

**Influence of insoluble fibre in poultry feeds on
energy utilisation and gut parameters in broiler
chickens**

By

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text.

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Janet Caritas Doru Pandi

06/03/06 /

Abstract

In Papua New Guinea (PNG), the major constraint limiting profitability of small-scale broiler producers is the high cost of commercial feeds. The high feed prices are due to the inclusion of a high proportion of imported grains in the feeds. Hence, economic viability of poultry production is more dependent on the price of imported grains than it is on growth rates of chickens. Current research in PNG funded by the Australian Centre for Agricultural Research (ACIAR) is targeted at utilising locally available resources so as to reduce the proportion of imports and thus the prices of feeds. However, many locally available feedstuffs contain high proportions of fibrous materials, which may impede performance of broiler chickens.

The overall aim of the ACIAR project is to examine the potential for improvement of the economic viability of small-scale broiler chicken production in PNG, by greater usage of locally-grown feed ingredients. The objective of my studies was to determine the consequences of high levels of insoluble fibrous materials on growth rate, feed efficiency, nutrient digestibility, digestive organ structure, and morphology of intestinal mucosa of broiler chickens.

The sources of insoluble fibre used in this project were cellulose, wheat straw, palm kernel expeller meal (PKE) and copra meal (CM) included at varying levels. The cellulose and wheat straw were included at levels up to 15% while palm kernel and copra meals were used at 30%. Cellulose and wheat straw were chosen for experiments in Australia to demonstrate the effects of relatively pure sources of insoluble fibre with minimum interference from other antinutritive factors often present in feed ingredients such as palm kernel meal and copra meal. The experiment in PNG studied the effects

of locally-grown fibrous ingredients on broiler production and to validate the findings from experiments in Australia.

Cellulose did not significantly affect performance, digesta passage time or relative organs weights. However, the apparent metabolisable energy (AME) and the dry matter digestibility (DMD) were significantly reduced ($P < 0.05$). Wheat straw had a significant effect on the gizzard weight, feed conversion efficiency as well as apparent metabolisable energy and dry matter digestibility of the diets.

Birds on diets with palm kernel meal showed significantly lower gains, and feed conversion efficiency. Apparent metabolisable energy and dry matter digestibility were also significantly reduced. Birds on the PKE diet also had significantly higher gizzard weights. No significant differences were observed on relative weight of the small intestines nor were there any significant differences observed on villus height, crypt depth and villus: crypt ratio. The relative size of the gizzard was increased by dietary fibre as shown by other researchers, but the effects of insoluble fibre at the mucosal level in this project were inconclusive.

In summary, I was able to show that inclusion of insoluble fibre at high levels diminished growth performance and nutrient digestibility, especially the AME value of the diet. However, on an economic basis, these reductions in performance may be offset by the cheapness of locally-grown feed ingredients in PNG. Further study of the effects of insoluble fibre on pancreas size and possibly its function is warranted.

Also, more work needs to be conducted to determine the effect of high dietary levels of insoluble fibre at the mucosal level in birds given diets containing tropical feed ingredients such as palm kernel meal and copra meal.

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

In Papua New Guinea (PNG), the major constraint affecting small-scale broiler producers is the high cost of commercial feeds. The high feed prices are due to the inclusion of a high proportion of imported grains in the feeds. Economic viability of poultry production is more dependent on the price of imported grains than it is on growth rates. Research currently is targeted at utilising locally available resources so as to reduce the proportion of imports and thus the prices of feeds. However, many locally available feedstuffs contain high proportions of fibrous materials.

Dietary fibre consists mainly of non-starch polysaccharides (NSP) and lignin, which are resistant to hydrolysis by the digestive enzymes of monogastric animals. The fibre fraction in the diets has been found to have anti-nutritive effects on chickens. Fibre, which is negligibly digestible, depresses performance of birds. The role of fibre in digestion by chickens is poorly understood because the focus of chicken husbandry in the developed countries has been to maximise energy intake using high quality feeds. Modern day husbandry practices are based on use of cereal-based feeds and profitability is dependent on access to cost-effective diets (Graham, 1991). Formulation of feeds is not simple as different feed ingredients are blended, and because each feedstuff has a given nutritive value, inclusion of one will affect the nutritive value of the other (Graham, 1991). There are many factors that affect the capabilities of birds to digest and absorb nutrients. Hughes (2001; 2003) reviewed bird- and feed-related factors such as NSP content in feeds and gut morphology, digesta passage rate and microflora as some examples of factors that affect the capability of birds to digest and absorb nutrients.

This project aims to improve the economic viability of small-scale poultry production in PNG, with the objectives of determining the effects of high fibrous material on the functional capacity of the digestive organs in chickens and the digestibility of other nutrients present in diets.

The literature review for this project covers the small-scale broiler industry in PNG, its importance, the need to enhance its growth, the current constraints facing it and the measures taken to improve its viability. This section leads into the indigenous feed resources available in the country and the limitations for their use as ingredients in broiler rations. Because these limitations involve the high fibre content in the indigenous ingredients, carbohydrates are discussed in general, including the types of carbohydrates, the soluble and insoluble fractions of the NSP and their effects on the nutritive value of broiler diets. It then briefly describes the digestive system in broilers, the effect of NSP with regard to the gizzard and the small intestinal mucosa of chickens and gut microflora in general.

1.2 Small scale broiler chicken production in Papua New Guinea

1.2.1 Brief history

The broiler industry (especially the frozen chicken meat market) in PNG is well established. The commercial poultry industry was established in the early 1980s and has since grown due to an import ban on other competing products, which in 1992 was replaced by a high specific tariff (ACIAR, 2000). Niugini Table Birds, the largest company involved in the frozen chicken meat market, has contract farmers who produce chickens in rural villages. This smallholder scheme involves the company selling day-old chicks and feed to the growers with a promise to buy back fully-grown

chickens for processing. The net profit received by the contracted farmers is the price paid by the company less the cost of feeds, chickens and services (e.g., the provision of advice from extension officers to teach growers about poultry husbandry and management).

In addition to the fresh and frozen commercial markets, there are smallholder producers who sell live broiler chickens in local markets. There are about 50,000 small-scale poultry farmers who are traditional subsistence farming families with this enterprise as their sole or major source of income. These farmers operate independently from the commercial frozen carcass industry, and are currently producing about 6.7 million birds per year. These small-scale farming families are scattered through out the country, with most living in the peri-urban (fringe) areas of major cities and towns, in squatter settlements, along the main highways, and in rural villages. These farmers purchase broiler day-old chicks (a batch of 52), and raise them for 6-8 weeks on commercial feeds. For most, this is the only source of income, which contributes to payment of school fees, provision of food for the families, meeting of social obligations such as bride price, and every day costs. Due to economic pressures, these small-scale broiler farmers are threatened by the rising commercial feed prices, and may either cease or limit production.

1.2.2 Major constraints

The major constraint facing smallholder broiler farmers is the high cost of commercial broiler feeds. The largest commercial feed company in the country relies heavily on imported feedstuffs, especially grains, for use as ingredients in the manufacture of feeds. For farmers living in remote areas where accessibility is either by plane or ship, the costs of feeds are twice that of the costs in major towns and cities due to transportation fees. Thus availability and access to these feeds can be as much a

problem as feed costs. Despite high input costs and feed availability due to remoteness and lack of road access, these farmers continue to raise broiler chickens, which is possible due to high demand for live chickens.

Smallholder broiler chicken production with a gross value of AUD\$67M per annum was identified as a sector where feed costs could be reduced and profitability increased if there was greater use of local feed resources (ACIAR Poultry Feeding Systems Project, 2002). Utilising locally available materials as alternatives for imported grains can help alleviate the high feed prices. Emphasis on using available resources in areas where these resources are in abundance is now the current trend in research in the livestock sector.

Many of these locally available resources can be used as ingredients in the development of poultry feed, however, their chemical composition, based on nutritive values, must first be determined. Apparent metabolisable energy (AME) values and the dietary fibre contents of these resources must be analysed. This is currently being undertaken as part of a bigger project titled “Poultry Feeding Systems in PNG”, project number AS2/2000/077 that is funded by the Australian Centre for International Agricultural Research (ACIAR).

1.2.3 Potential feed resources

Some of the potential local food resources in PNG which can be utilised as poultry feed ingredient for least cost or lower density diets are as classified below;

1. Crop and By-products

These comprise the major cash crops such as oil palm, coconut, cocoa and coffee. Others include rice, wheat and pyrethrum.

2. Grains and Legumes

The major types of grains in the country are maize, sorghum, wheat and sunflower. Legumes include foliage as well as grains; including soybean, cowpeas, mung beans, jack beans and velvet beans. The foliages of these plants are also used.

3. Traditional Staple Crops

This group includes plantains, sago and coconut and traditional root and tuber crops such as sweet potatoes, cassava and yams. In some cases both the fruit/tubers as well as leaves could be utilised as feed ingredient.

4. Animal Proteins

Currently there are two large tuna canneries in the country providing fishmeal as a rich source of protein. Meat and bone meal as well as other abattoir by-products are also available resources for poultry feeds.

1.3 Dietary fibre and carbohydrates

1.3.1 Dietary fibre

Trowel (1996) defined dietary fibre as all polysaccharides and lignin that are not digested by the endogenous secretions of the human digestive tract. The major constituents of dietary fibre are carbohydrates such as sugar alcohols, oligosaccharides, polysaccharides and lignin (Annison, 1993). Although not hydrolysed by the endogenous secretion of monogastric animals, this fraction of a feedstuff must not be considered as inert. This fibre fraction, depending on its solubility, can either have beneficial or non-beneficial effects on the animal. Although most studies in poultry indicate that insoluble fibres are inert and therefore act only as nutrient diluents (Angkanaporn *et al.*, 1994; Carre, 2002), some other workers have suggested that fibre in general has a negative effect on nutrient digestion and absorption in chickens

(Krogdahl, 1986). It has been suggested that fibres encapsulate nutrients in the plant cell, becoming a physical barrier to digestive enzymes to slow down nutrient digestion. Several fibre sources have been shown to cause pancreatic enlargement, indicating increased secretion (Kratzer *et al.*, 1976).

1.3.2 Carbohydrates

Carbohydrates by definition consist of several series of carbon compounds characterised by having two or more hydroxyl (-OH) groups and one or more other functional groups such as aldehydes (-CHO) or ketones (-C=O), which usually exist as acetals, ketones, hemi-acetals or hemi-ketones. The main subdivisions of carbohydrates, based on molecular weight, are monosaccharides, oligosaccharides (degrees of polymerisation (DP) from 2-10) and polysaccharides (DP>10) (Carre, 2002).

1.3.3 Polysaccharides

This group of carbohydrates is found in plants, micro-organisms and animals and fulfil a wide variety of functions in living organisms. Compounds such as starch and glycogen effectively store sugars in plants and animals. Others, such as cellulose, chitin or polysaccharides of bacterial cell walls, are structural materials analogous to the structural proteins. Polysaccharides can also be referred to as glycans and are formed when ten or more monosaccharides residues are joined through glycosidic bonds between the hemi acetal or hemi ketal group of one sugar and the hydroxyl group of another, resulting in high molecular weight compounds. Polysaccharides are classified into two groups called homo- and hetero-polysaccharides depending on the type of monosaccharides they contain. As the name implies homopolysaccharides are glycans containing only one type of monosaccharide. Cellulose and starch are homopolysaccharides as they consist entirely

of glucose, and are also termed glucans (Figure 1.1). Heteropolysaccharides contain two or more monosaccharides, such as galactomannan or guar gum, which contain galactose and mannose (Figure 1.1).

Polysaccharides are either linear or branched. Branched polysaccharides contain a long main chain onto which one or more side chains are attached. Heteropolysaccharides with uronic acids as main sugars are called uronans. Some of the heteropolysaccharides contain non-carbohydrate substitutes such as acetyl ($-\text{COCH}_3$) and methyl ($-\text{CH}_3$) groups. Polysaccharides then are either starch or non-starch polysaccharides.

