EVALUATION OF THE ANTI-NUTRITIVE AND HYPOCHOLESTEROLEMIC EFFECTS OF A β-GLUCAN PREPARATION EXTRACTED FROM NEW ZEALAND BARLEY

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ABSTRACT

The present study evaluated the anti-nutritive as well as the hypocholesterolemic effects of barley β-glucan through the use of a commercially prepared barley β-glucan extract. Two experiments with broiler chickens were performed. In the first experiment (Chapter 3), a commercially prepared barley β-glucan extract was added (15 g/kg diet) to a synthetic diet based on cornstarch and casein. In addition, the diet was heated to evaluate the effects of heat treatment on the anti-nutritive effects of β-glucan. The diet was fed to twenty 15-day-old male broilers for 7 days. Diets containing β-glucan reduced ($P<0.01$) nitrogen (N) and carbon (C) digestibility, increased in the full weight of the whole gut ($P=0.08$) and the caeca ($P<0.05$), and increased ($P<0.01$) the insoluble solids volume (ISV) and water holding capacity (WHC) of digesta of birds. Heating the diets decreased ($P<0.01$) N digestibility and increased the ISV of the digesta.

In the second experiment (Chapter 4), different amounts of the β-glucan extract were added to a barley-based diet to provide three different levels of dietary β-glucan (low (19.8 g β-glucan /kg diet), medium (50.7 g β-glucan/kg diet), and high (68.3 g β-glucan/kg diet)). In addition, a β-glucanase was added to each of the diets to assess its influence on digestibility, performance, and digesta physico-chemical properties. The diets were fed to 36, 15-day-old male broilers for seven days. Results showed variable responses. The values obtained for gross energy (GE) digestibility, and the weights of the whole gut empty and the second part of the small intestine full and empty were similar between diets of low and medium β-glucan content, but lower ($P<0.05$) than the values obtained with diet high. In the case of WHC and ISV, the values obtained with diets medium and high were similar, but higher ($P<0.01$ for WHC; $P<0.05$ for ISV) than the values obtained with diet low. For viscosity, the higher ($P<0.01$) values were obtained with diet low, while the values obtained with diets medium and low were similar. The variable responses were attributed to the soft gelatinisation of β-glucan when dispersed in water, which could have encapsulated the native β-glucan from barley, hindering any anti-nutritive effect it could have. Another possibility suggested was in terms of the threshold level of β-glucan needed to elicit its anti-nutritive effects, which was not reached in some of the diets. The β-glucanase

inclusion improved N, C ($P<0.05$), and GE ($P<0.01$) digestibility, decreased the viscosity of the digesta ($P<0.01$) and the weights of the whole gut and the second part of the small intestine ($P<0.01$), and improved the weight gain and feed conversion ratio (FCR) of the birds ($P<0.05$). The experiment also suggested that viscosity is not the main mechanism needed for the anti-nutritive effects of β-glucan because although the viscosity of most of the diets was low, the β-glucan still elicited some anti-nutritive effects. It is possible that the gelling capacity of the β-glucan extract was one of the main factors influencing the anti-nutritive effects of the extract.

In addition to these two studies, an *in vitro* digestibility method simulating the chicken's gut was developed to evaluate the anti-nutritive effects of β-glucan on N and C digestibility as well as on the physico-chemical properties of the digesta (Chapter 5). The method showed acceptable accuracy ($r=0.93$, $P<0.01$) in the prediction of N digestibility *in vivo* in a wide range of diets. In the case of C digestibility, although a significant correlation ($r=-0.64$, $P<0.05$) between the *in vitro* and *in vivo* values was found, the *in vitro* digestion did not reflect what happened during the *in vivo* digestion. This was possibly due to the fact that the *in vitro* assay was not designed to analyse C digestibility, and the results obtained were in fact an artifact of the data set. The physico-chemical properties of the digesta were not predicted accurately by the *in vitro* method because conditions such as the churning effect of the intestine and the action of the microorganisms present in the intestine, which greatly affect digesta, could not be replicated in the method.

The hypocholesterolemic effects of the β-glucan extract were evaluated in growing male rats through the inclusion of β-glucan in synthetic diets based on cornstarch and casein (Chapter 6). In addition, the effects of coconut oil (rich in saturated fatty acids (SFA)), and flax oil (rich in polyunsaturated fatty acids (PUFA)) in blood lipids were also evaluated. The diets were fed to 36 28-day-old male rats for 28 days. The results of this experiment showed a reduction ($P=0.07$) in total cholesterol (TC) levels by the inclusion of β-glucan. The kind of oil used in the diets did not affect TC levels ($P>0.05$). Serum TG levels were decreased ($P<0.01$) by inclusion of β-glucan. When coconut oil was used, β-glucan inclusion decreased ($P<0.05$) serum TG levels. However, when flax oil was used, β-glucan inclusion did not have any effect on TG ($P>0.05$). In addition, it is suggested that the hypocholesterolemic effects of the β-

glucan extract used were due to a decrease in lipid absorption in the small intestine, which was caused by the β-glucan inducing a gel formation, which delayed nutrient absorption. This gel formation induced by the β-glucan extract is considered to be one of the main factors responsible for the hypocholesterolemic effects of the β-glucan extract.

It is concluded that the anti-nutritive and hypocholesterolemic effects of barley β-glucans were demonstrated through the use of a commercially prepared barley β-glucan extract, and that the gel formation induced by the β-glucan extract could be one of the main mechanisms responsible for the anti-nutritive and hypocholesterolemic effects of this NSP, which could be equally important to increases in viscosity induced by intact β-glucan.

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LIST OF ABBREVIATIONS

ADG	AVERAGE DAILY GAIN
C	CARBON
DFI	DAILY FEED INTAKE
DHA	DECOSAHEXAENOIC
EMP	EMPTY
EPA	EICOSAPENTAENOIC
FA(S)	FATTY ACID(S)
FCR	FEED CONVERSION RATIO
GE	GROSS ENERGY
HDL	HIGH DENSITY LIPOPROTEIN
HMG CoA	β-HYDROXY-β-METHYLGLUTARYL COENZYME A
INT	INTESTINE
ISV	INSOLUBLE SOLIDS VOLUME
LDL	LOW DENSITY LIPOPROTEIN
Lp (a)	LIPOPROTEIN (a)
MUFA(S)	MONOUNSATURATED FATTY ACI(S)
N	NITROGEN
NSP	NON-STARCH POLYSACCHARIDES
PUFA(S)	POLYUNSATURATED FATTY ACID(S)

RO	REVERSE OSMOSIS
SFA(S)	SATURATED FATTY ACID(S)
TC	TOTAL CHOLESTEROL
TG	TRIGLYCERIDES
UFA(S)	UNSATURATED FATTY ACID(S)
UWL	UNSTIRRED WATER LAYER
VISC	VISCOSITY
VLDL	VERY LOW DENSITY LIPOPROTEIN
WHC	WATER HOLDING CAPACITY
β-GLUC	β-GLUCAN

CHAPTER 1

GENERAL INTRODUCTION

Barley is one of the four major grains produced in the world, which contributes significantly to the food supply as human food, malt products, and livestock feed (Nilan and Ullrich, 1993). It is extensively used in stockfeed formulations for ruminants, pigs, and poultry. However, when fed to poultry it is less digestible and yields less energy than when fed to pigs or ruminants. In addition, the low nutritional value of barley in poultry diets is accompanied by the production of wet droppings or sticky fecescan contribute to health and environmental concerns. Both low nutritive value and wet droppings have been attributed to the β-glucan content of barley (MacGregor and Fincher, 1993). β-glucan is a non-starch polysaccharide (NSP) found in the endosperm of grain walls (Newman *et al.*, 1989; Annison and Choct, 1991; MacGregor and Fincher, 1993), forming about 70% of the barley wall (Newman *et al.*, 1989), and cannot be digested by vertebrates (MacGregor and Fincher, 1993). It has been suggested that β-glucan impairs digestion through an increase in the viscosity of the digesta of chickens, which limits the diffusion and mixing of enzymes and degradation products. The overall effects are a lower nutrient digestibility and a slower rate of bird growth (MacGregor and Fincher, 1993). In addition to viscosity, Choct (1997) has proposed other mechanisms for the anti-nutritive effects of β-glucans such as changes in gut functions by modifications of the endogenous secretions of water, proteins, electrolytes, and lipids, as well as interactions with the gut microflora.

The anti-nutritive effects of barley β-glucans can be reduced through the use of enzymes, specifically β-glucanase (Wood, 1984; Campbell and Bedford, 1992; Bhatty, 1993). It has been suggested that β-glucanase hydrolyses β-glucan thereby decreasing its molecular weight and its viscosity (Bhatty, 1993), which is regarded as one of the main anti-nutritive factors of β-glucan (Annison and Choct, 1991).

In contrast to its anti-nutritive effects in chickens, β-glucan has demonstrated to possess hypocholesterolemic properties in both experimental animals and in humans

(Klopfenstein, 1988; Hecker *et al.*, 1998). The mechanisms proposed for this hypocholesterolemic action include bile acid binding and alterations in intestinal digestibility and absorption through an increase in the viscosity of the digesta (Klopfenstein, 1988).

