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EFFECT OF NON-STARCH POLYSACCHARIDES ON BLOOD LIPID METABOLITES, ORGAN WEIGHTS, INTESTINAL MUCIN PRODUCTION AND ENDOGENOUS LOSSES IN WEANER PIGS, AND PROTEIN DIGESTION IN BROILER CHICKENS

A thesis presented in partial fulfilment of the requirements for the degree of Master in Applied Science (Animal Science) at Massey University, Palmerston North, New Zealand

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2002

ABSTRACT

This study was undertaken to examine the anti-nutritional influence of soluble non-starch polysaccharides (NSP) in two monogastric species. In experiment 1, the influence of NSP on blood lipid metabolites, organ weights, growth performance, mucin production, and endogenous nitrogen and amino acid flows were evaluated in pigs. In experiment 2, the influence of NSP on ileal nitrogen digestibility and flow were determined in broiler chickens.

In experiment 1, different levels of purified maize arabinoxylan (AX) and barley β-glucan extract Glucagel™ (BG) were substituted for cellulose in enzymatically hydrolysed casein-based (EHC) diets. Five experimental diets consisting of different levels (4% and 7.5%) of AX and BG and EHC, wheat starch, sugars and coconut oil were formulated. These diets contained titanium oxide as an indigestible marker. Each experimental diet was fed to five 3-wk old LWxLR pigs for 21 days. The results showed that AX and BG did not significantly influence ($P>0.05$) the levels of blood metabolites measured after 21 days in fasted and fed states. Some blood metabolites showed significant changes over time. The levels of total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were significantly increased ($P<0.05$) after 21 days. On the 21st day, fasted and fed states were compared. Fasting significantly increased TC and some HDL levels, but LDL levels were not affected. The increase of blood metabolites over time was attributed to the interplay between increased synthesis in the liver and other tissues or decreased catabolism. Similar LDL values indicated differences of LDL metabolism between humans and pigs, which lack cholesteryl ester transfer protein (CETP) activity. Thus, very little HDL cholesteryl ester is transferred to LDL.

The values obtained for empty organ weight were similar ($P>0.05$) between different diets although gut fill was significantly greater ($P<0.01$) with dietary inclusion of 7.5% BG, indicating the presence of gelling property of BG. Carcass weight and liveweight were similar ($P>0.05$) between diets. Daily feed intake (DFI) was also similar due to the restricted feeding scheme. However, weight gain ($P<0.05$) and feed conversion ratio (FCR) were improved ($P<0.01$) with dietary inclusion of 7.5% AX and BG, indicating high degradation rates of AX and BG molecules in pigs. This improvement was not due to the

difference in gut fill. It could also be proposed that the threshold levels or length of time to initiate increased organ weights and affect growth performance was not achieved.

Evaluation of crude mucin (CM) indicated a significant numerical increase in CM associated with increased level of AX, but not with BG. In the same trial apparent nitrogen digestibility (AND) ranged from 73.1% to 80.9% across diets. When corrected for endogenous losses, the range of true nitrogen digestibility (TND) across all diets became closer (88.36% to 90.7%). However, AND and TND were similar ($P>0.05$) in pigs fed different NSP. The endogenous nitrogen flow (ENF) showed numerical significant increase with increased level of AX, but not with BG. It is possible that the branched structure of AX molecules, which is difficult to breakdown, and its ability to hold water hampers digestion and absorption process and consequently leads to increased ENF and CM flow. BG may not be an anti-nutritional factor in pigs as implied by its high mechanical breakdown by microbes colonising the pig gut.

Numerical increase in endogenous amino acid flows (EAAF) was observed with increased levels of AX but no definite trend with BG. In fact, EAAF in mixed NSP diets (4%BG and 3.5% cellulose) was even significantly higher than 7.5% BG. When pure NSP diets were compared, EAAF was highest in 7.5% AX ($P<0.05$), intermediate in BG, and lowest in control diet. Specifically, EAAF was highest for glutamic acid. Significant increased flow ($P<0.01$) for amino acid threonine, proline and serine with 7.5% AX are consistent with the high level of crude mucin found for this diet (i.e. those amino acids are abundant in mucin).

In the second experiment, different levels (3% or 6%) of purified maize arabinoxylan (AX) and barley β-glucan extract Glucagel™ (BG) were substituted for wheat starch in enzymatically hydrolysed casein-based (EHC) diets. Five experimental diets consisting of EHC, cellulose, wheat starch, dextrose and vegetable oil were formulated. These diets contained titanium oxide as an indigestible marker. Each experimental diet (control, 3% and 6% of BG or AX) was fed for 7 days to 27-day old birds in cages, with 4-5 birds/cage. Inclusion of AX and BG did not significantly influence feed intake ($P>0.05$). AND was numerically depressed at 90.37% and 90.4% for 6% AX and BG as compared to 91.1% for control diet. Ileal nitrogen content and endogenous nitrogen flow were numerically increased with increased levels of AX and BG, though statistically significant differences were not observed due to high variations among the replicates. Inclusion of 6% BG significantly depressed dry matter digestibility ($P<0.05$), suggesting preservation of hydration property of gelling BG.

It is then concluded that the anti-nutritional effect of soluble NSP was evident in chicken as indicated by decreased dry matter digestibility ($P<0.05$) and the extent of increase in ileal flow of nitrogen in chicken. The cause of increased nitrogen flow with increased levels of NSP is not clear, but could be due to increased secretion of endogenous protein, decreased reabsorption, or combination of both. In pigs, dietary inclusion of arabinoxylan promotes anti-nutritional activity through its influence in nutrient digestion and absorption. This is shown by the increase of CM, ENF and EAAF ($P<0.05$) with increased level of dietary AX. This effect can be related to the ability of AX to hold water and their branched structure, which is difficult to degrade. Further, it would appear that gelling BG extract is likely well tolerated and its dietary inclusion seemed not a factor to negatively influence pig nutrition, at least with the levels used in this trial. The increase in ENF and EAAF associated with dietary inclusion of mixed NSP (4%BG and 3.5 % cellulose) is difficult to comprehend and is open for speculation. It is indicated that further research is needed to better understand the dynamics of cholesterol-lowering effect of NSP, and the effects of NSP on organ weights, ENF and EAAF. Such experiments should be conducted using relatively older animals and for a longer period of time.

ACKNOWLEDGEMENT

My sincere gratitude is expressed to my supervisor, Dr. Patrick C.H. Morel for his untiring commitment, guidance and encouragement throughout the study.

My heartfelt appreciation is extended to the Governments of New Zealand and the Philippines through the New Zealand Official Development Assistance program for providing the scholarship funding.

Special appreciation to the Davao Oriental State College of Science and Technology faculty and administrative staff, led by Dr. Jonathan A. Bayogan for supporting this academic advancement.

I am grateful for the technical expertise offered by Dr. Ravi Ravindran, Dr. Rana Ravindran and Mr. Shane Rutherfurd.

Special thanks to Don Thomas, Blake Camden, Ed James, Graham Pearson, Yvette Cottam, for their technical assistance throughout the course of the experiment.

Thanks to the staff of the nutrition laboratory in the Institute of Food Nutrition and Human Health for their help offered in the chemical analysis.

Special gratitude is expressed to my international friends, Slumber Badubi from Botswanna, George Reyes and Carlos Ramirez from Colombia and Jeff Yu from China. Thank you for your company.

Special gratitude to the Carambas family, Manong Angel, Manang Myrna and Gigi; to Eric Bergonio, Mayette Emana, Ponchie Santos and Mona de los Reyes for the "many help" they offered.

Thanks to Jacy, Gladys, Danny and Gina, Jerry and Marivic, and John for the company.

I am grateful to my tennis mates Indra, Norman, Leo, Mayette and Paco.

I am very grateful for the love, support and prayers of my parents, sisters and brother.

Appreciation is also expressed to my wife's family for their encouragement and for being there always.

My fondest gratitude is expressed to my wife, Anne Catherine, and son, Roy Angelo, for their love, inspiration, support and understanding and to whom this piece of work is humbly dedicated.

To the Lord Almighty for making things possible, thank you.

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LIST OF ABBREVIATIONS

AA	**Amino acid**
ADG	**Average daily gain**
AFDC	**Apparent faecal digestibility coefficients**
ANF	**Anti-nutritional factor**
AX	**Arabinoxylan**
BG	**Betaglucan**
CAS	**Casein**
CDC	**Chenodeoxycholic acid**
CMC	**Carboxymethylcellulose**
CETP	**Cholesteryl ester transfer protein**
CHD	**Coronary heart disease**
CM	**Crude mucin**
Da	**Daltons**
DE	**Digestible energy**
DFI	**Daily feed intake**
DM	**Dry matter**
DMD	**Dry matter digestibility**
DMI	**Dry matter intake**
EAAF	**Endogenous amino acid flow**
EHC	**Enzymatically hydrolysed casein**
ENL	**Endogenous nitrogen losses**
EPL	**Endogenous protein loss**

FCR	**Feed conversion ratio**
GIT	**Gastro-intestinal tract**
HDL	**High-density lipoprotein**
HMG CoA	**3-hydroxy-B-methylglutaryl Coenzyme A**
IRA	**Ileo-rectal anastomosis**
LCAT	**Lecithin:cholesterol acyl transferase**
LDL	**Low-density lipoprotein**
N	**Nitrogen**
NSP	**Non-starch polysaccharides**
PVTC	**Post-valve T caecum**
TC	**Total cholesterol**
TRL	**Triglyceride-rich lipoprotein**
TND	**True nitrogen digestibility**
UWL	**Unstirred water layer**
VLDL	**Very low-density lipoprotein**

CHAPTER I

GENERAL INTRODUCTION

Pigs require diets that liberate adequate levels of essential nutrients (energy, proteins and amino acids) necessary for maintenance, growth, production and reproduction. As nutrition, particularly dietary factors, covers 60 – 70% of the total cost of pig meat production (Noblet and Henry, 1993), it is then essential that accurate evaluation of nutrients in feedstuff and optimal utilisation of feed by the animal be given preferential attention.

To date, approximately two billion tonnes of cereal grains and 140 tonnes of legumes and oil seeds are produced throughout the world every year that yields 230 million tonnes of fibrous materials as part of variety of by-products (Choct, 1997). Barley, wheat and corn are the major grains used in pig production. The fibrous component of the grain consists primarily of non-starch polysaccharides (NSP), which in cereals forms part of the cell wall structure (Choct, 1997). Barley β-glucan and wheat arabinoxylan are the major soluble NSP's comprising 77% and 88%, respectively, of the endosperm. Arabinoxylan can also be found in the wheat aleurone (25%) (Newmann *et al.*, 1989; Fincher and Stone, 1986), and it consists of a complex chemical structure composed of two sugars (arabinose and xylose) in a branched structure (Han, 2000).

It is well known that there is a negative relationship between dietary fibre and digestibilities of nutrients (Jorgensen *et al.*, 1996; Yin, 1994). This anti-nutritive property is related to the ability of soluble NSP to increase digesta viscosity in chickens resulting in impairment of nutrient digestion and absorption (Choct and Cadogan, 2001) by increasing endogenous losses of protein (Angkanaporn *et al.*, 1994) and interference with gut microflora (Choct *et al.*, 1996). However, this effect of viscosity from soluble NSP is ineffective in pigs, which can be related to anatomical and physiological differences of the two species (Thacker, 2000; Pluske *et al.*, 1999).

Several authors demonstrated that the amount of endogenous losses in thedistal ileum of pigs depends on dietary factors, specifically the level and type of fibres (Zebrowska and Kowalczyk, 2000; Leterme *et al.*, 1998; de Lange *et al.*, 1989), but how fibre components influences the increase in endogenous secretion remains poorly elucidated. It is, however, hypothesized that fibre may directly stimulate secretion of nitrogenous compounds into the digestive tract (Zebrowska and Low, 1987), and reduce endogenous amino acid reabsorption related to the physiochemical properties of fibre (Bergner *et al.*, 1981).

The determination of undigested mucin in ileal digesta has nutritional importance because it may represent a considerable loss of endogenous amino acids (Lien *et al.*, 2001).

However, limited studies have examined the effects of individual dietary constituents on mucin secretion, and dietary fibre is of interest since it induces structural and morphological changes in the digestive tract leading to increased mucin secretion (Vahouny and Cassidy, 1986).

On the other hand, several authors have shown that soluble NSP's, particularly β-glucans, demonstrate hypocholesterolemic factor in humans and experimental animals (AACC Report, 2001; Klopfeinstein, 1988; Hecker *et al.*, 1998). The exact mechanism proposed for this action is not clear, but several theories had been elucidated, namely bile acid binding and disturbances in digestion and absorption in the intestine brought about by increased digesta viscosity (Klopfenstein, 1988).

The series of experimental studies embodied in this thesis evaluate the anti-nutritive effects of soluble (arabinoxylan and β-glucan) and insoluble (cellulose) NSP, in pigs and chickens, as well as hypocholesterolemic effect in pigs. Chapter 3 assesses the anti-nutritive effects (i.e. growth performance and endogenous nitrogen losses) of β-glucan and arabinoxylan. It also includes assessment of the hypocholesterolemic effect of both soluble NSP, as well as the effects of the same in the secretion of mucin. Chapter 4 evaluates the anti-nutritional effects (i.e. ileal nitrogen digestibility and endogenous nitrogen losses) of betaglucan and arabinoxylan in chickens.

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CHAPTER II

REVIEW OF LITERATURE

2.1 Introduction

Today's swine industry necessitates development of efficient and site-situation specified feeding schemes due to the expanding range in quality and type of feedstuffs used for diet formulation. It is well known that cereals, particularly wheat and barley, and their by-products, contain non-starch polysaccharides (NSP) that form part of the cell wall (Englyst, 1989). The soluble NSP are known for their anti-nutritive factors and inhibition of availability and utilization of nutrients by directly acting on the nutrient itself or initiating physiological changes in the gastro-intestinal tract.

β-glucan and arabinoxylan are known to increase digesta viscosity attributed to their highly complex and branched structure (Thacker, 2000). The anti-nutritive effects are very prominent in chickens, but less evident in pigs because of anatomical and physiological differences between the two species (Pluske *et al.*, 1999). The major impact of soluble NSP to the said species is directed towards depressed nutrient digestibilities and increased endogenous nitrogen losses associated with increased mucus production (Satchithanandam *et al.*, 1990). Equally important, however, is the property of soluble NSP to modify blood or serum cholesterol levels in experimental animals and humans (Newman *et al.*, 1989).

This chapter describes the definitions, structures and classifications of soluble NSP, it also explains the dietary influence on endogenous nitrogenous losses from the ileum, as well as hypocholesterolemic properties and mucin secretions related to dietary fibres.

2.2 Dietary Fibre – definition, development and classification

The term "dietary fibre" had been in vogue as early as 1800s but confined largely in reference to animal forages. Later, crude fibre had been described referring to the residue leftover after treatment with acid or alkali. Until the observation of the pattern of disease prevalence of people in developed countries, whose diets composed mainly of whole plant materials, minimal attention had been paid to elucidate its physical, chemical and physiological properties (Potty, 1996). Dietary fibre was first used in 1953 to refer to the non-digestible residue in food, and later became an acceptable term in place of crude fibre (Spiller, 1993). At this stage, the importance of fibre was recognised as a preventive measure against a variety of disorders (Burket and Trowell, 1975 cited by Southgate, 1982). Southgate (1982) hypothesized that a diet having a high content of cell wall materials protects against a range of diseases (constipation, diverticular disease, coronary heart disease) whereas, a diet having a low component of plant cell wall is a causative

source for the occurrence of diseases. The hypothesis definitely refers to the type of diets and presence of protective capacity of plant cell wall in developing disease resistance. Trowell (1974, cited by Spiller, 1993) described dietary fibre as the skeletal remains of plant cells that is resistant to the enzymatic digestion, and this was later magnified as plant polysaccharides and lignin that can withstand hydrolysis by the digestive enzymes of man. Such definitions revealed that dietary fibre is non-starch polysaccharides (NSP) plus lignin, though it may better apply to the dietary fibre complex (Spiller, 1993), and it was Englyst (1989) who identified the principal component of dietary fibre as non-starch polysaccharides.

Non-starch polysaccharides (NSP) originate from dietary fibre, and in this context, it constitutes the chemical portion of polysaccharides and other plant materials in the diet which are not digested by the normal endogenous secretion nor absorbed by the small intestine (Ravindran 2001; Englyst, 1989), although degraded to a variable degree in the large intestine of the mammalian tract (Asp, 1996). The new concept of NSP that refers to plant cell wall materials, less lignin, is considered as a better definition of dietary fibre providing a best index of plant cell wall polysaccharides aside from being chemically precise and preserving the old concept of dietary fibre (Englyst, 1989).

Therefore dietary fibres can be summed up as the edible parts of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. These include polysaccharides, oligosaccharides, lignin and associated plant substances. They promote beneficial physiological effects including laxative effects, and/or blood cholesterol level attenuation, and/or blood glucose level attenuation. These definitions, therefore, clearly delineate the meaning of dietary fibre and analogous fibre, and define the important and relevant functional properties of all dietary fibres (AACC report, 2001).

2.3 Chemical Composition, Classification and Structure of Non- Starch Polysaccharides (NSP)

There are about nine monosaccharides that serve as predominant building blocks of NSP (Smits and Annison, 1996). These are the pentoses (arabinose and xylose), hexoses (glucose, mannose and galactose), 6-deoxyhexoses (rhamnose and fucose) and hexauronic acids (galactoronic and glucoronic). These sugars are joined by the glycosidic bonds between the hemi-acetal group of one sugar and the hydroxyl group of another. The bonds

are identified by the carbon atoms of each sugar that are involved in the bond (1 to 6 for hexose and 1 to 5 for pentose) and the orientation of the oxygen atom in the hemi-acetal group (primary B) (de Lange, 2000; Smits and Annison, 1996). There are numerous numbers of potential polysaccharides structures, which are considered as heterogeneous and complex (Ravindran, 2001; de Lange, 2000). The complexity is increased by covalent bonding to non-carbohydrate compounds, such as methyl and acetyl groups, phenolic acids, proteins and lignins, and non-covalent bonding within and between polysaccharides. Such diversity in chemical structure, both within and between dietary source, is implied in the physical structure and physiological activities of various NSP fractions (de Lange, 2000). The main NSP structures common in feed ingredients of plant origins are cellulose, β-glucans, arabinoxylans, arabinogalactan, galactomannan, xyloglucan and rhamnogalactorounans (de Lange, 2000), but only cellulose, arabinoxylan and β-glucan are described herein.

Generally, plant polysaccharides can be grouped broadly into two distinct and chemically well-defined types, the storage polysaccharides starch (α-glucan) and the cell wall polysaccharides (non-α-glucan), which are otherwise known as non-starch polysaccharides (Englyst, 1989). Classification of NSP can be based in variety of ways depending on the purpose of the investigator, but it is originally based on methods used for extraction and isolation of polysaccharides (Choct, 1997). NSP can be segregated on the basis of function in the plant either as storage (mannans, guar gum, galactans and xyloglucans) or structural components (cellulose and hemicellulose) and non-structural like gums and mucilages associated with the endosperm and intercellular spaces (Ravindran, 2001). The glucans and arabinoxylan from cereals are also known to have structural and protective roles (Baghurst *et al.*, 1996). Several conditions separate NSP according to being neutral containing neutral sugar residues, and acidic having uronic acid residues or pectic substances. Other criteria classify them into three groups: A, B and C depending to their solubility in various pH (Asp, 1996). The NSP can be further separated based on solubility to water, alkali solutions, alcohols (Ravindran, 2001) or plainly soluble and insoluble fractions (Choct, 1997). Another classification divides NSP into three groups: cellulose, non-cellulosic polymers and pectic polysaccharides, the former containing arabinoxylan, mixed linked β-glucans and its derivatives ((Figure 1) (Choct, 1997).

2.3.1 Structure and Chemistry of Major NSP

The major NSP discussed herein are cellulose, β-glucan and arabinoxylan. The cell walls of most cereal grains are composed of cellulose microfibrils associated with glucomannan

and are embedded in a matrix of hemicellulose-like arabinoxylan and β-D-glucan cross linked with phenolic esters (Potty, 1996).

In most plants, cellulose is present as the structural polysaccharides in the cell wall. In cereals' endosperm wall, β-glucan in barley and arabinoxylan in wheat, sorghum, corn and triticale, are the major NSP (de Lange, 2000; Choct, 1997; Potty, 1996; Smits and Annison, 1996).

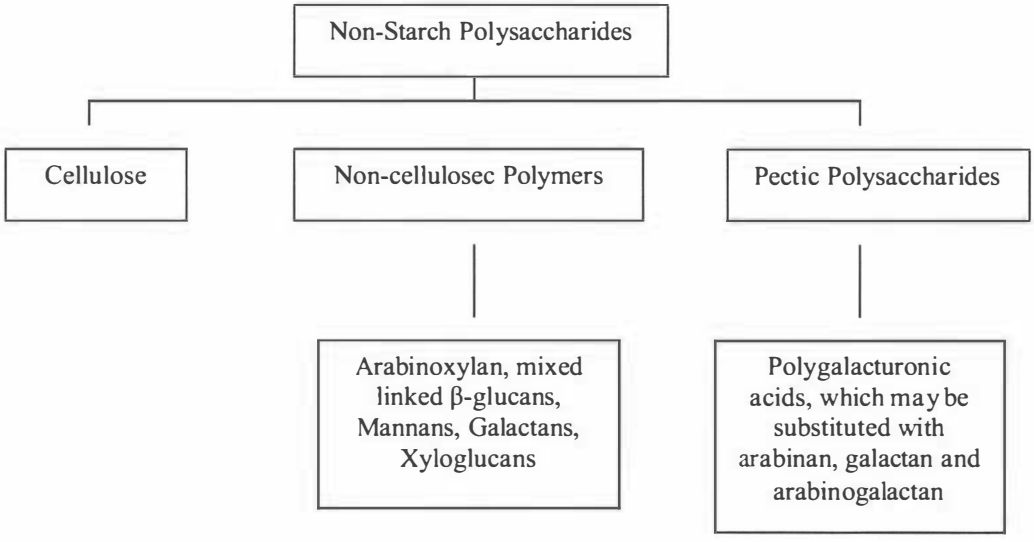


Figure 1. NSP classification (Choct, 1997)

2.3.1.1 Cellulose

Cellulose is the major structural and fibrous constituent of plants found in the walls of cellular tissue that is usually combined with xylans and lignin, and by far the most abundant molecule in nature. It is a (1-4)-β-glucan containing up to several thousand β-gluco-pyranosyl residues linked through (1-4) β-linkages to form very long, linear chains (MacGregor and Fincher, 1993). Individual cellulose chains tend to align and aggregate which leads to the formation of crystalline microfibrils, which have been stabilised by extensive intermolecular hydrogen bonding and which gives considerable tensile strength to the cell wall (Fincher and Stone, 1986). Their polymers are essentially insoluble in water due to difficult access to its crystalline region. However, finely segregated cellulose can readily take up water (0.4 g water/g cellulose), and increase faecal weight when added to diets (Southgate *et al.*, 1993). The presence of beta linkages and the crystalline nature of cellulose provides resistance from enzymatic and chemical actions thereby making it partially degradable in the monogastric digestive system (Ravindran, 2001).

2.3.1.2 Beta-glucans

β-glucans are of intermediate solubility, partly water-soluble but often require alkali for complete extraction (Ravindran, 2001). They represent essential components of the cell walls of wheat aleurone (25%), and are the principal soluble NSP of barley comprising 70% of the endosperm. The other remaining components are arabinoxylans, glucomannans, cellulose, protein, and phenolic constituents (Newmann *et al.*, 1989; Ficher and Stone, 1986). β-glucans are also noted as cell wall components in sorghum, millet and corn, but mostly left uncharacterised (Ravindran, 2001; Fincher and Stone, 1986; Wood, 1984). Similar to other β-glucans, the cereal β-glucans are composed entirely of glucose units and their distinction between other polymers lies between the nature of the linkages between units, which usually consist of a linear chain of cellotriosyl and cellotetraosyl units joined by both β-(1-3) and β-(1-4) linkages (Choct, 1997; Smits and Annison, 1996; Newmann *et al.*, 1989; Wood 1984). Approximately 70% of β-glucan in barley is (1-4) linked and 30% are (1-3) linked, and usually segments of two or three (1-4) linkages are separated by (1-3) linkages, although up to five contiguous (1-3) linkages which have evolved as a minor structural feature have been reported (Choct, 1997). Further, β-glucan links may appear as extended, wormlike chains in solutions, and the immersion of the (1-3) linkages in the said molecules causes breakage of the regular structure of the β- (1-4) chains preventing close packing, and thereby giving a more soluble polymer (Klopfenstein, 1988). The molecular weight reported for water-soluble β-glucan estimated by ultra centrifugation ranges from 200,000 to 300,000 corresponding to a degree of polymerisation of 1200 – 1850 monomers (Choct, 1997).

2.3.1.3 Arabinoxylan

Arabinoxylan is particularly abundant in the walls of the aleurone cells, in the starchy endosperm, in the husk and presumably in the wall remnants that make-up the other material tissues surrounding the grain. It consists of pentoses, arabinose and xylose and is therefore referred as pentosans (Fincher and Stone, 1986; Southgate *et al.*, 1993). In wheat, arabinoxylan contains phenolic acid (ferulic acid) as substituent, and it is esterified into the primary alcoholic group of the arabinose side chain (Amado and Neukom, 1985).

Arabinoxylan consists of a complex chemical structure composed of two sugars, arabinose and xylose in a branch structure. The arabinose originates from pectin and xylose comes from hemicellulose. It has been reported that arabinoxylan contains a backbone structure of D-xylopyranose residues linked by β-(1-4)- glycosidic bonds with units such as L-

arabinofuranose attached as branches by β-(1-2)- or β-(1-3)- linkages. Typically, L-arabinofuranose or other side chains are carried on the main chain as non-reducing end groups (Han, 2000).

In wheat, arabinoxylan is predominantly located in the endosperm cells (88%), one third of it is soluble in water and the remaining two thirds is soluble in alkali agents. The water-insoluble arabinoxylan is anchored in the cells by alkali label ester-like cross-link rather than by simple physical entrapment. In contrast, most water-soluble arabinoxylan is not bound to the cell wall and can form a highly viscous solution. It can absorb water ten times its weight. In the presence of peroxidase (H_2O_2), arabinoxylan accelerates the formation of a gel network as a result of a covalent cross-linking reaction (Choct, 1997). This gel-like interaction in the matrix of the wall is related in the structural feature of this polysaccharide that permits some intermolecular alignment in the junction zones. The gelling property, together with viscosity, strength and porosity of the wall matrix, could be altered through changes in the degree and spatial arrangement of arabinosyl substitution along the xylan backbone (Fincher and Stone, 1986).

2.4 NSP: Physical Properties, General Anti-nutritive Effects and Effects on Animal Performance

Inclusion of NSP in the diet of monogastric animals has variable and diverse effects, which are mainly attributed to a wide range of compounds possessing different physiochemical properties. Most NSP are part of the cell wall and are closely associated with other materials (polysaccharides and non-carbohydrates substances), such as protein and lignins. Such association is essential for it influences the manner NSP behaves when ingested (Smits and Annison, 1996; Choct, 1997).

For barley to be completely digested, an exogenous enzyme β-glucanase is necessary to hydrolyse β-glucan, since monogastric animals do not have the maximum capacity for its synthesis. Besides, endogenous sources of β-glucanase found in barley kernel are not sufficient to hydrolyse β-glucan found in grains leading to unavailability of the nutrients found in the cells (Danielson *et al.*, 1997). Further, the nutrients of the cells are only available once digestive actions and processing disrupt the integrity of the cell wall liberating the said nutrients, and this usually occurs late during digestion process. Moreover, hydration of the cell wall during processing, cooking and consumption leads to

high digesta viscosity and inhibition of diffusion resulting in delayed release of nutrients (Wood, 1994).

Table 2.1 NSP content of barley, wheat and maize (Smits and Annison, 1996)

Cereal	Soluble NSP	Insoluble NSP	Total NSP	Main NSP	Composition (%DM)
Wheat	2.4	9.0	11.4	Arabinoxylan	6.0
				β-D-Glucan	0.5
				Cellulose	2.0
Barley	4.5	12.2	16.7	Arabinoxylan	3.3
				β-D-Glucan	7.6
				Cellulose	3.9
Maize	-	-	-	Arabinoxylan	4.2
				β-D-Glucan	0.1

The deterrent effects of NSP are inclined towards their viscous nature, physiological and morphological effects on the digestive tracts and the interaction of the microflora of the gut. The mechanisms include altered intestinal transit time, modification of intestinal mucosa, and changes in hormonal regulation due to a varied rate of nutrient absorption (Vahuony, 1982). The majority of these effects are dependent to the physical properties of NSP that includes viscosity, water-holding capacity and their ability to absorb or bind to small molecules and ions, and surface activity.