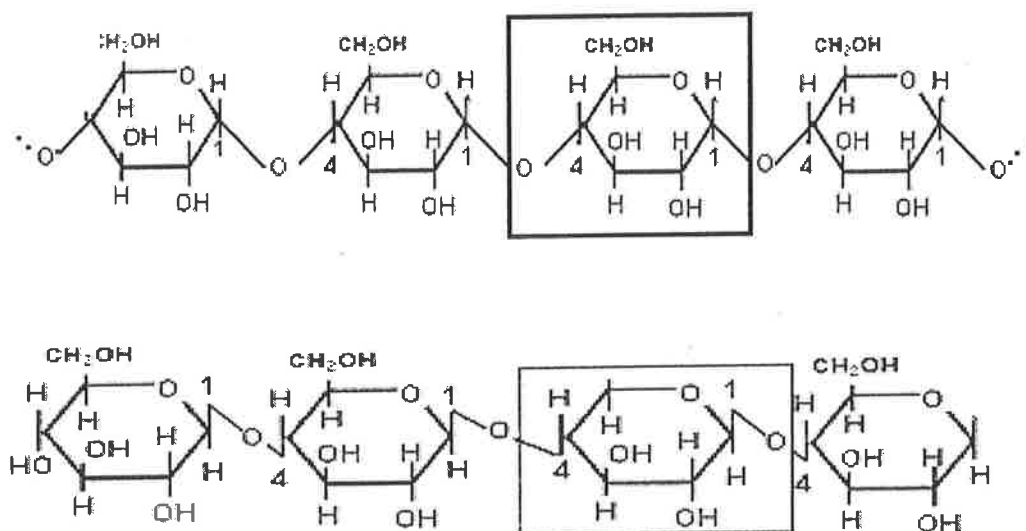


Figure 1.1 Structures of starch and cellulose

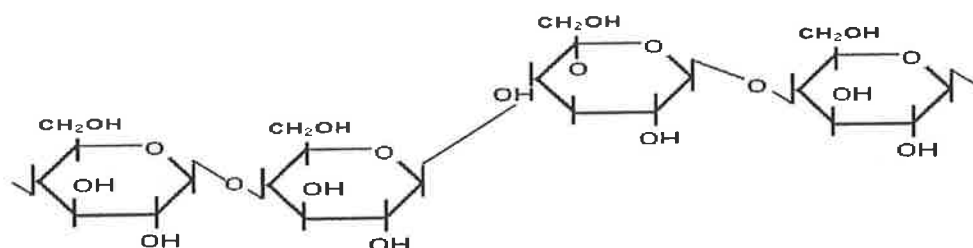


Figure 1.2 Structure of galactomannan (β - (1 $\rightarrow$ 3), (1 $\rightarrow$ 4)-D-glucan)

Starch (Figure 1.1) consists entirely of glucose units, and is therefore a glucan. Native starch is found in many plant tissues (tubers, roots, fruits, seeds) as a reserve of biological energy. Starch consists of two polymers of glucose called amylose and amylopectin. Amylose is a linear α -(1 $\rightarrow$ 4)-glucan while amylopectin is a branched α -(1 $\rightarrow$ 4)-glucan with α -(1 $\rightarrow$ 6) links at branch points (Annison, 1993). Examples of other polysaccharide compounds with similar structures are glycogen and dextrin.

1.4 Non-starch polysaccharides

Polysaccharides present in feedstuff include starch and NSP (Choct, 2002). NSP can be hydrophilic or hydrophobic depending on their molecular weights and their intermolecular bonds. NSP can either be intra cellular or extra cellular. Fructans are a major example of intra-cellular components as they act mainly as storage components, while celluloses are main examples of cell wall components, which contribute to the cell wall rigidity, form and regulation of permeability (Carre, 2002). NSP are present in several feed ingredients, such as cereal grains and some legume seeds (Choct and Annison, 1992). Different ingredients contain not only different amounts of soluble and insoluble NSP, but also the structure and the physio-chemical characteristics differ widely (Choct, 2002). Most are part of the cell wall and are closely associated with other polysaccharides, protein and lignin (Smits and Annison, 1996).

Smits and Annison (1996) noted that plant cell walls are highly ordered and consist of different polysaccharides, polyphenols, glycoproteins and glycolipids. These components are arranged in three main patterns to give fibrillar polysaccharides (mainly cellulose), matrix polysaccharides (mainly hemicelluloses and pectin) and encrusting substances (mainly lignin), (Smits and Annison, 1996). They reported that the structural arrangements within the cell wall would affect the physical and chemical

properties of the polysaccharides and this may in turn affect their physiological action on nutrient digestibility in diets and gut modification in monogastric animals. Common properties of soluble NSP are their resistance to the animals' digestive enzymes, and their tendency to create a viscous environment within the intestinal lumen (Iji, 1999).

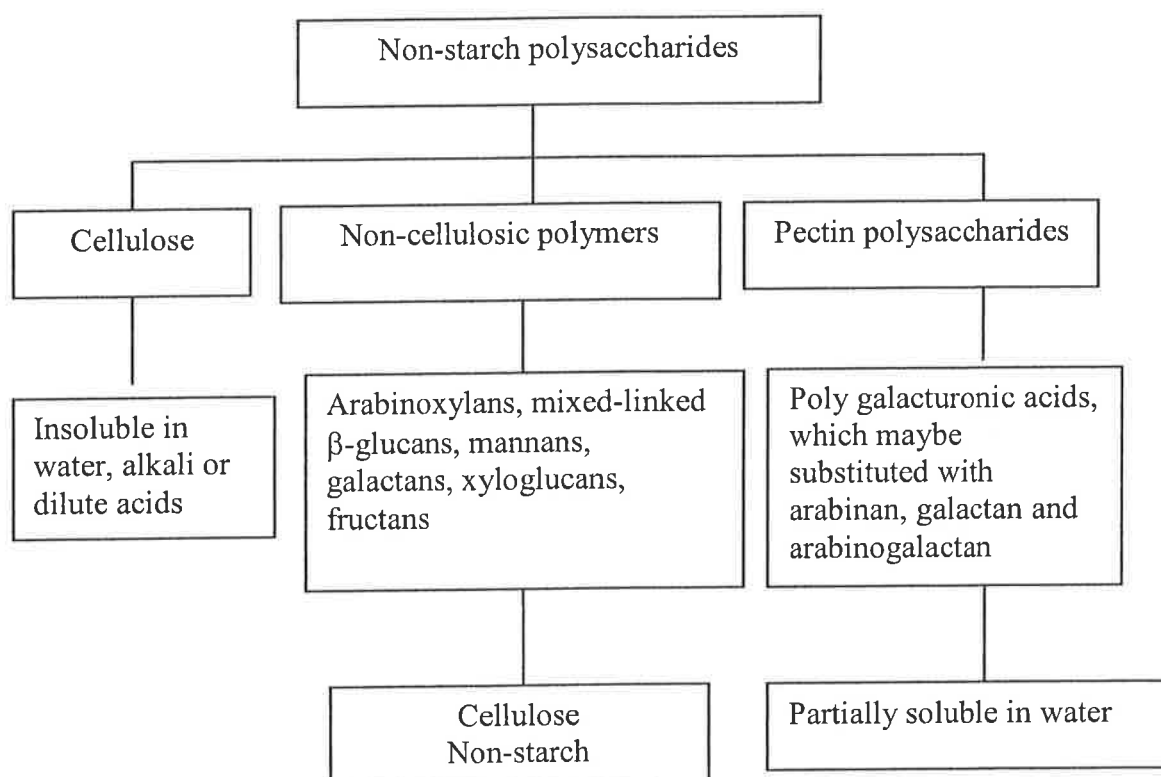


Figure 2 Classification of NSP (from Choct, 2002)

NSP are classified into three main groups (Figure 2). Cellulose is a structural NSP and is the major component of cell walls where it represents 5-95% of all polymers (Carre, 2002). It is a major portion of the dietary fibre component of feedstuffs. It is a linear compound with a chemical composition entirely of very long chains of β - (1→4)-glucopyranose units. This regular extended chain structure of polymers allows molecules to pack closely, giving rise to crystalline lattice or micro fibrils. The crystalline lattices prevent penetration of water molecules thus making celluloses insoluble. It is indigestible in monogastric animals and requires very strong concentrated acids for hydrolysis and its solubilisation is even difficult with hot alkali (Carre, 2002).

Non-cellulosic polymers (Figure 2), can either be storage such as mannans, xyloglucans and galactans or structural such as arabinoxylans or β -(1 $\rightarrow$ 3), (1 $\rightarrow$ 4)-D-glucan (β -glucans).

Pectins or pectic polysaccharides (Figure 3) contain high proportions of galacturonic acid. Most are water soluble due to their branched nature. They have a main chain of α -(1 $\rightarrow$ 4)-D-galacturonic acids with varying amounts of α -(1 $\rightarrow$ 2)-L-rhamnosyl residues. Apple and citrus pectins are constituted of main chains of galacturonic acids with very low amounts of rhamnose. Many of these NSP are esterified with methoxy groups on up to 80% of uronic acid C6 carboxyls (Annison, 1993).

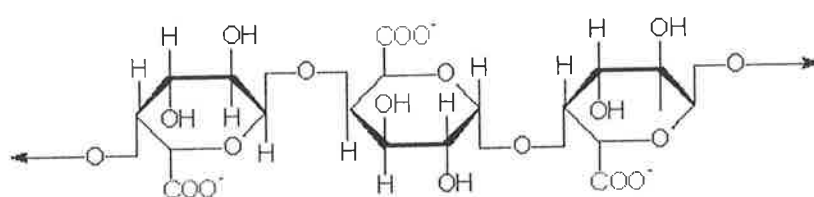


Figure 3 Pectic acid (α -1,4-galacturonic acid)

As shown in Figure 2, soluble NSP are the non-cellulosic polymers like arabinoxylans, mixed β -linked-glucans, mannans, galactans, fructans, and pectic polysaccharides, which are soluble in water (Choct, 2002).

1.4.1 Soluble non-starch polysaccharides

The major factor responsible for the solubility of polysaccharides is the length of their molecules. These structures can be linked to several compounds (up to four) through glycosidic ether or ester linkages to form arborescent structures (Carre, 2002). The long length of their molecules results in them being hydrophilic which in turn gives rise to their viscous nature, which is the main anti-nutritive factor in chickens.

In recent years, due to findings that soluble NSP have anti-nutritive effects on digestion and absorption in poultry, most research has focused on that fraction of NSP, (Smits and Annison, 1996; Choct and Annison, 1990; Carre, 1995; Choct *et al.*, 1996, 1999, *et al.*, 1999; Iji, 1999, *et al.*, 2001). The net effects of these anti-nutritive factors include altered intestinal transit time, increased endogenous losses of nutrients and a change of nutrient digestion and absorption patterns (enzymatic digestion vs. microbial fermentation) (Choct, 2002).

1.4.2 Insoluble non-starch polysaccharides

Insoluble NSP are extra-cellular component and are involved in cell structure, mostly cellulose and lignin (Carre, 2002). Cellulose and lignin are found in plant structural components such as oat and rice hulls, straw, hay wheat bran, and wood shavings. Insoluble fibres such as cellulose and wheat bran affect digestion and nutrient absorption in poultry and are not extensively degraded by bacterial fermentation, thus the microflora composition and quantity is not significantly influenced in the gastrointestinal tract (Choct *et al.*, 1996). Moreover, insoluble NSP were often regarded as an inert nutrient diluent (Angkanaporn *et al.*, 1994; Carre, 2002). However, this fibre fraction is found at high levels in feed ingredients of plant materials that are fed to monogastric animals and have physiological and nutritional implications for the monogastric feed industries. Currently work done on the insoluble NSP has shown that moderate inclusions in diets can improve starch digestibility with no effects on performance in birds fed wheat based and potato diets.

1.5 Features of insoluble NSP

1.5.1 Water-holding capacity

Insoluble fibre is negligibly digested in the small intestine and the obvious effect is increased bulk of digesta in the gut. This increased bulk of digesta in the gut triggers an increase in the small intestinal transit rate and influences excreta consistency in monogastric animals (Choct, 2002).

1.5.2 Gut transit time

The amount of time a food material takes to pass through the gastro-intestinal tract is called the feed or digesta passage time. Inclusion of coarsely ground oat hulls in wheat-based diets has been shown to reduce the feed passage time through the gastro-intestinal tract of the chicken (Hetland and Svihus, 2001). Rogel *et al.* (1987a) showed a similar result in gut transit time when finely ground oat hulls were included in the diets. These works demonstrate that the faster rate of digesta through the small intestine is dependent on the water-holding capacity of insoluble NSP, which is a function of particle size (Choct, 2002). The water-holding capacity of insoluble NSP may possibly have prevented the solubilisation of the normally soluble fraction of NSP, leading to an increase in transit time of digesta through the small intestine, thus eliminating the prospect of establishment of fermentative organisms in the gut (Choct, 2002). Smit and Annison (1996) assumed that this feature of insoluble NSP may alleviate the detrimental effects of viscous NSP (increased digesta viscosity leading to proliferation of gut microflora) and this may explain the results obtained by Rogel *et al.* (1987a) where an improvement was noted in the starch digestibility of low apparent metabolisable energy (AME) wheat after inclusion of oat hulls in the diet.

The feed passage time through the gastro-intestinal tract of chickens is affected by the feed composition. Work by Hetland and Svihus (2001), showed a decrease in passage time with addition of coarsely ground oat hulls in wheat-based diets. Larger particle size is linked with increased gizzard activity signified by an increase in gizzard weight due to increased grinding activity to reduce larger particle size to a certain size ($<100\ \mu$) before leaving the gizzard.