The studies described in this thesis evaluated the anti-nutritive actions of barley β-glucan in broiler chickens, as well as its hypocholesterolemic effects in rats, through the use of a commercially prepared barley β-glucan extract derived from New Zealand barley. In order to do this, the thesis was divided into four experiments. The Experiments in Chapters 3 and 4 evaluated the anti-nutritive effects of β-glucans in chickens as well as the effects of heat treatment of the diet and the influence of a β-glucanase, on the anti-nutritive properties of β-glucan. Chapter 5 describes an *in vitro* digestibility method simulating the chicken's gastrointestinal tract, to predict the anti-nutritive effects of β-glucan on N and C digestibility *in vivo*, and on the physico-chemical properties of the digesta. Finally, in Chapter 6, an evaluation of the hypocholesterolemic effects of β-glucan, as well as the effects of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) on blood lipid levels in rats was conducted.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Each year, an estimate of two billion tonnes of cereal grains are produced throughout the world (Choct, 1997). Barley is one of the four major grains produced worldwide (Bhatty, 1993; Nilan and Ullrich, 1993). It contributes significantly to the food supply around the world as human food, malt products, and livestock feed (Nilan and Ullrich, 1993). For livestock, its use varies between 47 and 90% of the total production in different countries, and can be used as a major energy and protein source for swine, as a roughage source for ruminants, and to support egg production in laying hens. For broiler chickens, barley has to be treated to eliminate the β-glucan (Bhatty, 1993), which is a polysaccharide found in starchy endosperm of grain walls, and in barley it forms about 70% of this wall (Newman *et al.,* 1989). Vertebrates are incapable of degrading plant cell wall polysaccharides such as β-glucan. In broiler chickens, this polysaccharide impairs digestion by increasing digesta viscosity, which limits the diffusion and mixing of enzymes and degradation products. The overall effects are a lower digestibility of nutrients, and slower growth rates. B-glucan depolymerisation with enzymes can overcome the undesirable effects of barley (MacGregor and Fincher, 1993).

In contrast to their detrimental effects in poultry feeding, β-glucans are highly successful in lowering serum cholesterol concentrations (Newman *et al.,* 1989). Several human and animal studies have confirmed a hypocholesterolemic action of β-glucan. Various mechanisms have been proposed to account for the cholesterol-lowering effects of β-glucan, but none has neither been proved nor discarded.

The present work reviews the definition, classification, structure, and properties of barley β-glucans, as well as its anti-nutritive effects in poultry and its cholesterol-lowering properties in humans and experimental animals.

2.2 Non-Starch Polysaccharides: definition and classification

In 1976, Trowell (cited by Southgate, 1993; Spiller, 1993) defined dietary fibre based on the physiological characteristics of some of the plant cells as "the polymers of plants that cannot be digested by the endogenous secretions of the human digestive tract". Plant polysaccharides can be broadly separated into two distinct and chemically well-

defined types; the storage polysaccharide starch (α-glucan) and the cell wall polysaccharides (non α-glucan), which may conveniently be called non-starch polysaccharides (NSP) (Englyst, 1989). Thus, in 1987 Englyst and co-workers (cited by Southgate *et al.,* 1993) suggested the term NSP for the carbohydrate plant cell wall material originally called dietary fibre, less the lignin. This definition was considered to be a better definition than dietary fibre, and Trowell (cited by Englyst, 1989) considered it gives the best index of plant cell-wall polysacccharides, it is chemically precise, and it keeps with the original concept of dietary fibre.

The non-starch polysaccharides (NSP) are a group of complex carbohydrates, which excludes the α-glucan polysaccharides or starch (Kopinski *et al.,* 1995; Choct, 1997). They are largely plant cell wall polysaccharides, and have a structural function (Englyst *et al.,* 1983; Asp, 1996), thus, plant cell walls are the main source of dietary NSP. The NSP include cellulose, hemicellulose, and uronic acids, which together with lignin are sometimes referred to as dietary fibre. They contain arabinose, xylose, mannose, glucose and galactose residues as the main constituents sugars (Englyst *et al.,* 1983). Non-starch polysaccharides are resistant to enzymatic digestion in the small intestine of mammals (Englyst *et al.,* 1983; Baghurst *et al.,* 1996; Englyst and Hudson, 1996,), but in some animals, a considerable amount of NSP may be fermented in the small intestine (Asp, 1996). Most of the NSP can be classified according to many different criteria, for example neutral, which contain mainly neutral sugar residues, and acidic, containing mainly uronic acid residues or pectic substances. Another criteria classifies them in into three groups, A, B, and C, depending on their solubility at various pH (Asp, 1996). Non-starch polysaccharides can also be roughly separated into soluble and insoluble fractions depending on their solubility in water or weak alkali solutions (Choct, 1997). They also can be classified into storage NSP which include mannans, guar gum, galactans and xyloglucans, and those NSP performing a structural/protective role from which cellulose is the best known. Glucan and arabinoxylans from cereals also perform structural roles (Baghurst *et al.,* 1996). Another very similar classification groups the NSP into three categories; the cellulosic polysaccharides from which cellulose is the best known, the non-cellulosic polysaccharides which include uronic acid polymers, galactose polymers, arabinose polymers, xylan polymers, branched and substituted glucose polymers and mannose polymers, and the third group called the non-structural NSP which includes

NSP not present in the cell walls including galacturonans, and a range of gums and mucilagues (Johnson and Southgate, 1994). Another classification catalogues the NSP into three groups: cellulose, non-cellulosic polymers and pectic polysaccharide.

The non-cellulosic polymers include arabinoxylans, mixed linked β-glucans, mannanas, galactans, and xyloglucan, while the pectic polysaccharides include polygalacturonic acids, which may be substituted with arabinan, galactan, and arabinogalactan (Choct, 1997). This is summarised in Figure 2.1.

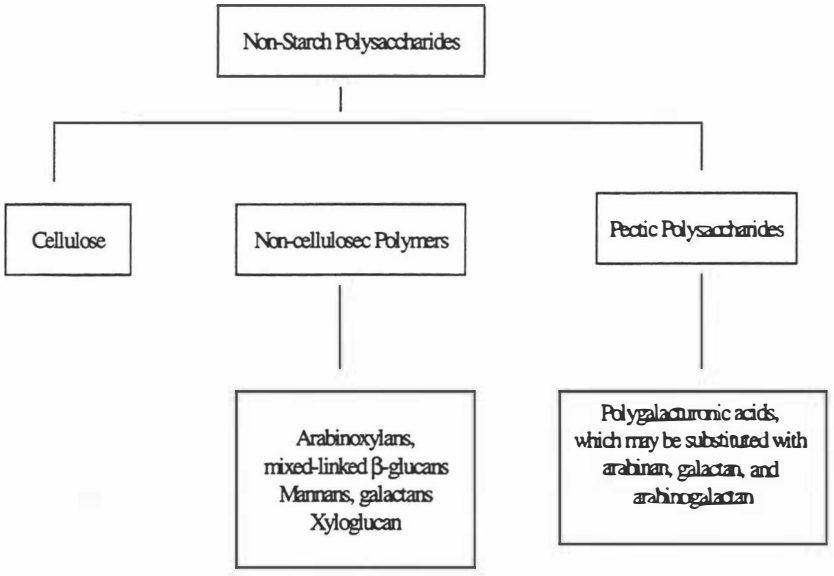

Figure 2.1. NSP Classification

Source: Choct, (1997).

2.3 Structure and chemistry of barley β-glucans

The primary cell walls of most cereal grains are composed of cellulose microfibrils, which are associated with glucomannan and are embedded in a matrix of hemicelluloses like arabinoxylan and/or β-D-glucan, cross linked by phenolic esters and /or proteins (Potty, 1996). Parenchymatous tissues of cereals have practically no pectin (Lineback and Rasper, 1988; Annison, 1993; Annison and Choct, 1994; Choct, 1997), except for

rice in which pectic substances have been reported to be present (Potty, 1996). In barley, the main NSP present are arabinoxylans or pentosans, and β-glucan (Bacic and Stone, 1981; Vietor *et al.,* 1992; Annison, 1993; Annison and Choct, 1994; Potty, 1996; Choct, 1997).

In cereals, a polysaccharide similar to glucose, which has some β-(1→3)-linkages, is found. This β-(1→3), (1→4)-D-glucan is commonly referred to as β-glucan (Linebabck and Rasper, 1988). Although this polysaccharide is ubiquitous amongst the cereals, it occurs in highest amounts in the endosperm of oats and barley (Wood, 1984; Klopfenstein, 1988; Choct, 1997), with concentrations values ranging from less than 2 percent to 11 percent in barley, depending on factors such as cultivar, method of determination, and growing conditions (Klopfenstein, 1988; Bhatty, 1993).

In barley, β-glucans represent a major component of the cell wall material (Lineback and Rasper, 1988), about 70 percent of the endosperm wall; the remainder consists mainly of arabinoxylans, glucomannans, cellulose, protein, and phenolic constituents (Newman *et al.,* 1989). β-glucans comprise a large portion of the aleurone and endosperm cell walls (Danielson *et al.,* 1997). Cereal β-glucan, like other β-glucans, is composed entirely of glucose units. The distinction among these polymers lies in the nature of the linkage between the units (Wood, 1984). β-glucans consist of a linear chain of cellotriosyl and cellotetraosyl units joined by both β-(1→3) and β-(1→4) linkages (Wood, 1984; Klopfenstein, 1989; Newman *et al.,* 1989; Oscarsson *et al.,* 1996; Choct, 1997; Danielson *et al.,* 1997). The β-glucans of barley contain approximately 70 percent (1→4) linkages and 30 percent (1→3) linkages, in which segments of two or three (1→4) linkages are separated by (1→3) linkages. However, it has been reported that up to five contiguous (1→3) linkages exist as minor structural features (Choct, 1997). In addition, it has been reported that they appear to exist in solution as extended, wormlike chains (Klopfenstein, 1988), and that the incorporation of the (1→3) linkages in this molecule breaks the regular structure of the β-(1→4) chains, preventing close packing of the chains to give a more soluble polymer.

The molecular weight reported for the water soluble β-glucans estimated by ultracentrifugation ranges from 200,000 to 300,000 corresponding to degrees of polymerisation of 1200 to 1850 monomers (Choct, 1997). Figure 2.2 shows the chemical structure of β-glucan.