2.4.1 Physical Properties

2.4.1.1 Viscosity

Most polysaccharides when dissolved in water produce viscous solutions. Viscosity is dependent on solubility of NSP, molecular weight and size, structure (branched or linear), presence of charged group, and concentration (Bach Knudsen, 2001; Moughan *et al.*, 1999; Plaami, 1997; Choct, 1997; Annison and Choct, 1994). At low concentration, the viscosity of water is increased by direct interaction between polysaccharides and water molecules, and as concentration increases, the polysaccharide molecules interact among themselves and become entangled in a network, increasing viscosity. This process is dependent in the formation of the junction zones between the polysaccharide molecules (Moughan *et al.*, 1999; Oakenfull, 1993). Further, gel formation can occur when the interaction of the polysaccharide molecules becomes intense (Moughan *et al.*, 1999). In particular, the soluble forms of NSP (pectin and β-glucan) can develop significant viscosity (Annison and

Choct, 1994; Plaami, 1997), which is related to physical interaction between polysaccharide molecules found in the solution (Englyst, 1989). β-glucan has a propensity to form aqueous solution of high viscosity. Its structural character includes irregularity in shape, which in turn, reduces its tendency to pack into stable, regular molecular aggregates. Thus, it remains relatively soluble in water. This high intrinsic viscosity is mainly attributable to the molecular asymmetry in combination with high molecular weight (MacGregor and Fincher, 1993).

Viscosity is a major factor in the anti-nutritive effects of NSP in monogastric diets (Choct, 1997). Its effects include anti-motility action in the intestine that can influence absorption by delaying gastric emptying, impairing the mixing process in the upper small intestine, altering absorptive sites, and delaying small bowel transit time. It also increases the rate of diffusion of substrates and digestive enzymes and hinders effective substrate-enzyme interaction at the mucosal surface (Choct, 1997). Soluble NSP interacts with the glycocalyx of the brush border of the small intestine that results in thickening of the unstirred water layer of the mucosa, thus reducing the efficiency of nutrient absorption through the intestinal wall (Choct, 1997). These effects are all detrimental to nutrient absorption and utilisation that decrease optimum growth and production performance. As indicated in broilers, ingestion of wheat arabinoxylan resulted in increased digesta viscosity, but such effects should still be investigated in pigs.

2.4.1.2 Water-holding capacity

Dietary fibre components like cellulose, hemicellulose, β-glucans and pectins (all non-starch polysaccharides) have the properties usually associated with hydrocolloids being long chain polymers that thicken or gel in the aqueous system (Potty, 1996). They are hydrophilic molecules that possess the ability to hold water, specifically those that contains sugar residue with free polar groups. Further, the water holding capacity of fibre is greatly influenced by particle size, which determines the volume of the interstitial space within fibre matrix available for water entrapment (Kay, 1982). Processing feedstuff, like grinding wheat bran to fine, decreased the ability to uphold water up to 26% than unprocessed bran (Kritchevsky, 1988). This condition suggests that the physical structure of fibre is the most important determinant of hydrability (Kay, 1982).

Soluble NSP entraps water by forming a network. Their intense molecular interaction may progress into gel formation, thus affecting the pattern of nutrient absorption, post- prandial satiety and intestinal motility (Kay, 1982). As a result from formation of networks with

water, the viscosities and water holding capacities of soluble NSP are relatively higher compared to insoluble NSP (Smits and Annison, 1996).

In pigs, it has been shown that the water-holding capacity of fibrous feed ingredients is associated with increased endogenous gut nitrogen losses of both secretory and bacterial origin. It is also evident that the water-holding capacity of dietary fibre elicited effects on *ad libitum* food intake in pigs (Moughan *et al.*, 1999).

2.4.1.3 Ability to absorb or bind to small molecules or ions

Organic materials such as bile acids, other steroids, various toxic compounds, and bacteria may be reversively bound to fibre as it passes along the gastro-intestinal tract (Kay, 1982). Many polysaccharides have the ability to bind other polar molecules and ions (Oakenfull, 1993). For instance, polyuronic acids with free carboxyl groups are known to form a coordination complex with di- and trivalent metal ions (Kay, 1982). Some NSP such as pectins may have a high charged density at some pH values due to the presence of acidic groups. Apart from the association of cations with the negatively charged groups, the stereo-structure of the polysaccharides in some NSP allows the chelating of ions to occur. Indeed, cations can form ionic bridges between NSP molecules, profoundly influencing their viscosity and gel forming properties (Moughan *et al.*, 1999; Smits and Annison, 1996).

2.4.1.4 Surface activity

Polysaccharides can present charged (negative and less commonly positive) as well as weakly hydrophobic and hydrophilic surfaces. When in solution, they tend to associate with other surfaces. When ingested, these may be the surfaces of food particles, the surface of lipid micelles or the glycocalyx surface of the gut. Once again, molecular mass and chain length play a major role as allosteric effects are almost certain. This may influence nutrient digestibility (Moughan *et al.*, 1999).

2.4.2 General Anti-nutritive Effects of NSP in Monogastric Animals

2.4.2.1 Physiological effects of viscosity and water-holding capacity

In chickens, there appears to be prominent evidences that soluble NSP components of barley (β-glucan) and wheat (arabinoxylan) influenced the digestion of nutrients such as starch, protein, fat (Choct and Annison, 1992), and the apparent metabolisable content (Annison, 1993; Bedford and Schulze, 1998), affecting the litter quality (Classen, 1996).

Choct and Annison (1990) provided a relationship between energy metabolisability and NSP composition (β-glucan and arabinoxylan) of cereals. The relationship signifies that an increasing concentration of NSP in cereals had deterrent effects in metabolisable energy in chicks. Results from trials conducted by Choct and Annison (1992) revealed that inclusion of pentosan (40g AX as AEP/kg) decreased the ileal digestibility of starch, protein and lipids by 14.6 %, 18.8% and 28.8 %, respectively. The well-known negative effects on poultry productivity were derived mainly from increase in viscosity of digesta of birds fed on diets containing soluble viscous NSP (Choct and Annison, 1992; White *et al.*, 1983). There are several mechanisms involved that promote viscosity in monogastric animals (Smits and Annison, 1996). This viscous property can impair the diffusion and convective transport of lipase, oils, and bile salts within the cells, as well as transport of glucose and sodium (Edwards *et al.*, 1988). More so, viscosity may reduce the contact intensity between potential nutrients (e.g. fats) and digestive secretions (e.g. lipase, bile salts), impair transport to the epithelial surface (Smits and Annison, 1996), and depress the rate of passage of feeds increasing the microbial population in the colon (Bedford, 1996). For absorption to take place, the nutrients must cross the aqueous barrier, the unstirred water layer (UWL) adjacent to the intestinal mucosa. Gel forming gums had been shown to increase the thickness of the UWL. Viscosities of the intestinal fluid also modify and increase the thickness of the layer (UWL) decreasing the rate of diffusion of nutrients (White *et al.*, 1983). Mucus produced by goblet cells participates in the formation of the UWL by increasing the volume of the adherent mucosal fluid and its viscosity. It had been known that dietary fibre enhanced secretion of mucus (Sachithanandam, *et al.*, 1990), which increases digesta viscosity. This condition also enhanced the increase of resistance for transport of nutrients through UWL adjacent to the epithelial surface by increasing the thickness of the mucus layer or by changing the physicochemical properties of the mucus (Smits and Annison, 1996).

In young chicks, the increase in viscosity in the small intestine associated with solubilization of β-glucan and arabinoxylan is thought to be a major factor contributing to their anti-nutritive effects, although intact endosperm cells may also act as physical barriers to the endogenous enzymes, and hence, reduce utilisation of nutrient that are encapsulated within endosperm cells (Campbell and Bedford, 1992; Classen, 1996; Bedford and Schulze, 1998).

In pigs, where wheat and barley are major dietary components, minimal work had been conducted related to viscosities compared to trials undertaken in young birds (Pluske *et al.*,

1999). Thus, the relationship between solubility or viscosity and the anti-nutritive property in pigs is not as clearly established as compared to domestic birds (Graham and Aman, 1991). This might be attributed to the differences in digestive physiology between pigs and poultry. Lewis *et al.* (1998) investigated the relationship between wheat quality on ileal and faecal nutrient digestibility. The results showed no significant relationship between viscosity and either ileal or faecal digestibility, which suggests that viscosity alone cannot provide a suitable basis for predicting the nutritive value of wheat for pigs, and thus, pigs and poultry have different responses to NSP from cereals.

Similar with broiler chicken, isolated soluble NSP sources increased luminal viscosity which has been considered a major determinant of delayed absorption (Pluske *et al.*, 1999), though several trials like that of Johansen *et al.* (1997) demonstrated low viscosities in jejunal contents, the region where absorption is most active. It is possible that dilution with pancreatic and bile juice may partly explain the condition. But same as with poultry, the low and variable viscosity found in the digesta of pigs was attributed to enclosure in intact cell walls leading to only partial solubilization, and by depolymerisation of β-glucan (Pluske *et al.*, 1999).

2.4.2.2 Effects of NSP in gut morphology and digestive processes

Fibre is usually an integral part of the diet in pig, and an increase in fibre content in the pig diet is directly related to the amount of available nutrients and energy. Usually, a diet high in fibre content contains less metabolisable energy than diets low in fibre. This implies that a diet with high fibre content causes earlier satiety due to gastric signals (Wenk, 2001).

Dietary fibre influenced intestinal morphology as well as the rate of intestinal turnover in pigs (Jin *et al.*, 1994) that eventually can affect nutrient digestion, absorption and metabolism. Moreover, it can also initiate significant increase in the secretion of endogenous fluids, as shown by increased secretions of saliva and gastric juice, pancreatic juice and bile in pigs fed with 180g/kg dietary fibre (Zebrowska *et al.*, 1983). The increased amount of secreted digestive fluids means an extra metabolic effort or demand for the pig, though a more efficient digestibility of the feeds is expected. Presumably, the increased secretion of digestive fluid is associated with higher activity of secretory organs resulting in enlargement of such organs (Wenk, 2001). In growing-finishing pigs fed with diets high in dietary fibre (268 g kg^{-1} DM), there were significant increase in the weight of stomach, caecum, and colon (Jorgensen *et al.*, 1996). Pluske *et al.* (1998) demonstrated a positive linear relationship between the weight (full or empty) of the large intestine and

the daily intake of NSP and resistant starch. Nonetheless, improved digestibilities of protein, dry matter and energy were not associated with this modification, the values, in fact, decreased (Jorgensen *et al.*, 1996).

Conversely, the presence of NSP in the digesta may also directly influence gut function that affects the digestion of nutrients. In humans, the presence of viscous NSP delayed the gastric emptying and absorption of nutrients from the small intestine is also slowed (Annison, 1993). The decreased rate of absorption is not a consequence from inhibition of diffusion of nutrients, but rather related to a decrease in the convective transport of nutrients resulting from a decrease in the effectiveness of peristalsis (Edwards, 1990). In pigs, there is a significant variation in the pre-caecal digestion of β-glucan with values ranging from 0.170 – 0.820 and 0.70 – 0.90 from oats (Bach Knudsen *et al.*, 1993; Johansen *et al.*, 1997) and barley (Fadel *et al.*, 1988), respectively. It was primarily the soluble β-glucan fraction that is degraded (0.67) compared to insoluble components (0.21) (Bach Knudsen and Canibe, 1997). This high degradation is caused presumably by the relatively high solubility of the linear β-glucan and the high density of micro-organisms present in the distal region of the small intestine. In contrast, there is a quantitative recovery of arabinoxylan from wheat and oats, an effect contributed to cross-linking and highly branched structure of arabinoxylan that requires a more complex enzyme system for degradation (Back Knudsen, 2001; Back Knudsen and Canibe, 1997).

Membrane-bound enzymes in the small intestine influenced the final stage of digestion of protein and carbohydrates. It can be theorised that a viscous environment would restrict the access of these enzymes to their dietary substrate that constrained digestion (Iji, 1999). The digestibility of lipids in pigs fed on diets high in wheat and oats (Bach Knudsen *et al.*, 1991) and in broiler chicken fed with non-fermentable NSP, carboxymethylcellulose (Smits *et al.*, 1998), was impaired. In broiler chicken, this was related to the reduction of the concentration of bile acids, which aid in the digestion of lipids. In pigs, it is accounted according to the increase in the rate of fat excretion resulting from the increased loss of bile acids caused by extensive fermentation of carbohydrates and low luminal pH (Iji, 1999). Nevertheless, the reduction of the activity of the intestinal enzymes in the presence of soluble NSP may, however, be compensated for the increased cellular hypertrophy and hyperplasia (Ikegami *et al.*, 1990) resulting in an enlargement of the organs involved in response to amount to the work performed in drying, mixing, shaping, moving and expelling undigested dietary residues (Pluske *et al.*, 1999). Changes in the organ size in response to feeding high levels of NSP are likely to have an impact on energy metabolism.

This is indicated in the high rate of energy expenditure of visceral organs relative to their size, as shown by disproportionately high amount (0.25) of whole-body maintenance expressed as oxygen consumption of organs drained by hepatic-portal vein including the large intestine (Yen *et al.*, 1989 cited in Pluske *et al.*, 1999). With this, some of the reductions of feed efficiency associated with high fibre feeding may be related to increased basal heat production in addition to reduced energy and amino acid digestibility (Varel and Yen, 1997; Pluske *et al.*, 1999).

Soluble non-starch polysaccharides depressed the activity of several pancreatic enzymes *in vitro*, namely amylase, lipase, trypsin and chymotrypsin. The cellulose and xylan reduce the activity of the said enzymes to less than half of their original activity (Dunaif and Schneeman, 1981). Reduction of enzymatic activity is related to non-specific binding of enzyme to NSP polymers, but, due to the large volume of enzymes present in pancreatic secretions, the reduction effect is prevented (Selvendran *et al.*, 1987). The presence of NSP tends to increase water content of the gut and leads to increased endogenous secretion of enzymes. Also, it elevates secretory output of salivary glands, stomach, liver, pancreas and intestinal wall that results in increased secretion of water, protein, lipids and electrolytes (Low, 1989). Likewise, increased viscosity causes an increase in intestinal mass and pancreatic size. One response of the animal to increased viscosity is greater endogenous losses due to the attempt of the animal to overcome poor mixing of enzymes and food in viscous digesta. At high viscosity, the animal has no further adaptive capabilities and so nutrients pass through the intestine undigested. Thus, prolonged consumption of NSP causes adaptive changes in the digestive system characterised by enlargement of digestive organ and increased secretion of digestive juices, accompanied by a decrease of nutrient digestion (Low, 1989; Choct, 1997).

Water-holding capacity, brought about by soluble NSP, markedly influenced voluntary feed intake. Kyriazakis and Emmans (1995) mentioned that voluntary feed intake of young pigs decreased in a quadratic manner as the water-holding capacity of the diet is increased. It is postulated that the presence of large amount of NSP in the digesta could trigger a feedback loop for "gut fill". This might occur by one, or both, of the following two mechanisms: (1) high digesta viscosity resists the propulsive actions of the intestinal contraction and thus reduce digesta flows, and (2) increasing the physical bulk of the digesta increases the ability to hold large amount of water (Choct and Cadogan, 2001).

Generally, it is usually agreed that dietary fibre can modify the intestinal morphology by affecting appearance, length and number of the villi, cell proliferation, mucosal cell

division and absorptive functions, but the results are equivocal (Mosenthin *et al.*, 1999). The studies conducted by Jin *et al.* (1994) indicated that feeding high fibre diet to piglets altered intestinal morphology and increased the rate of mucosal turnover. In contrast, Moore *et al.* (1987) showed that addition of different fibre sources to a basal diet with relatively high fibre content did not affect intestinal morphology of the pigs. It is then suggested that dietary fibre influences intestinal morphology through various mechanisms, many of which are interactive.

2.4.2.3 Microflora implication and microbial fermentation

In pigs microbial fermentation occurs, to some degree, in all regions of the digestive tract. The extent is dependent on the region of the gastro-intestinal tract, the age of the pig and the composition of the diet (Mosenthin *et al.*, 1999). It is known that the structure and function of the microflora can be influenced by environmental conditions within the gut ecosystem. Further, the composition of the microflora can be altered by modifying the diet, and diet composition can be referred as the 'probable single most important control factor for microbial activity' (Mosenthin *et al.*, 1999). In particular, diet abundant in dietary fibre help promote the presence of cellulolytic bacteria without changing the total number of micro-organisms (Moore *et al.*, 1987; Varel, 1987). This implies that these micro-organisms are important in the digestion of NSP in the large bowel. Dietary fibre is not digested by the endogenous processes of the pig, but efficiently by the microbial flora. Bach Knudsen (1991) observed that a greater proportion of the wheat soluble fibre passes the ileum of the pigs without being digested, and therefore, represents a good substrate for micro-organisms in the large intestine. Wenk (2001) mentioned several authors who reported similar observation on other feedstuff. Those authors showed that in diets containing a high soluble fibre content, the microbial activity in the large intestine is generally increased. The increased microbial activity did not only indicate better utilisation of nutrients, but also increased secretions of microbial substances that ultimately resulted in reduction of absorption of nutrients by soluble dietary fibre (Rainbird *et al.*, 1984). Further, microbial activity can be gauged using ATP concentration in the digesta as affected by dietary fibre. Diets that contained wheat bran had sustained ATP concentration in the large intestine compared to diets with wheat flour. This indicates that microbial activity with the diet containing wheat bran was far greater than the diet with low fibre content (Jorgensen and Just, 1988; Wenk, 2001).

In poultry, although intensive microbial degradation is limited by small size and high rate of passage in the small intestine and faster rate of passage in the large intestine as

compared to pigs (Wenk, 2001), gastrointestinal microflora may partially influence detrimental effects on nutrient digestibility, particularly of fats (Smits and Annison, 1996). This was illustrated by the less pronounced effect on digestibility of long chain fatty acid in caecectomized than in intact birds (Choct *et al.*, 1992) with pronounced improvements in the performance of the former after supplementation of rye-based diets with antibiotics (Choct 1997), and in germ-free chicks where fibre viscosity has least pronounced effects on fat digestibility. Further, soluble NSP increased the residence time of digesta, which may decrease oxygen tension and favour the development of anaerobic microflora. The proliferation of these microbes can lead to production of toxin and deconjugation of bile salts that result to depressed fat digestion (Choct 1997).

Therefore, significant evidence showed that viscous NSP modify bacterial activity in order to lower fat digestibility (Smits and Annison, 1996). Viscous polysaccharides increase residual time of ingesta, decrease oxygen tension and favour increased microbial activity. Bacteria colonise the proximal small intestine and affect the host digestion mechanism by three means (Bedford, 1996):

1. Invasion/Disease - they alter performance status of animal and make them prone to E. coli infection resulting in diarrhoea in pigs and wet and sticky droppings in poultry.

2. Competition for resources - with altered rates of digestion, there is greater opportunity for gut micro-organism to metabolise nutrients. Intestinal viscosity delays the rate of digestion where gut micro-organism can pursue their effect by contesting absorption of nutrients resulting in decreased live weight gain and feed conversion ratio.

3. Secondary effects of metabolites - effects of secondary secretion of metabolites due to NSP may result to increased concentration of luminal bacteria. This event causes proliferation of mucosal growth posing detrimental effects to body maintenance and growth rate.

2.4.2.4 NSP absorption and interaction of other molecules

Presence of NSP alters fat digestion by failure of pancreatic lipase to act on the lipid/water interface, which is already occupied by NSP (Annison, 1993). Also, it has the ability to bind to bile salts, lipids and cholesterol. Viscous NSP enhances bile acid secretion that leads to secretion of this acid in the faeces. This, in turn, can result in increased hepatic synthesis of bile acid from cholesterol to re-establish the composite pool of these metabolites in the entero-hepatic circulation (Choct, 1997). This influenced the absorption

of lipids and cholesterol in the intestine, leading to major changes in the digestive and absorptive mechanism of the gut affecting overall efficiency in nutrient synthesis. It is accepted that NSP inhibits digestion by directly complexing digestive enzymes and enzymes co-factor (Ikeda *et al.*, 1989; Annison, 1993). Interactions of protein and polysaccharides are common because the two molecules have both hydrophobic and hydrophilic surfaces. As mentioned, direct complexing of the co-factors inhibits actions of enzymes as shown by the inability of the amylase to bind to calcium ions, which was already attached to NSP (polyuronates such as pectins), thereby inhibiting amylase activity (Annison, 1993).

2.4.3 Anti Nutritive Effects: differences between species

Though both are monogastric species, pigs differ from poultry relative to size and anatomical features of the gastro-intestinal tract. The pig's stomach represents about 30% of the volume of the digestive tract where limited mixing of food occurs. The small intestine is long and permits intensive endogenous digestion at an almost neutral pH, and it is the site of absorption of most available nutrients. In the large intestine, undigested feed components and endogenous secretions are fermented by micro-organisms, and it is the site of absorption for short chain fatty acids and some vitamins. Chicken, on the other hand, has a crop before and a gizzard (muscular stomach) below the true stomach (proventriculus), in which both glands release endogenous secretions. Microbial activity is important if the feed remains for a longer period in these organs. In poultry, microbial activity is limited in the small intestine, due to its small size and the high rate of passage. In the large intestine, the rate of passage is also faster, thus, limiting intensive microbial degradation (Wenk, 2001).

Similar to poultry, numerous researches have been conducted in pigs to enhance utilisation of NSP in diets by use of enzymes on the basis that soluble dietary fibre form viscous solution in the small intestine. The results in general are equivocal, and if positive, have largely been restricted in younger pigs (Pluske *et al.*, 1999). It can be noted that the digesta from weanling pigs was more watery compared to chicken, suggesting that diffusional constraint, if present, is less severe in pigs. It has been shown that there is an increased viscosity in the presence of added pentonase in rye-fed weanling pigs, but the levels were far less than those reported in chickens. Pig differs physiologically from young chicks, for which the digesta tends to have lower dry matter content at 800g/kg compared to 900g/kg for pigs. Since viscosity initiated by soluble dietary fibre is logarithmically related to

concentration, simple dilution can essentially eliminate the viscosity problem and the associated constraint on luminal dilution (Campbell and Bedford, 1992).

The difference in anti-nutritive effect of NSP is also shown in the influence of enzyme supplementation in both species. Thacker (2000) reported that in poultry, dietary treatment with β-glucanase dramatically improved the nutritive value of barley as indicated by improvements of weight gain (over 50%) and feed conversion efficiency (by 15%). It is recognised that β-glucan also poses a problem for swine, and recent reports regarding supplementation of enzymes to pig diets failed to show improvements in performance in similar magnitude to those observed in poultry for either growing/finishing or starter pigs (Thacker, 2000). Oftentimes, combinations of enzymes in pig diets had been practiced making it difficult to define the specific activities that are responsible for any increase in growth performance (Choct and Cadogan, 2001). The less consistent response to enzyme supplementation of pigs compared to poultry can be accounted by the differences in physiology, specifically in the difference of the gut pH. In pigs, feed remains in the stomach for approximately 4 –12 hours in a relatively low pH, whereas in chicken, feed passes through the low pH of the proventriculus in 20 – 40 minutes. It is therefore evident that longer exposure to low gut pH of the pig lowers enzyme activity, and the simultaneous presence of gastric proteolytic enzymes may hasten inactivation (Thacker, 2000).

Another aspect of difference is on digesta viscosity, which plays a major part in the anti-nutritive property. In pigs, the effect of digesta viscosity is unclear, but the water content of the pig digesta is very high and, therefore, the effect of soluble NSP is not of the same magnitude as in poultry (Choct and Cadogan, 2001). Viscosity measured in the pig intestinal tract is almost 100 fold less than what have been reported in chicken. Moreover, another attribute to varying levels of anti- nutritive effect is the innate characteristics to degrade β-glucans and pentosans. These soluble NSP are extensively degraded in the small intestine of pigs. In the review of Thacker (2000), the degradation rates of β-glucan prior to terminal ileum ranges from 79 to 95%, while that of pentosans are relatively low but still in excess of 65%. The aforementioned conditions relatively showed the differing response of the two monogastric species to diets that contained high amount of soluble non-starch polysaccharides.

2.4.4 Anti-Nutritive Effects in Pigs

2.4.4.1 Anti-nutritive effects of arabinoxylan

Pentosans or arabinoxylan are more common in rye, wheat and triticale. They consist of a backbone of (1,4) linked xylopyranosyl residues with terminal 1,2 and 1,3 arabinofuranosyl substitutions. This substitution reduces the ability for bonding between carbohydrate chains and consequently results in fractions, which are water-soluble and highly viscous (Thacker, 2000).

After ingestion, arabinoxylan becomes soluble resulting in increased digesta viscosity. This viscous nature of NSP is the primary cause of their anti-nutritive effect in monogastric animals. Increased viscosity leads to increased diffusion rate of substrate and digestive enzyme and hinders effective interaction in mucosal surfaces (Ikegami *et al.*, 1990).

If the potential of improving the nutritive value of rye, a source of arabinoxylan, can be substantiated by enzyme supplementation, then it can be an alternative feed resource in swine feeding. However, glucanase supplementation to pig diets elicited variable responses paving the way to practise the usage of multi-enzymes (Choct and Cadogan, 2001). In the review of Choct and Cadogan (2001), several studies demonstrated that when different wheat samples were added to a balanced, commercial type weaner diet, the feed intake and growth rate of the pigs varied by 47% and 48%, respectively. Enzyme supplementation (with affinity to either soluble or insoluble NSP) to these wheat samples indicated varied response. When glucanase with affinity for only the insoluble NSP was added to these wheat-based diets, the enzyme reduced the daily gain and feed intake of the low quality wheat but tended to increase voluntary feed intake and weight of pig offered with good quality wheat. Further, when xylanases (with affinity to soluble and insoluble arabinoxylan) was added to wheat with pre-determined wheat quality, the enzymes had marked effects on all samples specifically for wheat sample with lowest quality. The results of these studies clearly indicate that both soluble and insoluble NSP have negative effects on feed intake affecting growth rate. However, such effect is, in part, alleviated by enzyme supplementation. Similar results were presented by Gill *et al.* (2000) that showed a significant improvement in the conversion of food to weight gain in piglets given wheat, barley or sugar beet pulp supplemented with enzymes. Although results from enzyme supplementation have been consistent in younger pigs, recent studies have shown large improvement in growth performance of older pigs fed with diets based in low quality wheat supplemented with xylanases (Choct and Cadogan, 2001). The preceding results,

showing positive influence in growth parameters, had been argued in the review of Thacker (2000). This review indicated that enzyme supplementation to swine diets showed modest or no improvements, since appreciable quantities of β-glucans and pentosans are degraded prior to the terminal ileum in pigs, presumably by endogenous feed enzymes or intestinal bacteria. Therefore, it would be difficult to justify routine inclusion of β-glucanase or pentonase in diets fed to swine.

Studies in rats showed that increasing wheat bran in the diet cause sloughing of the intestinal mucosal cells that enhanced mucus production. The addition of 6% methylcellulose in a barley-soybean diet decreases apparent ileal nitrogen digestibility in pigs, and adding gel–forming polysaccharides impairs protein digestion (Murray *et al.*, 1977). Further, there is a linear regression effect of apparent faecal digestibility coefficient (AFDF) with dry matter, nitrogen and energy in piglets fed with increasing amount of wheat middlings. Reduction of AFDF for dry matter and energy was attributed to failure of the piglets to digest fibre present in the wheat middlings. Additional inclusion of wheat bran in the diet causes increased water content in the faeces due to the water-holding capacity of fibre (Graham *et al.*, 1989). The above-mentioned studies showed the nutritional effects of arabinoxylan.

2.4.4.2 Anti-nutritive effects of β-glucan

β-glucan is a polymer of glucose consisting of a β-1,4 linked backbone with β-1,3 linkages. The presence of the β-1,3 linkages differentiates β-glucan from cellulose and results in soluble components which are viscous in solution. The pattern of β-1,3 and β-1,4 linkages is not always the same but approximately 85% of barley and oat β-glucan is made up of two or three β-1,4 bonds separated by a single β-1,3 linkage. The remaining betaglucan contains longer sequences of β-1,4 bonds interrupted by a single β-1,3 connection (Thacker, 2000).

