EVALUATION OF CHAYA (*Cnidoscolus aconitifolius*) LEAF MEAL AS AN INGREDIENT IN POULTRY DIETS: ITS AVAILABILITY AND EFFECT ON THE PERFORMANCE OF CHICKENS

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Declaration

I declare that the work presented herein is my own and all contribution of other authors is properly acknowledged. This thesis has not been submitted elsewhere for any other degree or professional qualification.

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Abstract

A series of experiments were carried out with the aim of evaluating chaya (*Cnidoscolus aconitifolius*) leaf meal (CLM), a fibrous protein-rich foodstuff, in terms of both digestibility and poultry performance as a potential ingredient to be utilised in poultry diets. In the first experiment, chaya plants at either 4, 8 or 12 weeks of re-growth were harvested. With the exceptions of ether extract and oxalic acid, no differences in chemical composition of the leaves were found between the different ages of re-growth for the contents of dry matter, crude protein, neutral detergent fibre, acid detergent fibre, lignin, ash, or hydrogen cyanide. In experiment two, true metabolisable energy (TME) content as well as TME corrected to nitrogen equilibrium (TME_N) and dry matter and nitrogen digestibilities in CLM were evaluated in commercial broilers and criollo chickens. Neither CLM inputs nor genotype of the bird significantly affected the TME_N values. The collection period of droppings and the body weight of the bird significantly ($P<0.05$) affected TME_N. In experiment three, the effect of different fibre sources on endogenous losses of nitrogen, uric acid and amino acids as well as on energy balance and body weight loss were evaluated in cockerels. The different fibre sources caused significant ($P<0.05$) differences in all the dependent variables. An important fact, demonstrated by experiments 2 and 3, was that the endogenous losses of birds showed a very high variation; however, possible explanations for those findings were put forward. In experiment four, TME, net energy and heat increment values of CLM and wheatfeed were determined using cockerels in calorimeter chambers. Lower ($P<0.05$) values of TME and net energy were found in CLM compared with wheatfeed, and that was attributable to the different chemical composition of the ingredients evaluated and to the different properties of their fibre. In experiment five, the effect of different amounts of dietary CLM on apparent metabolisable energy (AME) of diets and on dry matter, nitrogen and amino acid digestibilities in broilers was evaluated. There were three experimental diets (0, 150 and 250 g/kg CLM) which were fed to the broilers from 1 to 21 days of age. AME and all the dependent variables were significantly ($P<0.05$) affected by dietary CLM contents, in both 7- and 21-day-old birds. There were higher values for all dependent variables in birds aged 21 days than in those aged 7 days. There were no differences between the control diet and CLM 150 g/kg diet for amino acid digestibility, with the exceptions of alanine, arginine and proline. However, amino acid digestibility was lower ($P<0.05$) in the diet containing 250 g/kg CLM than in either control or 150 g/kg CLM diets. In experiment six, the effect of adding the enzymes ß-glucanase and pectinase on amino acid digestibility of CLM was evaluated in broilers. Ileal samples were collected from birds given two doses of CLM by tube feeding. Only the pectinase treatment increased ($P<0.05$) lysine and total amino acid digestibility. In experiment seven, the performance of broilers fed different CLM dietary amounts was evaluated. Two studies were carried out in this experiment. The performance parameters of birds decreased as CLM in diets increased to 350 and 250 g/kg in the first and second studies respectively, and this was attributable to the fibre content of CLM rather than to its oxalic acid and hydrogen cyanide contents. The length and weight of caeca were greater ($P<0.05$) as dietary CLM increased, but the weight of

the gizzard showed the opposite tendency. It was concluded that CLM is an alternative ingredient for inclusion in diets for broilers, mainly in small-farm systems. It represents an acceptable source of amino acids but it contains low concentrations of both metabolisable and net energy as would be expected in a high-fibre foodstuff.

Abbreviations

ADF	Acid detergent fibre
AME	Apparent metabolisable energy
AME_N	Apparent metabolisable energy corrected to nitrogen equilibrium
CFE	Chaya fibrous extract
CLM	Chaya leaf meal
CP	Crude protein
Cps	Centipoise
DM	Dry matter
d	Day
dl	Decilitre
g/kg	gram/kilogram
GE	Gross energy
GLC	Gas-liquid chromatography
N	Nitrogen
NDF	Neutral detergent fibre
NE	Net energy
SE	Standard error
SEM	Standard error of means
TME	True metabolisable energy
TME_N	True metabolisable energy corrected to nitrogen equilibrium
UA	Uric acid
w/v	weight/volume

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Chapter one
INTRODUCTION

Fibre is present in all diets fed to farmed animals and its inclusion in poultry diets has been studied in several laboratories. Most, irrespective of the chemical composition of the fibre, emphasise its deleterious effects on diet digestibility and acceptability and on bird performance as the amount of dietary fibre is increased. However, fibre is a significant constituent of a large number of ingredients, many of which are included in commercial poultry diets. In fact, all poultry diets contain some fibre, thus research into both the use of new fibrous foodstuffs and better approaches to the understanding of the effects of fibre on the nutrition of poultry are worthy of investigation and likely to lead to a better understanding on how best to feed poultry with the resources (feedstuffs) available.

The effects of dietary fibre on the digestibility of poultry diets and consequently on the performance of the birds is highly variable, presumably because the chemical composition and structure of the fibre is also highly variable. Dietary fibre is modified mainly by fermentation in the bird's lower gut and caeca. Fermentation of fibre may produce useful amounts of energy in the form of volatile fatty acids. Ingestion of high amounts of dietary fibre (above around 5% of crude fibre) may have more negative effects than any benefits obtained from energy released in the form of volatile fatty acids both in chickens and probably in other non-herbivorous species.

The developing countries, most of them situated in tropical areas, lack the necessary funds to obtain cereals from other countries for human and livestock feeding. There are now 800 million people in the world suffering from malnutrition. Inadequate food distribution and incomes too low to acquire food of satisfactory quantity and quality to satisfy the nutritional needs of the human population are the main explanations for this state of affairs.

Telek and Martin (1983) have pointed out that there are available more species of plant in the tropics than in temperate zones, for livestock food. It is

estimated that there are 3 million of species of plant in the tropics is in contrast to the 1.5 million species found in the temperate zones. They also pointed out that it is unlikely to be possible to improve the world food deficiency, mainly in the tropics, by conventional agricultural practises alone, and they proposed the utilisation of tropical leaves as a source of protein for feeding animals.

Like in the most important areas of intensive animal production in the world, the poultry industry in Mexico is increasing in size. However, the diets for poultry and pigs in that country are based on cereals, most of which are imported from other countries (mainly the USA). This state of affairs increases the cost of production as well as the prices of meat and eggs. Moreover, the dependence on the supply of imported foodstuffs for animal production in Mexico results in capital leaving the country. This has a profound effect on the economy of a developing country like Mexico. The existence of a growing poultry industry in a country where the majority of the population is having difficulties in affording a balanced and adequate diet, is apparently contradictory and a situation that can only be resolved by the development and application of considerable innovative practices.

The use of local foodstuffs to sustain non-ruminant enterprises on small farms in the tropics may help to alleviate some of the difficulties that the population experience in gaining access to high quality animal protein as part of its diet. Speedy (1998) suggested that one way to improve the food supply, its sustainability and the long-term stability of society can be through the small farmer. Integrated small-farming systems are known to be capable of producing food for the family under sustainable conditions. Poultry as a livestock, because of its ability to be exploited under minimal inputs, becomes an ideal species for small farming systems giving eggs and meat as the main products (Rushton and Ngongi, 1998).

Research into the utilisation of fibrous foodstuffs is of particular importance in the tropics, where many local foodstuffs and by-products have high fibre contents but also are good sources of protein and other nutrients like xanthophyll. Forages such as sugar cane *(Saccharum officinarum)*, chaya (*Cnidoscolus aconitifolius*), leucaena (*Leucaena leucocephala*), and ramon (*Brosimum alicastrum*), are all known to small farmers in the tropics but their potential use has been underestimated by scientists and technicians because they aim to "improve" the efficiency of animal

production in the developing countries by using conventional and profitable foodsuffs for animal feeding. In the tropical areas of Mexico there are many plant resources with great feeding potential. These are currently under-exploited because of a lack of basic information on both their chemical compositions and nutritive values to animals. One of these plants, chaya (*Cnidoscolus aconitifolius*) is a possible cheap renewable source of protein and should be examined for use in diets for both humans and animals. Diaz-Bolio (1974) mentioned that in the backyards of Yucatan, Mexico, chaya is consumed by poultry. Donkoh *et al.* (1990, 1999) emphasised the great potential of chaya as a protein source in poultry diets. However, because of the relatively high fibre content of chaya its inclusion into western-type poultry diets represents a challenge for nutritionists.

The overall objective of this thesis is to assess the nutritive value of chaya leaf meal (CLM) as a fibrous protein-rich foodstuff for poultry. The digestibilility of CLM as well as the performance of chickens fed on different dietary concentrations of CLM will be evaluated. A critical assessment will also be made of how effectively the current techniques used to evaluate conventional feedstuffs are able to cope with diets/feedingstuffs high in fibre.

Chapter two
LITERATURE REVIEW

2.1 Fibre in the diets of monogastric animals

It seems that fibre ingested by monogastric animals can be one of the most important dietary components influencing metabolism of other nutrients in the gastrointestinal tract, despite almost invariably representing only a small proportion of the total diet. The different effects of dietary fibre on the gastrointestinal tract depend on its chemical and physical characteristics as well as other inherent features of the animal, such as species or age.

The beneficial effects of dietary fibre and its physicochemical properties have been widely studied in humans and have been related to the diminution or prevention of some chronic diseases (*e.g.* bowel cancer) of Western societies (Smits and Annison, 1996; Rowland, 1999). These beneficial effects have still to be confirmed in birds, where the feeding of fibre has tended to focus on its antinutritive properties (Klasing, 1998).

Fermentability is one significant characteristic of the fibre, and it refers to the capability of fibre to be utilised as a substrate by the micro-organisms in the gut (Coon *et al.*, 1990). The energy from enzyme-resistant polysaccharides that constitute fibre (see later for a description of what fibre comprises, pp 7-9) becomes obtainable during the fermentation process (Muramatsu *et al.*, 1991). That energy is mainly in the form of volatile fatty acids (principally acetic, propionic and butyric acids) which are absorbed and used by the animals' metabolic processes, although other waste-products in small amounts are derived from these processes *e.g.* hydrogen, methane and carbon dioxide. However, it is now known that the extent of fibre fermentation is determined by the characteristics of the fibre. For instance, insoluble hemicellulose and cellulose, in association with lignin and cutin, seem to be less fermentable than soluble hemicellulose and pectin in both ruminant and monogastric animals (Van Soest, 1978, Kritchevsky, 1988).

Monogastric animals, such as pigs, poultry, rats, and including humans, have less capacity to ferment dietary fibre than ruminants (Jung, 1997). There are some monogastric animals such as *Equidae* and rabbits who can ferment some more fibre in their hindguts, but always less than that by ruminants (Russell and Gahr, 2000). Even in ruminants, there are some limitations on the extent whereby fibre is digested. These are attributed to physical and structural barriers in addition to the lignification of plant tissues. The plant species, the stage of maturity and the leaf : stem ratio are considered to be the most important features (Buxton and Redfearn, 1997). However, Varga and Kolver (1997) have focused attention to other aspects derived from processes in the animal which influence microbial growth and consequently the extent by which fibre is digested, such as the rates of food passage, particle size and the chemical and/or biological treatment it has been subjected to, as well as the strategies applied (for example meal feeding, adaptation the animal to the feed, and processing of the food, such as pelleting or grinding).

Van Soest (1978) claimed that while monogastrics are able of utilise hemicellulose better than cellulose, ruminants can use both classes of polysaccharide to approximately the same degree. Only small quantity of fibre is invariably digested by poultry (Grosjean *et al.*, 1999). This is in contrast to the higher amounts of fibre considered to be digested by pigs, rats and humans (Carré *et al.*, 1984; Carré and Leclercq, 1985; Longstaff and McNab, 1986, 1989; Slominsky *et al.,* 1994). Some avian species can ferment fibre in their caeca more extensively than others. Cilliers *et al.* (1994) reported that ostriches were capable of digesting lucerne meal, an ingredient high in fibre, much more effectively than adult cockerels. Hence, the decision to include of fibrous plant-protein sources in diets for poultry could depend on the target avian species (Cilliers *et al.,* 1999). The soluble (see below) components of dietary fibre are the most susceptible to fermentation by birds and provide most energy, but in some species cellulose can also be fermented (Carré *et al.*, 1990; Cilliers *et al.*, 1994). Sibbald *et al.* (1990) reported that pigs acquire more energy from fibrous foodstuffs than chickens, even although the efficiency with which they do so is only 40-70% of that made available by enzymatic digestion of starch.

Dietary fibre can exert nutritional effects in addition to the provision of energy. These are related to its physicochemical properties. Glitsø *et al.* (1998) found that, in pigs, the chemical composition and quantity of the dietary fibre result in variations in its extent and rate of fermentation. As a consequence variables such as the transit time of the digesta, its pH and the property of faecal bulking are modified. Faecal bulking is considered the property of the faeces to be voluminous when fibre is added into the diet. From the physiological point of view dietary fibre has been classified into soluble and insoluble fractions (Prosky *et al.*, 1988). Pectins, β-glucans, gums, some hemicelluloses and arabinoxylans are generally considered to constitute the soluble fraction; the insoluble fraction comprises mainly cellulose, lignin and some insoluble hemicelluloses (Roehrig, 1988). This partition of the different polysaccharide classes is useful to nutritionists, because the physiological and nutritional effects of fibre in the gut largely depend on its solubility (Carré and Leclercq, 1985).

Because of its greater chemical reactivity, it is the soluble fibre that is most likely to be implicated in the anti-nutritive effects (Klasing, 1998). Soluble fibre frequently increases the viscosity of the gut contents, an increase mainly correlated with its solubility rather than the nature of the non starch-polysaccharides present. Soluble non starch-polysaccharides can produce extraordinarily viscous solutions which greatly influence the properties of the digesta (Choct and Annison, 1992 a,b; Choct *et al.*, 1995; Smits *et al.*, 1997, 1998). High viscosity of the intestinal contents makes the interaction between the digestive enzymes and the substrates difficult (Önning and Asp, 1995). This is believed to result in decreases in both the rate and extent of the digestion of the food as well as the diminished absorption of the end products of digestion (Choct *et al.*, 1996). The primary effect of the water-holding capacity of insoluble polysaccharides might be to increase the bulk of the digesta and decrease the time taken for the chyme to pass through the intestine. Consequently, the digestibility of nutrients may be reduced (Smits and Annison, 1996). Published data, however, tends to be contradictory.

Lindberg and Cortova (1995) and Andersson and Lindberg (1997a,b) fed pigs on diets containing different forages and found that both the digestible and metabolisable energy of the diets were reduced as the dietary inclusion of the

forages increased. In contrast, Sauer *et al.* (1991) reported that the inclusion of 100 g/kg powdered cellulose or 100 g/kg barley straw in pig diets did not affect the ileal digestibilities of the indispensable amino acids, with the exception of leucine.

Morphological changes in the digestive tract of monogastric animals fed on high-fibre diets are to be expected (Savory and Gentle, 1976a). A "fibre" effect on the morphology and gut function in rats has been reported by Roehrig (1988); thus, a diet containing wheat bran increased colon muscle cell size, whereas oat bran and pectin decreased jejunum muscle cell size. It has been pointed out that any fibre regimen modifying mucosal cell hypertrophy or cell hyperplasia and the rate of digesta transit, would be expected to be associated with modifications to the enzyme systems operating in the gut. Buddington and Weiher (1999) reported longer intestines with greater mucosal mass among dogs fed on diets containing fermentable fibre compared with the intestines of dogs fed on a diet with cellulose, which is less fermentable. They concluded that increases in intestinal dimensions and functional capacities provided strong evidence for the existence of a relationship between the properties of the fibre in a diet, the nature of the resident bacteria and the characteristics of the gastrointestinal tract in mammals.

2.2 The definition of fibre

Dietary fibre has diverse definitions that are constantly changing as analytical techniques and the knowledge of the physical and chemical properties of fibre develop. The term "fibre" was initially referred to as the materials from plant cell walls that were resistant to hydrolysis by the digestive enzymes of man and animals. The concept of dietary fibre was further developed to include all the polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes, and the term non-starch polysaccharides has come to be used instead of fibre in the past decade. In poultry nutrition the main components of dietary fibre have gradually come to be recognised as the non-starch polysaccharides present in plants (Selvendran *et al.*, 1987; Kritchevsky, 1988; Englyst, 1989). Furthermore, Burton-Freeman (2000) described fibre as diverse plant substances that are resistant to digestion by

alimentary enzymes in humans and most animals, and attributed to that fibre some physical properties such as bulk-volume, viscosity and water-holding capacity.

Trowell (1976) suggested that the definition of dietary fibre should be restricted to the structural polymers (cellulose, other polysaccharides and lignin) of the plant cell wall. He also introduced a new term, "dietary fibre-complex", which included all the structural polymers of dietary fibre together with all the associated chemical substances. Selvendran *et al.* (1987) described the fibre components as complex polysaccharides, some of which are associated with polyphenolics (which include lignin) and proteins. The non-carbohydrate components, for instance polyphenolics, protein, cutin, waxes, suberin, phenolic esters and inorganic compounds, are present in low amounts in foods of plant origin. However, lignin and phenolic esters in the lignified tissues of wheat bran and cutin, the waxes in leafy vegetables and suberin in roots and tubers all contribute importantly to the effects to the physiological properties of dietary fibre. Theander *et al.* (1995) defined dietary fibre as the sum of the non-starch polysaccharides and Klason lignin, which included native lignin, tannins, cutins, and some proteinaceous products, as well as Maillard reaction products, formed on heat processing and also unable to be digested. The tissues from the parenchyma of plants constituted the bulk of dietary fibre from fruits, vegetables, the cotyledons of seeds and the endosperm of cereals. Substantial amounts of lignified tissues are present in many vegetable foods; however, most of these tissues are consumed when the degree of lignification is low.

The polysaccharides contained in plants can be classified into two chemical groups; the first comprises starch, the storage polysaccharide and an α-glucan, which is susceptible to digestion by the pancreatic enzymes. The second group is made up of the cell wall polysaccharides or non-starch polysaccharides, which do not contain α-glucans and cannot be hydrolysed enzymatically. However, although those carbohydrates are to some extent susceptible to fermentation by the intestine microorganisms, they are not considered to be completely indigestible (Van Soest, 1978; Englyst, 1989).

The chemical components of fibre vary widely across plant species and between tissues within a plant. Although the dietary fibre is found in the cell walls in most foods, legumes deposit important amounts of non-starch polysaccharides

within the cells of their seeds. Klasing (1998) classified the different polymers of fibre found in vegetable foods according their origin (Table 2.1).

Table 2.1 Primary dietary fibre polymers found in major food groups (Klasing, 1998)

Food group	Polymers present
Legumes	Celluloses, pectins, xyloglucans, galactomannans
Cereals	Celluloses, arabinoxylans, β-glucans, lignin
Fruits	Celluloses, pectins, xyloglucans, cutin, waxes, lignin
Grasses	Celluloses, hemicelluloses, pectins, lignin

The polysaccharides present in dietary fibre comprise cellulose, hemicelluloses, pectic substances, neutral arabinans and arabinogalactans. Cellulose is the polysaccharide most commonly found in nature and it is a polymer of linearly linked β-1,4 glucose molecules (of several thousand units), packed together in compact aggregates and organised as microfibrils. Hemicellulose is frequently associated with cellulose but is solubilised by aqueous alkali after the removal of water-soluble and pectic polysaccharides. Xylans and xyloglucans are the most important polysaccharides forming the hemicelluloses (Theander *et al.,* 1989). A main chain of D-xylopyranose residues joined by β(1-4) links is the characteristic of xylans. Xyloglucans contain a cellulosic β-D-glucan backbone to which short side chains of β-1,4-linked xylose molecules are attached at C-6 of at least one-half of the glucose residues (Northcote, 1972; Selvendran *et al.,* 1987).

Pectic substances constitute a mixture of colloidal polysaccharides, which can be partially extracted from the cell walls with hot water (which partially solubilises them) or with hot aqueous solutions of chelating agents. Neutral pectic arabinans and arabinogalactans may originate from the degradation of more complex pectic

polysaccharides; however, Selvendran *et al.* (1987) suggested that some of them exist as such from the cell wall.

The epidermal cell of leaves, fruits and other aerial organs of plants are protected with a layer of waxes and cutin, a relatively nonpolar complex mixture of alcohols, ketones and lipids. Because cutin and waxes are resistant to microbial fermentation, cutinized tissues constitute an important restriction to bacterial fermentation in the gut, mainly when those tissues are not cooked (Selvendran *et al.,* 1987).

The development of the plant cell walls is very well structured and their formation includes fibrillar polysaccharides (predominantly cellulose), matrix polysaccharides (hemicellulose and pectin) and encrusting substances (lignin). Fibrillar and matrix polysaccharides are formed simultaneously during cell wall formation while the encrusting substances are deposited in the secondary thickening phase of specialised cells. The proportion of these constituents changes greatly as the plant matures (Klasing, 1988). Kritchevsky (1988) has summarised the chemical structures of the different fibre polysaccharides and these are presented in Table 2.2.

Table 2.2 Chemical composition of different fibre polysaccharides (Kritchevsky, 1988)

Component	Principal component		Features
	Primary chains	Secondary chains	
Cellulose	Glucose	—	Linear polymer with β-(1,4) linkages
Hemicellulose	Mannose, glucose, galactose, xylose, arabinose	Arabinose, galactose, glucuronic acid	Mainly β-(1,4) pyranosides
Pectins	Galacturonic acid	Rhamnose, fucose, arabinose, xylose	Mainly β-(1,4) galacturonans; with varying degrees of methylation
Mucilages	Galactose-mannose, glucose-mannose, arabinose-xylose, galacturonic acid	Galactose	—
Gums	Galactose, glucuronic acid-mannose, glucuronic acid- glucose	Xylose, fucose, galactose	—
Algal polysaccharides	Mannose, xylose, glucuronic acid	Galactose	Contain sulphate
Lignin	Sinapyl alcohol, coniferryl alcohol, p-coumaryl alcohol	—	Complex, crosslinked, phenylpropane polymer

Northcote (1969, 1972) argued that the lignification process starts in the primary wall region and then penetrates the wall at an early period of secondary thickening. The lignified walls have cellulose microfibrils dispersed in hemicellulose and lignin. The most common polysaccharides found in the cell walls of the parenchymatous tissues of dicotyledons are the pectic substances, hemicelluloses (*e.g.* xyloglucans) and cellulose, whereas those found in lignified tissues are lignin, hemicelluloses (*e.g.* glucuronoxylans) and cellulose. Pectic substances dominate the cell wall of parenchymatous tissues, and lignin is the major component of the cell walls of lignified tissues.

Techniques based on the extraction of the plant materials with acid and base detergents have been the preferred procedures for measuring the fibrous components in foods for over 30 years (Klasing, 1998). Longstaff and McNab (1989) have proposed the application of analytical methods such a gas-liquid chromatography (GLC) for the better understanding of the relationship between polysaccharide structure and its digestion. These methods rely on the quantification of the constituent neutral sugars released by the hydrolysis of the component polysaccharides and determined by GLC. In this context, fibre represents the sum of the individual neutral sugars together with the uronic acids from pectic substances and acidic xylans.

According to its functional properties, dietary fibre can be divided into a soluble fraction (pectins, β-glucans, gums and some hemicelluloses) and an insoluble fraction (lignin, cellulose and some hemicelluloses). This physiological classification is convenient for nutritional purposes because the fibre solubility determines the main effects on and in the gastrointestinal tract (Carré and Leclercq, 1985).

2.3 Effects of fibre in and on the gastrointestinal tract of poultry

The effects of dietary fibre on the gastrointestinal tract of poultry are variable and generally they may be expected to be the same as in any other monogastric animal. They depend on the properties and chemical structure of the components. However, Jorgensen *et al.* (1996) noted that the microbial degradation of dietary fibre in the caecum and colon of pigs and rats seems to be higher than that occurring in poultry. Other factors such as species, age and diet adaptation may also be important factors.

Fibre sometimes is credited with the prevention of the complete digestion and absorption of other dietary components by encapsulating potentially digestible ingredients (*e.g.* starch and protein). Experiments have demonstrated that appropriate enzyme supplementation of diets improves the performance of chickens through solubilisation of the non-starch polysaccharides and consequently better utilisation of those diets (Theander *et al.*, 1989; Chesson, 2000; Choct and Kocher, 2000). Dietary supplementation with enzymes has also been shown to result in a reduction in the viscosity of the digesta in birds fed on grain-based diets (Friesen *et al.*, 1992; Choct *et al.*, 1995).

Carré *et al.* (1990) reported a higher digestibility of the water-soluble non-starch polysaccharides than of the water-insoluble fraction in both cockerels and ducks. Carré *et al.* (1995b) further noted that the digestibility of water-soluble pectin at a low concentration in a diet (6 g/kg) was not significantly different in mature cockerels and broiler chickens. However, this does not exclude the possibility that greater differences may exist between mature birds and broilers when higher dietary concentrations of pectin are fed.

Duke *et al.* (1984) found that preconditioning turkeys to high-fibre diets resulted in at least a four-fold increase in cellulose utilisation. The caeca of the turkeys fed on the high-fibre diet were about 25 % longer than those fed on the low-fibre diet, on an equivalent body weight basis. A possible explanation for that fact is the trophic effect of the short chain fatty acids (which come from the fermentation process of the fibre) on the proliferation of the cells of intestinal tissues (Sakata, 1987). Savory (1992a) suggested that the degradation of cellulose by the intestinal

microflora occurred normally in conditioned (for 3 weeks before the sampling period) fowls, the extent of the degradation depending on the duration of the preconditioning to the high-fibre diets. Sarmiento and Belmar (1998) fed commercial Hubbard and naked-neck criollo chickens on diets containing different amounts of crude fibre. They concluded that a dietary concentration of 106 g/kg crude fibre resulted in the lowest apparent dietary dry matter digestibility in both types of birds, when compared that value with those obtained from lower concentrations of dietary fibre.

Kritchevsky (1988) and Zyla *et al.* (2000) have drawn attention to the exchange capacity of fibre for calcium, iron and zinc, bile acids and bile salts as an other important effect of dietary fibre. The combination of calcium with some of the components of fibre leads to the formation of insoluble complexes and an increasing intestinal viscosity.

2.3.1 Size of the gastrointestinal tract

There are several studies that show that dietary fibre results in the enlargement of the bird's gut. Longstaff *et al.* (1988) found heavier and longer caeca in chicks fed on diets containing pentoses and uronic acids (which usually come from non-starch polysaccharides), than those from chicks fed on a glucose-based diet and they attributed this elongation to the process of fermentation.

Abdelsamie *et al.* (1983) reported that the relative lengths and weights of the intestines as well as the lengths of the caeca were increased in broilers fed on diets with high concentrations of dietary fibre. Savory (1992b) fed fowls on diets containing 0, 100, 200 or 400 g/kg dried grass, 200 g/kg powdered cellulose, or 200 g/kg dried grass with a polysaccharidase supplement. Compared to the basal diet, additions of 100, 200 or 400 g/kg dried grass or 200 g/kg cellulose caused significant increases in the lengths of the small intestine, while 200 g/kg dried grass with supplementary enzyme did not.

Jorgensen *et al.* (1996) found that the consumption of diets high in fibre caused increases in the lengths of the gastrointestinal tracts of broiler chickens. They

emphasised the impact that this response would have on energy metabolism, as visceral organs have a high rate of energy consumption relative to their size. Fuente *et al.* (1998) reported an enlargement in the empty weights of the intestinal tracts of chickens fed on barley-based diets because of the effect of fermentation of the non-starch polysaccharides from barley.

2.3.2 Changes in the intestinal microflora as a result of the consumption of fibre

Wagner and Thomas (1978) indicated that the nature of the microflora in the small intestine of chickens can be markedly modified by the composition of their diet. Their observations indicated that depressions in the growth of chicks fed on diets containing rye or pectin was a result of the propagation of detrimental microbes within the intestine. Increases in both butyric acid and gas production were related to the flora present in the chicks fed on the diets containing those ingredients. Bedbury and Duke (1983) fed turkeys on diets with low or high dietary fibre contents and found no significant difference in the mean colony counts between the two groups of birds. The predominant microorganism was *Eubacterium,* but *Lactobacillus, Peptostreptococcus, Escherichia coli, Propionibacterium* and *Bacteroides* were also isolated. The percentage of *Peptostreptococcus* was significantly greater in the turkeys fed on the high-fibre diet and that of *Escherichia coli* was significantly greater in the turkeys fed on the diet low in dietary fibre.

Klasing (1998) pointed out that the type and quantity of the dietary fibre can clearly affect the types of microflora that colonise the gut. The viscosity of the soluble components of the fibre produces an increase in the residence time of the digesta in the small intestine resulting in lower oxygen tension and modifications to the types of bacterial populations. Some microbial populations can induce the production of bacterial toxins and cause mucosal immune responses, both of which affect the bird negatively

Zubair *et al.* (1996) fed turkeys on diets with dietary crude fibre concentrations of between 25 and 90 g/kg. The results showed that there were no significant effects of dietary fibre on the rate of excretion and amount of caecal

droppings from the turkeys. Neither was there any effect of dietary treatment on the number of microbes in the caeca. Leeson *et al.* (1997) fed turkeys on diets with different amounts of fibre and concluded that fibre *per se* did not influence caecal activity in the turkey (microbial count, caecal weight, caecal droppings). Furthermore, it did not seem to contribute to the production of greater or more viscous caecal contents.

2.3.3 Viscosity of the digesta

The structural pentosans present in rye and wheat endosperm cell walls as well as the β-glucans present in barley produce viscous solutions when dissolved in water. The decreased diffusion of both substrates and digestive enzymes as well as diminished rate of nutrient absorption are the main effects of the gut viscosity. The poor performance of chickens through reduced nutrient utilisation is therefore the final effect (Bedford and Classen, 1992, 1993; Choct and Annison, 1992b; Zyla *et al.*, 2000).

Bedford and Classen (1992) described intestinal viscosity as an underestimated contributory factor to growth reduction in birds, because lower viscosity and improvement in gain were observed with enzyme supplementation of wheat-based diets. Van der Klis *et al.* (1993) reported that the dietary addition of the indigestible soluble polysaccharide carboxymethyl cellulose in chickens, affected retention time of the diet through an increase in the gastrointestinal tract viscosity. As the intestinal viscosity was increased the net sodium absorption from the intestinal lumen was reduced, and consequently a lower rate of water absorption resulted.

Fuente *et al.* (1998) pointed out that digesta viscosity explained 38 % of the reduction in apparent metabolisable energy (AME) in chickens fed on barley-based diets. They found that for each centipoise unit of increase in digesta viscosity, the dietary AME was reduced by 64.4 kJ/g.

2.3.4 Water-holding capacity and bulking properties

Soluble polysaccharides such as pectins, gums, β-glucans and some hemicelluloses have very high water-holding capacities. In the event of quantities of these components not being fermented, they produce more volume or bulkiness to the faeces. However that volume can also arise as a result of both an increase in the bacterial mass caused by fermentable dietary fibre or by the ability of the digesta to absorb water, a characteristic of poorly degradable dietary fibre (Glitso *et al.*, 1998). Components of the insoluble fibre fraction can absorb considerable amounts of water without causing a marked increase in the viscosity of the digesta. The insoluble fibre (and the water and bacteria associated with it) are the primary contributors to the greater volume of faeces in birds. The bulk caused by insoluble fibre reduces the residence time of the digesta in some avian species, and this may produce lower nutrient digestibilities. In others, however, insoluble fibre has little effect on the utilisation of starch, protein or lipids, apart from nutrient-dilution effects (Cilliers *et al.*, 1994; Klasing, 1998).

Insoluble polysaccharides such as cellulose and xylans can hold water rather like sponges, but their viscosities are relatively low. The primary effect of the water-holding capacity of insoluble polysaccharides might be to increase the bulk of the digesta and decrease the time taken for the chyme to pass through the intestine and consequently reduce the digestibility of nutrients (Robertson and Eastwood, 1981 a,b; Smits and Annison, 1996). Reducing particle size results in increased water holding capacity. A fibre that is completely degraded cannot hold water in the colon, but it can contribute to a fecal bulk by increasing the mass of the gut microflora (Roehrig, 1988).

2.4 Alternative feeding resources for small farming systems

The world population is predicted to increase from 5.4 billion in 1990 to about 7.2 billion in 2010. This increment will occur mostly in the urban areas of developing countries. The effects of this increase will be most intense on the patterns of food production, marketing and consumption. Policies are needed to secure the stock of food for the growing population to maintain economic development and to preserve the environment (Sansoucy *et al.*, 1995). As a consequence of the growth in the world population, the problem of malnutrition is also an important and worrying concern. However, malnutrition in both rich and poor countries is mainly a matter of adequate food distribution rather than one of deficient availability (Ford, 2000; Smil, 2000). Evans (1998) has pointed out "hunger and plenty co-exist today as they have throughout human history, but with less reason. The difference is that there is enough food for all of us now produced in the world, even in times of local famine, yet the poorest of the poor, up to 800 millions of them, still suffer chronic under-nutrition". In spite of that situation, the worldwide farming industry is increasing in size every year and it might give the impression that its only (or at least main) purpose would be to maximise profits and without any concern about the important issue relating adequate food distribution.

Poultry production in Mexico has been growing very rapidly over the last few years. During the period between 1990 to 1998 the production of eggs and chicken meat has increased to between 1.0 and 1.5 million metric tons (Chavez, 1999). In world terms Industria Avicola (1997) asserted that Mexico was the fourth biggest chicken grower and the fifth biggest producer of eggs in the world. As in many other Western countries the poultry industry is controlled by a few huge combines, who produce about 50% of Mexico's broiler meat. Balconi (1998) has observed that those large producers were growing in significance because of the Mexican economic crisis in 1994, which led to the collapse of many of the country's smaller poultry producers. Treviño (1999) reported that the volume of poultry meat produced in Mexico was only below those from the USA, China, Brazil and France. The estimated poultry meat production, poultry meat imported and poultry meat consumption in Mexico in the year 1998 were 1.7, 0.23, and 1.96 million metric tons

respectively. Egg production in Mexico in 1997 was 1.59 million metric tons from a flock estimated at 290.77 million birds. Balconi (1998) reported that poultry meat consumption in Mexico in 1998, at 1.75 million metric tons, had increased by 6% compared with the previous year. Consumption of poultry meat has been increasing as a consequence of higher beef and pork prices. The annual *per capita* consumption of eggs and chicken meat in Mexico were 15.8 and 16.0 kg respectively during 1997 (Industria Avicola, 1997) and they were 18.8 and 21.6 respectively during 2000 (CANACINTRA, 2001).

In 1997 the Yucatan State of Mexico occupied sixth and ninth places, respectively, in terms of the country's egg and poultry meat production (Balconi, 1998). The Yucatan State of Mexico, because of its unique geographical situation, plays an important role in the poultry industry in that country. The most important foodstuffs for feeding to poultry are imported from the USA and arrive in Yucatan more cheaply than at other locations in Mexico. As a result the production costs of chickens and eggs in that state are lower than in other parts of the country.

Sansoucy *et al.* (1995) have pointed out that food balance in the countries, irrespective of the size, is not an adequate measure of its food security. In the developing countries availability does not assure access, because of poor distribution or lack of purchasing power. Perez (1998) and Diario de Yucatan (1998) reported that the child malnutrition in the Yucatan State is still an important social problem and also drew attention to the fact that the Yucatan State came in the first five places in the country in terms of population malnutrition. Balam-Pereira *et al.* (1997) observed that poverty and child malnutrition are still serious problems in the Yucatan State of Mexico. In 1998 it occupied second place in the country with 61.6 % of children aged 5 years or less suffering from malnutrition. In Mexico, the existences of a large and growing poultry industry and of a situation is there the majority of the population have difficulties in reaching a balanced and adequate diet, is certainly a contradiction.

In this context, Preston (1992) has pointed out that poultry must be evaluated as food producers in terms of their impact on the sustainability of the system in which they play a part, and described the following aspects where the modern poultry industry is failing:

• Issues relating environmental sustainability and economic aspects. The production units are almost never integrated with the end use of the excreta. Consequently, there is little or no recycling of wastes. In most developing countries the poultry industry uses cereals as the basis for the feeding system and has to compete with the demands of the human population, but world grain production is decreasing as a result of other economical and environmental issues. A strategy must be developed to search out robust and sustainable alternative sources of energy.

• Animal welfare and sociological concerns. Poultry are kept in "unfriendly" housing systems which, in the majority of the developed countries, will soon be prohibited. Those systems are dependent on the mechanical provision of food and medication. In the so-called welfare friendly systems likely to be demanded in future alternatives to automation may need to be developed. The modern poultry industry almost excludes the possibility of job opportunities for women and children, or their mixture, with household and family activities.

• Concerns on energy consumption. The modern poultry industry, like other current agricultural processes depend on high energy inputs, which is mainly from non-renewable sources. In order to alleviate the energy crisis worldwide, the developed of low-input and renewable-energy farming systems will constitute alternative and sustainable agricultural methods.

In contrast, small agricultural systems, which are self-sufficient, low-energy demanding and sustainable can provide grains and vegetables as well as animal products such as meat, milk and eggs for the family and produce for sale. In the event of failure of specific crops or markets they have more security because of the diversity of their products. They employ family labour mainly in the form of women and children (Preston, 1992; Speedy, 1998). Ford (2000) pointed out that, in the future, small farmers will re-emerge and increase in number responding to the increased requirement of food by the society. In this context, Rushton and Ngongi (1998) emphasised the importance of poultry as a livestock species for many poor, rural families worldwide, and how, in many cases, they are exploited under garbage-based conditions. The small size of poultry and their ability to survive on minimal inputs make their management a relatively easy activity for any household to

practise, and they asserted that rural poultry development might be one way to reduce rural poverty.

Tropical plant resources as food for livestock are abundant but information is required on how they can best be utilised. Over 40 years ago Duckworth and Woodham (1961) pointed out that leaf protein concentrates represented a potential ingredients in the diets of pigs, poultry and man, and the amino acid contents of those concentrates resembled that of soyabean. For animal feeding Samarasinghe and Rajaguru (1992) have recomended the use of non-conventional ingredients which are not directly consumed by man (and consequently are non-competitive), as a means to partially solving the deficiency of concentrate foodstuffs in developing countries. Nagy *et al.* (1978), in a study with different tropical leafy protein sources, proposed their utilisation as another mean of alleviating the shortage of foodstuffs.

The use of non-conventional ingredients in poultry feeding is not a common practice in commercial poultry production for various reasons. Most of these ingredients have not yet been evaluated under standard commercial conditions, or contain some anti-nutritive factors such as toxic amino acids, lectins or saponins, which make them difficult to use in poultry feeding (Limcangco-Lopez, 1989; Belmar *et al.*, 1999).

Other non-conventional ingredients, such as those containing high amounts of fibre, have not been accepted for poultry feeding because of their low available energy contents and consequently the poor poultry performance obtained from them (Slominsky and Campbell, 1990; Panigrahi and Powell, 1991; Sobamiwa and Longe, 1994). This is despite the fact that many of them are readily available in sufficient quantities to meet potential demand. They are frequently cheaper than conventional feed ingredients, particularly in tropical areas where much of the growth in the production of poultry is taking place and where there is often a lack of access to conventional commercial products.

Some questions arise when considering the aspects described above in this section. How much poorer is poultry performance under the conditions prevailing on sustainable, tropical, small farms? Are any reduced economical benefits in the efficiency of the production of poultry under these systems more important than allowing the socially poor population access to high quality animal protein? How

important is it to improve the nutritional status of the lower-income sectors of the population, by searching for cheaper and less high-yielding foodstuffs than maximising animal production? These questions should not only be analysed from the points of view of economics and the high-performance of animals, but the sociological and equitable food-distribution contexts have also to be considered.

In addition, Smil (2000) has raised another question of particular importance concerning the current food production industries. Can intensive agriculture continue producing enough food without inducing adverse environmental changes and without compromising many irreplaceable natural resources (*e.g.* plant, animals, oxygen, water)? The cost for the current agricultural activities has certainly included a great transformation of the natural ecosystems and a profound dependence on fossil fuels. Those actions have resulted in an already significant alteration of natural cycles and in an increase in environmental pollution.

2.5 Performance of poultry fed on different fibrous foodstuffs

The use of fibrous foodstuffs in poultry diets has been popular subject for research and most reports emphasise that the outcome is an invariably poor performance (Abdelsamie *et al.,* 1983; Aguilera *et al.,* 1984; Tillan *et al.,* 1986; Onifade and Babatunde, 1996, 1997; Udedibie and Opara, 1998; Farrell, *et al.,* 2000; Perez *et al.,* 2000). Chickens, which have high rates of growth and food consumption, are particularly susceptible to changes provoked by the inclusion of higher concentrations of fibre in the diet than those obtained from conventional (commercial) diets.

Apart from the fibre content in leaves, there are many other components originated from the metabolism of plants which may be toxic and consequently impair the performance of animals consuming them. Those compounds such as phyto-estrogens, saponins, tannins and lectins, among many others are associated with anti-physiological effects which interfere with the metabolism of the animal basically by chemical mechanisms (D'Mello, 2000). However, a discussion of such factors and their mechanisms is beyond the scope of this thesis.

Dietary fibre, in contrast, influences the utilisation of most of the nutrients in the food through both physical and chemical mechanisms which take place in the lumen of the gastrointestinal tract and eventually produce changes in food intake, digesta transit time, absorption rates and, therefore, digestibility.

