34.56 |
| 1 | 8 | 0.0427 | 0.427 | 5.247 | 52.47 |
| 2 | 1 | 0.077 | 0.77 | 6.465 | 64.65 |
| 2 | 2 | 0.2488 | 2.488 | 10.458 | 104.58 |
| 2 | 3 | 0.0288 | 0.288 | 13.451 | 134.51 |
| 2 | 4 | 0.2122 | 2.122 | 2.731 | 27.31 |
| 2 | 5 | 0.0154 | 0.154 | 8.519 | 85.19 |
| 2 | 6 | ** | ** | 6.827 | 68.27 |
| 2 | 7 | 0.2068 | 2.068 | 2.828 | 28.28 |
| 2 | 8 | 0.1677 | 1.677 | 5.959 | 59.59 |
| 3 | 1 | 0.0421 | 0.421 | 11.922 | 119.22 |
| 3 | 2 | ** | ** | 12.082 | 120.82 |
| 3 | 3 | 0.2094 | 2.094 | 5.386 | 53.86 |
| 3 | 4 | 0.1316 | 1.316 | 5.057 | 50.57 |
| 3 | 5 | 0.0034 | 0.034 | 4.323 | 43.23 |
| 3 | 6 | 0.0229 | 0.229 | 7.026 | 70.26 |
| 3 | 7 | 0.0557 | 0.557 | 7.269 | 72.69 |
| 3 | 8 | 0.0311 | 0.311 | 8.226 | 82.26 |
| 4 | 1 | 0.0069 | 0.069 | 10.155 | 101.55 |
| 4 | 2 | ** | ** | 8.726 | 87.26 |
| 4 | 3 | ** | ** | 7.103 | 71.03 |
| 4 | 4 | 0.0054 | 0.054 | 4.573 | 45.73 |
| 4 | 5 | ** | ** | 8.303 | 83.03 |
| 4 | 6 | ** | ** | 9.152 | 91.52 |
| 4 | 7 | 0.0217 | 0.217 | 7.499 | 74.99 |
| 4 | 8 | 0.0416 | 0.416 | 10.093 | 100.93 |

*) the sample too few (not enough for analysis)
**) not detectable

## APPENDIX 11.

## SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENT 1.

1. Correlation

| Combination | N | Pearson Correlation | Prob>\|R\| under Ho:Rho=0 |
|---|---|---|---|
| SI.1 x NDF | 12 | -0.4096 | 0.1871 |
| SI.1 x ADF | 12 | -0.4678 | 0.1251 |
| SI.2 x NDF | 12 | -0.4170 | 0.1774 |
| SI.2 x ADF | 12 | -0.4329 | 0.1598 |
| SM x NDF | 12 | 0.2694 | 0.3971 |
| SM x ADF | 12 | 0.3717 | 0.2341 |
| SI.1 x SM | 12 | -0.3836 | 0.2183 |

note:
SI.1 = Swelling index obtained from the 1st 12 hours
SI.2 = Swelling index obtained from the 2nd 9 hours
SM = Storage modulus (G')

2. One-way analysis of variance

| | DF | SS | MS | Fvalue | Pr>F |
|---|---|---|---|---|---|
| Model<br>SI.1 = Feedstuffs | 11 | 88.0007 | 8.0001 | 2.21 | 0.0365* |
| Error | 35 | 130.5090 | 3.6252 | | |
| Model<br>SI.2 = Feedstuffs | 11 | 211.5719 | 19.2338 | 1.11 | 0.3797 |
| Error | 35 | 604.1019 | 17.2601 | | |
| Model<br>SM = Feedstuffs | 11 | 27175.36 | 2470.49 | 13.22 | 0.0001 |
| Error | 35 | 6541.24 | 186.89 | | |

*) Significant (P<0.05)
**) Significant (P<0.01)