Moreover, improved starch digestibility has also been attributed to the action of the gizzard when whole wheat or insoluble fibre materials are given to birds (Hetland *et al.*, 2002). They suggested that in addition to the grinding effect due to presence of these materials, an active gizzard also mixes digestive juices and nutrients, which is beneficial for digestion.

1.5.3 Larger gut capacity

Structural components such as oat hulls, rice hull, wood shaving, cellulose, have been shown to be beneficial to poultry at moderate levels, however, the mechanism by which inclusion of insoluble fibre in poultry diets improves nutrient digestion and absorption is yet to be clearly understood (Hetland *et al.*, 2003). Hetland *et al.*, (2003) investigated the physical presence of oat hulls and wood shavings in whole or ground wheat based diets on the gut physiology of birds and the influences on the digestibility of other nutrients. Their results showed that starch digestibility was significantly increased by the inclusion of oat hulls for broilers and wood shavings for layers. They concluded that the presence of insoluble fibre in oat hulls and wood shavings might be associated with improved digestibility and absorption of starch present in other feed ingredients. This finding led to their proposal of a possible mechanism by which the presence of insoluble matter in the gut might trigger the secretion of digestive enzymes

and bile salt into the digestive tract (anterior duodenum), which might lead to improve starch digestibility. They found that the bile acids in the gizzard increased in proportion to gizzard contents, indicating gastroduodenal reflux.

Bile acids enter the intestine in the posterior duodenal loop, so the presence of these acids in the gizzard contents is an adequate indicator of gastroduodenal reflux and supports the hypothesis that chyme reflux between gizzard and duodenum is increased with inclusion of insoluble fibre. Bile acids because they are strong emulsifiers facilitate nutrient solubilization by effective emulsification of the liberated lipids. Incomplete lipid emulsification leads to impaired solubility and hence decreased nutrient digestibility (Hetland *et al.*, 2003). Hetland *et al.*, (2003) suggested that the observed starch digestibility might in part be due to the enhanced lipid emulsification due to increased bile acid concentration. Furthermore, observed improvement in starch digestibility may also be caused by increased amylase in the chyme of the jejunum in the presence of insoluble fibre (Hetland *et al.*, 2003). Based on work done by Malagelada *et al.* (1973), which showed that bile acids were found to have stabilising effects on digestion of proteases, preventing autolysis, Hetland *et al.* (2003) suggested that if that was the case then an improvement in the enzyme - substrate interactions, leading to an increased nutrient digestibility is expected. However, their work on amylase activities was not conclusive due to wide individual variations but it is possible that digestive enzyme concentration in the upper gut may also be elevated by increased reflux (Hetland *et al.*, 2003). The enlargement of the gizzard due to inclusion of insoluble fibre may suggest an increased digestive capacity of birds (Hetland *et al.*, 2003).

1.5.4 Improved starch digestibility

Past research have shown that starch digestibility was improved by presence or inclusion of low levels of insoluble fibre such as oat hulls, rice hulls, barley hulls and cellulose in wheat-based diets and with raw potato starch in broiler diets (Rogel *et al.*, 1987a, b; Hetland and Svihus, 2001; Cao *et al.*, 2003; Hetland *et al.*, 2003). Inclusions of insoluble fibre in diets were associated with increased feed intake (Newcombe and Summers, 1985; Hetland and Svihus, 2001), signifying the birds' ability to increase consumption when given low-energy diets (Rogel *et al.*, 1987a, b). Hetland *et al.* (2003) also observed positive effects of wood shavings to layers fed wheat-based diets.

1.6 Effects of NSP on the digestive organs of broiler chickens

1.6.1 Digestive organ development and function

Generally the avian digestive tract includes a beak, oesophagus, crop, proventriculus, gizzard, small intestine, paired caeca, large intestine, and cloaca. A food particle is pecked by the beak and swallowed whole. The food material passes to the crop, which is a storage organ via, the oesophagus. The crop is present in many but not all species of birds. In chickens, it is a ventral diverticulum of the oesophagus. From the crop the food material is passed into the proventriculus, which is the glandular stomach and is the site of acid secretion. Here the food material is mixed with the secreted digestive juices and acid. The food then enters the gizzard where mechanical digestion occurs. The chyme is then moved into the small intestine. The small intestine is divided into three sections, beginning with the duodenum, which is a u-shaped tube and encloses the pancreas, the jejunum and the ileum. The jejunum and the ileum are both the same width and form short coils held in position by the mesentery. The jejunum leaves the duodenum and is continued distally by the ileum which opens into the large intestine. Digestion of food material continues in the small intestine and major absorption occurs

here as well. The pancreas secretes enzymes such as amylase and trypsinogen into the duodenum while the liver secretes bile salts through the duct. These enzymes and bile salts aid in the digestion of protein and fats.

Digestion occurs along the whole length of the small intestine. The small intestine contains lightly folded linings of digestive cells. The folds are prolonged into microscopic finger like projections called villi. The villi greatly increase the surface area for absorption. The large intestine consists of the colon, the paired caeca and the cloaca. The shape, size and capacity of the caeca vary amongst birds and the structure is most developed in granivorous species whose diet consists of high levels of plant fibre and chitin (Jozefiak *et al.*, 2004). The role of caeca is the absorption of water and microbial synthesis of vitamins. This organ also plays a major role in the microbial degradation of some carbohydrates.

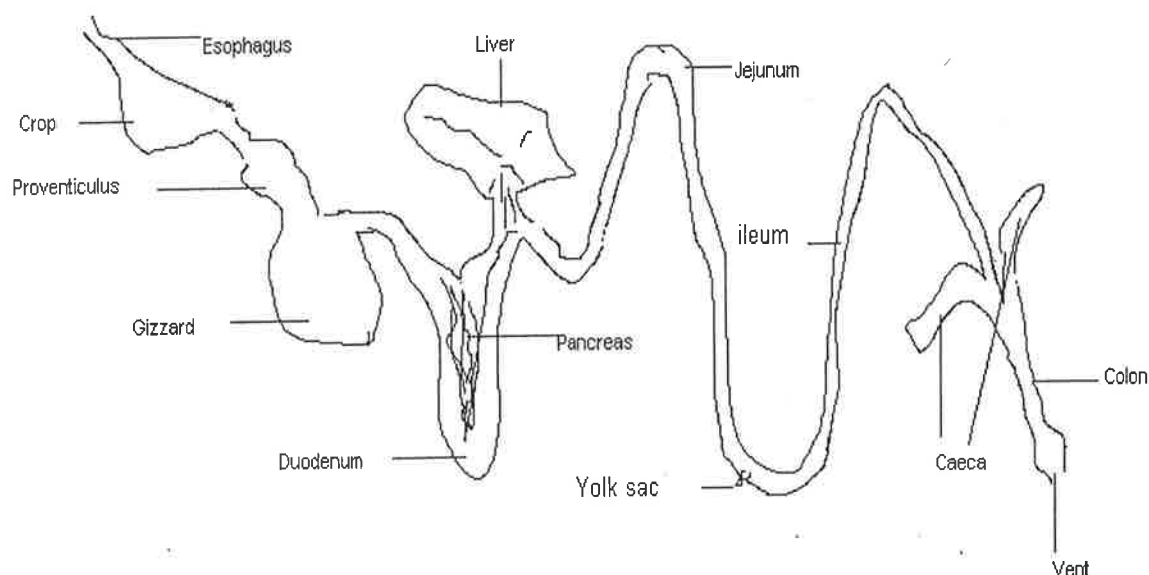


Figure 4 Digestive tract of a broiler chicken

1.6.2 Gizzard and the small intestinal mucosa

It has been reported that most NSP digestion in poultry occurs in the distal tract under the influence of enzymes secreted by the resident microflora (Pawlik *et al.*, 1990; Friesen *et al.*, 1992).

The literature abounds with information on the effect of viscous NSP in poultry through viscous digesta and sticky droppings. Moreover, Iji (1999), in his review on the impact of cereal NSP on intestinal development, outlined the likely effects of these viscous NSP on the digestive tract of chickens. Some of the effects of soluble NSP include an increase in the full, empty and relative weights and lengths of the gastrointestinal tract (Nestor *et al.*, 1981; Siri *et al.*, 1992; Jorgensen *et al.*, 1996; Smits *et al.*, 1997;). Iji (1999) stated these mentioned effects could be an indication as to what actually occurs at the intestinal mucosa. Iji (1998) found a significant increase in crypt depth in the jejunum and the ileum, an increased villi height in the ileum and a reduced villi height in the duodenum of chickens supplemented with the more viscous NSP (guar gum and gum xanthan). These changes were accompanied by a reduction in the cell population and size in the jejunum (Iji and Tivey, 1998). Iji (1998) suggested that dietary fibre may also indirectly change the intestinal growth through interactions with mucosal growth factors, therefore dietary factors that influence structural changes in the intestine would be capable of regulating dietary functions.

As mentioned earlier structural NSP may improve nutrient solubilisation especially of lipids through the secretion of more bile salts, which aid lipid digestion (Hetland *et al.*, 2003). The presence of bile acid or salt in the gizzard is linked with duodenal gut reflux, which may be due to the presence of fibre in the gizzard. The presence of coarse fibre in diets may be an influencing factor in increased gizzard weights. An

increased gizzard weight is an indication of a larger gut capacity due to dietary influences.

1.6.3 Gut microflora

As previously stated, insoluble fibre is not extensively degraded by bacterial fermentation hence microbial composition and population are not significantly influenced (Choct *et al.*, 1996). Degradation of NSP in poultry varies due to lack of digestive enzymes (Jozefiak *et al.*, 2004). Soluble NSP create a viscous environment within the gastro-intestinal tract thus slowing the digesta passage rate leading to proliferation of microflora in the gut. With the insoluble NSP, inclusion of cellulose at higher level increased faecal excretion of urinary nitrogen, suggesting caecal reflux where the reverse flow of urinary nitrogen was increased due to increased levels of cellulose contributing to increased microflora and ammonia levels (Cao *et al.*, 2003). Though inclusion of insoluble fibre at moderate levels did not influence gut microflora, inclusion at higher levels may influence gut microbial population and composition.

1.7 Summary

Past studies have shown that insoluble fibres in poultry diets were inert and acted as nutrient diluents (Angkanaporn *et al.*, 1994; Carre, 2002) some, however, suggested that fibre in general had a negative effect on nutrient digestion and absorption in chicken (Krogdahl, 1986; Angkanaporn *et al.*, 1994; Carre, 2002). Research to date has shown that insoluble fibre at moderate levels has positive effects on nutrient absorption and digestion through larger gut capacity and faster digesta transit time (Hetland and Svihus, 2001; Svihus and Hetland, 2001; Hetland *et al.*, 2002; Hetland *et al.*, 2003). As stated by Hetland *et al.* (2003), the mechanisms by which the inclusion of insoluble fibre in poultry diets improves nutrient digestion is not yet clearly

understood. The effect of insoluble fibre at the mucosal level and its interactions with nutrient digestion and absorption functions need to be investigated. Moreover the effects of insoluble fibre on the gizzard and gut refluxes also need investigation.

Inclusion of insoluble fibre at higher levels in non-wheat based diets and the proliferation of gut microflora in the gut especially the caeca warrant investigation, as moderate levels have proven beneficial in wheat-based diets. Digestibility of nutrients such as protein and fat apart from starch in the in the presence of high levels of insoluble fibre if proven to improve nutritional status of diet may assist a lot in the development of least cost rations based on high fibre materials in third world countries like PNG where the small-scale production of broiler chickens is impeded due to high cost of productions.

The aim of the project is to improve the economic viability of small-scale poultry production in PNG, with the objectives to determine the effects of high levels of fibrous material on the functional capacity of the digestive organs in chickens and the digestibility of other nutrients especially the apparent metabolisable energy of the diets.

The project hypotheses are

- 1 The presence of high levels of insoluble NSP increases apparent metabolisable energy.
- 2 The presence of high levels of insoluble NSP shortens digesta passage time.
- 3 The presence of high levels of insoluble NSP results in an increase in the gizzard, and relative weights of the pancreas and small intestinal segments.

Most of the methods used to test these hypotheses were selected based on available facilities in Papua New Guinea. The first hypothesis was tested using the apparent

metabolisable energy (AME) bioassay with young broiler chickens at 21 days of age. This method is currently used at South Australian Research and Development Institute (SARDI) - Poultry Unit at the University of Adelaide Roseworthy Campus. Similar feed testing facility is available in Papua New Guinea.