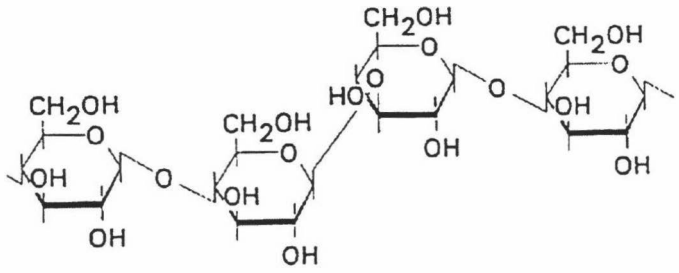

Figure 2.2. β-glucan structure

Source: Smits and Annison (1996).

2.4 Relationships between NSP chemistry and anti-nutritive effects

Originally, it was considered that NSP were fermented to a limited extent in the lower bowel of chickens and other monogastric species, making minor contributions to the nutrition of these animals. However, in recent years considerable evidence has been gathered indicating that cereal NSP possess anti-nutritive effects when present in broiler diets (Annison and Choct, 1991; Annison and Choct, 1994; Smits, 1996; Choct, 1997). Annison and Choct, (1994) reported that research with broiler chickens in the 70's and 80's showed that NSP could depress macronutrient digestion which, in turn, resulted in performance losses In 1983, White *et al.* noted a depression in the growth of broilers as well as increases in the viscosity of digesta samples when isolated barley β-glucan was added to the birds' diets. The anti-nutritive activity of barley was confirmed by further studies, which also demonstrated that the polymer passed through the chicken unchanged (Annison and Choct, 1991).

Monogastric animals are incapable of synthesing endogenous β-glucanase, which is necessary to hydrolyse the β-glucans found in barley. Although barley kernels contain endogenous β-glucanase, its amount is not sufficient to completely hydrolyse the β-glucans found in the grain (Danielson *et al.,* 1997).

β-glucan must influence any process requiring rupture of the cell wall. For example, when the intake of whole grains occurs, where the cell wall is not broken, the nutrients of that cell will be less available until digestive action or further processing disrupts the cell wall integrity. This may occur late in the digestive process, if it occurs at all. This phenomenon may contribute to slow the release of nutrients. Hydration of cell walls during processing, cooking or consumption will lead to local high viscosity and inhibition of diffusion, again, delaying nutrient release (Wood, 1984).

It has generally been conceded that the main anti-nutritive effects of NSP are associated with their viscous nature, their morphological and physiological effects on the gastrointestinal tract, and their interaction with the gut microflora (Choct, 1997). Many of these effects depend on the physical properties of the NSP, the most relevant being solubility, viscosity, water holding capacity, and their ability to adsorb small molecules and ions (Oakenfull, 1993). In turn, the physico-chemical properties and functions of NSP vary considerably, depending on their composition and structure (Potty, 1996).

2.4.1 Solubility

Some of the NSP are water-soluble. Solubility is determined by the presence of branches in the polysaccharide structure (Oakenfull, 1993). Solubility in β-glucans is dependent upon the number and distribution of the (1→3) linkages, whose presence causes an irregularity in the chain. This prevents them from associating closely, which allows water molecules to come between them, thus making β-glucans water soluble (Annison, 1993; Bhatty, 1993). β-glucans exhibit differential solubility. Differences are apparent in the ratio of (1→3) to (1→4) linkages in each of the fractions. However, no relationships have been defined yet between the proportion of (1→3) or (1→4) linkages and lower or higher solubility. Other factors affecting β-glucan solubility include molecular size differences, and associations between β-glucans and proteins (Bacic and Stone, 1981).

Soluble NSP interact with the glycocalyx of the brush border in the small intestine, thickening the rate-limiting unstirred water layer of the mucosa, and thus reducing the efficiency of nutrient absorption through the intestinal wall (Choct, 1997).

2.4.2 Viscosity

Soluble NSP such as pectins and β-glucan can develop significant viscosity (Annison and Choct, 1994; Smits, 1996; Plaammi, 1997). Viscosity is caused by physical interactions between the polysaccharide molecules in solution (Englyst, 1989), and is dependent in a number of factors such as the polysaccharides solubility (Groff *et al.*, 1995; Smits, 1996; Choct, 1997), the size of the molecule, whether it is branched or linear, the presence of charged groups (Annison and Choct, 1994; Smits, 1996), its molecular weight distribution (Plaammi, 1997), the surrounding structures (Smits, 1996), and the concentration. At low concentrations the viscosity is increased by a direct interaction between the polysaccharide and the water molecules. With increasing concentrations, the polysaccharide molecules interact themselves, becoming entangled in a network, which can cause great increases in the viscosity. This process depends on the formation of junction zones between the polysaccharide molecules. (Oakenfull, 1993; Smits, 1996).

β-glucan also has a propensity to form aqueous solutions of high viscosity. These polysaccharides have an irregular shape overall, which reduces its tendency to pack into stable, regular molecular aggregates. Therefore, they remain relatively soluble in water. The high intrinsic viscosities are attributable mainly to the molecular asymmetry in combination with their high molecular weight (MacGregor and Fincher, 1993).

Viscosity has been recognised as a major factor in the anti-nutritive effects of NSP in monogastric diets (Choct 1997). It has an antimotility action in the intestine, which can influence absorption by delaying gastric emptying, impairing convection or mixing in the upper small intestine, altering the absorptive site, and delaying small bowel transit time (Plaammi, 1997). As well as decreasing the rates of diffusion of substrates and digestive enzymes, high gut viscosity also hinders the effective substrate-enzyme interaction at the mucosal surface (Choct, 1997). All these effects are further reflected as

changes in postprandial sugar and lipid levels, and in reduced bioavailability of micronutrients (Plaammi, 1997).

In general, the relationship between β-glucan content and extract viscosity can vary because of factors such as different cereal grains or cultivar, variations in structure and molecular weight of the polysaccharide, and different proportions of soluble and insoluble forms (Wood, 1984).

2.4.3 Water holding capacity

The water holding capacity of fibre in general has important physiological effects in both the upper and lower intestine. Hydration of fibres occurs by adsorption to the surface of the macromolecules and by entrapment within the interstices of the fibrous or gel matrix. The fibre saturation capacity or upper limit of water held is determined by the chemistry and morphology of the macromolecules and by the pH and electrolyte concentration of the surrounding medium (Kay, 1982).

Polysaccharides are hydrophilic molecules, thus, they have the ability to hold water, particularly those polysaccharides containing sugar residues with free polar groups (Oakenfull, 1993). Soluble NSP can entrap water molecules through the formation of networks (Annison and Choct, 1994). When the interactions of the polysaccharide molecules become great, gel formation can occur (Smits, 1996). The presence of sugar residues with free polar groups confers a significant hydrophilic capacity to polysaccharides. In the upper intestine, the water-holding capacity of fibre may affect the pattern of nutrient absorption, postprandial satiety, and intestinal motility (Kay, 1982).

Particle size influences the water-holding capacity due to the fact that it determines the volume of the interstitial space within the fibre matrix available for water entrapment (Kay, 1982). In a review of dietary fibre, Kritchevsky (1988) mentioned that finely-ground wheat bran holds 26 percent less water than unground bran. Actually, Kay (1982) suggests that the physical structure of fibre is the most important determinant of hydration.

2.4.4 Binding of ions and small molecules

Polysaccharides have the ability to bind other polar molecules and ions (Oakenfull, 1993). Organic materials that can be bound to fibre include bile acids, other steroids, various toxic compounds, and bacteria (Kay, 1982). The major factors determining their ability to bind metal ions are the number of free carboxyl groups and particularly their uronic acid content (Oakenfull, 1993). Likewise, in some NSP, the three-dimensional molecule structure allows a chelation of ions to occur. Cations can form ionic bridges between NSP molecules. This in turn can influence their viscosity and gel formation properties. In addition, small molecules may be weakly bound to NSP through both hydrophobic and hydrophylic bond interactions (Smits, 1996; Choct, 1997).

Although the chemical structure of β-glucans plays an important role in conferring its physico-chemical and anti-nutritive properties, other factors are also important. These include molecular weight, protein concentrations, protein effects on molecular weight, the sequence of linkages (Wood, 1984), environmental growing conditions and barley variety (Wood, 1984; Newman *et al.,* 1989).

2.5 Anti-nutritive effects of NSP in chickens

In poultry, the ingestion of cereals with a high content of cell wall NSP inhibits the digestion of the major nutrients, which results in a decrease in the apparent metabolisable energy (AME) values of the cereals (Annison, 1993). Several research groups have characterised the anti-nutritional effects of barley β-glucans. Most studies report that these compounds cause growth depression accompanied by sticky droppings (Choct and Annison, 1990; Choct *et al.,* 1992; Choct and Annison, 1992A; Choct and Annison, 1992B; Annison, 1993; Kopinski *et al.,* 1995; Choct *et al.,* 1996). These effects have been associated with an inhibition of starch and nitrogen digestibility, fat absorption and energy digestibility, which results in depression of growth and feed conversion efficiency (Annison, 1993).

Although the exact mechanism of action of the NSP is unclear, several possible effects in the gut, which can be responsible for their anti-nutritive properties, have been described. These include alterations in the viscosity of the digesta, changes in intestinal

physiology, microfloral implications and NSP adsorption of molecules and interactions with other molecules.

2.5.1 Viscosity alterations

There is evidence that the ability of the cereal NSP to increase the viscosity of the digesta of chickens is a major factor in the mechanism of action of NSP anti-nutritive effects (Annison, 1993). This is supported by work in which it has been demonstrated that the adverse effects of soluble NSP diminishes when these polymers are partially hydrolysed using enzymes capable of degrading β-glucans (White *et al.,* 1983; Choct and Annison, 1992a; Choct and Annison, 1992b; Annison, 1993; Annison and Choct, 1994; Kopinski, 1995; Bedford, 1996; Choct *et al.,* 1996; Choct, 1997). It has been observed that even relatively small reductions in viscosity can lead to significant improvements in nutrient digestion (Bedford, 1996). Other works supporting the hypothesis that viscosity is involved in the anti-nutritive effects caused by NSP in poultry diets have shown that barley with high extract viscosity are more detrimental to chickens than those with low extract viscosity (Choct and Annison, 1992A; Annison, 1993).