## APPENDIX 12.

## SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENT 2.

1. TWO-WAY ANALYSIS OF VARIANCE

| | DF | SS | MS | F value | Pr>F |
|---|---|---|---|---|---|
| Model | | | | | |
| % N = fl ra fl*ra | 11 | 0.0388 | 0.0035 | 1.55 | 0.1647 |
| Error | 31 | 0.0706 | 0.0023 | | |
| Type III-SS | | | | | |
| fl | 2 | 0.0032 | 0.0016 | 0.71 | 0.4982 |
| ra | 3 | 0.0184 | 0.0061 | 2.69 | 0.0635 |
| fl*ra | 6 | 0.0190 | 0.0032 | 1.39 | 0.2486 |
| Model | | | | | |
| % CR = fl ra fl*ra | 11 | 0.0463 | 0.0042 | 2.19 | 0.0426* |
| Error | 31 | 0.0595 | 0.0019 | | |
| Type III-SS | | | | | |
| fl | 2 | 0.0008 | 0.0004 | 0.20 | 0.8225 |
| ra | 3 | 0.0407 | 0.0136 | 7.07 | 0.0009** |
| fl*ra | 6 | 0.0041 | 0.0007 | 0.36 | 0.8989 |
| Model | | | | | |
| $N_{tot}$= fl ce fl*ce | 11 | 3438.3870 | 312.5806 | 2.45 | 0.0244* |
| Error | 31 | 3949.1415 | 127.3917 | | |
| Type III-SS | | | | | |
| fl | 2 | 215.1069 | 107.5535 | 0.84 | 0.4395 |
| ce | 3 | 2009.6131 | 669.8710 | 5.26 | 0.0047** |
| fl*ce | 6 | 1043.5714 | 173.9286 | 1.37 | 0.2594 |

*) = significant (P<0.05)
**) = highly significant (P,0.01)

note:
% CR = the percentage of chromium in the digesta
% N = the percentage of nitrogen in the digesta
$N_{tot}$ = the total nitrogen/intubation
fl = feeding level
ra = dietary ratio
ce = intake of cellulose

## APPENDIX 12.

## SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENT 2.

### 1. TWO-WAY ANALYSIS OF VARIANCE

| | DF | SS | MS | F value | Pr>F |
|---|---|---|---|---|---|
| Model | | | | | |
| % N = fl ra fl*ra | 11 | 0.0388 | 0.0035 | 1.55 | 0.1647 |
| Error | 31 | 0.0706 | 0.0023 | | |
| Type III-SS | | | | | |
| fl | 2 | 0.0032 | 0.0016 | 0.71 | 0.4982 |
| ra | 3 | 0.0184 | 0.0061 | 2.69 | 0.0635 |
| fl*ra | 6 | 0.0190 | 0.0032 | 1.39 | 0.2486 |
| Model | | | | | |
| % CR = fl ra fl*ra | 11 | 0.0463 | 0.0042 | 2.19 | 0.0426* |
| Error | 31 | 0.0595 | 0.0019 | | |
| Type III-SS | | | | | |
| fl | 2 | 0.0008 | 0.0004 | 0.20 | 0.8225 |
| ra | 3 | 0.0407 | 0.0136 | 7.07 | 0.0009** |
| fl*ra | 6 | 0.0041 | 0.0007 | 0.36 | 0.8989 |
| Model | | | | | |
| $N_{tot}$= fl ce fl*ce | 11 | 3438.3870 | 312.5806 | 2.45 | 0.0244* |
| Error | 31 | 3949.1415 | 127.3917 | | |
| Type III-SS | | | | | |
| fl | 2 | 215.1069 | 107.5535 | 0.84 | 0.4395 |
| ce | 3 | 2009.6131 | 669.8710 | 5.26 | 0.0047** |
| fl*ce | 6 | 1043.5714 | 173.9286 | 1.37 | 0.2594 |

*) = significant (P<0.05)
**) = highly significant (P,0.01)

note:
% CR = the percentage of chromium in the digesta
% N = the percentage of nitrogen in the digesta
$N_{tot}$ = the total nitrogen/intubation
fl = feeding level
ra = dietary ratio
ce = intake of cellulose

2. Analysis Regression

| | DF | SS | MS | F value | Pr>F | Parameter estimates | | Prob>\|T\| |
|---|---|---|---|---|---|---|---|---|
| 1. For All observation | | | | | | | | |
| Model | | | | | | | | |
| $N_{tot}$ = ce | 1 | 1750.2437 | 1750.2437 | 12.73 | 0.0009** | Intercept | 12.0407 | 0.0108* |
| Error | 41 | 5637.2849 | 137.4948 | | | ce | 1.1536 | 0.0009** |
| 2. For fl=15 g only | | | | | | | | |
| Model | | | | | | | | |
| $N_{tot}$ = ce | 1 | 293.3010 | 293.3010 | 6.24 | 0.0280* | Intercept | 12.4886 | 0.0214* |
| Error | 12 | 564.0786 | 47.0066 | | | ce | 0.8700 | 0.0280* |
| 3. For fl=20 g only | | | | | | | | |
| Model | | | | | | | | |
| $N_{tot}$ = ce | 1 | 1424.9355 | 1424.9355 | 7.945 | 0.0145* | Intercept | 5.6417 | 0.5128 |
| Error | 13 | 2331.6464 | 179.3574 | | | ce | 1.6995 | 0.0145* |
| 4. For fl=25 g only | | | | | | | | |
| Model | | | | | | | | |
| $N_{tot}$ = ce | 1 | 223.7847 | 223.7847 | 1.191 | 0.2965 | Intercept | 20.3011 | 0.0532 |
| Error | 12 | 2253.8890 | 187.8241 | | | ce | 0.7211 | 0.2965 |