Ameenuddin *et al.* (1983) stated that forages like alfalfa and clover have great potential for producing high quality protein. However, the fibre contents of such ingredients and their negative effects on diet digestibility and poultry performance have been described in many studies (Piliang *et al.*, 1982; Carre *et al.*, 1984; Van der Klis *et al.*, 1993; Sarmiento and Belmar, 1998).

Savory and Gentle (1976b) found that adult Japanese quail fed on a diet high in fibre had lower body weights than those fed on a diet low in fibre. Importantly, body weight changed rapidly when the diets were interchanged.

Ricke *et al.* (1982) studied responses of chickens to the inclusion of different fibre sources in the diets. Consumption of a diet containing the cell walls of alfalfa resulted in a higher growth rate and better feed efficiency than of one with polyethylene (both "fibre" sources were included at 80 g/kg in the diets). Chickens fed on a diet containing 80 g/kg pectin grew more slowly than birds fed on the control diet (one with no added pectin).

Abdelsamie *et al.* (1983) fed broilers on diets of equal energy content but with acid detergent fibre (ADF) contents varying from 50 to 100 g/kg. The dietary ADF content did not affect the food consumption of the broilers. However, the dietary fibre negatively affected both growth rate and food conversion efficiency. Because the chickens in all treatments consumed equal amounts of metabolisable energy, it seems probable that the fibre may have affected the efficiency of protein utilisation.

Newcombe and Summers (1985) fed broilers on diets containing 0 to 600 g cellulose/kg. They found that the birds were incapable of compensating for the effects of dietary dilution by increasing the food intake of any of the diluted diets sufficiently to maintain the nutrient intake achieved by the birds fed on the basal diet. This inability to consume a constant amount of energy may mainly be a direct consequence of the physical limitations imposed on food intake by the capacity of the gut.

Van der Klis *et al.* (1993) fed broilers from 25 to 32 day of age on diets with increasing concentrations of carboxy methyl cellulose (CMC), an indigestible and soluble polysaccharide, and found that their food : gain ratio was significantly increased with increasing dietary concentrations of CMC. The diet with 5.0 g/kg CMC did not affect body weight gain nor food intake, while that with 10.0 g/kg reduced both significantly by between 20 and 25%. The authors attributed the responses to the effects of the viscosity of the CMC at the luminal side of intestinal wall.

Sarria and Preston (1995) fed chicks on diets into which two tropical fibrous plants had been incorporated, and concluded that either plant could replace up to 15% of the protein normally derived from soyabean meal without affecting their performance.

Choct *et al.* (1996) reported that the addition of the equivalent of 40 g/kg soluble non-starch polysaccharides to a commercial-type broiler diet reduced weight gain, food conversion efficiency and dietary apparent metabolisable energy in chickens. The authors explained the poor performance in terms the large reductions that were observed in the digestibilities of the starch, protein and lipid by the birds fed on the diet containing the added soluble non-starch polysaccharides.

Onifade and Babatunde (1998) fed broiler chicks on diets with different amounts of three fibrous agro-industrial by-products, palm kernel meal, brewers' dried grains and maize offal. They found that the broilers fed on the control diet had higher body weights, the lowest food consumption and the best efficiency of food utilisation. This higher performance was attributed to the higher nutrient density of the diet, a slower rate of food passage and a better apparent dry matter retention. The authors concluded that circumstances other than production efficiency may determine whether ingredients high in fibre are included in broiler diets.

Donkoh *et al.* (1999) evaluated performance when diets containing 0, 25, 50 and 75 g chaya leaf meal/kg were fed to broilers from 1 to 56 days of age. The concentration of chaya leaf meal in the diet had no effect on feed consumption. However, chicks fed on the diets containing 50 and 75 g chaya leaf meal/kg had significantly lower weight gains. It was concluded that chaya leaf meal could be included in diets for chickens at concentrations up to 25 g/kg without any adverse

effect on performance. However, increased concentrations of red blood cells, haemoglobin, haematocrit and decreased total serum cholesterol, as well as increased liver and heart weights were observed among birds fed on the diets containing the higher amounts of chaya leaf meal. The authors assumed that the hydrogen cyanide and oxalate present in the chaya leaf meal produced the effects observed on the blood parameters.

Slominsky and Campbell (1990) fed laying hens on diets containing 400 g/kg canola meal (*Brassica campestris*), an ingredient relatively high in fibre. In an initial trial they found low digestibility of the non-starch polysaccharides from canola meal and after the addition of cell-wall degrading enzymes in the diet the digestibility of these polysaccharides was increased by 37 %.

Panigrahi and Powell (1991) reported that palm kernel meal could be incorporated at up to 500 g/kg diet without depressing the growth of broilers. However, they pointed out that, because of the high fibre content of the palm kernel meal, a low metabolisable energy value of the diets for chickens was to be expected.

Sobamiwa and Longe (1994) found a diminished performance in broilers fed on diets containing increased concentrations of cocoa-pod husk and they attributed those response to some of the fibre components present in that foodstuff rather than the fibre content *per se*. The authors claimed that lignin and pectin were more deleterious than cellulose, even although the latter was the most abundant component in cocoa-pod husk.

Chapter Three
CHAYA (*Cnidoscolus aconitifolius*) AND THE CHEMICAL COMPOSITION OF ITS LEAVES

3.1 The description of chaya (*Cnidoscolus aconitifolius*) plant

The Chaya (*Cnidoscolus aconitifolius*) plant belongs to the *Euphorbiaceae* family, and is a member of the *Cnidoscolus* genus. McVaugh (1944) and Standley and Steyermark (1949) described members of the genus *Cnidoscolus* as an extremely homogeneous, strictly American group of plants consisting of between 40 or 50 species. The genus *Cnidoscolus*, whose name is derived from the Greek word referring to the irritant spines found on the species, is prevalent throughout Central America and the Western Caribbean. Directly related to the genus *Jatropha*, chaya is distinguished by the possession of a single white floral envelope, distinctive petiolar glands and stinging epidermal hairs. There are two closely related species *Cnidoscolus chayamansa* and *Cnidoscolus aconitifolius,* the former being less hairy than *C. aconitifolius*. For the purposes of the present study the chaya referred to will be *Cnidoscolus aconitifolius* [(Mill.) Johnston].

Chaya is a fast-growing small tree, which is well adapted to the tropics. It is a green perennial shrub found in the Yucatan Peninsula of Mexico and it grows well in both the humid and the dry tropical climates, suggesting a wide adaptability. Chaya grows to become a small and strong tree and its foliage can be harvested all the year round as an edible and fresh crop. It is high in nutrients and can be used in a number of different dishes (Martin and Ruberté, 1978; Peregrine, 1983). Diaz-Bolio (1974) mentioned that the word "chaya" comes from the Mayan language "chay", which was the local Indian name of the plant.

Chaya is also found along the pacific coast of Mexico, where it normally grows at low elevations. There is good evidence to show that chaya is a plant with excellent nutritional properties (Martin *et al.*, 1998). It is of unusually high nutrient content for a spinach-type vegetable, and in this context it compares favourably with

many legumes, *e.g.* cowpea, jackbean and ricebean. Nagy *et al.* (1978) have referred to some important horticultural advantages of chaya, such as the ease with which it can be propagated and pruned, its tolerance to both heavy rainfall and drought, and its high productivity. However, information relating specific conditions of rainfall for the chaya crop could not be found. It has been suggested that, because chaya produces abundant edible forage in such a small spaces and with no special conditions for its growth, further research into its nutritional value is merited. A two-year old Chaya plant is shown in Figure 3.1

Figure 3.1 Two-year old Chaya (*Cnidoscolus aconitifolius*) plant located in the Yucatan State of Mexico.

3.1.1 Botanical description

Martin and Ruberté (1978) and Peregrine (1983) have provided a botanical description of chaya and advice on its cultivation and this is presented below. The chaya plant is a big shrub normally growing to 3 m in height, but can reach 5 m under optimum environments. It branches easily and new branches tend to grow upwards. The foliage is particularly noticeable because of its dark-green colour. The central stem is about 10 cm in diameter, sometimes being wider in older plants, while the branches are 2 to 3 cm in diameter. The trunk is large and divided into transverse white plates. Its wood is soft, easy to break and susceptible to decomposition. The leaves are alternate, simple and hairless, except for some hairs on the margin, and invariably palmately lobed. The leaves are more wide than long and can reach dimensions of 22 by 18 cm.

The chaya plant flowers frequently on a 3- or 4-forked inflorescence 2 to 10 cm in diameter. Female flowers are produced in the lowest (proximal) forks of the inflorescence. The ovary is 3 mm long and the styles are 3 to 4 mm long. The white flowers, sustained distally, are frequently 6 to 7 mm long. Its odour tends to the unpleasant.

Figure 3.2 Three-month old re-grown stem with leaves from chaya plant.

3.1.2 Cultivation

The chaya plant can be established all the year round, but it is easier to propagate at the beginning of the rainy season than at other times of the year. There is practically no seed production, stem cuttings being the favoured approach to propagation, which is very simple. Stem cuttings taken at any stage from 10 to 120 cm long dried in the shade for 3 days produce the best results. It is important to dry off the cut wound, otherwise fungi or bacteria can cause the stems to decompose. Cuttings can be planted directly into well-drained soil where they are required to grow. When cuttings are solidly established, they resist considerable rain and can

adapt to faulty drainage. When the plants are producing new shoots they can be planted in the field.

The most convenient soil for chaya is a well-drained loam, because of its relative intolerance to waterlogged soil, which can quickly kill off the plants. Chaya can also prosper in sand and heavy clays and is also tolerant of many other soil types. The chaya plants can also be used as a long-lived hedge. The plantings usually consist of only a few isolated plants as a backyard crop.

After planting, the initial plants are usually healthy, but tend to grow slowly, new growth taking between 2 to 6 weeks. Edible leaves are available within 4 or 5 months after planting, but only after the first year of growth the plants can be severely pruned to which they respond with rapid new growth. During severe periods of drought, growth may stop and some leaf fall may occur. During the early stages of cultivation, chaya must be protected from weeds. Large plants must be protected from vines, which can break the branches by their weight and destroy the foliage by shading.

The effects of fertiliser application are not generally known. Manure or mineral fertilisers can be useful in accelerating growth and increasing yields; however, satisfactory growth has been observed in the home garden under adverse conditions and without the application of fertiliser.

A harvest of 60 to 80% of the leaves and branches is adequate to permit chaya to re-grow rapidly. In large plants, even when cut 40 cm from the ground only a few weeks are necessary before the harvesting process can be repeated. At the household level, only a small proportion of the foliage is removed at any one time. With that approach one plant can be harvested several times a week on a continuous basis.

Some pests have been observed to affect chaya. Larvae of the *Lepidopterous*, *Dasychira sp.* nr. *osseata* (Walker) complex, caused little damage, while another moth larva [*Spodoptera litura* (F.)] again is not of economic significance. However, in Central America the tomato horn-worm [*Manduca sexta* (Joh.)] can defoliate entire plants within a few days. Younger plants seem to be more susceptible to the horn-worm than older plants. After defoliation spontaneous new growth appears rapidly. Several fungi have been observed on the crop, but never on established

bushes. Chaya is almost free from diseases in Puerto Rico. Elliot and Zettler (1987) reported that cassava common mosaic virus was detected by serology in 23 of 33 samples (69.7%) of chaya (*Cnidoscolus aconitifolius*) collected in the Yucatan State in Mexico. However, viral symptoms were not obvious in most cultivated samples.

3.1.3 Uses

Booth *et al.* (1992) reported that chaya was consumed, by the indigenous people in Guatemala, as young leaves boiled, drained and usually, fried. The chaya plant is mainly consumed as a spinach-like vegetable. The younger leaves and about 20 cm of the stem are harvested usually for this purpose. Chaya is also used to some extent as an animal feed. Although recommended as a possible animal food, is it not clear if it has been used for swine or cattle. Chaya has also been used as a folk medicine. With diets based mainly on maize, when problems related to niacin and lysine deficiency can be expected, then chaya has been claimed to help alleviate such deficiencies. The use of chaya has also been reported to alleviate some health complains *e.g.* kidney problems and relief from constipation (Diaz-Bolio, 1974).

The presence of proteolytic activity in the latex of species from the *Euphorbiaceae* family reported by Lynn and Clevette-Radford (1988) was confirmed particularly in chaya by Iturbe-Chiñas and López-Munguia (1986) who considered the possible commercial importance of the chaya proteases. In an investigation of enzyme extraction and the hydrolysis of some food proteins, they found high proteolytic activity in chaya leaves towards haemoglobin and lower activities towards commercial casein, soyabean flour and fish protein isolate.

3.1.4 Production

There are few reports on the yields achieved from chaya. Peregrine (1983) found that an 18- to 24-month-old tree produced 4.9-7.4 kg of fresh leaves per month under the conditions prevailing in Brunei and, at a population density of 1111 trees

per hectare, chaya gave yields ranging between 4999 and 8333 kg/ha. Sandoval (1990) reported that under the tropical conditions prevailing in the Yucatan State of Mexico (with watering during the dry season) at a population density of 4356 trees/ha, yields of 5889 and 5776 kg dry matter (DM)/ha /year, respectively, were obtained when harvesting was carried out every 4 and 8 weeks. When the leaves and young stems were harvested together from the trees after 8 or 12 weeks of re-growth, the yields were 6512 and 10,058 kg DM/ha/year, respectively.

3.2. Chemical composition of chaya leaves

The chemical compositions of chaya reported by Munsell *et al.* (1949), Martin and Ruberté (1978) and Booth *et al.* (1992) are shown in Table 3.1. They all found chaya to be an important source of protein, carotene, B-vitamins, ascorbic acid, calcium and iron.

Donkoh *et al.* (1990; 1999) have also reported on the composition of chaya and their data are presented in Table 3.2. Reyes *et al.* (1991) analysed the long chain fatty acid content of three chaya species, *Cnidoscolus chayamansa, Cnidoscolus aconitifolius and Cnidoscolus souzae;* the last is a wild species found in the Yucatan State in Mexico. The total fat contents found for the above 3 respective species were 79.3, 79.3 and 53.5 g/kg. Myristic, stearic, oleic, linoleic and arachidonic acids were detected at the concentrations shown in Table 3.3 where, as is usual with vegetable fats, those of the unsaturated long chain fatty acids (18:2 and 20:1) were higher than those of the saturated counterparts (16:0 and 18:0).

Table 3.1 Chemical compositions of two species of chaya (*Cnidoscolus aconitifolius* and *Cnidoscolus chayamansa*) leaves

Component, per kg of edible portion	Species			
	C. chayamansa[1,2,3]			*C. aconitifolius*
	1	2	3	4
Water (g)	790.0	811.0	917.0	764.0
Protein (g)	82.0	62.0	22.0	81.0 *
Carbohydrate (g)	72.0	61.0	27.0	NR
Ether extract (g)	19.0	19.0	5.0	1.6
Fibre (g)	19.0	26.0	10.0	23.0
Ash (g)	17.0	21.0	15.0	26.0
Calcium (g)	4.21	2.26	0.9	3.34
Iron (mg)	120	50	20	110
Phosphorus (mg)	630	540	390	82
Potassium (g)	NR	NR	2.71	NR
Magnesium (mg)	NR	NR	310	NR
Ascorbic acid (g)	2.44	1.96	NR	1.76
Carotene (mg)	85	80	NR	60
Niacin (mg)	17	15	NR	20
Riboflavin (mg)	3.0	4.0	NR	5.0
Thiamine (mg)	2.0	3.0	NR	2.0
Carotenes (mg)	NR	NR	27	NR

*reported as nitrogen by 6.25
NR, not reported
1. Souza-Novelo, cited by Martin & Ruberté (1978)
2. Diaz-Bolio, cited by Martin & Ruberté (1978)
3. Booth *et al.* (1992)
4. Munsell *et al.* (1949)

Table 3.2 Composition of *Cnidoscolus aconitifolius* leaf meal

Component	Reference	
	Donkoh *et al.* (1990)	Donkoh *et al.* (1999)
Dry matter (g/kg as received basis)	894.9	902.4
g/kg dry matter		
Crude protein	296.2	269.5
Ash	139.5	143.7
Ether extract	40.7	38.9
Crude fibre	101.3	116.4
Neutral detergent fibre	150.7	158.3
Acid detergent fibre	123.4	131.6
Hemicellulose	27.4	26.7
Calcium	93.4	87.5
Phosphorus	8.1	7.7
Magnesium	4.3	4.8
Sodium	2.0	2.9
Potassium	8.2	7.0
Manganese	0.2	0.1
Iron	20.8	18.6
Zinc	0.1	NR
Copper	0.5	NR
Metabolisable energy (MJ/kg)	8.88	5.52

NR, not reported

Table 3.3. Relative composition of long chain fatty acids in three species of chaya (Reyes *et al.*, 1991)

	Species		
Fatty acid (% of total fatty acid content)	*C. chayamansa*	*C. aconitifolius*	*C. souzae*
Myristic (16:0)	26.89	26.27	33.22
Stearic (18:0)	3.36	1.46	2.22
Oleic (18:1)	5.88	2.92	2.44
Linoleic(18:2)	10.08	11.68	10.0
Arachidonic (20:1)	53.78	57.66	52.11

Table 3.4 Amino acid compositions of chaya leaf and soyabean meals

Amino acid	Species			
	C. chayamansa [1]	*C. aconitifolius* [2]	*C. aconitifolius* [3]	Soyabean [4]
Alanine	4.6	14.6	NR	NR
Arginine	11.2	14.5	21.5	35.6
Aspartic acid	13.7	28.6	NR	NR
Cystine	1.7	3.8	4.1	7.4
Glutamic acid	19.0	34.5	NR	NR
Glycine	3.5	15.4	NR	21.5
Histidine	NR	6.3	7.2	13.2
Isoleucine	3.1	12.1	10.5	22.2
Leucine	5.8	22.2	18.6	38.4
Lysine	4.9	19.8	14.5	30.5
Methionine	1.2	4.8	3.6	7.0
Phenylalanine	6.0	13.8	14.3	24.4
Proline	1.0	13.9	NR	NR
Serine	3.3	12.4	NR	25.9
Threonine	3.3	11.18	10.5	19.5
Tryptophan	NR	3.2	2.4	8.3
Tyrosine	3.5	8.4	NR	21.6
Valine	4.9	15.4	16.2	23.4

1. Martin & Ruberté (1978), percentage of total amino acids
2. Donkoh *et al.* (1990), g/ kg dry matter
3. Donkoh *et al.*(1999), g/ kg dry matter
4. NRC (1994), g/ kg dry matter; 498 g/kg crude protein (on dry matter basis)

NR, not reported

Donkoh *et al.* (1990) reported that the overall mean availability of the amino acids in chaya for chickens is 84%, and, according to Table 3.4, chaya contains 68.5, 64.9 and 57.3%, respectively, of the amounts of methionine, lysine and threonine found in soyabean meal. In this respect it compares favourably with soyabean meal,

and this suggests that chaya is worthy of investigation as far as a potential feed ingredient for poultry.

Regarding the chemical composition of chaya at different stages of maturity, Sandoval (1990) found 209 and 178 g/kg, respectively, of crude protein in samples of leaves with petioles together, at 8 and 12 weeks of regrowth. In a further report Sandoval *et al.* (1990a) found 235 ± 29 and 225 ± 19 g/kg, respectively, of crude protein in samples of leaves and petioles at 4 and 8 weeks of regrowth. In a third study Sandoval *et al.*(1990b) reported 212 ± 32 and 238 ± 34 g/kg, respectively, of crude protein in samples of leaves and petioles at 8 and 12 weeks of regrowth.

There are some toxic substances in chaya. National Academy of Sciences (1975), Martin and Ruberté (1978) and Peregrine (1983) have all reported that chaya contains glucosides of hydrocyanic acid in the leaves. Although the concentrations were not reported, it was noted that they were destroyed by exposure to heat. Rivas (1985), however, reported a concentration range of between 1.10 and 1.58 g of hydrogen cyanide in fresh chaya leaves per kg of dry matter; in dried leaves the hydrogen cyanide content was reduced to 0.088-0.099 g/kg of dry matter. Donkoh *et al.* (1990) reported that there were 1.98 and 1.07 g/kg, respectively, of hydrogen cyanide and oxalate in the sample of their chaya leaf meal and, in a second study Donkoh *et al.* (1999) reported 1.02 and 0.85 g/kg for the same respective components.

Some features of dietary fibre and its influence on the digestive physiology of poultry have been described in the present review of the literature. Aspects relating to the issues of inadequate food distribution in the world, despite the growing farming industry of which poultry is a good example and how the use of non-conventional foodstuffs like forages and leaves by small-farming systems would alleviate that situation have also been discussed. The description of the chaya plant and some of its chemical characteristics were part of this review of the literature as well. However, neither information on the effect of the harvesting period on the chemical composition of the chaya leaves nor on how chickens are able to cope with dietary chaya leaf meal (nutrient utilisation and digestive physiology) have been established. Therefore, in order to provide that information several experiments were

performed to determine the effects of feeding diets containing chaya leaf meal on digestibility and on the the performance of chickens.

3.3 Experiment 1. The effect of different ages of re-growth on the chemical composition of chaya leaves

3.3.1 Materials and methods

The effect of three different harvesting periods on the chemical composition of chaya was examined. Twenty-four chaya plants from a 2-year-old plot (20 m x 30 m) were chosen at random. The climate where the plot was located is tropical, sub-humid with an annual precipitation ranging from 900 to 1100 mm and a temperature average of 26°C. The soil of the plot was shallow (10 cm to 30 cm), calcareous, clay loam and with fine clay sediment, *i.e.* a mixture of litosol and luvisol soil types (INIFAP-CIRSE, 1999). The selected plants were free from any apparent diseases and were established at 1.5 m x 1.5 m (4356 plants/ hectare). When rain was not present, they received watering twice a week.

After harvesting all plants at the beginning of the Spring season, 8 plants were each harvested again after either 4, 8 or 12 weeks of re-growth. The harvesting consisted of cutting all the leaves first, and then the young stems until the plants were approximately 1 m in height. The leaves from each plant were stored in plastic bags and frozen at -10°C to await chemical analyses. Analyses for dry matter (DM), crude protein (CP), ash, ether extractives, lignin, hydrogen cyanide and oxalic acid were carried out according to the methods outlined by AOAC (1980). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) analyses were performed sequentially according to Van Soest *et al.* (1991) and using a fibre analyser (ANKOM, 1997). Data were subjected to analysis of variance with age of re-growth as the only factor.

Additionally, a pool sample of chaya leaves from 8 and 12 week-old of re-growth was taken for uronic acid and for non-starch polysaccharides analyses. Uronic acid was performed by colorimetric determination (Theander *et al.*, 1995) and non-starch polysaccharides was determined according to the gas-liquid chromatographic procedure outlined by Englyst *et al.* (1994).

3.3.2 Results

Table 3.5 Chemical compositions of chaya leaves harvested after either 4, 8 or 12 weeks of re-growth

Parameter	Age of re-growth, weeks			SEM	Probability
	4	8	12		
Moisture (g/ kg When harvested)	795.0	787.8	788.5	11.22	0.882
g/ kg dry matter					
Crude protein	276.6	278.3	263.3	5.00	0.092
Ether extractives	71.3a	76.1ab	88.5b	3.51	0.007
Neutral detergent fibre	172.2	175.4	172.9	3.93	0.833
Acid detergent fibre	121.8	119.5	118.9	3.54	0.834
Lignin	33.2	39.6	38.7	2.42	0.153
Ash	100.9	101.2	102.6	2.25	0.979
Oxalic acid	8.0a	8.1a	6.1b	0.57	0.037
Hydrogen cyanide	1.3	1.2	1.3	0.09	0.717

SEM = standard error of mean
Different letters *a, b* in the same row are indicative of values that significantly differ at $P<0.05$

There were no significant differences between plants with different age of re-growth for the parameters analysed with exception for ether extractives and oxalic acid contents (Table 3.5). The ether extractives content was higher ($P<0.05$) in plants with 12 weeks than in those with 4 weeks of re-growth. The oxalic acid content was

lower ($P<0.05$) in those plants with 12 weeks than in those either with 4 or 8 weeks of re-growth.

The results of non-starch polysaccharydes (as neutral sugars and uronic acid) are shown in Table 3.6. Galactose, glucose and xylose were the most important sugars found, and also uronic acid represented a significant component. However, galactose and uronic acid were the main components in the soluble portion. There was a total content of 197.2 g/kg non-starch polysaccharides, where 68.72 % and 31.26 % corresponded to the insoluble and soluble components respectively.

Table 3.6 Non starch polysaccharides content as neutral sugars and uronic acid (g/kg) of chaya leaves

Sugar	Insoluble	Soluble	Total
Xylose	25.33	2.77	28.10
Arabinose	21.11	2.31	23.42
Mannose	14.27	1.56	15.83
Galactose	25.04	12.98	38.02
Glucose	34.27	3.74	38.01
Uronic acid	15.5	38.3	53.80
TOTAL	135.52	61.66	197.2

3.3.3 Discussion

The chemical composition of chaya leaves in the current study in general agreed with the earlier findings of Donkoh *et al.* (1990; 1999) with plants grown in Ghana, except for oxalic acid which was higher in the present study. The higher amounts of oxalic acid found at either 4 or 8 weeks of re-growth could be explained in terms of a response to cutting which may influence new growth containing higher amounts of antinutrients than uncut leaves. Lowry (1989) pointed out that the concentration of some toxic substances is higher in new or developing leaves than in mature leaves, as a response to the effects of browsing.

It is difficult to explain the higher amounts of ether extractives accumulated in chaya leaves at 12 weeks of re-growth, but it could simply be a consequence of the normal accumulation of some substances such as oil, lignin or fibre that occurs with the maturity of the tissues of the plant.

The CP concentrations in chaya found in this study are higher than those reported by Sandoval (1990) and Sandoval *et al.* (1990a; 1990b) probably because the inclusion of the petioles, which usually contain more fibre, together with leaves in the latter three studies decreased CP. Factors such as plant parts, soil type, climate and season of the year are all possible sources of variation in the chemical composition of forages (Ivory, 1989).

It is possible that the fact that no effect of age was observed on the chemical composition of the chaya leaves could be explained because chaya, like any other tree, has a slower rate of growth in comparison to grasses. Probably periods of harvesting every 4 weeks were too close together to allow different chemical compositions to manifest themselves on most of the parameters evaluated, even between 4 to 12 weeks of re-growth. Lowry (1989) reported that only a small change in the chemical composition of tree leaves occurs during maturation, in comparison to the great decrease that takes place in tropical grasses at the same stage of development. Camacho-Morfín *et al.* (2000) reported no differences in chemical compositions of the leaves of the tree *Acacia saligna* harvested every 30 days throughout the four seasons of the year. Pretel *et al.* (2000) reported similar contents of crude protein in the leaves of *Brosimum alicastrum* trees, which never had been harvested, at 4.5, 6.5 and 8.5 years of age.

The insoluble non-starch polysaccharides content found in the sample of chaya leaves from Table 3.6, plus the lignin content (from Table 3.5), corresponded approximately to the neutral detergent fibre reported for those samples (between 8-12 week of regrowth), which was according to the expected findings. From the content of neutral sugars and uronic acid found, it could be possible to estimate the polysaccharides contained in the fibre portion of chaya leaves. Considering the insoluble component it is possible to assume that xylose, arabinose and mannose were originated from hemicellulose; galactose, uronic acid and also some arabinose were originated from pectin and glucose from cellulose. On the other hand, the

soluble component was mainly conformed by soluble pectin and some residual soluble hemicellulose (Selvendran *et al.*, 1987; Van Soest, 1994b).

Chapter four
DIGESTIBILITY AND ENERGY VALUES OF CLM IN CHICKENS AND COCKERELS

4.1 Introduction

The efficiency of utilisation of the gross energy contained in the feedingstuffs ingested by animals depends on diverse factors such as their age, of breed and the development and stage of maturity of the gastrointestinal system. It also, to a large extent, depends on the chemical composition of the foodstuff (Jadhao *et al.*, 1999; Sulistiyanto *et al.,* 1999). Food intake affects daily heat production in chickens. The heat increment of feeding in poultry has been reported on many occasions (Li *et al.*, 1991; MacLeod, 1991a; Zhou and Yamamoto, 1997; Koh and MacLeod, 1999). The heat increment produced by the food depends on its chemical composition; fat produces a lower heat increment than protein or carbohydrate (Shannon and Brown, 1969). The effect of different fibre sources on heat production may have different effects on birds and consequently on the net energy values of fibrous foodstuffs. The nutritional value of fibrous foodstuffs may be altered when their inclusion in poultry diets is expressed in the form of net energy.

The aims of the experiments reported in this chapter were to study the effects of some factors on the digestibility of chaya leaf meal (CLM), and to determine its true metabolisable energy (TME) and net energy (NE) values as well as the digestibilities of the constituent amino acids. It was also planned to investigate the effect of different fibre sources on the endogenous losses in chickens. Five experiments were carried out using both chickens and cockerels and they were done either at Roslin Institute or the Faculty of Veterinary Medicine of the University of Yucatan, Mexico.

The CLM was harvested from two-year-old chaya plants (with between 8 and 10 weeks allowed for re-growth) produced under the tropical conditions prevailing in

the Yucatan State of Mexico. The selected plants came from similar plots as those described in section 3.2.1.

The harvesting consisted of cutting all the leaves and stems down to a height of 1 m. All the leaves were then separated and dried in an oven at 60 °C for 48 h. The dried leaves were ground through a 1 mm-mesh sieve on an electric mill, and the CLM obtained was then stored in plastic bags and frozen at -10°C until required.

4.2 Experiment 2. Effect of different inputs (by tube-feeding), different collection periods of excreta, bird genotype and body weight on the digestibility of CLM

4.2.1 Materials and methods

Three studies were carried out to examine the effects of feeding different amounts of CLM and of different periods of excreta collection. In addition, the effects of bird genotype and body weight on the digestibility of CLM was investigated. True metabolisable energy (TME), TME corrected to nitrogen equilibrium (TME_N), dry matter (DM), gross energy (GE) and nitrogen (N) digestibilities were the dependent variables. TME_N was calculated according to the following formula proposed by Parsons *et al.* (1982b):

$$TME_N \text{ (MJ/kg)} = \frac{FEf - [EEf + 34.4\ Nf] + [EEu + 34.4\ Nu]}{FC}$$

Where: FEf (MJ/kg) is the gross energy of total feed consumed

EEf (MJ) is the energy in the excreta collected from the fed birds

EEu (MJ) is the energy in the excreta collected from the fasted birds

Nf (g) is the nitrogen retained by the fed birds

Nu (g) is the nitrogen retained by the fasted birds

FC (g) is the feed consumed.

Each g of nitrogen is assumed to generate 34.4 kJ of additional urinary energy in the excreta (Parsons *et al.,* 1982b).

The effect of genotype on the water intake and on the DM content of the excreta was also determined. The averages of the maximum and minimum temperatures and relative humidity in the poultry house were 38.2 °C, 23.8 °C and 82%, respectively and a cycle of 14 h light : 10 h dark was maintained. All the birds were allocated to individual wire pens (40 x 50 cm), with steel trays placed

underneath for the collection of droppings. A repeat tube-feeding scheme over two days was applied in this experiment (McNab and Bernard, 1997). Fresh water was available to the birds at all times. The droppings were collected quantitatively every 24 h, frozen (-10°C) until each collection period was finished, dried at 60°C and finely ground. Analyses for N and ash were carried out according to AOAC (1980). GE was determined by adiabatic bomb calorimeter (Roslin Nutrition, 2000). The CLM used in this experiment contained 297 g/kg crude protein, 94 g/kg ash, 153 g/kg crude fibre, and 16.9 MJ/kg gross energy. A MINITAB (1999) software package was used to perform all the statistical analyses.

In the first study, the effects of tube feeding three different amounts of CLM and of using three different periods over which to collect the droppings were examined in broilers. Thirty-six male commercial Hubbard broilers with an average body weight of 1.8 $\pm$ 0.15 kg were used. The birds were fasted for 24 h after which they received 50 ml of glucose solution (50% w/v) by tube. Three inputs of CLM (25, 35 and 45 g) were fed by tube and three droppings collection periods (48, 56 and 72 h after the last tube feeding) were distributed in a factorially arranged design. Nine birds were fed twice (24 and 48 h after being given the glucose solution) per input of CLM. Additionally, three birds were tube-fed 50 g of glucose for each collection period to determine the endogenous DM, N and GE losses observed after 48, 56 and 72 h of food withdrawal.

The procedure described above was repeated one week later on the same group of birds in order to collect 18 observations per treatment. In the intervening period the birds were fed *ad libitum* on a commercial pelleted diet (180 g/kg crude protein and 12.6 MJ/kg ME).

The general linear model procedure was applied to the analyses of variance on the data as a 3 x 3 factorial design. The interaction food input x collection period was included in the model. When significant effects were identified by analysis of variance Tukey's multiple comparison was used. Regression analyses were performed between factors affecting significantly dependent variables. Also, linear regressions of DM, GE and N excretions on CLM input were determined. The intercept values from these equations were also used to calculate the dependent

variables instead of endogenous losses obtained from fasted birds (Sibbald and Morse, 1983a,b).

In the second study the effect of body weight of broilers was evaluated. There were three groups (G) of twelve male Hubbard broilers weighing 645 ± 23 g (G1); 1020 ± 16 g (G2); and 1501 ± 49 g (G3). The birds were fasted for 24 h and then received 30, 40 and 50 ml, respectively, of glucose solution (50% w/v) by tube. Eight birds per group were fed twice (24 and 48 h after being given the glucose solution) by tube either 15, 25 or 35 g of CLM. Additionally, 4 birds from each group were tube fed 30, 40 and 50 g of glucose respectively, to determine the endogenous losses of DM, N and GE. Analyses of variance were performed on the data with body weight as the only factor.

In the third study the effect of genotype was determined using Hubbard and naked neck criollo chickens. Twelve Hubbard chickens with an average weight of 1.8 ± 0.2 kg and 12 naked neck criollo chickens with an average weight of 1.8 ± 0.18 kg were used. The birds were fasted for 24 h and then received 50 ml of glucose solution (50% w/v) by tube; 24 h after being given the glucose solution, 8 birds per genotype were given 25 g of CLM by tube at 08:00 h, and at 16:00 h, for two days. There were two droppings collection periods of 64 or 72 h after the last tube-feeding. Additionally, 4 birds per genotype were tube-fed 50 g of glucose to determine the endogenous losses of DM, N and GE per collection period. The procedure described above was repeated one week later using the same birds in order to collect 16 observations per treatment. The water intake, after correction for evaporative losses, and the DM contents of the excreta from four birds per genotype were also determined. In between the assays, the birds were fed *ad libitum* on a commercial pelleted diet (180 g/kg crude protein and 12.6 MJ/kg ME). The data were analysed statistically as a 2 x 2 factorially arranged design.

4.2.2 Results

In study one, the CLM input significantly increased ($P<0.05$) the DM and N digestibilities (Table 4.1) and the linear regressions of those variables on CLM input

were significant (Figure 4.1). The CLM input did not affect the mean of GE digestibility and consequently neither TME nor TME_N were affected. There were no effects of the collection periods on any of the dependent variables, although the mean N digestibility showed a tendency to decrease as the collection period was increased. No feed input x collection period interaction was detected for any of the dependent variables.

The mean endogenous losses (± standard deviation) from the fasted birds for DM, GE and N at 48 h were 6.18 ± 1.42 g, 73.7 ± 14.7 kJ and 0.92 ± 0.21 g respectively. The corresponding means found at 56 h were 8.97 ± 1.5 g, 101.6 ± 36.2 kJ and 1.27 ± 0.51 g respectively, and at 72 h were 9.71 ± 1.9 g, 124.3 ± 26.2 kJ and 1.67 ± 0.47 g, respectively.

The regression lines of DM, GE and N excretions on CLM input were all significant ($P<0.01$), and the equations for 48 h collection period were:

DM (g) = 16.9 + 1.25 input $R^2 = 0.684$

GE (kJ) = 146 + 19.5 input $R^2 = 0.911$

N (g) = 1.80 + 0.05 input $R^2 = 0.524$

For 56 h collection period the equations were:

DM (g) = 20.1 + 1.16 input $R^2 = 0.840$

GE (kJ) = 193 + 18.6 input $R^2 = 0.965$

N (g) = 2.34 + 0.05 input $R^2 = 0.608$

And for 72 h collection period they were:

DM (g) = 20.0 + 1.27 input $R^2 = 0.683$

GE (kJ) = 90.3 + 22.1 input $R^2 = 0.946$

N (g) = 2.70 + 0.06 input $R^2 = 0.465$

The intercept values from those equations were numerically higher than the values derived using fasted birds. When those intercepts were used as endogenous losses, in order to calculate the dependent variables, none of them was affected ($P>0.05$) by CLM input, although these means from Table 4.2 show an improvement with respect to the same values from Table 4.1.

However, after using those intercepts as endogenous losses, GE digestibility, TME and TME_N (Table 4.2) all decreased ($P<0.05$) as the collection period was increased.

In study two, one bird from G1 was excluded because it showed symptoms of illness. The mean TME_N values increased with increasing average body weight ($P<0.05$). However, TME and the digestibilities of DM, GE and N were not affected ($P>0.05$) by the average body weight of the birds (Table 4.3). However, with the exception of N digestibility, which showed the largest variation, there were slight tendencies for all the dependent variables to increase with increasing body weight.

Table 4.1 Least square means of true metabolisable energy corrected to nitrogen equilibrium (TME_N), true metabolisable energy (TME) and true digestibilities of dry matter, gross energy and nitrogen of CLM by broilers at three feeding inputs and over three droppings collection periods

			True Digestibility (%)		
	TME_N MJ/kg	TME MJ/kg	Dry Matter	Gross energy	Nitrogen
Input (g)					
25	6.05	6.06	14.33a	35.89	1.04a
35	6.20	6.24	17.24ab	36.94	4.36a
45	6.16	6.44	23.72b	38.10	17.24b
Probability	0.711	0.231	0.008	0.232	0.002
Collection period (h)					
48	5.89	6.09	17.18	36.08	12.89
56	6.15	6.26	21.29	37.03	7.37
72	6.36	6.39	16.82	37.81	2.38
Probability	0.054	0.399	0.249	0.399	0.077
SEM	0.13	0.15	2.07	0.89	3.18

Different letters *a, b* in the same column are indicative of values that differ significantly ($P<0.05$)

Table 4.2 Least square means corrected[1] of true metabolisable energy corrected to nitrogen equilibrium (TME_N), true metabolisable energy (TME) and true digestibilities of dry matter, gross energy and nitrogen of CLM by broilers at three feeding inputs and over three droppings collection periods

	TME_N MJ/kg	TME MJ/kg	True Digestibility (%)		
			Dry matter	Gross energy	Nitrogen
Input (g)					
25	6.23	6.93	36.89	41.01	42.44
35	6.32	6.85	33.35	40.57	34.20
45	6.26	6.92	36.25	40.94	40.46
Probability	0.873	0.935	0.445	0.936	0.173
Collection period (h)					
48	6.53a	7.19a	34.25	42.54a	40.81
56	6.97a	7.64a	39.02	45.20a	41.27
72	5.31b	5.87b	33.21	34.77b	35.02
Probability	0.001	0.001	0.119	0.001	0.312
SEM	0.13	0.15	2.07	0.89	3.18

1.Intercept values from linear regression of excretions on CLM input were used to calculate the dependent variables as described above in the text.

Different letters *a, b,* in the same column are indicative of values that differ significantly ($P<0.05$)

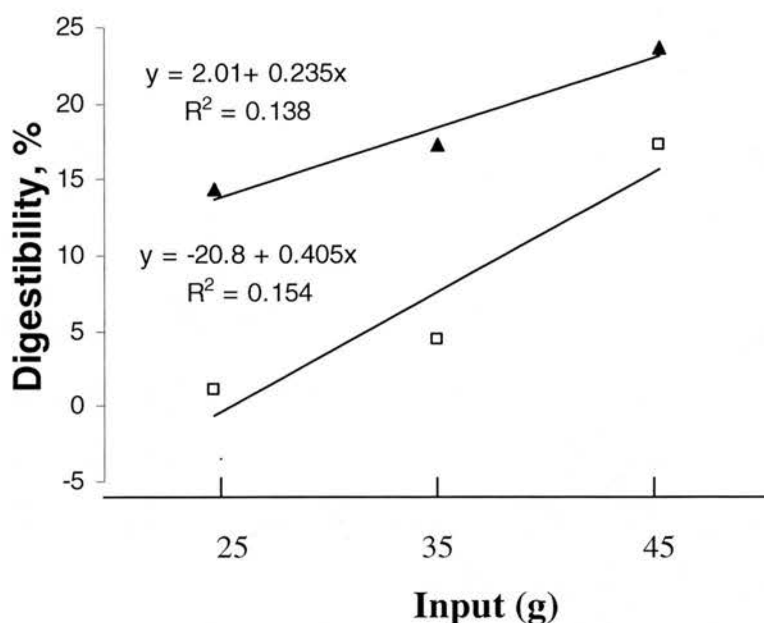

Figure 4.1 Linear relationship between dry matter (▲) and nitrogen (□) digestibilities and CLM input.