The mechanism by which viscosity affects nutrient digestion and absorption is not totally understood. However, several hypotheses have been proposed (Choct and Annison, 1992a). These include the effect of viscosity on mixing of intestinal contents and the altered transport of nutrients at the mucosal surface (White *et al.,* 1983).

The increase of the viscosity in the intestinal contents caused by the presence of NSP in the diet will slow the rate of mixing of digestive enzymes with the substrate (White *et al.,* 1983; Choct and Annison, 1992a; Morris, 1992; Read and Eastwood, 1992; Bedford, 1996), and reduce the contact intensity between potential nutrients and the digestive secretions (Morris, 1992; Read and Eastwood, 1992; Smits, 1996), hindering their interaction at the mucosal surface (Choct and Annison, 1992a; Morris, 1992; Read and Eastwood, 1992), depressing feed passage rate, and increasing microbial populations in the small intestine (Read and Eastwood, 1992; Bedford, 1996).

The viscosity of the intestinal fluid is also likely to alter the transport properties of nutrients at the mucosal surface. For absorption to take place, nutrients must cross an

aqueous barrier, the unstirred water layer (UWL), which is the fluid surface tangent to the apex of the villus. The highest concentration of nutrients in the lumen is outside of the UWL. The rate of nutrient diffusion to the apices of the villi is a function of the thickness of the UWL. As the thickness increases, the rate of diffusion decreases. The UWL is reduced by flow near the surface of the mucosa, and this flow varies due to the continuous churning of the intestinal contents. The viscosity of the intestinal fluid also alters the thickness of the layer. High viscosity of the intestinal fluid augments the thickness of the UWL, decreasing the rate of diffusion of nutrients (White *et al.*, 1983; Morris, 1992; Smits, 1996). Mucus also participates in the formation of the UWL by increasing the volume of adherent mucosal fluid and its viscosity, and it has been observed that the viscosity caused by NSP induces a secretory response of mucus which may increase the resistance for transport of nutrients through the UWL by increasing the thickness of the mucus layer and/or by changing the physicochemical properties of the mucus (Smits, 1996).However, according to Morris (1992), the inhibition of nutrient diffusion across the UWL is likely to be significant only at very high polymer concentrations.

Intestinal viscosity is dependent upon many interacting factors (Bedford, 1996a; Bedford, 1996b), the most important being listed in table 2.1. In addition, it should be considered that viscosity may or may not be detrimental to performance depending on the prevalence of risk factors which respond to increased viscosity (Bedford, 1996b). Some of the risk factors which augments the negative effects of high viscosity are listed in table 2.2.

Table 2.1. Factors affecting intestinal viscosity

FACTOR	EFFECT ON VISCOSITY
Grain variety	+/-
Climate of production of grain	+/-
Processing the diet	Higher temperature and shear increase viscosity
Inclusion level of grain	Higher inclusion levels increase viscosity
Breed of bird	Breeds differ in intestinal viscosity on identical diets
Age of bird	Viscosity falls with age

Source: Bedford (1996).

Table 2.2. Risk factors, which will augment the negative effects of high viscosity

RESPONSE	DIRECTION OF RESPONSE WITH INCREASED VISCOSITY	CONSIDERATIONS
Protein/starch digestibility	Negative	More apparent in younger birds when digestive capacity is limited
Fat digestibility	Negative	Risk greatest with older birds since fat level increases and more saturated fats used
Microbial competition for ileal nutrients	Increased	Risk greatest in older birds with more mature flora and in dirty environment
Microbial provision of energy due to caecal VFA's	Depressed	Older birds with more mature caeca more sensitive. Use of enzymes provides oligosaccharides for fermentation

Source: Bedford (1996).

2.5.2 Changes in intestinal physiology

Feeding diets based on viscous grains, *i.e.* rye, barley, oats, triticale and wheat, results in significant increases in the relative size of the digestive tract (Bedford, 1996a). As the viscosity increases, the bird adapts to the perceived deficiency in digestive capacity by increased pancreatic enzyme output and villus surface area/gut weight (Bedford, 1996b). This is reflected by an increased intestinal mass and pancreatic size. However, enzyme output is modulated before changes in size of the digestive tract occur. One of the first responses to increased viscosity is greater endogenous losses due to attempts of the bird to overcome the poor mixing of enzymes and food in the viscous digesta. At higher viscosities, even these attempts are overwhelmed and diffusion constraints will slow digestion as a whole (Bedford, 1996a). In other words, with excessively high viscosity the bird has no further adaptive capabilities, so nutrients pass through the small intestine undigested (Bedford, 1996b).

It is presumed that gross changes in intestinal size become apparent at great extremes in viscosity (Bedford, 1996a). Viscous gums increase stomach size and intestinal length in

rats and decrease the density of crypts and villi (Annison, 1996; Bedford, 1996a). Similar results have been observed in rye-fed and barley-fed chicks. In both cases, xylanase and β-glucanase supplementation respectively reversed the adverse effects of the NSP.

The mechanism by which these changes are brought about might well involve microbial interaction (Bedford, 1996a), and is discussed in the following section.

2.5.3 Microflora implications

Although the effect of viscosity appears to be a major factor responsible for the anti-nutritive properties of NSP, considerable evidence suggests that gut microflora of chickens might also be directly or indirectly involved (Choct and Annison, 1992A; Annison, 1993; Kopinski *et al.,* 1995; Bedford, 1996a; Bedford and Morgan, 1996; Choct *et al.,* 1996; Smits, 1996; Choct, 1997). This evidence includes work in which it has been demonstrated that the addition of antibiotics to diets containing rye and barley improves the performance of chickens (Choct and Annison, 1992A; Annison, 1993; Bedford, 1996a; Choct *et al.,* 1996; Smits, 1996; Choct, 1997). It has also been reported that the deleterious effects of wheat pentosans on the digestibility of long chain fatty acids was less pronounced in caecectomised chickens than in intact chickens. In another study cited by Smits (1996), it was reported that in germ-free chicks, fibre viscosity had negligible effects on fat digestibility, and the authors assumed that viscous NSP must modify bacterial activity in order to lower fat digestibility.

It has been proposed that as viscous polysaccharides increase residence time of digesta, the oxygen tension is decreased and this favours an increased microbial activity (Choct and Annison, 1992A; Bedford, 1996a; Choct *et al.,* 1996; Smits, 1996). Bacteria then colonise the proximal small intestine (Smits, 1996). According to Bedford (1996a), the bacterial population in the intestine can have an effect on the efficiency of host digestion by one of three mechanisms:

1. Invasion/Disease: Disease alters the energy status of the bird, which mounts an immune response which increases maintenance costs reducing feed efficiency. Evidence exists that ingestion of wheat and barley can predispose birds to diseases like

coccidiosis and necrotic enteritis. The mechanisms involved have yet to be proposed, but it may be linked with an increase in the viscosity of intestinal contents.

2. Competition for resources/Provision of resources: Ideally, the bird should extract as much of the nutrients as it can before the small intestinal bacteria are exposed to the digesta. The slower the rate of host digestion, the greater the opportunity for ingested nutrients to be metabolised by bacteria. Intestinal viscosity has a significant effect on rate of digestion, and this ability can markedly influence the partitioning of energy between the bird and the microflora. Gut bacteria can take advantage of the viscous environment and this has as a net result a depression in weight gain and feed conversion ratio.

3. Secondary effects of metabolites: The fermentation of substrates by bacteria may well result in the production of many metabolites or products, which may directly or indirectly affect the digestive system. Rye-based diets and isolated wheat arabinoxylans have been shown to significantly increase the concentration of short-chain fatty acids in the lumen of the intestine. These fatty acids can reduce the pH of the gut, and this has a profound negative effect on pancreatic secretion. Luminal bacteria can also produce polyamines, and when bacterial concentration increases, polyamine concentration increases as well, which augments the small intestinal and colonic mucosal growth rate.

Whether the effects of the metabolites are beneficial or detrimental depends very much upon the status of the intestinal tract. If the bird is coping well, stimulation of intestinal growth will only increase maintenance costs and thereby reduce feed efficiency (Bedford, 1996a).

2.5.4 NSP adsorption of molecules and interactions with other molecules

Some polysaccharides exhibit strong surface activities and thus adsorb to particles and interact with other molecules. The first step in fat digestion is the formation of an emulsion in the lumen of the gut. Many NSP are able to stabilise oil/water emulsions by interacting with the oil/water interface. Pancreatic lipases act only at the lipid/water interface and it is possible that the presence of NSP at this interface might be the mechanism by which they inhibit the digestion of fats (Annison, 1993). Also, lipid metabolism in the intestine can be influenced by the fact that NSP can bind bile salts,

lipids and cholesterol. Viscous NSP can enhance bile acid secretion and subsequently result in significant loss of these acids in the faeces (Choct, 1996; 1997). This, in turn, can result in increased synthesis of bile acids from cholesterol in the liver to re-establish the composite pool of these metabolites in the enterohepatic circulation. All these may ultimately influence the absorption of lipids and cholesterol in the intestine, leading to major changes in the digestive and absorptive dynamics of the gut with consequent poor overall efficiency in nutrient assimilation by the animal (Choct, 1997).

It has also been suggested that NSP may inhibit digestion by directly complexing digestive enzymes. Protein/polysaccharide interactions are common as both types of molecules present both hydrophobic and hydrophylic surfaces. Enzymes may also be inhibited by the direct complexing of their co-factors. The activity of amylases, for example, is dependent on the presence of Ca^{++} ions, and some NSP bind cations very strongly and thus may inhibit amylase activity (Annison, 1993).