Monogastric animals cannot synthesise β-glucanase. The amount of β-glucanase derived from barley grain and bacteria in gastro-intestinal tract is insufficient for hydrolysis of β-glucan, thus β-glucan creates a viscous environment in the digestive tract causing poor absorption of dietary nutrients and reduced growth rate (Wang *et al.*, 1992). In poultry, β-glucan and pentosans lower the value of cereal grains through an increased intestinal viscosity that impedes enzyme substrate association, affecting the process of absorption (White *et al.*, 1983). It has also been suggested that β-glucan allows the microbial population to assimilate a greater proportion of the nutrients contained in the feed into their

own system, thereby reducing the availability of these nutrients to the host (Thacker, 2000).

The influence of barley β-glucan can be best described by studies involving supplementation of enzymes. β-glucanase supplementation of 0.5 % in barley-based diets did not produce improvement in the process of absorption in 80-kg pigs (Graham *et al.*, 1989). Thacker (2000) stated that enzyme supplementation to barley-based diets in growing/finishing pigs had no effect on nutrient digestibilities, weight gain and feed intake, but showed improvements in feed conversion ratio. Thus, the aforementioned results provide little justification for inclusion of enzymes for pigs diets of this weight range. A supplementation of 0.1% of β-glucanase in 50-kg pigs failed to significantly improve apparent digestibility of nutrients in the ileum or faeces, even though it showed some enzymatic dynamics in the said site (Graham *et al.*, 1986). Further, Jensen *et al.* (1998) stressed out that the effect of β-glucanase supplementation on digestive enzymes activity in the intestinal contents and pancreatic tissues were not significant, though supplementation of β-glucanase increased the digestibility of β-glucans and reduced digesta viscosity in the upper gastro-intestinal tract without affecting digestibility of starch and nitrogen, liveweight gain, and feed gain ratio. Moreover, supplementation of 0.25% β-glucanase in diets of 25-kg grower pigs showed that there is an increased dry matter digestibility coefficient, but crude protein and energy values are not affected (Thacker *et al.*, 1992).

Results of enzymes supplementation in younger pigs are also equivocal. In 10.8-kg piglets, processing (like fine grinding) and enzyme supplementation did not improve growth performance, and seems not economical, in piglets fed with barley (Chu *et al.*, 1998). However, in 12-kg piglets, where 1.25% β-glucanase was added, the ileal digestibility, growth rate and feed conversion ratio were improved (Bedford *et al.*, 1992). Thacker *et al.* (1992) also noted that 0.25% β-glucanase supplementation in barley-based diet for 8kg piglets resulted in increased growth rate and feed efficiency. With these variable results, it can be assumed that enzyme supplementation is ineffective in mature pigs for enzyme activity is neutralized by hostile low pH of the gut and the presence of a larger lactobacilli population, (associated with increasing age) capable of degrading β-glucans. The presence of fibre-degrading bacteria in the foregut of pigs can partially disrupt the cell wall prior to the ileum that probably release nutrients encapsulated from this components and render these nutrients available for digestion within the small intestines (Graham *et al.*, 1989). The said fibre-degrading capacity of pigs is positively related to age (Graham *et al.*, 1986). Younger pigs have less mature digestive tract and lower endogenous secretion as indicated

by production of positive results in ileal digestibility of nutrients and growth performance when enzymes were added to the diets. Thus, anti-nutritive effect is greater in younger pigs and where enzyme supplementation is relatively advantageous.

2.4.5 Effects of NSP on Digestion of Other Nutrients

2.4.5.1 General effects

NSP has a negative effect on the precaecal digestion and absorption of essential nutrients. It causes reduction of absorption of nutrients that reduces the true nutrient digestibility. Another factor is increased secretion of digestive juices or an increased microbial synthesis of fat and protein (Low, 1989), which reduces apparent nutrient digestibility. The presence of NSP reduced nutrient digestion by reducing the retention time of the digesta in the gastro-intestinal tract. The effect of retention time is manifested only in the hindgut, thus precaecal absorption is either not affected or prolonged (Bakker *et al*, 1998).

2.4.5.2 Effects in minerals

NSP lowers absorption of minerals. The digestibility experiments showed that the fibrous diets reduced the total tract absorption of ash by 8% units. This is associated with a reduction in tract absorption of calcium (6%), phosphorous (6%) and potassium (8-14%). Theoretically, requirements for calcium and phosphorous might not be met when they are supplied at marginal levels in NSP-rich diets (Bakker *et al*., 1998).

2.4.5.3 Effects on Starch and Sugar

In general, no effect of NSP is found on starch digestibility at the terminal ileum, but it has a profound negative effect of some disaccharases. However, in the relatively long small intestine this effect is corrected for, prior to the terminal ileum (Bakker *et al*., 1998).

2.4.5.4 Effects on fats

The presence of NSP significantly reduced apparent fat digestibility both at the terminal ileum and over the total tract (Shi and Noblet, 1993). The NSP affects apparent fat digestion in two main ways:

(1) Indirect mechanism- there is increased secretion of fat into the lumen of the gastro-intestinal tract, as a consequence of increased excretions of bile acids in the small intestine (endogenous fat) and bacterial synthesis of fatty acids in the hindgut (microbial fat). It is expected that 4.7 g of fat (endogenous fat) is lost per kg DM intake. Specifically, a net

excretion of 4.7 g non dietary fat per kg cellulose diet, 8.9 g per kg maize starch diet and 9.7 g per kg soya bean hull diet were observed, which comprises both endogenous and microbial fat (Bakker *et al.*, 1998).

(2) Direct mechanism- there is reduced dietary fat digestion by affecting the bile acids and other micellar components. Experiments from Bakker *et al.* (1998) revealed that NSP reduces ileal fat digestibility by 4–7% units. Further, purified cellulose had a higher negative effect than soya bean hull, but both sources affect apparent fat digestibility.

2.4.5.5 Effects on protein

Presence of fermentable carbohydrates significantly reduces apparent protein digestibility (Shi and Noblet, 1993). In endogenous N losses, there is a linear correlation between amount of secreted nitrogen and amount of consumed NDF or DM intake. Usually, the amount of endogenous secreted protein ranges from 16- 39 g/kg feed (de Lange *et al.*, 1990). Further, the presence of active microflora in the hindgut leads to reduction of absorption of protein at around 49–62 g/kg fermented NSP, and thus, reduces secretion of nitrogen in the urine and ultimately decreases apparent total tract protein digestibility (Bakker *et al.*, 1998)

In general, it is evident that dietary NSP levels had negative influence on the digestibility and rate of absorption of nutrients from starch, protein and fats. There are several mechanisms involved in reducing the rate and extent of apparent nutrient digestion and absorption (de Lange, 2000). First, the endogenous secretion and losses of enzymes and mucus and the sloughing of mucosal cells are likely increased when intake of NSP is increased. Endogenous secretions such as bile acids, can be bound, particularly by viscous or gelling and lignified NSP; thus reducing the extent of recycling. These effects resulted in reduction of nutrient digestion. Second, viscous NSP in particular, will interfere with digesta movement and the mixing of digestive enzymes and nutrients in the intestinal lumen. Combined with increase mucus production, NSP can also increase the resistance of the unstirred water layer at the intestinal surface. Third, NSP from cell wall can physically hinder the access of digestive enzymes to nutrients that are enclosed inside the cell walls. Fourth, soluble NSP in particular may stimulate microbial growth and increase the amount of microbial protein and fat at the terminal ileum or in faeces. Selected NSP may also stimulate the growth of toxin-producing microbes, which may affect gut health directly and digestive function indirectly. And fifth, feeding animals with NSP may alter intestinal morphology and the capacity of the gut to absorb nutrients.

2.5 Dietary Fibre and Hypocholesterolemic Effects

Dietary fibre is basically known to attenuate blood cholesterol, and researches over the past several decades showed that increased consumption of dietary fibre and high fibre foods produced a positive adjustment in the level of serum cholesterol in man (AACC Report, 2001). Such findings established a driving force on the impact of dietary fibre consumption against risk of coronary heart disease (CHD). In man, total plasma cholesterol and low-density lipoprotein-associated cholesterol (LDL) are accepted biomarkers indicative of the changes in the risk level of the said disease, and reductions of such metabolites towards a prescribed norm are considered acceptable measures of reductions of risk of CHD. Scientific consensus on the evidence of the role of dietary fibre in reducing the risk of CHD has been acknowledged as shown by the ability of oat products, psyllium, guar gum and legumes to reduce blood cholesterol. In human studies, Davidson *et al.* (1991 cited in AACC report, 2001) reported that a diet containing β-glucan (from oatmeal and oat bran), in conjunction with a low fat diet, is effective in lowering blood cholesterol levels in a dose dependent manner. A diet that contains 50 g/day of oat bran and 42.5 g/day of processed oat bran demonstrated a significant reduction in serum cholesterol in hypocholesterolemic individuals. Fibre–based supplements consisting of 75% soluble fibre and 25% insoluble fibre given in 20 and 40 grams doses showed decreases in total cholesterol, LDL-cholesterol and LDL/HDL ratio compared to a placebo group (Hunninghake *et al.*, 1994).

Studies in rats also showed a significant influence of dietary fibre on serum cholesterol. Anderson *et al.* (1994) reported that rat fed psyllium, guar gum, oat gum and pectin, all rich in soluble fibre, have significantly lower serum and liver cholesterol concentrations than rats fed with cellulose. The study in which oat gum was fed to rats suggests that β-glucan is an active agent for reducing serum and liver cholesterol levels in rats. Similar results were obtained in chicken. When oat bran and waxbar barley were fed to chickens, a significant hypocholesterolemic response was generated. The chicks exhibiting hypocholesterolemia had significantly greater lipid content in their excreta, an indication of interference with absorption possibly due to intestinal viscosity (Newman *et al.*, 1992).

Furthermore, there were many studies which revealed that the hypocholesterolemic effects of β-glucan are not always accompanied by significant changes in total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and total triglycerides contents both in serum or plasma. The AACC report (2001) revealed that dietary fibre reduced total body cholesterol, even when a significant reduction in serum cholesterol did not occur. Oda *et al.* (1993 cited in AACC report, 2001) showed that rats

on diets supplemented with soluble fibre fractions from oat, barley or wheat had lower liver cholesterol even though their plasma cholesterol was not significantly lowered. Topping (1991) noted that soluble NSP isolates from barley produced reductions of 6% to 30% in plasma total and LDL cholesterol in subjects with different degree of hyper-cholesterolemia. In another trial, Hecker *et al.* (1998) disclosed that LDL-cholesterol was significantly lowered in rats fed with β-glucan tortillas, but there were no differences found in levels of total cholesterol, HDL-cholesterol or triglycerides. Further, in the review of Jenkins *et al.* (1996), they noted that the cholesterol lowering effects of guar gum were predominantly reflected in the LDL-fraction with much less or no change in the HDL-cholesterol fraction.

2.5.1 Possible mechanisms of action of dietary fibre

Dietary fibre has long been recognized to have detrimental nutritional effects in poultry and in some aspects, piglet nutrition. But, it was proven to be highly successful in depressing serum cholesterol, as indicated in the aforementioned studies using human and different animal models. The mechanisms by which β-glucan acts as cholesterol-lowering agent is unclear, although intestinal viscosity created by the hydrophilic nature of β-glucan has been suggested as the mechanism of action (Newman *et al.*, 1989). Moreover, AACC report (2001) indicated that high molecular weight β-glucan is more effective than low molecular weight β-glucan in increasing bile acid excretion. Further, a number of possible mechanisms are likely to be involved in the hypocholesterolemic effect of dietary fibre, but none of it is mutually exclusive in offering an explanation for the mechanisms in individual food, and more likely that for a given diet or food, a complex mechanism is functional (Jenkins *et al.*, 1996).

2.5.1.1 Bile acid sequestration

Various food fibres, grains and isolated fibre components absorbed bile or bound to bile salts with considerable degree of variations, as shown from results of several *in vitro* studies (Vahouny, 1982; Kritchevsky and Story, 1993). The amount of bile adsorption or binding can be affected by differences in bile salts or bile acid employed, incubation conditions, pH and osmolality, structure of the bile acids, the nature of the micelles, and the physical and chemical form of the fibre. At low pH, the bile acid binding seems prominent. Further, the presence of mucilaginous and gel-forming polysaccharides and lignin promotes bile acid binding (Kay 1982).

There are two different important factors in bile acid binding. First, bile acid binding results in reduced bile acid absorption. This increases the amount of cholesterol to maintain the bile acid pool, which increases faecal excretion, thereby reducing the cholesterol content in the body and less cholesterol is available for incorporation into lipoprotein for release into venous circulation (Gallaher and Schneeman, 1986; Anderson and Chen, 1979). Second, bile acid binds to fibre making it unavailable for micelle formation, resulting in a decreased amount of cholesterol solubilized in the intestine and reduced lipid absorption (Gallaher and Schneeman, 1986). Thus, in the presence of fibre, more bile is lost to the faeces, creating more needs to be made in the liver from cholesterol, thereby reducing the concentration of circulating cholesterol (Gurr and Asp, 1994). But this is not always the case. Story (1985) concluded that the changes in bile acid excretion alone are neither consistent nor large enough to account changes in serum cholesterol. In some instances, dietary fibre in the diet elevated bile acid excretion but no changes in levels of cholesterol were observed. In other cases, a substantial change in bile acid excretion has consistently accompanied the hypocholesterolemic effects. This clearly indicates that complex mechanisms are involved for all sources of fibre and the hypothesis involving absorption-excretion is not valid for all conditions.

Further, bile acid metabolism can be influenced by dietary fibre through alteration of the spectrum of bile acid present in bile and faeces (Kritchevsky and Story, 1993). An increase in chenodeoxycholic acid (CDC) derivatives can alter bile saturation, cholesterol absorption, and reabsorption of bile acids from small intestines (Story and Thomas, 1982). These authors mentioned that some dietary fibre (alfalfa and oat bran) seemed to cause a consistent increase in CDC, whereas other sources did not. As such, most dietary fibres cause an increase in primary bile acid excretion, possibly as a result of changes in the environment of the large intestine. In general, dietary fibre effects on bile acid metabolism appeared at best to comprise only part of the overall mechanism related to fibre influences on serum or plasma and liver cholesterol levels (Kritchevsky and Story, 1993).

2.5.1.2 Altered lipid digestion and absorption

The soluble dietary fibre has been thought to influence the absorption of nutrients from the small intestine through its effect on luminal viscosity that may reduce the rate of nutrient absorption (Bach Knudsen, 2001), and in long terms, may alter intestine morphology. According to Anderson and Chen (1979) cholesterol may bind to certain fibre that may retard its absorption. The formation of gel in the intestine and colon may partition cholesterol in such a manner that gut absorption is reduced. Alteration in the rate and site

of lipid absorption may alter the pattern of lipoprotein secretion and catabolism (Jenkins *et al.*, 1993). It is well documented that soluble dietary fibre delay gastric emptying (Vahouny, 1982) and is possibly involved in the modification of activities of key gastro-intestinal enzyme resultant to decrease in the diffusion process of substrate and hinder their effective interaction at the mucosal surface (Ikegami *et al.*, 1990). But, Jenkins *et al.* (1993) noted that there were discrepancies in the changes in the activity of small intestine brush border enzymes with fibre treatments. The discrepancy is most likely due to factors like feeding high levels of fibre, the use of weanling animals that are unable to adapt to high fibre intakes, not fasting animals prior to killing for mucosal sampling, and differences in the site of tissue sampling that may lead to differences in observation in examining enzyme adaptation and activity.

As noted, viscous fibre derivatives inhibit transport rates of water soluble molecules, and the presence of gelling fibres may modify the resistance of the surface-associated, unstirred water layer (Jensen *et al*, 1993) that in turn, influence nutrients flux and absorption in the intestine. Specifically, Cassidy and Calvert (1993) mentioned that viscous fibre like guar gum can interfere with bulk phase diffusion of lipids and may also have a residual effect in limiting transmural transport of lipids. In the review of Cassidy and Calvert (1993), dietary fibre not only reduced the amount of cholesterol and triolein in the small intestinal wall, but it also significantly delayed the disappearance of both compounds (through radioactivity) from the small intestine lumen. This suggests that gelling fibre interferes with the diffusion of lipid-containing micelles within the small intestines.

The influence of dietary fibre on reduction of blood/serum cholesterol can also be assessed based on lipid recoveries in the thoracic duct and mesenteric lymph. The presence of soluble and insoluble fibres significantly decreased the rate of cholesterol and fatty acids recovery. Further, Cassidy and Calvert (1993) revealed that residual or adoptive response to fibre supplementation could result to impaired lymphatic absorption of luminal cholesterol.

Therefore, the preceding evidences suggest three important considerations, namely: (a) viscous fibre can modify the rate of absorption of fatty acid and cholesterol through disruption of micellar solubility of lipids by both bile acid sequestration and interference with the bulk-phase diffusion of lipids, (b) prolonged intake of dietary fibre may modify the nutriture of upper intestine that may result in morphological and functional changes in the intestine with respect to lipid absorption, and (c) lymphatic absorption of lipid suggests that either direct or adoptive response to fibre intake may interfere with triglyceride

digestion and delayed absorption of unesterified fatty acid, thereby reducing absorption of cholesterol.

2.5.1.3 Reduction of hepatic cholesterol synthesis by propionate

In the large intestine, colonic bacteria ferment and absorb dietary fibre to short chain fatty acids such as acetic, propionic and butyric, and many experiments then suggest that propionic acid may mediate hypocholesterolemic effects (Anonymous, 1987). The same study demonstrated that diet inclusion of propionic acid based on dry matter (from 3.4% to 10.4%) decreased serum HDL-cholesterol up to 14%, but LDL-cholesterol levels were not affected. Further, it is theorised that the mechanism responsible for the reduction of cholesterol concentration revolves on the ability of propionate to inhibit the activity of 3-hydroxy-3-methylglutaryl CoA (HMG COA) synthase, the rate-limiting enzyme in cholesterol synthesis in animal tissue. In particular, short chain fatty acid decreased HMG CoA reductase activity. The same author also demonstrated that propionate may influence cholesterogenesis by inhibition of enzymes HMG CoA reductase and synthase activity affecting cholesterol synthesis.

Furthermore, substances from barley and oats, like tocotrienol, repress the enzyme HMG COA reductase, a rate-limiting enzyme for synthesis of cholesterol (Peterson and Querishi, 1997; Newman *et al.*, 1989). However, the tocotrienol concentration in barley is too low to initiate cholesterol-lowering effect. Besides, the presence of α-tocopherol in the diet reduces the effect of tocotrienol on cholesterol levels (Querishi *et al.*, 1996). These authors concluded that it is the β-glucan found in oat and barley that lowers HMG CoA reductase activity and the lowered activity of the rate-limiting enzyme for cholesterol synthesis may partly explain the influence of betaglucan in cholesterol levels.

In another aspects, there are several mechanisms by which plant fibre may influence hepatic lipid metabolism, particularly the short chain fatty acid products derived from dietary fibre (Anderson and Chen, 1979). The hepatic fatty acids and triglycerides metabolism can be altered by a steady rate of entry of small quantities of short-chain fatty acids into the portal vein through the following: altering the oxidation reduction state, inhibiting pyruvate dehydrogenase via formation of enzyme acetyl CoA, and by influencing hepatic ketogenesis by providing excessive amount of ketone precursor. These complex mechanisms may decrease triglyceride synthesis or release of very low-density lipoprotein (VLDL) leading to depressed value of fasting or post-prandial triglycerides (Anderson and Chen, 1979).

Based from preceding experiments, not only the vital function of dietary fibre in reducing various types of cholesterol was clearly illustrated. It also showed that the mechanisms by which dietary fibre influences lipid metabolism are complex, and might be interactive. Vahouny *et al.* (1982) summarized the direct and indirect mechanisms by which dietary fibre may influence lipid metabolism.

2.6 Gut Nitrogen Losses and Protein Evaluation in Pigs

Significant quantities of endogenous nitrogen compounds enter into the pig digestive tract, particularly stomach and small intestine, in the form of pancreatic juice, bile, mucus glycoprotein, lymph, plasma urea and proteins, and shedding of epithelial cells which line the intestinal mucosa. The endogenous protein is subjected to digestion and many of the secreted compounds are reabsorbed. Others escape digestion and absorption and are degraded by large intestinal bacteria to ammonia or voided in the faeces and in urine as urea if reabsorbed, but not re-utilised (Zebrowska and Kowalczyk, 2000). The dietary factors affecting endogenous losses were also discussed herein.

The nutritive value of the diet is largely determined by the available nutrient contained in the feedstuff, particularly amino acids, which are by concept of availability of nutrients in feedstuff, is incompletely digested and metabolised by animals. The digestibility of amino acid is a critical factor in formulation of diets, and effective evaluation of amino acid digestibilities can be translated into significant improvement in production parameters. The recent methods used to differentiate between endogenous protein and feed protein in the gastro-intestinal tract are presented hereto. In earlier studies, conventional methods like feeding protein-free diets and feeding diets containing 100% digestible protein, and mathematical regression have their own merits and demerits. Recently, several other alternative methods have been developed like homo-arginine method, isotope dilution technique and enzymatically hydrolysed casein (EHC) technique, which is also known as peptide alimentation technique. The advantages and drawbacks of these methods are presented.

2.6.1 Concept of Protein and Amino Acid Digestibility

2.6.1.1 Faecal vs ileal digestibilities

Apparent faecal amino acid digestibility is the simplest and traditional way of determining amino acid digestibility. In this method, the materials, both dietary and endogenous proteins, unabsorbed in the small intestine are subjected to microbial metabolism in the

large intestine making measurement ambiguous (Moughan, 1991). During digestion, some protein, peptides and free amino acid pass through the hindgut unaltered and are excreted in faeces, but a considerable proportion of nitrogenous materials entering the hindgut is metabolised by microflora. Also, non-protein nitrogen may also be used for synthesis of microbial protein and amino acids. As a result, measured amino acid value is likely to be far from the true amino acid digestibility value. Further, 80% of nitrogen found in faeces is of bacterial origin and thus a very small proportion of faecal amino acid flows directly relate to undigested dietary amino acid entering the hindgut and this generates erroneous values resulting to faulty conclusions (Hodgkinson and Moughan, 2000; Moughan, 1991). As a consequence, digestibility obtained from total tract collection is considered to overestimate the actual digestibility of dietary protein (Sauer and Ozimec, 1986).

The ileal analysis is the preferred method for the determination of amino acid digestibility for pigs (de Lange *et al.*, 1990). However, it should be considered that there is also a significant microbial activity in the small intestine (Dierick *et al.*, 1986), which has implication for the meaningfulness and accuracy of ileal amino acid digestibility coefficients (Hodgkinson and Moughan, 2000). Apparent, true and net ileal amino acid digestibilities are the most frequently used systems for amino acid digestibility determination. As the term implies, each method includes the removal of the digesta from terminal ileum to prevent bacterial degradation and fermentation reaction in the lower digestive tract. As described later, cannulation and slaughter techniques are used to measure the said values. An indigestible marker is mixed in the feed with which the animal is given a standard feed volume. In principle the amino acid digestibility is considered as the difference between the amount of amino acid in the feed and the amino acid found in the ileal digesta. The total ileal flow (TIF) of amino acid (in g/d) is calculated as:

$$TIF = (K/k) \times (aa)$$

where: K- quantity of marker in the diet; k- concentration of marker in the digesta; and aa- concentration of amino acid in the digesta.

From this, the apparent amino acid ileal digestibility (AAAID) can be calculated as:

$$AAAID = I - [(K / aad) \times (aa / K)]$$

where: aad - daily amino acid concentration in the feed (g); K - quantity of marker in the diet; and aa - concentration of amino acid in the digesta.

2.6.1.2 Apparent digestibility of amino acid

This is a measure of protein digestibility based on feeding the animal a test protein and a semi-synthetic protein-free mixture. This method of estimating amino acid digestibility does not take into consideration endogenous losses of amino acid, but it is rather reflected in digestibility coefficient (Boisen and Moughan, 1996b), thus the term apparent ileal digestibility existed. This leads to a source of error by making an underestimation of the amino acid absorbed in the diet (Fuller, 1988). Apparent digestibility for relatively low protein feedstuff is determined by feeding the animal with the feedstuff alone. For protein-rich sample, it is diluted with N-free mixture to give the diet a protein content of about 18%. Further, apparent digestibility is influenced by the protein content of the test diet (Boisen and Moughan, 1996a).

2.6.1.3 Net digestibility

This is a measure of protein digestibility relating to the feedstuff itself rather than to a test diet. For low protein feedstuff, net digestibility is determined directly, while for protein-rich feedstuff, it is determined by extrapolation of relationship with apparent digestibility and the level of inclusion of protein-rich feedstuff in the diet. Further, net protein digestibility is calculated by measuring the changes of amino acid content of the terminal ileum. As the ratio of total diet is increased, the ileal crude protein flow increase linearly, and this relationship is extrapolated to the point where the test protein is the only dietary constituent (Boisen and Moughan, 1996b). These indicate that there are no changes in the endogenous amino acid losses as feeding levels increase and that the increased amino acid content in the ileum is related entirely to the increase in undigested amino acid (Fuller, 1988). This theory is relatively faulty, for the level of dietary protein influences the rate of protein secretion to the digestive tract. Thus, the increase in amino acid content is due to an increase in endogenous protein losses (Fuller, 1988). Some findings showed contradictions, recommending that there is no relationship between protein level of the diet and endogenous losses because these losses might be a manifestation of non-protein constituent of the diet.

2.6.1.4 True ileal amino acid digestibility

This is a measure of protein digestibility where flow of undigested protein at the terminal ileum is corrected by using an estimate of endogenous ileal protein flow (from cell debris, gastric secretion, urea in blood, etc) and where the latter flow is determined by giving pigs N-free diet or enzymatically hydrolysed casein (Boisen and Moughan, 1996b). Feeding N-

free diet assumed many criticisms like being unphysiological, and the absence of protein alters body metabolism and therefore endogenous losses does not resemble to the losses from animal given the test diets (Fuller, 1988). Further, calculations assumed that endogenous protein losses is influenced only by food dry matter intake, which is not so. Moreover, different researchers used different N-free mixtures that markedly influenced endogenous losses and true ileal protein digestibility (Boisen and Moughan, 1996b).

2.6.1.5 Real digestibility

Real digestibility is a measure of protein digestibility of the feedstuff itself in which all component of endogenous ileal protein flow had been corrected. This method separates the amino acid from endogenous and dietary origin and considered as the most accurate system in determining the digestibility of amino acid in the diet. Oftentimes, the value is also referred as 'true digestibility' where a constant value (DE intake) is used to correct endogenous losses. But with real digestibility, the digesta flow is corrected for actual endogenous protein flow, which is variable (Boisen and Moughan, 1996b).

2.6.2 Methods of Digesta Collection

There are several methods wherein digesta can be colected from the terminal ileum, whereby the effects of the hindgut microbes is minimised. These methods will be compared based on their benefits, drawbacks and validities.

2.6.2.1 Slaughter technique

As the term implies, this technique involves slaughter of experimental animals to remove a section of the terminal small intestine from anaesthetised animal. Such procedure prevents sloughing off of mucosal cells that increase the nitrogen content (Nyachoti *et al.*, 1997). This technique, being simple and ethically acceptable, provides minimal interference with animal digestive tract, and there is no limitation on the type of diet given that can cause potential digesta blockage, as observed in cannulated pigs. More so, digesta sampling can be obtained from different parts of the digestive tract (Hodgkinson and Moughan, 2000). This technique takes a shorter time to complete an experiment with less labour input and no metabolic crates needed (Nyachoti *et al.*, 1997). It often uses a non-digestible dietary marker, with limited amount of digesta collected, by which there are some conditions of variable results due to minimal amount of samples.

In comparison, no significant differences were noted between ileal digestibilities obtained from this technique and from pigs fitted with simple-T cannulas (Hodgkinson and

Moughan, 2000; Nyachoti *et al.*, 1997). The slaughter technique is the method of choice for small animals, as well as pigs.

2.6.2.2 Anastomosis

In this method, the small intestine is completely transected, either at the terminal ileum or just after the ileo-caecal sphincter and then joined to the rectum, such that ileal digesta are easily collected from the anus. A more recent method, the ileo-rectal anastomosis involves transection of the distal ileum anterior to the ileo caecal valve where the large intestine is left intact and ileum is exteriorised from the animal and attached to the skin by preparation of a stroma. The ileo-rectal anastomosis (IRA) can be modified by insertion of cannula into the isolated colon to evacuate both the materials present and fermentation gases from the tract (Hodgkinson and Moughan, 2000). Its advantages include easy maintenance of animals, and animals can be given diets of any texture. Further, it eliminates the reliance of a dietary marker due to the fact that the digesta can be collected quantitatively via the anus. The absence of digesta leakage and problems with blockage that occur with some cannulation methods can be inhibited. The IRA method has several setbacks. As with most surgical procedures, the impact of IRA on the physiology and nutrition of the animals may pose some concerns. Further, it includes complete transection of the ileum that may affect gut motility and digesta flow rate, and it allows proliferation of bacteria in the small intestine affecting adaptation of the said organ. And finally, large intestine exerts control over digestive function and affects mineral and water uptake (Moughan, 1991).