The second hypothesis was tested by the whole tract transit time (WTTT) method using ferric oxide in gelatine capsules. This method is correlated to the oro-caecal transit time (Hughes 2003) and preferred because facilities were not available in PNG for the hydrogen breath analysis tests or the titanium oxide as an indigestible marker.

The third hypothesis was tested by assessing the relative organ weights (g/kg live weight) as well as histological studies on the villi and crypt morphometry to determine any effects on the small intestinal mucosa in relation to high levels of insoluble fibre. Tissue sections were brought back to Australia for analysis using equipment at the Histology Laboratory of the University of Adelaide, Roseworthy campus.

There were a total of three experiments conducted to test these hypotheses, in two different locations. The first two experiments, which are discussed in Chapters 3, and 4, were conducted at Roseworthy Campus of the University of Adelaide at the SARDI-Poultry Unit using a standard source of NSP.

The third experiment was conducted in Lae, Papua New Guinea at the National Agricultural Research Institute's (NARI) Livestock Program facility. This experiment was conducted using test ingredients that were high in fibre under a natural environment. This experiment was conducted in PNG using local ingredients due to quarantine restrictions involved in bringing these ingredients into Australia.

CHAPTER 2: MATERIALS AND METHODS

2.1 Introduction

This chapter describes the methods used to test the hypotheses presented in the previous chapter. The AME bioassay procedure and the Whole Tract Transit Time (WTTT) measurements are similar to those used by Hughes (2003). The methods for tissue collection for histological purposes, tissue processing, embedding and sectioning were similar to those described by Iji (1998) and Hughes (2003). These methods were common to all three experiments conducted in this project.

A basal diet with sorghum as the main energy source was used in all three experiments. Cellulose powder (Ace Chemical Ltd - South Australia) was used as the standard source of insoluble NSP in the first and second experiments while copra meal and palm kernel meal were the sources of insoluble NSP in the experiment conducted in PNG.

There were two different breeds of broiler chickens used depending on where the experiments were conducted. The experiments in Australia used Cobb 500 broiler chickens while Ross broiler chickens were used in the experiment conducted in PNG. A total of 288 day-old broilers were used.

2.2 Experimental

2.2.1 Location

The first two experiments were conducted at the SARDI Poultry Unit at Roseworthy Campus, University of Adelaide, South Australia. These experiments were conducted in a temperature-controlled shed. Roseworthy is located at 34°S and 138°E and is about 68m above sea level. It has an average annual rainfall of approximately 40mm. The average minimum and maximum temperatures for the month of November (experiment 1) were 11.5°C and 25.3°C, respectively and 10.4°C and 27°C, respectively, for March 2005 (experiment 2).

The third experiment was conducted at the Poultry Feed Testing facility at Labu, Morobe Province, PNG. This experiment was conducted at local ambient conditions in an open-sided shed in April 2005. Lae is located at 6°S and 147°E on the map and is about 8m above sea level. It has an average annual rainfall of 2000mm with temperatures ranging from 21-31°C. The minimum and maximum temperatures for the month of April were 23 and 30°C, respectively, with an average rainfall of 366mm.

2.2.2 Birds, diets and experimental design

The experimental design for all of the experiments was a complete randomised block design. There were a total of four blocks with twelve experimental units. There were a total of six dietary treatments with each offered to separate cages of male and female chickens in each block.

The basal diet composition is shown in Table 1. The diet consisted of sorghum as the main source of energy. Other diet components included soybean meal, sunflower oil, limestone, dicalcium phosphate, salt and a mineral and vitamin supplement.

Table 1 Diet Composition

Ingredient	g/kg
Sorghum	575
Soybean meal	320
Sunflower oil	60
Dicalcium phosphate	20
DL-methionine	7
Limestone	11
Vitamin and mineral premix	2
Choline chloride (60%)	2
Salt	3

2.2.3 Rearing and management

Broiler chickens were raised on a floor pen and given commercial starter crumbles from day old to 21 or 22 days of age. Birds had free access to the experimental diets and to water.

2.3 Apparent metabolisable energy

At 21-22 days of age the experimental chickens were randomly allocated to metabolism cages.

For the first two experiments at Roseworthy, single-bird metabolism cages (260 x 360 x 400mm) were used. Birds were placed two per cage for two days prior to the start of the bioassay. For the experiment in PNG, four birds were placed in a multiple-bird metabolism cage, at the start of the bioassay. The dimensions of the metabolism cages were 600 x 450 x 380mm in both countries.

At the start of the bioassay, the average weight of the flock was determined so that the individual weight of the experimental birds was within $\pm 200\text{g}$ of the average weight of the flock.

The AME bioassay was conducted over a seven-day period in both countries. After the experimental chickens were weighed, the experimental diets were introduced to the birds. The birds were given three days to adapt to the cages and the diets. Each experimental diet with the feed hopper (1200g for single bird and 2500g for the grouped trial) was weighed, and the feed hopper replaced on the cage.

After the adaptation period, the trays were thoroughly cleared and feed residues for the adaptation period weighed and recorded for each diet. The feed was then topped up to 1000g for single bird trials and 2000g for the group trial. Approximately 200g of each feed sample were collected in plastic bags as back-up samples, whilst a 70ml screw-capped jar was used to take a representative sample for each diet. These were then stored at 4°C until required. Additional samples were collected as back-ups for use in case extra material was needed for repeated analysis.

The collection period began after the trays were cleared at the end of the adaptation period. At the start of each day for four consecutive days, excreta were collected from each cage after carefully removing any feed or feathers, and dried overnight at 90°C, in

a fan-forced oven. At mid-week, diets were again topped up with a weighed amount of feed after excreta collection. Excreta from each cage were pooled at the end of the bioassay and weighed. Each pooled sample was finely ground using a grinder with a 2mm mesh in a cross beater mill (Glen Creston Ltd) and a representative sample was placed in a screw-capped jar. The representative samples of each experimental diet and excreta were then pelleted and used for gross energy measurement with a Parr isoperibol bomb calorimeter (Parr 1261 and 1281 model -Australia and Parr 1266 model -PNG).

2.4 Whole tract transit time measurements

Whole Tract Transit Time is an indication of the digesta transit time through the digestive tract of the chicken. This measurement was done in the three experiments on the second day after the first excreta collection. Birds from selected cages were taken out and a gelatine capsule containing 200mg of ferric oxide was inserted into the crop of the bird and the bird was then returned to the cage. The time when the capsule was given to the bird was recorded.

Whole Tract Transit Time was calculated as the time difference in minutes from when the capsule was introduced and time of the first sighting of red droppings in the excreta tray.

2.5 Analytical procedures

2.5.1 Dry matter analysis

Samples to be tested were removed from the refrigerator and allowed to reach room temperature before the test was conducted. Each crucible was weighed, and then

approximately 2g of the test diet or ingredient were placed into the empty crucible and the total weight taken. After weighing, the crucibles containing the sample were dried overnight in an oven at 105⁰C. The crucibles and the sample were weighed the next day. All analysis of ingredients and diets samples was done in duplicate.

Dry matter (DM) was calculated as such:

$$DM = (W1-W2) / (W3-W2)$$

Where

DM - dry matter

W1 - dry weight of crucible and sample

W2 - weight of crucible

W3 - wet weight of crucible and sample

2.5.2 Gross energy analysis

Gross energy analysis was performed to calculate the energy of a sample and is measured in mega joules per kilogram (MJ/kg). Gross energy (MJ/kg) is defined as the heat of combustion that is given off when a sample is completely oxidised in the bomb calorimeter.

Samples to be tested were removed from the refrigerator and allowed to reach room temperature before the test was conducted. The sample was then placed into a tared crucible and the weight recorded. Poultry feed samples (approximately 1.7g) were weighed to 0.0001 and were done in duplicate. If the difference between the duplicates was greater than 0.05 MJ/kg, an additional sample was tested. Dried poultry excreta samples were pelleted prior to analysis. A single measurement of gross energy was done in Australia, and in duplicate in PNG. All bomb calorimeters were standardised

using benzoic acid according to the manufacturer's specifications before analysis of any samples was done.

2.5.3 AME calculation

The AME of an experimental diet on a dry matter basis was calculated as follows: -

$$\text{AME of diet} = (\text{DietGE} \times \text{Feed} - \text{ExcretaGE} \times \text{Dry excreta}) / \text{Feed} / \text{Pellet DM}$$

Where

DietGE - Gross energy of diet

ExcretaGE - Gross energy of excreta

Feed - Feed intake over the collection period

Dry excreta - total dry excreta collected over the collection period

PelletDM - Dry matter of pelleted diet

2.6 Histology

2.6.1 Tissue collection

At the end of the AME bioassay trial, chickens were euthanased with 0.5ml of pentobarbitone injected into the brachial vein, and then weighed. An incision was made in the skin below the breastbone and the skin pulled back towards the head to expose the breast. The breast was cut on each side through the ribs and then lifted to expose the intestines.

For histology studies, the intestines were excised at the exit of the gizzard and at the cloacal end and then removed and placed on paper towels. These were then spread out as shown in Figure 4, and sections of 50mm in length were taken at the midpoints from

the duodenum, jejunum and the ileum (Figure 4). These tissue samples were cut open longitudinally, rinsed in 10% buffered formalin to remove contents and placed into labelled vials containing buffered formalin solution. These were then transferred into 70% ethanol after 24 hours and stored until needed.

For gross organ and small intestinal section weights, the digestive tract was excised at the exit of the proventriculus and at the cloacal end. The intestines were then taken out and spread out on paper towel as shown in Figure 4. The gizzard was detached, emptied of contents then weighed. The pancreas was dissected from the duodenum and weighed. The small intestine was excised and each segment (duodenum, jejunum and ileum) was identified as shown in Figure 4, and separated. The contents were gently squeezed from each of these sections. The sections were then rinsed with water; patted dry with paper towel and weighed.

2.6.2 Tissue processing and embedding

Small intestinal sections that were preserved in 70% ethanol were placed into labelled tissue cassettes and processed using a Shandon Automated Tissue processor. The tissues were processed for a total of 16.5 hours through the following steps.

1. 70% ethanol for one hour
2. 80% ethanol for one hour
3. 95% ethanol for 30 mins
4. 95% ethanol for one hour
5. Absolute ethanol for two hours
6. Absolute ethanol for two hour
7. Histolene/Ethanol (50:50) for one hour
8. Histolene for two hours

9. Histolene for two hours
10. Wax paraplast for two hours
11. Wax for two hours

After processing, the tissue cassettes were removed and placed in hot wax in a Shakira tissue-embedding machine. Tissues were removed from the cassette one at a time, and placed in a longitudinal orientation in the mould containing hot wax. The mould is used to embed the tissue and when the wax is cooled, provides necessary support for microtomy.

The top of the cassette was removed while the bottom half was placed over the sample in the mould. The mould containing the tissue and the cassette was placed on the cooling surface (-4°C). The wax block with the embedded tissue was removed from the mould after the wax had cooled.

2.6.3 Tissue sectioning, staining and mounting

After the tissue was embedded, sections were made using a Leitz microtome-1512. The sectioning was done at 7μ . The tissue ribbons were carefully teased from the blade of the microtome with a brush and placed in a water bath ($45-50^{\circ}\text{C}$). Then the sections were mounted on poly-L-lysine coated slides and dried at room temperature.

The tissues were stained with Haematoxylin and Eosin and mounted with a drop of a Depex (VWR International Ltd-England). A cover slip was placed over the sections and allowed to dry.

2.6.4 Image analysis

The slides were viewed at the Adelaide Microscopy Centre - University of Adelaide. Imaging of the stained sections was done using a Nikon Eclipse TE300 light microscope in bright field mode, with Nikon DXM 1200 camera and ACT-1 image acquisition software. Digital images were taken with the 4X objective lens and with the eyepiece at 10X magnification. Each individual image was calibrated accordingly and measurements of villus height and crypt depth were made using AnalySIS 5 software (Soft-Imaging System, Germany). The villus: crypt ratio for each section from each bird was later calculated in Microsoft Excel.

2.7 Statistical analysis

Growth performance data, relative organ weights, apparent metabolisable energy values, and villus and crypt measurements were analysed using the General Linear Model procedure in the Statistical Analysis System®. Due to imbalances in numbers of birds of each gender or numbers of birds in each dietary treatments, least square means are shown in the tables of results. Differences in least square means were determined by paired *t*-tests when effects were significant according to the analysis of variation.

2.8 Animal ethics

The Animal Ethics Committees of the Department of Primary Industries and Resources South Australia and the University of Adelaide approved all the experiments conducted. Approval numbers were 15/04 and S-54-2004, respectively. All procedures complied with the "Australian code of practice for the care and use of animals for scientific purposes" (Australian Agricultural Council, 1997) and "Australian model code of practice

for the welfare of animals. Domestic Poultry" (Standing Committee on Agriculture and Resource Management, 1995).