2.6 The use of β-glucanases in poultry

The beneficial effects observed with the inclusion of dietary enzymes in barley-based diets for poultry have been known for many years, are well documented, and have been the subject for several comprehensive reviews (Annison and Choct, 1991; Campbell and Bedford, 1992; Bedford and Morgan, 1996). The use of enzymes in poultry diets is now a common strategy in many countries where wheat and barley are the predominant grains fed to poultry (Bedford and Morgan, 1996). Research in this area has concentrated mainly on improving the nutritive value of barley (Annison and Choct, 1991).

2.6.1 Mechanism of action of enzymes

Although the use of enzymes has a widespread acceptance by the industry, there is little agreement on the mechanism (s) by which these products function in the birds' intestinal tract (Bedford and Morgan, 1996). One of the original theories was that β-glucanases helped disrupt the cell wall structure of the barley endosperm, allowing more rapid access of the bird's endogenous amylases and proteases to the cell contents. However, subsequent work demonstrated that disruption of plant cell walls could not explain all

the responses observed (Bedford, 1996b). Further research led to the concept that performance improvement in relation to enzyme supplementation is not due to polysaccharide hydrolysis and subsequent absorption of the released sugars, but to a break down of the β-glucans into smaller polymers. This would alter β-glucans' ability to form highly viscous solutions, which inhibit nutrient diffusion and transport (Annison and Choct, 1991). However, chicks fed low-viscosity barleys frequently respond to dietary enzyme supplementation, which is difficult to explain by viscosity reduction alone. In this case, maybe breakage of the cell wall by the enzyme and the subsequent exposure of nutrients such as starch and protein to the digestive enzymes within the gut lumen can explain the improvement observed (Campbell and Bedford, 1992).

2.6.2 Enzyme sources

Enzymes capable of hydrolysing grain cell walls are found in a wide range of microbial sources. Dietary enzymes are mainly derived from fungal and bacterial fermentation. Actually, fungal fermentation is the source of many commercial supplements (Classen and Bedford, 1991). Characterisation of enzyme sources is difficult due to the fact that there are multiple enzyme forms and that there is an uncertainty regarding their substrate specificity. Also, multiple forms having the same activity are common, but they frequently have different characteristics such as temperature stability and pH optimum. Many enzyme activities are not characterised or are of unknown significance with regard to improvements in the nutritional value of grain (Classen and Bedford, 1991).

However, the increased knowledge of the structure of grain cell walls allowed scientists to tailor-make specific enzymes that can act on the substrates of interest in commercial supplements, even though the enzyme additives destined for animal feeds are crude preparations that generally exhibit activity towards a range of substrate materials. This is not necessarily undesirable since most animal feeds are not based on a single cereal or protein source (Campbell and Bedford, 1992). The enzyme activities necessary for the hydrolysis of β-glucan are shown in Table 2.3

Beta-glucanase has been broadly accepted in poultry feeds. It is normally added in a dry form to the feed and acts upon hydration within the digestive tract. This application has

led to widespread adoption of barley into poultry feeds, an area where barley had previously been used to a very limited extent (Campbell and Bedford, 1992).

Table 2.3 Primary enzyme activity required for hydrolysis of cereal grain cell wall β-glucan

ENZYME	
Endo-1,3;1,4- β-glucanase	(EC 3.2.1.73)*
Endo-1,4- β-glucanase	(EC 3.2.1.4)*
β-glucosidase	(EC 3.2.1.21)*
β-glucan solubilase	

*Enzyme nomenclature. Academic Press, New York (1978).

Source: Classen & Bedford (1991).

2.6.3 Substrate hydrolysis

It is important to know the level of hydrolysis required to improve the nutritional value of cereal grains because it determines the type and amount of enzyme to be used. In the case of β-glucan, it is presumed that a total hydrolysis is of some nutritional significance because of the ready absorption of glucose. However, it is improbable that complete hydrolysis to monosaccharide constituents occurs within the time frame of feed passage through the digestive tract in poultry. This in turn may cause growth depression and an increase in fecal moisture. A partial hydrolysis of arabinoxylan, a NSP found in wheat yields undigested oligosaccharides, which may also lead to unfavourable microbial growth in the hind gut.

The hydrolisis of β-glucan has primarily been accomplished by the use of enzyme sources containing endo- β-glucanase activity, which is the most cost effective and acceptable method. This enzyme cleaves the 1→4 linkages where the glucosyl residue on the reducing side is joined to the next glucosyl by a 1→3 bond (Classen and Bedford, 1991).

2.6.4 Enzyme stability

During feed processing and passage through the digestive tract, dietary enzymes are exposed to hostile environments such as high temperatures during pelleting, low pH in the proventriculus and gizzard, and the effect of proteolytic enzymes. For this reason it is important to select sources with enzymes capable of surviving to their site of activity, the small intestine (Classen and Bedford, 1991).

2.6.5 Factors influencing enzyme response

2.6.5.1 Genotypic and environmental differences in barley

The response to dietary β-glucanase is not uniform among barleys. This is due to the fact that β-glucan level and viscosity are affected by both environment and barley genotype (Campbell and Bedford, 1992). Genotypic effects on β-glucan content are well established, and regional variations in the nutritional barley are known to occur. These regional variations appear to be influenced by climatic factors. Moisture stress during crop maturation increases β-glucan content and viscosity. Maturity stage also plays an important role, with β-glucan content increasing during kernel development and solubility decreasing with maturity (Classen and Bedford, 1991; Campbell and Bedford, 1992).

2.6.5.2 Bird age

The negative effects observed in chickens fed barley–based diets are age-dependent (Classen and Bedford, 1992). It has been reported that the major adverse effects occur during the first four weeks of age, and that these effects are related to feed transit time, with older birds transporting viscous materials in the gastrointestinal tract more readily than young birds. However, research indicates that some beneficial effects are still seen beyond four weeks of age (Classen and Bedford, 1991).

2.7 Effects of β-glucan on plasma cholesterol and phospholipids

2.7.1 Dietary fibre and lipid metabolism

Several sources of dietary fibre have been demonstrated to alter lipid metabolism in both human and animal studies (Katzumi and Sugano, 1986, Schneeman and Lefevre, 1986). In different animal models, the direct effects of dietary fibre supplements on certain aspects of fat digestion and on the absorption of fatty acids and cholesterol have been assessed (Cassidy and Calvert, 1993). In a review on the effects of different dietary fibres on cholesterol metabolism in experimental animals, Kritchevsky and Story (1992) mentioned that different types of fibre exert different effects. For example, pectin and other gelling fibres like mannan or guar gum can reduce serum cholesterol levels in rats regardless of whether the diet contains cholesterol or not. They also mention that cellulose, gum arabic, and agars do not have hypocholesterolemic effects. Actually, the different physicochemical properties of each fibre determine at which extent cholesterol absorption is inhibited (Ikeda *et al.,* 1989). The water-soluble fibres have been described as the most effective in lowering plasma cholesterol levels (Chen and Anderson, 1986; Gallaher and Schneeman, 1986; Schneeman and Lefvre, 1986; Mazur *et al.,* 1990; Newman *et al.,* 1992; Hecker *et al.,* 1998). These fibres may include pectins, several gums, and legumes. Among the cereal grains, oats and barley appear to be the most effective. Both grains appear to have a high proportion of soluble dietary fibre, specially mixed linked (1→3) (1→4) β-D glucan (Hecker *et al.,* 1998; Newman *et al.,* 1992), and have been demonstrated to possess hypocholesterolemic effects in both experimental animals and in humans (Klopfenstein, 1988; Hecker *et al*, 1998).

Not all studies have reported whether the cholesterol-lowering effects of β-glucan observed occur specifically in total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), or in very low-density lipoprotein cholesterol (VLDL-C). Topping (1991) reported one study in which NSP isolates high in soluble NSP reduced TC and LDL-C between 6 and 30% in hypercholesterolemic subjects. In another study by McIntosh *et al.* (1991), the authors reported a hypocholesterolemic effect of barley β-glucan with slight reductions observed for LDL-C in mildly hypercholesterolemic men. In rats fed tortillas supplemented with β-glucan, the LDL-C reported was significantly lower compared to the control group,

but no differences for TC, HDL-C or triglycerides were observed (Hecker *et al.,* 1998). In contrast to all these studies, Mazur *et al.* (1990) fed a highly fermentable carbohydrate to rats and found that the experimental diet markedly reduced the proportion of HDL_1 subpopulation in HDL-C isolated by centrifugation. Schneeman *et al.* (cited by Mazur *et al.,* 1991) also reported modifications in HDL-C composition when various dietary fibres were fed to rats.

2.7.2 Mechanisms of dietary fibre influencing lipid absorption and metabolism

The mechanisms whereby fibre exerts its effects on lipids and cholesterol absorption and metabolism are not fully understood and have been the subject of intensive investigations (Ikeda *et al,* 1989; Mazur *et al.,* 1990). Several mechanisms have been proposed. Amongst these the best studied include A) Binding of bile acids, B) Altered intestinal digestion and absorption, and C) Reduced hepatic cholesterol synthesis by propionate.

However, it is possible that multiple mechanisms are involved in the hypo-cholesterolemic effects of dietary fibre and that these mechanisms vary among different fibre sources, because none of the three hypotheses previously mentioned have been entirely consistent with the observations accumulated (Gallaher *et. al.*, 1992). Even though the evidence is not entirely satisfactory, it is important to outline the ways each of these mechanisms are thought to work.