2.6.2.3 Cannulation technique

In using cannulation technique, thorough considerations must be made to the physiological function of the animals. Several observations revealed that pigs cannulated at the terminal ileum have parallel voluntary feed intake to non-cannulated animals but showed less growth rate and less efficiency in feed utilisation. Furthermore, cannulation resulted to disruption of the normal digesta flow (Moughan, 1991).

There are several cannulation techniques used to collect ileal digesta. These are:

- **T-Cannula**- is considered as the least invasive cannulation technique. It involves surgical implantation of a T-shaped cannula in the terminal ileum, without disrupting the migrating myo-electric complexes responsible for intestinal motility. This method divides intestinal flow to both the intestine and cannula assuming that the portion collected in the cannula is representative of the ileal flow without fractionation of components due to divided flow,

but it is not always the case specifically when feeding high-fibre diets (Hodgkinson and Moughan, 2000). As indicated, collecting representative ileal flow relied with the indigestible marker found in the diet, by which, recovery values of 60% - 95% is relatively variable (Butts *et al.*, 1993b). When this method is of frequent use for a long time, the cannula might be outgrown by the animals, and its stem is prone to blockage with fibrous material. Further, amino acid digestibility values from this method and from slaughter method are comparable, but statistically different when compared with the IRA technique for amino acid, methionine, proline and tryptophan (Leterme *et al.*, 1990).

- **Re-entrant cannulation technique-** the ileo-ileo and ileo-caecal technique were criticized for total transection of the ileum. This is totally undesirable (Moughan, 1991) because it disrupts the normal function of digestion (Fuller, 1991; Sauer and de Lange, 1992). Aside from a relatively complex surgical intervention, complete transection of the intestine disrupts the transmission of migrating myoelectrical complexes for normal digesta flow, and, being in re-entrant mode, propulsion of digesta flow into the cannula is based in the entrance of more digesta to the cannula making it more prone to blockage, unless feed sample is finely ground (Hodgkinson and Moughan, 2000). The new method modified by Darcy *et al.* (1980 cited in Hodgkinson and Moughan, 2000), whereby the proximal cannula is located immediately distal to the ileo-caecal valve, is an advantage because it maintained the integrity of the small intestine and preserved the functional role of the ileo-caecal sphincter, thus maintaining a more physiological state. But this method requires more labour like continuous collection of digesta, which should be returned to hindgut at regular interval. Conversely, it has the advantage like maximum quantity of digesta collected and it does not rely on the use of digestibility marker (Nyachoti *et al.*, 1997).

- **Post valve T caecum cannula (PVTC)**- involves the removal of approximately two-thirds of the whole caecum, with the insertion of cannula into the remaining caecum opposite the ileo-caecal valve (van Leeuwen *et al.*, 1991). This technique is favourably used because it permits almost complete collection of digesta, and surgical intervention of the small intestine is prevented, thereby minimising the effects in ileal muscle movement. The cannula opening is large enough to prevent blockage, and coarser diets, like dietary fibre, can be offered to animals without restriction (Hodgkinson and Moughan, 2000). The major concern of this method relates to the removal of substantial part of the caecum, which alters the metabolism of the animals, and its superiority over the T-cannula is not well established (Moughan, 1995). However, Kohler *et al.* (1992) compared the growth performance, nitrogen retention and weights of internal organs of cannulated and intact

pigs. These authors found no significant differences between the parameters compared. Their findings suggested that PVTC method is suitable for collection of digesta for metabolic studies, and considered by many to be the current method of choice, which will likely give acceptable results (Hodgkinson and Moughan, 2000).

- **Steered ileo-caecal valve cannula** – this technique is a modification of the PVTC cannula (Mroz *et al.*, 1996) wherein metal rings are placed around the terminal ileum and a cord attached to the rings is placed through the cannula. The digesta can be collected by gently pulling the cord that moves the terminal ileum to the mouth of the cannula. This method is more advantageous over PVTC method because the caecum of the animal is not removed, but the effect of the presence of the metal rings inside and outside of the terminal ileum on the movement of the terminal ileum is not clear (Hodgkinson and Moughan, 2000).

2.6.2.4 Use of experimental animals

Another alternative method is using laboratory animal as model for pigs (usually rats). The rats showed general agreement with pig in apparent ileal digestibility with several feed ingredients, except legumes and plant food having high anti-nutritional factors. Comparative results showed no significant differences in amino acid values obtained from rats and pigs (except for methionine) (Moughan, 1991).

2.6.2.5 Nylon bag technique

Nylon bag technique is a rapid ileal amino acid digestibility assay that includes rapid measurement in a large number of samples. Its drawback includes interruption of hydrolysis of amino acid and interaction between anti-nutritive factors in the digestive tract (Sauer *et al.*, 1989).

2.6.3 Determination of Endogenous Nitrogen and Amino Acid Losses

Several conventional methods have been used to quantify endogenous nitrogen flow at the terminal ileum of pigs and poultry, including feeding a protein-free diet, which is dubious. Alternative approaches, then, have been developed.

2.6.3.1 Feeding protein-free diet

Using a protein-free diet, which assumed that all nitrogen containing compound in the ileum is of endogenous origin, is criticised as being unphysiological, and can cause reduced gastric and pancreatic enzymes secretion that, in turn, reduced the rate of protein

synthesis in the body and gut. As a consequence, lowered endogenous protein losses may occur (Hodgkinson and Moughan, 2000). When an animal is fed a protein-free diet, the metabolism of enzyme alanine or glutamine and, consequently, proline is affected resulting in high secretion of the latter (Sauer and de Lange, 1992). Further, dietary fibres and anti-nutritional factors associated with dietary proteins may enhance endogenous losses (Nyachoti *et al.*, 1997). Moreover, a protein-free diet may lack the stimulatory effect on endogenous gut protein secretion (Donkoh *et al.*, 1995). The afore-mentioned studies, therefore, clearly indicate that protein-free state leads to substantial alteration of whole body and gut metabolism, and most likely, the estimate of endogenous flow is misleading (Hodgkinson and Moughan, 2000). This method is modified by de Lange *et al.* (1989b) whereby pigs fed with protein-free diet were intravenously infused with either saline (negative body nitrogen) or balanced mixture of free amino acid (positive nitrogen balance). The results of this study showed no significant differences except that pigs infused with saline significantly excreted more proline. These findings confirmed that negative body nitrogen balance of pigs fed with protein-free diet does not cause a lowered ileal endogenous amino acid loss, and the high proline losses is indicative of negative body nitrogen balance (Leterme *et al.*, 1996).

2.6.3.2 Natural protein devoid of specific amino acid

This method involves intravenous infusion of dietary essential amino acid not present in the diet. And in such case, the animal is in positive body nitrogen balance. An example of this material is zein, a purified protein from maize, which is almost completely lacking in lysine. The administration of zein to animals allows a direct measure of endogenous lysine loss. Feeding zein, infused intravenously with lysine, to young pigs had significantly higher ileal lysine flows compared to protein-free control. This result, in the absence of fibre or anti-nutritional factors in a purified zein protein diet, is direct evidence for an effect of protein on endogenous ileal lysine flow (Hodgkinson and Moughan, 2000; Butts *et al.*, 1993b).

2.6.3.3 Homoarginine method

In this method, the specific feed-induced protein loss can in principle be measured using the homoarginine method wherein the lysine in the dietary protein is guanidinated by treatment with o-methylisourea forming homoarginine that is assumed to be liberated and absorbed at the same rate of original lysine would have been. This technique enables the loss of endogenous lysine to be measured together with other essential amino acids on the

assumption that composition of amino acids for endogenous losses of protein is constant (Boisen and Moughan, 1996a). This assumption is not fully established as indicated by the decrease in protein digestibility of different milk products after guanidination. Further, if guanidination is incomplete, the unreacted lysine might have a different digestibility compared to modified lysine. This method has several drawbacks, namely: it provides information about endogenous flow of lysine only, and the flow of all other amino acids need to be estimated by assuming a constant relationship between the concentration of lysine and those of other amino acids in the endogenous protein (Hodgkinson and Moughan, 2000). It should be noted that absorbed homoarginine maybe toxic and so guanidinated proteins can not be fed for long periods because homoarginine may accumulate in the body due to slow rate of conversion of the same to lysine, and may interfere with the urea cycle resulting to accumulation of urea in the body. In fact, Moughan and Rutherfurd (1990) observed a decrease in feed intake over the entire experimental period, making derived ileal values relatively variable. On the other hand, this method offers considerable promise in determining endogenous ileal amino acid flow because it estimates the latter in a physiologically normal state (Hodgkinson and Moughan, 2000). This method is considered to be superior over protein-free and regression methods as indicated by studies showing 90% of the amino acid found in the ileum were of endogenous origin (Siriwan *et al.*, 1987), leading to dramatic increase of ileal endogenous loss of lysine over protein-free technique.

2.6.3.4 Isotope dilution technique (tracer technique)

The tracer technique allows determination of amino acids of endogenous and dietary origin. The technique enables labelling of dietary or body protein with radioactive isotopes usually ^{15}N, and endogenous protein losses is calculated based on the calculation of tracer in ileal digesta (de Lange *et al.*, 1990). This is considered as a promising technique, which allows a direct measurement of the feed-dependent endogenous ileal protein loss, but does not allow direct estimation of all the respective amino acid flows. There is also disagreement on the constitution of an appropriate amino acid pool (Boisen and Moughan, 1996a), and the preference of precursor pool had a significant effect on the dilution factor (Moughan *et al.*, 1992). The study of de Lange *et al.* (1990), using the ^{15}N dilution method, showed significantly higher endogenous losses when feeding common vegetable feedstuff with values ranging from 25.5 to 35.5g protein/kg DM intake. Further, when using ^{15}N stable isotopes in diets with increased dietary fibre, the nitrogen endogenous flow was considerably enhanced (Schulze *et al.*, 1995b).

This method enables the researcher to segregate protein from non-digested dietary and endogenous origin, and values obtained are expressed in real or true digestibility coefficient, for it measures the endogenous protein loss is specific to the feedstuff investigated. Usually, endogenous losses measured and calculated from this approach are considerably higher compared to traditional methods (Boisen and Moughan, 1996a). As earlier mentioned, endogenous protein losses increased when animals were fed with a diet high in dietary fibre and vegetable feedstuff. Huisman *et al.* (1992) added that endogenous loss value as high as 107g/kg DM intake was observed in toasted beans, and as low as 31 and 34 g/kg DM for two pea varieties. This information clearly manifests the essential impact endogenous losses may offer on values of apparent digestibility (Boisen and Moughan, 1996a). In the experiment by Schulze *et al.* (1995a), which differentiates endogenous nitrogen losses using EHC technique and tracer method, the results were similar, though the ^{15}N endogenous flow was lower than EHC value by 15%.

2.6.3.5 Enzymatically hydrolysed protein method

This method, also called as peptide alimentation method, allows endogenous ileal nitrogen and amino acid flows to be determined in animals fed diet containing dietary peptides and free amino acids as sole source of nitrogen (Boisen and Moughan, 1996a). Digestion in test animals is stimulated by feeding enzymatically-hydrolysed casein, containing mixtures of free amino acids and tri-peptides, with no peptides being larger than 5000 Daltons (Da). To remove the undigested dietary peptides and free amino acid, with molecular weight lower than filtration cut-off of 10,000 Da, the ileal digesta is centrifuged and repeatedly ultrafiltered. The endogenous material is then contained in the high molecular weight fraction (>10,000 Da) (Hodgkinson and Moughan, 2000). In many instances, small peptides and amino acids from endogenous sources are removed with the supernatant, which is likely to result in slight underestimation of ENL (Nyachoti *et al.*, 1997). Moughan and Schuttert (1991) reported a small portion of free and peptide bound amino acid is found in ENL. Leterme *et al.* (1996) found out that around 22% of the total digesta nitrogen were present in the <10,000 Da, and Butts *et al.* (1992) reported values of 21%. These information suggest considerable variations in the contribution of free and peptide bound amino acids to ENL.

This method also allows a direct determination of endogenous flow of total nitrogen and all other amino acids, as opposed to others where only lysine or nitrogen are measured directly. Further, dietary factors, like fibre and anti-nutritional factors, lead to increase endogenous amino acid losses above basal levels, and such effects cannot be readily

determined using the enzyme hydrolysed protein method (Nyachoti *et al.*, 1997). But when true digestibility coefficient of the feedstuff are known through their basal values, the effects of these factors are correctly costed in the feedstuff itself (Hodgkinson and Moughan, 2000). Generally, this appears to be an excellent method in determining the endogenous nitrogen and amino acid flows because it allows direct determination of the same in animals of apparently normal physiological state, with slight underestimation in the ultra-filtrate. Further, this technique is applied across varieties of species with sufficient results and clear evidence suggesting a direct effect of peptides present in the gut on endogenous protein secretion or reabsorption (Boisen and Moughan, 1996a).

2.6.3.6 In vitro / In vivo digestibility techniques

Alternatively, endogenous nitrogen losses can be estimated from the difference between *in vivo* values of apparent ileal digestibility and *in vitro* digestibility values (Boisen and Eggum, 1991). *In vitro* technique includes conditions simulating the digestion processes in the stomach, small intestine and hindgut using enzymes pancreatin, pepsin and multi-enzyme complex through specified incubation steps (Boisen, 2000; Boisen and Moughan, 1996a). This method is not influenced by endogenous protein loss and should be ideally related to the real digestibility of the feed protein, but in practice, validation of *in vitro* results of protein digestibility should be based on *in vivo* apparent digestibility (Boisen, 2000).

As mentioned earlier, the endogenous protein loss (EPL), classified into basal and extra, had significant effects to ileal flow of protein and amino acids, and apparent nitrogen digestibility needs to be corrected for basal EPL in order to obtain a 'true' standardised value for digestibility (Boisen and Verstegen, 1998). It should be noted that extra EPL is a specific cost associated with the feed, and thus values of digestibility should be corrected for the extra-specific EPL (Boisen, 2000) In the assumption that the difference between *in vitro* and *in vivo* apparent digestibility of protein is caused by EPL, the apparent digestibility *in vivo*, can in principle, be predicted from *in vitro* digestibility after correcting for total EPL (Boisen, 2000).

Moreover, Boisen and Moughan (1996a) summarized the endogenous protein loss in growing pigs, and their results showed a high variation in published results, attributed to the differences among methods and real differences between feedstuff. However, Boisen (2000) suggested that *in vivo* digestibility of amino acids can be predicted with acceptable accuracy in a simple system in which the *in vitro* (real) digestibility of nitrogen and amino

acid within the sample, as well as the amino composition of EPL, is assumed to be constant. Such method showed that predicted values of standardised digestibility of amino acid in common feedstuff are in good agreement with corresponding tabulated values from NRC and CVB (Boisen, 2000)

2.6.4 Endogenous Protein Loss and Dietary Factors

Considerable amounts of endogenous protein enter to the digestive tracts of monogastric animals from various sources. These are secretions from the saliva, stomach, small intestines, mucopolysaccharides from epithelial cells, and slough off epithelial cells. According to Boisen and Moughan (1996a), endogenous protein losses seemed to be increased in protein-containing diet than protein-free diet, and it is influenced by several dietary factors brought by feed-induced variations or indirectly by changes in intestinal bacterial activity.

2.6.4.1 Dry matter intake

In specific live weight, there seems to be a significant influence of feed dry matter intake on endogenous loss as indicated by the studies of Butts *et al.* (1993b) that clearly demonstrated a linear relationship between endogenous protein losses and dry matter intake. But other literature, like Furuya and Kaji (1992), showed contradicting evidence as illustrated by no influence on EPL when dry matter intake (DMI) was increased. The differences herein mentioned can be attributed, in part, by the differences in methodology and/or diet composition. EHC method was used by Butts *et al.* (1993b), which is more reliable because it is carried out in more physiological condition, while Furuya and Kaji (1992) used protein-free diets. Thus, for feed evaluation purposes, it is suited to express endogenous loss in relation to dry matter intake. Therefore, nutrients, like protein, had influence on endogenous secretion in the gut and reutilisation of endogenous protein. But generally, undigested dry matter can be a better predictor in a feed–induced endogenous protein loss, on the assumption that endogenous loss throughout the digestive tract is mainly influenced by undigested portion of the diet (Boisen and Moughan, 1996a). Nevertheless, the studies of Butts *et al.* (1993b) and Furuya and Kaji (1992) demonstrated that the response to the intake of dry matter depends on the level of protein in the diet.

2.6.4.2 Protein

It is widely known that increased dietary protein levels substantially increased the amount of endogenous nitrogen secreted in the gut lumen (Moughan and Rutherfurd, 1990), as

indicated by the potent stimulation of endogenous secretion in the gut by both protein and peptides (Butts *et al.*, 1993a). The high ileal endogenous protein losses were also evident in the diets containing peptides as compared to protein-free methods (Boisen and Moughan, 1996a). Similarly, endogenous ileal lysine loss was also increased from feeding intact pigs with lysine-deficient protein zein (Butts *et al.*, 1993b). The mechanisms for the influence of these dietary peptides on EPL have yet to be elucidated. However, the aforementioned results suggested a common effect for added peptides or those arising from natural digestion of protein. Generally, the presence of exogenous protein in the gut appears to slow down the breakdown of endogenous protein, though this alone may not explain the observed increase in ileal endogenous protein secretion. There is a possibility that dietary protein stimulates endogenous protein secretion and reduces digestion and absorption of endogenous protein (Nyachoti *et al.*, 1997). Moreover, Ikegami *et al.* (1975) mentioned that feeding low quality protein resulted in accumulation of substantial quantities of digestive enzymes in the gut due to low rate of breakdown that, in turn, increased ENL. On the other hand, Boisen and Moughan (1996a) concluded that there seems no direct relationship between dietary protein and endogenous ileal protein losses because these losses are induced primarily by the non-protein parts of the feed. This had been indicated by almost identical quantities of endogenous loss brought about by the different levels of inclusion of EHC in the diet (10% by Moughan and co-workers, and 18% by Schulze *et al.*, 1995a). However, Hodgkinson *et al.* (2000) contradicted such finding, and these authors showed a significant effect of dietary peptide concentration on the endogenous ileal flows of N and all of the amino acids, with a linear increase in endogenous ileal amino flow with increasing dietary EHC concentration.

2.6.4.3 Anti-nutritional factors

Ileal endogenous nitrogen flow is also affected by the presence of anti-nutritional factors (ANF) naturally found in the feedstuff itself (Schulze, 1994; Huisman *et al.*, 1992). ANF bind to various nutrients or damage the gut wall and, thereby, reduce digestive efficiency. The main ANF that interfere with nutrient digestion and absorption are lectins, protease inhibitors, tannins, antigenic protein, phytic acid, gloconulates and gossypol (de Lange *et al.*, 2000), but only few are mentioned herein.

Generally the effects of ANF are even more complex than the effect of fibre and are more variable for various ANF (Boisen and Moughan, 1996a). Taking into account the experiment of Schulze (1994) that presented the effects of purified soya bean trypsin inhibitor in the flow of nitrogen in the ileum of young pig, it is concluded that ileal flows

of endogenous and exogenous nitrogen are significantly increased by trypsin inhibitor in a linear manner. Trypsin inhibitor binds specifically to enzymes at their active sites that, in turn, leads to stimulation of pancreatic secretion (Boisen and Moughan, 1996a), and feeding protease inhibitor may also result to pancreatic hypertrophy (de Lange *et al.*, 2000). Moreover, at high levels of inhibitors in the ingested diets, an increased secretion may not completely compensate for the inhibition leading to decreased digestion and absorption of both endogenous and feed proteins (Boisen and Moughan, 1996a). However, Grala *et al.* (1998), in studies on pigs fed diets with different soybean products and rapeseed cake, showed that reabsorption rather than secretion of endogenous nitrogen along the small intestinal tract seemed to be influenced by anti-nutritional factors present in those feeds. Tannins, which form complexes with carbohydrates and proteins in the feed and with digestive enzymes, are known to interact to both dietary and endogenous nitrogen that caused a decrease in true amino acid digestibility (Jansman, 1993). Other ANF, like soya bean lectins, when present in the diet, can also cause increased endogenous gut nitrogen losses, probably arising from a loss of mucus (de Lange *et al.*, 2000).

2.6.4.4 Dietary fibre

In many studies, NSP significantly influenced endogenous ileal protein losses, though it has a wide range of chemical components with unique physical properties and physiological influence (Graham *et al.*, 1991). This had been demonstrated by the experiment of de Lange *et al.* (1989a), Furuya and Kaji (1992), and Leterme *et al.* (1992), where they clearly showed that with purified cellulose as dietary fibre source, no effects on ileal endogenous nitrogen were observed. This result may be erroneously interpreted as fibre had less influence on endogenous protein losses. However, the addition of pectins, but not cellulose, in the diet of pigs increased the endogenous nitrogen in the terminal ileum (de Lange *et al.*, 1989a). It can be noted that in diets having compounds of high viscosity, the ileal endogenous nitrogen losses increased with increasing viscosity (Larsen *et al.*, 1993). Further, in the study of Leterme *et al.* (1998) on the effect of pea fibre on protein and amino acid losses in pigs, results indicated that fibre with high water-holding capacity increased ileal endogenous nitrogen. Souffrant (1999) mentioned that inner fibres of barley, not barley hulls, added to nitrogen-free diet produced an increase in ileal nitrogen flow. Therefore, the results of aforementioned studies demonstrated that different fractions of fibre might affect the flow of endogenous nitrogen in the distal ileum to different extent.

2.7 Mucin and Dietary Fibre

Mucus is a large molecular weight glycoprotein that covers the entire luminal surface of the gastro-intestinal tract. This mucus layer served various functions primarily related to the protection of the underlying epithelium (Lien *et al.*, 2001). These are: mucus, along with bicarbonate, protects the epithelium from vigorous digestive processes and corrosive gastric juices by creating unstirred layer and by acting as diffusion barrier, preventing large molecular weight compounds from reaching the epithelium. Mucus traps toxins and bacteria preventing infection. Adherent and soluble mucus in the intestinal lumen act as lubricant providing protection from mechanical damage caused by the passing of food. Further, it also played an important role in digestive processes by creating a digestive zone in which enzymes are immobilised near the epithelial surface, preventing their rapid removal by peristalsis and placing them in a more favourable form for digestion. These factors subjected mucus to all chemical and physical forces of digestion. In fact, the presence of mucus in the lumen of digestive tract is primarily attributed to proteolysis, with physical abrasion (Allen, 1981). Thus, recovering the ungraded mucin in the ileal digesta is of utmost importance because it represents an essential quantity of endogenous protein and carbohydrates retrieved at this portion (Lien *et al.*, 2001).

2.7.1 Mucus Structure and Properties

Mucins are high molecular weight glycoproteins (2 x 10^6 daltons) consisting of 65-85% carbohydrates and 20-30% protein, with variable amounts of sulphate and water (Lien *et al.*, 2001; Satchithanandam *et al.*, 1990). It consists of four sub-units, weighing approximately 5 x 105 daltons each, that are linked by disulphide bonding and are arranged into the 3-dimensional polymeric structure necessary for gel formation. Mucins are backboned by protein, which are surrounded by oligosaccharides chains and resembles a 'bottle brush' structure (Allen, 1981). Its protein core is divided by two distinct regions, the glycosylated region and the nonglycosidic region. The glycosylated region of the mucin molecule represents more than 95% of the glycoprotein and is termed as 'native protein' mucin. Protein in this region accounts for 65% of the total protein mucin and is rich in serine, threonine and proline. This site is resistant to proteolytic attack due to tight packing of oligosaccharides. The second region, the nonglycosidic or naked region, which is accessible to proteolytic attack, represents about 3.5% of mucin protein or 4-5% of total glycoprotein. This site has an amino acid composition similar to that of an average globular protein, which is consistent in its role in the formation of polymeric structure of mucus via the joining of mucus subunits by disulphide bridges (Lien *et al.*, 2001). With

these characters, the most remarkable property of mucin is its ability to form a gel (viscoelastic semisolid material) that adheres to the epithelial surface and provides a physical barrier between underlying cell surface and lumen (Satchithanandam *et al.*, 1990).

2.7.2 Mucin Degradation in the Gastro-intestinal Tract

The mucus gel is a dynamic balance between erosion and secretion demonstrated by the ability of enzymes pepsin, pronase, papain, and trypsin to proteolytically digest mucin into its component subunits (Pearson *et al.*, 1980, Lee *et al.*, 1987). This proteolytic degradation weakens mucus gel, and thus succumbs to intense physical force of digestion. In rats, when pepsin is introduced to the stomach, there is a linear increase in the recovery of mucin up to a pepsin concentration of 1 mg/mL (Munster *et al.*, 1987). Mucin in ileal effluent consists of mixtures of gastric and intestinal mucin, the proportion of which is determined by diet consumed. In pigs fed protein–free diet, ileal mucin originated largely from the small intestines (Lien *et al.*, 1997), while those pigs fed bean-containing diet, higher contribution of gastric to total mucin was observed (Lien *et al.*, 2001). It is also noted that mucin from ileal digesta is of low solubility probably influenced by, among other factors, bile and luminal pH (Lien *et al.*, 2001).

2.7.3 Attributes of Mucin to Endogenous Protein

Protein in mucin totalled only 5 to 11% of endogenous protein, subject on the infusion treatment and the degree of proteolytic degradation from feeding pigs protein-free diet containing either a complete amino acid mixture or saline, given intravenously. The three predominant amino acids in mucin are threonine, serine and proline, which are represented in relatively higher proportion compared to other amino acids. The values of these amino acids were even underestimated as indicated in high content of threonine in the soluble non-mucin fraction of the diet (Lien *et al.*, 1997). The underestimation is based on the assumption that mucin oligosaccharide chain is in complete elongation, however, in elevated mucin secretion, elongation of the same chain is incomplete (Allen, 1981). This result suggests that threonine is the predominant indispensable amino acid in endogenous protein, and therefore, the presence of mucin in ileal digesta will explain the low digestibilities of this amino acid in many feedstuffs fed to pigs (Sauer and Ozimec, 1986).

2.7.4 Diet and Mucin Secretion

Diet composition had considerable influence on mucin secretion since it is stimulated by similar neural and hormonal factors that control digestive processes (Allen, 1981; Lien *et*

al., 1997). The effects can be categorised as indirect (via influence on digestive processes), or direct (via interaction of mucosal gel). The significance of the indirect influence is exemplified in the secretion of proteolytic enzymes as demonstrated by the increase in mucin output and the proportion of mucin subunits in the presence of pepsin following administration of stimulants. Lien *et al.* (2001) mentioned several authors that indicated insulin, secretin and gastrin as a stimulant for mucus secretion. Mucus synthesis is also elevated by meal consumption, as shown by a 50% increase in glycoprotein carbohydrate in canine heidenhein pouches (Kowalewski *et al.*, 1976 cited in Lien *et al.*, 2001). This action is associated to an increase in proteolytic degradation indicated by 300% increase in pepsin secretion (Lien *et al.*, 2001).

The influence of dietary constituents, primarily dietary fibre, on mucus secretion is of greater significance. Diets high in fibre tended to induce structural, morphological and cytokinetic changes in the digestive tract that indicates a capacity for high mucin secretion (Vahouny and Cassidy, 1986; Jacobs, 1986). The types of dietary fibre influence synthesis and secretion of mucus, suggesting that insoluble fibre in diet initiates increased mucus secretion as compared with soluble fibre. This is shown by the dramatic increase in the radioactivity incorporation into glycoprotein in rats fed with cellulose (10%) or wheat bran (10%) compared to rats fed fibre free diet (Vahouny *et al.*, 1985). Further, Sachithanandam *et al.* (1990) found out that the amount of luminal immuno-reactive mucin was significantly increased in rats fed with wheat bran (10% or 20%) and citrus fibre (5%) as compared to fibre-free rats. In pig, Lien (1995 cited in Lien *et al.*, 2001) found a linear trend in mucin output associated with fibre intake after exclusion of one pig that showed variable results. These said results showed the influence of fibre types on gastro-intestinal tract and mucosal response, which likely demonstrate that insoluble fibre has more abrasive action by scraping mucin from mucosa as they pass through the digestive tract (Lien *et al.*, 2001). In insoluble fibre-fed animals, the mucus layer may be maintained by increasing goblet cells activity and thus increasing the capacity for mucin synthesis. The increase in goblet cell activity may account for the increased amount of adherent mucus and increased incorporation of labels associated with insoluble fibres (Vahouny *et al.*, 1985; Satchithanandam *et al.*, 1990). On the other hand, soluble fibre is more damaging to intestinal mucosa which must respond by re-establishing both the mucus and epithelial layers. The epithelial layer is repaired by increasing the rate of cell replacement, while the mucus layer may be re-established primarily using stores in crypt cells rather than by increased mucin synthesis (Lien *et al.*, 2001).