CHAPTER 3: EFFECT OF CELLULOSE ON PERFORMANCE, APPARENT METABOLISABLE ENERGY, WHOLE TRACT TRANSIT TIME AND GUT PHYSIOLOGY OF BROILER CHICKENS

3.1 Introduction

Dietary fibre is defined as all polysaccharides and lignin which are not digested by the endogenous secretions of the human digestive tract (Trowell, 1976). However, although not hydrolysed, this fraction of a feedstuff must not be considered as inert.

Past and current work done on insoluble NSP have shown that moderate inclusions in diets have been associated with improved starch digestibility in birds (Rogel *et al.*, 1987a,b; Hetland and Svihus, 2001; Cao *et al.*, 2003; Hetland *et al.*, 2003). Structural components such as oat hulls, rice hull, wood shaving and cellulose have shown to be beneficial to poultry diets at moderate levels. However, the mechanism by which inclusion of insoluble fibre in poultry diets improves nutrient digestion and absorption is yet to be clearly understood (Hetland *et al.*, 2003).

Cellulose has been used in poultry diets as a source of dietary fibre for nutritional studies over the past decades (Siri *et al.*, 1992; Siri *et al.*, 1994; Svihus and Hetland 2001; Cao *et al.*, 2003). Siri *et al.* (1992) investigated cellulose using semi-purified diets that were iso-caloric and iso-nitrogenous to determine optimum cellulose level for best performance of broiler chicks. Their work showed that feed intake, weight gains and feed conversion ratios increased with an increase in cellulose level. Cao *et al.* (2003) also studied the effects of cellulose on growth, nitrogen utilisation, and retention time in the digestive tract and caecal microflora of chickens. Their work showed that

these parameters were dependent on inclusion rates. They observed positive effects on growth when the level was at 3.5%, however, at 10% inclusion, the effects were detrimental.

The aim of the present trial was to use cellulose as a standard NSP, to study the effect of high level of insoluble fibre on AME of diets, WTTT, gizzard weight and gut morphology of broilers. The results were used as baseline data for subsequent experiments.

3.2 Experimental

3.2.1 Location

The experiment was conducted at the SARDI's Poultry Unit at Roseworthy Campus, University of Adelaide, South Australia.

3.2.2 Birds, diets and experimental design

The experiment was conducted using 48 single bird metabolism cages. The six dietary treatments were replicated eight times (four males and four females).

The experimental diets were prepared by diluting the sorghum component of the basal diet (Table 1) with cellulose powder (Ace Chemicals Ltd, South Australia) at the rate of 0, 2.5, 5.0, 7.5, 10 and 12%, respectively. The diets were then pelleted, air-dried and placed into labelled bins. The diets were introduced to the birds at 22 days of age. Birds were given three days to adapt to their respective diets and the collection of excreta commenced on the fourth day.

3.2.3 Rearing and management

The birds were raised on a floor pen and given a commercial starter crumbles from day old to 20 days of age. Two birds were placed in a single cage according to their gender, two days before the start of the trial and given pelleted finisher feeds. At day 22, one bird from each cage was removed and discarded. The remaining birds were weighed and then given the experimental diets. Birds had free access to feed and water.

3.2.4 General procedures

The general procedures, AME bioassay procedures and digesta passage time measurements, using ferric oxide gelatine capsules, were as described in Chapter 2.

At the end of the trial, each bird was killed by intravenous injection of pentobarbitone and weighed. Half of the total number of birds in the trial was used for gross digestive tract measurements. However, samples collected for histological examination were not processed due to lack of time.

3.3 Results

There was no diet x gender interactions for any of the parameters measured and the significant differences between genders in start weights and end weights were expected in chickens, with male birds tending to be heavier than female birds.

There was a significant ($P < 0.001$) diet effect on AME and dry matter digestibility of the diets.

Table 2. Effects of gender and diet on growth, feed intake, feed conversion, whole tract transit time, apparent metabolisable energy and dry matter digestibility.

Effect	Level	BWEND	BWGAIN	FBD	FCR	WTTT	ME-2	DIG-DM
Gender	Female	1152b	450.6a	100.0a	1.557a	194.6a	15.96a	0.687a
	Male	1213a	469.1a	104.9a	1.579a	203.7a	15.86a	0.682a
Diet	Description							
1	Basal diet (B)	1203a	482.2a	106.0a	1.572a	159.3a	16.23a	0.736a
2	B + 2.5 % Cellulose	1181a	469.2a	101.5a	1.525a	149.3a	16.12a	0.719ab
3	B + 5.0 % Cellulose	1229a	500.6a	109.0a	1.526a	211.5a	16.22a	0.707b
4	B + 7.5 % Cellulose	1168a	435.3a	99.3a	1.602a	210.1a	16.10a	0.674c
5	B + 10 % Cellulose	1186a	462.2a	103.2a	1.566a	225.0a	16.02a	0.650d
6	B + 12 % Cellulose	1136a	409.7a	95.4a	1.617a	239.8a	14.75b	0.623e
	Pooled SEM	14.1	11.2	1.8	0.017	17.0	0.07	0.004

Means with a common letter within a main effect are not significantly different ($P < 0.05$); BWGAIN = live weight gain (g/bird); FBD = feed intake (g/bird); FCR = Feed conversion ratio (g feed: g gain); ME-1 = AME of diet (MJ/kg as- fed basis); ME-2= AME of diet (MJ/kg dry matter basis); DIG-DM = Dry matter digestibility coefficient; Pooled SEM= Pooled Standard error of mean.

The least square means for weight gain, feed intake and feed conversion ratio did not show any significant gender effect. However, as shown in Table 2, there was significant ($P<0.01$) gender effect on the end weights. There was no significant gender or diet effect observed on the WTTT of birds on the dietary treatments in this experiment (Table 2).

AME values were significantly different ($P<0.001$) due to dietary treatments. The test diet with 12% cellulose had significantly lower ($P<0.001$) AME values compared to other diets (Table 2). The dry matter digestibility of the diets also showed significant differences due to dietary treatments (Table 2). The digestibility of the basal diet was not significantly different to that of the 2.5% cellulose diet, but was significantly higher ($P<0.001$) than those of 5, 7.5, 10 and 12% cellulose diets. No effects due to gender were observed in AME values or digestibility of the diets.

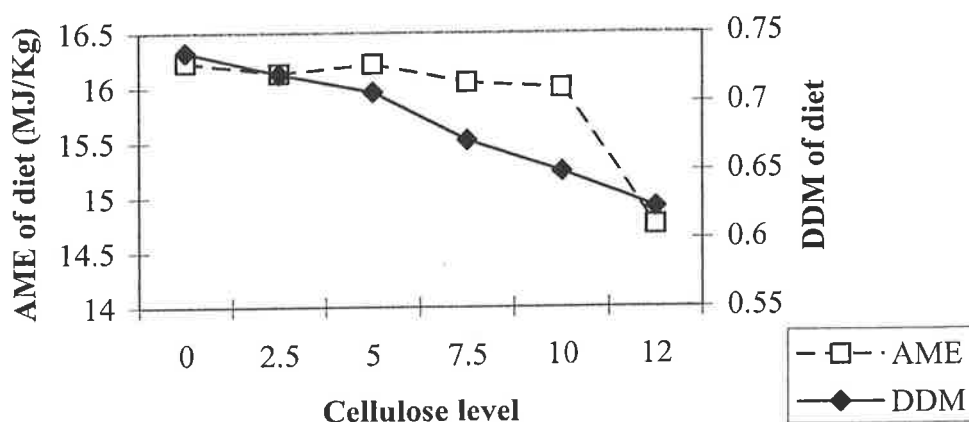


Figure 5 Relationship between cellulose inclusion rates and AME and DDM of diets.

As shown in Figure 5, the relationship between the AME of the diets and their digestibility is in direct proportion to the cellulose inclusion in this experiment. The

AME and digestibility of the diets decreased as the percentage inclusion of cellulose in the diet was increased.

The relative gizzard and intestinal section weights did not show any significant diet or gender effects. There was no diet x gender interaction for the relative weight of the gizzard, jejunum or the ileum; however, there was a significant ($P < 0.05$) diet x gender interaction observed in the relative duodenal weight of chickens.

Table 3. Effect of gender and diet on the relative gizzard and small intestinal weights (g/kg body weight) of broilers.

Effect	Level	Gizzard	Pancreas	Duodenum	Jejunum	Ileum
Gender	Female	23.8	3.27	9.14	16.0	11.6
	Male	24.0	2.81	8.44	14.9	12.2
Diet						
1	Basal diet (B)	24.1	3.26	8.44	15.4	11.7
2	B + 2.5 % Cellulose	24.5	2.87	9.39	14.8	12.4
3	B + 5 % Cellulose	21.9	2.99	10.3	16.2	10.9
4	B + 7.5 % Cellulose	25.4	2.97	6.73	15.0	11.3
5	B + 10 % Fine straw	23.2	3.05	8.20	14.8	12.3
6	B + 12 % Coarse straw	24.2	3.10	9.71	16.4	12.7
	Pooled SEM	0.82	0.161	0.524	0.97	0.34

No significant differences ($P>0.05$).

3.4 Discussion

The aim of this experiment was to study the effect of a standard insoluble NSP on performance, digesta transit time, AME values, dry matter digestibility and gross organ weights of broiler chickens.

There were no diet effects for any parameter measured except for AME and dry matter digestibility. This may have been due to the percentage inclusion of cellulose and the short feeding duration. Moreover, only the sorghum component was diluted, thus the rates used here may not have been high enough to influence growth rates, digesta transit time or relative organ weights. The inclusion of cellulose in the diets, however, did influence the AME and dry matter digestibility. Diets with higher inclusion of cellulose had lower AME and dry matter digestibility values. The WTTT of birds on these diets although not significant, tended to increase with decrease in AME and digestibility. Despite these significant reductions observed in the AME and dry matter digestibility, performance of birds on these diets on average was good.

Svihus and Hetland (2001) showed that a 1:10 cellulose inclusion with wheat, stimulated feed intake of birds as well as the relative weight of the intestines and a beneficial effect was found on starch digestion in birds. Starch analysis was not performed in this experiment, however, if bird performance on average was good despite reductions in AME and digestibility, I suggest that cellulose at 12% inclusion may still be beneficial to chickens. This needs to be studied further.

The gender effect in this experiment might not have been obvious due to sampling size. However, the relative pancreas weight was marginally affected by treatment in this experiment. The female birds tended to have higher pancreas weight compared to the

male broiler birds. The increase in pancreas weight may have been due to two possible factors. The increased weight in the pancreas may have been an indication of enlarged glands in the pancreas due to increased secretion of amylase to better digest starch or an increase in secretion of other enzymes. Hughes (2003) observed similar results between genders in his study. Hence it is possible that a larger sample size may have revealed a difference, and that males and females may differ significantly in digestive functions to dietary treatments in their digestive functions. Further study is required to elucidate the underlying mechanisms.

3.5 Summary

The results of this experiment suggest that 10- 12% cellulose inclusion in the diets may be beneficial, as performance of birds was not significantly affected. While increased inclusion of cellulose decreased AME of the diets as expected, performance was not significantly affected. This may be because cellulose aided utilisation of other dietary components such as starch, protein and fat.

Relative weights of gizzard, pancreas and small intestinal sections did not increase possibly because cellulose was too finely ground or perhaps the sampling population may have been too small to detect any significant changes associated with cellulose inclusion.

CHAPTER 4: EFFECT OF CELLULOSE ON GROWTH PERFORMANCE, WHOLE TRACT TRANSIT TIME, APPARENT METABOLISABLE ENERGY AND THE DIGESTIVE PHYSIOLOGY OF BROILER CHICKENS

4.1 Introduction

Cellulose is an insoluble NSP and is mostly found in structural carbohydrates. In most plant species, cellulose is the major cell wall component. It has been used in many nutritional studies as a source of fibre (Siri *et al.*, 1992; Siri *et al.*, 1994; Svihus and Hetland 2001; Cao *et al.*, 2003).

Coarse and finely ground ingredients have been shown to have an effect on the gizzard and the digesta passage time (Rogel *et al.* 1987b; Hetland and Svihus, 2001). In Australia wheat straw (AME=0.08MJ/kg) was used to study the effect of particle size on the relative organ weight of broilers and WTTT of chickens fed on dietary treatments containing cellulose and wheat straw at differing levels (R.J. Hughes, personal communication).

The feed passage time through the gastro-intestinal tract of chickens is affected by the feed composition. Hetland and Svihus (2001) showed a decrease in passage time by addition of coarsely ground oat hulls in wheat-based diets. Larger particle size is linked to increased gizzard development, signified by an increase in gizzard weight due to the need for increased grinding activity to reduce larger particle size to a certain size (<100 μ) before leaving the gizzard.