2.7.2.1 Bile acid binding

Several *in vitro* techniques have demonstrated the binding of bile acids to various grains, food fibre sources, and isolated fibre components (Kritchevsky, 1982; Vahouny, 1982; Ebihara and Schneeman, 1989; Gallaher and Schneeman, 1986; Cassidy and Calvert, 1992). Factors that influence the absorption of bile acids by dietary fibre include pH, osmolality, the bile acid structure, the nature of the micelles, and the physical and chemical form of the fibre. In a relatively low pH the binding of bile acids is greater. In addition, it is probable that the adsorption of bile acids has a hydrophobic nature. Among the different fibres studied, those that appear to be most effective in bile acid sequestration are the mucilaginous and gel-forming polysaccharides, as well as the

lignins (Vahouny, 1982). The type of bile acid or salt used can also affect the extent of binding (Kritchevsky, 1982). Other factors affecting the response include the length of the treatment period, and specially the type of population studied (Miettinnen, 1987).

There are two different factors that could be important as a consequence of bile acid binding by fibre. First, the bile acid binding could lead to reduced bile acid absorption, increasing its excretion. This in turn would increase the amount of cholesterol needed to maintain the bile acid pool (Story, 1985; Gallaher and Schneeman, 1986). Thus, if cholesterol synthesis could not compensate for the increased rate of catabolism, the cholesterol pool would be reduced (Gallaher and Schneeman, 1986). Second, the bile acids bound to fibre would be unavailable for micelle formation, and as a consequence the amount of lipid solubilised within the intestine, including cholesterol, could be decreased, leading to a reduced lipid absorption (Story, 1985; Gallaher and Schneeman, 1986).

If these hypotheses were valid, an increase in faecal steroid excretion in response to ingestion of dietary fibre, which adsorbs bile acids *in vitro*, would be expected (Kritchevsky and Story, 1993). However, in a review of the modification of steroid excretion in response to dietary fibre, Story (1986) concluded, "changes in bile acid excretion alone are neither consistent nor large enough to account for changes in serum cholesterol". In some cases there is an increase in bile acid excretion when feeding fibre, and no changes in cholesterol levels are registered, while in other cases a lowering of cholesterol levels is not accompanied by an increase in bile acid excretion. These data indicates that the hypothesis concerning absorption-excretion is not valid for all situations.

A second possible effect of fibre on bile acid metabolism could be the alteration of the spectrum of faecal bile acids by a change in their concentration (Story, 1986). This change could be in response to either absorption of the salts or to water absorption due to the water holding capacity of the fibres (Story, 1985). It has been observed that an increase in CDC (Chenodeoxicholic salts) especially, occurs in response to many hypocholesterolemic fibres. However, there are some notable exceptions like oat bran in humans. All these changes can alter bile saturation, cholesterol absorption, and bile acid reabsorption from the small intestine.

According to Story (1986), the entire hypothesis of bile acid binding is yet to be defined by investigating the changes in relative amounts of bile acids affected by the amount of bile acid in each pool, not only the amount in the intestine.

2.7.2.2 Alterations in intestinal digestion and absorption

It has been demonstrated that NSP can affect lipid digestion and absorption mainly through an increase in the viscosity of the digesta (Topping, 1991). Ingestion of viscous fibre components has been shown to delay gastric emptying (Vahouny, 1982), and to decrease the diffusion process of substrates and enzymes in the small intestine hindering their effective interaction at the mucosal surface (Ikegami *et al.*, 1990). These fibres reduce rapid accessibility of the micelle to intestinal absorptive surface, decreasing lipid absorption (Ikeda *et al.*, 1989; Newman *et al.*, 1992; Cassidy and Calvert, 1993; Imazumi and Sugano, 1986). It has also been suggested that the gelling fibres may modify the unstirred water layer, influencing nutrient flux and absorption in the intestine (Vahouny, 1982; Cassidy and Calvert, 1993). Upper small intestine delay, whether by an increased transit rate or by a limited micellar lipids diffusion, will ultimately result in decreased overall lipid absorption, which can be explained, at least in part, by two major influences on lipid absorption: The first involves the continued efficiency of micellar solubilisation. During the delayed lipid absorption the phospholipids and the bile acids components of micelles are lost and this, in turn, alters solubility and equilibrium characteristics of cholesterol, fatty acids, and monoglycerides in the small bowel. The second effect on lipid absorption includes an alteration of the normal route of absorption of long chain fatty acids, as well as an influence on the overall efficiency of absorption of lipids in general due to a delay in lipid absorption in the upper intestine (Vahouny, 1982).

The hypothesis that viscosity and lipid malabsorption are responsible for the hypocholesterolemic effects of fibre is supported by a number of studies. In a review of the effects of dietary fibre on intestinal absorption, Cassidy and Calvert (1993) mentioned that from several studies measuring lipid recoveries in the thoracic duct or mesenteric lymph in animal models, it can be suggested that the presence of either soluble or insoluble fibre can slow the lymphatic absorption of both cholesterol and fatty acids. However, other authors consider that the evidence linking NSP chemical

structure, physical properties, and physical action is "rather slight". Topping (1991) mentioned some studies in which viscous fibres had hypocholesterolemic effects, but in a study in which galactomanns of different viscosities were fed to rats, the gum with the lowest viscosity had the best hypocholesterolemic effect. He suggested that while viscosity might be involved in a modification of fat absorption and in cholesterol reduction, the relationship is not necessarily a simple one. Clearly, more research is needed.

2.7.2.3 Reduced hepatic cholesterol synthesis by propionate

Dietary fibres that decrease plasma and liver cholesterol concentrations are fermented almost completely by colonic bacteria to short-chain fatty acids such as acetic, propionic, and butyric, which are then absorbed almost completely by the large intestine. It has been suggested that some of these short-chain fatty acids, especially propionic acid, may be the product that mediates these hypocholesterolemic effects (Anonymous, 1987; Bergman, 1990; Gallaher *et al.*, 1993; Thomas *et al.*, 1984; Miettinen, 1987; Truswell, 1993).

A number of experiments have been conducted to study the effects of propionate in cholesterol homeostasis. Experiments with rats have shown that the inclusion of propionate in purified diets lowers cholesterol concentrations in both serum and liver (Anonymous, 1987; Bergman, 1990). The same effects have been observed in pigs (Anonymous, 1987). Inclusion of propionic acid at different concentrations between 3.4 and 10.4 percent of the diet dry matter reduced serum cholesterol concentrations up to 14%. From these studies it was also observed that serum cholesterol decrease occurred at the expense of HDL-cholesterol and that LDL-cholesterol concentrations were not affected. However, earlier studies showed a decrease in both, HDL- and LDL-cholesterol with diets containing propionic acid or calcium propionate.

Several hypotheses have been proposed in an attempt to explain cholesterol reductions by propionate or propionic acid, although none have been proved. It has been suggested that the activity of 3-hydroxy-3-methylglutaryl CoA (HMG CoA) synthase, probably the rate-limiting enzyme of cholesterol synthesis in animal tissues, is affected by propionate. This hypothesis is supported by studies in which inhibited HMG CoA

synthase activity in bovine liver (Anonymous, 1987). Also, Ide *et al.* (cited by Anonymous, 1987) found that short-chain fatty tended to decrease HMG CoA reductase activity while long-chain fatty acids tended to produce greater enzyme activity.

Anonymous (1987), also suggested that propionate may be a substrate for cholesterogenesis, but the precise pathway for incorporation of propionate on cholesterol metabolism depends on the balance between its conversion to cholesterol and its regulatory effects on cholesterol synthesis, probably through inhibition of HMG CoA synthase and HMG CoA reductase.

Anderson and Chen (1979) proposed that lipid metabolism in the liver can be altered in several ways by the short-chain fatty acid products of fibres. They mention that a steady rate of entry of small quantities of short-chain fatty acids into the portal vein might alter hepatic fatty acids and triglyceride metabolism by a) altering the oxidation-reduction state, b) inhibiting pyruvate dehydrogenase via the formation of acetyl CoA, and c) an influence in hepatic ketogenesis by providing excessive amounts of ketone precursors. As a consequence of the interaction of these factors, the triglyceride synthesis or the release of VLDL would tend to decrease, having as a result lower fasting and postprandial triglyceride values.

However, in some studies no evidence of the hypocholesterolemic effects of propionate have been found. Gallaher *et al.* (1993) found no significant differences in hepatic cholesterol synthesis among diets containing fibres of different fermentabilities. In the same study they also found that hamsters fed cellulose had significantly lower cholesterol synthesis than hamsters fed psyllium mucilloid, a fermentable fibre. They proposed that in hamsters the amount of propionate produced by fibre fermentation was too low to inhibit hepatic synthesis. Actually, Truswell (1993) mentioned that VFA feedback has never been directly demonstrated, and Anonymous (1987) proposed that since fibre fermentation to propionate accounts for less than 2 percent of calories in humans, the relevance of animal studies in which 6 to 10 percent of calories is replaced by propionate is not clear, and that obviously, additional research is required.

In addition to these three hypotheses, which have been extensively studied, a fourth hypothesis exists. This hypothesis proposes that tocols (tocopherols and tocotrienols), which are fat-soluble compounds present in barley and oats, can lower cholesterol in

animal and human studies through an inhibition of cholesterol synthesis by repressing β-hydroxy-β-methylglutaryl coenzyme A (HMG CoA) reductase, which is the rate-limiting enzyme for cholesterol synthesis (Newman, 1989; Peterson and Qureshi, 1997).

However, in a study by Peterson and Qureshi (1997) in which they tried to find whether there would be an additive or synergistic response in cholesterol reduction with β-glucan and tocotrienols acting together, or whether the effect would be no greater than that achieved by either compounds acting alone, the authors concluded that β-glucans but not tocols affected HMGCoA activity, and that the tocols present in barley had very little, if any, effect on serum lipids in the presence of β-glucan.