Table 4.3 Least square means (± SE) of true metabolisable energy (TME), true metabolisable energy corrected to nitrogen equilibrium (TME_N) and true digestibilities of dry matter, gross energy and nitrogen of CLM by broilers at three different average body weights

Parameter	Average body weight (kg)			Probability
	0.6	1.0	1.5	
TME (MJ/kg)	6.04 ± 0.12	5.73 ± 0.10	6.84 ± 0.10	0.050
TME_N (MJ/kg)	5.74a ± 0.10	5.70ab ± 0.09	6.67ac ± 0.91	0.026
Digestibility (%)				
Dry Matter	26.82 ± 0.80	23.95 ± 0.70	28.36 ± 0.70	0.308
Gross Energy	35.76 ± 0.73	33.91± 0.63	40.49 ± 0.63	0.051
Nitrogen	18.10 ± 2.61	1.95 ± 2.28	10.35 ± 2.28	0.255

Different letters *a, b, c* in the same row are significantly different at $P<0.05$

In study three, there were no significant differences between genotypes for any of the studied variables (Table 4.4). Higher ($P<0.05$) TME and TME_N values, and higher DM and GE digestibilities were found at the 64 h collection period than after 72 h. Because of the high variation of the data neither genotype nor collection period affected N digestibility, though there was a tendency for it to be lower in Hubbard chickens and at the 72 h collection period. The statistical analysis indicated that collection period affected the two genotypes differently leading to significant genotype x collection period interactions ($P<0.05$) for TME, TME_N and for DM and GE digestibilities.

Table 4.4 Least square means of true metabolisable energy (TME), true metabolisable energy corrected to nitrogen equilibrium (TME_N)and true digestibilities of dry matter, gross energy and nitrogen of CLM by Hubbard and criollo chickens over two collection periods

	Genotype			Collection period			
Parameter	Hubbard	Criollo	Proba bility	64 h	72 h	Proba bility	SEM
TME (MJ/kg)	6.59	6.35	0.395	7.42	5.52	0.001	0.20
TME_N (MJ/kg)	6.69	6.27	0.085	7.25	5.70	0.001	0.16
Digestibility (%)							
Dry Matter	30.09	30.20	0.970	37.71	22.59	0.001	1.99
Gross Energy	39.01	37.55	0.397	43.88	32.68	0.001	1.19
Nitrogen	-9.5	1.22	0.520	5.7	-14.0	0.244	11.69

When data were analysed by genotype, the mean DM digestibility (Table 4.5) was higher ($P<0.05$) at the 64 h than at the 72 h collection period in both Hubbard and criollo genotypes, although both TME and TME_N values and the digestibility of the GE were different between collection periods only in the Hubbard chickens.

Nitrogen digestibility did not differ between collection periods and it was negative at 72 h in both genotypes, although the trend showed that Hubbard had lower N digestibility than criollo chickens.

Table 4.5 Least square means of true metabolisable energy (TME), true metabolisable energy corrected to nitrogen equilibrium (TME_N) and true digestibilities of dry matter, gross energy and nitrogen of CLM by genotype over two collection periods

	Hubbard				Criollo			
Parameter	64 h	72 h	Probability	SEM	64 h	72 h	Probability	SEM
TME (MJ/kg)	8.16	5.03	0.001	0.26	6.67	6.02	0.151	0.30
TME_N (MJ/kg)	8.01	5.36	0.001	0.20	6.49	6.03	0.251	0.26
Digestibility (%)								
Dry matter	41.05	19.13	0.001	3.08	34.35	26.04	0.038	2.54
Gross energy	48.27	29.74	0.001	1.56	39.48	35.62	0.152	1.79
Nitrogen	4.23	-23.34	0.352	20.2	7.14	-4.7	0.493	11.87

The means of water intakes (Table 4.6) during both the fed and starved periods were higher ($P<0.05$) in the Hubbard than in the criollo chickens, and the means of DM contents of the excreta from the criollo was consequently higher ($P<0.05$) than that from the Hubbard chickens.

Table 4.6 Mean water intakes and dry matter contents of excreta from Hubbard and criollo chickens as a result of tube-feeding CLM

Parameter	Genotype		SEM	Probability
	Hubbard	Criollo		
Water intake (ml/day)				
After tube-feeding	406.2	240.0	24.82	0.003
When starved	171.8	56.8	20.39	0.007
Dry matter in excreta (g/kg)	224.8	453.4	23.49	0.001

4.2.3 Discussion

The endogenous losses observed in the first study were similar to those reported by Askbrant and Khalili (1990) for a 48 h collection period and lower than those values found by Farrel *et al.* (1991) from starved birds receiving glucose. However, the large variation in endogenous losses found in this study agreed with that reported by Parsons *et al.* (1982b). McNab and Blair (1988) have pointed out that, in general, it is their experience that endogenous losses are quite variable with coefficients of variation of around 10%.

The CLM input did not affect GE digestibility, consequently neither TME_N nor TME were affected. These results are in agreement with Sibbald (1975) and Sibbald and Morse (1983a) who reported that the TME value of a foodstuff is independent of the amount eaten, even when fibrous foodstuffs are evaluated. Furthermore Wolynetz and Sibbald (1984) claimed that TME_N values are the more reliable measures of the bio-available energy of foodstuffs at all intakes, because the

variation is lower than that of the uncorrected values for both growing and adult birds, and laying hens. The correction to zero N balance reduces the variation for energy excreted and consequently improves the precision of the values generated in the assays (Sibbald and Morse, 1983b; McNab and Blair, 1988).

The positive linear relationship between nitrogen digestibility and CLM input could be partially explained by the fact that the proportion of excreted nitrogen emanating from the catabolism of body protein is likely to be reduced (and consequently, more diluted) as input is increased. Sibbald and Morse (1983a) have pointed out that the effects of fasting may be overcome if larger inputs are used in the TME bioassay. They found that the nitrogen concentration in the excreta was higher in fasted than in fed birds, whereas the opposite relationship applied for energy concentration. Sibbald (1975) also claimed that, at high feed intakes, endogenous losses had little effect on the metabolisable energy values derived. Muztar and Slinger (1980a) reported that crude fibre consumption increased the loss of faecal nitrogen from animals as the result of mechanical abrasion of the digestive tract. However, Jonsson and McNab (1983), after feeding broilers on different amounts of grass meal, found a negative N retention with some of the inputs and argued that this resulted from low food intakes *per se* rather than the concentration of the grass meal (and, hence, fibre) in the diets.

Similar effects to those observed on N digestibility would explain the tendency for DM digestibility to increase as the CLM input was increased. An additional effect could be caused by not digested material, such as CLM's fibre, increasing endogenous losses as it passes through the gastrointestinal tract (Tenesaca and Sell, 1981). Moreover, the proliferation of the intestinal microflora may contribute to the mass of excreta voided by birds fed on high fibre-containing diets, in this case CLM, and could play an important role in the derivation of digestibility coefficients (Parsons *et al.,* 1982c; Iji, 1999).

All the means of dependent variables, calculated again with the intercepts of the regressions of DM, GE and N excretions, were numerically higher (Table 4.2) than those derived from balance experiments and corrected for endogenous losses using values measured in fasted birds (Table 4.1). The likelihood that the latter approach underestimates endogenous losses and results in the generation of low

digestibility coefficients when practical diets high in fibre are fed merits serious consideration. Although Sibbald and Wolynetz (1985) pointed out that differences between obseved endogenous losses from fasted birds and the corresponding intercepts might be associated with tissue nitrogen catabolism between fed and fasted birds, controversy has surrounded whether or not the dietary fibre content increases endogenous losses. Farrell (1981), using cockerels, evaluated over 32 h balance periods (*i.e.* excreta collection), derived TME values for a number of ingredients with different NDF contents and established that increasing dietary NDF increases endogenous losses. Sibbald (1982) argued that Farrell's (1981) finding was biased because 32 h was insufficient time for all the indigestible residues of the ingesta to clear the gastrointestinal tract, and that, consequently, intercepts of linear regressions of data from the same experiment would produce higher values. In the present study the endogenous losses were calculated from excretions made in the 72 h after feeding, a time likely to be long enough to allow for the complete clearance of all indigestible feed residues. Moreover, in the same paper Sibbald (1982) acknowledged that both assumptions are crucial to the validity of the TME assays as a means of evaluating the energy status of feeds (*viz.* that there is a linear relationship between the energy voided as excreta and the energy input as feed in previously fasted birds and that the intercept of this regression line is a valid estimate of endogenous loss). In this study, however, these assumptions were of doubtful validity and need to be confirmed in experiments with more birds.

Moreover, Farrell *et al.* (1991) also indicated that endogenous losses are influenced by the food intake and particularly, that the crude protein and crude fibre concentrations of the input do affect the size of the endogenous losses. CLM, in the present study, was especially high in both crude protein and crude fibre, which may have contributed to increase the sizes of the endogenous losses. Also, Härtel (1986) reported that the intercepts found in regression equations relating energy excretions to food intake were too high and equivocal. He argued that that the application of the wrong intercepts of endogenous energy losses could lead to the misinterpretation of results, because in daily fed animals the endogenous energy losses are very low and might be ignored.

The length of the collection period reduced GE digestibility, and both the

TME and TME_N values (P<0.05), derived using the regression intercepts for endogenous losses (Table 4.2). This could arise with CLM because its high fibre content is likely to mean that a clearance time of up 72 h will be required to recover all its indigestible residues (Lessire, 1990). Sibbald (1979b) pointed out the need to extend the excreta collection period in the evaluation of foodstuffs having slow rates of passage through the digestive tract, although it may result in reductions on both nutrient digestibility and TME values. Sibbald (1979a) also indicated that both high fibre and ash contents in foodstuffs may be responsible for slow rates of passage and it may prove necessary to extend the collection period when evaluating such materials. Sibbald (1980) referred to the important role fibre plays in causing a slower rate of passage of some foodstuffs (like oats) compared to ingredients low in fibre (such as maize or wheat). McNab and Blair (1988) recommended extending the excreta collection period unilaterally to 72 h in order to minimise between-bird variation.

Dale and Fuller (1982) found that the endogenous energy loss was inversely proportional to the energy intake and argued therefore, that the use of starved birds to derive endogenous energy values in TME assays could not be totally correct. After all, in the study reported herein, "fasted" birds (*i.e.* receiving 50 g glucose) had an ME input of 780.3 kJ (NRC, 1994) whereas fed birds given 25 or 45 g CLM twice received only 263 or 474 kJ of ME respectively (from Table 4.2, TME_N value at 72 h collection). Thus the critical question is, which birds were more severely starved? It is worth cautioning, however, that the results from the first study should be taken as suggestive rather than conclusive. Therefore, in order to improve knowledge when evaluating fibrous materials for ME with poultry as well as selecting the most appropriate control, additional research into the effects of different procedures is required.

In study two, TME_N, which is claimed to be the most precise measure of metabolisable energy (Wolynetz and Sibbald, 1984), increased as the body weights of the birds increased. Sibbald (1978), despite not detecting a consistent effect of bird age on the TME values over different diets, reported a tendency for TME values to be higher in older birds. This seems reasonable given that it is generally accepted that birds improve their capacity to digest feed as they mature (Zelenka, 1968).

Sibbald (1982) reported that AME values of foodstuffs increase with bird age, most noticeably when fibrous materials are being evaluated. Sibbald (1975) had proposed earlier that part of the variation in apparent metabolisable energy values found in birds of different ages could be caused by variations in their endogenous energy losses relative to their food intake. Kussaibati *et al.* (1983) estimated that younger birds excreted three or two fold more endogenous energy than older birds. Bourdillon *et al.* (1990), evaluating a number of different diets, reported higher apparent metabolisable energy values in cockerels than in chickens.

In the third study genotype did not affect any of the dependent variables whereas the collection period affected them all with the exception of N digestibility. The effect of longer collection periods has already been discussed. However, the collection period affected the two genotypes differently and resulted in a significant genotype x collection period interaction. Hubbard chickens showed lower TME, TME_N, DM and GE digestibilities at 72 h than at 64 h, whereas criollo chickens did not. Hubbard chickens consumed almost twice as much water as criollo chickens and this could have led to more dilution of the pectin content of CLM. Pectin usually increases the viscosity of the intestinal content, and consequently the rate of passage of the digesta is reduced (Bedford and Classen, 1992). Van der Klis *et al.* (1993) reported a lower net sodium absorption from the intestinal lumen as consequence of the increased intestinal viscosity, resulting in a reduced rate of water absorption. This hypothesis was supported by the observation that the dry matter content of the droppings from the Hubbard chickens was lower than that from the criollo. The study was conducted under a high ambient temperature prevailing in the tropics where it has been demonstrated that criollo (naked-neck) are better adapted to the natural environment than commercial chickens (Fraga *et al.*, 1994; Yunis and Cahaner, 1994; Segura, 1998). Deeb and Cahaner (1994) reported that the attribute of the naked-neck gene could be to allow criollo chickens better control of thermoregulation at high ambient temperatures (above 24°C) than normal fully feathered birds. In contrast, Belay *et al.* (1993) asserted that fecal dry matter and fecal nitrogen were not influenced by environmental temperature (24 vs 35 °C) in broilers, and suggested that digestion efficiency is little influenced by ambient temperature.

Although N digestibility was very low by both genotypes, there was a tendency for the values to be higher in the criollo than in the Hubbard chickens. This could suggest different energy requirements in those genotypes, even when they have the same average body weight, but differences in body composition could be found between those genotypes. Bonnet *et al.* (1997) found that the N retention by commercial chickens maintained at a constant ambient temperature of 32°C was lower than by those birds maintained at 22°C. Plavnick and Hurwitz (1982) reported that energy requirements and carcass composition are both affected by the strain of bird. Fraga *et al.* (1994) have reported that when naked neck chickens are fed on low-protein diets, they showed either lower protein requirements or better efficiency in protein utilisation than commercial chickens. Segura and Loria (1994) reported on the performance of naked neck criollo chickens raised under commercial conditions in the tropics, where a slow grow rate (average body weight of 1.6 kg at 21 weeks of age) was found. The selection of an appropriate bird type for the evaluation of fibrous foodstuffs is required, and the results obtained may well depend on which bird type has been chosen for the assay. In future studies, therefore, it will be important to establish the selection of the foodstuffs for nutritional evaluation, which finally depends on the strain of bird used for specific production system.

4.3 Experiment 3. The effect of different fibre sources on endogenous losses in cockerels

4.3.1 Materials and methods

Two studies were carried out to investigate the effect of different source of fibre given by tube on the endogenous losses of N, amino acids and uric acid (UA), as well as on energy balance and body weight loss in mature cockerels. The birds were housed in individual wire cages with polymethacrylate trays placed underneath to collect droppings. The birds were fasted for 24 h when they were each given 50 ml of glucose solution (50% w/v) by tube. Fresh water was available to the birds at all times. The excreta was collected every 24 h until 72 h after the last tube feeding and care was taken to remove all contaminating feathers. After each collection, the samples of excreta were freeze-dried and finely ground. Determinations of N, UA and GE in excreta were made on a N analyser (LECO FP-328), TRAACS–800 and bomb calorimeter (Parr-1261), respectively. Amino acid analyses were performed by high performance liquid chromatography (Roslin Nutrition, 2000). Analysis of variance was carried out on the data using the General Linear Models procedure (MINITAB, 1999).

In study one, 42 Isa Brown cockerels (30-month-old and 3.5 ± 0.2 kg in weight) were used. The average temperature in the poultry house during the experimental period was 20°C and a 14 h light:10 h dark cycle was maintained throughout. Twenty-four hours after giving the glucose solution 6 birds per treatment were fed by tube over two days, and were fasted for 72 h after the last tube feeding. The treatments were as follows:

G 25 g glucose

P10 5 g pectin plus 25 g glucose

P20 10 g pectin plus 25 g glucose

CE10 5 g cellulose plus 25 g glucose

CE20 10 g cellulose plus 25 g glucose

CE30 15 g cellulose plus 25 g glucose

CFE 10 g chaya fibrous extract plus 25 g glucose

The pectin was citrus in origin (Pectin Citrus, Biomedicals Inc). Commercial cellulose for animal feeding was acquired from an UK provider. Considering the hypothesis that fibre from ingredients produce different physiological responses in the animal than purified fibre, chaya fibrous extract (CFE) was tested as fibre source. The CFE was obtained from CLM which had been boiled (100 g/l) for 1 h in a solution of cetyltrimethylammonium bromide (0.1% w/v) in 1N sulphuric acid, and then filtered hot through filter paper (Whatman No.1) washed with boiling distilled water and finally with acetone. The extract was dried and boiled for 1 h with 6N hydrochloric acid (300 g/l), and subsequently washed thoroughly 5 times with distilled water and drained. Thereafter, the extract was dried in an oven at 70°C. The final extract had 23 g/kg N and 702 g/kg acid detergent fibre. Two excreta samples from birds fed on G, P20, C20 and CFE were analysed for their contents of amino acids. In order to correct to zero N and amino acids intake, before statistical analysis, the contents of N and amino acids in CFE were subtracted from the droppings of birds fed on the CFE. The data were analysed as a completely randomised design with treatment as the only factor using the general linear model procedure (MINITAB, 1999). When significant treatment effects were observed the Tukey test was used to distinguish differences between means. Dry matter output data from birds fed on G, P20, CE20 and CFE were also analysed by variance analysis and Tukey's test was used to differentiate differences between means.

In the second study 48 five-month-old cockerels (24 Leghorn, 1.7 ± 0.17 kg and 24 naked-neck criollo, 2.2 ± 0.3 kg) were used. The average maximum and minimum temperatures and relative humidity in the poultry house were 38.2°C, 23.8°C and 82%, respectively, and a 14 h light: 10 h dark cycle was maintained. All birds were weighed at the beginning and end of the trial and body weight losses were recorded. Twenty-four hours after giving the glucose solution, 5 birds per genotype were fed by tube over two days either 10, 15, 20 or 25 g CFE plus 25 g glucose and then were fasted for 72 h after the last tube feeding. Four birds per genotype were fed 25 g glucose only. Two-way analysis of variance with genotype and treatment as factors was performed on the data using the general linear model procedure (MINITAB, 1999). The initial body weight as covariate was included in the model.

When significant treatment effects were observed the Tukey test was used to distinguish differences between means. Linear regressions of dependent variables on CFE input were calculated. And also linear regressions of dependent variables on initial body weight were determined.

4.3.2 Results

In the first study, 1 bird fed on CFE was excluded from the analysis of variance because it showed symptoms of illness. There were no differences between treatments (Table 4.7) in N and UA excretion. Particularly, means of UA from cockerels fed on G and CFE were numerically higher than those from cockerels fed on the other fibre sources. Means of energy balance in cockerels fed on P (10 and 20 g) and CFE were higher ($P<0.05$) than those fed on G.

The dry weight of excreta for G, P20, CFE and CE20 were 2.41 ± 0.88, 10.8 ± 0.88, 20.7 ± 0.88, and 21.8 ± 0.97 g, respectively, with treatment G giving the lowest ($P<0.05$) and treatment CE20 the highest ($P<0.05$) values.

Means of excretion of alanine, valine, methionine, isoleucine, leucine and phenylalanine from birds fed on CFE were lower ($P<0.05$) than those from birds fed on other fibre sources (Table 4.8). However, there was a tendency for all the amino acid excretions to be higher in birds fed on P20 and lower on those fed on CFE.

Table 4.7 Least square means (± SE) of excretion of nitrogen and uric acid, and energy balance[1] by cockerels fed on different fibre sources

Fibre source	Nitrogen (g)	Uric acid (g)	Energy balance (kJ)
Glucose	2.38 ± 0.07	7.36 ± 0.37	647.6a ± 5.46
Pectin			
10 g	2.12 ± 0.07	5.72 ± 0.37	718.6d ± 5.46
20 g	2.16 ± 0.07	6.51 ± 0.37	786.6c ± 5.46
Cellulose			
10 g	1.93 ± 0.07	6.92 ± 0.37	656.4a ± 5.46
20 g	2.01 ± 0.07	6.48 ± 0.37	692.2ad ± 5.46
30 g	2.09 ± 0.07	6.92 ± 0.37	677.9ad± 5.46
Chaya fibrous extract	2.61 ± 0.09	9.15 ± 0.44	884.6b ± 6.55
Probability	0.239	0.312	0.001

Different letters *a, b* on same column are significantly different at $P<0.05$
[1]Energy balance = gross energy input - gross energy output

Table 4.8. Least square means of amino acid excretion (mg/72 h) by cockerels fed on different fibre sources

	Fibre source				SEM	Probability
Amino acid	Glucose	Pectin	Cellulose	Chaya fibrous extract[1]		
Alanine	67.7ab	104.5a	62.1ab	-18.7b	15.5	0.021
Arginine	68.8	94.2	56.5	23.6	14.5	0.105
Aspartic acid	107.2	169.1	94.7	50.2	25.0	0.113
Cystine	60.2	75.8	49.4	54.5	4.9	0.068
Glutamic acid	165.2	249.0	146.9	74.8	37.1	0.119
Glycine	130.8	202.8	173.6	142.4	32.9	0.489
Histidine	46.9	55.3	46.6	41.6	6.96	0.615
Hydroxylysine	1.1	2.0	1.4	2.2	0.70	0.654
Hydroxyproline	8.3	15.4	9.9	12.8	1.69	0.130
Isoleucine	54.1ab	95.3a	45.2ab	-10.6b	16.0	0.042
Leucine	82.3a	132.2a	69.2a	-70.1b	22.0	0.011
Lysine	74.3	94.3	59.3	34.3	17.1	0.234
Methionine	24.8ab	33.8a	25.5ab	-4.6b	6.3	0.045
Phenylalanine	46.0a	67.6a	42.3a	-73.7b	14.6	0.008
Proline	73.9	118.8	69.1	65.0	10.4	0.062
Serine	77.4	125.0	70.5	70.8	15.8	0.177
Threonine	67.8	119.9	62.1	64.8	15.5	0.108
Tryptophan	12.1	24.6	21.3	34.3	4.23	0.084
Tyrosine	46.6	73.8	46.6	19.3	9.8	0.075
Valine	66.0a	104.5a	56.5a	-41.1b	16.6	0.014
TOTAL	1363.5	2106.5	1296.8	634.3	301.8	0.108

[1]After correction to amino acid consumed (see text)
Different letters *a, b* on same row significantly differ at P<0.05

In the second study, there was a significant difference between genotypes for body weight loss. The interaction input by genotype was not significant for any of dependent variables. The effect of initial body weight as covariable was significant (P<0.05) for weight loss. The regression lines of both N excretion and weight losses on initial body weight were positive (P<0.05) and the regression line of energy balance on initial body weight was negative (P<0.05).

The amount of CFE increased the excretion of N and UA significantly (Table 4.9) and the regression lines for both variables N and UA excretion were significant (Figure 4.2). The birds given only glucose had the lowest energy balance and the highest body weight losses (P<0.05).

Table 4.9 Least square means (± SE) of nitrogen and uric acid excretion, energy balance[1] and body weight loss by cockerels fed on different amounts of chaya fibrous extract

	Glucose	Chaya fibrous extract input (g)				Probability
		20	30	40	50	
Nitrogen (g)	2.15a ± 0.07	1.92ab ± 0.06	1.98ab ± 0.06	2.63a ± 0.06	2.90ac ± 0.05	0.001
Uric acid (g)	5.47a ± 0.23	4.56ab ± 0.19	4.96a ± 0.19	7.03ac ± 0.18	7.27ac ± 0.18	0.006
Energy Balance (kJ)	634.9a ± 5.59	723.9b ± 4.59	735.3b ± 4.54	742.3b ± 4.44	732.6b ± 4.41	0.001
Body weight Loss (g)	240.8a ±10.01	183.5ab ± 8.22	109.3b ± 8.13	228.3a ± 7.96	176.4ab ± 7.90	0.013

Different letters *a, b* on same row are significantly different at P<0.05
[1]Energy balance = energy input - energy output

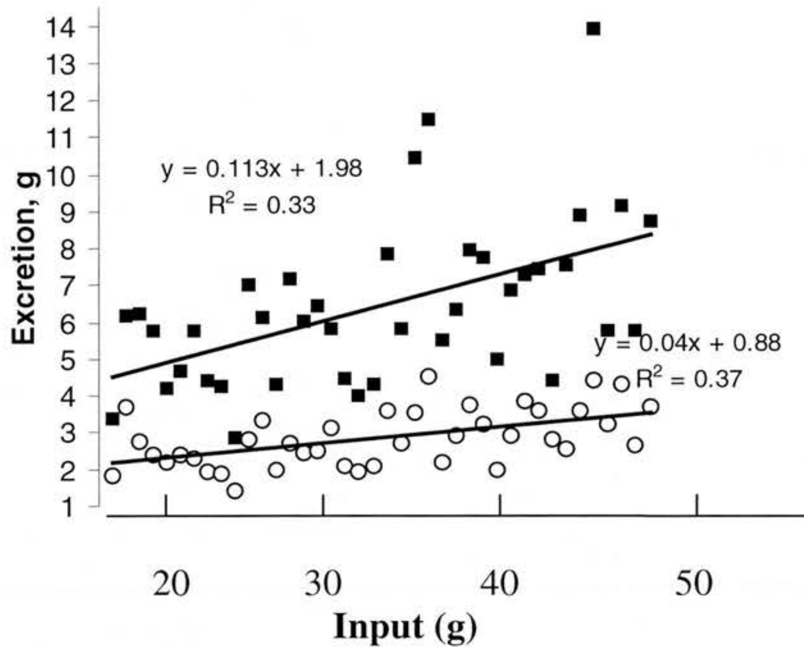

Figure 4.2 Uric acid (■) and nitrogen (○) excretion by cockerels fed on different amounts of chaya fibrous extract.

4.3.3 Discussion

The results of N excretion found in the first study agreed with that reported by other authors (Tenesaca and Sell, 1981; Green,1988; Askbrant, 1989). Green (1988) reported that increasing the intake of wood cellulose had no influence on the excretion of amino acids or N from the droppings in both caecectomised and intact birds. However both N and UA excretion from birds fed on CFE showed a tendency to be higher than the means from others treatments, and that was confirmed in the second study. In the second study CFE input increased linearly (Figure 4.2) both N and UA excretion. A possible explanation for this outcome has been put forward by Askbrant (1989), who suggested that one effect of the consumption of fibre is the prevention of retrograde peristalsis of urine from the cloaca into the colon and caeca. A consequence might, therefore, be that no (or less) fermentation of uric acid occurs in the hind gut. Data from Karasawa and Maeda (1995) supported this hypothesis

and asserted that avoiding the entrance of urine containing urea and uric acid into the caeca and the colon results in marked decreases in the degradation of urea and uric acid to ammonia at these sites, resulting in increased uric acid excretion by birds. Karasawa *et al.* (1988) discussed the importance of nitrogen recycling in the nitrogen economy of the fowl, and how it depends on the quantity of urinary uric acid that flows into the caeca by means of reverse peristalsis and on the retention of the degradation products, mainly ammonia, by the body. However, the same effect might be expected in birds fed on cellulose; Muztar and Slinger (1980a) suggested that fibre from different ingredients generally differ substantially in their physico-chemical properties from pure cellulose and would be expected to have different effects on the digestive tract.

Sibbald (1981) did not find any differences in the energy balance between birds fed on cellulose and those fed on glucose only. In the first study, the higher energy balance found in birds fed on the treatments P (10 or 20 g) than in those fed on glucose indicated that pectin may have been partly fermented in birds' gut and thereby, contributed some energy towards the metabolic processes. Nyman and Asp (1982) and Carré *et al.* (1995a), respectively, have provided evidence to support the view that pectin is partly fermented and utilised by both rats and birds. Nyman *et al.* (1990) also mentioned that pure pectin was easily fermented in the gut of rats.

Higher energy balances were found among the birds fed on the CFE than in those birds fed on glucose only, and this suggests that CFE must have undergone some fermentation. Consequently, it seems probable that an important amount of energy for metabolism was produced from this process. The data from the weight losses by birds fed on CFE in the second study offers some support for this hypothesis.

Neither pectin nor cellulose increased the endogenous nitrogen or uric acid losses from cockerels in this study. However, cockerels fed on pectin excreted the highest amount of amino acids, and those fed on CFE excreted the lowest amount of amino acids. Parsons *et al.* (1982b) did not find that cellulose affected apparent amino acid digestibility coefficients by adult cockerels. Although Parsons *et al.* (1983) reported that cockerels fed on a high fibre diet containing cellulose and pectin excreted larger amounts of amino acids than their fasted counterparts. They

suggested that increased amino acid excretion originated as a result of bacterial synthesis in the large intestine and caeca and consequently from the greater amounts of endogenous protein. Because alanine and aspartic acid are the main amino acids in microbial cells, the excretions of these amino acids were found to be significantly increased.

It has been emphasised that the microbial contribution to the amino acid content of excreta (Kessler *et al.,* 1981; Parsons *et al.*, 1982a,c) is greatest when fermentable fibrous material is available. The birds fed on the different dietary fibre sources all seemed to obtain some energy as a result of microbial fermentation. CFE increased the endogenous losses of both nitrogen and uric acid, but not those of the amino acids. The significant regression lines of nitrogen excretion, weight losses and energy balance on initial body weight agreed with the positive relationship between body weight and endogenous losses mentioned by Sibbald (1982) when the variation in body weight is large, as was the case in the data from this study.

4.4 Experiment 4. True metabolisable energy, heat increment and net energy values of CLM in cockerels

4.4.1 Materials and methods

The present experiment was conducted to determine the heat increment after feeding cockerels with either wheatfeed, as a conventional fibrous ingredient commonly used in poultry diets in the UK, or CLM, and to derive the true metabolisable and net energy values of both ingredients. CLM was obtained as described in section 4.1. Wheatfeed was obtained from a commercial supplier in the UK. The chemical composition of the ingredients is shown in Table 4.10.

Table 4.10 Chemical composition (g/kg) and gross energy (MJ/kg) of chaya leaf meal and wheatfeed on an as received basis

	Chaya leaf meal	Wheatfeed
Dry matter	880.0	875.7
Ether extract	66.6	36.0
Crude protein	268.6	158.1
Ash	143.6	44.9
Crude fibre	163.9	68.8
Neutral detergent fibre	202.1	296.4
Hemicellulose	62.0	179.9
Acid detergent fibre	140.0	116.5
Density (g/cm^3)	0.386	0.429
Gross energy	16.75	16.83

Ten Isa Brown cockerels (3.15 ± 0.1 kg live weight) were used to derive the net energy (NE) value of the two fibrous foodstuffs, wheatfeed and CLM. The birds were housed in individual calorimeter chambers (Lundy *et al.*, 1978; MacLeod *et al.*, 1985) with polymethacrylate trays placed underneath for excreta collection.

Five calorimeter chambers were available. Two groups of 5 birds were used over 4 weeks, so that 3 and 2 birds were fed on wheatfeed and CLM respectively the first week and *vice versa* the following week. The birds were placed in the calorimeter chambers for 7 d, with the first 2 d being used for adaptation and the remaining 5 d for measurement. During the adaptation period the birds were fasted for 2 d to determine their endogenous energy losses. During the measurement period the birds were tube-fed either 25 g wheatfeed or 25 g CLM in mash form on two occasions, at 09.00 h on days 4 and 5. The birds had free access to water at all times. Body weights and water consumptions were recorded daily. The lighting cycle was 14 h light:10 h dark; lighting was switched on and off at 04.00 and 18.00 h respectively. The calorimeter measurements were performed for the last 5 d of each week. Heat production was calculated from oxygen consumption and carbon dioxide production measurements made by the computer system described by MacLeod *et al.* (1985).

The excreta samples collected from both the fasted and feeding periods were freeze-dried for gross energy analysis. Determination of gross energy in both ingredients and droppings was made in an adiabatic bomb (Parr-1261) calorimeter (Roslin Nutrition, 2000). Water holding capacity (Robertson and Eastwood, 1981b) was also measured in 6 samples of each material: the dried samples (1 g) in a flask were added 50 ml distilled water, the mixtures shaken by hand, left 30 min at room temperature and filtered through Whatman No.1 paper until no more water dropped through the paper. The fresh weight was determined and, after oven drying at 60°C water holding capacity was calculated.

True metabolisable energy (TME) was derived according to the method of Longstaff and McNab (1986). TME, total heat increment, net energy, and the efficiency of utilisation of TME (k), were calculated according to the formulae quoted by MacLeod (1991a).

TME was calculated as:

$I_{TME} = I_E$ – (faecal energy + urinary energy) + (endogenous faecal energy + endogenous urinary energy)

where I_E is gross energy intake.

Total heat increment was estimated as the difference between heat production during feeding and fasting periods.

Net energy was calculated as:

$$I_{NE} = I_{TME} - \text{Total heat increment}$$

Net efficiency of utilisation of energy was calculated as:

$$k = I_{NE} / I_{TME}$$

The data were analysed as paired comparisons so that wheatfeed and CLM measurements on the same bird were compared (MINITAB, 1999).

4.4.2 Results

Despite very similar gross energy contents (Table 4.10), chaya yielded significantly less TME (Table 4.11; $P<0.01$) than wheatfeed. The total heat increment associated with the feeding of chaya leaf meal was 1.7 times greater than that from the intake of wheatfeed, although high variability across birds prevented` this result reaching statistical significance. However, the net efficiency of the utilisation of TME (k) of CLM was significantly lower than that for wheatfeed ($P<0.05$). The combination of lower TME and lower k resulted in CLM having a NE value only 0.53 that of wheatfeed ($P<0.01$). Water intake was higher after the input of CLM than after giving wheatfeed ($P<0.05$). There was a pronounced diurnal rhythm in heat production (Figure 3.3), associated with both raw materials and when the birds were fasted. As would be expected from the overall heat increment, there was some indication that the highest values were observed among the birds fed on CLM. Body weight loss was not affected ($P>0.05$) by the foodstuff. The water-holding capacity value of CLM was higher ($P<0.05$) than that of wheatfeed; the means were 4.61 ± 0.48 and 3.28 ± 0.31 g water/g dry matter respectively.

Table 4.11 Means (± SE) of true metabolisable energy, heat increment and net energy values of two fibrous foodstuffs in cockerels

Parameter	Chaya leaf meal	Wheatfeed	Probability
True metabolisable Energy (MJ/kg)	5.76 ± 0.35	8.39 ± 0.24	0.001
Total heat increment (kJ/d)	46.3 ± 10.9	27.1 ± 12.2	0.212
Efficiency of utilisation of TME (k)	0.64 ± 0.08	0.86 ± 0.06	0.035
Net energy (MJ/kg)	3.86 ± 0.62	7.26 ± 0.61	0.002
Water consumption when fed (ml/d)	137.0 ± 12.2	101.6 ± 15.2	0.031
Body weight loss (g)	88.4 ± 20.5	67.4 ± 14.1	0.179
Water holding capacity (ml/g)	4.61 ± 0.19	3.28 ± 0.12	0.001

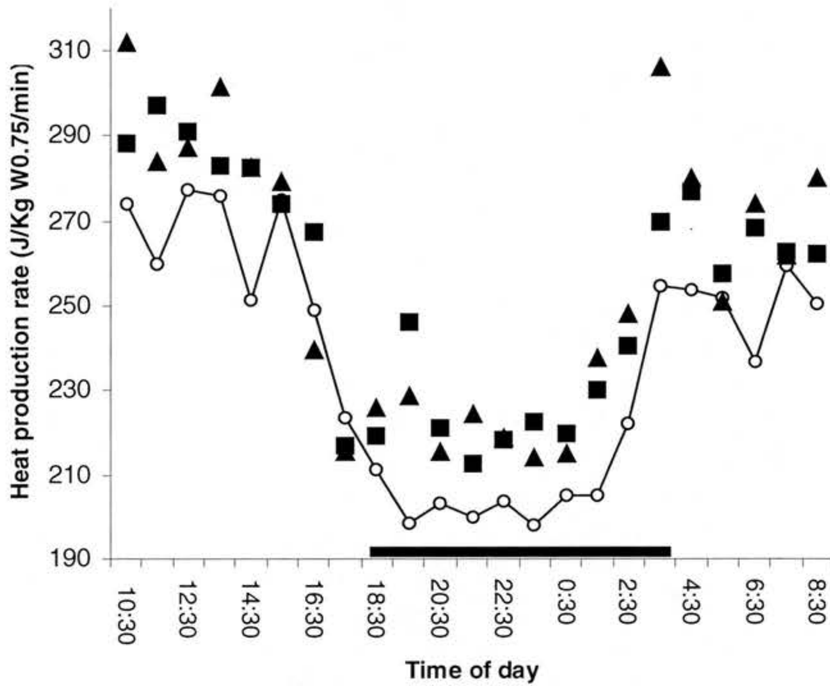

Figure 4.3 Patterns of variation in the heat production of cockerels fed on chaya leaf meal (▲) or wheatfeed (■). The continuous line (○) represents fasting heat production. The black horizontal bar indicates the dark period. Each point represents the mean of ten birds.

4.4.3 Discussion

The different chemical compositions of wheatfeed and CLM probably explain the differences in energy utilisation found in this study. The relative proportions of the different chemical components vary widely across plant species and between tissues within a plant. CLM contained greater amounts of crude fibre, ADF and ash than wheatfeed, although the gross energy content of both foodstuffs was similar. ADF consists mainly of the water-insoluble components cellulose and lignin (Van Soest *et al.*, 1991). Carré *et al.* (1984) claimed that both the NDF and ADF components of feedstuffs were detrimental to energy utilisation and good predictors of the apparent metabolisable energy value of poultry diets. It was

revealed above (chapter 3), from GLC analyses, that CLM contains also pectin, and this is known to depress nutrient digestibility, the metabolisable energy content of the diet and consequently growth rate in chickens (Vohra and Kratzer, 1964; Wagner and Thomas, 1977; Bishawi and McGinnis, 1984; Langhout *et al.*, 1999).

Wheatfeed contains higher concentrations of hemicellulose than CLM; hemicellulose is water-soluble to some extent and consequently is better digested than cellulose. Chesson and McNab (1990) found 289.7 g/kg of total non-starch polysaccharides in wheatfeed with a relative content of 0.67 of arabinoxylans and 0.24 of cellulose. Longstaff and McNab (1989) discussed the restricted ability of chickens to digest fibre, with water-soluble fibre being more digestible. In three varieties of wheat, Longstaff and McNab (1986) found an average of 78 g/kg of hemicellulose, 12% of which was soluble hemicellulose. They reported that adult cockerels digested 24% of the arabinoxylan or pentosan derived from wheat hemicellulose.

Choct and Annison (1990) found 520 g/kg of pentosans in the water-soluble preparation from a wheat milling by-product. That fraction did not show detectable anti-nutritive effects in adult birds, which may be less sensitive to the anti-nutritive effect of soluble non-starch polysaccharides than young birds. Carré *et al.* (1990) mentioned that the digestibility of the water-soluble components in wheat was higher than that of the water-insoluble components in both cockerels and ducks. In this study the hemicellulose from wheatfeed may have been better utilised by cockerels, as the result of microbial fermentation in the gut, than the cellulose from the CLM. This hypothesis may help to explain the higher TME value found in birds fed on wheatfeed than in those birds fed on CLM. Jorgensen *et al.* (1996) concluded that dietary fibre may be responsible for between 86 and 96% of the variation in dietary metabolisable energy concentration. And that is because fibre constitutes an entity with very variable physico-chemical characteristics and properties.

However, the TME values found in the present study for both wheatfeed and CLM were low, as would be expected for high-fibre ingredients. The TME found in the present work for wheatfeed agreed with that reported by Chesson and McNab (1990) of 7.88 MJ/kg. Donkoh *et al.* (1999) published a TME value for CLM of 5.52 MJ/kg which was similar to that found in the current study.

CLM also contained a higher concentration of crude protein than wheatfeed. Musharaf and Latshaw (1999) in a review of the literature showed that when protein is used as an energy source for maintenance or production, increased heat production results from the excretion of nitrogen from dietary amino acids. There is also increased heat production if the amino acids are used for protein synthesis (MacLeod, 1997). MacLeod (1991b) fed broiler chickens on diets containing 130 or 210 g/kg of crude protein and found that heat production was higher on the diet with the higher protein content.

The most important sources of heat increment in the gut are associated with digestion, intestinal secretions, absorption and metabolism of the digesta (Blaxter, 1989; Li *et al.,* 1992; Zhou and Yamamoto, 1997). The protein content and the metabolisable energy as a proportion of the total energy of diets were considered in equations developed by Blaxter (1989), to predict the k coefficient. Those results showed that k was always higher in highly digestible ingredients such as grains than in less digestible ones such as roughage. The physico-chemical properties of CLM fibre may have increased energy costs, because of the increased work of peristalsis to transport the fibre through the intestine and, because of the increased bulk of the digesta attributable to the high fibre content, mainly from ADF (Jorgensen *et al.,* 1996). Also, CLM has a lower density than wheatfeed and that offers another obvious explanation for the increased bulk of digesta in birds fed on that material.

Although there was no significant difference between the effects of CLM and wheatfeed on the total heat production, probably because of the large variation between birds and the small sample size, a trend was maintained throughout the day for there to be greater heat production from cockerels fed on CLM (Figure 4.3). That tendency was easily explained by the fact that the heat increment produced by birds fed on wheatfeed was proportionately only 0.58 that of birds fed on CLM.

As result of the differences in the TME values and heat productions between cockerels fed on wheatfeed and CLM, lower NE and k values were found for CLM than for wheatfeed. Stevens and Hume (1995) noted that the digestive tract was the most metabolically active organ in terms of both protein synthesis and energy expenditure. Different dietary characteristics produce different degrees of heat increment, which lead to different efficiencies in the utilisation of metabolisable

energy (k). McBride and Kelly (1990) and many previous authors have stated that the retention of energy (or net energy) is the difference between metabolisable energy consumption and the heat increment of the food.

The higher water consumption by cockerels fed on CLM may have resulted from the greater water-holding capacity of the CLM fibre compared with wheatfeed fibre. Robertson and Eastwood (1981a, b) reported that cereal fibre, such as bran, has a lower water-holding capacity than fibre from potato; they argued that bran was able to bind less water and contained lower amounts of ADF than potato fibre. Also, they pointed out that the anatomical and the structural differences in fibre were partially responsible for the quantity of water which can be trapped. Insoluble polysaccharides such as cellulose and xylans retain water by physical mechanisms, but the viscosity of the liquid is relatively low. The primary effect of the water-holding capacity of insoluble polysaccharides may be to increase the bulk of the digesta, thereby decreasing the time taken for the chyme to pass through the intestine and leading to reduced nutrient digestibility (Smits and Annison, 1996; Klasing, 1998). The ingredient fed did not affect the changes in body weight of the cockerels in this experiment, probably because the measurement periods were not long enough to show differences.