Mucin output can also be changed by secondary factors, as shown by the influence of dietary fibre on the distribution and activity of proteolytic enzymes that may induce degradation of mucus gels. Feeding wheat bran, as compared to fibre-free diet, elevated trypsin and chemotrypsin activity (Schneeman *et al.*, 1982). In like manner, feeding oat bran, pectin and cellulose supplemented diets to rats increased small peptidase activity (Farness and Schneeman, 1982). But, the extent to which changes in proteolytic activities influences the changes in mucin secretion remains to be elucidated. Increase in protein content, seemed to increase mucin output, resultant from improvements in the proteolytic capacity of the digestive tract (Lien *et al.*, 2001; Lien *et al.*, 1997; Mariscal-Landin *et al.*, 1995).

Swine feedstuff like cereals, soya beans and legumes contain lectins, which have the capacity to bind to glycoproteins. Moreover, lectins induced histamine release, a known mucus secretagogue (Haas *et al.*, 1999). In pigs fed with peas and beans, mucin output was significantly increased, which is not surprising due to well-documented toxic effects of lectins. However, the high mucin output resulted from feeding peas need to be validated because of high trypsin inhibitor content of peas used in this study (Lien *et al.*, 2001).

In general, the source of diet may also affect the relative proportions of gastric and intestinal mucins. In barley-based pig diets, mucin from ileal digesta was derived primarily from gastric mucosa (63.6%). In pig fed diets having peas and lentils, the proportions of gastric and intestinal mucus in ileal digesta were comparable (49.1% to 54.9% gastric mucin). In pigs fed protein-free diets, gastric mucin totalled to approximately 25% of mucin in ileal digesta, while in pigs fed a wheat diet with or without pea inclusion, gastric mucin represents 50% of mucin in ileal digesta (Lien *et al.*, 2001).

2.8 Conclusion

It has been established that dietary NSP, both soluble and insoluble, have considerable influence on digestibility and metabolisms of other nutrients during absorption process that can be attributed to their physical properties, effects on gut micro flora, viscosity, solubility and water-holding capacity. Specifically, β-glucans and arabinoxylans have anti-nutritional effects on monogastric species that are associated to their hydrophilic properties, highly complex and branched structure and their interaction with other cell wall components. Further, the response of anti-nutritive effects of NSP differs across monogastric species. It seemed to appear that poultry are more vulnerable to inclusion of soluble NSP in the diet as compared to pigs, as indicated by their lowered animal performance and increased

endogenous nitrogen losses. Such is not surprising due to anatomical and physiological differences of the two species.

In addition, soluble NSP, like β-glucan, is known to show cholesterol lowering properties both in experimental animals and in humans. However, the mechanisms for the hypocholesterolemic effects of β-glucan are not clear, but are believed to be related to the highly soluble fraction of the same. Several hypotheses have been presented, but one cannot pinpoint the exact mechanism. As such, the said theories cannot be scientifically proven nor can be disregarded.

Ileal digestibility provides better estimates of nitrogen absorption. With the several methods used to measure endogenous nitrogen losses, the level of agreement for the magnitude of ileal nitrogen and amino acid flows is surprisingly high (Hodgkinson and Moughan, 2000). However, these methods open the opportunities for major advances to be made in the measurement and understanding of mammalian endogenous nitrogen secretion, reabsorption, flow and excretion. It is evident that ENL in the ileum of pig is influenced by animal factors and dietary constituents, the most important of which are dry matter intake, level and quality of feed protein, presence of anti-nutritional factors and dietary fibre. These factors lead to an increased endogenous nitrogen flow, and proper identification of such may be beneficial in finding means of reducing endogenous losses and increasing dietary nutrient utilisation.

Mucus is a large molecular weight glycoprotein that provides a protective lining in the gastrointestinal tract. Its recovery in the ileum can provided baseline information into the effects of diet and dietary constituents on the gastro-intestinal tract, particularly dietary fibre that has apparent direct effects on mucus secretion. Moreover, measurement in the recovery of mucin in ileal digesta is essential in the assessment of endogenous protein losses and its effect on true digestibility.

Therefore, this research had been conducted to investigate the influence of NSP namely: β-glucan and arabinoxylan, and cellulose on the production performance, endogenous nitrogen losses in two monogastric species (chicken and pigs). The hypocholesterolemic effect and the influence of soluble NSP on the ileal mucin content in pigs were also evaluated.

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CHAPTER III

INFLUENCE OF ARABINOXYLAN AND BETAGLUCAN ON BLOOD METABOLITES, ORGAN WEIGHTS, INTESTINAL MUCIN PRODUCTION, NITROGEN DIGESTIBILTY AND ENDOGENOUS ILEAL AMINO ACID LOSSES IN WEANER PIGS

3.1 Introduction

The soluble and insoluble dietary fibres play a pivotal role in monogastric nutrition mainly because of their anti-nutritional factors, poor utilisation as feed material, and effects on the level of blood metabolites.

Recently, there is an increased interest in the use of fibrous feeds for monogastric animals, as indicated by the use of cereals as one of the most important ingredients of pig diets in Europe. However, pigs do not produce the endogenous enzyme required to breakdown cell wall non-starch polysaccharides (NSP). Many researchers (Low, 1982; Jorgensen *et al.*, 1996; Yin, 1994; Graham *et al.*, 1986; Fernandez and Jorgensen, 1986) established that the addition of dietary fibre in the diet resulted in a negative correlation between apparent ileal digestibility of starch, crude protein, fat and minerals. The said relationship can be associated with changes in the rate of absorption of different feed nutrients and from increased endogenous nitrogen secretion. It can be noted that the level and source of dietary fibre are the most important factors influencing the amount of endogenous nitrogen (N) and of amino acid present in the ileal digesta (Sauer and Ozimik, 1986).

In addition, dietary fibres have a profound effect on the weight of the gastrointestinal tract as well as size of the visceral organs. According to Anugwa *et al.* (1989) and Pond *et al.* (1988), the feeding of fibrous feeds to pigs resulted to increased relative and absolute stomach weight but decreased liver, kidney and large intestine weights. These organs are considered as metabolic tissues having a higher energy expenditure than the carcass, which may indirectly affect energy partitioning, thereby affecting growth performance.

The effects of dietary fibres on lipid metabolism had been studied intensively. To date, hypertension leading to cardiovascular diseases had been associated with persons having high levels of plasma cholesterol. Fortunately, some sources of dietary fibres had been indicated to alter lipid metabolism in both humans and animals (Izumi and Sugano, 1986; Schneeman and Lefevre, 1986). β-glucan, a water-soluble fibre from barley, is found to be effective in lowering plasma cholesterol level (Schneeman and Lefevre, 1986). On the other hand, the presence of dietary fats affects level of blood lipids, and coconut oil, an unsaturated fatty acid, appears to raise blood cholesterol level that might be detrimental to human health. The presence of coconut oil in this trial offered a challenge to the cholesterol-lowering characteristics of β-glucan.

Mucus secretion from the gastro-intestinal tract consists, among others, of mucin with a high molecular glycoprotein responsible for the gel nature of the mucus (Lien *et al.*, 2001; Satchithanandam, 1990.). Mucin appeared to have been scarcely digested prior to the large intestine (Lien *et al.*, 1997), and its presence provides an aqueous "coat" along the gastro-intestinal tract that provides a surface barrier to the intestinal absorption of nutrients. Moreover, the presence of mucin had been inferred by the threonine content of endogenous protein and lower threonine digestibility of many feedstuffs. It is also a source of primary endogenous carbohydrates from ileal digesta. In addition, feeding a fibre-rich diet can influence mucus secretion. Thus, an estimate of the ileal recovery of mucin provides information on the content of endogenous protein and carbohydrates, and changes in mucin content or composition at the mucosal surface might be involved in the alteration of nutrient absorption in the small intestine as induced by fibre feeding.

Also, dietary fibre had profound effects on growth parameters as indicated by the animal's feed intake, feed conversion ratio and average daily gain. Insoluble fibre appeared to slow down growth, while soluble fibres enhanced better growth performance.

In the present experiment with pigs, an investigation was undertaken to determine the nutrient digestibilities and flow of endogenous nitrogen at the terminal ileum consequent upon giving different levels and types of soluble (arabinoxylan, β-glucan) and insoluble (cellulose) dietary fibres. It is designed to determine the effects of soluble NSP on growth performance (body weight gain and feed utilisation) and organ weights. It is also performed to monitor the influence of the dietary fibres (β-glucan, arabinoxylan and cellulose) on levels of blood cholesterol, triglycerides, high-density lipoprotein and low-density lipoproteins in weaner pigs. The influence of dietary fibre in the secretion of crude mucin in the small intestine is also examined.

3.2 Materials and Methods

3.2.1 Animals and Management

Twenty-five 3-week old LW x LR crossbreed piglets selected from five litters were used in the study. The piglets were obtained from a commercial farm in New Zealand. The animals were individually penned in smooth edge metal cages and had free access to fresh water through nipple drinker. Using heat lamps, the experimental animals were maintained in a 25°C temperature-controlled room with relative humidity of 50– 70%. The trial was

conducted at the Animal Physiology Unit, Massey University, Palmerston North, New Zealand.

3.2.2 Experimental Designs and Diets

This experiment followed a completely randomised design with five treatment diets as follows:

Control = 7.5% Cellulose (100% insoluble fibre)

4% AX = 4% Arabinoxylan + 3.5% Cellulose (50% soluble and 50% insoluble fibre)

7.5% AX = 7.5% Arabinoxylan (100% soluble fibre)

4% BG = 4% β-glucan + 3.5% Cellulose (50% soluble and 50% insoluble fibre)

7.5% BG = 7.5% β-glucan (100% soluble fibre)

Five piglets, one from each litter, were assigned to each experimental diet. The treatment groups were, at start, balanced for weight and sex so that each treatment groups had either 3 males:2 females or 3 females:2 males with similar average liveweight at the start of the experiment.

Five experimental synthetic diets, consisting of different levels (4% and 7.5%) of soluble dietary fibres, based on wheat starch, sugars and coconut oil were formulated. Casein (CAS diets) and enzymatically-hydrolysed casein (EHC) were the sole source of protein. The diets contained titanium oxide as indigestible marker and its concentration was used as a reference value from which digestibility coefficients were calculated. The percentage composition of the experimental CAS and EHC diets are presented in Tables 3.1 and 3.2. In both, casein and EHC diets, diet 1 contained no β-glucan (BG) and arabinoxylan (AX), diet II contained 4% arabinoxylan, diet III had 7.5% arabinoxylan, diet IV contained 4% β-glucan and diet V had 7.5% β-glucan. The β-glucan was sourced from a commercial brand Glucagel™ (Gracelink Ltd, Lower Hutt, New Zealand) having 70% purity, with low molecular weight, partially depolymerised, and when dispersed in water forms a soft gel, instead of a viscous solution. The arabinoxylan was extracted from maize and supplied by Limagrain SA Clermont-Ferrand, France. The dry matter, nitrogen, amino acids, and NSP composition of the experimental diets is presented in Table 3.3.

Table 3.1 **Composition of the casein-based diets expressed in percent.**

Diet Composition	CAS 1	CAS 2	CAS 3	CAS4	CAS5
Wheat starch	56.55	56.55	56.55	56.55	56.55
Sugar	7	7	7	7	7
Cellulose	7.5	3.5	0	3.5	0
Casein	15	15	15	15	15
Maize arabinoxylan (Ax)	0	4	7.5	0	0
Glucagel™	0	0	0	4	7.5
Common Ingredients[1]	13.95	13.95	13.95	13.95	13.95

[1]Coconut oil- 10; Dicalcium phosphate- 3; Sodium chloride- 0.15; Vit/min premix- 0.25; Methionine; 0.35; Threonine- 0.2;

Table 3.2 **Composition of the enzymatically hydrolysed casein diets expressed in percent.**

Diet Composition	EHC1	EHC2	EHC3	EHC4	EHC5
Wheat starch	56.15	56.15	56.15	56.15	56.15
Sugar	7	7	7	7	7
Cellulose	7.5	3.5	0	3.5	0
EHC	15	15	15	15	15
Maize arabinoxylan (Ax)	0	4	7.5	0	0
Glucagel™	0	0	0	4	7.5
Common Ingredients	14.35	14.35	14.35	14.35	14.35

Coconut oil- 10; Dicalcium phosphate- 3; Sodium chloride- 0.15; Vit/Min premix-0.25; Titanium oxide- 0.4; Methionine- 0.35; Threonine- 0.2;

3.2.3 Experimental Procedures

The experimental procedures of this study were approved by the Massey University Animal Ethics Committee in compliance with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

3.2.3.1 Feeding schedule

The feeding schedule is presented in Table 3.4. From day 1 to day 4 of the experiment, each piglet received a daily allocation of 600 grams of a commercial weaner diet given twice (0900h and 1600h) daily to minimize changes from feed transition and to improve the adaptation process. From day 5 to day 19, piglets were fed with corresponding casein-based experimental diets. Each piglet received a daily feed allowance of 600 grams, given twice daily, for the first four days. Feed increments of 50grams were allocated every four days thereafter. The refusals were collected, weighed and recorded before new feeds were given. On day 20, the piglets were fed with enzymatically hydrolysed casein-based diets

given twice daily at 0900 and 1700 hour. This diet substitution was done to allow the measurement of endogenous losses, as well as keeping the experimental cost down. Piglets fed with CAS 1, CAS II, CAS II, CAS IV, and CAS V experimental diets were switched to EHC 1, EHC II, EHC II, EHC IV, and EHC V experimental diets, respectively. From day 21 to day 25, the piglets were fed five times a day (0800h, 1000h, 1200h, 1400h, 1600h) to accustom piglets to regular feeding and to ensure an adequate amount of digesta was present in the intestine to obtain a representative sample. The feed refusals were collected once a day (before 0800h feeding) when pigs received five hourly feeding.

Table 3.3 **The dry matter (DM) content (g/100g), nitrogen (g/100), amino acid (g/100g) and NSP (g/100g) composition of experimental diets on as is basis. Results of the chemical analysis.**

	Control	4%AX	7.5% AX	4% βG	7.5% βG
Dry matter	90.02	90.86	91.29	90.81	90.45
Nitrogen content	2.21	2.40	2.40	2.33	2.43
Amino acids					
Aspartic acid	0.951	0.979	0.981	1.011	1.011
Threonine	0.530	0.578	0.597	0.628	0.563
Serine	0.631	0.618	0.682	0.639	0.663
Glutamic acid	2.757	2.769	2.943	2.808	2.859
Proline	1.331	1.333	1.402	1.363	1.352
Glycine	0.237	0.255	0.282	0.259	0.271
Alanine	0.386	0.400	0.433	0.415	0.423
Valine	0.824	0.816	0.854	0.838	0.854
Isoleucine	0.660	0.677	0.702	0.683	0.678
Leucine	1.190	1.207	1.247	1.217	1.236
Tyrosine	0.330	0.335	0.359	0.350	0.349
Phenylalanine	0.608	0.615	0.640	0.633	0.626
Histidine	0.348	0.351	0.385	0.373	0.386
Lysine	1.051	1.004	1.067	1.031	1.023
Arginine	0.447	0.449	0.480	0.484	0.485
Total	12.279	12.386	13.055	12.733	12.778
NSP					
Insoluble	7.83	1.37	-	1.5	-
Soluble	0.71	5.33	4.0	3.2	4.7
Total	8.09	6.7	4.0	4.7	4.7

AX- arabinoxylan; βG- β-glucan; NSP- Non-starch polysaccharides

3.2.3.2 Collection of blood samples, organ weights, and digesta

On day 5 of the trial just before starting the casein-based diets, piglets were anaesthetised using halothane/oxygen (Fluothane, Imperial Chemical Industries Ltd, Cheshire, England).

From each piglet, a 10 ml blood sample was withdrawn from the jugular vein into a plain vacutainer. The blood samples were processed into serum and stored at –85°C for further analysis. Animals were weighed after blood samples were taken. On the 25th day, feed was withdrawn at 1700 hours and piglets were fasted overnight. With the same procedure for blood collection, a second 10ml blood sample were withdrawn into plain vacutainer, processed and stored at –85°C for further analysis. Individual piglets were then given five one-hourly feeds. Thirty minutes after the last of the five hourly diets was given, the piglets were weighed, again anaesthetised and blood samples were collected from the jugular vein. The piglets were then euthanased by intracardiac injection of 10ml sodium pentobarbitone (Pentobarb 300- P.A.R. Class II Chemstock Animal Health, Ltd, Christchurch, New Zealand), weighed, and the gastro-intestinal organs were exposed. The gastro-intestinal tracts were apportioned into stomach, small intestine, large intestine, caecum and colon, and were weighed full. The small intestine was divided in three equal anatomical parts and labelled as S1 (duodenum), S2 (jejunum), and S3 (ileum). The total digesta content of each part was collected, weighed and frozen to –20°C for consequent titanium oxide determination and digestibility analysis. Digesta samples were also taken from the stomach, caecum and colon, and their weights were recorded before the samples were frozen. The gastro-intestinal tissues were emptied and weighed. The organs like heart, liver (gall bladder removed), lungs, kidneys and spleen were removed, blotted dry and their corresponding weights were recorded. Digesta samples from stomach, small intestines, colon and caecum were frozen, freeze-dried and ground to pass a 5mm sieve.

Table 3.4. **Diet and amount of feed offered during trial.**

Day of Trial	Diet	Daily Amount Offered	Number of feeding/ day
1 – 4	Farm	600	2
5 – 9	CAS	600	2
10 – 14	CAS	650	2
15 – 19	CAS	700	2
20	EHC	750	2
21 – 23	EHC	750	5
24 - 26	EHC	800	5

The daily feed intake (DFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated. The blood samples collected were prepared and assayed for total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). The contents of small intestine, particularly from S3 digesta, was

analysed for N digestibilities and endogenous nitrogen and amino acid losses, while S2 portion was analysed for crude mucin content.

3.2.4 Chemical and Analytical Procedures

3.2.4.1 Processing of digesta samples for endogenous losses determination

To remove any undigested amino acids that originate from diet, 1.5g freeze-dried digesta samples were placed in 50ml centrifuge tube, which were then added with 20ml distilled water. The ileal digesta samples were further added with 3 drops hydrochloric acid, and were homogenized for 1 minute using the vortex mixer. The samples were then stored at refrigeration temperature (0-10°C) overnight before being centrifuged at 7740 rpm (rotor no. 876) for 20 minutes at 0°C. The supernatant was then decanted into a separate container and the precipitate was washed with 5-10 ml deionised water, and was homogenized for one minute before being centrifuged again for 20 minutes at 7740 rpm at 0°C. The second supernatant was gently poured off and added to the first supernatant. The mixed supernatant was ultra-filtered using the molecular weight cut-off filters in centrepep-10 concentrators (Amicon, W.R. Grace Company, Denver, Massachusetts, U.S.A) by centrifuging the ultrafiltration tube at 3000 rpm at 4°C for 45 minutes. The ultra-filtration process was repeated as needed, at least five times. Following ultra-filtration, the high molecular weight fraction (MW > 10,000 Daltons) was added to the precipitate (from centrifugation) and the samples were frozen, freeze-dried and ground finely to pass a 0.5mm sieve before being analysed for titanium, nitrogen and amino acid contents.

3.2.4.2 Nitrogen, titanium and dry matter analyses

Freeze-dried ileal digesta samples, together with the representative test diets, were analysed for nitrogen content in a LECO FP-2000 analyser (LECO Corporation, 3000 Lakeview Ave. St. Joseph 49085-2396 USA) using the Dumas process (Granger, 1997). The dry matter (Harris, 1970) and titanium oxide contents were determined according to the methods of Short *et al.* (1996) by the Nutrition Laboratory, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.

3.2.4.3 Amino acid analysis

Amino acid analysis was conducted according to the procedures provided by the Nutrition Laboratory, Institute of Food Nutrition and Human Health. Amino acids were determined following hydrolysis of duplicate samples (5-7mg) in 1ml of 6mol/L glass distilled hydrochloric acid (HCl) containing 0.1% phenol in glass tubes sealed under vacuum, for

24 hours at 110 ± 2°C. Amino acid concentrations were measured using a Waters ion exchange HPLC system calibrated against a reference amino acid mixture of known concentration. The peaks of the chromatogram were integrated using the dedicated software Maxima 820 (Waters, Millipore, Milford, MA), which identify the amino acid by retention time against a reference amino acid mixture. Norleucine and Lysozyme were used as internal and external standards, respectively, and the weights of each amino acid were calculated using free amino acid molecular weights. No corrections were made for losses of amino acid during hydrolysis. Cysteine and methionine are destroyed during acid hydrolysis, so were determined by oxidation of duplicate samples (3-4mg) with 1ml performic acid (1part 30% H2O2 to 9 parts 88% formic acid) for 16 hours at 0^0C. The samples were then neutralised with 0.15 ml of 50% (w/w) Hydrogen Bromide (HBr) prior to acid hydrolysis. Tryptophan, which is also destroyed during acid hydrolysis, was not determined.

The endogenous flow were then calculated as follows:

Endogenous nitrogen flow (ENF)

= Nitrogen in endogenous losses x (TiO_2 in diet / TiO_2 in endogenous losses)

Endogenous amino acid flow (EAAF)

=Amino acid in endogenous losses x (TiO_2 in diet / TiO_2 in endogenous loses)

The nitrogen digestibility was the calculated as follows:

Apparent nitrogen digestibility (AND)

=[(N diet/ TiO_2 in diet) – (N digesta/ TiO_2 in digesta)] / (N diet/ TiO_2 in diet)

True nitrogen digestibility (TND)

= AND + [ENF / N diet]

3.2.4.4 Lipoprotein cholesterol concentration analysis

The level of blood metabolites was calculated by formulas of Friedewald *et al.* (1972) modified by Allan *et al.* (2000), whereas the lipoprotein concentrations were calculated by simple methods. The Friedewald formulas necessitate the evaluation of three parameters namely; the total serum cholesterol concentration, the serum triglyceride concentration and

the high-density lipoprotein (HDL) cholesterol concentration. Total cholesterol and triglyceride concentrations were determined by enzymatic methods. In human beings, HDL cholesterol concentrations are measured in the supernatant following precipitation of apolipoprotein-B containing lipoproteins (predominantly LDL and very low-density lipoprotein (VLDL)), or by direct assay (Allan *et al.*, 2000, Friedewald *et al.*, 1972).

The HDL cholesterol was determined by precipitating the ApoB-containing lipoproteins (LDL and VLDL) from 1 ml of serum at 4°C by the addition of 50:L of sodium heparin at a concentration of 4000 USP/ml and 100:L of 1 mol/L $MnCl_2$. Precipitation methods for determining the HDL content of pig sera have been previously validated (Allan *et al.*, 2000; Calvert and Scott, 1975; Fidge, 1972). After mixing, the sample was allowed to stand for 30 min at 4°C and then centrifuged at 15,000g for 5 min in a microcentrifuge. The supernatant was removed and its cholesterol concentration was determined by the automated enzymatic method described above using a cholesterol reagent modified by the further addition of EDTA to a final concentration of 8 mmol/L (Allan *et al.*, 2000).

The serum triglyceride concentration was determined by the enzymatic method of Roche (formerly Boehringer Mannheim) using lipase, glycerol kinase, glycerol phosphate oxidase and peroxidase. LDL-Cholesterol and HDL-Cholesterol were determined by applying a modification of the Friedewald formula (Allan *et al.*, 2000) using the equation:

[LDL-C] = [Total cholesterol] - [HDL-C] – ([Triglycerides] x 0.25)

where: [LDL-C], low-density lipoprotein cholesterol concentration; [HDL-C], high-density lipoprotein cholesterol concentration

The triglycerides concentration (Triglycerides) multiplied by 0.25 provided an estimate of the very low-density lipoprotein cholesterol concentration (VLDL-C).

The results for all blood metabolites analyses (Total cholesterol, Serum triglycerides, High-density lipoproteins and Low-density lipoproteins) were expressed in mmol/L.

3.2.4.5 Analysis for crude mucin content

The five EHC-based diets and ground S2 digesta samples were analysed for crude mucin content according to procedures modified by Lien *et al.* (1997); Allen (1981). Into a 50ml polystyrene test tube that contained approximately 3 grams of fresh diet or digesta sample, a pre-cooled 25ml of 0.15M sodium chloride containing 0.02M sodium azide were added. The samples were homogenised for 1 minute using vortex mixer before being centrifuged

at 12000g, 4°C for 30 minutes. The aqueous layer was decanted into another labelled 50ml polystyrene test tube. The decanted layer was centrifuged as before to complete the removal of insoluble material. A 15ml of the aqueous fraction was pipetted to a pre-weighed test tube previously cooled in an ice bath. The solution was dispersed for 1 minute using a vortex mixer and 25.7ml of ice-cooled ethanol (99%) was added to a final concentration of 60% by volume. The samples were allowed to precipitate overnight at –20°C before being centrifuged at 1,400g, at 4°C for 10 minutes. The precipitate was recovered by decanting the supernatant and was solubilized in 15ml of 0.15M sodium chloride. The solution was then dispersed before a 25.7ml of pre-cooled ethanol (99%) was added to a final concentration of 60% by volume. The samples were allowed to precipitate overnight before being re-centrifuged at 1400g, 4°C for 10 minutes. The precipitate was recovered and rinsing procedures were continued until a clear supernatant was obtained. The final residue was dissolved in 10ml RO water, freeze-dried, weighed and analysed for nitrogen content.

3.2.5 Statistical Analysis

Data for blood metabolites were analysed using the GLM procedure of SAS version 6.12 (SAS 1997), using the repeat measure model.

$$Y_{jkl} = \mu + D_j + P_k(Dj) + T_l + D_j \times T_l + + E_{jkl}$$

Where: Y_{jkl} = dependent variable; μ = general mean; D_j = the fixed effect of jth diet treatment; $P_k(D_j)$ = the random effect of the k^{th} pig within the j^{th} diet treatment; T_l = the fixed effect of l^{th} time of sample; $D_j \times T_l$ = interaction between diet and time of sampling; E_{ijk} = residual error.

The model permits fixed effects of diet, fixed effects of different collection time, and their possible interactions, random effect of pigs within the treatments to be assessed, the level of significance was set at α=0.05. Significant differences between means were determined through the least significant difference (LSD) method from GLM procedure of SAS (SAS Institute 1996). No difference between sexes were observed.

Data for growth performance were analysed in the GLM procedure of SAS using the following model:

$$Y_{ijk} = \mu + L_i + D_j + Eijk$$

where: Y_{ijk} = dependent variable; μ = general mean; L_i = the fixed effect of litter of origin; D_j = the fixed effect of diet; E_{ijk} = residual error

Data for organ weights were analysed with GLM procedure of SAS with empty body weight as covariant factor. The model is:

$$Y_{ijk} = \mu + b\ (x_{ijk}-X) + L_j + D_k + E_{ijk}$$

where: Y_{ijk} = dependent variable; μ = general mean; $b\ (x_{ijk}-X)$ = empty body weight as covariate; L_j = the fixed effect of litter of origin; D_k = the variable effect of diet; E_{ijk} = residual error

Data for mucin content were analysed in the GLM procedure of SAS using the following model:

$$Y_{ijk} = \mu + L_i + D_j + Eijk$$

where: Y_{ijk} = dependent variable; μ = general mean; L_i = the fixed effect of litter of origin; D_j = the fixed effect of diet; E_{ijk} = residual error

Data for endogenous nitrogen and amino acid losses were analysed in the GLM procedure of SAS using the following model:

$$Y_{ijk} = \mu + L_i + D_j + Eijk$$

where: Y_{ijk} = dependent variable; μ = general mean; L_i = the fixed effect of litter of origin; D_j = the fixed effect of diet; E_{ijk} = residual error

3.3 Results

Two piglets were excluded from samplings of blood metabolites and organ weights due to death. One animal was not included for evaluation of growth parameters because of its poor performance (average daily gain during the trial = 46 g/d)

3.3.1 Blood Metabolites

The significant level of the effect of litter, diet, piglets and time of sampling on blood metabolites are given in Table 3.5. The mean values of blood metabolites collected over three different periods are presented in Table 3.6.