From all the data available, it is clear that all the potential mechanisms by which fibre influences lipid absorption and metabolism are complex, and all may have more or less importance (Vahouny, 1982), but what is clear is that all these mechanisms need to be studied in more detail. Table 2.4 presents a summary of some of the possible mechanisms of dietary fibre that may influence lipid absorption.

Table 2.4 Possible mechanisms of dietary fibre influences on lipid absorption

Direct effects
Gastric emptying
Altered transit times
Interference with bulk phase diffusion and availability to intestinal surface
Binding of fibre to intestinal surface coat
Sequestration of bile acids and other micellar components
Indirect effects
Effects on bile acid pool size and composition
a. Increased faecal excretion of acidic and neutral steroids
b. Increased 7α-hydroxylation of cholesterol
Altered responses of gut glucagon and pancreatic insulin
Adaptive changes in intestinal structure and function

Source: Vahouny (1982).

2.7.3 Effects of barley β-glucans on cholesterol concentrations

Barley β-glucans have long been recognised as a detrimental factor for both beer filtration and poultry feeding (Newman *et al.,* 1989). However, they are proving to be highly successful in depressing serum cholesterol (Newman *et al.,* 1989, Hecker *et al.,* 1998). In several studies it has been confirmed that β-glucans are the fraction responsible for the hypocholestrolemic effects of barley. Such studies demonstrate that supplementing barley-based diets with β-glucanase to chickens reverse the hypocholestrolemic effects achieved with diets not containing β-glucanase (Newman *et al.,* 1989, Newman *et al.,* 1992).

Although the evidence to consider β-glucans as to be cholesterol lowering agents is strong, there is no agreement as to their mechanism of action (Klopfstein, 1988). The intestinal viscosity created by the hydrophilic nature of the β-glucans has been suggested as their mechanism of action (Newman, 1989). However, as it has been demonstrated that genetic differences of barley cultivars can cause considerable variations in extract viscosity (Newman, 1992), the variability among barley cultivars must be considered. For instance, certain cultivars of barley have been identified as being more effective than others. Waxy cultivars in particular have high β-glucan concentrations. However, a high amount of β-glucan is not always correlated with a stronger hypocholesterolemic effect, as demonstrated by McIntosh *et al.* (1991) in a study where feeding a barley cultivar with a very high β-glucan content to hypercholesterolemic men did not have a greater response to that obtained feeding barley bran containing less than half the amount of β-glucan. These authors suggest that it might be the soluble fraction of β-glucan rather than the total β-glucan the fraction more biologically relevant with regard to plasma cholesterol.

2.8 Effects of dietary fatty acids in cholesterol metabolism

Dietary fatty acids (FA) have been classified into four groups: (1) Saturated fatty acids (SFA), (2) Monounsaturated fatty acids (MUFA), and the Polyunsaturated fatty acids (PUFA) which are divided into (3) n-6 PUFA and (4) n-3 PUFA. The chain length of the SFA can vary from 4 to 20 carbons, but the 12-18 carbon SFA'S are predominant in the diet.

Dietary SFA are provided by consumption of dairy and animal products as well as vegetable oils such as coconut, palm, and palm kernel oils. The main dietary sources of MUFA and n-6 PUFA are vegetable oils such as olive, soybean, corn and safflower oils. The principal dietary MUFA is oleic acid, and linoleic acid is the main n-6 PUFA, with palmitic and oleic acids being the most abundant fatty acids in nature.

Since the 1950's, Hegsted, Keys, and their colleagues (cited by Khosla and Sundram, 1996) have studied extensively the ability of dietary fats to influence total cholesterol (TC) concentrations in blood (Khosla and Sundram, 1996). From these studies, it was concluded that replacing dietary carbohydrate isoenergetically with SFA increases TC, whereas PUFA replacement lowers TC. Monounsaturated fatty acids were considered "neutral" because no effects were observed. However, clinical studies later showed that replacement of SFA with MUFA lowers low-density lipoprotein cholesterol (LDL-C) specifically, and epidemiological data provide support for high intakes of MUFA. It is also important to mention that meta-analyses and literature review have led to the conclusion that dietary cholesterol itself contributes to increased TC, mainly LDL-C (Grundy, 1989, Khosla and Sundram, 1996).

From the 1950's studies, it was concluded that the SFA and PUFA effects were mediated by changes in LDL-c. Latter studies showed that FA's appear to increase high-density lipoprotein cholesterol (HDL-C) whereas only SFA and dietary cholesterol increase LDL-C. PUFA lowers LDL-C while only one study mentioned that MUFA had a LDL-C lowering effect.

More recently, as the knowledge of lipoprotein metabolism increased, and with the advent of newer technology, attention has shifted from the effects of dietary fatty classes on TC to the effcets of specific FA on specific lipoprotein fractions (Khosla and Sundram, 1996). The major dietary FA and their structure are listed in table 2.5.

Most of the information regarding the effects of dietary fat on plasma lipids and lipoproteins comes from the fatty acids listed on Table 2.5. Even though the mechanisms by which dietary FA affect serum lipid concentrations are still under study, there is enough data which allows scientists to predict how the amount and quality of fat in the diet will affect plasma lipids and lipoproteins in humans (Katan *et al.*, 1994). The effects of the main dietary FA on blood lipids and lipoproteins are reviewed below.

Table 2.5. Major dietary FA

Trivial name	Structure (Chain length and number of unsaturated bonds)
Saturated FA	
Lauric	12:0
Myristic	14:0
Palmitic	16:0
Stearic	18:0
Monounsaturated FA	
Oleic	18:1n-9
Trans-palmitoleic	16:1
Trans-oleic	18:1
Polyunsaturated FA	
Linoleic	18:2n-6
α linoleic	18:3n-3
Eicosapentaenoic acid (EPA)	20:5n-3
Decosahexaenoic acid (DHA)	22:6n-3

Source: Katan *et al.* (1994).

2.8.1 Palmitic acid

Palmitic acid is the main saturated fatty acid in most human diets (Grundy, 1994, Katan *et al.*, 1994). From metabolic ward studies as well as from epidemiological evidence, there is strong evidence to affirm that palmitic acid raises the serum TC concentration, as compared to unsaturated fatty acids (UFA) or carbohydrates. Actually, palmitic acid is considered the main cholesterol-raising FA of all the SFA. It has been observed that this cholesterol increase occurs mainly in the LDL-C fraction, although slight increases can also occur in HDL and very low-density lipoprotein (VLDL) (Grundy, 1994).

However, the cholesterol-raising potential of palmitic oil has been controversial (Katan et. al., 1994; Grundy, 1994; Khosla and Sundram, 1996). When the cholesterol-raising effects of palmitic oil were directly compared with the effects of other acids, it was concluded that it is hypocholesterolemic or similar in relation to myristic, hypocholesterolemic relative to lauric plus myristic, hypercholesterolemic or similar compared to lauric and oleic, and hypercholesterolemic in relation to stearic acid (Khosla and Sundram, 1996). Other evidence includes the fact that in some animals, including primates, diets rich in palmitic acid have smaller cholesterol-raising effects than the ones reported in humans. In addition, in studies where the experimental design was not rigorous, little cholesterol-raising effects of palmitic acid were detected (Grundy, 1994). Nonetheless, in well-controlled studies, palmitic acid has been proved to be hypercholesterolemic in relation to UFA and carbohydrates (Grundy, 1994, Katan *et al.*, 1994).

It has been suggested that the variation in the cholesterol-raising effects of palmitic acid may have two reasons. First, it has been observed that the metabolic status of the subjects studied has a big effect on the response observed. Older subjects require smaller changes in dietary fat saturation to achieve a change in LDL-C levels, while in younger subjects the hypercholesterolemia attributed to palmitic acid is either muted or disappears. Second, the animal studies have frequently examined the effects of palmitic acid at very high concentrations, and the results have been extrapolated for all concentrations of palmitic acid (Khosla and Sundram, 1996).

2.8.2 Myristic acid

It has been suspected for a long time that myristic acid is the most cholesterol raising of all FA, being four to six times more hypercholesterolemic than palmitic acid (Katan *et al.,* 1994). However, the direct data used to claim such an effect is less strong than that for palmitic acid (Grundy, 1994). Various research groups have reported different effects. Keys *et al.* (cited by Grundy, 1994) reported that myristic acid has a similar hypercholesterolemic effect to that of palmitic acid, while Hegsted *et al.* (cited by Grundy, 1994) suggetsed that myristic acid is more hypercholesterolemic than palmitic acid, and Katan *et al.* (1994) found that myristic acid raised cholesterol concentrations 1.5 times more than palmitic acid does. In addition, he reported that the raising effects were observed on the HDL fraction.

2.8.3 Lauric acid

Lauric acid is an intermediate length SFA, and as such, it enters the circulation partly as a component of chylomicron triglycerides and partly as a free FA. It was previously thought that medium-chain SFA did not raise cholesterol concentrations. However, the effect of lauric acid was re-evaluated (Grundy, 1994, Katan *et al.,* 1994). Keys *et al.* (cited by Grundy, 1994) reported that lauric acid is as hypercholesterolemic as palmitic and myristic acids. Temme *et al.* (cited by Katan *et al.,* 1994) concluded that a diet rich in lauric acid can raise HDL, LDL, and TC concentrations more than a diet rich in palmitic acid can. However, on the other hand, Grundy (1994) reported that lauric acid appears to increase LDL concentrations only two-thirds as much as palmitic acid does, but when compared with oleic acid, it has a marked hypercholesterolemic effect.

2.8.4 Stearic acid

The effect of stearic acid is much lower than that observed for lauric, myristic and palmitic acids (Katan *et al.,* 1994). Actually, it has been established in several studies that stearic acid does not affect serum cholesterol concentrations, thus it is considered neutral (Khosla and Sundram, 1996; Grundy, 1994), or to have a similar effect to that of oleic acid (Khosla an Sundram, 1996; Katan *et al.,* 1994). From the data available it has been suggested that the neutral effects of stearic acid apply to LDL as well as to

triglycerides, but it might have a mild lowering effect on HDL. However, the effect on HDL is uncertain.