In the present study, the higher heat production found during the light period than during the dark one agreed with the findings of Lundy *et al.* (1978), Li *et al.* (1991, 1992) and and Buyse *et al.* (1993) and was considered to be largely an effect of light on the physical activity of the birds.

This experiment confirms previous findings on the detrimental effect of fibre on metabolisable energy. It is concluded that the chemical composition and amount of fibre influence both the metabolisable energy values derived from high-fibre foodstuffs and the efficiency with which such energy is utilised by the birds. The energetic losses associated with high-fibre ingredients may constitute a very large proportion of their gross energy. In this study, the metabolisable energy and net energy derived from CLM represented, respectively, 0.34 and 0.23 of its gross energy content.

4.5 Experiment 5. Effect of different amounts of dietary CLM on the apparent metabolisable energy values and on the dry matter, nitrogen and amino acid digestibilities of the diets

4.5.1 Materials and methods

This experiment was conducted to study the effect of different amounts of dietary CLM on the apparent metabolisable energy (AME) and AME corrected to nitrogen equilibrium (AME_N) values and on the DM, N and amino acid digestibilities of the diets.

One hundred and forty-eight one-day-old male Ross chickens were used. The birds were allotted to brooders in which the temperature and light were automatically controlled. The average temperature in the brooder during the experimental period was 25.5°C and a 23 h light : 1 h dark cycle was maintained. At one day of age, 6 birds were allocated to each of 24 brooder quadrants. There were 3 experimental diets, based on maize and soyabean meal, containing 0, 150 and 250 g /kg of CLM. The chemical composition of the CLM is shown in Table 4.12. All diets were formulated to contain the same concentrations of metabolisable energy and protein. The diet composition, chemical analyses and amino acid compositions of the diets are shown in Tables 4.13 and 4.14. The diets, which also contained 4 g of titanium dioxide/kg as a dietary marker (Peddie, *et al.*, 1982) were pelleted. Diets and fresh water were offered *ad libitum* to the birds.

Each experimental diet was allocated at random to eight brooder quadrants. The experimental diets were fed from 1 to 21 days of age. At day 7, two birds (the lightest and the heaviest) were removed from each brooder quadrant.

On days 7 and 21, dropping samples were taken from all the quadrants, freeze dried and stored at -20°C. Gross energy and titanium (Roslin Nutrition, 2000) were determined on the diets and droppings to calculate the AME values of the diets.

At day 21, two birds from each quadrant were selected for the collection of digesta from the small intestine. The birds were killed by an intravenous injection of

Euthetal (sodium pentobarbitone). Immediately after injection, the digesta samples were collected from the region between Meckel's diverticulum and the ileocaecal junction for both amino acid and titanium analyses. Every four ileal samples from the same diet were pooled for both amino acid and titanium analyses.

Gross energy and titanium in both diets and droppings were determined using a bomb calorimeter and by colorimetry respectively. Determinations of amino acid composition were performed by high performance liquid chromatography [HPLC] (Roslin Nutrition, 2000). Statistical analyses were carried out on the data using MINITAB (1999) software. Analysis of variance was performed on data by the general linear model procedure with diet as the only factor and Tukey's multiple comparison was used when significant effects were identified. Also, the data were analysed as paired comparisons so that 7-day-old and 21-day-old dependent variables on the same quadrant were compared (MINITAB, 1999).

Table 4.12 Chemical composition of chaya leaf meal

Component	Concentration (g/kg)
Crude protein	305.0
Crude fibre	140.0
Ether extract	55.2
Ash	98.7
Phosphorus	3.1
Calcium	15.3
Amino acids	
Alanine	15.0
Arginine	15.2
Aspartic acid	25.7
Cystine	3.1
Glutamic acid	31.6
Glycine	12.0
Histidine	5.8
Hydroxyproline	0.7
Isoleucine	10.9
Leucine	19.2
Lysine	12.7
Methionine	4.3
Phenylalanine	13.8
Proline	9.8
Serine	8.8
Threonine	10.5
Tryptophan	4.9
Tyrosine	8.9
Valine	15.0

Table 4.13 Composition of the diets containing different amounts of chaya leaf meal

Ingredient	Diet (g/kg)		
	Control	CLM150	CLM250
Maize meal	722.6	607.7	531.0
Soyabean oil	1.4	38.0	62.5
Maize gluten meal	34.8	35.0	35.0
Soyabean meal	200.0	135.2	92.2
CLM	-	150.0	250.0
Premix			
Vitamins-minerals[1]	5.0	5.0	5.0
Limestone	11.4	5.5	1.5
Sodium chloride	2.5	2.5	2.5
Aliphos 50	14.8	12.9	11.6
Choline chloride	0.3	0.3	0.3
Lysine	2.4	3.0	3.4
Methionine	0.8	0.9	1.0
Titanium dioxide	4.0	4.0	4.0
Chemical analyses (g/kg)			
Crude protein	184.6	190.5	186.2
Crude fibre	24.1	36.9	45.4
Phosphorus	6.6	6.5	6.4
Calcium	10.9	10.6	10.1

[1]Supplied per kg of diet: vitamin A, 12,000 iu; vitamin D_3, 5,000 iu; vitamin E, 50 iu; vitamin K, 3 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 5 mg; vitamin B12, 15 µg; nicotinic acid, 50 mg; pantothenic acid, 15 mg; folic acid, 1mg; biotin, 200 µg; iron, 80 mg; copper, 10 mg; manganese, 100 mg; cobalt 0.5 mg; zinc, 80 mg; iodine, 1 mg; selenium, 0.2 mg; molybdenum, 0.5 mg.

Table 4.14 Amino acid composition of the diets contained different amounts of chaya leaf meal

Amino acid	Diet (g/kg)		
	Control	CLM150	CLM250
Alanine	10.1	10.7	10.3
Arginine	10.9	9.9	9.4
Aspartic acid	16.1	15.8	15.0
Cystine	3.1	2.8	2.6
Glutamic acid	35.0	30.8	27.2
Glycine	7.0	7.0	6.7
Histidine	4.8	4.4	4.1
Isoleucine	7.1	6.9	6.5
Leucine	17.7	17.8	16.5
Lysine	10.4	10.7	10.3
Methionine	3.6	3.8	3.8
Phenylalanine	9.5	9.4	8.8
Proline	11.9	10.9	9.8
Serine	7.4	6.9	6.3
Threonine	6.4	6.5	6.2
Tryptophan	2.2	2.2	2.2
Tyrosine	6.1	5.9	5.9
Valine	8.7	8.8	8.5

4.5.2 Results

As the dietary concentration of CLM increased the AME and AMEn values and the DM, GE and N digestibilities in both bird ages, 7d and 21d, decreased ($P<0.05$, Table 4.15). Furthermore, all dependent variables were higher ($P<0.05$) in 20-d-old birds than in 7-d-old birds (Table 4.16).

Table 4.15 Least square means of apparent metabolisable energy and dry matter, gross energy and nitrogen digestibilities of diets containing different amounts of CLM by chickens at 7- and 21-day-old

Parameter	Diet			Probability	SEM
	Control	CLM150	CLM250		
7-d-old					
AME (MJ/kg)	12.36a	11.55b	11.23b	0.001	0.14
AME_N (MJ/kg)	11.73a	10.94b	10.66b	0.001	0.14
Digestibility (%)					
Dry matter	74.71a	69.06b	65.15c	0.001	0.70
Gross energy	75.17a	67.53b	63.03c	0.001	0.86
Nitrogen	62.27a	58.55ab	56.13b	0.017	1.37
21-d-old					
AME (MJ/kg)	13.01a	12.18b	12.46b	0.001	0.10
AME_N (MJ/kg)	12.33a	11.57b	11.86b	0.001	0.09
Digestibility, %					
Dry matter	77.38a	70.73b	69.10b	0.001	0.51
Gross energy	79.13a	71.26b	69.92b	0.001	0.59
Nitrogen	67.28a	59.46b	58.67b	0.001	1.05

Different letters *a, b, c* in same column significantly differ at $P<0.05$

Table 4.16 Means of apparent metabolisable energy and dry matter, gross energy and nitrogen digestibilities of the diets by chickens aged 7- and 21-d-old

Parameter	7-d-old		21-d-old		Probability
	Mean	SEM	Mean	SEM	
AME (MJ/kg)	11.71	0.13	12.55	0.09	0.001
AME_N (MJ/kg)	11.11	0.12	11.92	0.08	0.001
Digestibility (%)					
Dry matter	69.63	0.96	72.40	0.79	0.001
Gross energy	68.58	1.16	73.44	0.91	0.001
Nitrogen	58.98	0.97	61.80	0.99	0.012

The amino acid digestibility in birds fed CLM250 was lower ($P<0.05$) than that from birds fed on either control or CLM150 (Table 4.17). However, there were no differences in amino acids digestibility between control and CLM150, with the exception of alanine, arginine and proline digestibilities which resulted in lower values ($P<0.05$) in birds fed on CLM150. N digestibility was lower ($P<0.05$) in birds fed on CLM diets than in those fed on control diet, and it was numerically lower from excreta samples (Table 4.15) in all the diets than from ileal samples (Table 4.17) in 21-d-old birds. Because of technical difficulties, tryptophan analysis could not be determined in ileal samples.

Table 4.17 Least square means of ileal nitrogen and amino acid digestibility coefficients (%) of the diets containing different amount of CLM by chickens aged 21days

Ileal digestibility	Diet			Probability	SEM
	Control	CLM150	CLM250		
Nitrogen	79.7a	76.3a	70.8b	0.001	0.94
Alanine	96.0a	90.9b	86.6c	0.001	0.56
Arginine	91.7a	87.8b	83.1c	0.001	0.92
Aspartic acid	87.8a	86.9a	76.7b	0.001	0.72
Cystine	86.7a	84.0a	69.8b	0.001	1.06
Glutamic acid	89.3a	86.5a	78.3b	0.001	0.87
Glycine	80.8a	77.5a	66.2b	0.001	0.99
Histidine	89.6a	87.3a	73.3b	0.001	0.85
Isoleucine	85.3a	83.5a	72.9b	0.001	0.83
Leucine	87.1a	86.1a	76.5b	0.001	1.05
Lysine	90.7a	89.0a	81.9b	0.001	0.64
Methionine	93.4a	94.9a	82.0b	0.001	1.22
Phenylalanine	87.7a	85.3a	77.1b	0.001	0.88
Proline	85.4a	80.3b	71.5c	0.001	1.00
Serine	82.2a	78.6a	63.4b	0.001	1.28
Threonine	79.3a	79.0a	64.2b	0.001	1.13
Tyrosine	89.6a	82.9a	73.6b	0.002	2.24
Valine	87.0a	85.9a	73.4b	0.001	0.82
Total amino acids	87.6a	85.1a	74.7b	0.001	0.87

Different letters *a, b, c* in same row significantly differ at $P<0.05$

4.5.3 Discussion

The effect of dietary concentration of CLM on the AME and AME_N values agreed with the findings of Farrell *et al.*(1991) who, in experiments of a similar type reported that, as the concentration of wheat bran in the diet increased, dietary AME values decreased. Chickens can digest a small quantity of fibre in comparison to the higher amounts digested by pigs, rats and humans (Carré *et al*, 1984; Carré and Leclercq, 1985; Longstaff and McNab, 1986, 1989). Sibbald *et al.* (1990) also reported that pigs are able to acquire more energy from fibrous foodstuffs than chickens. The effects related to fibre depend on its physicochemical properties. Glitso *et al.* (1998) mentioned that the characteristics and quantity of the dietary fibre result in variations in the extent to which it is fermented. The action of fermentation modifies other variables such as the transit time of the digesta, its pH and the faecal bulking.

Lindberg and Cortova (1995) and Andersson and Lindberg (1997a,b) fed pigs on diets containing increasing amounts of different forages. They found a reduction in both digestible and metabolisable energy values of the diets with increasing inclusion of all the forages evaluated. A probable explanation for the result seen in the present study is that dietary fibre could prevent (or at least reduce) the digestion and absorption of dietary components such as starch and protein by means of encapsulating those nutrients. Furthermore, components of the insoluble fibre in CLM (*e.g.* fractions like NDF) can trap considerable quantity of water. The insoluble fibre, the water and the bacteria associated with it are the main components of the bulk of digesta, and are the factors likely to influence the time taken for the digesta to pass through the gastrointestinal tract. (Cilliers *et al.*, 1994; Smits and Annison, 1996; Klasing, 1998). They may therefore, also be the factors that influence the extent to which the diet is digested. Freire *et al.* (2000) asserted that insoluble fibres, which have low degradation, are able to increase the passage of the digesta through the gastrointestinal tract, due to their high water-holding capacity, and consequently reduced dietary values of AME migh be expected.

The effect of age on metabolisable energy values of diets and nutrient digestibility has already been discussed above in section 4.2.3. In this study the results show the beneficial effect of the maturity of the digestive tract of the bird on nutrient digestibility (*e.g.* nitrogen, gross energy and dry matter). A possible additional effect may have been the longer period of adaptation to the diets in older birds (Duke *et al.,*1984). Savory (1992a) mentioned that the extent by which cellulose is degraded by fowls depends on the duration of preconditioning to the high-fibre diets. However, Bartov (1995) found a negative effect of age on dietary AME_N when feeding chicks on high protein-low energy diet, arguing that the finding was consequence of the intrinsic characteristics of such a diet.

The differences between the nitrogen digestibility from excreta samples and ileal samples found in this study confirm previous findings by other authors regarding the significance of microbial contribution to the nitrogen content in excreta and consequently its effect on reducing apparently nitrogen digestibility. (Raharjo and Farrel, 1984; Bielorai *et al.*, 1991; Sauer *et al.*, 1991) Ravindran, *et al.* (1999) pointed out that determination of amino acid digestibility by excreta analysis might not be a valid method for all feedstuffs because of the important role that nitrogen metabolism plays in the hindgut of the bird. Nitrogen metabolism includes both degradation of nitrogenous substances and synthesis of microbial proteins. The process of microbial deamination of amino acids conduces to the synthesis of ammonia, which is absorbed in great proportion but not utilised by the bird, therefore, it is excreted in the urine in the form of uric acid. Results from Green *et al.* (1987a,b) comparing endogenous amino acid output between intact and caecectomised birds have not showed statistical differences, suggesting that endogenous amino acid excretion was the most important source of variation between type of bird. However, he also found differences between bird type for digestibility of particular amino acids such as threonine, glycine and lysine, but those differences were reduced when apparent digestibility coefficients were adjusted to true digestibility values. This suggest that the problems associated with apparent digestibility trials based on excreta collection samples might be overcome if adjustments made by endogenous losses were carried out in these samples. Nevertheless, Ten Doeschate *et al.* (1993) pointed out that amino acid digestibility

based on dropping collection may overestimate the availability of amino acids because of their disappearance after terminal ileum is caused by microbial fermentation rather than by absorption by the host.

In the current study the digestibilities of the amino acids of the control diet are in agreement with the results reported by Raharjo and Farrell (1984) evaluating different vegetable protein meals, Summers and Robblee (1985) feeding broilers on wheat and soyabean meal-based diets, and with Ravindran *et al.* (1999) evaluating maize and soyabean meals. Peisker (1999) mentioned that the mean digestibility of the amino acids in corn-soyabean diets for poultry was 88%, a value which is in agreement with the average of 87.6% found in this study.

Despite the similar amino acid composition in the different diets (Table 4.14), there were differences in amino acid digestibility resulting from the inclusion of CLM. The increasing concentration of dietary fibre as the CLM content of the diets increased could be the reason of the lower significantly amino acid digestibilities found in birds fed on the diet with the highest amount of CLM (CLM250). Green (1997a) pointed out that dietary carbohydrate influence microbial activity in the hind gut in poultry, however this, there is still controversy on this matter. Raharjo and Farrell (1984) declared that the outputs of both the nitrogen and amino acids augmented in ileal digesta with increasing ADF in the diets, and consequently their digestibility coefficients were reduced. Iji (1999) pointed out that as a result of high amounts of non-starch polysaccharides in the diet, microbial fermentation activity is increased in chickens. This result in the production of large quantities of volatile fatty acids, which reduce the quantity of carbohydrate that are indispensable to activate the digestive enzyme function. Angkanaporn *et al.* (1994) mentioned that impairment of protein digestion, inhibition of amino acid absorption or an increase in the secretion of endogenous protein could all be possible causes for the derivation of low ileal protein digestibility coefficients associated with non-starch polysaccharides in diets. Nyman *et al.* (1990) also proposed that the appearance of high amounts of crude protein in the excreta and, consequently, the derivation of lower crude protein digestibility coefficients in diets high in fibre may result from undigested cell wall proteins, unabsorbed intestinal secretions, dead mucosal cells and microbial protein. Schulze *et al.* (1994) found that both

endogenous and exogenous nitrogen in the ileal chyme of pigs were linearly increased with increased amount of dietary purified NDF, and that was attributable to increased ileal losses of both endogenous and exogenous protein.

The lower alanine, arginine and proline digestibilities found in birds fed on CLM diets could at least partly, be explained in terms of the hypothesis of Parsons *et al.* (1983) who reported that aspartic acid and alanine are the main amino acids in microbial cells and proline is an important component of endogenous protein. Thus any increase in the excretion of those amino acids, as a result of microbial activity in the intestine arising from the presence of the CLM fibre, would be expected to result in an apparent decrease in the extent to which they were digested. It must be correct to debate the impact that microbial activity in the upper regions of the gastrointestinal tract of chickens has on the way the diet is utilised. On this subject, Boorman (1999) has argued that bacterial activity in the small intestine should not continue to be disregarded.

The arginine digestibility also decreased significantly as the dietary CLM concentration in the diets increased. A possible and simple explanation for this response could be the fact that, as the amount of CLM in the diet increases, so the amino acid concentration in both diet and digesta decreases. A consequence of this effect is that exogenous amino acid concentrations exert a more influential effect on the amino acid fluxes existing in the gastrointestinal tract (Short *et al.* 1999; Edwards *et al.* 2000). Thus, if exogenous amino acid concentrations are unaffected by the nature of the diet, while the changes in the compositions of the diets are reducing the input of exogenous amino acids, a consequence of this combination would be to reduce the (apparent) digestibility of the amino acids at both the ileal and faecal levels. This raises the difficult issue of the difference between apparent and true digestibility coefficients.

4.6 Experiment 6. Effect of adding enzymes on ileal amino acid digestibility of CLM in chickens

4.6.1 Materials and methods

Sixty 4-week-old Ross male chickens were used to evaluate the effect of adding enzymes on the apparent ileal amino acid digestibility of CLM. The birds were fasted for 30 h and then 15 birds received twice (14.00 h and 09.00 h) 15 g CLM by tube as one of four treatments. The treatments were:

CLM without enzyme (control)

CLM with added β-glucanase

CLM with added pectinase

CLM with added enzymes pectinase plus β-glucanase

A commercial supplier in the UK provided the enzymes. The supplier informed that the pectinase enzyme had been derived from the organism *Aspergillus* and contained an activitity of 3756 units/g as measured using pectin as the substrate. The β-glucanase enzyme, derived from the organism *Trichoderma*, had an activity of 5417 units/g as measured using β-glucan as the substrate. The enzymes were added at concentrations of 250 units of pectinase/kg and 1000 units of β-glucanase/kg.

The chemical composition of the CLM is presented in Table 4.12 and it contained 4 g/kg titanium dioxide as an inert dietary marker. Four hours after having been given the last doses of CLM all the bird were killed by an intravenous injection of Euthetal (sodium pentobarbitone) for digesta collection (Kadim and Moughan, 1997). The small intestines were removed and their contents (between Meckel's diverticulum and the ileocaecal junction) were collected. Every three samples from each treatment were pooled and the resultant five samples per treatment were freeze-dried and sieved.

Determinations of titanium and amino acids were made by a colorimetric method and by high performance liquid chromatography (HPLC), respectively

(Roslin Nutrition, 2000). Data were subjected to analysis of variance with dietary treatment as the only factor.

4.6.2 Results

Because of technical difficulties, tryptophan analysis could not be determined in ileal samples. The results are shown in Table 4.18. There were no differences between treatments for amino acids digestibility except for lysine and for total amino acid digestibility. The birds fed on the CLM with added pectinase had the highest ($P<0.05$) digestibility values for lysine and for the total amino acids than those fed on the other treatments. The methionine digestibility coefficient resulted 100% in all treatments.

Table 4.18 Least square means of ileal nitrogen and amino acid digestibilities (%) of CLM and CLM with different added enzymes by 4-week-old chickens

Ileal digestibility	Enzyme				Probability	SEM
	Control	β-glucanase	Pectinase	β-glucanase + pectinase		
Nitrogen	51.2	51.2	53.9	49.8	0.557	2.02
Alanine	55.6	57.9	60.7	55.2	0.102	1.48
Arginine	80.3	96.0	94.4	85.6	0.094	4.26
Aspartic acid	54.8	56.8	59.2	53.5	0.139	1.60
Cystine	51.4	44.3	50.2	45.7	0.620	4.33
Glutamic acid	56.7	60.6	64.1	58.2	0.055	1.63
Glycine	50.4	52.2	55.7	48.9	0.070	1.56
Histidine	52.4	54.6	57.3	52.1	0.178	1.66
Isoleucine	58.3	61.2	64.6	59.8	0.129	1.67
Leucine	63.3	66.0	69.0	65.1	0.139	1.52
Lysine	48.2a	53.7ab	58.5b	53.0ab	0.037	1.96
Methionine	100	100	100	100	1.0	0
Phenylalanine	62.0	64.8	67.8	63.1	0.156	1.67
Proline	53.4	57.1	60.6	47.5	0.081	3.09
Serine	52.7	53.4	55.5	51.0	0.366	1.69
Threonine	50.9	52.9	54.9	50.2	0.199	1.51
Tyrosine	66.2	69.4	71.0	67.4	0.218	1.54
Valine	55.3	57.8	61.3	56.7	0.098	1.48
Total amino acids	59.5a	62.3ab	65.0b	59.6a	0.040	1.23

Values with different letters *a, b, c* in the same row are significantly different at $P<0.05$

4.6.3 Discussion

There are diverse benefits to be gained from the use of enzymes in poultry diets. Some of the benefits influencing the performance of poultry are the increased feeding value of the dietary raw materials, the reduction in the variation in the nutrient quality of the ingredients, leading to increased nutrient digestibility, and the reduction in the water content of excreta (Marquardt *et al.*, 1996; Bedford, 2000; Smulikowska and Mieczkowska, 2000). However, the effectiveness of enzymes depends on many factors such as the ingredient that is being evaluated, the microbial population in the gut (and, consequently, the age of the bird) and the characteristics and amounts of the enzymes used. The detrimental effect of pectin, because of its viscosity, on the digestive and absorptive functions in poultry is well known (Classen , 1996; Iji, 1999).

The mean overall amino acid digestibility of CLM was somewhat low, and this was in agreement with Reverter *et al.* (1999) who fed pigs on diets containing different forage meals to evaluate the apparent ileal digestibilities of amino acids contained in those forages. They found that the lowest and highest coefficients of digestibility of essential amino acids were 0.31 and 0.74 for methionine and tyrosine respectively in lucerne meal; 0.44 and 0.76 for threonine and arginine respectively in white clover; 0.55 and 0.76 for tyrosine and lysine respectively in red clover, and 0.49 and 0.68 for threonine and valine respectively in perennial ryegrass.

However, the results found in the present work differ with those values reported by Donkoh, *et al.* (1990) for CLM in chicks. They found coefficients of amino acid availability ranging from 66.7 to 92.1% for cystine and tyrosine respectively utilising excreta digestibility, and those differences might be attributable to the method used. Ravindran *et al.* (1999) argued that for poorly digestible ingredients (such as those high in fibre), differences between ileal and excreta digestibility for amino acids are largely high. The indigestible amino acids at the ileum will reach the hindgut turning in substrate for microbial fermentation and eventually disappear in the excreta.

In the present work, higher lysine and total amino acid digestibilities evidenced the beneficial effect of pectinase on the pectin content of CLM. The

hydrolysis of the cellulose contained in CLM by the enzyme β-glucanase could have been less efficient than that of pectin by pectinase. The hydrolysis of the water soluble fraction of non-starch polysaccharides is carried out rapidly, in contrast to the slow degradation of the water insoluble component (Choct and Kocher, 2000). Van Soest (1994b) has mentioned that soluble polysaccharides like pectin or β-glucans are more susceptible to the action of enzymes than the insoluble polymers such as cellulose. That could partially explain the fact that no effect was found on CLM cellulose by the enzyme β-glucanase. Although Philip *et al.* (1995) and Yu *et al.* (1998) reported a beneficial response in the performance of broilers fed on diets containing barley when they were supplemented with β-glucanase, the effect was attributed to the activity of that enzyme on the β-glucan component of barley, a soluble polysaccharide which is more easily cleaved by the enzyme than insoluble cellulose. Enzyme utilisation for hydrolysis of non-starch polysaccharides even might work well mainly on soluble polysaccharides, it not necessarily help on improve the digestibility of other nutrients. Kocher *et al.* (2000) added different enzymes in diets for broilers containing lupins, and they found that enzyme addition significantly reduced the concentration of insoluble non-starch polysaccharides in the ileum, even although no benefical effects on protein digestibility were found.

The methionine digestibility coefficient in all treatments was too high, and that was a possible overestimation. It probably arised as result of a very low amount of methionine flowing to the ileum and consequently it was not detectable by laboratory analysis. Siriwan *et al.* (1993), measuring the endogenous amino acid losses in broilers, reported low flows of methionine in the ileal digesta.

Chapter five
PERFORMANCE OF CHICKENS FED ON DIETS CONTAINING DIFFERENT AMOUNTS OF CLM

5.1 Material and methods

The aim of this chapter is to describe the effect of different dietary amounts of CLM on the performance of chickens. The experiment 7 was carried out, and it included two studies, one at FMVZ-UADY in Mexico and one at Roslin Institute.

The first study was carried out with maize only as the diet control, reflecting the feeding conditions that prevail for poultry in rural backyards in the Southeast regions of Mexico, where maize usually represents the main or unique component of the diet (Rejon *et al.,* 1996; Trejo, 1998). The experimental diets which were based on maize only were different in energy, nitrogen and amino acid concentrations; these depended on the amount of CLM included in the diet. The second study was performed under commercial conditions using balanced diets containing similar concentrations of nitrogen, metabolisable energy and amino acids; CLM was mixed with several ingredients.

In the first study, sixty Hubbard 3-week-old chickens, 30 males (547 ± 40 g) and 30 females (514 ± 27 g), were allocated to individual wire pens (40 cm x 50 cm), with individual feeders and drinkers. The birds were fed *ad libitum* and had free access to water during the two-week trial. There were five diets based on maize and different amounts of CLM (0, 150, 250 and 350g/kg) and a final diet containing soyabean meal. All diets were fed in mash form and they contained a mineral and vitamin premix, as well as the same concentrations of calcium and phosphorus. The composition of the diets are shown in Table 5.1.

Six birds of each sex were distributed at random to one of the five diets. Food consumption was recorded daily, and after two weeks, the birds were weighed. The data on food intake, body weight gain, food : gain ratio and final body weight were analysed by two-way analysis of variance with diet and sex as factors. Initial body

weight was included as covariable. Tukey's test was used to distinguish mean differences (MINITAB, 1999).

In the second study, one hundred and forty-eight one-day-old male Ross chickens were used to investigate the effect of different dietary amounts of CLM on bird performance, gut size and the viscosity of the digesta, as well as on some blood parameters. The birds were allotted to brooders fitted with automatic controls for temperature and light. At one day of age, 6 birds were allocated to each of 24 brooder quadrants. There were three treatment diets based on maize and soyabean meals: control, 150 and 250g /kg CLM. The composition and chemical analyses of the diets were shown in Chapter 4 (Table 4.13). The pelleted diets and fresh water were offered *ad libitum* to the birds. Each experimental diet was allocated at random to eight brooder quadrants per experimental diet. The experimental diets were fed from 1 to 21 days of age.

In order to reduce variation of dependent variables, at day 7, two birds (the lightest and the heaviest) were removed from each brooder quadrant. The chickens were weighed individually and food consumption per quadrant was recorded on days 7, 14 and 21 of the experiment, and also calculations of food : gain ratio were carried out. At day 21, two birds per quadrant were bled from the brachial vein. Red blood cell count, haemoglobin and haematocrit (packed cell volume) were determined as blood parameters by an electronic particle counter (Coulter Elecronics), cyanmethaemoglobin method and microhaematocrit method, respectively described by Maxwell *et al.* (1990a).

At day 21, sixteen birds from each treatment were killed by an intravenous injection of sodium pentobarbitone (Euthetal). After injection, the digestive tract was removed including the gizzard and caeca. The contents between the gizzard and Meckel's diverticulum were collected by gently squeezing with the fingers and their viscosities determined (per duplicate) using an automatic viscometer (Brookfield Model DV-II +). The weights of empty gizzards and small intestines were recorded individually as well as the weights and lengths of the caeca. Data were subjected to analysis of variance with diet as the only factor.

Table 5.1 Composition and chemical analyses of the diets containing different amounts of CLM in the first study

Ingredient	Diet (g/kg)				
	Maize	Maize-Soyabean	C150	C250	C350
Maize meal	961.6	828.3	821.2	727.5	633.9
Soyabean meal	0.0	135.4	0.0	0.0	0.0
CLM	0.0	0.0	150.0	250.0	350.0
Vitamins[1]	0.5	0.5	0.5	0.5	0.5
Minerals[1]	1.0	1.0	1.0	1.0	1.0
Sodium chloride	2.5	2.5	2.5	2.5	2.5
Calcium phosphate	20.4	17.6	16.3	13.6	10.9
Calcium carbonate	14.0	14.6	8.6	4.9	1.3
Chemical analyses (g/kg, as fed basis)					
Crude protein	81.7	130.0	110.8	130.2	149.6
ME (Mj/kg)	13.47	12.87	12.48	11.82	11.15
Crude fibre	21.1	27.7	36.0	46.0	55.9
Phosphorus	6.5	6.5	6.5	6.5	6.5
Calcium	10.0	10.0	10.0	10.0	10.0
Lysine	2.5	5.8	4.1	5.1	6.2
Tryptophan	0.5	1.5	0.8	1.0	1.1
Threonine	2.7	4.7	3.8	4.4	5.1
Cystine	1.7	2.4	2.0	2.2	2.4
Methionine	1.7	2.3	1.9	2.1	2.2

[1] Supplied per kg of diet: vitamin A, 12,000 iu; vitamin D_3, 5,000 iu; vitamin E, 50 iu; vitamin K, 3 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 5 mg; vitamin B12, 15 μg; nicotinic acid, 50 mg; pantothenic acid, 15 mg; folic acid, 1 mg; biotin, 200 μg; iron, 80 mg; copper, 10 mg; manganese, 100 mg; cobalt 0.5 mg; zinc, 80 mg; iodine, 1 mg; selenium, 0.2 mg; molybdenum, 0.5 mg.

5.2 Results

In experiment one the results of body weight gain, food : gain ratio and final body weight were different (P<0.05) between diets. The highest values for those variables were obtained with the maize-soyabean meal and the C250 diets (Table 5.2). However, food intake means were not different between diets, although the

trend showed numerically higher values for the maize-soyabean meal and the C250 diets. Initial body weight as a covariable significantly affected the final body weight. There were no significant differences (P>0.05) between males and females for food intake (981.4 ± 28.7 g vs 940.5 ± 27.9 g), body weight gain (239.4 ± 15.8 g vs 220.9 ± 15.4 g), food: gain ratio (4.47 ± 0.26 vs 4.79 ± 0.25) and final weight (770.3 ± 15.8 g vs 751.8 ± 15.4 g).

Table 5.2 Least square means (± SE) of performance parameters of chickens fed on diets containing different amount of CLM in study one

Parameter	Diet					Probability
	Maize	Maize-Soyabean	C150	C250	C350	
Food Intake (g)	919.6 ± 12.6	1032.8 ± 12.6	921.7 ± 12.6	1005.5 ± 11.5	925.2 ± 14.0	0.171
Body weight gain (g)	172.8a ± 6.94	310.1b ± 6.95	194.2a ± 6.95	257.5ab ± 6.35	216.2ab ± 7.72	0.001
Food: gain ratio	5.67ab ± 0.11	3.68ac ± 0.11	4.84a ± 0.11	4.19a ± 0.10	4.73a ± 0.12	0.011
Final body weight (g)	703.7a ± 6.94	841.0b ± 6.95	725.1a ± 6.95	788.4ab ± 6.35	747.1ab ± 7.72	0.001

Different letters *a, b, c* in same row significantly differ at P<0.05

Table 5.3 Least square means (± SE) of the performance parameters of the chickens fed on the diets containing different amount of CLM in the second study

	Diet			Probability
	Control	CLM150	CLM250	
Weight gain (g)				
1-7 d	72.9a ± 0.52	77.1ab ± 0.45	64.4ac ± 0.45	0.003
8-14 d	172.2a ± 1.08	183.6a ± 0.94	128.8b ± 0.94	0.001
15-21 d	214.7a ± 1.33	213.8a ± 1.16	137.3b ± 1.16	0.001
1-21 d	459.8a ± 2.58	474.6a ± 2.25	330.6b ± 2.25	0.001
Food intake (g)				
1-7 d	105.6 ± 2.35	116.6 ± 2.05	107.6 ± 2.05	0.389
8-14 d	262.6a ± 4.30	295.8ab ± 3.75	239.5ac ± 3.75	0.005
15-21 d	322.8a ± 4.29	349.2a ± 3.74	260.0b ± 3.74	0.001
1-21 d	691.1a ± 9.82	761.8ab ± 8.82	607.0ac ± 8.82	0.001
Food : gain ratio				
1-7 d	1.46a ± 0.019	1.54ab ± 0.016	1.68b ± 0.016	0.014
8-14 d	1.53a ± 0.013	1.60a ± 0.011	1.90b ± 0.011	0.001
15-21 d	1.50a ± 0.013	1.63b ± 0.011	1.90c ± 0.011	0.001
1-21 d	1.50a ± 0.010	1.60b ± 0.008	1.84c ± 0.008	0.001

Values with different letters *a, b, c* in same row are significantly different at $P<0.05$

In the second study, birds from one quadrant fed on the control diet were removed from the experiment because they showed very poor performance. Body weight gain, food intake (with exception of those at 7 d) and food : weight gain ratios of the birds fed on the CLM250 diet at 7, 14 and 21 d were all lower (P<0.05) than those birds of fed on either the control or CLM150 diets (Table 5.3). Consequently, the body weights of the birds fed on the CLM250 diet were all lower (P<0.05) than those of the birds fed on either the control or CLM150 diets at 7, 14 and 21 d (Table 5.4). The results of the blood components in this study (Table 5.5) showed no effects (P>0.05) of the different diets for haematocrit and red cell count. However, birds fed on CLM250 diet showed a lower (P<0.05) value of haemoglobin than those chickens fed on CLM150.

Table 5.4 Least square means (±SE) of the body weights (g) of the chickens fed on the diets containing different amount of CLM in the second study

	Diet			
Age (d)	Control	CLM150	CLM250	Probability
7	114.1a ± 0.52	118.3ab ± 0.45	105.6ac ± 0.45	0.003
14	286.3a ± 1.48	302.0a ± 1.29	234.5b ± 1.29	0.001
21	501.0a ± 2.58	515.8a ± 2.25	371.8b ± 2.25	0.001

Values with different letters *a, b, c* in same row are significantly different at P<0.05

Table 5.5 Least square means of the blood components of 21-d-old chickens fed on the diets containing different amount of CLM in the second study

	Diet			SEM	Probability
Parameter	Control	CLM150	CLM250		
Haemoglobin (g/100ml)	8.00a	8.18ab	7.65ac	0.137	0.031
Haematocrit (%)	28.53	28.00	26.81	0.532	0.076
Red blood cell count (millions/ml)	1.56	1.53	1.67	0.079	0.418

Values with different letters *a, b, c* in same row are significantly different at P<0.05

There were differences (P<0.05) between diets for the weights of the gizzards both in terms of absolute weight and in terms of body weight (Table 5.6). The lowest values were in birds fed on CLM250 diet. The intestinal weights did not differ between birds fed on the three diets in both measurements. The values of length and weight of caeca in both absolute and relative measurements from birds fed on the control diet were lower (P<0.05) than those of birds fed on either of the CLM diets. The body weight as covariable was significant for the absolute weights of the gizzard and the intestine. In contrast to what was anticipated, the viscosity of the digesta decreased (P<0.05) as the amount of CLM in the diet increased (Table 5.6).

Table 5.6 Least square means of the weights of gut compartments and digesta viscosity in 21-d-old chickens fed on diets containing different amounts of CLM in the second study

	Diet			SEM	Probability
	Control	CLM150	CLM250		
Gizzard.					
(g)	14.32a	12.42b	11.78b	0.397	0.002
(g/kg[1])	26.18a	22.99b	20.91b	0.725	0.002
Intestine					
(g)	19.51	20.29	23.92	0.694	0.083
(g/kg[1])	36.08	37.62	44.85	1.30	0.062
Caeca					
(g)	4.78a	6.93b	8.33b	0.494	0.002
(g/kg[1])	9.06a	12.65b	16.17c	0.973	0.005
length, cm	9.90a	11.50b	12.42b	0.261	0.001
Digesta viscosity (centipoise)	2.23a	1.49b	1.23c	0.062	0.001

Values with different letters *a, b, c* in same row are significantly different at $P<0.05$
[1]Referred as g/kg body weight.

5.3 Discussion

The poor performance of the birds fed on only maize in the first study was probably the consequence of the low crude protein and amino acid contents of this diet, even although it had the highest estimated metabolisable energy concentration (Boorman, 1999). Those findings agreed with those of Bielorai *et al.* (1991), who fed chickens with maize as the sole source of protein and attributed low weight gain to

both the low dietary concentration of amino acids and their relatively low but highly variable absorption.

The lack of any effect of gender on bird performance found in this study agrees with the conclusions of Sibbald (1982) who in a review of the literature showed, that there is no difference in the ability of the two sexes to obtain bioavailable energy from food, when the birds are sexually immature.

The results on bird performance found in this study are also in good agreement with other workers who found that the use of fibrous foodstuffs in poultry feeding is associated with poor performance (Abdelsamie *et al.,* 1983; Aguilera *et al.,* 1984; Tillan *et al.,* 1986; Perez *et al.,* 2000). This is most noticeable in chickens which have the genetic potential to achieve high levels of growth and food consumption and are, therefore, very susceptible to changes provoked by the inclusion of fibre in the diet. Savory and Gentle (1976b) reported that adult Japanese quail fed on a diet high in fibre weighed less than those fed on a corresponding diet low in fibre, and suggested that the difference in body weight was caused mainly by the difference in energy utilisation. It was argued that the birds fed on the diet high in fibre had expended more energy on feeding than those fed on the low-fibre counterpart.

In this study, CLM contained a high concentration of crude protein, but NDF and crude fibre were also present in high amounts. As a result, increasing the concentration of CLM in the diets, the crude fibre and NDF contents were increased and the metabolisable energy concentration was decreased in both studies. Moreover, in the second study, even although soyabean oil was added to the diets in an attempt to equalise the concentrations of metabolisable energy, the apparent metabolisable energy found in those diets decreased as the CLM concentration increased at both ages 7 and 21 day-old. This could explain fairly strong the tendency for the body weight gain of birds fed on diets containing CLM to be poor (Panigrahi and Powell, 1991). Newcombe and Summers (1985) and Onifade and Babatunde (1998) fed broilers on diets containing different fibre sources. They found that the physical limitation imposed by the capacity of their guts meant that the birds were incapable of reaching nutrient intakes equal to those of the birds fed on control diets. Consequently a lower performance, attributed to low nutrient density was

found in those birds. Likewise, Sobamiwa and Longe (1994) reported reduced performances in chickens fed on diets containing increased amounts of cocoa-pod husk, an extremely fibrous material. They attributed this to some of the fibre component in that ingredient rather than to the fibre content itself. The authors considered that lignin and pectin were more deleterious than cellulose, even although that was most abundant in the husk.

The effect of fibre on the digestibility of dietary protein and its utilisation is another possible explanation for the lower performance of the birds fed on diets containing CLM. Abdelsamie *et al.* (1983) claimed that dietary fibre could affect the efficiency of protein utilisation in broilers. Angkanaporn *et al.* (1994) postulated that impaired protein digestion, inhibition of amino acid absorption or the increased secretion of endogenous protein could all be possible reasons for the low protein digestibility associated with the presence of non-starch polysaccharides in diets. Also Nyman *et al.* (1990) suggested that the lower digestibility of crude protein observed in high-fibre diets may have its origins in undigested cell wall protein and reduced digestion and absorption of protein overall.

The results of food intake found in the current study differ from those reported by Donkoh *et al.* (1999) who evaluated broiler performance from 1 to 56 days of age, when diets containing 0, 25, 50 and 75 g CLM /kg were fed. In that study the concentration of CLM in the diet had no effect on feed consumption, probably because lower concentrations of CLM were used. However in the same report (Donkoh *et al.*, 1999) chicks fed on the diets containing 50 and 75 g CLM /kg gained significantly less weight. This was attributed not to the fibre content of diets but to the oxalates and cyanogenic glucosides present in the CLM. In contrast to those results, in this study the weight gains of the chickens fed on the diets containing up to 250 and 150 g /kg CLM in experiments 1 and 2, respectively, did not differ significantly from those fed on maize-soyabean diets. Moreover, heat-processing of CLM (like in the current study) has been reported to reduce the content of cyanogenic glucosides (Herrera *et al.*, 1993).