The mean blood metabolites values for total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) taken from fasted pigs before start of feeding treatment diets (day 0) were similar ($P<0.05$) indicating comparable basal levels of blood metabolites. The basal levels for TC, TG, HDL and LDL ranged from 2.18 to 2.32 mmol/L, 0.36 to 0.56 mmol/L, 0.88 to 0.94 mmol/L and 1.12 to 1.16 mmol/L, respectively. The consumption of different levels of arabinoxylan and β-glucan did not have significant effect ($P>0.05$) on blood levels of TC, TG, HDL and LDL measured on day 21 fasted, with blood metabolites values ranging from 3.16 to 3.36mmol/L, 0.43 to 0.51mmol/L, 1.54 to 1.68mmol/L and 1.36 to 1.52mmol/L for TC, TG, HDL and LDL, respectively. Similarly, dietary inclusion of arabinoxylan and β-glucan had no significant depressant effects ($P>0.05$) on the levels of TC, TG, HDL and LDL measured on day 21 fed state (post-prandial; 2hours after the last feeding). The values of blood metabolites, in the five treatment diets, measured post-prandial ranged from 2.95 to 3.18mmol/L, 0.37 to 0.54mmol/L, 1.41 to 1.65mmol/L and 1.16 to 1.48mmol/L for TC, TG, HDL and LDL, respectively.

Table 3.5 **Significance level for blood metabolites**

	Diet	Pig (Diet)	Time	Time x Diet	R^2	RSD
Cholesterol	NS	***	***	NS	0.90	0.261
Triglycerides	NS	**	NS	NS	0.62	0.12
High-density Lipoprotein	NS	***	***	NS	0.92	0.131
Low-density Lipoprotein	NS	***	***	NS	0.81	0.202

Furthermore, repeat measure analysis was used to illustrate changes in the levels of blood metabolites measured by comparing the three different periods of collection namely fasted basal values (taken on day 0) against day 21 fasted values, and fasted against post-prandial values (collected 2h after feeding) in blood samples collected on day 21 (see Table 3.5). Some blood lipids showed significant changes over time ($P<0.001$). In pigs fed different levels of arabinoxylan and β-glucan, the levels of TC, HDL and LDL, measured after on day 21 fasted, were significantly increased ($P<0.001$), as compared to fasted basal values (see Table 3.6). There were no significant differences ($P>0.05$) for TG values for the two periods being compared. Moreover, when blood metabolites values from fed and fasted state measured on 21st day were compared, TC values were significantly higher ($P<0.001$) in fasted state as compared to fed state, except for 7.5% β-glucan. HDL values were relatively higher in fasted state as compared to fed state, but significant differences ($P>0.05$) were observed only in 7.5% arabinoxylan and mixed NSP (4% BG and 3.5%

cellulose). LDL values were similar in the two periods (P>0.05), except for mixed NSP diet (4%BG and 3.5% cellulose), although levels of the samples taken on fasted state tended to be higher than fed state. TG values were similar (P>0.05) between the two periods.

Table 3.6 **Effects of arabinoxylan (AX) and ß-glucan (BG) fed to weaner pigs on levels of blood metabolites measured during day 0 fasted, day 21 fasted and day 21 fed (post-prandial) state.**

	TREATMENT					
	Control	**4%AX**	**7.5%AX**	**4%BG**	**7.5%BG**	**Pooled SE**
Cholesterol (mmol/L)						
Day 0 fasted	2.21^{a}	2.29^{a}	2.32^{a}	2.26^{a}	2.18^{a}	0.122
Day 21 fasted	3.36^{c}	3.23^{c}	3.31^{c}	3.16^{c}	3.23^{bc}	
Day 21 fed	3.18^{b}	3.03^{b}	3.12^{b}	2.95^{b}	3.16^{b}	
Triglycerides (mmol/L)						
Day 0 fasted	0.36	0.56	0.49	0.45	0.45	0.056
Day 21 fasted	0.49	0.46	0.45	0.51	0.43	
Day 21 fed	0.37	0.49	0.54	0.48	0.43	
HDL (mmol/L)						
Day 0 fasted	0.89^{a}	0.88^{a}	0.94^{a}	0.90^{a}	0.88^{a}	0.061
Day 21 fasted	1.61^{b}	1.68^{b}	1.55^{b}	1.54^{b}	1.55^{b}	
Day 21 fed	1.55^{bc}	1.65^{bc}	1.46^{c}	1.41^{c}	1.48^{bc}	
LDL (mmol/L)						
Day 0 fasted	1.16^{a}	1.16^{a}	1.15^{a}	1.16^{a}	1.12^{a}	0.094
Day 21 fasted	1.52^{ab}	1.36^{b}	1.55^{b}	1.38^{b}	1.50^{b}	
Day 21 fed	1.46^{bc}	1.16^{c}	1.43^{bc}	1.32^{bc}	1.48^{bc}	

^{a,b,c,}- values with different superscript within diet and blood metabolites are different from each other (LSD, P<0.05); HDL- high density lipoprotein; LDL- low density lipoprotein.

3.3.2 Organ weights

Organ weights, as influenced by different types of dietary fibres, were analysed with empty body weight as covariate. The result is presented in Table 3.7. The addition of 4% or 7% β-glucan or arabinoxylan to pig diet did not significantly influence (P>0.05) organ weights in weaner pigs, although organ weights for stomach, small intestine, lung and liver were relatively heavier in weaner pigs fed with highly soluble dietary fibres (7% of arabinoxylan or β-glucan). Significant litter differences (P<0.05) were also noted in organ weights of stomach, colon, small intestine, lung, kidneys and spleen suggesting maternal and genetic variations between litters.

Table 3.7. **Organ weights in weaner pigs fed different levels (4%, 7.5%) of arabinoxylan (AX) and β-glucan (BG).**

	T R E A T M E N T S								
Organs (g)	**Control**	**4%AX**	**7.5%AX**	**4%BG**	**7.5%BG**	**SEM**	**EBW**	**Litter**	**Diet**
N	4	5	4	5	5				
Stomach	115.2	126.73	127.2	135.7	140.4	115.3	*	*	NS
Caecum	41.4	49.0	48.7	43.0	44.0	3.3	**	NS	NS
Colon	234.4	220.5	184.6	215.7	246.5	21.5	**	NS	NS
Small intestine	370.0	396.4	360.5	394.6	430.8	6.12	***	NS	NS
Heart	97.8	104.9	102.3	96.8	97.0	7.5	*	NS	NS
Lung	231.86	235.2	238.3	225.0	233.3	13.9	**	*	NS
Liver	462.5	458.1	508.3	486.8	510.6	24.6	***	NS	NS
Kidney	105.7	112.7	102.3	103.8	103.3	4.5	***	**	NS
Spleen	58.0	54.7	59.4	70.9	58.3	7.3	NS	*	NS

Significantly different; *-(P<0.05);**- (P< 0.01); ***- (P<0.001); NS- not significant

3.3.3 Growth performance

The effect of dietary fibres in growth performance of weaner pigs is presented in Table 3.8. Inclusion of 7.5% arabinoxylan and β-glucan in diet fed to grower pigs significantly improved the average daily gain (ADG) (P<0.01) and feed conversion ratio (FCR) (P<0.05) as compared to pigs fed with control diet and 4% levels of NSP. The daily feed intake was similar in pigs fed with control and different levels and types of soluble NSP. An ADG of 373.7g and 331.0g was observed in pigs fed with 7% β-glucan and arabinoxylan as compared to 234.4g, 264.3g and 256.2g for pigs fed with control, 3% betaglucan or arabinoxylan, respectively. Similarly, a feed conversion ratio of 2.7kg and 2.3kg was markedly improved in pigs fed with 7.5% β-glucan and arabinoxylan, respectively. Further, gut fill was similar in pigs fed with control, arabinoxylan and 3% β-glucan, but was significantly increased when 7.5% β-glucan was included in the diet fed to pigs. However, feeding weaner pigs with different levels and types of soluble NSP did not produce significant improvements in liveweight and carcass weight at the end of the trial. Some litter effects in final liveweight (P<0.05), average daily gain (ADG) (P<0.05), and carcass weight (P<0.01) were observed, indicating genetic differences. Differences in FCR and growth rate were not due to difference in gut fill (ie. using gut fill as a covariate in the model did not remove the significant diet effect).

Table 3.8. **Influence of different levels of arabinoxylan (AX) and β-Glucan (BG) on carcass weights and growth parameters in weaner pigs.**

	T R E A T M E N T S							
	Control	**4%AX**	**7.5%AX**	**4%BG**	**7.5%BG**	**SEM**	**Litter**	**Diet**
N	3	5	4	5	5			
LW start (kg)	14.7	14.3.	14.6	14.1	14.7	0.58	NS	NS
LW end (kg)	18.3	18.1	19.6	18.1	20.3	0.87	*	NS
Carcass weight (kg)	15.5	14.6	15.8	14.7	16.0	0.61	**	NS
DFI (g/d)	772.4	779.2	841. 9	786.3	832.2	36.12	NS	NS
FCR (g/g)	3.4^{a}	3.1^{ab}	2.7^{bc}	3.0^{ab}	2.3^{c}	0.21	NS	*
Daily gain (g)	234.4^{c}	256.2^{c}	331.0^{ab}	264.3^{bc}	373.0^{a}	29.08	*	**
Gutfill (g)	518.4^{b}	687.2^{b}	675.9^{b}	512.8^{b}	1045.8^{a}	123.07	NS	**
% Gutfill	2.8^{b}	3.8^{ab}	3.3^{b}	2.9^{b}	5.1^{a}	0.58	NS	**

^{abc}- rows with different letter superscript are significantly different; *-(P<0.05);**- (P< 0.01); NS- not significant; LW- liveweight; DFI-daily feed intake; FCR-feed conversion ratio.

3.3.4 Mucin content

The results on the influence of different types and levels of dietary fibre on crude mucin (CM) secretion in weaner pigs are presented in Table 3.9. The ileal crude mucin content, expressed in mg/g digesta DM, was not influenced by addition of β-glucan but was significantly increased (P<0.01) with dietary inclusion of 4% and 7.5% arabinoxylan (42.0 mg/g digesta DM and 61.7mg/g digesta DM for 4% and 7.5% arabinoxylan, respectively), as compared to control (22.9mg/g digesta DM). There were 83% and 169% increase in CM secretion associated to 4% and 7.5% inclusion level of arabinoxylan compared to control diet. The nitrogen content in crude mucin was also determined, however, the crude mucin nitrogen content was similar in pigs fed with diet supplemented with different types and levels of soluble NSP (P>0.05). Further, the types of soluble NSP influenced ileal flow of crude mucin. The ileal crude mucin flows, expressed in mg/g DMI, were significantly increased (P<0.05) with the dietary inclusion of 4% and 7.5% arabinoxylan (12.2 mg/gDMI and 16 mg/gDMI), but were similar in pigs fed with control (12.2 mg/g DMI) and β-glucan supplemented diets (12.2 mg/g DMI and 10 mg/g DMI for 4% and 7.5% β-glucan levels, respectively). The flow of nitrogen in crude mucin was similar in pigs fed diets with different levels of betaglucan or arabinoxylan. Furthermore, significant litter

differences were observed in all parameters measured suggesting genetic or maternal effect.

Table 3.9. **Influence of different levels (4% and 7.5%) arabinoxylan (AX) and β-glucan (βG) on crude mucin (CM) secretion in weaner pigs.**

	T R E A T M E N T S							
	Control	**4%AX**	**7.5%AX**	**4%BG**	**7.5%BG**	**SEM**	**Litter**	**Diet**
Crude Mucin (mg/g dig DM)	22.9[a]	42.0[b]	61.7[c]	32.5[a]	23.8[a]	3.48	*	**
CM Nitrogen (mgN/g dig DM)	1.16	1.13	1.61	1.84	1.0	0.86	*	NS
CM Flow (mg/g DMI)	12.2[a]	16.0[b]	24.4[c]	12.2[a]	10.0[a]	2.38	*	*
CM Nitrogen Flow (mg/g DMI)	0.634	0.447	0.650	0.692	0.428	0.59	*	NS

Means with different superscript are significantly different, *-(P<0.05); **-(P<0.01); NS- not significant; mg/g dig DM- mg/g digesta dry matter; mgN/g dig DM- mg Nitrogen/g digesta dry matter.

3.3.5 Endogenous nitrogen and amino acid losses

The influence of soluble NSP on protein digestibilities and endogenous nitrogen and amino acid flows in the ileum is shown in Table 3.10. Apparent nitrogen digestibility (AND) and true nitrogen digestibility (TND) were similar (P>0.05) in pigs fed with different types and levels of soluble NSP. The lack of significant differences in AND and TND can be ascribed to high between animal variation. Apparent nitrogen digestibility ranged from 73.44% to 80.95%. Pigs fed with the mixed soluble and insoluble NSP (4%BG and 3.5% cellulose) diet had the lowest value and pigs fed the control diet had the highest value (80.95%). TND values ranged from 89.14% to 90.7% in pigs fed different levels of arabinoxylan and β-glucan.

Endogenous nitrogen flow was significantly increased (P<0.05) with dietary inclusion of NSP. Significant numerical increase (P<0.05) was observed in endogenous nitrogen flow associated with increased dietary levels of AX. When comparing the purified levels, endogenous flow of nitrogen was significantly higher in dietary inclusion of 7.5% AX, intermediate in 7.5% BG and lowest in control diets (P<0.05). However, ileal endogenous nitrogen flow was not influenced by dietary inclusion of β-glucan (P>0.05). Dietary inclusion of mixed NSP (4% BG and 3.5% cellulose) and 7.5% arabinoxylan resulted to significantly hgher endogenous nitrogen flow (4.03 g/kg DMI and 3.75 g/kg DMI) than

those fed with control diet (2.27 g/ kg DMI). Inclusion of 7.5% BG, mixed NSP (4% AX and 3.5% cellulose) had similar endogenous nitrogen flow in pig fed control diet (P>0.05).

Table 3.10. **The influence of arabinoxylan (AX) and B-glucan (BG) on Apparent nitrogen digestibility (AND), True nitrogen digestibility (TND), ileal endogenous nitrogen flow (ENF, g/kg DMI), and ileal flow of amino acid (g/kg DMI) in weaner pig.**

	T R E A T M E N T S						
Digestibilities	**Control**	**4%AX**	**7.5%AX**	**4%BG**	**7.5%BG**	**SE**	**DIET**
APN (%)	80.95	80.90	74.13	73.44	78.81	2.6	NS
TND (%)	90.22	90.70	88.36	89.14	89.21	1.3	NS
Flow (g/kg DMI)							
Nitrogen flow	2.27^{c}	2.58^{bc}	3.75^{ab}	4.03^{a}	2.79^{bc}	0.37	*
Aspartic acid	0.90^{c}	0.99^{c}	1.53^{ab}	1.51^{a}	1.11^{bc}	0.144	*
Threonine	0.80^{b}	0.92^{b}	1.27^{a}	1.32^{a}	0.85^{b}	0.118	*
Serine	0.66c	0.79bc	1.18a	1.10ab	0.78c	0.105	*
Glutamic Acid	1.86^{b}	1.82^{b}	3.27^{a}	2.20^{b}	2.21^{b}	0.257	*
Proline	0.83^{b}	0.92^{b}	1.73^{a}	1.25^{ab}	0.90^{b}	0.168	*
Glycine	1.47^{bc}	1.60^{ab}	2.05^{ac}	2.21^{a}	1.28^{b}	0.227	*
Alanine	0.48c	0.58c	0.89ab	0.90a	0.63bc	0.087	*
Valine	0.59^{b}	0.65^{b}	0.92^{ab}	1.03^{a}	0.67^{b}	0.101	*
Isoleucine	0.35^{c}	0.43^{bc}	0.65^{a}	0.61^{ab}	0.48^{ac}	0.065	*
Leucine	0.60^{c}	0.66^{c}	0.99^{ab}	1.61^{a}	0.70^{bc}	0.128	*
Tyrosine	0.27^{c}	0.30^{bc}	0.48^{ab}	0.55^{a}	0.32^{bc}	0.061	*
Phenylalanine	0.26^{b}	0.29^{b}	0.43^{ab}	0.56^{a}	0.32^{b}	0.061	*
Histidine	0.27^{b}	0.32^{b}	0.50^{a}	0.47^{a}	0.32^{b}	0.039	*
Lysine	0.28^{b}	0.40^{b}	0.65^{a}	0.64^{a}	0.44^{ab}	0.073	*
Arginine	0.30^{b}	0.36^{b}	0.55^{ab}	0.69^{a}	0.42^{b}	0.082	*
Amino acid (total)	9.91^{b}	11.04^{b}	17.09^{a}	16.20^{a}	11.43^{b}	1.501	*

^{abc}- rows with different letter superscript are significantly different; *-(P<0.05);

Endogenous ileal amino acid flow determined through EHC methods were significantly higher (P<0.05) in pigs fed diet containing 7.5% AX and mixed NSP (4%BG and 3.5% cellulose). The endogenous flows of all amino acid, except glycine, valine, phenylalanine and arginine was significantly greater in pigs fed with 7.5% AX compared to pigs fed with control diets. Surprisingly, the endogenous flow of aspartic, threonine, serine, glycine, alanine valine, leucine, tyrosine, phynelalanine histidine and arginine were significantly higher (P<0.05) in pigs fed with mixed NSP (4% BG and 3.5% cellulose), as compared

pigs fed with 7.5% BG and control diets. Endogenous amino acid flows were comparable (P>0.05) in pigs fed with 7.5% AX and mixed NSP (4%BG and 3.5% cellulose), except for glutamic acid. Comparing the three pure NSP levels, greater endogenous amino acid flows were observed in pigs fed with 7.5% AX, intermediate flow for 7.5% BG and lowest for pigs fed with control diets. It is also surprising that the endogenous amino acid flow were significantly higher in pigs fed mixed NSP (4%BG and 3.5% cellulose) as compared to pigs fed 7.5% BG, in majority of the amino acids examined. The difference in the secretion of endogenous amino acids was also manifested in the total endogenous amino acid flow. Feeding 7.5% AX had significantly higher (P<0.05) endogenous total amino acid flows (17.09 g/kg DMI) than those fed 7.5% BG (11.43 g/kg DMI) and control diets (9.91 g/kg DMI). Total amino acid flow was increased with increasing levels of AX, but no trend was observed in β-glucan inclusion.

3.4 Discussion and Conclusions

3.4.1 Blood metabolites

Allan *et al.* (2000) clearly validated the methodology for determining blood lipids from porcine serum. HDL cholesterol concentration was determined using precipitation methods, while LDL was determined by modification of Friedewald formula. The resulting formula closely approximate the values obtained from ultracentrifugation.

The absence of significant differences in the basal levels of total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) values in pigs assigned to different treatments indicate uniformity and homogeneity of basal levels. Inclusion of arabinoxylan and β-glucan in diet fed to pigs did not demonstrate significant differences (P>0.05) in TC, TG, HDL and LDL values measured during fasting and during post-prandial, after 21 days consumption of experimental diets. This result is in contrast to the hypocholesterolemic effect of β-glucan and other soluble fibres, which have been demonstrated in different models, like rats, chickens, pigs, and even humans. In human studies, dietary fibre supplementation (composed of 75% soluble fibre and 25% insoluble fibre) given in 10g and 20g dosages showed decrease in TC and LDL/HDL ratios compared to placebo groups over a 15–week trial (Hunninghake *et al.*, 1994). McIntosh *et al.* (1991) demonstrated that β-glucan lowered serum TC and LDL cholesterol in hypercholesterolemic men as compared to wheat food given for 11 weeks. Klopfenstein (1988), in her review in the role of β-glucan in nutrition and health, showed rats offered with bread rich in β-glucan for 35 days had lower TC that rat fed control bread. In the same

study, a slightly greater fall was observed with LDL cholesterol, which is a major risk factor lipoprotein for coronary disease. Newman *et al.* (1989) showed that β-glucan from barley caused significant reductions in both TC and LDL cholesterol. Anderson *et al.* (1993) reported depressed serum cholesterol in rats fed psylium, oat gum guar gum and pectin (all rich in soluble fibre) after 3-week feeding. Maqueda (1999) indicated that β-glucan extract depressed TG levels in rats fed diet added with coconut oil, which has hypercholesterolemic actions (McNamara, 1992). All of these studies contrast the result of the current trial. However, Pond *et al.* (1981) showed that serum cholesterol was not influenced by inclusion of a 20% alfalfa in diets fed to pigs. Hecker *et al.* (1998) revealed that in rats fed with β-glucan tortillas for 25 days, TC levels were similar to control group. These results conform with the output of the current trial.

There are various mechanisms proposed on the way β-glucan promotes hypocholesterolemic effects. The major theories relate to modification of lipid absorption and bile acid sequestration, both consequently lead to reduction of cholesterol absorption. It is well known that increased viscosity, as enhanced by soluble fibre, influenced lipid absorption (Newman *et al.*, 1989 and 1992). Viscous NSP delays gastric emptying and small bowel transit, traps water in the gel matrix, and impairs absorption of nutrients by reducing mixing movements and convective diffusion (Spiller, 1996). Further, AACC report (2001) indicated that high molecular weigh β-glucan is more effective than low molecular weight β-glucan in increasing bile acid excretion. However, Topping (1991), in his review suggested that results on hypocholesterolemic effects of NSP due to increased level of dietary fibre are equivocal. Though the majority showed cholesterol-lowering effects, other trials in rats involving NSP of different viscosities showed absence of significant differences in reduction of cholesterol levels. Thus, hypocholesterolemic effect and depressed fat absorption associated with increased viscosity involved a complex process, and none of these is mutually exclusive in offering explanation for the mechanism in individual food (Jenkins *et al.*, 1996). The other mechanism proposed involved gel formation. Fibre gelation can alter resistance of unstirred water layer resulting to impede nutrient flux and absorption. It can also disturb micelle formation in the small intestine (Rieckhoff *et al.*, 1998; Cassidy and Calvert, 1993). This factor delayed lipid absorption and excretion of other nutrients. Maqueda (1999) concluded that the soft gel formation, not increase in viscosity, of β-glucan extract, when mixed in water, was responsible for the reduction of TC and TG in rats.

In this trial, the absence of significant differences in levels of TC, TG, HDL and LDL in pigs fed with different levels of arabinoxylan and β-glucan can be partly explained by the duration of feeding the experimental diets. The significant hypocholesterolemic effects were observed in humans after consuming experimental diets up to 12 weeks, while in rats up to 35 days. In this trial, experimental diets were offered for only 21 days. Thus, it can be questioned if the time period was too short to measure stimulatory effect of dietary fibre on lowering blood lipids. Second, β-glucan and pentosans were extremely degraded in the intestinal tract of pigs (Graham *et al.*, 1989; Thacker, 2000), as compared to poultry (Maqueda, 1999). Thus, the hypocholesterolemic property can be partially neutralised due to high degradation and solubilisation of NSP. The high degradation rate of β-glucan and pentosans can be shown by the increased growth rate and feed conversion efficiency in this trial. Thus, cholesterol-lowering property can be partly inhibited. Another possible explanation for lack of dietary effects of blood metabolites levels is that the concentration of dietary fibre was not sufficient to induce hypocholesterolemia in pigs, and the age of the experimental animal itself. Allan *et al.*, (2000 and 2001) observed significant changes in blood metabolites level in 8-week old pigs fed commercial diets or supplemented with different cholesterol sources. In contrast, the current trial used 3-week old pigs fed experimental diets for 3 weeks, which pehaps explained the lack of significant responses in blood metabolites levels.

The levels of TC, HDL and LDL were significantly increased ($P<0.05$) over time (21 days after). No significant effects in TG levels were observed after 21 days. Furthermore, when the 21st day fasted and fed metabolites values were compared, it was shown that feeding significantly depressed TC levels. HDL levels were also depressed in 21st day fed state, however, significant lower levels ($P<0.05$) were noticed only in dietary inclusion of 7.5% AX, and 4% BG and 3.5% cellulose combination. On the other hand, LDL levels were similar in the two periods being compared, except for dietary inclusion of mixed NSP (4%AX and 3.5% cellulose), which had significantly higher fasted level as compared to fed level. No significant difference was observed for TG values in the two periods compared.

The lack of significant depressant effects on TG levels observed in different periods can be related to the inefficiency in lowering either the chylomicrons or the triglyceride-rich lipoprotein (TRL) triglycerides response after a test diet, such that dietary fibre may be unable to alter the digestion and absorption process of dietary fat present in coconut oil. This finding agreed to the finding of Anderson and Clark (1986) that long-term studies in

human showed that various soluble fibres hardly modify fasting serum triglycerides. Only when large amount of dietary fibre and low amount of dietary fat are fed for a long period, some effects can be observed in laboratory animals (Judd and Truswell, 1985; Mazur *et al.*, 1990) and in humans (Cara *et al.*, 1992). Further, a hypocholesterolemic effect of dietary fibre is not always accompanied by significant changes on blood metabolites in serum or liver. AACC Report (2001) showed that fibre feeding reduced total body cholesterol, even when a significant reduction of serum cholesterol did not occur. This may be the case of the absence of significant changes of blood metabolites fed different levels and types of dietary fibre measured during day 21 fasted and day 21 fed state.

Significant increases in fasted levels of TC, HDL and LDL after 21 days feeding indicate that pigs obtained cholesterol from dietary origin and from increased synthesis (liver, adipose tissue, intestine and central nervous system) than body catabolism. The resultant levels of blood metabolites are a measure of the interplay between supply by the liver and demands for their lipid constituents by the tissue (Kingman *et al.*, 1993). In humans, a balance is reached by adjustment of endogenous synthesis to the dietary cholesterol intake (Mersmann and Pond, 2001).

Feeding significantly reduced TC in this trial. This was in agreement with the results of Allan *et al.* (2000) and Baetz and Mengeling (1971). Cara *et al.* (1992) mentioned that ingesting a meal containing 750mg cholesterol with different fibre sources decreased cholesterolemia for 6h compared to fasting values, and thus fibre sources had cholesterol-lowering effect to a variable extent. The said effect involved several complementing mechanisms like a signal based on the amount of cholesterol, of intestinal origin, enters into the liver via the bloodstream is enough to quickly reduce (within 1h) the endogenous synthesis of cholesterol, which lowers post-prandial cholesterolemia early after meal intake. This resulted from the inhibition of the activity of the key enzyme, HMG CoA reductase, involved in the cholesterol synthesis as shown in laboratory animals (Brown and Goldstein, 1983). Moreover, oat bran, which contain high amount of soluble fibre, displayed a hypocholesterolemic effect after chronic intake, and it also exhibited the most marked influence of post-prandial cholesterolemia (Cara *et al.*, 1992). The result of this trial is in contrast to that of Luhman *et al.* (1992). These authors found out that feeding had no effect on TC and LDL values. The two trials differ in the types of feed offered to experimental animals. The current trial utilised highly soluble fibres as main factor in the diet, while the latter used high fat diet. This may justify the differences in the response of TC and LDL levels on the two trials.

In the current trial, fasting increased HDL level as compared to fed values. However, significant increased levels were only observed in dietary inclusion of 7.5% AX, and 4% AX and 3.5% cellulose combination. Metabolism of chylomicron in the blood after a meal may change significantly the composition and metabolism of HDL. This may be an important physiological phenomenon of the body in the control of post-prandial lipemia. Chylomicron particles are thought to transfer phospholipids, apo A-I and A-II to HDL, and HDL in contrast, transfer cholesteryl ester and apo E and C to chylomicron remnants. It had been suggested that 5-10 hours after a meal might be required to observe major alteration in HDL composition. Thus, at least a 14h fast is suggested before a meal when observing post-prandial lipoprotein (Tall, 1986).