It was hypothesised that presence of cellulose will improve AME values and dry matter digestibility, increase digestive organ and intestinal weights, and increase villus height and villus height to crypt ratio and that these effects will differ according to gender.

4.2 Experimental

4.2.1 Location

The experiment was conducted at the SARDI's Poultry Unit at Roseworthy Campus, University of Adelaide, South Australia.

4.2.2 Animals, diets and experimental design

A sorghum basal diet was used (Table 1) and five experimental diets were made by diluting this basal diet with cellulose at inclusion rates of, 0, 5, 10, and 15%. The remaining two diets were prepared by diluting the basal diet with wheat straw at 15% inclusion rate. The straw was either very fine (0.5mm) or was coarsely grounded (5mm) using the cross beater mill (Glen Creston Ltd).

The dietary treatments were fed to 48 birds (24 males and 24 females) housed in single metabolism cages in a temperature-controlled environment from 21 to 28 days. At 28 days, birds were slaughtered, weighed and dissected for measurements of digestive organs and collection of gut tissue samples for histology.

4.2.3 Rearing, management and general procedures

Rearing and management, AME bioassay procedures, digesta passage time measurements using ferric oxide gelatine capsules, and analytic procedures were as

described in Chapter 2. At the end of the trial, each bird was killed by intravenous injection of pentobarbitone and weighed. Half of the total number of birds in the trial were used for digestive tract measurements and the other half for gut histology studies. Tissue collection and fixation were also described earlier in Chapter 2.

4.3 Results

There was no diet x gender interactions for any parameters analysed, however there was a significant diet effect ($P < 0.05$) on the feed conversion ratio of birds. The AME and the digestibility of diets also showed significant diet effects ($P < 0.001$). Gender effects were observed on the start and end weights and also on the weight gains.

There were no diet effects on weight gain and feed intake for birds (Table 4). Diet significantly affected FCR ($P < 0.05$). Birds on the straw diets had significantly higher FCR values compared to other diets. The FCR of birds on the diets containing 10 and 15% cellulose did not differ significantly from the straw diets except that of the 5% cellulose which was significantly lower ($P < 0.05$) than these diets.

No gender effect was observed in FCR and intakes or AME and digestibility of diets as shown in Table 4.

There were no significant gender or diet effects on the WTTT of diets through the gastro-intestinal tract of birds on the different levels of cellulose diets and the wheat straw diets (Table 4). Mean passage time for the diet containing coarsely ground wheat straw was 180 minutes whilst that of the finely ground wheat straw was 252 minutes; however, this difference was not significant.

AME values for the different experimental diets were significantly different ($P<0.001$) from each other as shown in Table 4. There is a reduction in the AME value of diets with increased cellulose levels. Wheat straw diets had lower AME values but were not significantly different from each other and the 10 and 15% cellulose diets and all these were significantly ($P<0.001$) from the basal and the 5% cellulose diets. The digestibility coefficient of these diets also showed significant differences ($P<0.001$). The digestibility coefficient was significantly ($P<0.001$) lower in the diets with higher cellulose and wheat straw (Table 4). The relative weights of gizzard showed a significant diet effect ($P<0.05$) but no gender effect was detected, while the small intestine segment weights were not influenced by the dietary treatments. However, there was a significant gender effect ($P<0.05$) on the relative duodenal weight.

The weights of the intestinal sections were not significantly different among birds in all treatment diets (Table 5). The empty gizzard weights showed significant differences ($P<0.05$) between the wheat straw, basal and cellulose diets (Table 5). The gizzards were heaviest in the birds on the 15% finely ground straw. The 15% ground had the second highest gizzard weight.

Table 4. Effects of gender and diet on growth, feed intake, feed conversion, whole tract transit time, apparent metabolisable energy and dry matter digestibility of diets.

Effect	Level	BWEND	BWGAIN	FBD	FCR	WTTT	ME-2	DIG-DM
Gender	Female	1253b	516b	118b	1.63a	217a	14.32a	0.658a
	Male	1476a	610a	141a	1.63a	242a	13.87a	0.637a
Diet								
1	Basal diet (B)	1354a	576a	126a	1.57b	206a	15.09 a	0.701a
2	B + 5 % Cellulose	1407a	605a	129a	1.50b	259a	14.83 a	0.677ab
3	B + 10 % Cellulose	1370a	580a	133a	1.61ab	251a	14.00 b	0.643bc
4	B + 15 % Cellulose	1376a	563a	129a	1.61ab	233a	13.50b	0.608c
5	B + 15 % Fine straw	1354a	526a	130a	1.75a	252a	13.61b	0.630c
6	B + 15 % Coarse straw	1325a	526a	131a	1.74a	180a	13.53 b	0.627c
	Pooled SEM	15.6	12.5	2.25	0.02	12.8	0.12	0.007

Means with a common letter within a main effect are not significant ($P < 0.05$)

BWGAIN = live weight gain (g/bird); FBD = feed intake (g/bird); FCR = Feed conversion ratio (g feed: g gain); ME-1 = AME of diet (MJ/kg as- fed basis); ME-2= AME of diet (MJ/kg dry matter basis); DIG-DM = Dry matter digestibility coefficient; Pooled SEM= Pooled Standard error of mean

Table 5. Relative gizzard and small intestinal weights (g/kg body weight) of broiler chickens on different treatment diets

Effect	Level	Gizzard	Pancreas	Duodenum	Jejunum	Ileum
Gender	Female	18.4a	2.68a	9.10a	16.3a	10.8a
	Male	16.3b	2.52a	7.67b	15.4a	11.3a
Diet						
1	Basal diet (B)	16.7b	2.53a	8.70a	14.0a	10.2a
2	B + 5 % Cellulose	15.7b	2.77a	8.24a	16.0a	10.7a
3	B + 10 % Cellulose	17.3ab	2.65a	8.30a	15.7a	10.4a
4	B + 15 % Cellulose	15.5b	2.54a	8.97a	16.6a	12.7a
5	B + 15 % Fine straw	20.3a	2.71a	8.71a	16.9a	10.9a
6	B + 15 % Coarse straw	18.5ab	2.42a	7.39a	16.0a	11.5a
	Pooled SEM	0.54	0.13	0.34	0.48	0.51

Means with the common letter are not significantly different ($P < 0.05$)

Table 6 Measurements of villus height, crypt depth and villi: crypt ratio (V: C) of small intestinal sections of birds

Villus height and crypt depth (μ)	Dietary treatments						
	Basal diet	5 % Cellulose	10 % Cellulose	15 % Cellulose	15 % Finely ground straw	15 % Coarsely ground straw	Pooled
Duodenum							
Villus height	1513	1401	1539	1262	1518	1514	1513
Crypt depth	335.4	364.8	219.5	206.4	240.1	241.2	190.1
V: C ratio	5.15	3.89	7.19	6.02	6.66	5.90	0.81
Jejunum							
Villus height	1148	1094	1051	1158	1113	1127	770.1
Crypt depth	177.0	238.4	179.7	171.7	160.1	263.4	280.1
V: C ratio	6.40	5.08	5.95	6.57	6.66	5.54	0.61
Ileum							
Villus height	722.5	701.0	802.7	673.2	777.9	600.5	400.1
Crypt depth	164.5	158.6	181.0	190.5	130.2	186.3	220.1
V: C ratio	4.52	4.47	4.51	3.32	5.72	3.45	0.51

Means are not significantly different ($P>0.05$).

4.4 Discussion

The gender effects observed on the start and end weights and on the weight gains were expected as male broiler chickens have faster growth rates and are heavier compared to female broiler chickens (Hughes, 2003). Birds on the cellulose diets showed no ill effects of cellulose on performance. Cao *et al.* (2003) showed that cellulose at 3.5 % level had positive effects whilst at 10% substantial negative effects were observed on performance, nitrogen utilisation and retention time in chicks. The results here are consistent with findings by Cao *et al.* (2003) in that the mean weight gain and mean FCR decreased with increasing levels of cellulose, however, these differences were not significant. Birds on the basal diets were able to convert more efficiently the consumed food into live weight. Birds on the straw diets had higher FCRs compared to that of the other diets, thus were unable to efficiently utilise the consumed food. An increased feed intake, which was not significant, was also observed here with increasing cellulose level as also reported by Siri *et al.* (1992). Despite lower AME values of straw diets overall bird performances between all treatment diets were not significant. Based on my hypothesis, at 15% inclusion insoluble fibre in the form of cellulose showed no negative effect on bird performance and may indicate improved nutrient availability.

Svihus and Hetland (2001) noted an increase in relative intestinal weight (with digesta) when wheat diet was diluted with cellulose powder in the ratio of 1:10 (w/w). There was a significant effect due to gender on the duodenal weights in this study. There was a difference between the genders with female birds showing higher duodenal weights than the male chickens. Again these differences can be attributed to variability among individual birds.

Birds on the straw supplemented diets, however, showed significant differences in the empty gizzard weights. This is indicative that wheat straw was rough enough to stimulate gizzard activity and may have been retained longer in the gizzard, thus eliciting increased gizzard weights due to increased grinding activity. Whether the straw was fine or coarse and its particle size had an effect on the gizzard is not conclusive as the difference in weights were negligible. This result confirms that gizzard weight is affected by higher inclusion of insoluble fibre due to more grinding activity. The increased gizzard weight may be a positive effect as birds were able to change their digestive capabilities in the presence of insoluble fibre to cater for discrepancies in the feed matrix. There were no observed significant diet or gender effects on the villus height, crypt depth and villus height to crypt ratio of birds which may have been due to a smaller sample size in this experiment.

Inclusion of both cellulose and wheat straw in the diets reduced the AME values of these diets compared to the basal diet. This reduction in AME values is consistent with the low AME of these two ingredients. The inclusion of these two ingredients in the diets have influenced digestibility. Although these two ingredients significantly lowered AME values and digestibility, birds were able to have good growth performances.

The lower AME of diets with wheat straw may have led to energy dilution, however, based on bird performance we can assume that the reduction in AME may be less than expected which may indicate improved digestibility of energy from ingredients other than the straw. If this is the case then, based on my hypothesis the presence of higher levels of insoluble fibre, such as wheat straw in the diets may have had an influence on the digestibility of other nutrients in the feed matrix.

4.5 Summary

Cellulose at 15% had no ill effects on the performance of birds. On a 7-day trial the effects of cellulose on the digestive organs of birds especially the gizzard was less noticeable. Wheat straw as a source of insoluble fibre had increased gizzard weight thus had influence on the digestive capacity of the birds and may have also improved energy digestibility in other ingredients due to it being present in the feed matrix.

Particle size effect on the gizzard with wheat straw is not conclusive in that the differences between the coarsely ground and the finely ground were negligible and the time for the birds to adapt to the diets may also be a factor in the outcome of this trial.

CHAPTER 5: EFFECTS OF HIGH LEVELS OF COPRA AND PALM KERNEL MEALS ON PERFORMANCE, WHOLE TRACT TRANSIT TIME, APPARENT METABOLISABLE ENERGY AND GUT PHYSIOLOGY OF BROILER CHICKENS

5.1 Introduction

Based on results obtained for bird performance and apparent metabolisable energy values of local feed resources from studies conducted through the established feed testing facility in PNG, palm kernel cake and copra meal are potential poultry feed ingredients (unpublished data). Palm kernel expeller meal (PKE) and copra meal (CM) are by-products of the oil extracting industries and are produced in huge quantities annually in PNG. These two resources are available in abundance and accessible to small-scale broiler farmers throughout PNG.

PKE despite having high fibre content is moderately high in protein and can be utilised for animal feeding. PKE because of its high fibre content, grittiness and unpalatable nature has been generally considered to be indigestible to non-ruminants.

Gohl (1981) indicated that PKE although seldom used in poultry diets is palatable to poultry and can be included up to 20% in diets with good results. Studies by Onwudike (1986 a, b, c, d) showed that groundnut cake can be replaced with PKE in diets of broiler chicks, starter and grower pullets, broilers and layers. Onwudike (1986c) found that PKE could effectively replace groundnut cake up to 28% for starting birds and 35% for finishing birds without any significant effects on bird performance. In finishing birds as the level of PKE increased there was a decrease in abdominal fat and

an increase in gizzard weights. He observed that birds appeared to adjust better to the higher levels of PKE the longer they stayed on the diets (Onwudike, 1986c). Panigrahi (1991) obtained similar results with broiler chickens and concluded that the maximum inclusion rate of PKE is determined largely by the high fibre and low ME values rather than by toxic or anti-nutritional factors.

Gohl (1981) stated that copra meal can be included up to 40% in poultry diets if other protein sources such as fish meal or soybean meal can be supplemented to increase the content of lysine in the formulation. Panigrahi *et al.* (1987) and Sundu *et al.* (2005a,b) showed good results with copra meal inclusion and both suggested that the performance observed might have been due to the physical structure of the ingredient provided all dietary nutrient requirements are met. Sundu *et al.* (2005) suggested crumbling the copra meal or an inclusion of an enzyme to improve performance.