There must be differences between animal species for tolerating the toxic substances contained in plants. Sowls (1996) asserted that ruminants are able to adapt to diets high in oxalates, however, the extent to which nonruminants are able

to do so, is not known. Jacob *et al.* (1994) found oxalate concentrations as high as 10% of the dry matter content in *Maireana breviflora* grazed by sheep and they reported no acute oxalate toxicity in those animals.

The haematological results found in the present study also disagreed with those reported by Donkoh *et al.* (1999). They mentioned increasing values of haemoglobin, haematocrit and red blood cell count in broilers as the amount of dietary CLM increased, even although the birds in the current study were fed on diets containing higher concentrations of CLM than those from the aforementioned study. The haemoglobin results found in this study agreed with values reported by Hodges (1977) who, in a review of the literature, quoted concentrations 7.1, 7.4 and 7.4 g/dl in White Rock chickens aged 1, 4 and 9 weeks, respectively, and concentrations ranging from 8.61 to 13.19 g/dl in New Hampshire mature chickens. However, Maxwell *et al.* (1990a,b) reported that broilers fed *ad libitum* and kept under normal environmental conditions had higher values of haemoglobin, haematocrit and red blood cell count than those found in the current study.

Hodges (1977), who reported higher red blood cell count values than those found in this study, also mentioned that there was a high variation in the red blood cell between individual birds and some factors, such as age, sex and environment, may affect that count. Fajimi *et al.* (1993) reported slight higher haematological values, for 10-week-old broilers raised under tropical conditions, than those reported herein. However, the haematocrit values in the present study agreed with those reported from several other laboratories (Hodges, 1977; Gentle *et al.,* 1989; Maxwell, 1990).

The results of digestive organ measurements found in the present study agreed with various studies showing that dietary fibre results in the enlargement of the bird's gut (Abdelsamie *et al.*, 1983; Savory, 1992c; Fuente *et al.*, 1998) and of the rat's bowel (Wyatt *et al.*, 1988; Goodlad and Mathers, 1990; Zhao, *et al.,* 1995). Longstaff *et al.* (1988) found heavier and longer caeca in chicks fed on diets containing pentoses and uronic acids (which usually come from the degradation of non-starch polysaccharides), than those from chicks fed on a glucose-based diet and they attributed this elongation to the process of fermentation. Sakata (1987) asserted that short chain fatty acids, which are derived from fibre fermentation are

responsible for the elongation of the gut, arguing that a short chain fatty acid trophic effect stimulates the proliferation of intestinal epithelial cells. However, Wyatt *et al.* (1988) have stated that, in rats fed on high-fibre diets, the enlargement of the gut is a simple adaptive response to the increased bulk of the digesta, and it occurs due to the accumulation of the undigested material. Jorgensen *et al.* (1996) found that the consumption of diets high in fibre caused an increase in the length of the gastrointestinal tract among broiler chickens. They emphasised the impact that this response would have on energy metabolism, as visceral organs have a high rate of energy consumption relative to their size.

It seemed that, contrary to expectations, the inclusion of CLM in the diets did not increase the viscosity of the digesta, even although it contains some soluble fibre mainly in the form of pectin. Langhout and Schutte (1996) pointed out that, although pectins are widely variable in chemical structure and it is their viscous property that is the main factor that accounting their anti-nutritive effects. Characteristics of the pectin such as its dietary concentration, its origin and the degree to which it is esterified, are all likely to determine its effects on digestion and, consequently, on the performance of broilers. Indeed, the viscosities in the gut of the birds fed on both diets containing the CLM were lower than that found in those of the birds fed on the control diet. Philip *et al.* (1995) reported averages of intestinal viscosities of 93.2, 28.5 and 17. 2 cps in 14-, 21- and 35-day-old birds, respectively, fed on diets based on barley, much more higher than the range of values (1.23-2.23) found here.

The results under the conditions of the current experiment suggest that CLM does represent an alternative source of protein for poultry diets, despite its high fibre content. It appears that it may be included at up to 150 g/kg in commercial diets without exerting an adverse effect on the performance of broilers. Also, CLM mixed with maize at up to 250 g/kg would probably improve the performance of chickens fed on low-protein diets such as those used in rural backyards in developing countries, but this needs confirmation in scavenging birds.

Chapter six
GENERAL DISCUSSION

The small farming systems practised in many of the tropical regions of the world offer a realistic alternative means of providing cheaper animal products to rural families, thereby improving their nutritional status. Furthermore, those small-scale systems are more sustainable than large commercial farming enterprises, many of whose operations rely on the use of imported foodstuffs. As has already been mentioned in the Introduction to this thesis, a paradoxical state of affairs exists in the Yucatan state of Mexico in so far as it possesses the most advanced poultry industry in the country yet also unenviably manages to occupy the leading place in population malnutrition, mainly in rural areas.

CLM is not yet available on a commercial scale nor would it currently be considered an acceptable ingredient for inclusion in commercial diets by the poultry industry. Nevertheless, the results discussed in the present thesis provide some support for the view that CLM is a suitable ingredient for feeding to poultry in low input-output systems, such as is practised by small-scale farmers in the tropics. The composition of CLM has been fully described and some information on its utilisation by poultry has been reported. The results from these experiments support in part the above assertion.

6.1 Chemical composition and digestibility of CLM

It has been shown that the chemical composition of chaya leaves did not change significantly during the 4- and 12-week-period of re-growth. This strongly suggests that the leaves can be harvested at 4, 8 or 12 weeks and the preferred time will largely be determined by the yields of dry matter of CLM collected at the various times.

CLM contains acceptable if somewhat lower concentrations of the essential amino acids (Table 4.12), when these are compared to the amino acid content of soyabean meal, the conventional source of protein found in almost all commercial poultry diets (Table 3.4). Relative to soyabean meal, the proportion of amino acids present in CLM ranged from 0.416 for lysine to 0.641 for valine. However, CLM comes out in a rather better light when comparisons are made with another fibrous foodstuff, dehydrated alfalfa meal (NRC, 1994), a widely acceptable ingredient in poultry diets. Without exception the concentrations of all the essential amino acids in CLM were higher than the corresponding values in alfalfa.

The relatively high content of oxalic acid found in CLM appears to decrease as the plant matures. Given the potential toxicity of oxalic acid, this may be an important factor that will need to be taken into account when developing a harvesting plan. The management strategies for cutting or browsing the forage from the trees as a means of eliminating or minimising some of its toxic components are likely to be critical, considering that the browsing intervals might influence the severity of the toxicity. Ultimately, however, as mentioned by Lowry (1989) the presence of toxic substances or anti-nutrients in plants should be confirmed from the results of feeding trials rather than from chemical analyses alone.

Radeleff (1970) has pointed out that oxalates occur in plants in the form of salts of calcium, sodium or potassium and declared that it is not uncommon to find relatively high amounts of oxalic acid in the plant kingdom. Telek (1983) suggested that 5 g represented the fatal dose of oxalic acid in man. Sowls (1996), who reported concentrations of oxalic acid ranging from 3.69 to 5.39 g/kg in samples of cactus, qualified those amounts as "not being extremely high in oxalic acid, compared to common forage for livestock". Given that oxalic acid has the capacity to bind calcium, and that man is able to adapt to low intakes of dietary calcium, Liener (1980) concluded that, at least in humans, there is actually only a minimal risk associated with the consumption of oxalate-containing plants. For chronic intoxication with oxalic acid to occur requires both a very high rate of consumption of oxalic acid and very low calcium and vitamin D intakes over long periods of time. In this context, because commercial poultry diets are usually balanced to contain adequate amounts of both calcium and vitamin D, there is, consequently, only a low

probability of toxicity symptoms being observed among poultry consuming oxalic acid. Moreover, Samarasinghe and Rajaguru (1992) fed broilers on diets containing 100 g/kg of colocasia (*Colocasia esculenta*) which contained calcium oxalate ranging in concentration from 10 to 40 g/kg, and reported a very "low contribution" from the calcium oxalate in depressing the dietary palatability in and growth of those birds.

In the plant kingdom, when oxalic acid is present in a species then more often than not hydrogen cyanide is also to be found (Telek, 1983). This axiom has been shown to hold true for chaya in both this study and that of Donkoh (1990, 1999). Liener (1989) has stated that from 2.0 to 3.0 g of hydrogen cyanide/kg of dry matter is the amount likely to cause intoxication in humans. Furthermore, he defines the acceptable concentration of hydrogen cyanide in a foodstuff, for example in lima beans, to range from 0.1 to 0.2 g/kg of dry matter. Montgomery (1980) has reported that the minimal lethal dose of hydrogen cyanide ranges from 0.5 to 3.5 mg/kg body weight, but has also pointed out that well-nourished cattle can tolerate the continuous consumption of grasses containing up to 50 mg/kg. Whether these same limits are applicable to humans is not known. Likewise, caution should be exercised before assuming that other monogastric animals such as poultry can tolerate these doses. It is on this topic that responses recorded in this thesis are strikingly different from those reported earlier by Donkoh (1999). In the present study haematological data from birds fed on diets containing 150 and 250 g/kg CLM provided no evidence to support the view that either hydrogen cyanide or oxalic acid was detrimental to the birds. In contrast, Donkoh (1999) attributed the higher haematocrits and red cell counts which he observed in birds fed on diets containing up to only 75 g/kg CLM as indicative of a toxic response to the consumption of hydrogen cyanide and oxalic acid.

The term true metabolisable energy is distinguished from apparent metabolisable energy basically by application of a correction factor to compensate for the endogenous energy voided by fasted birds. Dale and Fuller (1982) pointed out that, unlike mammals, fasted birds cannot survive on stored glycogen to meet their maintenance energy requirements and have to rely on the catabolism of body

reserves of fat and protein. The catabolism of protein results in the birds having to eliminate the nitrogen-containing end products of this process (mainly uric acid) and consequently the endogenous losses from fasted birds is almost always bound to be higher than those from their fully fed counterparts. Therefore, TME values, which are almost invariably derived using the mean value found for the endogenous energy excreted by starved birds, will also be overestimated. Furthermore, in order to reduce the range in values observed for the endogenous energy losses (this could arise from differences in the birds' body compositions, ratio of fat to protein), they recommended the use of fed (with highly digestible foods such as glucose or starch) rather than fasted birds as negative controls (to provide a value for the endogenous loss). Intercepts of regression lines, which relate energy output and energy input, is also an alternative approach capable of providing acceptable estimates of endogenous losses (Sibbald, 1982).

In the same way as endogenous energy losses are important in the derivation of meaningful TME values, so too are endogenous nitrogen and dry matter losses crucial in deriving valid true nitrogen and dry matter digestibility coefficients. However, in the course of this study it was demonstrated that the true nitrogen and dry matter digestibilities of CLM increased linearly as the CLM input increased (Figure 4.1). This result has probably arisen because of the important role played by endogenous losses when high fibre foodstuffs are evaluated over different collection periods by the method of tube-feeding practised in the TME procedure.

From the data generated in the second study of the experiment 3, it was noted that the controversy related to the calculation of endogenous losses – whether to derive from starved birds or from the intercepts of regression lines - was unresolved. The data from this experiment were used to calculate the endogenous energy lost over 72 h from birds fed on glucose only, and the intercept of the linear regression equation of the energy output against the input of the chaya fibrous extract. The mean value of the endogenous energy lost from the birds fed on glucose only was 146.2 ± 31.4 kJ, a similar value to that found in experiment 2, when using different inputs of CLM. The equation of the regression was as follows:

Gross Energy output (kJ) = 47.2 + 17.5 CFE input ($R^2 = 0.94$)

The intercept value of that equation (47.2 kJ) was numerically much lower those (for 48, 56 and 72 h of collection of droppings) found in the aforementioned experiment 2, even although chaya fibrous extract had a higher concentration of fibre than CLM. It seemed that endogenous energy lost from a bird was being influenced by the amount of energy supplied to the birds. Birds in experiment 3 were all given, in addition to the chaya fibrous extract, some extra energy in the form of glucose, which must have helped to reduce the endogenous energy losses, so that fasted birds catabolise more body protein than the fed birds. Although, different strains were used in those experiments, the intercepts of the regression lines of energy output on chaya fibrous extract input were similar (48.5 and 46.0 kJ for criollo and leghorn genotypes, respectively).

When the TME method is to be applied in practice, therefore, it would seem sensible to provide the birds with an additional source of energy in order to reduce the variation in and the size of the endogenous energy loss. This would be particularly important when ingredients (such as CLM) that are high in fibre and low in metabolisable energy content, are under evaluation. As a valid technique for measuring the TME values of high-fibre foodstuffs such as CLM it would appear that the tube-feeding method gives reasonable results. The TME_N of CLM, derived using adult cockerels, was 5.76 MJ/kg, a value lower than that found for wheatfeed (8.39 MJ/kg). Similar values of 6.53 and 5.31 MJ/ kg were found when 3-week-old broilers were given CLM (experiment 2) over balance periods of 48 and 72 h, respectively. Despite the finding that, when CLM replaced part (15 and 25%) of the control diet, significant reductions were observed in the dietary AME_N values, it appears that the AME_N value derived for CLM was higher when it was fed as part of a diet than the corresponding TME_N value derived from tube-feeding CLM alone. From the figures presented in Table 4.15 for 3-week-old birds, it is possible to calculate the AME_N values of the CLM by subtracting the proportional contributions in AME_N made by the control diet (10.48 and 9.25 MJ/kg, respectively) from the AME_N values of each of the diets containing CLM (11.57 and 11.86 MJ/kg, respectively) and dividing the results by the proportions of CLM in the test diets (0.15 and 0.25, respectively). Carrying out these calculations resulted in values of 7.26 and 10.44 MJ/kg, respectively, being derived for the CLM from the diets

containing 150 and 250 g/kg CLM. It seems probable that these values, which surprisingly were not significantly different from each other, are both overestimating the AME_N of the CLM. Alternatively, if the value derived for the TME_N content of CLM (5.76 MJ/kg) is (proportionally) subtracted (0.86 and 1.44 Mj/kg, respectively) from the AME_N values of each of the diets in which it is present, then the AME_N values of the control components of these diets can be calculated (10.71 and 10.42 MJ/kg, respectively). By dividing these values by the proportions of the control diet present in the two diets 0.85 and 0.75, respectively), AME_N values for the control diet can be derived (12.59 and 13.89 MJ/kg for CLM150 and CLM250, respectively). Both of those are higher than the value of 12.33 MJ/kg found for the control diet directly. These values can only be explained in terms of there being a positive interaction between CLM and one or more of the other dietary ingredients. However, it is also possible that the adaptation period (20 days) of the birds to the diets containing the CLM could partially explain the apparent discrepancies between the TME_N and AME_N values. Farrel *et al.* (2000) found similar results, when evaluating diets containing either lucerne or sweet potato vines meals (both ingredients high in fibre) and added 50 g/kg soyabean oil, resulted in "surprisingly" high values of AME in broiler chickens. Moreover, Dublecz *et al.* (2000) reported for alfalfa meal a higher AME_N value than its corresponding TME_N value. They argued that ingredients such as alfalfa, having extreme nutrient contents, are probably digested worse when they are fed alone due to the fact that in practical conditions they are included in low amounts into the diets.

As methods for determining the bioavailable energy in foodstuffs for poultry, the TME and AME procedures have both advantages and disadvantages. Strict comparisons between the two protocols are further complicated because there are two different experimental methods whereby AME can be estimated. The first is based on total food intake and total excreta collection measurements, while the second relies on the inclusion of an inert marker in the food to relate the amount of droppings produced to the food eaten. Sibbald (1982) stated that both types of AME assay tend to be slow and expensive. Even the so-called improved rapid AME assay promoted by Farrell (1981), which claimed to have some advantages such as speed and a requirement for a relatively small sample size over the classical methods, still

showed some weaknesses as far as training birds and obtaining adequate intakes of unpalatable ingredients were concerned. Sibbald (1982) also argued, on the basis of his own empirical data, that TME values of ingredients are additive, whereas it was difficult to establish how precisely additivity held for the corresponding AME values. Consequently, diet composition and the resulting interactions between ingredients may play an important role in the values attributed to the AME values of diets. In the current study, diets including CLM also contained higher amounts of soyabean oil, a factor which could have exerted a beneficial effect on the digestibility of the diets and consequently on their AME values. Dänicke *et al.* (2000) claimed that it is not always valid to assume that the AME values of raw materials are additive. They have found disproportionate increases in the AME_N values of broiler diets when 60 g of tallow/kg was added to diets containing the enzyme xylanase. They declared that as a result of interactions between dietary ingredients, estimated AME values are sometimes rather different from the values observed. Nitsan *et al.* (1997) also reported on an improvement in food utilisation by broilers as the result of the addition of soyabean oil to the diets, resulting in higher net energy deposition in the body, although, that beneficial contribution of fat to dietary AME_N is curvilinear.

Farrell (1981), a strong proponent of the AME procedure, has accepted some of the advantages of the TME method – the speed with which results of the test ingredients can be provided and the minimal amount of sample that is needed for their evaluation. Another advantage of the TME method is the precision with which it is able to evaluate ingredients which are poorly accepted (*e.g.* fibrous foodstuffs) by poultry, or when oils or fats (particularly mixtures) require to be assessed. However, Farrell (1981) has also drawn attention to the difficulties in collecting quantitatively the small amounts of excreta that are voided from birds given quite small amounts of food. He has expressed concern about the relatively long time (between 2 and 4 weeks) taken for the body weights of the birds to recover from the small amounts of food given before, during and immediately after force-feeding (most commonly 50 g over 4 days). However, my experience from the current study revealed that adult birds recovered to their original body weight within approximately one week and the problems in collecting small amounts of excreta

were not encountered.

An additional area of concern that is often expressed by animal welfare groups is the imposition on the birds of an unacceptable procedure frequently referred to as force-feeding. However, D'Alfonso *et al.* (1999) have recently suggested that the application of force-feeding birds is not absolutely necessary to calculate TME_N values accurately. They have found very little variation in the TME values derived from birds fed voluntarily, and have proposed their procedure as a valid alternative to tube-feeding. However, this approach needs to be carefully evaluated before it can be totally accepted, mainly with ingredients having low acceptability.

Without doubt and despite more than 25 years of research effort, the most overriding concern still associated with the TME method, and noted during the present study evaluating CLM, is to uncover a more reliable way to measure the endogenous losses used to correct apparent to true metabolisable energy/digestible nutrient values. A further complication in the values derived by the TME procedure and pointed out by Farrell (1981) is the possible variation caused by the composition of the diet. Because the composition of the diet may modify the time taken for the digesta to pass through the digestive tract, excreta collection periods may need to be modified. Therefore, it may be inappropriate to apply the same single value for endogenous loss derived from starved birds over a fixed period of time (mostly 48 h) to all foodstuffs. However more information is unfortunately still required to resolve this issue, before assuming that Farrell's hypothesis is completely true.

Scott (1996) has proposed that if a foodstuff or diet promotes additional endogenous losses then these should be charged against the feed rather than the bird. This thesis strongly implies that AME values yield more realistic measures of the amounts of energy that become available to the bird during the course of digestion and metabolism. Scott (1996) has also argued that the AME procedure is more relevant to commercial practice where birds are fed *ad libitum* under conditions which promote positive balances of both energy and protein.

The effect of chaya fibrous extract on the excretion of both nitrogen and uric acid by birds was notable (Figure 4.2). In chapter 4, it was discussed how both the microflora and the cells sloughed off from the intestinal mucosa could increase the

excretion of endogenous nitrogen when ingredients with high concentrations of different types of fibre are fed to chickens. The remaining question in this context could be: how much of the endogenous nitrogen excreted is from microbial sources, as a result of increased fermentation of dietary fibre, and how much of it is from the intestinal mucosa as a result of the abrasive effect of the fibre on those cells?

The effect of body weight on increasing the TME_N value of CLM was confirmed; this was attributed to the higher capacity of the bird's gut to absorb nutrients as the bird increases in weight. It has been demonstrated in different species of animals, particularly in ruminants, that the ability to utilise dietary fibre is related to body size. Van Soest (1994a) mentioned that solid organs such as those of the digestive tract tend to be in direct proportion to body mass. Ruminants and nonruminant herbivorous animals fall on the same regression line, all being similar in gastrointestinal capacity rclated to body size. However, the amino acid composition of CLM makes it more suitable for feeding to larger nonruminant animals than chickens [*e.g.* turkeys, ostriches and pigs (Duke *et al.*, 1984; Cilliers *et al.,* 1997; Cillers and Angel, 1999; Santos, 1999)]. Additionally, any difference in the ability between criollo and commercial chickens in utilising CLM needs to await the results of further research, as attempts are made to match the utilisation of such fibrous materials to the appropriate animal production system.

The total heat increment from feeding CLM was 1.7 times greater than that from feeding wheatfeed, and the net efficiency of the utilisation of the ME (k) from CLM was 0.64, while that from wheatfeed was 0.86. Despite both the CLM and wheatfeed having similar concentrations of gross energy the role of their different chemical components (especially the high fibre contents) was the determining factor in those results. The metabolisable and net energy values derived for CLM represented 0.34 and 0.23, respectively, of its gross energy content. The combination of its lower TME value and the lower net efficiency of utilisation led to CLM having a NE value of 3.86 MJ/kg, which was only 0.53 of that of wheatfeed. From these figures it is reasonable to assume that CLM cannot properly be considered a source of energy for poultry. NE determinations are expensive and complex to carry out, requiring either sophisticated equipment for whole body calorimetry or the complicated and careful methodology involved in comparative slaughter. However,

the benefit of having, for proposes of feed formulation and ingredient utilisation, several practical estimates of bird performance relative to one foodstuff rather than one single determination of NE from the same ingredient is worthy of consideration. After all, NE reflects the "productive energy" or the retained energy from an ingredient or diet. Determining directly the performance of poultry fed on potential dietary ingredients could be useful information in establishing the strategy for small farming systems, where maximising both the economical and animal efficiencies are not the most important objectives. Those systems consider animal production as just one constituent of several components conceived from an "holistic" point of view. In this context, possible beneficial interactions of CLM with other ingredients in the diet or even more importantly, with other components in the system are interesting and worthy aspects remaining to be investigated.

It was noted that both the metabolisable and net energy values of CLM for poultry were relatively low, as was to be expected for a fibrous foodstuff. Thus, although its nutritive value judged as a source of energy was poor, its negative nutritional properties were very obvious when the effects that CLM fibre might exert on the digestion of whole diets are considered. In this context, from the results of the non-starch polysaccharide analyses, the composition of the fibre in CLM could be deduced from the nature and quantity of the sugar residues released by acid hydrolysis. Uronic acids and galactose were the main components released from the soluble fibre fraction, while xylose, arabinose, mannose, galactose and glucose were present the principal components in the insoluble fraction. Uronic acid usually comes from pectins and less frequently from hemicelluloses (Theander *et al.*, 1995). On the other hand, xylose and arabinose are the main constituents of xylan polysaccharide or hemicellulose, which along with cellulose (consisting entirely of glucose) and lignin, forms the characteristic insoluble cellulose-hemicellulose-lignin complex. Insoluble polysaccharides constituted 135.5 g/kg of the CLM and, with the addition of lignin, this complex could represent 174.6 g/kg, a value that closely corresponds to the neutral detergent fibre content of CLM. Those polysaccharides were probably responsible for the effects observed in the gastrointestinal tract, attributable by many others to insoluble fibre (*e.g.* water holding capacity, bulking

and interference with both motility and enzyme function) on the digestion process and performance of the birds described earlier. Longstaff and McNab (1991) claimed that certain polysaccharides were able to adsorb the digestive enzymes with the result that their activities were compromised. The pectin content of CLM (the main soluble component found) by itself did not appear to play any important detrimental role in this study, neither affecting digestion nor the performance of the birds. There are different types of pectin found throughout the plant kingdom, their actual properties depending on the precise chemical structure particularly the degree of methylation. However, a chemical characterisation of the pectin of CLM was not carried out and its effects on the digestive processes in poultry remain to be investigated.

The derivation of apparent amino acid digestibility values of feedstuffs by analysis of the ileal contents taken from birds fed on the test ingredients is claimed to yield more acceptable values than those based on the recovery of amino acids from droppings. Furthermore, the difficulties in determining the effects of the ingested diet on the losses of endogenous amino acids, to allow corrections to be applied to produce true amino acid digestibility coefficients, have been pointed out by Green *et al.* (1987a, b) and Perez *et al.* (1993). This can be interpreted as suggesting that even when pure inputs (*e.g.* glucose) are used to generate estimates for the endogenous losses of amino acids in poultry, there are considerable doubts on the appropriateness of applying such values to birds being fed on balanced diets *ad libitum*. This was confirmed in this study from the variable amounts of amino acids found in the excreta of adult cockerels fed on different fibre sources. Although, Papadopoulos (1985) pointed out that results obtained from the faecal method are not "substantially different" from those using the ileal procedure.

Johns *et al.* (1986) reported that the gut microflora are able to change the composition of the excreta by both degrading and synthesising amino acids in the digesta in the hind gut. Any degradation of amino acids during the passage of the digesta down the gastrointestinal tract will result in their non-appearance in the faeces and lead to an overestimation in their digestibilities. Sebastian *et al.* (1997) stated that the recovery of amino acids from the ileum is a reliable measurement and

is not altered by caecal or intestinal bacteria. Ten Doeschate *et al.* (1993), however, have pointed out that in chickens there are differences between ileal and droppings digestibility coefficients for both nitrogen and amino acids, as a consequence of the contamination of faeces with urine. In contrast, Villamide and San Juan (1998) reported that assays based on excreta collection and corrected for endogenous losses of amino acids generated satisfactory values for the true digestibility coefficients of amino acids of feedstuffs in cockerels.

Whereas the inclusion up to 150 g/kg CLM in the diet did not affect the overall apparent amino acid digestibility, the incorporation of 250 g/kg CLM decreased that measurement. This reduction was attributed to the fibre content of the CLM. Methionine and alanine were the first and second most highly digestible amino acids, whereas glycine, serine and threonine were among those most poorly digested in the diets containing CLM. Although, the average amino acid digestibility, even for the diet containing 250 g/kg CLM, was considered acceptable for less demanding systems of broiler production, it would not meet the criteria demanded by modern intensive commercial enterprises which seek the greatest weight gains in the shortest times.

Furthermore, when CLM was evaluated on its own, with the exception of the digestibility coefficient of methionine, the value of which could have been overestimated (as has been explained earlier), arginine, tyrosine and leucine were the most highly digestible amino acid, whereas cystine was the most poorly amino acid digested. Those results were in good agreement and consistent with those reported by Villamide and San Juan (1998) who evaluated the amino acid digestibility of sunflower seed meal, an ingredient also high in fibre. However, when chaya fibrous extract was fed to adult cockerels (Table 4.8), the amounts of the endogenous amino acids in the excreta were all lower than those in the excreta of birds fed on glucose only. The only exception to this occurred with glycine, the concentration of which was probably influenced by its formation as a hydrolytic degradation product of uric acid (Villamide and San Juan, 1998). Moreover, if the small amounts of amino acids present in the chaya fibrous extract were reasonably considered to be indigestible and, hence, to be excreted, and these amounts subtracted from the totals excreted, then the contribution of alanine, isoleucine, leucine, methionine, phenylalanine and

valine from endogenous sources were all negative. This finding would mean either of two things. Firstly, that endogenous amino acid excretion is affected by the feedstuff being evaluated, *i.e.* the excretion of endogenous amino acids from birds fed different fibre (or perhaps even food) sources varies. It seems that variations in the endogenous losses of the amino acids is not only caused by the amount of fibre ingested, but also by the source (nature) of that fibre and/or its chemical composition. And secondly, that CLM, or one of its derivatives in this case, might modify the endogenous amino acid losses in birds favourably (compared to losses obtained from birds fed glucose only). Finally, as a matter of urgency, a preferred method should be established for the evaluation of amino acid digestibility on the basis of its ability to predict bird performance under specific dietary conditions.

Muztar and Slinger (1980b) pointed out that there will always be distortions in calculating true amino acid digestibility whereas fecal and endogenous urinary amino acid losses in the fed and fasted birds will not be equal. Whether the values of apparent digestibility of amino acids for both CLM and diets containing CLM may have been improved by correcting to true amino acid digestibility coefficients (by the application of some measurement for the endogenous amino acid components of the droppings), must unfortunately remain a matter for speculation at this stage. However, the formulation of diets containing cheap and highly-variable composition ingredients for poultry might have more beneficial effect when those diets are balanced with respect to digestible amino acids rather than to total amino acids (Khatum *et al.*, 1999).

The use of enzymes as a means of improving the feeding value of foodstuffs is increasing in importance. Their inclusion in poultry diets under commercial conditions is now commonplace and the benefits they have brought to the performance of poultry has been notable (Frigard *et al.,* 1994). In this study enzymes were used only as tools to demonstrate the presence in CLM and the possible effects exerted by some non-starch polysaccharides, particularly pectin, which had already been shown to be present in CLM by chemical methods. The action of pectinase significantly improved the overall amino acid digestibility of CLM, and that finding alone would represent an interesting topic of investigation when CLM is able to catch the attention of the animal feed industry as potential ingredient for inclusion in

poultry diets. Only the apparent amino acid digestibilities values of cystine and arginine in CLM were higher than the true digestibility values reported in alfalfa (NRC, 1994). However, apart from histidine, the contents (g/kg) of all the digestible essential amino acids in CLM were higher than those in alfalfa, because of their higher total concentrations in CLM.

6.2 Performance of broilers fed on different dietary concentrations of CLM

Birds fed on diets containing CLM showed a depressed performance, the extent of which depended on the nature of the diet and the availability of its energy and protein. However, perhaps a more important conclusion to be drawn from this experiment is that all birds fed on diets containing CLM were capable of producing satisfactory weight gains, even those fed on the diet containing 350 g/kg CLM. Searching agricultural systems or strategies, capable of exploiting the inclusion of CLM in diets in a complementary or holistic way rather than simply excluding "non profitable" diets, will be essential if nutritious animal protein products are to become more widely available to the poorer populations of the world.

In the current studies with chickens, despite the fact that CLM contains hydrogen cyanide and oxalic acid, nothing was uncovered that suggested that the consumption of CLM resulted in any toxic effects. In contrast, however, the inclusion of CLM in poultry diets did increase the size of some parts of the birds' guts. This was attributed to both the increased gut fermentation as consequence of higher fibre content of the diet and to the adaptive response to the physical presence of the same fibre. Similar findings to this have previously been reported in both chickens (Dänicke *et al.,* 2000; Iji *et al.,* 2001) and pigs (Freire *et al.,* 2000) and some suggestions have been put forward to explain these observations, *i.e.* large quantities of undigested material in the intestine provoking a trophic effect on the intestinal mucosa, variations in the rate of cell proliferation and increases in cell size and/or protein synthesis. The same adaptive responses to dietary fibre are known to occur in the young ruminant (at the pre-ruminant stage), when their gastrointestinal tracts are developing (Lasley, 1981). Cherry and Siegel (1978) suggested that the

long-term process of selecting chickens for maximum growth rate has altered the weight of the gastrointestinal tract, in particular some specific compartments. It will continue to be debated whether the enlargement of the gastrointestinal tract should be accepted as a natural adaptive response to the environment (*i.e.* fibre ingestion), and that birds should be allowed to make adjustments for the natural adaptation to the diet, or it corresponds to poor performance, and consequently it should be avoided as a less profitable characteristic of the poultry production system.

It is reasonable to assume that increased gut size will eventually lead to losses in the ratio carcass yield : body weight. However, if profitability as a criterion of performance is going to prevail, it should be taken into account in any cost-benefit analysis of diets containing CLM. The diet containing 250 g/kg CLM was able to increase body weight gain in broilers up to 49% greater than that achieved by feeding maize only. On the other hand, utilising only 0.85 and 0.75 of the conventional ingredients usually employed in commercial diets for chickens and complementing these with CLM, the body weight gains were 100% and 72%, respectively, of that obtained from the control diet (100% conventional ingredients). From this point of view, CLM might represent an alternative ingredient for poultry feeding. The most important task remaining is the calculation of the cost of obtaining CLM from different models of production and harvesting.

Several concerns have been raised in recent years about modern agricultural methods around the world. Those practices, such as the damage to the environment and the overproduction of food (mainly in developed countries), sometimes lead to reductions in the quality of food and can even constitute a potential health threat. The application of energy-intensive systems frequently using unfriendly animal production methods, have all drawn attention to the obligation to search for alternative agricultural systems (Lampkin, 1999).

In contrast, small farming systems, because of their small scale and the careful use of available resources, are usually more ecologically considerate, utilising practices that are less threatening to both human health and the environment. However, there is a lack of information on the resources required for those systems and on how best to utilise and preserve them. Branckaert *et al.* (2000) suggested the identification and use of the locally available feed resources to

formulate diets as balanced as possible as strategy for feeding the family poultry system. Chaya and many other vegetable resources in tropical areas are currently being under-utilised by farmers because information is required on how best to exploit their strengths in both human and animal nutrition. Particular attention should be paid to the fact that chaya plants, for example, are present in almost every rural backyard in the south-east of Mexico. In this context, chaya for animal feeding does not present us with the same dilemma over whether to feed to humans as cereals and most grains do. In order to alleviate the difficulties for sustainable and cheap food production, attempts at both encouraging chaya cultivation (in suitable areas) and continuing to search for how it can best be used in the context of animal feeding should be given high priority by governments and research institutions in particular.

Moreover, it should be remembered that most consumers would prefer to consume meat obtained from low input systems, employing forages and different natural resources. It is claimed to be more natural, sustainable, extensive, welfare-friendly and to use much less energy than meat obtained from animals eating diets manufactured from processed cereals and other industrial products and by-products.

6.3 General conclusions and proposals for further research

The chemical analysis of CLM showed that it had acceptable concentrations of essential amino acids, and higher than those in many other fibrous ingredients such as alfalfa. There was no evidence of toxicity from the oxalic acid or hydrogen cyanide present in the CLM throughout a 21-day feeding trial with broilers.

As a means of assessing the metabolisable energy content of CLM the TME procedure gave credible data. However it was less satisfactory in providing reasonable measures of the endogenous energy, nitrogen and amino acids losses in birds. Consequently, when the correction factors were applied to data generated from balance trials with birds fed on CLM highly variable results were obtained that pointed to the fact that endogenous losses were being affected by diverse factors beyond the control of the experiment.

The values for both the metabolisable and net energy contents of CLM for chickens were relatively low, as was to be expected from an ingredient high in fibre.

The apparent digestibility coefficients of the amino acids of diets containing up to 150 g/kg CLM did not differ significantly from those in the control diet.

The inclusion of enzymes, mainly pectinase, in diets containing CLM did result in a beneficial effect on amino acid digestibility of the CLM. Thus, the combination of enzymes and CLM might be a means whereby CLM could find an application in diets other than those used in small-farm systems such as the commercial feeding of poultry. Moreover, the essential amino acids in the CLM were shown to be highly available to chickens, more so than those in alfalfa, which is currently better known as a dietary ingredient.

From all the results found in the current study, it is possible to conclude that CLM is an alternative foodstuff suitable for inclusion in diets for broilers, principally in low-input systems. However its inclusion rate will depend not only on the characteristics of the production system but also on the objectives of production.

Some important further topics remain to be investigated on the use of CLM. From an agronomic point of view, it would be interesting to evaluate harvesting chaya at longer intervals than 12 weeks of regrowth in order to know if yields were improved, and whether its chemical composition, particularly the oxalic acid and hydrogen cyanide contents were affected. The possible benefits to be gained by associating the cultivation of chaya plants with other crops such as legumes would also be an interesting and worthwhile topic for research. The evaluation of an integrated agricultural system could improve the status of chaya as a species.

The establishment of a clearer understanding of the mechanisms involved in the physiological effects that the consumption of CLM exerts on endogenous losses would be a great help in developing a strategy for its use in diets for monogastric animals. It would also be useful to have a clearer picture of the possible interactions of the fibrous components of CLM with other nutrients in the diet, the microflora present in the gastrointestinal tract and the gastrointestinal tract itself. Greater knowledge of the limitations and advantages resulting from feeding chickens on diets containing CLM are likely to improve no only its utilisation in animal feeding,

but also would contribute to a better understanding of the nutritional physiology of digestion.

It is also important to establish which monogastric species is best able to cope with the fibre content of CLM and make best use of the nutrients it contains.

Over the past 3 or 4 decades there have been occasional flurries of interest in the possible extraction of protein components from leaves and forages. CLM is a protein-rich foodstuff, which undoubtedly would merit the attention of this important technology, and some investigations on this topic have already taken place.

The development of farming systems capable of using cheap and locally plentiful foodstuffs, where raw materials such as CLM would constitute important components in the diets for animals, nowadays represents a considerable challenge to the research communities in the tropical areas of developing countries.

Finally, the author acknowledges that hopefully there will be other work carried out in the future on this topic which will add to the small amount of knowledge generated in this thesis. He accepts responsibility and apologises for any oversights or omissions.

Chapter seven
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APPENDICES

Appendix 1. Laboratory Methods

Total Nitrogen. Crude Protein
Reference: AOAC, 1980

Reagents

a) Sulfuric acid. 93-98% H_2SO_4, N-free
b) Mercuric oxide or metallic mercury. HgO or Hg, reagent grade, N-free
c) Potassium sulfate (or anhydrous sodium sulfate). –Reagent grade, N-free.
d) Salicylic acid. –Reagent grade, N-free.
e) Sulfide or thiosulfate solution –Dissolve 40g com. K_2S in 1 Liter H_2O (Solution of 40g Na_2S or 80 g $Na_2S_2O_3.5H_2O$ in 1 liter may be used.)
f) Sodium Hydroxide –Pellets or solution, nitrate-free. For solution, dissolve about 450 g solid NaOH in H_2O, cool, and dilute to 1 liter
g) Zinc granules. –Reagent grade.
h) Zinc dust. –Impalpable powder.
i) Methyl red indicator. –Dissolve 1g methyl red in 200 ml alcohol.
j) Hydrochloric or sulfuric acid standard solution. –0.5 N, or 0.1 N when amount of N is small.
k) Sodium hydroxide standard solution 0.1 N

Apparatus

a) For digestion. Use Kjeldahl flasks of hard, moderately thick, well-annealed glass with total capacity 500-800 ml. Conduct digestion over heating device adjusted to bring 250 ml H_2O at 25°C to rolling boil in about 5 minutes or other time as specified in method. To test heaters, preheat 10 min if gas or 30 minutes if electricity. Add 3-4 boiling chips to prevent superheating.

b) For distillation. Use 500-800 ml Kjeldahl or other suitable flask, fitted with rubber stopper throughout which passes lower end of efficient scrubber bulb or trap to prevent mechanically, carryover of NaOH during distillation. Connect upper end of bulb tube to condenser tube by rubber tubing. Trap outlet of condenser in such way as to ensure complete absorption of NH_3 distilled over into acid in receiver.

%N = [(ml standard acid x normality acid) – (ml standard NaOH x normality NaOH)] x 1.4007/g sample

Crude Fat or Ether Extract

Reference: AOAC, 1980

Reagent

Anhydrous ether. –Wash commercial ether with 2 or 3 portions H_2O, add solid NaOH or KOH, and let stand until most of H_2O is abstracted from the ether. Decant into dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until H evolution ceases. Keep ether, thus dehydrated, over metallic Na in loosely stopper bottles.

Determination

(Large amounts H_2O-solvents components such as carbohydrates, urea, lactic acid, glycerol, and others may interfere with extraction of fat; if present, extract 2 gram sample on small paper in funnel with five 20 ml portions H_2O prior to drying for ether extraction.

Extract about 2 g sample, dried with anhydrous ether. Use thimble with porosity permitting rapid passage of ether. Extraction period may vary from 4 hr at condensation rate of 5-6 drops/sec to 16 hours at 2-3 drops/sec. Dry extract 30 min at 100°, cool and weigh.

Ash
Reference: AOAC, 1980

Weigh 3-5 g well mixed sample into shallow, relatively broad ashing dish that has been ignited, cooled in desiccator, and weighed soon after reaching room temp. Ignite in furnace at about 550° (dull red) until light gray ash results, or to constant weight. Cool in desiccator and weigh soon after reaching room temperature. Reignited CaO is satisfactory drying agent for desiccator.

Hydrocyanic Acid Formed by Hidrolysis of Glycosides. Alkaline Titration Method
Reference: AOAC, 1980

Place 10-20 g sample, ground to pass No. 20 sieve, in 800 ml Kjeldahl flask, add about 200 ml H_2O and let stand 2-4 hr. (Autolysis should be conducted with apparatus completely connected for distillation.) Steam distillation, collect 150-160 ml distillate in NaOH solution (0.5 g in 20 ml H_2O), and dilute to definite volume.

To 100 ml distillate (it is preferable to dilute to 250 ml titrate100 ml aliquot) add 8 ml 6N NH_4OH and 2 ml 5% Kl solution and titrate with 0.02N $AgNO_3$, using microburet. End point is faint but permanent turbidity and may be easily recognized, especially against black background.

1 ml 0.02 N $AgNO_3$ = 1.08 mg HCN. (Ag equivalent to 2 CN.)