Further, in the current trial, 21st day fed and fasted LDL levels were similar ($P>0.05$), except for dietary inclusion of mixed NSP (4%AX and 3.5% cellulose). This is in contrast to the works of Allan *et al.* (2001) in pigs and Cohn *et al.* (1988) in humans. Cohn *et al.* (1988) revealed significant reductions in LDL cholesterol 3 and 6 hours after eating a fat-rich meal. This resulted in decreased plasma concentration of apolipoprotein B. The post-prandial reduction of LDL can also be theorised that the post-prandial rate of catabolism of LDL exceeded its synthesis. It should be noted that the work of Cohn *et al.* (1988) used a fat-rich meal, while the current trial used highly soluble fibre diets, which have proven attenuation factor on cholesterol-rich food (Cara *et al.*, 1992). This may partly explain the difference of the result of the current trial with that of the work of Cohn *et al.* (1988). The absence of significant differences in LDL levels between fasted and fed state can be related in the differences of metabolism of LDL between pigs and humans. Porcine plasma lacks cholesteryl ester transfer protein (CETP) activity and consequently, minute amount of HDL cholesteryl ester is transferred to the LDL fraction. Further, only a small fraction (11%) of VLDL apolipoprotein B in pigs is converted into LDL. It is worthwhile to suggest that much of the cholesterol within porcine LDL is synthesised de novo by the activity of lecithin:cholesterol acyl transferase (LCAT) rather than via catabolism of VLDL (Knipping *et al.*, 1987; Birchbauer *et al.*, 1992 cited in Allan *et al.*, 2001). This may also explain the minimal differences observed in LDL values between fasted and post-prandial state.

Therefore, inclusion of different levels of arabinoxylan and β-glucan did not have significant influence on levels of blood lipids (TC, TG, HDL and LDL) measured during fasting and during post-prandial state. Several support statements have been detailed somewhere that include short duration of feeding regimen, age of experimental animal,

types of diet fed to animals (high-fat or high-fibre) and characters of purified extract. This study showed changes over time in post-prandial and fasted TC, HDL and LDL values in pigs fed different types and levels of soluble dietary fibres.

3.4.2 Organ weights

In this study, dietary inclusion of different levels of arabinoxylan and β-glucans did not demonstrate significant effects in organ weight of weaner pigs. However, the weights of heart, liver, lungs, stomach, small intestine and colon were quantitatively increased with increasing levels of betaglucan, though no trend was observed in organ weights for pigs fed with arabinoxylan. The result of this study is in agreement with the experiment of Anugwa *et al.* (1989), who showed that total gastro-intestinal tract (GIT), stomach, small intestine, heart and spleen of pigs failed to manifest hypertrophic effect in response to feeding high fibre diets for 17 days. However, significant hypertrophic effects were observed in organ weights measured beyond 17 days. Studies conducted by Pond *et al.* (1981) also showed non-significant effect on weights of empty stomach, small intestine caecum and colon in pigs fed with 20% alfalfa meal, which contain high amount of soluble NSP. The higher mean organ weights in pigs fed with 7.5% β-glucan in this trial, although not statistically significant, agrees with what would have been expected of pigs offered with high fibre diet (Pond *et al.*, 1981).

Several studies showed opposite results to the current experiment suggesting profound effects of NSP on gut size and gut development. Jorgensen *et al.* (1996) showed that pigs, weighing between 45 and 120kg, fed with high dietary fibre from pea and pectins had heavier stomach, caecum and colon weights compared to pigs fed with low fibre diets. The work of Pond *et al.* (1988) indicated that the relative weights (percentage of live body weight) of liver, heart, empty stomach and small intestines, caecum and colon were heavier in young adult pigs fed high lucerne than in those fed low lucerne. Further, Pluske *et al.* (1998) mentioned the presence of positive linear relationship between the weight of the large intestine and daily intake of NSP and resistant starch in pigs weighing between 35 and 55 kilograms.

The changes in size and weights of the GIT and other organs, associated with tissue hypertrophy, is a response of the work load of the involved organs in drying, mixing, shaping, moving and expelling large quantities of undigested dietary residues. It is also noted that visceral organs have a high-energy expenditure relative to their size (Pluske *et al.*, 1999). The presence of high correlation between empty visceral organ weight,

associated with feeding high fibre diet, and fasting heat production may increase basal heat production and reduce energy and amino acid digestibility resulting to decline in feed efficiency (Pluske *et al.*, 1999; Varel and Yen, 1997; Pond, 1989).

The non-significant influence of soluble NSP on organ weights, as shown in this trial, can be explained by the duration of feeding experimental diets. Anugwa *et al.* (1989) observed significant changes in organ weights only after 35 days feeding, but not in 17 days. In the current trial, experimental diets were offered for 21 days, thus expected significant changes in organ weights were not achieved. The lack of hypertrophy of the visceral organs associated with feeding different levels of arabinoxylan and β-glucan can also be associated to the high rate of degradation of soluble NSP in pigs. According to Thacker (2000), β-glucans and pentosans are extensively degraded in the intestinal tract of pigs. Ninety percent of dietary β-glucans and a relatively lower degradation rate for arabinoxylan at 65% were degraded prior to the terminal ileum. Thus, the high rate of degradation for β-glucan and arabinoxylan can influence an increase in nutrient digestibility and availability for absorption. Further, in this trial, soluble NSP used were of purified maize arabinoxylan and barley β-glucan extracts, which may behave differently in inducing digesta viscosity in the manner native arabinoxylan and β-glucan found in grains do. β-glucan extract in solution forms soft gel, instead of viscous environment, which may neutralise other anti-nutritive components, thus, resulting into a high degradation rate. Therefore, the absence of considerable increase in organ weights or organ hypertrophy is indicative of efficient functional capacity of the digestive organ due to the high degradability of soluble NSP in pigs, and thus, the extra functional capacity exerted by digestive organs, as indicated by increase in weight or hypertrophy, to enhance mechanical digestion is not manifested.

3.4.3 Growth performance

Initial live weights were similar to pigs assigned to different dietary treatments. This signifies uniformity of weights across all treatments, at the start of the experiment. At the end of the trial, final liveweight, as well as, carcass weight were similar to pigs fed with different levels of arabinoxylan and β-glucan. The range of final liveweight and carcass weight in pigs were 18.1 kg to 20.3 kg and 14.6kg to 16.0 kg, respectively, across all dietary treatments. Feed intake was similar in pigs fed with diet containing different levels (4% and 7.5%) of arabinoxylan and β-glucan. Furthermore, dietary inclusion of 7.5% arabinoxylan or β-glucan significantly increased average daily gain ($P<0.01$) and improved feed conversion ratio ($P<0.05$) in pigs, as compared to pigs fed with control diet. Gut fill

or percentage gut fill (PGF) were similar to pigs fed with control, arabinoxylan and 4% β-glucan, but was significantly increased in pigs fed with 7.5% β-glucan.

The non-significant differences observed in feed intake of the current trial is expected because the pigs were in restricted feeding program and pig gut capacity was higher than the given feed allowance of 600g/d to 800g/d, which is equivalent to only 8-9% of metabolic liveweight ($LW^{0.75}$). This result is in contrast to the work of Choct and Cadogan (2001), who showed depressed feed intake in pigs fed with low quality wheat as compared to high quality wheat. Gill *et al.* (2000) mentioned that in weaner pigs fed with wheat, which was partially substituted with unmolassesed dried sugar beet pulp or completely replaced by barley, the negative effect was restricted in the first week of weaning where the low-density high fibre diet tended to restrict voluntary feed intake. However, the said effect was transient as piglets adapted to the diets over time. The above results proved the ability of the pigs to get accustomed to low quality diet given in relatively longer duration. The heavier gut fill observed in pigs fed with 7.5% β-glucan can be related to the gelling characteristic of β-glucan extract resulting to increased water retention capacity. This may also be an implication of increased bulkiness. It is well known that soluble NSP increase physical bulk of the digesta that increases the ability to hold large amount of water (Choct and Cadogan, 2001). The increase in gut fill could also mean an increased amount of undigested nutrients present in the gut of pigs fed with 7.5% β-glucan, making it relatively heavier.

The significant improvements observed in daily weight gain and feed conversion ratio associated with increased dietary level of arabinoxylan and β-glucan were relatively surprising and in contrast with the common trend that production parameters (growth rate and fed conversion ratio) are negatively related to intake of soluble NSP. In most studies, like that of Cadogan *et al.* (2000), they showed decreased daily gain in pigs fed with low quality wheat as compared to pigs fed with high quality wheat control diet. Yin *et al.* (2001) demonstrated that overall weight gain and feed conversion ratio were significantly depressed in pigs that received buck barley (low quality) than in pigs fed falcon barley (high quality). The two barley varieties differed in their β-glucan content, 59.5g/kg and 45.6 g/kg for buck and falcon barley, respectively. Further, viscosity was highest in the distal part of the small intestines, and in most cases, higher than in pigs fed buck barley, and that explained the depressed digestibility and growth performance observed in pigs fed buck barley.

In the current trial, the highest level of arabinoxylan and β-glucan was 75g/kg diet, which was comparably higher than what is present in buck barley. Further, the improved growth rate and FCR of the current trial is difficult to reconcile to that of the common trend and results from previous studies. However, the improved FCR and growth rate associated with increased level of NSP in the current trial can be related to the differences in degradability between purified maize arabinoxylan and β-glucan extract to that of native β-glucan found in buck barley. β-glucan extracts forms a soft gel, instead of viscous solution, which is formed in buck barley, thereby increasing nutrient degradation, digestion and utilisation. The increased degradation of β-glucans in the small intestine reduced the physical barrier created by its gel-forming properties, therefore, providing ileal environment suitable for ileal enzyme substrate interactions (Yin *et al.*, 2001), indicating improved digestibility of nutrients and improved feed utilisation and growth rate. Further, Partridge (2000 cited in Yin *et al*, 2001) mentioned that pig digesta viscosity is likely to remain relevant to performance but have lower order of importance than for broilers. Inborr *et al.* (1991) suggested that viscosity in the small intestine has not been proven to be a factor influencing the performance of barley-fed pigs.

Therefore, the current results suggest that, though purified maize arabinoxylan and barley β-glucan possessed some anti-nutritive properties, as shown in their soft-gelling property and increased gut fill, the highest level of inclusion were not sufficient to decrease efficiency of digestion (as indicated by similar organ weights) and depress production parameters. In fact, values in the latter were improved. This result may also suggest that the duration of the experiment was not long enough to inflict significant changes in organ weights.

3.4.4 Mucin and dietary fibre

Mucin is a high molecular weight ($(0.5 - 2.0) \times 10^2$ Da) glycoprotein substance that contains a large amount of carbohydrates connected by O-glycosidic bonds to an inner peptide backbone. The viscous property of mucus is believed to be derived from composition of mucin (Pestova *et al.*, 2000), and mucus is thought to play a major role that provides a protective lining for the gastro-intestinal tract against harsh gut environment and absorption of gut bacteria (Lien *et al.*, 2001; Pestova *et al.*, 2000). Despite the importance of mucin to gastro-intestinal health, there were scarce information available relating the effects of diet, particularly dietary fibre, on the quantity of intestinal secretion.

In this trial, dietary addition of 4% and 7.5% arabinoxylan significantly increased ileal crude mucin content by 83% and 169%, respectively. Several authors have suggested that dietary fibres elicit an increase in intestinal mucin secretion. Satchithanandam *et al.* (1990) demonstrated that inclusion of wheat bran in diet fed to rats increased the immuno-reactive mucin by 210% higher than rats fed with fibre-free diet. Lien *et al.* (1995 cited in Lien *et al.*, 2001) found out a linear increase in mucin output associated with increasing fibre intake in pigs fed with different levels of pea fibre. Schneeman *et al.* (1982) reported that inclusion of dietary wheat bran, an insoluble NSP, to diet fed in rats increased both sloughing of intestinal mucosal cells and mucus production. The above experiments suggest that the insoluble fibre components exert influence that resulted to increased mucin secretion. However, a controversy still existed as to whether water-insoluble fibre affects secretion of mucus (Leterme *et al.*, 1992).

Moreover, Cassidy *et al.* (1981) observed that mucus secretion was elevated by supplementing diets with soluble fibre. In their study, rats fed with diets supplemented with either 15% pectin or lucerne showed increased percentage of intestinal villi exhibiting structural deviations by about 300% compared to rats fed chow. It was shown that increased crypt cell production is part of the response of small intestine brought about by the damage due to consumption of fibre such as guar gum and pectin (Lien *et al.*, 2001). The presence of soluble fibre may cause damages in intestinal mucosa, which may respond by re-establishing both the mucus and epithelial layer (Lien *et al.*, 2001). This consequently leads to increased mucin secretions. Further, in the study of Pestova *et al.* (2000), they found the possibility of inclusion of small amount of fibre in the starter diets fed to pigs may be responsible in the increase of mucin after weaning, though the said trial was not meant to test such hypothesis. These data, together with the results of the previous experiments, give an indication of the manner these different types of fibre influence an increase in mucin secretion. This is exemplified by the works of Huang *et al.*, (2001), who showed that pigs fed with wheat short, partially substituted by wheat bran and wheat flour enhanced crude mucin content ranging from 10.9g/d to 14.26 g/d, as compared to 7.71g/d mucin losses in pigs given diet consisting 95% barley or barley-legumes mixture (Lien *et al.*, 2000 cited by Huang *et al.*, 2001).

The flow of crude mucin was significantly increased with increasing levels of arabinoxylan added to pig diets. It is considered that proteolysis and physical abrasion were the primary factors influencing the presence of mucin in the gastro-intestinal tract (Montagne *et al.*, 2000), and presence of different types of dietary fibre may increase the activity and

distribution of proteolytic enzymes in the lumen of the small intestine that, in turn, enhances increase in mucin flow.

Several studies showed an increase in proteolytic enzymes activity associated with feeding dietary fibre. Schneeman *et al.* (1982) showed that the activity and distribution of proteolytic enzymes in the intestinal lumen were affected by diets supplemented with different types of dietary fibre. In rats fed diet containing wheat bran, the trypsin and chemotrypsin activity were higher in intestinal content (Schneeman *et al.*, 1982). Zebrowska *et al.* (1983) showed an increase in pepsin content in duodenal digesta of pigs fed on barley-based diet as a result of high fibre content of barley-soya meal diet. Total protease activity and increase in peptidase activity in the small intestine have been reported in rats fed with diet containing guar gum (Poksay and Schneeman, 1983), and oat bran, pectin and cellulose. These aforementioned studies showed the effects of different types of dietary fibre on proteolytic secretion, which in turn, resulted in increased mucin secretion and flow. However, the extent of flow of mucus or its recovery in the ileum as influenced by changes in proteolytic activity, which had been elevated by inclusion of different levels of dietary fibre, remains to be elucidated.

Leterme *et al.* (1998) showed that the linear increase in mucus flow in pigs that consumed diet high in inner pea fibre is positively related to water holding-capacity in the diet, rather than the feed intake. Thus, the increase in flow of crude mucin in the current trial, as shown by inclusion of arabinoxylan, can be partly explained by the ability of arabinoxylan to absorb water ten times its weight and bind it in its structure, and in the presence of peroxidase, accelerates formation of gel network (Choct, 1997). This condition ultimately leads to increased mucin flow, as shown in this trial. Further, arabinoxylan polysaccharide structure cannot be easily degraded or broken down due to its branched structure that requires cooperation of several enzyme systems (arabinofurosidase, xylosidase, endoxylanase) for complete degradation. The relatively shorter transit time in the small intestine (4-8h) is presumably not enough for the xylanases to cleave the bonding of the arabinoxylan polymers (Bach Knudsen and Canibe, 2000).

Other factors, including the increased abrasive nature of the grain-based diet may also influence mucin production. A diet of more abrasive nature may increase goblet cells numbers and activity and increased mucin production as compared to control diet (Pestova *et al.*, 2000). Schneeman *et al.* (1982) revealed an increase in the proportion of goblet cells, mucus-producing cells, in the duodenum and colon when 20% wheat bran is supplemented

to fibre-free diet. This abrasive effect of dietary fibre on the cells of the intestinal wall may result to increased ileal recovery of nitrogen (Huang *et al.*, 2001).

The results of the previous experiments and of this trial suggest that the most probable components implicated in the increase in the mucin flow might be dietary fibre and in some part, undigested protein components. However, different types of dietary fibres, like soluble and insoluble, might illicit varied actions to influence increase in mucin flow, which remains to be further investigated. Therefore, feeding high fibre diet may impair nutrient absorption as indicated by increased mucin secretion. The recovery of ileal mucin in the ileal digesta can provide important insights into the effects of diets and dietary constituents on the gastro-intestinal tract, and its measurement is essential in assessing endogenous protein recovery, which is important in determining true digestibility.

3.4.5 Endogenous Nitrogen and Amino Acid Losses

In this work, the enzyme hydrolysed protein method, which is considered as an alternative method for the determination of ileal endogenous nitrogen and amino acid flows, was used. In this method, the test animals were given diet composed of peptides and amino acids, which simulate the common substrate and products of protein digestion. The experimental animals were given purified diet that contains enzymatically-hydrolysed casein (EHC) as sole source of protein. The EHC contains a mixture of free amino acids and small peptides, having all the dietary peptides being less than 5,000 Da in size. The digesta were collected from the pigs and centrifuged before being ultrafiltered. This removed any compounds smaller than the filtration cut-off of 10,000 Da, thereby separating any unabsorbed amino acid and peptides of dietary origin. The retentate (greater than 10,000 Da) was added to the precipitate in the centrifugation steps and this material was used to determine the endogenous nitrogen and amino acids. This method, therefore, was used to determine endogenous nitrogen and amino acid losses in various types of monogastrics (Butts *et al.*, 1993ab; Moughan *et al.*, 1992; Schulze *et al.*, 1995; Donkoh *et al.*, 1995; Leterme *et al.*, 1996).

The apparent nitrogen digestibility (AND) was similar ($P>0.05$) in pigs fed with different levels and types of NSP, however, pigs fed with mixed NSP (4%BG and 3.5% cellulose) had the lowest AND value. Comparing the purified sources, 7.5% AX had the lowest AND (74.1%), 7.5% BG was intermediate (75.8%) and control diet had the highest AND (80.95%). The APN of pigs fed with 7.5%AX was similar to that of pigs fed with taiga and grete barley (Leterme *et al.*, 2000). However, the range of APN of this trial was

comparably lower as compared to pigs fed with wheat bran without purified NDF (Schulze, 1994). The lowered apparent digestibility in pigs fed with 7.5% AX, though the difference was insignificant, can be partly explained by increased endogenous nitrogen flow in pigs fed with the same diet. Similar condition had been reported in the studies of Yin *et al.* (2000) and Leterme *et al.* (2000). When corrected for the endogenous nitrogen losses, the true nitrogen digestibility (TND) values were similar but the range becomes relatively closer across all the pigs (88.3% to 90.7%) fed with different levels and types of NSP.

Endogenous nitrogen (EN) flow was significantly increased with dietary inclusion of mixed NSP (4%BG and 3.5% cellulose) and with 7.5% AX. The significant difference in the EN flow in pigs fed with mixed NSP (4%BG and 3.5% cellulose) is difficult to explain and is open for further speculation. The highest endogenous flows in this trial (4.07 g/kg DMI and 3.7 g/kg DMI) for pigs fed with mixed NSP (4%BG and 3.5% cellulose) and 7.5%AX were relatively lower as compared to pigs fed with barley and wheat (4.4 g/kg DMI; de Lange *et al.*, 1990). The endogenous flow (control diet) of this trial (2.274 g/kg DMI) was relatively lower than those reported by Hodgkinson *et al.*, (2000) (2.85 g/kg DMI), and Butts *et al.*, (1993ab) (3.7g/kg DMI, 2.7g/kg DMI). These completed studies used 30-50g cellulose/kg diet as sole source of fibre. Though there were relatively greater differences in the compared ileal nitrogen flow, the results of those studies and the current trial were relatively comparable. Increased soluble dietary fibre levels resulted in a significant increase in ENF, as shown by statistically significant increase in ENF in pigs fed with increasing levels of arabinoxylan. The significant increase in EN flow in pigs fed with 7.5% AX can be associated with the physicochemical property of AX that require multi-enzyme complex to be completely degraded and the short transit time makes enzymes inefficient in cleaving the bond (Bach Knudsen and Canibe, 2000). Arabinoxylan is known for its water- holding capacity, and this feature is responsible for the increase in ileal endogenous secretion of protein in pigs fed peas (Leterme *et al.*, 1996; 1998). The increased EN flow in pigs fed with 7.5% AX can also be associated to increased secretion and flow of crude mucin (see Table 3.9), which contain nitrogenous component (Lien *et al.*, 2001), as shown in pigs fed with the same diet. Linear increase in mucin secretion and flows were observed in feeding diet to pigs supplemented with increasing levels of arabinoxylan. In contrast, the lack of significant differences in EN flow in pigs fed 7.5% BG is an indication of significant degradation of β-glucan in the small intestine (Bach Knudsen and Canibe, 2000). These authors mentioned that soluble β-glucan fraction is degraded by the high microbial colonisation in the stomach and proximal small intestine.

Thacker (2000) showed the high degradation rate of β-glucan making it unlikely a factor in promoting anti-nutritive characters in pigs. Kim (1999) even reported a positive correlation (0.52) between barley soluble β-glucan and apparent ileal digestibility of amino acids, this may partly explain the lack of significant effect on ENF with 7.5% β-glucan.

The trend of the endogenous amino acid flow (EAAF) of this study were comparable to those reported by Hodgkinson *et al.* (2000), Kim (1999) and Butts *et al.* (1993ab). Studies to determine ileal endogenous amino acid flow (EAAF) using EHC are limited, and so, baseline information was sourced by comparing EAAF values in pigs fed with control diets to other previously completed EHC studies. The EAAF of the current work was relatively lower compared to works of Schulze *et al.* (1995). The two studies differed in the level of inclusion of EHC, which was higher in Schulze (18%), and that explained the difference. There was a slight difference between the EAAF (control diet) of the current work, which used 15% EHC level, to the EAAF of pigs fed with 10% EHC (Hodgkinson *et al.*, 2000). The differences may be related to the methods of digesta collection. The current work used slaughter technique, while the other used PVTC cannulation method providing total ileal collection.

Endogenous flow of amino acid, except arginine, phenylalanine, valine and glycine were statistically significantly higher ($P<0.05$) for pigs fed with 7.5% AX than those given with control diet. The EAAF were similar ($P>0.05$) for pigs fed 7.5% β-glucan and control diet, though majority of the EAAF was greater in 7.5% β-glucan, except glycine. EAAF were numerically increased, except for glutamic acid, which is associated with increased level of AX in the diet. As mentioned earlier, significantly higher ($P<0.05$) EAAF for aspartic acid, threonine, serine, glutamic acid, proline, alanine, isoleucine, leucine, histidine and lysine were observed for 7.5% AX as compared to mixed NSP (4%AX and 3.5% cellulose). No definite trend was manifested with dietary inclusion of β-glucan. In fact, EAAF of all amino acids, except lysine, isoleucine, proline, and glutamic acid, was significantly increased in pigs fed mixed NSP (4% BG and 3.5% cellulose) than in pigs fed with 7.5% β-glucan. Comparing the pure NSP levels, EAAF was highest in 7.5% AX, intermediary for 7.5% BG and lowest in pigs fed with control diet.

Mammalian gastrointestinal tracts contained considerable amounts of endogenous nitrogenous compounds (Souffrant, 1991), which comprise of nitrogen derived from various sources like enzymes, mucoproteins, desquamated epithelial cells, serum albumin, peptides, amino acids, amine and urea (Moughan *et al.*, 1992) and their secretions and re-utilisation can be influenced by dietary factors, specifically dietary fibre (Nyachoti *et al.*,

1997; Souffrant, 2001: Leterme *et al.*, 2000). The process where dietary fibre stimulates endogenous amino acid secretion was poorly explained. Nevertheless, soluble fibre had significant effects in the process of digestion and absorption (Johansen *et al.*, 1996). In this study, soluble dietary fibre influenced the flow of endogenous amino acid in the terminal ileum. The significant increase in EAAF in pigs fed with arabinoxylan can be related to its physicochemical property, specifically the water-holding capacity. Arabinoxylan is known to hold water ten times its weight and, in the presence of peroxidase, accelerates the formation of gel network (Choct, 1997). It is well established that fibre with high water-holding capacity induced increases in ileal secretion of protein and amino acids (Souffrant, 2001; Leterme *et al.*, 1996 and 1998; Leterme *et al.*, 2000). Leterme *et al.* (1998) showed a high correlation of EAAF with that of water-holding capacity (r = 0.996), and of fibre intake (r = 0.76). Further, increased in EAAF was observed in pigs fed with different levels of peas (Leterme *et al.*, 1998) and arabinoxylan (this trial), both have known water-holding capacity.

The statistically significant increase in EAAF, in some amino acids, with dietary inclusion of 7.5% arabinoxylan compared to 7.5% β-glucan can be associated to the differences in the polysaccharide structure that influenced their respective degradability. Arabinoxylan had a highly branched structure requiring several enzymes for complete degradation. In contrast, β-glucan due to its linear polymer can be easily degraded by the exo- and endo-enzymes (Bach Knudsen and Canibe, 2000). Leterme *et al.* (2000) reported that 49 – 90% of the total β-glucan is hydrolysed in the small intestine by microbial and endogenous barley β-glucanase. Moreover, pig digesta has high water content, precluding any marked depressive effects of β-glucan (Campbell *et al.*, 1992). Related to quantitative specific endogenous amino acid flow regardless of fibre levels, amino acids, glycine, glutamic acid, proline, threonine and aspartic acid predominated the endogenous flow amounting to more than 50% of the total EAAF. This agreed with the results of Hodgkinson *et al.*, (2000); Butts *et al.*, (1993a) and Moughan *et al.*, (1992). The said amino acids were able to resist acid and enzyme digestion in mucus glycoprotein, thus, decreasing their degradation rates (Kim, 1999; Allen 1981). Furthermore, glutamic acid was present at a highest concentration, as observed in other works (Hodgkinson *et al.*, 2000; Kim 1999; Butts *et al.*, 1993ab; Moughan *et al.*, 1992). This amino acids account for nearly 20% of the EHC (Leterme *et al.*, 1996). The increase in the endogenous flow of threonine, serine and proline associated with dietary inclusion of 7.5% arabinoxylan can be implied in the predominance of these amino acids in mucin (Lien *et al.*, 2001), which was also increased with dietary inclusion of 7.5% arabinoxylan.

Therefore, endogenous N and amino acid flows were significantly increased with dietary inclusion of arabinoxylan, which could be related to its physicochemical property, particularly its high water-holding capacity and its branched structure. In contrast, β-glucan, due to the presence of linear polymer and with its high degradation rate in pig, may be considered as unimportant factor in initiating the induction of ENL and EAAF or influencing protein digestion, at least at levels such as those used in this study. The increased in ENF and EAAF, in some amino acids, associated with dietary inclusion of mixed NSP (4%BG and 3.5% cellulose) is difficult to explain and is open for further speculation. It should be noted, however, that the relationship existing between chemical composition, physical properties and physiological effects of dietary fibre along the gastrointestinal tract is not a simple one. Clearly, research should be furthered to better understand the dynamics of β-glucan and other dietary fibres in affecting nutrient digestion and absorption in pigs.

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CHAPTER IV

ENDOGENOUS NITROGEN FLOW IN CHICKENS AS INFLUENCED BY LEVELS AND TYPES OF DIETARY FIBRE

4.1 Introduction

An estimate of 140 million tonnes of fibrous materials is produced from cereals and other sources, and such values are even increasing dramatically every year. In most grains or cereals, the fibre component is primarily consists of non-starch polysaccharides that form part of the cell wall structure (Choct, 1997).

Non-starch polysaccharides (NSP) played a crucial role in poultry nutrition due to the anti-nutritive effect elicited by soluble pentosans components (arabinoxylan and beta-glucan) and their effects on bird performance (Choct, 1997). The detrimental effects of NSP are believed to be associated to its viscous nature that has physiological, morphological and microbiological effects on the digestive tract. These events negatively impact nutrient digestion and also cause wet and sticky droppings (Bedford and Schulze, 1998).

It is established that NSP in wheat influence the apparent ileal digestibility of nutrients, including protein (Choct and Annison, 1992). The reduction in protein digestibility can be attributed to impaired digestion, inhibition of amino acid absorption or increased secretion of endogenous protein derived from gut secretion and sloughed off epithelial cells to the digesta. Using the guanidination method, Angkanaporn *et al.* (1994) found that the addition of isolated pentosans equivalent to 15 and 30 g wheat arabinoxylan per kg diet significantly increased endogenous amino acid flow and lowered overall digestibility of amino acids. In the present study, the influence of different types and levels of NSP on endogenous ileal nitrogen losses in broiler chickens was examined using the peptide alimentation method (Moughan *et al.*, 1990). Two types of soluble NSP, namely, viscous (barley β-glucan) and non viscous (maize arabinoxylan) were compared.