Previous research work on these two ingredients have involved copra meal and palm kernel meal from Asia and Africa, and it has been suggested that these two resources differ substantially in chemical composition, depending on the method of oil extraction and origin (Onwudike, 1986a; Thorne *et al.*, 1992; O' Doherty *et al.*, 2000; Sundu and Dingle, 2003). The major non-starch polysaccharides found in both copra and palm kernel meals are shown in Table 7.

Table 7 Percentages of the major NSPs present in copra meal (CM) and palm kernel meal (PKE)

Type of NSP	Mannan	Cellulose	Galactomannan	Reference
CM	26	13	61	Balasubramanian (1976)
PKE	78	12	-	Dusterhoft <i>et al.</i> (1992)

These ingredients are ideal for examining the effects of insoluble fibre and its influence in broilers, particularly on the digestibility of other dietary components and on gut capacity. Therefore the present trial was designed to investigate the effect of these two ingredients from PNG in broiler diets on performance, apparent metabolically energy, passage time, and gut physiological and histological changes.

5.2 Experimental

5.2.1 Location

The experimental work was conducted at the National Agricultural Research Institute's Livestock Program in Lae, Morobe Province, PNG.

5.2.2 Birds, diets and experimental design

The experimental design was a randomised complete block design, with four experimental diets and eight replicates of each diet. Thirty-two metabolism cages were used in this experiment.

The experimental diets were diluted with palm kernel meal obtained from New Britain Palm Oil Limited, Kimbe and the copra meal from the Goodman Fielders, Lae. These diets were prepared by diluting the basal diet (Table 1).

Table 8 Composition of experimental diets containing PKE and CM

Ingredient	Basal	30% PKE	30% CM	15% PKE
				+ 15% CM
Sorghum	57.50	40.25	40.25	40.25
Soybean meal	32.00	22.40	22.40	22.40
PKE	-	30.00	-	15.00
CM	-	-	30.00	15.00
Sunflower oil	6.00	4.20	4.20	4.20
Dicalcium phosphate	2.00	1.40	1.40	1.40
Limestone	1.10	0.77	0.77	0.77
DL-methionine	0.70	0.49	0.49	0.49
Vitamin and mineral premix	0.20	0.14	0.14	0.14
Salt	0.30	0.21	0.21	0.21
Choline chloride (60%)	0.20	0.14	0.14	0.14

Three diets were prepared; one with PKE at 30%, one with CM at 30% and the third with PKE and CM each at 15%. The micro-ingredients were hand mixed through bulking and then each diet ingredient was added to a cement mixer. The diets in mash form were stored in labelled bins, then introduced to the birds at 21 days of age.

A basic apparent metabolisable energy bioassay technique was employed (Chapter 2). At the end of the trial, birds were slaughtered, weighed and dissected for measurements of digestive organs and collection of tissue samples for gut morphology.

5.2.3 Rearing and management

Two hundred and sixty two broiler chicks (Ross) bought from Agmark Pacific (Agent-Niugini Table birds) were raised on two separate floor pens on a commercial starter crumbles (Lae Feed Mills-Goodman Fielders) from day old to 20 days of age. Weighing of birds and allocation to cages was as described in Chapter 2.

5.2.4 General procedures

The procedures were as described in Chapter 2.

At the end of the trial, 64 birds out of 128 birds were killed by intravenous injection of pentobarbitone and weighed. The first lot of 32 birds were used for digestive tract measurements, and the other lot of 32 for gut samples for histology work. For the first lot of 32 birds, the digestive tracts from the proventriculus to the caeca were excised. Empty gizzard and sections of the small intestine without contents were weighed and the weights recorded.

The second lot of 32 birds were also killed by intravenous injection and samples of about 50mm in length were collected from the midsections of the duodenum, the jejunum and the ileum. These were then rinsed and stored in formalin in labelled jars. The collected tissues were then transferred into 70% ethanol after 24 hours and stored until needed. Gross energy (GE) of diets and excreta were determined using the Parr bomb calorimeter-1266, standardised with benzoic acid.

5.3 Results

There was a significant diet effect on weight gain, FCRs and the AME and digestibility of diets.

Birds on the basal diets had significantly higher end weights than those on the 30% PKE and 15% CM- 15% PKE diets (Table 9). Birds on the 30% CM diets though had the second highest end weight were not significantly different to all the diets. Birds on the 30% PKE had the lowest end weights. Weight gains and feed conversion ratios did differ significantly ($P<0.001$) among treatment diets. Birds on the basal diet had significantly higher weight gains compared to the other diets. Birds on the 30% PKE had significantly lower gains compared to other diets. Birds on the 30% CM and the 15%CM-15%PKE diet had slight differences in their weight gains and were significantly different to the birds on the basal and the 30% PKE diets. Feed intakes of birds and the WTTT did not differ significantly.

The FCR of birds on the 30% PKE diets was highest ($P<0.001$) while birds on the basal diet had significantly lower ($P<0.001$) FCRs. The FCR of birds on the CM diet and the 15% CM-15%PKE diets were not significantly different from each other but were significantly higher than those on the basal diets and significantly lower than those on the 30% PKE diets.

Table 9. Effect of diets on growth, feed intakes, feed conversion, whole tract transit time, apparent metabolisable energy and dry matter digestibility

Diet	Description	BWEND	BWGAIN	FBD	FCR	WTTT	ME-2	DIG-DM
1	Basal diet (B)	862.5a	311.3a	85.6	1.66b	256	11.28a	0.554a
2	B + 30 % PKE	752.6b	216.8c	77.2	2.52a	253	9.049c	0.452b
3	B + 30 % CM	808.3a	261.7b	82.6	1.99b	257	9.864b	0.462b
4	B + 15 % CM + 15 % PKE	795.8a	264.7b	83.6	1.97b	222	9.801b	0.458b
	Pooled SEM	14.0	7.2	1.7	0.1	8.5	0.1	0.006

Means with the same letter are not significantly different ($P < 0.05$);

BWGAIN = live weight gain (g/bird); FBD = feed intake (g/bird); FCR = Feed conversion ratio (g feed: g gain);

ME-1 = AME of diet (MJ/kg as- fed basis); ME-2= AME of diet (MJ/kg dry matter basis); DIG-DM = Dry matter digestibility coefficient

PKE = Palm kernel expeller meal; CM = Copra meal; Pooled SEM= Pooled Standard error of mean

Small intestinal segment weights and the pancreas collected from birds on the different treatment did not display any diet effect, however, birds on the 30% PKE diets had higher pancreas, duodenal, jejunal and ileal weights compared to the birds on the basal and the 30% CM diets. Moreover, there was a significant ($P<0.05$) gender effect on the relative gizzard and pancreas weights.

Small intestinal segment weights and the pancreas collected from birds on the different treatment did not display any diet effect. There was a significant ($P<0.05$) gender effect on the relative gizzard and pancreas weights (Table 10).

As shown in Table 10, the relative weight of the gizzard of female broiler chickens was significantly higher ($P<0.05$) than that of the male broiler chickens. Pancreatic weights also showed a similar trend.

There was a significant difference in gizzard weights due to dietary treatments. As shown in Table 10, weights of empty gizzard and the gizzard with contents did differ significantly amongst treatment diets ($P<0.001$). Birds on the basal diet had significantly lower gizzard with and without contents. Birds on the 30% PKE and the 15%CM-15%PKE diets had higher gizzard with content weights but were not significantly different from each other and from the birds on the 30% CM diet but differed significantly ($P<0.001$) to those on the basal diets. Empty gizzard weights of birds on the 30% CM diet did differ significantly ($P<0.001$) from the 30% PKE and 15%CM-15%PKE diets but not from the basal diet.

Table 10 Effects of gender and diet on relative weights of gizzard and small intestinal weights of broilers

Effect		Gizzard	Pancreas	Duodenum	Jejunum	Ileum
Gender	Female	27.5a	3.74a	15.7a	29.8a	18.1a
	Male	24.7a	3.19a	14.4a	27.4a	18.0a
Diet						
	Basal (B)	20.9b	3.60a	15.2a	27.3a	18.5a
	B + 30 % PKE	31.0a	3.41a	14.9a	28.8a	18.0a
	B+ 30 % CM	23.2b	3.18a	14.0a	27.9a	17.9a
	B + 15% CM + 15 % PKE	29.4a	3.68a	16.1a	30.2a	17.9a
	Pooled SEM	0.6	0.1	0.5	0.8	0.7

Means with the same letter are not significantly different ($P < 0.05$).

PKE = Palm kernel expeller meal; CM = Copra meal; Pooled SEM= Standard error of mean.

Table 11 Effects of diet on villus height, crypt depth and villus: crypt ratio (V: C)
of small intestinal sections of broilers

					Pooled
	Basal	30% PKE	30% CM	15 PKE+15 CM	SEM
Duodenum					
Villus height	1398	883	1190	751	66.5
Crypt depth	356	263	385	321	26.3
V: C ratio	4.38	3.29	3.17	2.47	0.1
Jejunum					
Villus height	1183	976	1152	1070	103
Crypt depth	376	199	221	420	18.0
V: C ratio	3.14	4.70	4.63	2.80	0.5
Ileum					
Villus height	759	672	713	767	28.4
Crypt depth	219	294	449	447	65.0
V: C ratio	3.49	3.35	2.23	2.43	0.5

Means are not significantly different ($P>0.05$).

As shown in Table 11, there were no diet or gender effects on villus height, crypt depth and villi: crypt ratio of birds in this experiment.

5.4 Discussion

It is clearly evident here that PKE at 30% inclusion in diets of broiler chickens up to 28 days of age has significant effects on the growth performance of birds especially with the end weights, feed conversion efficiency and weight gains. Gohl (1981) indicated that PKE could be included up to 20% with good results. Onwudike (1986c) found that

PKE at 28% inclusion in starting broilers could be effective without having any negative effects. This contradiction may be due to the fact that in this case birds were only given the diets for a week where as in that experiment the trial period was six weeks. Onwudike (1986c) found that birds appeared to adjust better to the higher levels of PKE the longer they stayed on the diets. Similar to Onwudike (1986c) there were no significant differences in the intake of birds and the 30% PKE increased FCR and decreased weight gains. The lower intakes and poor feed conversion efficiency is reflected in the gains which could be a result of the high fibre content and grittiness (Gohl, 1981; Onwudike 1986c; Panigrahi and Powell, 1991; Onifade and Babatunde, 1997) and a deficiency in lysine and methionine contents (Onwudike, 1986a).

As opposed to the 30% PKE diet, birds on the 30% CM diet performed exceptionally well. Panigrahi (1987) and Sundu *et al.* (2005ab) showed good results with copra meal inclusion and both suggested that the performance observed may be due to the physical structure of the compound provided all dietary nutrient requirements are met. Gohl (1981) stated that copra meal can be included up to 40% in poultry diets if other sources such as fish meal or soy bean meal can be supplemented to increase the content of lysine in the formulation.

Work done at the National Agricultural Research Institute in PNG with dilution trial of a commercial broiler finisher pellet with copra meal at 20, 40 and 60% substitution can make a satisfactory low density feed for broiler chickens in the finishing stages. However the diet diluted with 60% CM need to be fed for an additional week to reach a marketable weight of 2 kg (unpublished data). Birds on 30% CM compared with 30% PKE had higher end weight, gain and better feed conversion efficiency and this may be attributed to the fact that copra meal contains less crude fibre than palm kernel meal.

Apparent metabolisable energy is important in chickens if an ingredient of very high fibre content is included in the diet. High fibre content decreases AME and digestibility of a diet and this in turn, is reflected in bird performance. As shown in the present study, inclusion of copra and palm kernel meals at 30% inclusion in the diets respectively lowered the AME and digestibility of the diets significantly. The lowered AMEs of these two diets could also be a factor in the lowered intakes, gains and poor feed conversion efficiencies, more so in 30% PKE diets than the 30% CM diets. Moreover, the digestibility coefficient likewise showed significant drops as well compared to that of the basal diet. This may all be linked to the high fibre content.

Coarse particles accumulate in the gizzard and are retained longer (Clemens *et al.*, 1975; Moore, 1999). Palm kernel and copra meal are coarse, thus the increased gizzard activity signified by an increased gizzard weight due to increased grinding activity to reduce larger particle size to a smaller size before leaving the gizzard.