*) significant (P<0.05)
**) significant (P<0.01)

## APPENDIX 13.

## SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENT 3.

1. TWO-WAY ANALYSIS OF VARIANCE

| | DF | SS | MS | F value | Pr>F |
|---|---|---|---|---|---|
| Model | | | | | |
| % N = fl ra fl*ra | 11 | 57.0233 | 5.1839 | 2.83 | 0.0107* |
| Error | 32 | 58.6851 | 1.8339 | | |
| Type III-SS | | | | | |
| fl | 2 | 7.5884 | 3.7942 | 2.07 | 0.1429 |
| ra | 3 | 39.8913 | 13.2971 | 7.25 | 0.0008** |
| fl*ra | 6 | 7.8964 | 1.3161 | 0.72 | 0.6382 |
| Model | | | | | |
| % Cr = fl ra fl*ra | 11 | 0.7741 | 0.0704 | 3.10 | 0.0060** |
| Error | 32 | 0.7255 | 0.0227 | | |
| Type III-SS | | | | | |
| fl | 2 | 0.1618 | 0.0840 | 3.71 | 0.0357* |
| ra | 3 | 0.4511 | 0.1504 | 6.63 | 0.0013** |
| fl*ra | 6 | 0.1909 | 0.0318 | 1.40 | 0.2438 |
| Model | | | | | |
| $N_{tot}$ = fl ra fl*ra | 11 | 2627322.337 | 238847.485 | 2.60 | 0.0172* |
| Error | 32 | 2938407.345 | 91825.230 | | |
| Type III-SS | | | | | |
| fl | 2 | 142508.215 | 71254.107 | 0.78 | 0.4687 |
| ra | 3 | 1625591.846 | 541863.949 | 5.90 | 0.0025** |
| fl*ra | 6 | 868634.637 | 144772.440 | 1.58 | 0.1861 |

*) significant (P<0.05)

**) highly significant (P<0.01)

note:
% CR = the percentage of chromium in the digesta
% N = the percentage of nitrogen in the digesta
$N_{tot}$ = the total nitrogen (mg)
fl = feeding level
ra = dietary ratio
ce = intake of cellulose

2. Regression

| | DF | SS | MS | F value | Pr>F | Parameter estimates | | Prob>F |
|---|---|---|---|---|---|---|---|---|
| 1. For all observations | | | | | | | | |
| Model | | | | | | | | |
| $N_{tot}$ = Pr | 1 | 1552489.821 | 1552489.821 | 16.25 | 0.0002** | Intercept | 128.7887 | 0.2597 |
| Error | 42 | 4013239.860 | 95553.330 | | | Pr | 34.3480 | 0.0002** |
| 2. For fl=15 g only | | | | | | | | |
| Model | | | | | | | | |
| $N_{tot}$ = Pr | 1 | 919124.7661 | 919124.7661 | 7.22 | 0.0198* | Intercept | -50.7272 | 0.8279 |
| Error | 12 | 1526772.6015 | 127231.0501 | | | Pr | 45.9289 | 0.0198* |
| 3. For fl=20 g only | | | | | | | | |
| Model | | | | | | | | |
| $N_{tot}$ = Pr | 1 | 824398.9697 | 824398.9697 | 8.74 | 0.0111* | Intercept | 99.1329 | 0.6156 |
| Error | 13 | 1225810.5421 | 94.293.1186 | | | Pr | 43.2855 | 0.0111* |
| 4. For fl=25 g only | | | | | | | | |
| Model | | | | | | | | |
| $N_{tot}$ = Pr | 1 | 87714.1308 | 87714.1308 | 1.34 | 0.2676 | Intercept | 330.2384 | 0.0603 |
| Error | 13 | 849953.7660 | 65381.0589 | | | Pr | 14.1191 | 0.2676 |

*) significant (P<0.05)
**) highly significant (P<0.01)