4.2 Materials and Methods

4.2.1 Animals, Housing and Management

Three-week old male Golden Coast Ross commercial broiler were selected for the experiment based on bodyweight, which ranged from 1.3 to 1.7 kilograms. The birds were housed in an electrically heated grower shed maintained at 22–24°C. All birds had *ad libitum* access to feed and water. The experiment was carried out at Poultry Research Unit, Monogastric Research Centre, Massey University, Palmerston North, New Zealand. Massey University Animal Ethics Committee granted the ethical standard for this trial.

4.2.2 Experimental Designs

One hundred twenty male birds were selected and allocated for 25 colony cages, with 4 to 5 birds per cage, so that all cages had similar body weights. The cages were then assigned at random to the five dietary treatments so that there were five replicates per group. The treatment diets were assigned as follows:

Control = 0% AX / BG

3% AX = 3% Arabinoxylan

6% AX = 6% Arabinoxylan

3% BG = 3% Beta-glucan

6% BG = 6% Beta-glucan

4.2.3 Experimental Diets

The diets formulated for this experiment contained 18% casein or enzymatically hydrolysed casein (EHC) as sole source of nitrogen. Different levels (3% and 6%) of arabinoxylan and β-glucan had been added to EHC diets as source of soluble dietary fibres. The composition of casein and EHC diets are shown in Table 4.1, respectively. The EHC-based diet contained titanium oxide as indigestible marker. The concentrations of titanium oxide were used to allow calculation of ileal digesta flow.

4.2.4 Experimental Procedures

The birds, housed in colony cages, were given a mash commercial-type diet from day 21 of age till day 27. Following overnight fasting at day 27, a casein-based diet was introduced and fed for the next three days to all the birds. The casein-based diet was similar to the control diet (Table 4.1) except that casein was used in place of EHC. The casein-based diet was withdrawn at 5pm of day 30, and the test diets (Table 4.1) were introduced on the morning of day 31. The EHC-based test diets were offered for 36 hours and feed intake during this period were monitored and recorded. The EHC-based diets were withdrawn at 5pm of day 32, and reintroduced in the morning of day 33 for 2-3 hours before slaughtering the birds. The birds were then euthanased with intra-cardiac injection of 1 ml sodium pentobarbitone. They were then dissected to reveal the lower gastro-intestinal tract between Meckel's diverticulum and the ileo-caecal-colonic junctions. The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a

point 40 mm proximal to the ileo-caecal junction. The contents of the posterior half of the ileum were collected by gently flushing with distilled water to labelled plastic bags. The digesta were pooled from birds within a pen. The samples were frozen immediately at –20°C for subsequent titanium oxide and nitrogen digestibility analyses.

4.2.5 Analytical Procedures

Digesta samples were freeze dried, ground to pass through a 0.5 sieve, and together with the diet samples, were analysed for nitrogen content through a LECO FT-200 analyser using the Dumas process (Granger, 1997). The samples were also processed for dry matter and titanium oxide content analyses (Short *et al.*, 1996) following the method developed in Nutrition Laboratory, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.

Nitrogen flow from the terminal ileum related to the ingestion of 1g of food dry matter was calculated using the following equation:

Nitrogen flow = Nitrogen in the digesta x (TiO_2 diet/TiO_2 digesta)

Apparent digestibility of nitrogen (ADN) was calculated using the formula:

ADN = [(N diet/TiO_2 diet) – N digesta/TiO_2 digesta)] / (N diet/TiO_2 diet)

The dry matter digestibility (DMD) was then calculated using the formula:

DMD= [(TiO_2 in ileal sample/TiO_2 in diet) –1]/ (TiO_2 in ileal sample/TiO_2 in diet)

In this study, the endogenous nitrogen (N) flow was calculated assuming a digestibility value of 100% for EHC. This assumption was based on previous trial where EHC had been shown to be completely digested by broiler chickens (Ravindran, 2000 unpublished data). Thus, based on the above assumption, endogenous N losses is, therefore, equal to nitrogen flow.

4.2.6 Statistical Analysis

Data were statistically analysed following the GLM procedure in SAS (1997), using the model: $Y_{ij} = \mu + D_i + E_{ij}$

where: Y_{ij} = observation of the j^{th} birds exposed to i^{th} treatment

μ = general mean

D_i= fixed effect of diet

E_{ij}= residual error

The above model allows dietary effects to be assessed. The significance level was set at $\alpha = 0.05$. Significant differences between means were assessed by the least significant differences (LSD) method.

Table 4.1 **Ingredient composition (g/kg) of the experimental diets.**

Ingredients	D Control	I 3% Ax[2]	E 6% Ax[2]	T 3% BG[3]	S 6% BG[3]
Starch	569.7	539.7	509.7	539.7	509.7
Dextrose	100.0	100.0	100.0	100.0	100.0
EHC[1]	180.0	180.0	180.0	180.0	180.0
Arabinoxylan	-	30.0	60.0	-	-
Beta-glucan	-	-	-	30.0	60.0
Vegetable oil	50.0	50.0	50.0	50.0	50.0
Cellulose	35.0	35.0	35.0	35.0	35.0
Titanium oxide	0.5	0.5	0.5	0.5	0.5
Common ingredients[4]	60.3	60.3	60.3	60.3	60.3

[1]-Enzymatically Hydrolysed Casein; [2]-Arabinoxylan; [3]-Beta-glucan; [4]- Dicalcium phosphate, 24.0; Dipotassium hydrogen phosphate, 14.3; Sodium bicarbonate, 12.0; Magnesium oxide, 2.0; Salt, 2.0; Trace mineral premix, 5.0 and vitamin premix, 1.0.

4.3 Results

The experiment involved feeding of birds with casein-based and enzymatically hydrolysed casein diets. The endogenous nitrogen flow, dry matter and nitrogen digestibilities, and dry matter and total feed intake (5 groups with 5 birds/group) are detailed in Table 4.2. Total feed intake for 36 hours were similar ($P>0.05$) in broiler fed different types and levels of non-starch polysaccharides. Though no significant differences in feed consumed over the specified period, a common trend was evident wherein total feed intake is quantitatively increased with addition of soluble NSP in the diet. Inclusion of soluble NSP did not influence nitrogen digestibility ($P>0.05$), at least in this trial, having nitrogen digestibility values in excess of 90% for different levels (3% and 6%) and types (soluble- β-glucan, arabinoxylan; insoluble- cellulose) of dietary NSP. However, it is interesting to note that the lowest values for nitrogen digestibility (90.4%) were observed in birds fed by the

highest inclusion level (6%) of arabinoxylan (AX) and β-glucan (BG), both were soluble NSP.

Table 4.2. **Influence of type and level of NSP on feed intake and endogenous nitrogen flow in broilers.**

DIETS	DMD[1]	IlealN (g/gTiO2)	ENF[2] (ug/gDMI)	ADN [3]	TFI[4]kg(36h)
Control	0.878[a]	0.457	2383	0.911	0.99
3.0% AX	0.882[a]	0.552	2549	0.906	1.07
6.0% AX	0.879[a]	0.605	2625	0.904	1.04
3.0% β-G	0.884[a]	0.506	2372	0.914	1.10
6.0% β-G	0.856[b]	0.535	2700	0.904	1.03
Pooled SEM	0.0055	0.055	269	0.010	0.055

[a,b] Values in the same column with different superscripts are significantly different at (P< 0.05), LSD; [1]-Dry matter digestibility; [2]-ENF Endogenous nitrogen flow; [3]- Apparent digestibility of nitrogen; [4]- Total feed intake for 36 hours; AX-Arabinoxylan; B-G-Beta-glucan.

Ileal nitrogen contents, expressed as g/g titanium (g/gT), were numerically increased with the addition of soluble NSP (0.605 g/gT against 0.552 g/gT for 6% and 3% arabinoxylan; and 0.535 g/gT against 0.506 g/gT for 6% and 3% β-glucan), but the influences were not statistically significant due to high variation among replicates. The same trend was also observed for endogenous nitrogen flow, where increase in the inclusion levels of soluble NSP in the diets resulted to quantitative increase of ileal nitrogen flow. Highest endogenous nitrogen flow was observed in 6% level of β-glucan (2700 ug/g DMI), followed by 6% level of arabinoxylan (2625 ug/g DMI), however, the said differences did not sustain statistical significance (P>0.05) due to greater variation among replicates.

On the other hand, the dry matter digestibility was not influenced by inclusion of arabinoxylan or 3% β-glucan (with values ranging from 0.879, 0.882 and 0.884 for 6% and 3% arabinoxylan, and 3 % β-glucan level of inclusion, respectively) but was significantly lowered (P<0.05) with 6% inclusion of β-glucan, with the dry matter digestibility value reduced to 0.856.

4.4 Discussion and Conclusion

In the peptide alimentation method (Moughan *et al.*, 1990), the animal is fed a purified diet containing enzymatically hydrolysed casein (EHC) (composed of free amino acids and peptides with a molecular weight of less than 10,000 Da) as sole source of nitrogen. The digesta are then collected from the animal and the endogenous protein (molecular weight, >10,000 Da) is separated from unabsorbed free amino acids and peptides by centrifugation

and ultrafiltration. In the present study, difficulties were experienced with the ultrafiltration step. The endogenous flow was therefore calculated assuming a digestibility value of 100% for EHC. This assumption was based on the previous work where EHC has been shown to be completely digested by broiler chickens (Ravindran, 2000 unpublished data). A similar approach has been used by Leterme *et al.* (1994) who employed the peptide alimentation method, but without the ultrafiltration of digesta. It should be considered, however, this approach may result in overestimation of the ileal flow if the EHC is not completely digested.

The influence of soluble NSP in bird performance can be manifested in the amount of feed consumed per unit time. It had been shown by Iji and Tivey (1997) that dilution of commercial diets with different types of NSP significantly influenced feed intake, expressed as g/100g body weight, in broilers thereby reducing growth rate and feed efficiency. In this trial, total feed intake were similar to birds fed diet with different levels, 3% or 6%, of arabinoxylan or β-glucan. Several studies in broilers showed non-significant effect of the dietary inclusion of soluble NSP on feed intake. Maisonnier *et al.* (2001) showed similar feed intake in male broiler chickens given diet based on wheat, and maize added with different levels of guar gum. However other parameters like feed conversion ratio (FCR) and growth rate were significantly influenced. Moreover, the experiment of Ouhida *et al.* (2000a) showed no difference in feed intake when broilers were fed NSP-rich diet (maize-barley-wheat based diets) with or without enzyme supplementation. The absence of differences in feed intake in this trial suggests that most of the differences in animal performance were related to impairment of the digestibility factors in the dietary constituents such as the like of NSP.

Apparent nitrogen digestibility were numerically depressed with increasing content of arabinoxylan and β-glucan in the diet, in which, greater depression at 90.3% and 90.4% were observed in 6% inclusion arabinoxylan and β-glucan, as compared to control diet (91.1%). However, such numerical depressions were not statistically significant due to high variations among replicates. This result is unexpected, and in contrast to common notion that soluble NSP negatively influenced protein digestibility in broilers, as shown by previously completed studies (e.g. Choct and Annison, 1990, 1992; Maqueda, 1999; Angkanaporn *et al.*, 1994; Hesselman and Aman, 1986). On the other hand, several studies reported non-significant effect of NSP on protein digestibilities. Kocher *et al.* (2000) showed that ileal nitrogen digestibility was not depressed by addition of lupin NSP, though the mean protein digestibility value of 84% was relatively lower than the values derived

from the current experiment (Table 4.2). The absence of depressant effects of soluble NSP in this trial can be explained, in part, by the high variation among replicates. It can also be explained by the manner the β-glucan extract react in water, forming a soft gel and not a viscous solution, thereby making the extract behave differently than native β-glucan in solutions of the same concentration and thereby neutralising some anti-nutritive effects (G.D. Coles pers comm., cited in Maqueda, 1999). Moreover, for nitrogen digestibility to be depressed, the concentration of soluble NSP should reach threshold levels, which were probably not achieved in this experiment. Also, Choct and Annison (1992) concluded that birds can tolerate minimal increases in gut viscosity brought by presence of soluble NSP, and birds performance was not depressed once viscosity is reduced to certain level.

Dry matter digestibility was significantly lowered with inclusion of 6% Betaglucan to broiler diet. This said result is in concordance to the fact that dry matter digestibility (DMD) was negatively related to intake of soluble NSP. The result of the current trial is in agreement with the experiment of Jorgensen *et al.* (1996) that increased fibre levels for pea, wheat bran and oat bran significantly depressed ileal digestibility of dry matter in diminishing sequence, as presented. Similar result was also indicated by Refstie *et al.* (1999), who concluded that non-starch polysaccharide content of different soya products significantly depressed organic matter digestibility.

The inclusion of increasing levels of NSP numerically increased the ileal nitrogen content (g/g Titanium) and calculated endogenous nitrogen flow (as ug/g DMI), though differences were not statistically significant due to high variation among replicates. Authors such as Angkanaporn *et al.* (1994) and Beames and Eggum (1981) demonstrated that wheat pentosans and some fibre sources depressed overall digestibility of amino acid and increased secretion of endogenous amino acid flow in chicken and rat, respectively. The said flow is consistent with the numerical increase of this study though no statistical significance was achieved. In general, the above results showed that increased levels of soluble NSP depressed dry matter digestibility. The present data also suggest that soluble NSP may influence the extent of the increase in the ileal flow of nitrogen in chicken.

It is a common knowledge that soluble dietary fibres possess anti-nutritive factors, particularly influencing the digestion process in poultry. There are proposed mechanisms that help to explain the anti-nutritive factors of NSP. Soluble NSP, like arabinoxylan and β-glucan, from endosperm wall hinders access of digestive enzymes to nutrients found in the cereal endosperm (Edwards *et al.*, 1988). However, evidence of depressed growth performance and digestibility in broiler fed carboxymethylcellulose (CMC) (Smits *et al.*,

1997), wheat pentosans (Choct *et al.*, 1996) and barley β-glucan (White *et al.*, 1981), and improvements noticed in fat digestibility, a non-endosperm-stored nutrients, raises doubt about this theory (Ouhida *et al.*, 2000b). The topic of increased digesta viscosity in chicken associated with soluble NSP is very well discussed and documented (Kocher *et al.*, 2000; Ouhida *et al.*, 2000ab; Iji, 1999; Choct, 1997; Choct *et al.*, 1996; Annison, 1993; Choct and Annison, 1992 and 1990). Increased digesta viscosity, caused by arabinoxylan from wheat and β-glucan from barley, influenced intestinal tract digestion by limiting the diffusion of nutrients and digestive enzymes (Choct *et al.*, 1996) restricting access of the these enzymes to their dietary substrate, and through primary and secondary increase of microbial activity of the small intestine (White *et al.*, 1981). Moreover, viscous digesta had been implicated with longer retention time in gastro-intestinal tract that increases microbial activity (Annison, 1993), which in part, inhibits digestibility of nutrients. Further, the increased digesta resistance to peristalsis, due to high viscosity, may cause a stimulation of endogenous secretions by a feedback mechanism (Angkanaporn *et al.*, 1994). These factors suggest increased nitrogen recovery and flow, as indicated in the current trial. Investigation done by Angkanaporn *et al.* (1994) on the influence of soluble fibre on endogenous nitrogen losses in chicken suggested that increased rate of dietary pentosans inclusion inhibited direct protein breakdown and amino acid absorption that leads to decreased nitrogen digestibilities and consequently caused an increase in endogenous amino acid losses.

In conclusion, increased level of soluble NSP significantly depressed dry matter digestibility. The current results also suggest that soluble NSP may increase ileal flow of nitrogen in chickens. The exact causes of the increased nitrogen flow with increased NSP levels are not known. It could be due to increased secretion of endogenous proteins into the gut, decreased re-absorption of endogenous protein or a combination of both effects. As suggested by Angkanaporn *et al.* (1994), it is possible that soluble NSP interacts with the gut wall modifying the actions of the peptide hormones, which regulate gut functions including stimulation of secretion of endogenous protein.

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CHAPTER V

GENERAL DISCUSSION AND CONCLUSION

The present study evaluated the influence of soluble NSP on endogenous nitrogen flow (chicken and pigs) and amino acid losses (pigs) through feeding purified diet based on enzymatically hydrolysed casein (EHC) as sole source of protein (i.e. peptide alimentation method). In pigs, the influence of soluble NSP on organ weights and growth parameters, as well as, the effects of the same on levels of blood metabolites and mucin secretion was examined. To evaluate the influence of soluble NSP on the parameters mentioned, a purified maize arabinoxylan and a commercially prepared barley β-glucan extract Glucagel™ were used.

In the pig trial, the influence of soluble dietary fibre on blood lipid metabolites was determined in three different times, day 0 fasted (start of the experiment), day 21 fasted and day 21 fed (end of experiment). Repeat measure analysis were used to compare blood metabolites measured over time. The two periods compared were fasted day 0 vs fasted day 21, and fasted day 21 and fed day 21. Levels of blood metabolites measured during day 0 (fasted) animals were similar ($P>0.05$) indicating homogeneity of blood metabolite levels. The levels of blood metabolites measured on day 21, either fasted of fed state, were not influenced ($P>0.05$) by dietary inclusion of arabinoxylan and β-glucan, contrasting the finding of McIntosh, (1991), Klopfrenstein, (1988) and Anderson *et al.* (1993). These authors reported significant changes in blood metabolites levels in human and rats, respectively. The result of this trial indicated that arabinoxylan and β-glucan failed to initiate hypocholesterolemic effect in pigs.

The absence of significant influence of soluble non-starch polysaccharides (NSP) to lower blood metabolites may be related to the duration of feeding experimental diets (21 days). It may be too short to initiate hypocholesterolemic effect. The concentration or levels of inclusion of arabinoxylan and particularly β-glucan extract (70% purity, Maqueda, 1999) may not be enough to initiate threshold level of cholesterol-lowering effect. The study also used three-week old piglets, which may be too young to be implicated in studies for hypocholesterolemic effect of dietary fibre. Comparing the three periods of collection using the repeat measure model, significant changes in levels of blood metabolites over time. Significant increase ($P<0.05$) in levels of TC, HDL and LDL were observed in day 21 (fasted) as compared to day 0 (fasted). When fasted and fed state (day 21) were compared, fasting significantly increased TC and some HDL levels. However, LDL levels were similar in the two periods being compared. No significant changes in TG levels for both periods compared.

The increase in levels of blood metabolites, except TG, after 21 days feeding suggested an increased accumulation of such metabolites over time. The increased levels were a result of interplay between supply from the liver and demand for their lipid constituents by the tissue (Kingman *et al.*, 1993), suggesting decrease in their catabolism or an increase in their synthesis. The increase in HDL in fasted period as compared to fed values (day21) implied an increase metabolism of chylomicron, which transfers apolipoprotein A-I and A-II to HDL. Such alteration of HDL composition is evident after 5–10 hours after a meal (Tall, 1986; Luhman *et al.*, 1991). The similar LDL values between fasted and fed state were attributed to the differences of metabolism of LDL between pigs and humans. Porcine plasma lacks cholesteryl ester transfer protein (CETP) activity and consequently, minute amount of HDL cholesteryl ester is transferred to the LDL fraction, and only a small fraction (11%) of VLDL apolipoprotein B in pigs is converted into LDL (Knipping *et al.*, 1987; Birchbauer *et al.*, 1992 cited in Allan *et al.*, 2001).

Dietary inclusion of arabinoxylan and β-glucan did not demonstrate significant increase ($P>0.05$) in empty organ weights, contrasting the results of Jorgensen *et al.*, (1996a) and Pond *et al.* (1988). The lack of influence of arabinoxylan and β-glucan on organ weights means that purified arabinoxylan and β-glucan extract, which produce gel-forming property, may not be a factor in disrupting the functional capacity of the organ involved particularly the gastro-intestinal tract, which has a direct involvement in mechanical digestion of food. However, inclusion of 7.5% β-glucan significantly increased ($P<0.05$) gut fill, showing the gelling influence of β-glucan extract and conserving some of its physico-chemical properties.

Further, the end liveweight and carcass weight were similar ($P>0.05$) in pigs fed arabinoxylan and β-glucan. Daily feed intake was similar ($P>0.05$), due to feeding restriction. In contrast, daily weight gain (331.09g for AX, 373.0 for BG vs 234.4g for control) and feed conversion ratio (2.7kg for AX, 2.3kg for BG vs 3.4kg for control) were significantly improved ($P<0.05$) in pigs fed AX and BG. These results suggested that the anti-nutritive effects of arabinoxylan and β-glucan could hardly be manifested in pigs probably due to high degradability of these NSP, as reported by Thacker (2000). Specifically, the soft-gelling property of β-glucan is not a factor in initiating anti-nutritive effect, at least with the levels used in this trial. In fact, values of FCR and ADG were significantly improved. This improvement was not due to the difference in gut fill.

The effect of dietary fibre in secretion and flow of crude mucin was also assessed. Increased level of arabinoxylan, but not β-glucan, resulted to significant numerical increase

($P<0.05$) in crude mucin secretion and flow. In contrast, crude mucin nitrogen secretion and flow were similar ($P>0.05$) in pigs fed arabinoxylan and β-glucan. These results, together with that of Leterme *et al.* (1998), suggested that the water-holding capacity of arabinoxylan and pea fibre was responsible for the increase in crude mucin flow and secretion. Increased crude mucin flow, caused by increased proteolytic activity, associated with increased dietary fibre feeding was also reported (Montagne *et al.*, 2000; Schneeman *et al.*, 1982). Abrasive effects of fibre may also increase ileal secretion of mucin and nitrogen (Huang *et al.*, 2001). The result suggested that dietary inclusion of arabinoxylan, but not β-glucan appeared to be effective in stimulation of crude mucin secretion and flow in the ileum. The two NSP differed in their physicochemical structures, arabinoxylan being branched and β-glucan being linear (Bach Knudsen and Canibe, 2000). Consequently, increased degradation rate of linear β-glucan may explain the significant differences in mucin secretion between the two NSP.

In the same trial, nitrogen digestibility and endogenous nitrogen and amino acid flows were examined. Apparent nitrogen digestibility (AND) was not significantly influenced with addition of dietary NSP, however, a relatively wider range was observed (73.13% to 80.95%) across all the diets. When AND was corrected for endogenous losses, the range of the true nitrogen digestibility (TND) across all the diets became narrower (88.36% to 90.7%), and no significant differences ($P>0.05$) for TND across the diets were observed.

Significant numerical increase was demonstrated in endogenous nitrogen flow (ENF) with increased dietary level of arabinoxylan, but not β-glucan. Arabinoxylan, which absorbs water ten times its weight, is known for its water-holding capacity, and this may be responsible for the increase in ENF. The same observation was reported in pigs fed peas (Leterme *et al.*, 1998). This condition could also be related to the increased secretion and flow of crude mucin, which contained essential quantities of nitrogenous components (Lien *et al.*, 2001; Satchithanandam *et al.*, 1990). The non-significant effect of dietary inclusion of 7.5% β-glucan implied a higher mechanical breakdown of β-glucan molecules, probably by high microbial colonisation in the pig gut (Bach Knudsen and Canibe, 2000). β-glucan extract concentration (70% purity; Maqueda 1999) may not be enough to initiate threshold reaction of ENF. This result made β-glucan not a factor of anti-nutritive property in pigs, at least with the level reported in this trial.

Dietary inclusion of soluble NSP significantly influenced ($P<0.05$) ileal endogenous amino acid flow (EAAF). Numerical increase in EAAF was observed with increased level of arabinoxylan, except for glutamic acid. With dietary β-glucan inclusion, no definite trend

could be observed, in fact values for mixed NSP (4% βG and 3,5% cellulose) was significantly higher than dietary inclusion of 7.5% β-glucan. EAAF was highest for 7.5% arabinoxylan intermediate for 7.5% β-glucan and lowest for control diet.

The anti-nutritive factor of NSP was manifested particularly by increased ileal endogenous amino acid flow in pigs fed arabinoxylan. This is related to the increase in ileal nitrogen flow. This result could be explained by the physicochemical properties of arabinoxylan: the water holding capacity and the branched structure. Specifically, significant increased endogenous flows of threonine, proline and serine with dietary inclusion of 7.5% arabinoxylan are consistent with the high level of crude mucin found for this diet (i.e. those amino acids are abundant in mucin). The absence of significant effect of dietary inclusion of 7.5% β-glucan indicated an efficient rate of breakdown of β-glucan component by digestive enzymes, thus soft-gelling property of β-glucan seemed to be well tolerated by pigs. Kim, (1999) reported a positive correlation (0.52) between barley soluble β-glucan and apparent ileal digestibility of amino acids, and this may partly explain the lack of significant effect on EAAF of 7.5% β-glucan diet. Moreover, the significant increase in EAAF related to dietary inclusion of mixed NSP (4%BG and 3.5% cellulose) is difficult to comprehend and is open for speculation.

In chicken, apparent nitrogen digestibility (AND) was numerically depressed at 90.3% and 90.4% for 6% dietary inclusion of arabinoxylan and β-glucan as compared to 91.1% for broiler chickens fed control diet. However, such numerical depressions were not statistically significant due to high inter replicates variation. The result of the current work contradicts to several previously completed studies (Maqueda, 1999; Choct and Annison, 1990 and 1992; Angkanaporn *et al.*, 1994). The lack of significant depressant effects for AND in this trial can be associated not only in the high inter replicates variation, but also with the behaviour of the extract when mixed in water forming a soft gel (Maqueda, 1999), instead of a viscous solution, that may react with other dietary components initiating neutralising effect of the anti-nutritive properties to some degree. In addition mature birds can tolerate minimal changes in gut viscosity and the growth performance may not be depressed once viscosity is lowered to a certain level (Choct and Annison, 1992).

Moreover in chicken, increased levels of NSP numerically increased ileal nitrogen content and calculated endogenous nitrogen flow, though, significant differences were not achieved due to high variation among replicates. Reports of Angkanaporn *et al.* (1994) and Beames and Eggum (1981) indicated that wheat pentosans and some sources of fibre significantly reduced overall digestibility of amino acids and consequently lead to

increased amino acid flow in chicken and rats, respectively. The numerical increase in ileal nitrogen content and calculated endogenous nitrogen flow in the current work is consistent to the results of the abovementioned studies, though statistically significant differences in the current trial were not achieved. Further, dry matter digestibility (DMD) was significantly lowered with inclusion of 6% β-glucan. Similar findings in peas, wheat bran, oat bran and different soya products were observed (Jorgensen *et al.*, 1996b; Refstie *et al.*, 1999).

The influence of soluble NSP to initiate anti-nutritional properties is diverse and involved several proposed mechanisms. Soluble NSP, like arabinoxylan and β-glucan, from endosperm wall hinders access of digestive enzymes to substances located in the cereal endosperm (White *et al.*, 1988), but most likely, not in the case of this experiment. NSP is well known to increase digesta viscosity, which limits diffusion of nutrients and digestive enzymes, causes longer retention time that increase microbial activity. These result to depressed digestion and absorption process in broiler chicken. Choct (1997) suggested that the resultant changes in the intestinal flora and in the levels of water and electrolytes are the secondary effects of β-glucan, complementary to its anti-nutritive factors. Maqueda (1999) found out a significant increase in water-holding capacity of β-glucan extract when fed to broilers, indicating preservation of hydration property, and thus promote anti-nutritive property. These factors consequently lead to significant lowering of dry matter digestibility, numerical depression in nitrogen digestibility, and numerical increase in nitrogen recovery and flow associated with dietary inclusion of different levels of arabinoxylan and β-glucan fed to broilers.

In conclusion, the anti-nutritive character of NSP is clearly demonstrated by the significant depressant effect on dry matter digestibility in broiler chicken fed increasing levels of soluble NSP. The current data also indicate that soluble NSP may influence the extent of increase in ileal flow of nitrogen in chickens. The definite cause of increased nitrogen flow is not clear but could be related to increased secretion of endogenous protein into the gut, decreased re-absorption of endogenous protein, or combinations of both effects had occurred. It is conceivable that the action of peptide hormones, which control gut functions (i.e. stimulation of secretion of endogenous protein), was modified by NSP through interaction with the gut wall. In pigs, inclusion of soluble NSP failed to increase organ weight, it even improved daily gain and feed conversion ratio. Arabinoxylan, but not β-glucan, seemed to contain effective anti-nutritive components, which was related to its physicochemical properties being branched and with greater capacity to hold water. β-

glucan seemed to preserve its physicochemical property, but its concentration was not enough to effect increased secretion and flow of nitrogen and amino acids. The soluble NSP was not effective to lower blood cholesterol, at least with the levels used. The duration of feeding and the age of animal for dietary fibre trial need to be re-evaluated. The significant influence of mixed NSP (4%BG and 3.5% cellulose) was difficult to explain and its dynamics is open for further studies.

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