Moreover high fibre diets appear to induce an increase in duodenal reflux (Sacranie *et al.*; 2005). Hetland *et al.* (2003) showed that chickens fed a high fibre diet had increased bile acid concentration in the gizzard signifying an increase in the gastroduodenal reflux. A well-developed gizzard and an increase in duodenal refluxes give a beneficial environment for the bird to maximise the nutritive value of the feed in that the feed is re-exposed to the digestive enzymes and an increase in bile acid concentration can also lead to enhanced lipid solubilisation and absorption.

Moreover, improved starch digestibility has also been linked with feeding whole wheat or insoluble fibre materials to birds (Hetland *et al.*, 2003).

Birds on the 30% PKE diets had significantly higher empty gizzard and gizzard with contents weights. The increase in gizzard weight is probably linked to an increase

grinding effect by the gizzard to reduce the fibre in the palm kernel meal, which is gritty and unpalatable. The gizzard content was also significant and may be due to an increase in gastrointestinal reflux due to increased gizzard contents, as reported by Hetland *et al.* (2003). Pancreatic weight was higher in birds on the palm kernel meal and may be indicative of enlarged glands to increase secretion of digestive juices. The intestinal segment weights of the small intestinal were higher in the same diet and may also suggest an increased tissue weight due to increased cell proliferation (Iji, 1999). There were no observed significant differences in the gut morphology and may be due to a smaller sample size.

5.5 Summary

This experiment signifies that copra meal and palm kernel can be used at 30% inclusion for low-density diets. However, the maximum inclusion rate of palm kernel meal is determined largely by the formulation constraints because of high fibre and low ME values rather than by toxic or anti-nutritional principles. Thus palm kernel meal cannot be ruled out completely.

Increased gizzard, pancreas and small intestinal segment weights observed in the palm kernel meal may be beneficial in that it influences a larger gut capacity in chicken.

Results of evaluation of the two ingredients suggest that palm kernel meal and copra meal need to be introduced to the diets over a period of time to assess the impact of these on the digestive capacity of birds, especially with the gizzard, gut reflux and intestinal mucosa which are all linked to nutrient digestion and absorption in the gut.

CHAPTER 6: GENERAL DISCUSSION

6.1 Introduction

The broad aim of the project was to obtain information on the use of relatively cheap, locally-grown feed ingredients in Papua New Guinea (PNG) in the expectation that reduced reliance on expensive imported feed ingredients could help improve economic viability of small-scale poultry production.

The main objectives of this project were to determine the effects of high inclusion of fibrous material on the performance and nutrient digestibility and the digestive capacity of broiler chickens.

The three hypotheses tested in this project were that the presence of a large amount of insoluble fibre could result in (i) an increase in the apparent metabolisable energy (AME) and dry matter digestibility (DMD) of the diets; (ii) a decrease in the rate of passage through the gastrointestinal tract and (iii) an increase in the digestive capacity of broiler chickens.

Each of the tests of hypotheses is discussed below in relation to findings of three experiments conducted during the course of my studies. This section also highlights the extent to which the project aim and objectives were achieved and includes recommendations for future research work.

6.2 Insoluble NSP and bird performance, apparent metabolisable energy and dry matter digestibility

In general, inclusion of cellulose in the first two experiments conducted in this project did not compromise the performance because weight gains and feed conversion ratios (FCR) were not drastically reduced. However, AME and DMD of the experimental diets were significantly reduced due to inclusion of cellulose. Other authors have also shown that moderate inclusion of cellulose have no ill effect on performance (Siri *et al.*, 1992; Cao *et al.*, 2003) and that inclusion of cellulose is associated with increased feed intakes linked with increased relative intestinal weights, improved starch digestibility and increased gizzard weight (Svihus and Hetland, 2001).

In broiler chickens, the relationship between AME and FCR is negatively correlated in that a reduced AME will increase FCR because birds tend to eat to meet their energy requirements. The results of my project are inconsistent with a simple negative relationship between AME and FCR, and hence some form of complex process must be occurring. A speculation as to why birds appeared to achieve good performance in the form of weight gains and FCRs despite apparent reductions in AME and DMD is that despite dilution of the diet by cellulose, other nutrients may have become more readily available to the birds.

Cellulose, however, when present in a natural ingredient such as palm kernel extract (PKE) and copra meal (CM) may have influenced weight gain and FCR differently as observed in this project with results of the experiment with PKE and CM diets.

PKE and CM are commercially available high-fibre by-products that could be included in broiler rations in PNG by small-scale broiler producers. In my study, PKE and CM were included in diets either individually at 30%, or in combination of 15% level for

each. Performance-wise, birds on the PKE diet had significantly lower gains (94.5 g/bird less than the basal diet and 44.9 g/bird less than CM diet) and higher FCR (0.86 a 52% increase and 0.53 which is a 27% increase compared to the basal and CM diet, respectively). The recommended level of inclusion of PKE as an ingredient in broiler diets is less than 30% (Gohl, 1981; Onwudike, 1986c). However, we could speculate that if the birds were given a longer period to adjust to the diets that there may not have been any significant differences in the end weights.

Birds on CM diets performed better than those on the PKE diets, which is consistent with the results of Gohl (1991) who indicated that CM could be included up to 40% in poultry feeds. These two ingredients could be used in differing combinations in broiler feeds.

Because the performance of the birds on diets incorporating the commercially-available high-fibre by-products did not match the performance of the birds on diets incorporating laboratory-grade cellulose, it is apparent that those by-products contained substances other than cellulose.

According to Choct (1999), there is a general inhibition of the digestibility of nutrients when diets with high level of soluble NSP are fed to chickens. These soluble NSP because of their viscous nature impede nutrient digestion by creating a viscous gut contents and thus reduce the mixing of digestive enzymes and their substrates. Choct *et al.* (1996) stated that poor bird performance is the result of reduced nutrient digestibility. Based on the results of the experiments conducted in this project, cellulose as a standard insoluble NSP seems to not have a detrimental effect on bird performance. However, in natural ingredients such as PKE, bird performance was poor compared to the birds on cellulose and CM diets. As indicated by Choct (1999), effects

of NSP on nutrient digestion are interrelated and dependent on solubility, molecular weights and configuration of the polymers, and that solubility of the NSP in turn is dependent on the chemical structure and their association with other cell wall components. This may be what is happening to birds on the PKE diets. Perhaps the percentage of cellulose in PKE is lower compared to other soluble NSP present in PKE or that because of its association with other cell wall materials, it is having a negative effect on growth performance that is soluble NSP compared to the effect of standard cellulose observed in this project.

Hughes and Choct (1999) provided evidence for the anti-nutritive effect of soluble NSP, which included a negative correlation between AME and soluble NSP, and that purified NSP depressed AME (which is evident in this project with cellulose). AME and DMD of diets were lowered due to inclusion of cellulose, CM and PKE. Bird performance as indicated by weight gain and FCR was better for birds on cellulose diets compared to the ones on the PKE diet. Cellulose as a standard insoluble NSP in this experiment may not have acted simply as a diluent but may have aided utilisation of other dietary components such as starch, protein and fat through alteration of the gut function, because the FCR was not impacted to an extent consistent with lowered AME.

The relative pancreas weight of birds on cellulose diets was marginally significant ($P = 0.06$) in the first experiment. This increase in pancreas weight may reflect two possible factors, namely, increased secretion of amylase improved digestion of starch, and increased secretion of lipase secretion improved fat digestion. Thus based on the pancreas weight of birds on cellulose diets, I could speculate that cellulose was not merely diluting AME but could have aided more complete digestion of other nutrients. Further study is required to elucidate the underlying mechanisms.

6.3 Insoluble NSP and digesta passage time

One of the hypotheses of this project was that digesta passage time is shortened due to the presence of a high level of insoluble fibre. The three experiments conducted demonstrated that digesta passage time measured as the whole tract transit time (WTTT) tended to increase although not significantly. Birds on 12 and 15% cellulose had longer WTTT than the birds on the basal diets. Digesta passage time for birds on PKE and the CM diets were similar to that of birds on the basal diet. On the other hand, birds on the coarsely ground straw, had the shortest WTTT (180 minutes) compared to the ones on the fine straw (252 minutes). Birds on the fine straw diets had similar WTTT compared with the 5 and 10% cellulose diets (259 and 251 minutes, respectively). These results are consistent with those of Hetland and Svihus (2001) who showed that coarsely ground oat hulls decreased the digesta passage time.

In both cases, the rate of passage of digesta decreased due to inclusion of coarsely ground materials. Therefore, the hypothesis that digesta transit time is shortened by the presence of high levels of insoluble fibre is supported when the particle size of the material is taken into account.

The tendency towards a shorter passage time of the coarsely-ground wheat straw in the birds in my trial was inconsistent with current understanding of the handling of fibre by the chicken gut, which is that fibre is reduced by the gizzard to a particle size of 100 μ before further passage. In rabbits, Hörnicke and Björnhag (1980) proposed that selective separation and excretion of insoluble fibre, while more readily digestible material is retained for longer periods, is a strategy used by rabbits for coping with diets of low digestibility. It allows higher intake and passage rates but still achieves reasonably efficient digestion of the higher quality components of the forage, and it may be that a similar mechanism could be present in broiler chickens particularly if the

gizzard was overwhelmed by the fibre after insufficient time to fully adapt to the coarse fibre diet.

6.4 Insoluble NSP and digestive capacity of broiler chickens

The hypothesis that the presence of a high level of insoluble fibre results in increase in relative weights of gizzard and small intestine was tested in this project.

Birds on the cellulose diets did not show any significant differences in the gizzard and small intestine weights. Birds on the 30% PKE diet and the combined 15% CM and 15% PKE diet, however, had significantly higher gizzard weights compared to the birds on the basal and the 30% CM diets. The level of inclusion of CM may not have been high enough to influence gizzard activity. The increase in gizzard weight is probably linked to an increase in grinding effect by the gizzard to reduce the fibre in the palm kernel meal, which is a gritty and an unpalatable ingredient.

It is possible that a larger sample size may have revealed a difference in organ weight, and that males and females may differ significantly in digestive function due to dietary treatments. Female birds tended to have higher pancreas weight compared to the male broiler birds. The increase in pancreas weight may have been due to two possible factors explained earlier in this chapter. Hughes (2003) observed similar results between genders in his study. Hence, further work is required to elucidate the underlying mechanisms.

The villus height, crypt depth and villus: crypt ratio did not show any significant differences due to insoluble fibre, in these experiments. Iji (1998) showed significant

increases in crypt depth in the jejunum and the ileum, an increased villi height in the ileum, and a reduced villi height in the duodenum of chicken supplements with viscous soluble NSP. Cellulose as a standard source of insoluble NSP in these experiments did not affect the intestinal morphology of the birds probably because insoluble fibre does not affect viscosity of the digesta.

I speculate that although PKE and straw had a significant effect on gizzard weight, which is indicative of larger gut capacity, PKE may have detrimental effects at the mucosal levels due to other antinutritive factors such as soluble NSP present in this ingredient. Wheat straw may or may not have detrimental effect at the mucosal level depending on its composition of soluble NSP. If wheat straw contained more structural insoluble NSP or lignin, then based on the results observed with cellulose here, I may speculate that cellulose and straw may have the same effect.

6.5 Conclusion

Cellulose at 15% inclusion did not shorten digesta passage time nor did it have significant effects on the digestive capacity of broiler chickens. Cellulose decreased the apparent metabolisable energy and dry matter digestibility; however, bird performance was not compromised as a consequence of these changes.

PKE at 30% as a source of insoluble fibre influenced gizzard weight. Performance of birds was not as good as the birds on the 30% CM diets. AME and dry matter digestibility were reduced due to the inclusion of both materials at 15%.

The two objectives of the project were achieved in that I was able to show that inclusion of insoluble fibre at high levels influenced nutrient digestibility, especially

the AME of the diets. The second objective was achieved in that the gizzard weights were increased by the inclusion of straw and PKE. However, the effects of insoluble fibre at the mucosal level in this project were inconclusive.

6.6 Future work

The birds were able to perform well despite a high cellulose inclusion although there were significant decreases in the AME and dry matter digestibility. I was not able to analyse the effect of insoluble fibre on other nutrients such as starch, protein or fat due to lack of time. Thus this work needs to be repeated to take into account a possible increase in digestibility of these nutrients as well as the AME of diets. Further study of the effects of insoluble fibre on pancreas size and possibly its function is warranted.

More work needs to be conducted to determine the effect at the mucosal level of high dietary levels of insoluble fibre. There were no significant differences observed here, however, a different result may have occurred had there been a higher degree of replication. There is a need to investigate further from an earlier age over a longer period of time, the effect of PKE and CM on the intestinal mucosa of broiler chickens.

An economic analysis of the use of PKE and CM in diets at varying inclusion rates needs to be done, as due to high prices of imported feeds in developing countries such as PNG, it might be economical to use a diet of lower nutrient content based on relatively cheap local ingredients and to accept a lower productivity in comparison with efficient but costly commercial broiler diets.

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