Oxalic acid
Determination by permanganate titration
Reference: AOAC, 1980

Heat sample and blank (5 ml H_2SO_4 (1 + 9) in 50 ml centrifuge tube) prepared solutions in boiling H_2O bath. Titrate hot solution with 0.01 N $KMnO_4$ until first pink persists $\geq$30 sec.

mg Oxalic acid / 100 g product =

ml 0.01 N $KMnO_4$ x 1350 x (net weight + 100 g) / (weight slurry taken x net weight)

where 1350 = 0.45 (mg anhydrous oxalic acid equivalent to 1 ml 0.01 N K MnO_4) x [(30 / 20) x (500 / 25) (dilution factors)] x 100 (to convert to 100 g product)

Calcium

Reference: Roslin Nutrition, 2000

Apparatus:

Atomic Absorption Spectrophotometer

Procedure:

Preparation of reagents and standars

Standard: Take 10ml Calcium Standard Solution and make up to 200 ml with deionised water.

To 2, 4, 6, 8 and 10ml of the above solution in volumetric flasks, add 1ml Lanthanum Chloride and make up to 100ml. These represent concentrations of approx. 1, 2, 3, 4 and 5ppm Calcium respectively.

Sigma: To 1ml of the Sigma standard (5mg/decilitre), add 1ml Lanthanum Chloride in a 10ml test tube and make up to 10ml with deionised water. This should give the same absorbance as the 5ppm standard.

Determination

Using the solution obtained in Test Method RNL/TM/04, place an amount depending on the ash level determined by experience with 1ml Lanthanum Chloride in a 10ml test tube and make up to 10ml with deionised water.

Run the 5 standards above through the auto analytical system and record results.

Run the samples(diets in duplicate, bone single) through the auto analytical system and record results.

The result as Calcium (ppm) is computed by calculator from the absorbance of the sample compared against a linear regression line for the standards.

$$\text{Calcium(\%)} = \frac{\text{Ca(ppm)} \times 50 \times 10}{\text{Solution Volume(ml)} \times 10000}$$

Note:

The amount of solution used varies according to the ash level in order to ensure that an appropriate absorbance is obtained:
For 1.5% ash, use 1ml, for 6% ash, use 0.1ml and for 12%, use 0.05ml.

Phosphorus
Reference: Roslin Nutrition, 2000

Apparatus

Bran & Luebbe Colorimeter (TRAACS 800)

Reagents

Preparation of ammonium molybdate / ammonium metavanadate

Dissolve 2g of ammonium metavanadate (Analar) in 800 ml deionised water contained in a 2 litre glass beaker by heating and stirring. Dissolve 40g ammonium molybdate in 800 ml deionised water contained in a 1 litre glass beaker by heating and stirring. When both solutions have cooled to ambient temperature, with the aid of stirring mix together and add carefully 280ml conc. Nitric Acid (Analar). The solution is then poured into a 2 litre volumetric flask, diluted to the mark with deionised water and mixed well by shaking and inverting. The reagent can be stored in a dark container indefinitely.

Before use add 0.1ml Aerosol 22 to about 100ml reagent.

Preparation of standard solution

Weigh accurately 21.94g of Potassium dihydrogen Phosphate (previously dried by oven at 100 ± 1°C) into a 250ml glass beaker. Dissolve the salt in about 150ml water, add 3-4 ml of conc. HCL and then carefully transfer the solution to a 1 litre volumetric flask and mix well by shaking and inverting. 1ml of this solution contains 5mg Phosphorus. Carefully dilute this solution with deionised water to give a range of standard solutions with 5, 10, 15, 20, 25mg Phosphorus per 100ml.

Analysis and standardisation

Using the solution obtained in Test Method RNL/TM/04, load colorimeter with, in order,

the ammonium molybdate/ammonium metavanadate reagent

the 25, 20, 15, 10 and 5mg P standard samples

the actual samples

Thereafter follow the Bran & Luebbe Manual

Calculation of results

Absorbances are automatically printed out by the colorimeter calibrated against the standards.

Uric acid

Reference: Roslin Nutrition, 2000

Apparatus

Bran & Luebbe Colorimeter (TRAACS 800)

Whirlymixer

Procedure

Preparation of standards and reagents

Stock: 200mg Uric Acid/100ml made up with 0.4% Li_2CO_3.

Take 0.25, 0.5, 1, 1.5 and 2.0ml stock to 100ml to give 0.5, 1.0, 2.0, 3.0 and 4.0mg Uric Acid/100ml.

Phosphotungstate: BDH dodeca Tungstophosphoric Acid diluted 1:1 with Deionised water.

Sodium Carbonate/Urea reagent: Take 150g of sodium carbonate and 200g urea and dissolve in deionised water and make up to 1 litre.

Sample extraction

Weigh 0.100g of sample into a 50ml centrifuge tube.

Add 10ml of 0.4% Li_2CO_3 solution.

Whirlymix for 10 seconds, then centrifuge at 6000rpm for 10 minutes.Decant the supernatant solution into a 100ml volumetric flask.

Add a further 10ml of LiCO3 to the tube, mix and spin as above and decant the supernatant liquor into the flask.

Repeat the procedure once more.

Finally make up to 100ml with deionised water.

Sample analysis and standardisation

Dip the relevant machine tubes into beakers containing the phosphotungstate and carbonate/urea reagents. The machine automatically uses these when needed.

Load the 4.0-0.25mg standards (2x 4.0 standard) in that order followed by the single actual sample.

Thereafter follow the Bran & Luebbe colorimeter (TRAACS 800) Manual

Calculation of results

The absorbances are automatically printed out by the colorimeter calibrated against the standards.

Nitrogen/ Crude protein

Reference: Roslin Nutrition, 2000

Apparatus

Leco Analyser FP-428/328

Procedure

Standardisation of Leco

Weigh 0.15-0.20g of EDTA and treat as faeces sample (see following). The result should be in range 9.51-9.63%. If outside this range, recalibrate the equipment according to the standard procedure in Leco Manual FP-428/328.

Analysis of sample

The appropriate weight of sample-0.2-0.3g, accurate to 0.0001g, is added to a tared tin foil cup which is then folded over to retain and exclude air from the sample.

Weigh and record. Enter ID and weight of sample. Press start. Machine will give either % nitrogen or % crude protein as selected.

Titanium dioxide

Reference: Roslin Nutrition, 2000

Apparatus

Colorimeter (TRAACS 800)

Hotplate

Muffle Furnace

Procedure

Preparation of reagents

Sulphuric Acid AR s.g. 1.84.

Sodium Sulphate Powder AR

Anti-bumping granules

Hydrogen Peroxide AR 30% w/v.

Titanium Dioxide AR

10% H_2SO_4: To 900ml of distilled water in beaker under cooling, add 100ml conc. Sulphuric Acid with continuous stirring.

4% H_2O_2: To 480ml of distilled water in beaker, add 20ml of 30% w/v H_2O_2 and mix.

Preparation of titanium dioxide standard solution

Accurately weigh 50+/-1mg TiO_2 (previously dried overnight at 100C) and transfer to 250ml glass beaker. Add about 5g Na_2SO_4, 50ml conc. H_2SO_4 and some anti-bumping granules. Cover beaker with a watch glass and heat to boiling on a hot plate to dissolve TiO_2. After 15 minutes, remove from hot plate, cool to ambient and cautiously add about 200ml distilled water. Cool, transfer with washings to a 500ml

volumetric flask and make up to volume with distilled water. This gives a final solution with a concentration of 10% H_2SO_4.

Take 20, 16, 12, 8, 4 and 2ml of the solution and make up to 20ml, where possible, with 10% H_2SO4 giving respectively concentrations of 10, 8, 6, 4, 2 and 1mg TiO_2/100ml.

Preparation of sample

For diets of 0.2% TiO_2 and subsequent fecal samples, weigh accurately about 3g diet (duplicate samples) or 1g fecal (single samples) and transfer to 100ml glass beakers. Ash overnight at 550C and to resultant white ash add about 1g Na_2SO_4 and 10ml conc. H_2SO_4. Add some anti-bumping granules, cover with a watch-glass and heat to boiling on a hot-plate until solution clarifies(usually within 15-30 minutes). Remove beaker from hot-plate, cool to ambient and cautiously add about 40ml distilled water. Transfer with washings to a 100ml volumetric flask and dilute to volume with distilled water. Transfer back to original beaker. This gives a final solution of 10% H_2SO_4.

Determination of titanium dioxide

The H_2O_2 reagent is fed continuously through the colorimeter.

Samples are added to the colorimeter in order

Standard TiO2 samples of concentrations 10 (2x), 8, 6, 4, 2 and 1mg TiO2/100ml.

Test sample

Thereafter follow the Bran & Luebbe Manual.

Calculation of results

The machine calculates and registers the % Titanium Dioxide automatically using linear regression analysis.

Gross energy

Reference: Roslin Nutrition, 2000

Apparatus

Bomb Calorimeter-Parr 1261

Temperature controlled water bath

Automated filling system

Sample pelleter

Procedure

Preparation of reagents

Standard: Dissolve 3.7600+/-0.0001g of Na_2CO_3 in 1 litre deionised water. 1ml is equivalent to 1 calorie.

Methyl Orange Indicator: Dissolve 0.0200+/-0.0001g of methyl orange in 100ml deionised water containing 0.6ml of 0.1N HCl.

Benzoic Acid pellets.

Fuse Wire: 23 calories "length".

Preparation of samples

Take approximately 1g of sample and pellet using the Parr pellet press and place into a stainless steel crucible of known weight (accurate to 4 decimal places). Weigh again to 4 decimal places to obtain weight of pellets. Use duplicate samples.

Standardisation of calorimeter

The energy equivalent value for each bomb is calculated using standard pellets of benzoic acid. The mean of 10 firings (as described below) is used. The allowable standard deviation for the equivalent energy value is 0.15%.

Determination of energy content

In dynamic mode, the calorimeter uses a sophisticated curve matching technique to compare the temperature rise with a known thermal curve to extrapolate the final temperature rise. The method is described in the Parr Bomb Calorimeter Operating Instructions.

Fill the water bucket, using the automated filling system, with water at 31°C. Insert into the bomb 10cm (23 calories) of pre-measured fuse wire to each of the two electrodes. Insert crucible with loop of fuse wire touching sample. Care must be taken to ensure the wire does not touch the sides of the crucible otherwise an erroneous result will be obtained. Place approx. 1ml of water in the base of the bomb, place sample in bomb, screw down tightly and close release valve. 20psi of oxygen is delivered to the bomb when the FILL button on the console is pressed. The bomb is placed into a pre-measured water bucket. The sample ID, calorimeter ID and sample weight is entered into the control unit.

The machine automatically calculates the (PRELIMINARY) gross energy of the samples by measuring the temperature rise due to combustion and incorporating the standard values.

The FINAL value is obtained using the following procedure.

Open control unit cover and remove bomb and bucket. Remove bomb from bucket and open valve SLOWLY to release residual gas pressure. Once pressure released and reading atmospheric, unscrew cap, wash all interior surfaces with distilled water and collect washings in a beaker. Titrate the bomb washings with the standard Na_2CO_3 solution using methyl orange indicator and record number of ml to neutralise. Remove all unburned pieces of fuse wire from the electrodes, straighten, measure and record length in cm. Subtract the length from 23 and record.

Re-enter data into the control unit using REPORT mode. The machine automatically recalculates data to give the FINAL value for Gross Energy.

Amino acid analyses by HPLC
Reference: Roslin Nutrition, 2000

Apparatus

Ion Exchange Chromatograph

Heating Block

Hydrolysis Tubes

Ice Bath

Ultrasonic Bath

Rotary Evaporator Tube

Procedure

Preparation of reagents and standards

Formic Acid-88% : Carefully add 880ml formic acid to about 100ml deionised water. Dilute to volume with deionised water in a 1000ml volumetric flask.

Performic Acid : Dissolve 0.25g phenol in 45ml 88% formic acid and add 5ml hydrogen peroxide(100 vol.). Allow to stand for 1 hour before using.

Hydrochloric Acid-6N : Carefully, with stirring, add 516ml concentrated hydrochloric acid to about 400ml deionised water. Cool and dilute to volume with deionised water in a 1000ml volumetric flask.

Hydrochloric Acid-7.5N : Carefully, with stirring, add 645ml concentrated hydrochloric acid to about 300ml deionised water. Cool and dilute to volume with deionised water in a 1000ml volumetric flask.

Sodium Hydroxide-4.2N: Dissolve 16.8g sodium hydroxide in about 50ml deionised water. Cool and dilute to volume with deionised water in a 100ml volumetric flask.

Acetic Acid-25mN: Add 1.45ml glacial acetic acid to about 500ml deionised water. dilute to volume with deionised water in a 1000ml volumetric flask.

Standards : Store at 0-5C.

Amino Acid Mixture-2.5mM: Purchased from Sigma Chemicals-Cat. No. AA-S-18.
Beckman Na-S Sample Dilution Buffer.

Cysteic Acid-4.0mM: Dissolve 169.2mg cysteic acid in about 50ml 0.01M HCl and make up to 250ml with 0.01M HCl in a volumetric flask.

Methionine Sulphone-4.0mM: Dissolve 181.2mg methionine sulphone in about 50ml M HCl and make up to 250ml with 0.01M HCl in a volumetric flask.

Calibration Internal Standard, nor-leucine 4.0mM: Dissolve 131.2mg nor-leucine in about 50ml 0.01M HCl. Dilute to volume with 0.01m HCl in a 250ml volumetric flask.

Analysis Internal Standard, nor-leucine 0.625mM : Dissolve 20.5mg nor-leucine in about 50ml 0.01M HCl. Dilute to volume with 0.01M HCl in a 250ml volumetric flask. 1ml of this is dried down with the sample aliquot and finally dissolved in 2.5ml of sample dilution buffer. The nor-leucine 0.625mM then gives a final concentration of 0.25mM.

Tryptophan-4.0mM: Dissolve 204.2mg tryptophan in about 50ml 0.01M HCl and make up to 250ml with 0.01M HCl in a volumetric flask.

Preparation of sample

All samples should be analysed in duplicate.

Hydrolysis

WARNING: The hydrochloric acid and sodium hydroxide solutions used in this hydrolysis procedure are corrosive. Protective clothing, gloves and eye protection MUST be worn at all times. The hydrolysis MUST be carried out in a fume cupboard.

Acid hydrolysis

Use this procedure to determine all amino acids except Methionine, Cysteine and Tryptophan.

Weigh accurately about 50-75mg sample into a screw capped glass hydrolysis tube. Add 5ml HCl (6N), placing the tube in an ultrasonic bath for 15 minutes in order to mix the contents. Flush the tube with oxygen-free nitrogen for 1 minute and seal. Hydrolyse for 24 hours by placing the tube in a heating block previously heated to

110+/-1C. After hydrolysis, remove the tube from the heating block and cool to room temperature. Quantitatively transfer the contents to a 50ml volumetric flask and dilute to volume with deionised water.

Acid hydrolysis with performic acid. Use this procedure to determine Methionine as Methionine Sulphone and Cysteine as Cysteic Acid.

Weigh accurately about 50-75mg sample into a screw capped glass hydrolysis tube and place the tube in an ice bath. Add 1ml COLD performic acid to the tube and place in an ultrasonic bath for 15 minutes in order to mix the contents. Cap and stand overnight at 0-5C. While the tube is still cold, carefully add 0.25g sodium metabisulphate (beware of frothing which may cause loss of sample). Mix contents immediately by vortexing. Add 4ml HCl(7.5N) and place the tube in a sonic bath for 23 hours.

Place the tube, unsealed, into a heating block previously heated to 110±1°C. After 1 hour, seal the tube and hydrolyse for 23 hours.

WARNING: A high pressure may develop in the tube causing the top to split open and the sample to be lost. It may be necessary to carefully release the pressure in the tube by carefully unsealing the tube about 3 hours after the tube is placed on the heating block. this should only be carried out with caution while wearing rubber gloves and a face shield with the tube pointed away from the body. This should not happen if the tubes are left unsealed for the first 2 hours.

After hydrolysis, remove the tube from the heating block and allow to cool to room temperature. Carefully open tube(see WARNING above), quantitatively transfer contents to a 50ml volumetric flask and dilute to volume with deionised water.

Alkaline hydrolysis

Use this procedure to determine Tryptophan.

Weigh accurately about 50-75mg sample into a glass hydrolysis tube. If sample is known or suspected to contain <20% starch, add 25mg hydrolysed potato starch or Dextrin. Alternatively it is acceptable to always add Dextrin. Add 5ml fresh Sodium Hydroxide(4.2N) and 1 drop octan-1-ol(anti-foam agent) and place the tube in an

ultrasonic bath for 15 minutes in order to mix the contents. Flush the tube with oxygen-free nitrogen for 1 minute and seal. Hydrolyse for 22 hours by placing tube on a heating block previously heated to 110+/-1C.

After hydrolysis, remove the tube from the heating block, cool to room temperature, quantitatively transfer contents to a 50ml volumetric flask containing 5ml HCl(6N) and, after allowing contents to cool to ambient, dilute to volume with deionised water.

Final preparation

Filter hydrolysate. Place an aliquot of filtrate(see Table below) and 1ml Analysis Internal Standard Solution into a rotary evaporator tube and dry at about 40C under vacuum until visually dry. Dissolve the residue in 2.5ml Beckman Na-S Sample Dilution Buffer. Store at 0-5C until analysis.

<u>Table of Volumes and Dilution Factors</u>

Crude Protein (%)	**Volume taken to dryness (ml)**	**Volume of sample dilution buffer (ml)**
<15	5	2.5
15-30	2.5	2.5
31-60	1.25	2.5
>60	0.5	2.5

A dilution factor can be calculated from the following formula. This will normally be done in the Excell spreadsheet:

$$\text{Dilution factor} = \frac{ab}{625c}$$

where:

a = total hydrolysate volume(50ml)

b = amount that the dried sample is diluted to(2.5ml)

c = amount of hydrolysate evaporated to dryness(see above)

625 = a factor converting the result to g/16g N

Calibration of HPLC equipment

Standards are sucked into sample coils and placed in the HPLC carousel.

Determination of amino acid

Samples are similarly loaded into the HPLC carousel.

The initiation, running and production of results are obtained by following the instructions in the Beckman 6300 System Gold Operation Manual.

Calculation of results

Chromatograph results are manually transferred from graphs to a spreadsheet in the computer where a special programme calculates(taking account of the standards results), displays and retains the amino acid concentration.

References

Hydrolysis: AOAC (1984) 43.263-43.264

Chromatography: Roth, M (1971) Anal.Chem. 43 880-882

Jones, B.N., Paabo, S., and Stein, S. (1981) J. Liq. Chrom. 4 565-586.

Alltech Data Sheet D28062

Proline Conversion: Cooper, J.H.T. et al(1984) J. Chromatography 285 484-489.

Beckman System 6300 Gold Operation Manual.

Neutral Detergent Fiber (NDF)

Reference: Ankom Technology, 1997

Apparatus

a) Digestion apparatus – ANKOM$^{200/220}$ FIBER ANALYSER
b) Filtration device – ANKOM TECHNOLOGY – F57 FILTER BAGS
c) Impulse bag sealer – Requires high enough temperature to melt and seal polymer in filter bags (ANKOM TECHNOLOGY – 1915/1920).
d) Desiccator

Reagents

a) *Neutral Detergent Solution (*ND) Add 30.0 g sodium lauryl sulfate; 18.61 g ethylenediaminetetraacetic disodium salt, dihydrate; 6.81 g sodium tetraborate decahydrate; 4.56 grams sodium phosphate dibasic, anhydrous; and 10.0 ml triethylene glycol, in 1 L distilled H_2O (ANKOM *TECHNOLOGY*, premixed chemical solution – FND20 or FND20C). Agitate and heat to facilitate solubility. Check pH range to 6.9 to 7.1.

b) *Alpha-amylase.* Heat-stable bacterial alpha-amylase:activity = 17,400 Liquefon Units / ml (ANKOM *TECHNOLOGY* – FAA).). One Liquefon Unit is the measure of digestion time required to produce a color change with iodine solution indicating a definite stage of dextrinization of starch substrate under specific conditions.

c) *Sodium sulfite* – Na_2SO_3, anhydrous (ANKOM *TECHNOLOGY* – FSS).

d) *Acetone* – Use grade that is free from color and leaves no residue upon evaporation (ANKOM *TECHNOLOGY* – FACE).

Safety Precautions

a) Acetone is highly flammable. Use fume hood when handling acetone and avoid inhaling or contact with skin. Make sure bags are completely dry and that all the acetone has evaporated before placing in oven.

b) Sodium lauryl sulfate will irritate the mucous membranes. A dust mask and gloves should be when handling this chemical.

Procedure

a) Prepare Sample

1) Weight Filter Bag (W_1) record weight and tare balance.
2) Weight 0.5g (± 0.05 g) of air-dried sample (W_2), ground to pass through a 1mm screen, directly into filter bag. Weight one blank bag and include in digestion to determine blank bag correction (C_1).
3) Seal the bags closed within 0.5cm from the open edge using the heat sealer.

4) Spread sample uniformly inside the filter bag by shaking and lightly flicking the bag to eliminate clumping.

5) A maximum of 24 bags may be placed in the bag suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. The weight is placed on top of the empty 9th tray to keep the bag suspender submerged.

b) When processing 24 sample bags add 1900 – 2000 ml of Neutral Detergent solution into ANKOM Fiber Analyzer vessel. If processing less than 20 bags add 100 ml/bag of detergent solution (minimum of 1500 ml (ensure Bag Suspender is covered)). Add 20 g (0.5 g/50 ml of ND solution) of sodium sulfite to the solution in the vessel and 4.0 ml of heat stable alpha-amylase.

c) Place bag suspender with samples into the solution in vessel. Turn *Agitate* and *Heat* ON and confirm that Bag Suspender is agitating properly. Set timer for 75 minutes and push *Start*. Close and seal lid of vessel.

d) After 75 minutes (timer will beep) turn *Agitate* and *Heat* OFF, open the drain valve and exhaust hot solution before opening lid. Warning: The solution in vessel is under pressure. The valve should be opened first to remove pressure before lid can be opened. Ensure exhaust hose is securely positioned for safe disposal of effluent.

e) After the solution has been exhausted close valve and open the lid. Add approximately 2000 ml of hot (90°-100°C) H_2O and 4.0 ml of alpha-amylase to the first and second rinses. Lower lid but do not tighten. Turn *Agitate* ON and leave *Heat* OFF. Each rinse should last 3-5 minutes. Exhaust water and repeat rinse two more times (total of three rinses).

f) Remove filter bags from bag suspender and gently press out excess water. Place in beaker and soak in acetone. Allow bags to soak 3 minutes then remove and lightly press out excess acetone.

g) Spread bags out and allow acetone to evaporate. Complete drying in oven at 105°C for at least 2 hours. Warning: Do not place bags in the oven until acetone has completely evaporated. Longer drying period may be required depending on oven and frequency of sample introduction into the oven.

Remove bags from oven, place directly into MoistureStop weigh pouch and flatten to remove air. Cool to ambient temperature and weight bags (W_3).

E. Calculate percent aNDF (as-is basis) = $\frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2}$

$$\text{aNDF (DM basis):} = \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2 \times DM}$$

$$\text{aNDF}_{OM} \text{ (DM basis):} = \frac{(W_4 - (W_1 \times C_2)) \times 100}{W_2 \times DM}$$

Where: W_1 = Bag tare weight

W_2 = Sample weight

W_3 = Weight after extraction process

W_4 = Weight of Organic Matter (OM) (loss of weight on ignition of bag fiber residue

C_1 = Blank bag correction (final oven-dried weight/original blank bag weight)

C_2 = Ash corrected blank bag (loss of weight on ignition of blank bag/original blank bag weight)

Acid Detergent Fiber (ADF)

Reference: Ankom Technology, 1997

Apparatus

a) Digestion apparatus – ANKOM$^{200/220}$ FIBER ANALYZER
b) Filtration device – ANKOM TECHNOLOGY – F57 FILTER BAGS
c) Impulse bag sealer – Requires high enough temperature to melt and seal polymer in filter bags. (ANKOM TECHNOLOGY –1915/1920).

d) Desiccator

Reagents

a) *Acid Detergent Solution* (Add 20 g cetyl trimethylammonium bromide (CTAB) to 1 L 1.00 N H_2SO_4 previously standardized. Agitate and heat to aid solution.

b) *Acetone* – Use grade that is free from color and leaves no residue upon evaporation

Safety precautions

a) Acetone is highly flammable. Use fume hood when handling acetone and avoid inhaling or contact with skin. Make sure bags are completely dry and that all the acetone has evaporated before placing in oven.

b) Rubber gloves and face shield should be worn when handling sulfuric acid. Always add sulfuric acid to water. If acid contacts skin wash with copious amounts of water.

c) CTAB will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.

Procedure

a) Prepare Sample

1) Weight Filter Bag (W_1) record weight and tare balance.

2) Weight 0.5g (±0.05 g) of air-dried sample (W_2), ground to pass through a 1mm screen, directly into filter bag. Weight one blank bag and include in digestion to determine blank bag correction (C_1).

3) Seal the bags closed within 0.5cm from the open edge using the heat sealer.

4) Spread sample uniformly inside the filter bag by shaking and lightly flicking the bag to eliminate clumping.

5) A maximum of 24 bags may be placed in the bag suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each

level rotated 120 degrees. The weight is placed on top of the empty 9th tray to keep the bag suspender submerged.

b) When processing 24 sample bags add 1900 – 2000 ml of ambient temperature Acid Detergent solution into ANKOM Fiber Analyzer vessel. If processing less than 20 bags add 100 ml/bag of detergent solution (minimum of 1500 ml (ensure Bag Suspender is covered)).
c) Place bag suspender with samples into the solution in vessel. Turn *Agitate* and *Heat* ON and confirm that Bag Suspender is agitating properly. Set timer for 60 minutes and push *Start*. Close and seal lid of vessel.
d) After 60 minutes (timer will beep) turn *Agitate* and *Heat* OFF, open the drain valve and exhaust hot solution before opening lid. Warning: The solution in vessel is under pressure. The valve should be opened first to remove pressure before lid can be opened. Ensure exhaust hose is securely positioned for safe disposal of effluent.
e) After the solution has been exhausted close valve and open the lid. Add approximately 2000 ml of hot (90º-100ºC) H_2O and lower lid but do not tighten. Turn *Agitate* ON and leave *Heat* OFF and rinse for 3-5 minutes. Exhaust water and repeat rinse to for a total of three times or until water is a neutral pH.
f) After final rinse remove filter bags from bag suspender and gently press out excess water. Place in beaker and cover with acetone. Allow bags to soak 3 minutes, then remove and lightly press out excess acetone.
g) Spread bags out and allow acetone to evaporate. Complete drying in oven at 105ºC for at least 2 hours. Warning: Do not place bags in the oven until acetone has completely evaporated**.** Longer drying period may be required depending on oven and frequency of sample introduction. Remove bags from oven, place directly into *MoistureStop* weigh pouch and flatten pouch to remove air. Cool to ambient temperature and weight bags (W_3).

Calculate percent ADF (as – is basis): $= \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2}$

$$\text{ADF (DM basis)} = \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2 \times DM}$$

$$\text{ADF}_{OM} \text{ (DM basis)} = \frac{(W_4 - (W_1 \times C_2)) \times 100}{W_2 \times DM}$$

Where: W_1 = Bag tare weight

W_2 = Sample weight

W_3 = Weight after extraction process

W_4 = Weight of Organic Matter (OM) (Loss of weight on ignition of bag and fiber residue)

C_1 = Blank bag correction (final oven-dried weight/original blank bag weight)

C_2 = Ash corrected blank bag (loss of weight on ignition of bag /original blank bag)

Determination of Uronic Acids by Colorimetry

Reference: Theander et al., 1995

a) *Sample preparation.*- In 20 ml glass tube, mix 250 µL duplicate sample hydrolysate (1 h at 125° C) with 250 µL boric acid-sodium chloride solution 2 g NaCl and 3 g H_3BO_3 in 100 ml H_2O). Prepare blank by mixing 250 µL 0.36M H_2SO_4 with 250µL boric acid-sodium chloride solution. (Note: If absorbance reading is too high, repeat analysis from this point, diluting hydrolysates with 0.36M H_2SO_4.)
b) *Hydrolysis.*- Add 4.0 ml 18M H_2SO_4 to tubes and mix thoroughly. Cover tubes with foil and place in 70°C water bath for 40 min. Cool tubes to room temperature in water bath and add 200 µL dimethylphenol solution (1 mg/ml). Thoroughly mix over 5 minutes interval by using Vortex mixer.
c) *Determination.*- Measure sample absorbance, A, at 400 and 450 nm against blank solution 10-25 min after addition of dimethylphenol. Subtract A at 400 nm to correct for interference of hexoses.
d) *Preparation of calibration curve.* Place two 10 ml aliquots of each galacturonic acid monohydrate standard solution, (20, 40, 70, 110, and 160 mg/100 ml galacturonic acid monohydrate stock solutions in H_2O) into separate 250 ml tall beakers and add 74.0 ml H_2O and 3.0 ml 12M H_2SO_4. Autoclave duplicate beakers separately for 1 h at 125°C. Dilute hydrolysate with H_2O to 100 ml at room temperature. Measure A of hydrolysates as in (**a**)-(**c**).

Calculations

Calculate uronic acid content from calibration curve and express as polysaccharide residues (anhydro-sugars) by multiplying by 0.830.

Calculate content (%) of uronic acid residues, UA, in sample, given as polysaccharide residues, as follows:

$$UA = \frac{W_u \times F_u \times F_C}{S}$$

Where S = weight (dry matter, mg) of original sample; W_u = weight (mg) of galacturonic acid monohydrate/100 ml hydrolysate, obtained from calibration curve; F_u = factor for recalculation of galacturonic acid monohydrate to polysaccharide residue (.0915 x 0.907=0.830); and F_c = compensation factor to adjust for greater degradation of free galacturonic acid as opposed to that of polygalacturonate, under conditions of uric acid calibration. (Note: F_c = 0.81 is recommended for use. Analysts should be aware that variations in experimental conditions and structure of various uronic acid-containing polysaccharides may influence the factor value. Therefore, it may be necessary to secure standardized pectin sample and determine the compensation factor.)

Measurement of constituent sugars of non-starch polysaccharides (NSP) by Gas-liquid chromatography (GLC)

Reference: Englyst et al., 1994

Reagents

Ammonia solution-soludium tetrahydroborate solution. Prepare 6 mol/dm^3 ammonia solution containing 200 mg/cm^3 of sodium tetrahydroborate ($NaBH_4$). Prepare immediately before use.

Bromophenol Blue solution, 0.4 g/dm^3. BDH.

GLC *internal standard solution,* 1 ml/cm^3. Weight 500 mg of allose (dried to constant mass under reduced pressure with phosphorus pentoxide) to the nearest 1 mg. Dilute to 500 cm^3 with 50% satured benzoic acid to give a 1 mg/cm^3 solution. The solution is stable at room temperature for several months.

GLC *stock sugar mixture.* Weight (all sugars dried to constant mass under reduced pressure with phosphorus pentoxide), to the nearest 1 mg, 0.52 g of rhamnose, 0.48 g of fucose, 4.3 g of arabinose, 4.45 g of xylose, 2.3 g of mannose, 2.82 g of galactose and 9.4 g of glucose. Place them in a 1 dm^3 calibrated flask and dilute to volume with 50% saturated benzoic acid. The solution is stable at room temperature for several months.

Sample preparation

All samples should be finely divided (to pass a 0.5 mm mesh) so that representative sub-samples may be taken. Foods with a low water content (<10g of sample) may be milled, and foods with a higher water content may be homogenized wet or milled after freeze-drying. Analysis of three sub-samples, A, B and C, allows separate values to be obtained directly for total NSP, insoluble NSP and cellulose, respectively. Soluble NSP is determined as the difference between total and insoluble NSP.

Weight, to the nearest 1 mg, between 50 and 1000 mg depending on the water and NSP content of the sample (to give not more than 300 mg of dry matter; *e.g.*, 300 mg are adequate for most dried foods but smaller amounts should be used for bran and purified fibre preparations) into 50-60 cm^3 screw-topped glass tubes. Add 300 (± 20) mg of acid-washed sand and approximately 15 glass balls to each. The sample must be dry (85-100 g of dry matter per 100 g of sample) and contains less than 10 g of fat per 100 g of sample. (It is recommended that all analyses be carried out in duplicate.) Add 40 cm^3 of acetone, cap the tubes and mix several times over a 30 minutes period. Centrifuge at 1000*g* for 10 minutes to obtain a clear supernatant and remove by aspiration as much of the supernatant liquid as possible without disturbing the residue. Vortex mix vigorously to ensure that the residue is dispersed thinly around the bottom 5 cm of the tube. Place the rack of tubes in a pan of water at 75°C in fume-cupboard. Remove the tubes singly and vortex mix vigorously at frequent intervals until the tubes and residues are dry.

Precipitation and washing of the residue for measurement of total NSP

Only sample portion A is given this treatment.

Cool the samples by placing in ice-water. Add 0.15 cm^3 of 5 mol/dm^3 hydrochloric acid and vortex mix thoroughly two or three times during a 5 min period with samples being replaced in the ice-water. Add 40 cm^3 of acidified absolute ethanol and mix well by repeated inversion, then leave in ice-water 30 min. Centrifuge at 1500*g* for 10 min to obtain a clear supernatant liquid. Remove by decanting or by

aspiration as much of the supernatant liquid as possible, without disturbing the residue, and discard it.

Add approximately 10 cm^3 of acidified 85% v/v ethanol to the residue and vortex mix. Dilute to 50 cm^3 with acidified 85% v/v ethanol and mix thoroughly by repeated inversion. Centrifuge and remove the supernatant liquid as above. Repeat this stage using 50 cm^3 of absolute ethanol.

Add 30 cm^3 of acetone to the residue and vortex mix thoroughly to form a suspension. Centrifuge and remove the supernatant liquid.

Vortex mix vigorously to ensure that the residue is dispersed thinly around the bottom 5 cm of the tubes. Place the rack of tubes in a pan of water at 75°C in a fume-cupboard. Remove the tubes singly and vortex mix vigorously at frequent intervals, to ensure that the residue in each tube is finely divided, until the tube and residue are dry. Place the rack of tubes in a fan oven at 80 °C for 10 min to remove any trace of acetone. It is essential that the residues and tubes are completely free of acetone.

Extraction and washing of the residue for measurement of insoluble NSP

Only sample portion B is given this treatment.

Add 40 cm^3 of sodium phosphate buffer. Place the capped tubes in a boiling water-bath for 30 minutes. Mix continuously or a minimum of three times during this period. Remove the tubes and equilibrate to room temperature in water. Centrifuge and remove the supernatant liquid.

Add approximately 10 cm^3 of water and vortex mix. Dilute to approximately 50 cm^3 with water and mix well by repeated inversion. Centrifuge and remove the supernatant liquid as above. Repeat this stage using 50 cm^3 of absolute ethanol.

Measurement of neutral NSP constituents by GLC

To prepare the standard sugar mixture, mix 1.0 cm^3 of the GLC stock sugar solution and 5 cm^3 of 2.4 mol/dm^3 sulfuric acid. Treat 2 x 1.0 cm^3 of this standard sugar mixture for calibration of GLC.

Prepare the alditol acetate derivatives for chromatography as follows. Add 0.50 cm^3 of internal standard (1 mg/cm^3 allose) to 1.0 cm^3 and to 2 x 1 cm^3 of the standard sugar mixture; vortex mix. Place the tubes in ice-water, add 4.0cm^3 of 12

mol/dm^3 ammonia solution and vortex mix. Test that the solution is alkaline (add a little more ammonia solution if necessary but replace the ammonia solution if more than 0.1 cm^3 extra is required), then add approximately 5 mm^3 of the antifoam agent octan-2-ol and 0.1 cm^3 of the ammonia solution-sodium tetrahydroborate solution and vortex mix. Leave the tubes in a heating block or in a water-bath at 40ºC for 30 min, then remove , add 0.2 cm^3 of glacial acetic acid, and mix again. Transfer 0.5 cm^3 to 30 cm^3 glass tubes, add 0.5 cm^3 of 1-methylimidazole to each. Add 5 cm^3 of acetic anhydride and vortex mix immediately. Leave the tubes for 10 min for the reaction to proceed (the reaction is exothermic and the tubes will become hot). Add 0.9cm^3 of absolute ethanol, vortex mix and leave for 5 minutes. Add 10 cm^3 of water, vortex mix and leave for 5 min. Add 0.5 cm^3 of Bromophenol Blue solution. Place the tubes in ice-water and add 5 cm^3 of 7.5 mol/dm^3 potassium hydroxide; a few minutes later add a further 5 cm^3 of 7.5 mol/dm^3 potassium hydroxide, cap the tubes and mix by inversion. Leave until the separation into two phases is complete (10-15 min) or centrifuge for a few minutes. Draw part of the upper phase into the tip of an automatic pipette; if any of the blue phase is included, allow it to separate, then run it out of the tip before transferring a portion of the upper phase alone into a small (autoinjector) vial.

Carry out conventional GLC measurement of the neutral sugars. At the beginning of each bath of analyses, equilibrate with the isothermal elution conditions for at least 1 h. Carry out several calibration runs to check that the response factors are reproducible. Inject 0.5-1 mm^3 of the alditol acetate derivatives.

GLC conditions

The following conditions are used: injector temperature, 275ºC; column temperature, 200ºC; detector temperature, 275ºC; carrier gas, nitrogen; and flow rate, 8 cm^3/min. Under these conditions, a chromatograph fitted with a flame-ionization detector and, preferably, autoinjector and computing integrator, using a Supelco SP-2380 wide-bore capillary column (30 m x 0.53 mm i.d.) at 210ºC will allow accurate determination of the individual sugars in the standard sugar mixture within 8 minutes.

Calculation of neutral sugars

The amount of individual sugars (expressed as grams of polysaccharide per 100 g of sample) is calculated as

$$\text{Amount} = \frac{A_T M_I R_F \times 100}{A_I M_T} \times 0.89$$

Where A_T and A_I are the peak areas of the sample and the internal standard, respectively, M_I is the mass (in mg; here 15 mg: total hydrolysate 30 cm^3 x 0.5 mg of allose) of the internal standard, M_T is the mass (in mg) of the sample, R_F is the response factor for individual sugars obtained from the calibration run with the sugar mixture and internal standard (allose) treated in parallel with the samples and 0.89 is the factor for converting experimentally determined values for monosaccharides to polysaccharides. All the calculations may be performed with a computing integrator.

Appendix 2. Analyses of Variance

Experiment 1.

Variables
Age; HCN; Oxalic Acid; ASH; WATER; FAT; LIGNIN; CRUDE PROTEIN; NDF; ADF

Age	HCN	Oxalic Acid	ASH	WATER	FAT	LIGNIN	CRUDE PROTEIN	NDF	ADF
4	1.34	9.9	100.9	809.5	70.4	33.6	290.9	185.9	128.2
4	1.15	8.3	104.1	815.2	68.5	33.4	271.8	171.4	135.5
4	1.83	7.3	112.3	793.1	81.2	27.7	294.0	179.9	128.7
4	1.28	6.6	98.2	811.3	83.7	29.3	275.0	163.9	118.1
4	1.21	8.6	89.8	797.0	68.2	21.2	274.1	164.9	114.6
4	1.53	8.6	104.2	768.0	61.3	38.6	264.5	173.8	118.4
4	1.14	8.2	93.1	762.7	66.4	40.5	248.5	168.3	115.1
4	1.12	7.0	104.8	803.2	71.1	41.4	293.9	169.2	115.8
8	1.21	8.9	97.6	818.5	74.3	39.4	283.9	175.2	124.5
8	0.91	8.3	107.9	810.8	72.4	37.4	301.5	186.9	126.5
8	1.88	7.0	103.8	811.1	76.8	32.1	281.6	177.7	120.7
8	1.14	6.6	100.5	808.0	97.6	56.3	272.6	168.2	117.1
8	1.11	7.7	103.3	753.9	70.1	42.7	288.0	176.4	123.1
8	1.18	10.3	92.2	745.5	69.9	32.5	277.0	163.1	107.1
8	1.08	7.0	99.9	739.0	77.0	42.5	258.0	194.4	124.1
8	1.29	9.3	105.0	815.8	71.1	34.4	264.3	161.2	113.3
12	1.69	4.6	108.7	810.5	72.9	41.9	261.2	161.5	105.1
12	0.97	6.6	105.9	802.1	93.1	45.1	273.4	180.1	128.2
12	1.36	6.3	95.4	801.6	90.0	38.2	268.8	166.9	118.5
12	1.47	5.3	98.7	819.4	98.3	43.3	273.6	166.6	101.0
12	1.70	6.0	91.0	796.2	65.5	30.5	241.1	168.4	118.5
12	0.97	4.0	97.9	709.1	95.9	38.9	251.0	156.0	107.5
12	1.16	5.0	97.1	810.7	92.2	30.4	278.1	189.6	132.1
12	1.24	11.3	110.3	758.5	100.4	41.5	259.6	194.2	140.4

```
Analysis of Variance for HCN
Source     DF      Seq SS      Adj SS      Adj MS        F       P
Age         2     0.05080     0.05080     0.02540     0.34   0.717
Error      21     1.57800     1.57800     0.07514
Total      23     1.62880

Analysis of Variance for Oxalic Acid
Source     DF      Seq SS      Adj SS      Adj MS        F       P
Age         2      20.563      20.563      10.282     3.89   0.037
Error      21      55.496      55.496       2.643
Total      23      76.060

Analysis of Variance for ASH
Source     DF      Seq SS      Adj SS      Adj MS        F       P
Age         2        1.69        1.69        0.85     0.02   0.979
Error      21      854.37      854.37       40.68
Total      23      856.06

Analysis of Variance for WATER
Source     DF      Seq SS      Adj SS      Adj MS        F       P
Age         2       250.8       250.8       125.4     0.13   0.882
Error      21     20809.3     20809.3       990.9
Total      23     21060.1
```

Analysis of Variance for FAT

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Age	2	1258.40	1258.40	629.20	6.37	0.007
Error	21	2075.82	2075.82	98.85		
Total	23	3334.22				

Analysis of Variance for LIGNIN

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Age	2	194.32	194.32	97.16	2.06	0.153
Error	21	991.42	991.42	47.21		
Total	23	1185.74				

Analysis of Variance for CRUDE PROTEIN

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Age	2	1076.7	1076.7	538.3	2.68	0.092
Error	21	4210.8	4210.8	200.5		
Total	23	5287.5				

Analysis of Variance for NDF

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Age	2	45.6	45.6	22.8	0.18	0.833
Error	21	2596.8	2596.8	123.7		
Total	23	2642.4				

Analysis of Variance for ADF

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Age	2	36.8	36.8	18.4	0.18	0.834
Error	21	2105.8	2105.8	100.3		
Total	23	2142.7				

Experiment 2. Study 1.