## APPENDIX 14.

## SUMMARY OF STATISTICAL ANALYSIS FOR EXPERIMENT 4.

1. ONE-WAY ANALYSIS OF VARIANCE

| | DF | SS | MS | F value | Pr>F |
|---|---|---|---|---|---|
| Model | | | | | |
| Cr1 = tr | 3 | 0.0428 | 0.0143 | 2.35 | 0.1017 |
| Error | 21 | 0.1275 | 0.0061 | | |
| Type III-SS | | | | | |
| fl | 2 | 0.1618 | 0.0840 | 3.71 | 0.0357* |
| tr | 3 | 0.4511 | 0.1504 | 6.63 | 0.0013** |
| fl*tr | 6 | 0.1909 | 0.0318 | 1.40 | 0.2438 |
| Model | | | | | |
| Cr2 = tr | 3 | 4.2790 | 1.4263 | 2.35 | 0.1017 |
| Error | 21 | 12.7541 | 0.6073 | | |
| Model | | | | | |
| N1 = tr | 3 | 70.8250 | 23.6083 | 3.41 | 0.0317* |
| Error | 27 | 186.9333 | 6.9235 | | |
| Type III-SS | | | | | |
| fl | 2 | 7.5884 | 3.7942 | 2.07 | 0.1429 |
| tr | 3 | 39.8913 | 13.2971 | 7.25 | 0.0008** |
| fl*tr | 6 | 7.8964 | 1.3161 | 0.72 | 0.6382 |
| Model | | | | | |
| N2 = tr | 3 | 7082.4989 | 2630.8330 | 3.41 | 0.0317* |
| Error | 27 | 18693.3297 | 692.3455 | | |
| Model | | | | | |
| $N_{tot}$ = tr | 3 | 11145575297 | 3715191766 | 5.15 | 0.0079** |
| Error | 21 | 15135109278 | 720719489 | | |
| Type III-SS | | | | | |
| fl | 2 | 142508.215 | 71254.107 | 0.78 | 0.4687 |
| tr | 3 | 1625591.846 | 541863.949 | 5.90 | 0.0025** |
| fl*tr | 6 | 868634.637 | 144772.440 | 1.58 | 0.1861 |
| Model | | | | | |
| Lys = tr | 3 | 45910760.19 | 15303586.73 | 1250.94 | 0.0001** |
| Error | 4 | 48934.79 | 12233.70 | | |

*) significant (P<0.05)
**) highly significant (P<0.01)

note:
CR1 = the percentage of chromium in the digesta
CR2 = mg of chromium per g digesta
N1 = the percentage of nitrogen in the digesta
N2 = mg of nitrogen per g digesta
$N_{tot}$ = the total nitrogen (mg)
Lys = the total of lysine (mg)
fl = feeding level
tr = dietary treatment

2. Regression

| | DF | SS | MS | F value | Pr>F | Parameter estimates | | Prob>F |
|---|---|---|---|---|---|---|---|---|
| 1. Model | | | | | | | | |
| $N_{tot}$ = tr | 1 | 9491663373 | 9491663373 | 13.00 | 0.0015** | Intercept | -22085.6302 | 0.1061 |
| Error | 23 | 16789021202 | 729957444 | | | tr | 3719.7102 | 0.0015** |
| 2. Model | | | | | | | | |
| Lys = tr | 1 | 30902644.42 | 30902644.42 | 12.31 | 0.0127* | Intercept | -2635.251 | 0.1031 |
| Error | 6 | 15057050.57 | 2509508.43 | | | Tr | 351.5830 | 0.0127* |
| 3. Model | | | | | | | | |
| $N_{tot-exc}$ = tr | 1 | 268499.4092 | 268499.4092 | 31.59 | 0.0049* | Intercept | -137.2010 | 0.2403 |
| Error | 4 | 33998.4073 | 8499.6018 | | | Tr | 51.8169 | 0.0049* |
| 4. Model | | | | | | | | |
| $Lys_{exc}$ = tr | 1 | 1729974332 | 1729974332 | 4.38 | 0.0500* | Intercept | -9615.0222 | 0.4124 |
| Error | 19 | 7500981970 | 394788525 | | | Tr | 2223.2372 | 0.0500* |

*) significant (P<0.05)
**) highly significant (P<0.01)

Note:
$N_{tot}$ = mg of nitrogen per g digesta
tr = dietary treatment
Lys = the total of lysine
$N_{tot-exc}$ = the total of nitrogen (mg) with excluding treatment 4.
$Lys_{exc}$ = the total of lysine (mg) with excluding treatment 4.