Variables.

Input; Collection period; Block; Gross Energy Digestibility; Gross Energy Digestibility corrected by regression; Dry Matter Digestibility; Dry Matter Digestibility corrected by regression; Nitrogen Digestibility; Nitrogen Digestibility corrected by regression; True Metabolisable Energy; True Metabolisable Energy corrected by regression; True Metabolisable Energy corrected by Nitrogen excretion; True Metabolisable Energy corrected to Nitrogen balance and corrected by regression.

50	48	1	37.19	45.74	10.17	32.74	16.33	53.04	6.29	7.73	6.02	6.86
50	48	1	28.46	37.01	0.55	23.12	25.86	62.57	4.81	6.26	4.38	5.22
50	48	1	31.39	39.94	1.60	24.17	-3.11	33.60	5.31	6.75	5.36	6.20
50	48	2	36.32	44.87	22.23	44.80	-0.87	35.84	6.14	7.58	6.15	6.99
50	48	2	30.35	38.90	14.23	36.80	-5.88	30.83	5.13	6.58	5.23	6.07
50	48	2	37.71	46.26	23.87	46.44	-3.10	33.61	6.37	7.82	6.43	7.27
50	56	1	32.80	43.61	15.35	38.78	9.61	54.19	5.54	7.37	5.39	6.48
50	56	1	38.64	49.46	21.16	44.59	18.76	63.35	6.53	8.36	6.22	7.31
50	56	1	32.33	43.14	2.82	26.25	-23.43	21.16	5.46	7.29	5.85	6.94
50	56	2	34.50	45.31	24.72	48.15	2.77	47.36	5.83	7.66	5.79	6.88
50	56	2	39.25	50.06	23.75	47.18	0.08	44.67	6.63	8.46	6.63	7.72
50	56	2	33.95	44.76	17.60	41.03	-23.04	21.55	5.74	7.57	6.12	7.21
50	72	1	43.57	39.54	2.13	23.79	16.68	59.60	7.36	6.68	7.09	5.70
50	72	1	37.69	33.66	9.07	30.74	12.26	55.18	6.37	5.69	6.17	4.78
50	72	1	44.95	40.92	36.59	58.25	35.09	78.01	7.60	6.92	7.02	5.63
50	72	2	34.63	30.60	15.14	36.80	-9.96	32.96	5.85	5.17	6.02	4.63
50	72	2	38.85	34.83	0.97	22.63	-19.39	23.52	6.57	5.89	6.89	5.50
50	72	2	33.57	29.55	16.11	37.77	-29.88	13.04	5.67	4.99	6.17	4.78
70	48	1	34.42	40.50	11.07	27.19	12.82	39.28	5.82	6.85	5.63	6.23
70	48	1	31.79	37.87	4.96	21.08	11.32	37.78	5.37	6.40	5.21	5.81
70	48	1	45.65	51.73	6.83	22.95	52.98	79.44	7.72	8.74	6.88	7.48
70	48	2	34.39	40.48	24.32	40.44	-8.27	18.19	5.81	6.84	5.97	6.57
70	48	2	33.17	39.26	22.39	38.51	3.03	29.49	5.61	6.64	5.58	6.18
70	48	2	43.22	49.30	35.29	51.41	9.52	35.97	7.31	8.33	7.18	7.78
70	56	1	34.81	42.51	14.59	31.32	10.41	42.54	5.88	7.18	5.74	6.52
70	56	1	36.79	44.48	16.69	33.43	2.92	35.05	6.22	7.52	6.20	6.98
70	56	1	33.38	41.07	9.86	26.60	26.19	58.33	5.64	6.94	5.24	6.02
70	56	2	37.93	45.63	32.42	49.16	19.92	52.05	6.41	7.71	6.11	6.89
70	56	2	37.83	45.52	25.02	41.76	-2.63	29.50	6.39	7.69	6.46	7.24
70	56	2	36.41	44.11	17.13	33.86	-7.25	24.88	6.16	7.46	6.30	7.08
70	72	1	38.61	35.74	-11.58	3.89	0.33	31.26	6.53	6.04	6.55	5.56
70	72	1	39.63	36.77	18.42	33.89	20.03	50.96	6.70	6.22	6.40	5.41
70	72	1	41.17	38.31	27.85	43.32	-2.33	28.60	6.96	6.48	7.03	6.04
70	72	2	32.82	29.95	14.33	29.80	-23.53	7.40	5.55	5.06	5.96	4.96
70	72	2	34.25	31.39	18.23	33.70	-22.11	8.82	5.79	5.31	6.18	5.18
70	72	2	38.66	35.80	22.63	38.11	-24.87	6.06	6.54	6.05	6.97	5.98
90	48	1	33.41	38.16	8.47	21.01	9.95	30.53	5.65	6.45	5.49	5.96
90	48	1	37.38	42.13	8.06	20.60	15.86	36.44	6.32	7.12	6.07	6.53
90	48	1	42.05	46.80	30.63	43.17	23.84	44.42	7.11	7.91	6.73	7.19
90	48	2	33.63	38.37	23.59	36.13	15.45	36.03	5.68	6.49	5.44	5.90
90	48	2	38.54	43.28	29.10	41.64	23.38	43.97	6.51	7.32	6.14	6.61
90	48	2	40.43	45.18	31.92	44.46	33.01	53.60	6.83	7.64	6.30	6.77
90	56	1	37.10	43.10	19.56	32.57	20.71	45.71	6.27	7.29	5.94	6.55
90	56	1	41.36	47.37	26.32	39.33	26.90	51.90	6.99	8.01	6.56	7.17
90	56	1	43.77	49.77	28.37	41.39	17.52	42.52	7.40	8.41	7.12	7.73
90	56	2	38.65	44.66	28.85	41.87	6.22	31.22	6.53	7.55	6.44	7.05
90	56	2	39.45	45.45	30.62	43.64	20.13	45.13	6.67	7.68	6.35	6.95

90	56	2	37.74	43.74	28.56	41.58	6.88	31.88	6.38	7.39	6.27	6.88
90	72	1	36.11	33.88	15.98	28.01	19.64	43.70	6.10	5.73	5.79	5.02
90	72	1	35.70	33.47	11.17	23.20	15.41	39.48	6.04	5.66	5.79	5.02
90	72	1	33.41	31.17	20.14	32.18	6.25	30.31	5.65	5.27	5.55	4.78
90	72	2	33.47	31.24	22.14	34.18	19.51	43.57	5.66	5.28	5.35	4.57
90	72	2	40.23	38.00	30.37	42.41	21.06	45.13	6.80	6.42	6.46	5.69
90	72	2	43.42	41.18	33.18	45.22	8.76	32.83	7.34	6.96	7.20	6.43

Analysis of Variance for Gross Energy Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	43.82	43.82	21.91	1.51	0.232
colecc	2	27.20	27.20	13.60	0.94	0.399
blo	1	1.92	1.92	1.92	0.13	0.718
Input*colecc	4	89.82	89.82	22.46	1.55	0.205
Error	44	638.20	638.20	14.50		
Total	53	800.97				

Analysis of Variance for Gross Energy Digestibility corrected by regression

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	1.93	1.93	0.96	0.07	0.936
colecc	2	1057.22	1057.22	528.61	36.45	0.000
blo	1	1.92	1.92	1.92	0.13	0.718
Input*colecc	4	18.23	18.23	4.56	0.31	0.867
Error	44	638.14	638.14	14.50		
Total	53	1717.43				

Analysis of Variance for Dry Matter Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	831.23	831.23	415.62	5.37	0.008
colecc	2	222.53	222.53	111.27	1.44	0.249
blo	1	1270.02	1270.02	1270.02	16.40	0.000
Input*colecc	4	31.44	31.44	7.86	0.10	0.981
Error	44	3407.29	3407.29	77.44		
Total	53	5762.52				

Analysis of Variance for Dry Matter Digestibility corrected by regression

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	127.76	127.76	63.88	0.82	0.445
colecc	2	345.56	345.56	172.78	2.23	0.119
blo	1	1270.41	1270.41	1270.41	16.40	0.000
Input*colecc	4	30.88	30.88	7.72	0.10	0.982
Error	44	3407.62	3407.62	77.45		
Total	53	5182.23				

Analysis of Variance for Nitrogen Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	2638.5	2638.5	1319.2	7.22	0.002
colecc	2	994.9	994.9	497.5	2.72	0.077
blo	1	2814.6	2814.6	2814.6	15.41	0.000
Input*colecc	4	887.0	887.0	221.7	1.21	0.319
Error	44	8038.1	8038.1	182.7		
Total	53	15373.1				

Analysis of Variance for Nitrogen Digestibility corrected by regression

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	667.4	667.4	333.7	1.83	0.173
colecc	2	437.0	437.0	218.5	1.20	0.312
blo	1	2814.5	2814.5	2814.5	15.40	0.000
Input*colecc	4	894.2	894.2	223.6	1.22	0.315
Error	44	8039.1	8039.1	182.7		
Total	53	12852.2				

Analysis of Variance for True Metabolisable Energy

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	1.2590	1.2590	0.6295	1.52	0.231
colecc	2	0.7794	0.7794	0.3897	0.94	0.399
blo	1	0.0561	0.0561	0.0561	0.14	0.715
Input*colecc	4	2.5586	2.5586	0.6396	1.54	0.207
Error	44	18.2637	18.2637	0.4151		
Total	53	22.9168				

Analysis of Variance for True Metabolisable Energy corrected by regression

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	0.0557	0.0557	0.0278	0.07	0.935
colecc	2	30.1849	30.1849	15.0924	36.49	0.000
blo	1	0.0554	0.0554	0.0554	0.13	0.716
Input*colecc	4	0.5223	0.5223	0.1306	0.32	0.866
Error	44	18.1990	18.1990	0.4136		
Total	53	49.0173				

Analysis of Variance for True Metabolisable Energy corrected to Nitrogen balance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	0.2163	0.2163	0.1081	0.34	0.711
colecc	2	1.9643	1.9643	0.9821	3.12	0.054
blo	1	0.4039	0.4039	0.4039	1.28	0.264
Input*colecc	4	2.4714	2.4714	0.6179	1.96	0.117
Error	44	13.8604	13.8604	0.3150		
Total	53	18.9163				

Analysis of Variance for True Metabolisable Energy corrected to Nitrogen balance and corrected by regression

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Input	2	0.0862	0.0862	0.0431	0.14	0.873
colecc	2	26.7097	26.7097	13.3549	42.29	0.000
blo	1	0.3987	0.3987	0.3987	1.26	0.267
Input*colecc	4	0.8349	0.8349	0.2087	0.66	0.622
Error	44	13.8961	13.8961	0.3158		
Total	53	41.9257				

Experiment 2. Study 2.

Variables
Body weight; Dry Matter Digestibility; Gross Energy Digestibility; True Metabolisable Energy; True Metabolisable Energy Corrected to Nitrogen balance

0.6	21.70	26.91	17.08	4.55	4.27
0.6	18.76	30.34	14.89	5.13	4.88
0.6	24.10	33.24	-10.51	5.62	5.79
0.6	25.37	34.83	26.14	5.89	5.46
0.6	29.12	39.77	7.73	6.72	6.60
0.6	28.27	37.13	23.08	6.28	5.90
0.6	40.42	48.10	48.34	8.13	7.34
1.0	29.17	37.74	16.39	6.38	6.11
1.0	21.03	28.36	11.72	4.79	4.60
1.0	27.01	33.69	15.14	5.69	5.45
1.0	18.55	28.96	-17.70	4.89	5.18
1.0	29.83	38.44	15.19	6.50	6.25
1.0	21.77	32.13	-5.82	5.43	5.53
1.0	26.84	36.81	5.49	6.22	6.13
1.0	17.45	35.22	-24.80	5.95	6.36
1.5	28.39	41.49	-4.44	7.01	7.09
1.5	21.50	34.75	-8.58	5.87	6.01
1.5	27.50	39.69	-4.09	6.71	6.78
1.5	22.12	34.75	-3.63	5.87	5.93
1.5	26.96	38.72	8.55	6.55	6.41
1.5	33.40	43.93	32.19	7.43	6.90
1.5	36.21	47.41	48.33	8.01	7.22
1.5	30.84	43.24	14.54	7.31	7.07

Analysis of Variance for Dry Matter Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BW	2	79.87	79.87	39.93	1.25	0.308
Error	20	639.13	639.13	31.96		
Total	22	719.00				

Analysis of Variance for Gross Energy Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BW	2	183.33	183.33	91.67	3.48	0.051
Error	20	526.93	526.93	26.35		
Total	22	710.27				

Analysis of Variance for Nitrogen Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BW	2	978.4	978.4	489.2	1.46	0.255
Error	20	6690.4	6690.4	334.5		
Total	22	7668.8				

Analysis of Variance for True Metabolisable Energy

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BW	2	5.2479	5.2479	2.6240	3.48	0.050
Error	20	15.0691	15.0691	0.7535		
Total	22	20.3170				

Analysis of Variance for True Metabolisable Energy corrected to Nitrogen balance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BW	2	4.7460	4.7460	2.3730	4.43	0.026
Error	20	10.7234	10.7234	0.5362		
Total	22	15.4694				

Experiment 2. Study 3.

Variables
Genotype; Collection period; Block, Dry Matter Digestibility; Gross Energy Digestibility; Nitrogen Digestibility; True Metabolisable Energy; True Metabolisable Energy corrected to Nitrogen balance

1	64	1	35.23	44.00	-18.17	7.44	7.66
1	64	1	45.24	48.14	41.67	8.14	7.39
1	64	1	53.36	55.90	79.70	9.45	8.07
1	64	1	44.27	48.67	29.17	8.23	7.68
1	64	2	34.03	46.23	-84.60	7.81	9.11
1	64	2	34.11	44.96	-34.12	7.60	8.08
1	64	2	43.86	48.18	33.94	8.14	7.52
1	64	2	38.34	50.11	-13.76	8.47	8.61
1	72	1	9.91	27.72	-91.67	4.6 9	6.12
1	72	1	4.01	21.11	-104.35	3.57	5.22
1	72	1	18.98	31.42	-42.98	5.31	5.96
1	72	1	8.34	24.94	-73.86	4.22	5.37
1	72	2	25.27	29.75	29.99	5.03	4.50
1	72	2	24.54	31.35	21.73	5.30	4.90
1	72	2	31.59	37.04	37.15	6.26	5.60
1	72	2	30.43	34.61	37.29	5.85	5.19
2	64	1	33.61	39.96	7.33	6.75	6.57
2	64	1	37.37	41.68	16.13	7.05	6.72
2	64	1	36.88	40.77	38.39	6.89	6.20
2	64	1	15.02	27.96	-71.08	4.73	5.83
2	64	2	35.51	38.44	11.66	6.50	6.25
2	64	2	40.84	45.11	16.75	7.63	7.28
2	64	2	41.17	43.31	28.88	7.32	6.78
2	64	2	34.45	38.65	9.09	6.53	6.32
2	72	1	34.43	41.33	-4.65	6.99	6.99
2	72	1	31.38	40.96	-2.60	6.92	6.90
2	72	1	30.74	39.78	37.27	6.72	6.06
2	72	1	23.58	33.02	9.42	5.58	5.38
2	72	2	22.77	37.00	-75.15	6.25	7.41
2	72	2	22.32	31.82	4.10	5.38	5.26
2	72	2	22.21	31.16	-1.34	5.27	5.24
2	72	2	20.95	29.90	-4.68	5.05	5.08

Analysis of Variance for Dry matter Digestibility, using the two genotypes

Source	DF	Seq SS	Adj SS	Adj MS	F	P
gen	1	0.09	0.09	0.09	0.00	0.970
colec	1	1827.71	1827.71	1827.71	28.56	0.000
blo	1	50.10	50.10	50.10	0.78	0.384
gen*colec	1	370.60	370.60	370.60	5.79	0.023
Error	27	1727.76	1727.76	63.99		
Total	31	3976.26				

Analysis of Variance for Gross Energy Digestibility, using the two genotypes

Source	DF	Seq SS	Adj SS	Adj MS	F	P
gen	1	16.94	16.94	16.94	0.74	0.397
colec	1	1003.07	1003.07	1003.07	43.85	0.000
blo	1	3.29	3.29	3.29	0.14	0.707
gen*colec	1	430.27	430.27	430.27	18.81	0.000
Error	27	617.59	617.59	22.87		
Total	31	2071.16				

Analysis of Variance for Nitrogen Digestibility, using the two genotypes

Source	DF	Seq SS	Adj SS	Adj MS	F	P
gen	1	929	929	929	0.42	0.520
colec	1	3107	3107	3107	1.42	0.244
blo	1	874	874	874	0.40	0.533
gen*colec	1	494	494	494	0.23	0.638
Error	27	59078	59078	2188		
Total	31	64481				

Analysis of Variance for True Metabolisable Energy, using the two genotypes

Source	DF	Seq SS	Adj SS	Adj MS	F	P
gen	1	0.488	0.488	0.488	0.75	0.395
colec	1	28.671	28.671	28.671	43.92	0.000
blo	1	0.091	0.091	0.091	0.14	0.711
gen*colec	1	12.264	12.264	12.264	18.79	0.000
Error	27	17.625	17.625	0.653		
Total	31	59.138				

Analysis of Variance for True Metabolisable Energy corrected to nitrogen balance, using the two genotypes

Source	DF	Seq SS	Adj SS	Adj MS	F	P
gen	1	1.4070	1.4070	1.4070	3.19	0.085
colec	1	19.3598	19.3598	19.3598	43.94	0.000
blo	1	0.0306	0.0306	0.0306	0.07	0.794
gen*colec	1	9.7130	9.7130	9.7130	22.04	0.000
Error	27	11.8971	11.8971	0.4406		
Total	31	42.4075				

Genotype Hubbard

Analysis of Variance for Dry matter Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	1922.2	1922.2	1922.2	25.21	0.000
blo	1	114.7	114.7	114.7	1.50	0.242
Error	13	991.3	991.3	76.3		
Total	15	3028.2				

Analysis of Variance for Gross Energy Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	1373.63	1373.63	1373.63	70.49	0.000
blo	1	25.83	25.83	25.83	1.33	0.270
Error	13	253.32	253.32	19.49		
Total	15	1652.79				

Analysis of Variance for Nitrogen Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	3040	3040	3040	0.93	0.352
blo	1	2707	2707	2707	0.83	0.379
Error	13	42462	42462	3266		
Total	15	48209				

Analysis of Variance for True Metabolisable Energy

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	39.219	39.219	39.219	70.48	0.000
blo	1	0.727	0.727	0.727	1.31	0.274
Error	13	7.234	7.234	0.556		
Total	15	47.179				

Analysis of Variance for True Metabolisable Energy corrected to nitrogen balance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	28.249	28.249	28.249	82.92	0.000
blo	1	0.000	0.000	0.000	0.00	0.987
Error	13	4.429	4.429	0.341		
Total	15	32.678				

Genotype Criollo

Analysis of Variance for Dry matter Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	276.14	276.14	276.14	5.35	0.038
blo	1	0.49	0.49	0.49	0.01	0.924
Error	13	671.38	671.38	51.64		
Total	15	948.00				

Analysis of Variance for Gross Energy Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	59.71	59.71	59.71	2.31	0.152
blo	1	6.34	6.34	6.34	0.25	0.628
Error	13	335.38	335.38	25.80		
Total	15	401.43				

Analysis of Variance for Nitrogen Digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	561	561	561	0.50	0.493
blo	1	105	105	105	0.09	0.766
Error	13	14678	14678	1129		
Total	15	15344				

Analysis of Variance for True Metabolisable Energy

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	1.7161	1.7161	1.7161	2.33	0.151
blo	1	0.1806	0.1806	0.1806	0.25	0.629
Error	13	9.5750	9.5750	0.7365		
Total	15	11.4717				

Analysis of Variance for True Metabolisable Energy corrected to nitrogen balance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
colec	1	0.8236	0.8236	0.8236	1.44	0.251
blo	1	0.0663	0.0663	0.0663	0.12	0.739
Error	13	7.4323	7.4323	0.5717		
Total	15	8.3221				

Variables

Genotype; Water intake after tube feeding; Water intake when starved; Dry matter in excreta

1	475.00	177.5	202.0
1	358.33	120.0	265.0
1	366.67	237.5	220.6
1	425.00	152.5	211.7
2	288.33	72.5	415.6
2	218.33	37.5	542.4
2	263.33	90.0	416.3
2	190.00	27.5	439.4

Analysis of Variance for Water intake after tube Feeding

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genot	1	55280	55280	55280	22.42	0.003
Error	6	14791	14791	2465		
Total	7	70070				

Analysis of Variance for Water intake when starved

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genot	1	26450	26450	26450	15.89	0.007
Error	6	9984	9984	1664		
Total	7	36434				

```
Analysis of Variance for Dry Matter content in excreta

Source     DF     Seq SS     Adj SS     Adj MS      F       P
Genot       1     104516     104516     104516   47.34  0.000
Error       6      13247      13247       2208
Total       7     117763
```

Experiment 3. Study 1.

Variables.
Fibre source; Energy Balance; Nitrogen excretion; Uric Acid excretion.

P10	696.78	2.46	8.50
P10	756.97	1.54	0.98
P10	658.38	3.06	8.91
P10	741.32	1.92	6.28
P10	714.49	1.84	4.36
P10	743.58	1.91	5.28
P20	806.15	2.62	9.03
P20	793.26	1.86	5.58
P20	787.85	1.97	5.69
P20	791.34	2.24	7.67
P20	786.75	1.99	4.73
P20	754.47	2.34	6.40
CE10	668.47	1.96	6.16
CE10	652.90	2.37	9.23
CE10	621.30	2.05	8.40
CE10	662.85	1.87	5.99
CE10	679.31	1.62	6.11
CE10	653.90	1.75	5.65
CE20	712.53	1.34	4.10
CE20	684.25	1.70	5.44
CE20	763.65	3.06	14.01
CE20	691.52	1.44	3.16
CE20	627.73	2.32	6.81
CE20	673.37	2.21	5.39
CE30	701.99	2.01	7.55
CE30	670.16	2.06	6.93
CE30	666.66	2.29	6.56
CE30	669.32	2.18	7.54
CE30	682.10	1.89	5.84
CE30	677.02	2.17	7.14
CFE	880.07	2.86	9.67
CFE	931.81	2.24	8.34
CFE	929.07	2.38	9.09
CFE	874.75	2.91	10.95
CFE	807.48	2.69	7.73
G	687.03	1.54	5.41
G	675.56	1.71	5.67
G	648.40	2.47	7.36
G	624.58	2.86	8.34
G	650.32	2.36	6.82
G	599.75	3.38	10.62

Analysis of Variance for Energy balance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Trat	6	233134	233134	38856	36.13	0.000
Error	34	36565	36565	1075		
Total	40	269699				

Analysis of Variance for Nitrogen excretion

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Trat	6	1.7914	1.7914	0.2986	1.41	0.239
Error	34	7.1950	7.1950	0.2116		
Total	40	8.9863				

Analysis of Variance for Uric acid excretion

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Trat	6	36.919	36.919	6.153	1.24	0.312
Error	34	168.996	168.996	4.970		
Total	40	205.915				

Experiment 3. Study 1.

Variables
Fibre source; Taurine; Hydroxyproline; Aspartic acid; Threonine; Serine; Glutamic acid; Proline

Glucose	49.72	11.30	98.31	65.54	71.19	153.68	74.58
Glucose	84.72	5.22	116.10	70.08	83.68	176.77	73.22
Pectin	69.53	16.36	161.55	116.56	124.74	249.49	124.74
Pectin	207.35	14.37	176.55	123.18	125.23	248.41	112.91
Cellulose	54.84	10.96	93.22	63.06	74.03	148.06	74.03
Cellulose	96.16	8.74	96.16	91.19	67.02	145.70	64.10
CFE	103.46	12.81	1.58	37.46	39.96	1.42	45.56
CFE	173.35	12.83	98.77	92.13	101.59	148.16	84.37

Glycine; Alanine; Cystine; Valine; Methionine; Isoleucine; Leucine; Tyrosine

128.82	62.15	56.50	59.89	20.34	48.59	75.71	42.940
132.84	73.22	63.80	72.17	29.28	59.62	88.91	50.200
216.77	102.25	77.71	102.25	30.67	89.98	130.88	73.620
188.87	106.75	73.90	106.87	36.95	100.59	33.44	73.900
178.23	63.06	49.35	57.58	24.67	46.61	71.29	46.610
169.01	61.19	49.53	55.36	26.22	43.71	67.02	46.620
78.29	-49.14	45.64	-73.84	-15.98	-41.74	-113.63	-0.059
206.59	11.83	63.48	-8.41	6.89	20.52	-26.60	38.700

Phenilalanine; Hydroxylysine; Histidine; Lysine; Arginine; Tryptophan; Total

Phenilalanine	Hydroxylysine	Histidine	Lysine	Arginine	Tryptophan	Total
41.81	1.13	50.85	72.32	63.28	15.82	1281.4
50.20	1.04	42.88	76.35	74.26	8.36	1445.5
67.48	2.04	55.21	87.93	94.07	20.45	2022.5
67.74	2.05	55.43	100.5	94.43	28.74	2190.5
43.87	2.74	46.61	60.32	57.58	19.19	1299.7
40.79	0.00	46.62	58.28	55.36	23.31	12.93.8
-102.63	1.90	28.23	0.65	-4.95	28.31	42.15
-44.85	2.54	54.94	67.98	52.22	40.40	1226.4

Analysis of Variance for Taurine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	9063	9063	3021	0.90	0.515
Error	4	13406	13406	3351		
Total	7	22469				

Analysis of Variance for Hydroxyproline

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	59.758	59.758	19.919	3.48	0.130
Error	4	22.928	22.928	5.732		
Total	7	82.686				

Analysis of Variance for Aspartic acid

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	14438	14438	4813	3.85	0.113
Error	4	4998	4998	1250		
Total	7	19436				

Analysis of Variance for Threonine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	3909.3	3909.3	1303.1	2.71	0.180
Error	4	1922.3	1922.3	480.6		
Total	7	5831.5				

Analysis of Variance for Serine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	4128.9	4128.9	1376.3	2.75	0.177
Error	4	2001.8	2001.8	500.5		
Total	7	6130.7				

Analysis of Variance for Glutamic acid

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	30736	30736	10245	3.71	0.119
Error	4	11036	11036	2759		
Total	7	41772				

Analysis of Variance for Proline

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	3757.6	3757.6	1252.5	5.74	0.062
Error	4	873.3	873.3	218.3		
Total	7	4630.9				

Analysis of Variance for Glycine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	6309	6309	2103	0.97	0.489
Error	4	8670	8670	2168		
Total	7	14980				

Analysis of Variance for Alanine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	16164.5	16164.5	5388.2	11.16	0.021
Error	4	1931.8	1931.8	483.0		
Total	7	18096.3				

Analysis of Variance for Cystine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	781.85	781.85	260.62	5.40	0.068
Error	4	193.05	193.05	48.26		
Total	7	974.91				

Analysis of Variance for Valine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	23060.4	23060.4	7686.8	13.79	0.014
Error	4	2229.1	2229.1	557.3		
Total	7	25289.5				

Analysis of Variance for Methionine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	1691.80	1691.80	563.93	7.00	0.045
Error	4	322.40	322.40	80.60		
Total	7	2014.20				

Analysis of Variance for Isoleucine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	11400.2	11400.2	3800.1	7.38	0.042
Error	4	2059.5	2059.5	514.9		
Total	7	13459.7				

Analysis of Variance for Leucine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	45086	45086	15029	15.47	0.011
Error	4	3887	3887	972		
Total	7	48973				

Analysis of Variance for Tyrosine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	2963.7	2963.7	987.9	5.08	0.075
Error	4	777.5	777.5	194.4		
Total	7	3741.2				

Analysis of Variance for Phenylalanine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	24455.1	24455.1	8151.7	19.08	0.008
Error	4	1709.2	1709.2	427.3		
Total	7	26164.4				

Analysis of Variance for Hydroxylysine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	1.7499	1.7499	0.5833	0.59	0.654
Error	4	3.9627	3.9627	0.9907		
Total	7	5.7126				

Analysis of Variance for Histidine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	194.58	194.58	64.86	0.67	0.615
Error	4	388.50	388.50	97.12		
Total	7	583.07				

Analysis of Variance for Lysine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	3832.0	3832.0	1277.3	2.17	0.234
Error	4	2356.7	2356.7	589.2		
Total	7	6188.7				

Analysis of Variance for Arginine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
FIBSOURC	3	5164.8	5164.8	1721.6	4.06	0.105
Error	4	1697.0	1697.0	424.3		
Total	7	6861.8				

```
Analysis of Variance for Tryptophan

Source      DF      Seq SS      Adj SS      Adj MS        F       P
FIBSOURC     3      507.10      507.10      169.03     4.70   0.084
Error        4      143.76      143.76       35.94
Total        7      650.86


Analysis of Variance for Total amino acid excretion

Source      DF      Seq SS      Adj SS      Adj MS        F       P
FIBSOURC     3     2175220     2175220      725073     3.98   0.108
Error        4      728834      728834      182209
Total        7     2904054
```

Experiment 3. Study 2.

Variables
Input; genotype; block; Initial Weight; Energy output; Energy Balance; Uric Acid excretion; Nitrogen excretion; Lost Weight

1	1	1	2850	176.8	606.66	5.86	2.50	300
1	1	1	2350	177.1	606.43	8.33	3.15	250
1	1	2	1925	132.9	650.56	5.01	1.95	184
1	1	2	2050	111.4	672.08	3.59	1.55	185
1	2	1	1700	117.7	665.81	5.01	1.81	175
1	2	2	1850	110.9	672.58	3.86	1.56	600
1	2	2	1475	158.8	624.65	4.52	1.68	117
1	2	2	1950	183.8	599.74	8.01	2.95	250
20	1	1	2150	391.9	740.40	2.88	1.35	175
20	1	1	2200	446.1	686.17	5.67	3.21	175
20	1	2	2075	426.9	705.36	5.73	2.28	170
20	1	2	2050	409.2	723.09	5.27	1.90	209
20	1	2	1550	409.0	723.28	3.70	1.68	149
20	2	1	1800	386.6	745.68	4.20	1.91	150
20	2	1	1950	377.0	755.24	5.25	1.81	200
20	2	2	1400	382.5	749.78	3.93	1.44	92
20	2	2	1550	389.5	742.77	3.75	1.37	58
20	2	2	1400	388.6	743.69	2.39	0.94	163
30	1	1	2250	677.4	629.31	6.51	2.32	150
30	1	1	2800	630.7	675.98	5.62	2.83	75
30	1	2	2100	467.4	839.56	3.84	1.49	159
30	1	2	2675	542.4	764.30	6.66	2.21	219
30	1	2	2250	568.4	738.24	5.56	1.98	171
30	2	1	1800	566.0	740.62	5.96	2.00	100
30	2	1	1800	585.1	721.55	5.34	2.61	100
30	2	2	1650	600.0	706.66	3.96	1.57	149
30	2	2	1600	533.4	773.24	3.49	1.46	121
30	2	2	1550	536.5	770.18	3.80	1.59	73
40	1	1	1700	804.5	676.60	7.36	3.09	100
40	1	1	2450	803.1	677.98	5.33	2.23	350
40	1	1	2150	733.4	747.67	9.92	3.07	350
40	1	2	2050	761.8	719.32	10.97	4.03	220
40	1	2	1950	682.6	798.47	5.04	1.69	148
40	2	1	1900	716.8	764.23	5.86	2.40	350
40	2	1	1850	703.5	777.55	7.46	3.23	125
40	2	1	2000	785.9	695.14	7.24	2.75	275

40	2	2	1450	660.6	820.46	4.48	1.50	88
40	2	2	1700	756.0	725.05	6.38	2.43	159
50	1	1	2300	1007.2	648.24	6.78	3.38	150
50	1	1	2200	1015.3	640.16	6.95	3.11	125
50	1	1	2350	920.9	734.53	3.92	2.32	200
50	1	2	2125	849.3	806.13	7.06	2.04	116
50	1	2	2650	980.6	674.87	8.41	3.09	298
50	2	1	1650	908.7	746.76	13.41	3.90	150
50	2	1	1850	893.6	761.85	5.30	2.75	200
50	2	1	1700	952.1	703.33	8.66	3.82	250
50	2	2	1550	819.5	836.02	5.26	2.19	140
50	2	2	1750	929.3	726.15	8.26	3.19	221

Analysis of Variance for Energy balance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
InitialWt	1	21055	1543	1543	0.80	0.376
input	4	59029	64295	16074	8.36	0.000
genot	1	9	510	510	0.27	0.610
block	1	9801	9336	9336	4.86	0.034
input*genot	4	4656	4656	1164	0.61	0.661
Error	36	69204	69204	1922		
Total	47	163752				

Analysis of Variance for Uric acid excretion

Source	DF	Seq SS	Adj SS	Adj MS	F	P
InitialWt	1	11.270	5.053	5.053	1.51	0.228
input	4	64.967	57.586	14.397	4.29	0.006
genot	1	0.611	0.956	0.956	0.28	0.597
block	1	2.336	1.220	1.220	0.36	0.550
input*genot	4	16.858	16.858	4.215	1.26	0.305
Error	36	120.859	120.859	3.357		
Total	47	216.901				

Analysis of Variance Nitrogen excretion

Source	DF	Seq SS	Adj SS	Adj MS	F	P
InitialWt	1	2.9340	0.8107	0.8107	2.57	0.118
input	4	8.7457	6.9728	1.7432	5.52	0.001
genot	1	0.2033	0.0775	0.0775	0.25	0.623
block	1	1.8200	1.3641	1.3641	4.32	0.045
input*genot	4	1.7668	1.7668	0.4417	1.40	0.254
Error	36	11.3661	11.3661	0.3157		
Total	47	26.8360				

Analysis of Variance for Lost Weight

Source	DF	Seq SS	Adj SS	Adj MS	F	P
InitialW	1	43207	86166	86166	14.00	0.001
input	4	89167	99129	24782	4.03	0.008
genot	1	24853	45118	45118	7.33	0.010
block	1	6042	8317	8317	1.35	0.253
input*genot	4	35314	35314	8828	1.43	0.243
Error	36	221584	221584	6155		
Total	47	420167				

Regression of energy output on chaya fibrous extract input
Descriptive Statistics

Variable	trat	N	Mean	Median	TrMean	StDev
Ebex(kJ)	1	8	146.2	145.9	146.2	31.4
	20	10	400.75	390.71	398.04	21.93
	30	10	570.7	567.3	570.4	57.9
	40	10	740.9	744.7	742.9	50.0
	50	10	927.7	925.2	930.3	64.0

Regression Analysis

The regression equation is
Gross Energy output (kJ) = 47.2 + 17.5 input

Predictor	Coef	StDev	T	P
Constant	47.18	25.93	1.82	0.077
Input	17.5094	0.7058	24.81	0.000

S = 49.91 R-Sq = 94.2% R-Sq(adj) = 94.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	1532901	1532901	615.48	0.000
Residual Error	38	94643	2491		
Total	39	1627543			

Experiment 4.

Variables
Heat Increment/day (CLM); Heat Increment/day (WF); TME/g CLM; TME/g Wf; Net energy CLM; Net energy Wf; k CLM; k Wf; Water Intake CLM; Water Intake Wf.

49.05	38.435	4.364	7.66	1.940	5.695	0.444	0.743	191.0	104.0
79.62	62.395	7.895	9.27	4.710	6.780	0.597	0.731	129.0	125.0
76.62	25.825	6.182	8.44	3.117	7.410	0.504	0.878	110.0	131.0
65.26	39.060	4.460	8.49	1.850	6.931	0.415	0.816	170.5	111.5
52.20	54.370	5.885	7.75	3.797	5.577	0.645	0.719	156.0	172.0
11.01	13.635	6.807	8.60	6.367	8.055	0.935	0.937	49.0	21.5
49.22	-49.935	5.374	9.88	3.405	11.883	0.634	1.202	145.0	33.5
85.69	60.915	4.774	7.83	1.346	5.399	0.282	0.689	141.5	120.5
-20.91	55.900	6.503	8.56	7.339	6.331	1.129	0.739	126.0	61.0
15.08	-29.250	5.370	7.39	4.767	8.563	0.888	1.158	151.0	135.5

Weight Loss CLM; Weight Loss Wf; Foodstuff; Water holding capacity

50	5	1	4.931
60	76	1	4.779
92	76	1	4.190
54	33	1	4.801
46	52	1	3.863
225	152	1	5.112
-6	71	2	3.078

136	93	2	3.223
140	105	2	3.802
87	11	2	2.980
		2	3.504
		2	3.142

Paired T-Test and Confidence Interval

Paired T for Heat Increment/d

	N	Mean	StDev	SE Mean
HeInc/dCLM	10	46.3	34.5	10.9
HaInc/dWf	10	27.1	38.8	12.3
Difference	10	19.2	45.1	14.3

95% CI for mean difference: (-13.1, 51.4)
T-Test of mean difference = 0 (vs not = 0): T-Value = 1.34 P-Value = 0.212

Paired T for TME/gCLM - TME/gWf

	N	Mean	StDev	SE Mean
TME/gCLM	10	5.761	1.121	0.355
TME/gWf	10	8.391	0.771	0.244
Difference	10	-2.629	1.044	0.330

95% CI for mean difference: (-3.376, -1.882)
T-Test of mean difference = 0 (vs not = 0): T-Value = -7.96 P-Value = 0.000

Paired T for Net energy

	N	Mean	StDev	SE Mean
NE CLM	10	3.864	1.965	0.621
NE Wf	10	7.262	1.934	0.612
Difference	10	-3.399	2.514	0.795

95% CI for mean difference: (-5.197, -1.600)
T-Test of mean difference = 0 (vs not = 0): T-Value = -4.28 P-Value = 0.002

Paired T for kCLm - kWf

	N	Mean	StDev	SE Mean
kCLm	10	0.6473	0.2635	0.0833
kWf	10	0.8612	0.1850	0.0585
Difference	10	-0.2139	0.2727	0.0862

95% CI for mean difference: (-0.4090, -0.0188)
T-Test of mean difference = 0 (vs not = 0): T-Value = -2.48 P-Value = 0.035

Paired T for Water Intake

	N	Mean	StDev	SE Mean
WaIntCLM	10	136.9	38.5	12.2
WaIntWf	10	101.6	47.9	15.2
Difference	10	35.4	43.9	13.9

95% CI for mean difference: (3.9, 66.8)
T-Test of mean difference = 0 (vs not = 0): T-Value = 2.55 P-Value = 0.031

Paired T for Weight Loss

	N	Mean	StDev	SE Mean
WtLstCLM	10	88.4	64.8	20.5
WtLstWf	10	67.4	44.6	14.1
Difference	10	21.0	45.6	14.4

95% CI for mean difference: (-11.6, 53.6)
T-Test of mean difference = 0 (vs not = 0): T-Value = 1.46 P-Value = 0.179

Analysis of Variance for Water holding capacity

Source	DF	SS	MS	F	P
Foodstuff	1	5.263	5.263	32.29	0.000
Error	10	1.630	0.163		
Total	11	6.893			

Level	N	Mean	StDev
1	6	4.6127	0.4805
2	6	3.2882	0.3083

Pooled StDev = 0.4037

Experiment 5.

Variables
Diet; Brooder; AME7d; AMEn7d; Gross energy digestibility 7d; Nitrogen digestibility 7d; Dry matter digestibility 7d; AME21d; AMEn21d; Gross energy digestibility 21d; Nitrogen digestibility 21d; Dry matter digestibility 21d

```
1 1 11.61 11.08 70.60 52.69 69.57 13.44 12.75 81.77 68.74 79.53
1 1 12.16 11.57 73.94 58.56 73.28 12.99 12.33 79.00 65.48 76.35
1 1 12.61 11.94 76.68 66.23 76.03 12.79 12.10 77.78 67.99 76.19
1 1 12.64 11.97 76.87 66.17 75.69 12.89 12.22 78.38 66.75 76.51
1 2 12.40 11.77 75.44 63.12 75.52 12.79 12.14 77.82 65.51 75.86
1 2 12.33 11.71 75.00 61.58 75.18 12.97 12.33 78.89 64.14 77.71
1 2 12.62 11.96 76.74 65.08 76.51 13.47 12.73 81.92 73.53 80.56
1 2 12.51 11.86 76.10 64.69 75.86 12.75 12.08 77.52 66.12 76.35
2 1 10.95 10.43 64.07 50.60 65.86 12.05 11.45 70.50 58.34 69.84
2 1 11.81 11.18 69.06 60.96 70.54 12.11 11.49 70.85 60.16 70.31
2 1 11.47 10.86 67.06 58.41 68.85 11.60 10.99 67.86 59.04 68.07
2 1 11.02 10.45 64.42 54.07 66.67 12.43 11.79 72.68 61.30 71.64
2 2 11.88 11.25 69.48 60.85 70.31 12.41 11.76 72.61 62.78 72.26
2 2 11.07 10.47 64.77 58.37 66.96 12.07 11.52 70.60 52.90 69.84
2 2 12.04 11.39 70.40 62.32 71.21 12.36 11.77 72.28 56.32 71.43
2 2 12.14 11.49 71.02 62.87 72.06 12.43 11.76 72.71 64.86 72.46
3 1 10.75 10.21 60.35 53.89 63.37 12.62 12.01 70.85 60.48 69.42
3 1 11.28 10.73 63.33 54.61 65.42 12.76 12.13 71.61 61.96 70.87
3 1 11.60 11.03 65.12 56.53 66.36 12.09 11.51 67.85 57.01 68.38
3 1 10.96 10.41 61.53 54.42 64.08 12.40 11.82 69.59 57.37 68.64
3 2 11.83 11.23 66.41 59.55 67.26 12.85 12.25 72.11 58.63 70.16
3 2 11.75 11.21 65.94 53.42 66.96 12.48 11.89 70.05 58.81 69.17
3 2 11.14 10.60 62.51 53.01 65.09 12.13 11.55 68.10 57.56 67.83
3 2 10.53  9.88 59.08 63.62 62.63 12.33 11.75 69.21 57.54 68.38
```

Analysis of Variance for AME7d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	5.4343	5.4343	2.7172	15.21	0.000
Brooder	1	0.4760	0.4760	0.4760	2.66	0.118
Error	20	3.5735	3.5735	0.1787		
Total	23	9.4839				

Analysis of Variance for AMEn7d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	4.9332	4.9332	2.4666	15.00	0.000
Brooder	1	0.3651	0.3651	0.3651	2.22	0.152
Error	20	3.2888	3.2888	0.1644		
Total	23	8.5871				

Analysis of Variance for Gross energy digestibility 7d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	602.38	602.38	301.19	50.09	0.000
Brooder	1	16.43	16.43	16.43	2.73	0.114
Error	20	120.26	120.26	6.01		
Total	23	739.08				

Analysis of Variance for Nitrogen digestibility 7d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	152.69	152.69	76.34	5.04	0.017
Brooder	1	71.21	71.21	71.21	4.70	0.042
Error	20	302.76	302.76	15.14		
Total	23	526.66				

Analysis of Variance for Dry matter digestibility 7d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	369.50	369.50	184.75	47.41	0.000
Brooder	1	16.38	16.38	16.38	4.20	0.054
Error	20	77.94	77.94	3.90		
Total	23	463.82				

Analysis of Variance for AME21d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	2.85091	2.85091	1.42545	17.17	0.000
Brooder	1	0.03154	0.03154	0.03154	0.38	0.545
Error	20	1.66065	1.66065	0.08303		
Total	23	4.54310				

Analysis of Variance for AMEn21d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	2.40416	2.40416	1.20208	16.42	0.000
Brooder	1	0.03682	0.03682	0.03682	0.50	0.486
Error	20	1.46376	1.46376	0.07319		
Total	23	3.90473				

Analysis of Variance for Gross energy digestibility 21d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	396.49	396.49	198.25	69.68	0.000
Brooder	1	1.08	1.08	1.08	0.38	0.544
Error	20	56.90	56.90	2.85		
Total	23	454.48				

Analysis of Variance for Nitrogen digestibility 21d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	362.55	362.55	181.27	20.42	0.000
Brooder	1	1.46	1.46	1.46	0.16	0.689
Error	20	177.55	177.55	8.88		
Total	23	541.56				

Analysis of Variance for Dry matter digestibility 21d

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Diet	2	307.67	307.67	153.83	72.34	0.000
Brooder	1	1.63	1.63	1.63	0.77	0.391
Error	20	42.53	42.53	2.13		
Total	23	351.83				

Paired T-Test and Confidence Interval

Paired T for AME7d - AME21d

	N	Mean	StDev	SE Mean
AME7d	24	11.713	0.642	0.131
AME21d	24	12.550	0.444	0.091
Difference	24	-0.838	0.559	0.114

95% CI for mean difference: (-1.074, -0.602)
T-Test of mean difference = 0 (vs not = 0): T-Value = -7.34 P-Value = 0.000

Paired T for AMEn7d - AMEn21d

	N	Mean	StDev	SE Mean
AMEn7d	24	11.112	0.611	0.125
AMEn21d	24	11.922	0.412	0.084
Difference	24	-0.810	0.541	0.110

95% CI for mean difference: (-1.038, -0.582)
T-Test of mean difference = 0 (vs not = 0): T-Value = -7.34 P-Value = 0.000

Paired T for Gross energy digestibility

	N	Mean	StDev	SE Mean
DGE7d	24	68.58	5.67	1.16
DGE21d	24	73.44	4.45	0.91
Difference	24	-4.859	3.196	0.652

95% CI for mean difference: (-6.209, -3.510)
T-Test of mean difference = 0 (vs not = 0): T-Value = -7.45 P-Value = 0.000

Paired T for Nitrogen digestibility

	N	Mean	StDev	SE Mean
DN7day	24	58.984	4.785	0.977
DN21d	24	61.805	4.852	0.991
Difference	24	-2.82	5.04	1.03

95% CI for mean difference: (-4.95, -0.69)
T-Test of mean difference = 0 (vs not = 0): T-Value = -2.74 P-Value = 0.012

Paired T for Dry matter digestibility

	N	Mean	StDev	SE Mean
DDM7d	24	69.636	4.491	0.917
DDM21d	24	72.407	3.911	0.798
Difference	24	-2.770	2.527	0.516

95% CI for mean difference: (-3.837, -1.704)
T-Test of mean difference = 0 (vs not = 0): T-Value = -5.37 P-Value = 0.000

Amino acid Digestibility

Variables

Diet; Nitrogen digestibility; Aspartic acid; Threonine; Serine; Glutamic acid; Glycine; Alanine; Valine; Isoleucine; Leucine; Tyrosine

1	80.97	87.12	78.13	81.31	89.61	79.35	96.18	86.34	84.98	87.92	85.32
1	81.05	87.94	78.83	82.42	89.92	81.01	96.30	86.95	85.54	88.07	100.00
1	79.20	88.49	80.18	83.13	89.49	81.84	95.87	87.72	85.89	87.09	87.43
1	77.83	87.67	80.18	81.75	88.25	81.01	95.58	86.93	84.81	85.47	85.56
2	76.32	87.09	79.28	78.85	86.21	77.33	90.91	85.46	82.95	85.45	82.02
2	74.97	86.64	77.89	77.84	85.68	76.29	89.98	84.68	82.36	85.17	82.34
2	76.50	86.04	78.23	77.67	85.93	77.35	91.05	86.13	83.48	85.95	82.40
2	77.55	87.96	80.63	80.23	88.17	79.01	91.55	87.27	85.16	87.68	84.81
3	68.57	75.66	62.31	61.05	76.21	64.84	86.00	71.71	71.07	73.98	71.33
3	70.02	75.33	62.22	61.24	78.14	64.24	86.62	72.86	72.68	76.85	73.33
3	70.00	75.68	62.84	61.49	76.54	65.04	84.72	71.79	71.22	74.06	71.72
3	74.72	80.14	69.59	69.66	82.13	70.84	89.03	77.17	76.63	80.94	78.16

Diet; Phenylalanine; Histidine; Lysine; Arginine; Proline; Methionine; Cystine; All average

1	87.72	89.34	90.80	90.50	86.43	94.38	86.71	87.18
1	88.13	90.05	90.95	90.65	86.65	93.63	88.02	88.53
1	88.44	90.12	90.52	95.50	85.06	94.05	86.48	88.08
1	86.47	89.04	90.58	90.13	83.54	91.39	85.68	86.71
2	84.44	87.08	89.49	87.81	80.31	92.80	82.63	84.71
2	84.97	86.84	87.99	87.41	80.20	92.19	83.46	84.23
2	84.91	86.67	88.93	87.25	79.36	95.59	84.93	84.82
2	87.06	88.53	89.64	88.89	81.53	98.88	85.01	86.59
3	75.03	71.91	80.72	81.24	69.22	79.43	67.33	72.88
3	77.29	72.08	80.73	82.66	71.11	81.81	67.83	73.94
3	75.25	71.65	81.16	82.96	69.65	81.08	69.39	73.31
3	80.90	77.44	85.00	85.53	75.86	85.65	74.66	78.78

Analysis of Variance for Nitrogen digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	162.499	162.499	81.250	22.74	0.000
Error	9	32.155	32.155	3.573		
Total	11	194.654				

Analysis of Variance for Aspartic acid

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	304.85	304.85	152.42	73.29	0.000
Error	9	18.72	18.72	2.08		
Total	11	323.56				

Analysis of Variance for Threonine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	594.62	594.62	297.31	58.09	0.000
Error	9	46.06	46.06	5.12		
Total	11	640.69				

Analysis of Variance for Serine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	799.02	799.02	399.51	60.89	0.000
Error	9	59.05	59.05	6.56		
Total	11	858.07				

Analysis of Variance for Glutamic acid

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	264.43	264.43	132.22	43.11	0.000
Error	9	27.60	27.60	3.07		
Total	11	292.04				

Analysis of Variance for Glycine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	466.34	466.34	233.17	58.94	0.000
Error	9	35.61	35.61	3.96		
Total	11	501.95				

Analysis of Variance for Alanine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	176.926	176.926	88.463	69.67	0.000
Error	9	11.427	11.427	1.270		
Total	11	188.353				

Analysis of Variance for Valine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	457.03	457.03	228.51	83.92	0.000
Error	9	24.51	24.51	2.72		
Total	11	481.54				

Analysis of Variance for Isoleucine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	359.04	359.04	179.52	63.96	0.000
Error	9	25.26	25.26	2.81		
Total	11	384.30				

Analysis of Variance for Leucine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	276.64	276.64	138.32	30.99	0.000
Error	9	40.17	40.17	4.46		
Total	11	316.81				

Analysis of Variance for Tyrosine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	512.75	512.75	256.37	12.68	0.002
Error	9	182.02	182.02	20.22		
Total	11	694.77				

Analysis of Variance for Phenilalanine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	246.74	246.74	123.37	39.03	0.000
Error	9	28.45	28.45	3.16		
Total	11	275.19				

Analysis of Variance for Histidine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	626.14	626.14	313.07	107.26	0.000
Error	9	26.27	26.27	2.92		
Total	11	652.41				

Analysis of Variance for Lysine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	174.722	174.722	87.361	53.42	0.000
Error	9	14.719	14.719	1.635		
Total	11	189.441				

Analysis of Variance for Arginine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	148.345	148.345	74.173	21.76	0.000
Error	9	30.679	30.679	3.409		
Total	11	179.024				

Analysis of Variance for Proline

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	399.57	399.57	199.79	49.47	0.000
Error	9	36.35	36.35	4.04		
Total	11	435.92				

Analysis of Variance for Methionine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	396.18	396.18	198.09	32.79	0.000
Error	9	54.37	54.37	6.04		
Total	11	450.55				

Analysis of Variance for Cystine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	660.72	660.72	330.36	73.10	0.000
Error	9	40.67	40.67	4.52		
Total	11	701.40				

Analysis of Variance for all average

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DIET	2	373.39	373.39	186.70	60.57	0.000
Error	9	27.74	27.74	3.08		
Total	11	401.13				

Experiment 6.

Variables
Treatment; Aspartic acid; Threonine; Serine; Glutamic acid; Glycine; Alanine; Valine; Isoleucine; Leucine

1	54.62	50.01	52.66	53.68	48.64	53.42	53.61	56.55	61.13
1	55.16	51.75	51.73	58.12	50.84	57.38	56.29	58.99	63.81
1	54.75	50.96	53.75	58.44	51.69	55.95	56.06	59.35	64.94
2	60.46	56.14	55.88	63.42	54.94	60.84	60.71	63.98	67.92
2	53.08	48.51	48.20	56.64	49.24	54.89	53.88	56.75	62.04
2	57.08	54.11	56.25	61.86	52.41	57.96	58.83	63.06	68.03
3	63.06	57.55	57.96	67.24	59.42	64.41	63.84	67.32	70.59
3	58.86	53.81	53.84	63.88	54.79	59.42	60.98	64.50	69.23
3	55.91	53.52	54.77	61.25	52.99	58.24	59.07	61.88	67.16
4	51.28	47.38	48.22	56.28	45.77	53.24	53.87	56.44	61.75
4	55.47	51.96	53.89	59.72	50.54	56.37	58.14	61.10	65.87
4	53.76	51.43	50.96	58.74	50.61	55.98	58.10	61.85	67.71

Treatment; Tyrosine; Phenilalanine; Histidine; Lysine; Arginine; Proline; Cystine; All average; Treatment; Nitrogen digestibility

1	63.82	60.07	49.83	48.83	76.66	52.50	47.74	57.87
1	66.90	61.82	54.54	52.90	76.68	54.23	55.70	60.40
1	68.02	64.21	52.85	42.81	87.62	53.53	50.91	60.34
2	71.63	66.64	57.04	55.94	88.01	57.71	51.89	64.30
2	64.68	60.63	51.03	50.69	100.00	52.43	40.53	59.01
2	71.77	67.28	55.94	54.54	100.00	61.14	40.60	63.58
3	72.38	69.68	59.56	62.04	100.00	64.08	53.69	67.81
3	70.73	67.98	56.15	57.99	100.00	57.99	40.46	64.15
3	69.84	65.90	56.24	55.59	83.33	59.70	56.49	63.05
4	64.74	59.29	50.80	51.15	80.08	54.05	54.86	58.19
4	68.35	63.90	49.23	54.38	84.61	51.53	46.87	60.70
4	69.23	66.22	56.27	53.27	92.27	36.95	35.61	59.94

1	45.34
1	49.60
1	57.76
1	51.90
1	51.60
2	54.92
2	48.75
2	55.62
2	47.65
2	49.01
3	57.45
3	51.76
3	54.51
3	60.65
3	45.16
4	55.53
4	48.03
4	50.63
4	48.88
4	45.95

Analysis of Variance for Aspartic acid

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	57.026	57.026	19.009	2.45	0.139
Error	8	62.154	62.154	7.769		
Total	11	119.180				

Analysis of Variance for Threonine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	40.711	40.711	13.570	1.96	0.199
Error	8	55.408	55.408	6.926		
Total	11	96.119				

Analysis of Variance for Serine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	31.288	31.288	10.429	1.21	0.366
Error	8	68.771	68.771	8.596		
Total	11	100.059				

Analysis of Variance for Glutamic acid

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	93.165	93.165	31.055	3.90	0.055
Error	8	63.685	63.685	7.961		
Total	11	156.850				

Analysis of Variance for Glycine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	76.813	76.813	25.604	3.49	0.070
Error	8	58.670	58.670	7.334		
Total	11	135.483				

Analysis of Variance for Alanine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	57.636	57.636	19.212	2.90	0.102
Error	8	53.022	53.022	6.628		
Total	11	110.659				

Analysis of Variance for Valine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	58.735	58.735	19.578	2.96	0.098
Error	8	52.877	52.877	6.610		
Total	11	111.613				

Analysis of Variance for Isoleucine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	64.635	64.635	21.545	2.55	0.129
Error	8	67.605	67.605	8.451		
Total	11	132.240				

Analysis of Variance for Leucine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	50.959	50.959	16.986	2.44	0.139
Error	8	55.741	55.741	6.968		
Total	11	106.700				

Analysis of Variance for Tyrosine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	39.322	39.322	13.107	1.84	0.218
Error	8	56.968	56.968	7.121		
Total	11	96.290				

Analysis of Variance for Phenilalanine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	57.919	57.919	19.306	2.28	0.156
Error	8	67.610	67.610	8.451		
Total	11	125.530				

Analysis of Variance for Histidine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	52.611	52.611	17.537	2.10	0.178
Error	8	66.735	66.735	8.342		
Total	11	119.346				

Analysis of Variance for Lysine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	161.93	161.93	53.98	4.65	0.037
Error	8	92.96	92.96	11.62		
Total	11	254.89				

Analysis of Variance for Arginine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	495.53	495.53	165.18	3.02	0.094
Error	8	436.97	436.97	54.62		
Total	11	932.49				

Analysis of Variance for Proline

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	281.24	281.24	93.75	3.26	0.081
Error	8	230.19	230.19	28.77		
Total	11	511.43				

Analysis of Variance for Cystine

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	105.34	105.34	35.11	0.62	0.620
Error	8	451.30	451.30	56.41		
Total	11	556.64				

Analysis of Variance for All average

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TreatAA	3	60.855	60.855	20.285	4.46	0.040
Error	8	36.365	36.365	4.546		
Total	11	97.220				

Analysis of Variance for Nitrogen digestibility

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treat	3	44.12	44.12	14.71	0.71	0.557
Error	16	329.18	329.18	20.57		
Total	19	373.30				

Experiment 7. Study 1.

Variables
Diet; sex; Total consumption; Final Weight; Weight gain; Food:Gain ratio; Initial weight

15	1	1083	760	233	4.65	527
15	1	856	671	148	5.78	523
15	1	850	727	169	5.03	558
15	1	872	700	146	5.97	554
15	1	977	756	240	4.07	516
15	2	612	627	139	4.40	488
15	2	1097	722	230	4.77	492
15	2	1004	791	251	4.00	540
15	2	864	670	170	5.08	500
15	2	855	709	183	4.67	526
15	2	984	770	232	4.24	538
25	1	911	783	185	4.92	598
25	1	977	773	249	3.92	524
25	1	1183	992	400	2.96	592
25	1	1001	834	270	3.71	564
25	1	1132	855	315	3.59	540
25	1	1098	788	311	3.53	477
25	2	735	643	102	7.21	541
25	2	1257	838	328	3.83	510
25	2	826	702	225	3.67	477
25	2	851	699	161	5.29	538
25	2	1156	778	285	4.06	493
25	2	940	777	259	3.63	518
35	1	876	765	195	4.49	570
35	1	800	685	108	7.41	577
35	1	1027	870	339	3.03	531
35	1	944	774	168	5.62	606
35	2	810	742	247	3.28	495
35	2	1175	826	305	3.85	521
35	2	1046	801	264	3.96	537
35	2	1003	787	237	4.23	550
35	2	857	680	171	5.01	509
35	2	726	575	98	7.41	477
M100	1	1123	803	192	5.85	611
M100	1	1090	760	201	5.42	559
M100	1	920	711	158	5.82	553
M100	1	985	725	246	4.00	479
M100	1	932	734	173	5.39	561
M100	1	994	738	208	4.78	530
M100	2	805	622	147	5.48	475
M100	2	899	698	203	4.43	495
M100	2	927	716	181	5.12	535
M100	2	850	684	127	6.69	557
M100	2	645	592	67	9.63	525
Soya	1	1131	1026	471	2.40	555
Soya	1	1178	920	380	3.10	540
Soya	1	1168	964	414	2.82	550
Soya	1	898	749	189	4.75	560
Soya	1	820	638	181	4.53	457
Soya	1	1177	751	157	7.50	594
Soya	2	1153	900	329	3.50	571

Soya	2	889	749	289	3.08	460
Soya	2	1115	931	431	2.59	500
Soya	2	863	806	294	2.94	512
Soya	2	967	804	290	3.33	514

Analysis of Variance for Total consumption

Source	DF	Seq SS	Adj SS	Adj MS	F	P
InicWgt	1	82719	39166	39166	2.05	0.159
level	4	136570	128136	32034	1.68	0.171
sex	1	17567	17567	17567	0.92	0.342
Error	48	916692	916692	19098		
Total	54	1153548				

Analysis of Variance for Final weight

Source	DF	Seq SS	Adj SS	Adj MS	F	P
InicWgt	1	61270	38421	38421	6.61	0.013
level	4	133793	131173	32793	5.64	0.001
sex	1	3588	3588	3588	0.62	0.436
Error	48	279189	279189	5816		
Total	54	477840				

Analysis of Variance for Weight gain

Source	DF	Seq SS	Adj SS	Adj MS	F	P
InicWgt	1	596	1696	1696	0.29	0.592
level	4	133793	131173	32793	5.64	0.001
sex	1	3588	3588	3588	0.62	0.436
Error	48	279189	279189	5816		
Total	54	417166				

Analysis of Variance for Food: gain ratio

Source	DF	Seq SS	Adj SS	Adj MS	F	P
InicWgt	1	5.543	5.566	5.566	3.41	0.071
level	4	24.128	23.760	5.940	3.64	0.011
sex	1	1.076	1.076	1.076	0.66	0.421
Error	48	78.374	78.374	1.633		
Total	54	109.121				

Experiment 7. Study 2.

Variables
Brooder; Diet; Body Weight (1st week); Weight gain (1st week); Body Weight (2nd week); Weight gain (2nd week); Body Weight (3rd week); Weight gain (3rd week); Total gain

1	2	98	56.8	227	129	360	133	318.8
1	2	102	60.8	275	173	453	178	411.8
1	2	94	52.8	236	142	409	173	367.8
1	2	106	64.8	287	181	562	275	520.8
1	3	95	53.8	207	112	315	108	273.8
1	3	101	59.8	236	135	390	154	348.8
1	3	102	60.8	251	149	395	144	353.8
1	3	91	49.8	212	121	260	48	218.8
1	3	116	74.8	224	108	330	106	288.8
1	3	104	62.8	232	128	391	159	349.8
1	3	101	59.8	263	162	457	194	415.8
1	3	102	60.8	220	118	336	116	294.8
1	2	103	61.8	300	197	561	261	519.8
1	2	118	76.8	312	194	544	232	502.8
1	2	98	56.8	253	155	454	201	412.8
1	2	108	66.8	275	167	494	219	452.8
1	1	98	56.8	253	155	451	198	409.8
1	1	86	44.8	254	168	476	222	434.8
1	1	138	96.8	368	230	684	316	642.8
1	1	119	77.8	293	174	519	226	477.8
1	1	136	94.8	368	232	658	290	616.8
1	1	129	87.8	355	226	582	227	540.8
1	1	135	93.8	337	202	560	223	518.8
1	1	144	102.8	385	241	613	228	571.8
1	3	109	67.8	242	133	378	136	336.8
1	3	122	80.8	269	147	411	142	369.8
1	3	106	64.8	253	147	448	195	406.8
1	3	110	68.8	258	148	395	137	353.8
1	2	108	66.8	252	144	397	145	355.8
1	2	118	76.8	244	126	422	178	380.8
1	2	110	68.8	293	183	492	199	450.8
1	2	110	68.8	315	205	517	202	475.8
1	2	115	73.8	306	191	492	186	450.8
1	2	103	61.8	299	196	540	241	498.8
1	2	125	83.8	276	151	466	190	424.8
1	2	115	73.8	310	195	550	240	508.8
1	3	106	64.8	235	129	388	153	346.8
1	3	120	78.8	267	147	422	155	380.8
1	3	112	70.8	258	146	423	165	381.8
1	3	112	70.8	256	144	415	159	373.8
1	1	134	92.8	362	228	624	262	582.8
1	1	142	100.8	322	180	546	224	504.8
1	1	93	51.8	236	143	470	234	428.8
1	1	132	90.8	324	192	517	193	475.8
2	3	119	77.8	256	137	387	131	345.8
2	3	117	75.8	255	138	379	124	337.8
2	3	97	55.8	247	150	405	158	363.8
2	3	105	63.8	266	161	408	142	366.8
2	2	143	101.8	356	213	594	238	552.8
2	2	125	83.8	285	160	451	166	409.8
2	2	107	65.8	264	157	459	195	417.8

2	2	135	93.8	346	211	600	254	558.8
2	1	110	68.8	239	129	392	153	350.8
2	1	120	78.8	280	160	450	170	408.8
2	1	108	66.8	265	157	500	235	458.8
2	1	90	48.8	195	105	379	184	337.8
2	2	127	85.8	313	186	533	220	491.8
2	2	115	73.8	314	199	516	202	474.8
2	2	120	78.8	326	206	576	250	534.8
2	2	116	74.8	261	145	430	169	388.8
2	3	105	63.8	256	151	430	174	388.8
2	3	91	49.8	193	102	319	126	277.8
2	3	94	52.8	169	75	261	92	219.8
2	3	111	69.8	256	145	395	139	353.8
2	1	106	64.8	228	122	362	134	320.8
2	1	94	52.8	252	158	474	222	432.8
2	1	92	50.8	235	143	397	162	355.8
2	1	97	55.8	264	167	509	245	467.8
2	1	124	82.8	293	169	502	209	460.8
2	1	94	52.8	244	150	413	169	371.8
2	1	110	68.8	291	181	470	179	428.8
2	1	131	89.8	318	187	542	224	500.8
2	2	152	110.8	362	210	619	257	577.8
2	2	149	107.8	393	244	648	255	606.8
2	2	140	98.8	378	238	630	252	588.8
2	2	135	93.8	332	197	579	247	537.8
2	3	97	55.8	196	99	276	80	234.8
2	3	94	52.8	228	134	401	173	359.8
2	3	100	58.8	210	110	346	136	304.8
2	3	84	42.8	169	85	269	100	227.8
2	1	97	55.8	242	145	451	209	409.8
2	1	123	81.8	304	181	548	244	506.8
2	1	105	63.8	272	167	472	200	430.8
2	1	110	68.8	220	110	436	216	394.8
2	2	118	76.8	314	196	565	251	523.8
2	2	124	82.8	316	192	536	220	494.8
2	2	122	80.8	313	191	507	194	465.8
2	2	127	85.8	330	203	550	220	508.8
2	3	116	74.8	197	81	286	89	244.8
2	3	129	87.8	312	183	529	217	487.8
2	3	106	64.8	214	108	352	138	310.8
2	3	106	64.8	197	91	302	105	260.8

Analysis of Variance for Body weight 1st week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder1	1	39.8	32.2	32.2	0.15	0.699
Diet1	2	2663.1	2663.1	1331.6	6.23	0.003
Error	88	18820.3	18820.3	213.9		
Total	91	21523.2				

Analysis of Variance for Weight gain 1st week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder1	1	39.8	32.2	32.2	0.15	0.699
Diet1	2	2663.1	2663.1	1331.6	6.23	0.003
Error	88	18820.2	18820.2	213.9		
Total	91	21523.2				

Analysis of Variance for Body weight 2nd week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder1	1	1173	1552	1552	0.90	0.346
Diet1	2	79139	79139	39570	22.92	0.000
Error	88	151947	151947	1727		
Total	91	232259				

Analysis of Variance for Weight gain 2nd week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder1	1	1645	2032	2032	2.21	0.141
Diet1	2	52924	52924	26462	28.81	0.000
Error	88	80836	80836	919		
Total	91	135404				

Analysis of Variance for Body weight 3rd week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder1	1	3608	5854	5854	1.12	0.292
Diet1	2	395103	395103	197552	37.94	0.000
Error	88	458166	458166	5206		
Total	91	856877				

Analysis of Variance for Weight gain 3rd week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder1	1	667	1377	1377	1.00	0.320
Diet1	2	123400	123400	61700	44.76	0.000
Error	88	121309	121309	1379		
Total	91	245375				

Analysis of Variance for total gain

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder1	1	3608	5854	5854	1.12	0.292
Diet1	2	395103	395103	197552	37.94	0.000
Error	88	458166	458166	5206		
Total	91	856877				

Variables

Brooder; Diet; Food intake/bird/ 1st week; Food:gain ratio 1st week; Food intake/bird 2nd week; Food:gain ratio 2nd week; Food intake/bird 3rd week; Food:gain ratio 3rd week; Total Food intake; Total Food:gain ratio

1	2	80.91	1.46	256.50	1.61	321.75	1.69	659.16	1.63
1	3	120.50	2.07	232.75	1.84	250.00	2.19	603.25	2.02
1	3	109.46	1.74	250.75	1.93	253.75	1.76	613.96	1.82
1	2	100.00	1.52	267.00	1.50	355.25	1.56	722.25	1.53
1	1	104.17	1.45	266.00	1.49	348.75	1.45	718.92	1.46
1	1	129.67	1.31	329.75	1.49	369.00	1.52	828.42	1.47
1	3	115.50	1.67	260.00	1.79	282.50	1.85	658.00	1.79
1	2	116.33	1.62	275.25	1.69	308.25	1.70	699.83	1.68
1	2	114.33	1.59	292.50	1.59	350.25	1.63	757.08	1.61
1	3	114.67	1.59	250.50	1.78	290.50	1.84	655.67	1.77
1	1	112.50	1.37	282.50	1.50	358.00	1.57	753.00	1.51
2	3	111.00	1.68	252.50	1.69	260.75	1.89	624.25	1.77
2	2	141.00	1.68	302.75	1.62	353.75	1.65	797.50	1.64
2	1	103.00	1.54	226.50	1.67	275.75	1.48	605.25	1.56
2	2	129.33	1.68	309.75	1.67	341.75	1.63	780.83	1.65
2	3	91.17	1.57	217.50	1.83	234.75	1.77	543.42	1.75
2	1	83.67	1.58	226.75	1.51	282.00	1.48	592.42	1.50
2	1	110.00	1.51	263.75	1.53	298.25	1.53	672.00	1.53
2	2	134.67	1.35	356.00	1.58	415.75	1.64	906.42	1.57
2	3	82.50	1.59	206.25	1.93	236.50	1.92	525.25	1.86
2	1	96.17	1.48	239.75	1.57	321.00	1.48	656.92	1.51
2	2	116.83	1.44	307.00	1.57	347.50	1.57	771.33	1.55
2	3	115.83	1.59	246.00	2.12	270.75	1.98	632.58	1.94

Analysis of Variance for Food intake 1st week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	7.4	3.1	3.1	0.01	0.915
diet	2	534.4	534.4	267.2	0.99	0.390
Error	19	5121.9	5121.9	269.6		
Total	22	5663.6				

Analysis of Variance for Food: gain ratio 1st week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	0.00314	0.00116	0.00116	0.06	0.803
diet	2	0.19483	0.19483	0.09742	5.35	0.014
Error	19	0.34588	0.34588	0.01820		
Total	22	0.54386				

Analysis of Variance for Food intake 2nd week

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	245.0	221.4	221.4	0.25	0.626
diet	2	12810.5	12810.5	6405.2	7.11	0.005
Error	19	17119.3	17119.3	901.0		
Total	22	30174.9				

Analysis of Variance for Food: gain ratio 2nd

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	0.00718	0.01289	0.01289	1.57	0.225
diet	2	0.46434	0.46434	0.23217	28.27	0.000
Error	19	0.15603	0.15603	0.00821		
Total	22	0.62755				

Analysis of Variance for Food intake 3rd

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	1106	1284	1284	1.43	0.247
diet	2	33550	33550	16775	18.64	0.000
Error	19	17098	17098	900		
Total	22	51754				

Analysis of Variance for Food: gain ratio 3rd

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	0.00791	0.00255	0.00255	0.31	0.587
diet	2	0.62132	0.62132	0.31066	37.28	0.000
Error	19	0.15832	0.15832	0.00833		
Total	22	0.78755				

Analysis of Variance for total food intake

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	2665	2755	2755	0.55	0.466
diet	2	96011	96011	48006	9.64	0.001
Error	19	94646	94646	4981		
Total	22	193322				

Analysis of Variance for total food: gain ratio

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	0.00060	0.00005	0.00005	0.01	0.918
diet	2	0.44717	0.44717	0.22359	47.27	0.000
Error	19	0.08987	0.08987	0.00473		
Total	22	0.53764				

Variables
Brooder; Diet; Haemoglobin; Haematocrit; Red cell count

1	1	7.5	28.0	1.86
1	1	7.5	28.0	1.05
1	1	8.4	28.5	1.51
1	1	7.5	28.5	1.21
1	1	7.6	27.0	1.00
1	1	7.8	29.5	1.76
1	1	7.6	27.5	1.51
1	1	8.1	30.0	1.30
2	1	8.4	29.0	1.53
2	1	8.0	28.5	1.54
2	1	8.2	30.0	1.80
2	1	8.7	27.5	1.71
2	1	8.8	32.5	1.63

2	1	8.0	28.0	2.10
2	1	8.0	27.0	1.56
2	1	8.0	27.0	1.98
1	2	8.1	27.0	1.10
1	2	8.4	27.5	1.06
1	2	8.2	27.0	1.22
1	2	7.8	25.0	1.71
1	2	9.3	31.0	1.92
1	2	8.8	30.5	2.23
1	2	8.1	31.5	1.36
1	2	8.7	28.5	1.46
2	2	8.2	29.5	1.34
2	2	7.8	27.0	1.87
2	2	8.1	26.5	1.41
2	2	8.7	27.5	2.13
2	2	7.6	26.5	1.33
2	2	8.6	31.5	1.64
2	2	7.5	27.5	1.05
2	2	7.0	24.0	1.68
1	3	7.3	23.5	1.82
1	3	8.3	29.0	1.53
1	3	6.3	22.5	1.66
1	3	7.0	23.5	2.20
1	3	8.1	29.0	1.57
1	3	8.1	29.0	1.21
1	3	8.2	29.0	1.64
1	3	7.4	25.5	1.57
2	3	7.8	27.0	1.60
2	3	7.5	27.0	1.99
2	3	8.7	31.5	1.54
2	3	7.5	27.0	1.27
2	3	8.4	27.0	1.84
2	3	6.9	25.0	1.98
2	3	7.4	25.0	2.02
2	3	7.6	28.5	1.36

Analysis of Variance for Haemoglobin

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	0.0352	0.0352	0.0352	0.12	0.735
Diet	2	2.2867	2.2867	1.1433	3.75	0.031
Error	44	13.3979	13.3979	0.3045		
Total	47	15.7198				

Analysis of Variance for Haematocrit

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	0.047	0.047	0.047	0.01	0.919
Diet	2	24.781	24.781	12.391	2.73	0.076
Error	44	199.625	199.625	4.537		
Total	47	224.453				

Analysis of Variance Red cell count

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	0.2465	0.2465	0.2465	2.45	0.125
Diet	2	0.1791	0.1791	0.0896	0.89	0.418
Error	44	4.4303	4.4303	0.1007		
Total	47	4.8560				

Variables

Brooder; Diet; Body Weight; Gizzard (g); Gizzard (g/kg); Small intestine (g); Small intestine (g/kg); Caeca (g); Caeca (g/kg); Caeca length average; Viscosity average

1	1	539	15.20	28.20	17.40	32.28	7.50	13.91	10.25	2.03
1	1	573	16.50	28.80	21.80	38.05	4.50	7.85	9.25	2.65
1	1	642	17.40	27.10	18.11	28.21	5.00	7.79	10.50	2.75
1	1	660	15.70	23.79	23.70	35.91	6.30	9.55	11.50	3.13
1	1	592	15.40	26.01	22.00	37.16	5.00	8.45	10.00	2.28
1	1	584	15.80	27.05	17.00	29.11	3.80	6.51	10.50	2.33
1	1	645	17.80	27.60	24.40	37.83	4.70	7.29	9.00	1.73
2	1	564	11.00	19.50	19.30	34.22	5.15	9.13	10.75	2.58
2	1	567	12.70	22.40	21.80	38.45	4.40	7.76	10.00	2.43
2	1	602	16.40	27.24	18.50	30.73	3.90	6.48	9.50	1.98
2	1	537	13.80	25.70	18.10	33.71	5.10	9.50	9.50	1.95
2	1	588	13.00	22.11	20.00	34.01	5.20	8.84	9.50	1.68
2	1	624	13.80	22.12	23.20	37.18	3.50	5.61	9.50	2.28
2	1	532	14.60	27.44	26.00	48.87	4.40	8.27	9.25	1.95
2	1	542	15.80	29.15	17.00	31.37	5.40	9.96	10.50	1.85
2	1	639	16.11	25.21	27.00	42.25	7.00	10.95	13.25	2.03
1	2	664	18.00	27.11	20.90	31.48	9.66	14.55	12.50	1.53
1	2	574	15.40	26.83	20.40	35.54	5.40	9.41	11.00	1.38
1	2	647	13.60	21.02	22.70	35.09	10.7	16.54	13.50	1.35
1	2	643	15.40	23.95	20.60	32.04	5.80	9.02	11.50	1.70
1	2	569	10.70	18.80	18.26	32.09	9.90	17.40	10.00	1.48
1	2	614	13.30	21.66	26.00	42.35	9.60	15.64	14.00	1.60
1	2	570	13.20	23.16	24.60	43.16	8.00	14.04	13.00	1.38
1	2	587	13.30	22.66	19.90	33.90	6.93	11.81	13.00	1.50
1	2	659	19.00	28.83	29.00	44.01	9.11	13.82	11.50	1.60
1	2	620	15.42	24.87	22.00	35.48	6.90	11.13	12.00	1.88
2	2	575	11.90	20.70	21.20	36.87	6.20	10.78	11.50	1.38
2	2	614	11.46	18.66	20.90	34.04	6.98	11.37	11.50	1.45
2	2	617	11.20	18.15	20.20	32.74	6.22	10.08	11.00	1.60
2	2	591	12.40	20.98	19.40	32.83	5.70	9.64	12.75	1.40
2	2	600	12.20	20.33	24.50	40.83	4.70	7.83	9.50	1.38
2	2	637	15.00	23.55	26.00	40.82	8.00	12.56	13.50	1.53
1	3	474	8.96	18.90	16.00	33.76	5.11	10.78	12.00	1.35
1	3	462	9.47	20.50	20.80	45.02	7.34	15.89	12.00	1.05
1	3	387	8.76	22.64	17.06	44.08	4.70	12.14	11.50	1.10
1	3	409	9.75	23.84	18.10	44.25	9.24	22.59	12.75	1.35
1	3	465	12.40	26.67	21.30	45.81	8.21	17.66	11.25	1.33
1	3	451	11.80	26.16	19.20	42.57	10.92	24.21	12.00	1.25
1	3	461	8.60	18.66	25.50	55.31	6.50	14.10	12.00	1.13
1	3	461	9.54	20.69	21.29	46.18	8.80	19.09	13.75	1.28
2	3	437	11.50	26.32	23.20	53.09	7.30	16.70	12.00	1.18
2	3	451	11.10	24.61	19.90	44.12	8.20	18.18	11.50	1.35
2	3	470	9.60	20.43	22.80	48.51	6.70	14.26	10.50	1.25
2	3	384	9.60	25.00	21.40	55.73	7.39	19.24	11.50	1.15
2	3	470	8.70	18.51	17.17	36.53	14.8	31.49	12.00	1.15

2	3	397	7.53	18.97	20.25	51.01	6.94	17.48	10.00	1.18
2	3	480	8.40	17.50	24.20	50.42	3.18	6.63	11.00	1.25
2	3	406	9.66	23.79	18.70	46.06	5.45	13.42	11.50	1.45

Analysis of Variance for Gizzard(g)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BodyWgt	1	255.753	21.638	21.638	8.54	0.006
Brooder	1	10.656	19.090	19.090	7.53	0.009
Diet	2	37.419	37.419	18.710	7.38	0.002
Error	43	108.997	108.997	2.535		
Total	47	412.825				

Analysis of Variance for Gizzard (g/kg)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BodyWgt	1	20.877	6.701	6.701	0.79	0.378
Brooder	1	27.651	54.970	54.970	6.52	0.014
Diet	2	122.989	122.989	61.495	7.29	0.002
Error	43	362.481	362.481	8.430		
Total	47	533.998				

Analysis of Variance for Intestine (g)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BodyWgt	1	48.945	61.200	61.200	7.92	0.007
Brooder	1	2.871	9.728	9.728	1.26	0.268
Diet	2	40.748	40.748	20.374	2.64	0.083
Error	43	332.312	332.312	7.728		
Total	47	424.876				

Analysis of Variance for Intestine (g/kg)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BodyWgt	1	1046.32	12.10	12.10	0.45	0.507
Brooder	1	10.99	37.80	37.80	1.40	0.244
Diet	2	161.07	161.07	80.54	2.98	0.062
Error	43	1163.60	1163.60	27.06		
Total	47	2381.98				

Analysis of Variance for Caeca(g)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BodyWgt	1	4.194	3.127	3.127	0.80	0.376
Brooder	1	14.712	4.752	4.752	1.22	0.276
Diet	2	55.969	55.969	27.984	7.16	0.002
Error	43	167.993	167.993	3.907		
Total	47	242.868				

Analysis of Variance for Caeca (g/kg)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BodyWgt	1	366.49	4.49	4.49	0.29	0.594
Brooder	1	47.79	14.38	14.38	0.92	0.342
Diet	2	184.30	184.30	92.15	5.92	0.005
Error	43	668.78	668.78	15.55		
Total	47	1267.36				

Term	Coef	StDev	T	P
Constant	17.517	9.114	1.92	0.061
BodyWgt	-0.00893	0.01663	-0.54	0.594

Analysis of Variance for Caeca lenght average

Source	DF	Seq SS	Adj SS	Adj MS	F	P
BodyWgt	1	0.217	2.648	2.648	2.42	0.127
Brooder	1	5.861	1.534	1.534	1.40	0.243
Diet	2	29.680	29.680	14.840	13.54	0.000
Error	43	47.116	47.116	1.096		
Total	47	82.874				

Analysis of Variance for Viscosity

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Brooder	1	0.0406	0.2094	0.2094	3.40	0.072
Diet	2	8.5322	8.5322	4.2661	69.25	0.000
Error	44	2.7104	2.7104	0.0616		
Total	47	11.2832				

PUBLICATIONS ARISING FROM THE THESIS WORK

Sarmiento-Franco, L., MacLeod, M.G. & McNab. J.M. (2000) True metabolisable energy, heat increment and net energy values of two high fibre foodstuffs in cockerels. *British Poultry Science* **41:** 625-629.

Sarmiento-Franco, L., McNab, J.M., Pearson, A. and Belmar, C. (2001) Performance of chickens fed on diets containing different amounts of chaya (*Cnidoscolus aconitifolius*) leaf meal. *Tropical Animal Health and Production*. In press.

Sarmiento-Franco, L., McNab, J.M., Pearson, A. and Belmar, C. L. Amino acid digestibility in chaya (*Cnidoscolus aconitifolius*) leaf meal and its effect on diets for broilers *British Poultry Science*. Submitted.