Possible explanations to the neutral effect of stearic acid on LDL include the suggestion that the FA is not well absorbed. However, recent investigations demonstrated that 90% of the diet stearic acid is absorbed. Another explanation is that in several studies with humans and animals it has been observed that stearic acid is rapidly converted into oleic acid (Grundy, 1994).

2.8.5 Oleic acid

In 1989, Grundy was the first to report that oleic acid lowered serum concentrations of TC when it was exchanged for SFA. It was observed that this change was due exclusively to a decrease in the LDL fraction of cholesterol. However, the hypocholesterolemic effects of oleic acid are registered only when it substitutes SFA. Grundy (1989) suggested that "from a mechanistic viewpoint, oleic acid may be truly neutral", and that the SFA is the active nutrient which raises cholesterol levels, while oleic acid allows for a return to normal LDL levels. More recently, Grundy (1994) also reported that the oleic acid neutrality extends to all the lipoprotein fractions: VLDL, LDL and HDL.

The neutral effects of oleic acid might be explained by the fact that this acid is the favoured substrate for cholesterol acyltranferase (ACAT), the enzyme that esterifies unesterified cholesterol in liver. When unesterified cholesterol amounts decrease the liver cell releases suppressive effects on LDL receptor synthesis. Other mechanisms proposed include an enhancement of the receptor LDL uptake by an enrichment of cell membranes with UFA, and a decrease on lipoproteins synthesis by the liver. However, these mechanisms have neither been proved nor ruled out. Overall, the major effect of oleic acid is that at high intakes it allows for normal LDL receptor expression (Grundy, 1994).

2.8.6 Trans-monosaturates

Trans-FA are geometrical isomers of UFA produced during the hydrogenation of linoleic and α-linoleic acids either in the rumen or in oil-hardening factories (Katan *et*

al., 1994). Before 1990, there was not enough evidence to conclude that trans-FA could adversely affect plasma lipids. In addition, collective evidence suggested that no hypercholesterolemic effects could be associated with trans-FA consumption (Khosla and Sundram, 1996). However, recent evidence suggests that these FA are not neutral (Grundy, 1994; Khosla and Sundram, 1996), affecting blood lipids by either increasing LDL and /or TC, decreasing HDL, and/or increasing lipoprotein A (Lp(a)) concentrations (Khosla and Sundram, 1996). Epidemiological reports have concluded that:

a) Trans-FA are positively correlated with TC and LDL, and negatively correlated with HDL.

b) These FA show a positive association with coronary heart disease (CHD) risk. In addition, in patients with coronary artery disease the levels of trans-FA have been found to be significantly higher than in normal subjects (Khosla and Sundram, 1996).

In contrast to the results obtained from human studies, a detailed metabolic study in which hamsters were used suggested that trans-oleic acid is biologically neutral (Khosla and Sundram, 1996). However, the empirical data available in humans do not support this statement.

The mechanism of action of trans FA is not clear yet. However, it has been suggested that like the SFA, trans FA may inhibit cholesterol esterifcation in liver, which in turn could suppress LDL receptor activity having as a final consequence an increase in LDL serum concentrations (Khosla and Sundram, 1996; Grundy, 1994).

2.8.7 Linoleic acid

The hypocholesterolemic effects of linoleic acid have been known for over 40 years (Khosla and Sundram, 1996). Early studies suggested that linoleic acid had a better hypocholesterolemic effect than oleic acid, when both acids are compared to SFA. This led to the belief that linoleic acid was the most cholesterol-lowering FA, making it the preferable FA for the diet. However, it has been observed that in experimental animals, high intakes of linoleic acid promote chemical carcinogenesis and tend to suppress the

immune system. In humans, high linoleic acid intakes can lower LDL concentrations and might increase the risk for cholesterol gallstones. It has also been reported that the presence of linoelic acid in LDL lipids makes them more prone to oxidation, which in turn could promote atherosclerosis development.

In addition, several studies have demonstrated that compared to oleic acid, linoleic acid has very little or no hypocholesterolemic effects, particularly for LDL, while it lowers HDL and VLDL (Grundy, 1994).

2.8.8 Omega-3 fatty acids (EPA & DHA)

Eicosapentaenoic and decosahexaenoic acids occur typically in fatty fish and unhydrogenated fish oil (Katan *et al.*, 1994). Recently, great interest in the possible benefits of these oils has developed. This is due to the fact that effects such as an improvement in the lipoprotein profile, prevention of thrombosis, and delayed development of atherosclerosis have been observed with high intakes of these FA (Grundy, 1994). It is believed that a decrease in VLDL-triglyceride concentrations is the primary effect of omega-3 FA, which occurs through an inhibition of the secretion of VLDL triglycerides (Grundy, 1994; Katan *et al.*, 1996). In addition, some studies have reported decreases in LDL, but others report raises in LDL and apoliporotein B (Katan *et al.*, 1994).

Grundy (1994) concluded that the hypocholesterolemic effect of omega-3 FA is not unique because when they are substituted for SFA, LDL concentrations fall as they do when other UFA are substituted, and that they do not have any unique action on HDL metabolism.

2.9 Concluding comments

β-glucan is a compound found in the cell-wall of grains. It is included in the definition of dietary fibre, because it is a non-starch polysaccharide (NSP). In oats and barley, it can make up to 70% of the plant cell wall, conferring these grains a number of properties.

It is well established that β-glucans can affect the performance of broiler chickens by decreasing the digestibility of nutrients, in particular, metabolisable energy. However, the mechanism(s) by which this occurs is/are not entirely understood. An increase in the viscosity of the digesta has been, so far, one of the major factors related to the anti-nutritive properties of NSP. In addition, it is well established that the use of β-glucanase, which breaks the polymer chain of the β-glucan, can overcome the undesirable effects of this cell-wall compound.

In contrast to its anti-nutritive effects in poultry, β-glucans have been shown to possess hypocholesterolemic properties in both experimental animals and humans. As in the case of its anti-nutritive properties, the mechanism(s) by which β-glucan exerts its hypocholesterolemic effect(s) are not well established. While a number of hypothesis have been proposed, the chief one being a sequestration of bile salts, which in turn increases the cholesterol metabolism, no one of these theories have been completely proven nor discarded.

Although much is known regarding the detrimental and beneficial effects of β-glucans, the fact that the mechanisms of action of this compound are not entirely clarified and understood creates a challenge to scientists, as well as the need for more research. The anti-nutritive effects of an extract of β-glucan derived from New Zealand barleys in chickens, plus its hypocholesterolemic effects in rats, are investigated in the work described in this thesis.

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CHAPTER 3

β-GLUCAN AND HEAT TREATMENT DECREASE NUTRIENT DIGESTIBILITY IN BROILERS

3.1 Introduction

Barley β-glucans are recognised to have anti-nutritive effects in poultry, which are manifested mainly as a decrease in energy and other nutrient digestibility, as well as the production of sticky faeces. These effects are due, in part, to an increase in the viscosity of the digesta of birds fed barley-based diets. In turn, the viscosity produced in the gastrointestinal tract by the β-glucan is dependent upon the solubility of this compound (Smits, 1996; Choct, 1997). Furthermore, Bhatty (1993) reported that the anti-nutritive activity of β-glucan is generally associated with its partial solubilisation in the gastrointestinal tract, which induces a viscous condition that interferes with nutrient absorption.

Several authors (Varo *et al.*, 1983; Bjorck *et al.*, 1984; Fadel *et al.*, 1989; Graham *et al.*, 1989) have reported an increase in the solubility of NSP such as β-glucan with heat-producing processes such as extrusion, cooking, baking, and pelleting. Moreover, Antoniou and Marquardt (1982; cited by Classen and Bedford, 1991) reported a decrease in the nutritional quality of rye and wheat when these grains were autoclaved. Classen and Bedford (1991) suggested that this could possibly be due to an increase in the solubility of arabinoxylans, the major NSP found in wheat and rye, which in turn would augment the viscosity of the digesta, decreasing nutrient digestibility.

In the present work, the anti-nutritive effects of barley β-glucans in broiler chickens were evaluated through the inclusion of a barley β-glucan extract in a synthetic diet. In addition, the effects of heat treatment of the diet and how this would affect the anti-nutritive effects of the barley β-glucan extract were also evaluated.

The hypotheses of this study were:

1. Barley β-glucans possesses anti-nutritive effects through a decrease in nutrient digestibility when fed to broiler chickens less than 4 weeks old.

2. Treatments involving heat increase the solubility of β-glucans, which in turn augments its anti-nutritive effects in broiler chickens.

These hypotheses were tested by adding a barley β-glucan extract to a synthetic diet

based on cornstarch and casein, to attribute the effects observed to the β-glucan. This diet was compared with another in which no β-glucan extract was added. In addition, water was added to the same diets, and they were heated. This allowed the influence of heat treatment on the anti-nutritive properties of β-glucan to be observed.

3.2 Material and methods

3.2.1 Animals and management

A total of 20 male Golden Coast commercial Ross broilers were used for this experiment. For the first two weeks of life, the birds were raised according to standard procedures in practice at the Massey University Poultry Research Unit in Palmerston North, New Zealand, where the experiment was carried out. The birds were fed a Tegel commercial broiler starter diet manufactured by Tegel Foods Ltd., Levin, New Zealand, until the trial commenced.

3.2.2 Experimental design

At the beginning of the experiment (Day 0), 20, 15-day-old male broilers were weighed and randomly allocated in a 2 X 2 factorial arrangement of treatments with the respective factors being "heat" or "no heat" treatment of the diet, and the absence or presence of a β-glucan extract added at 15% to the diet. Treatments were assigned as follows:

- A0 = Not heated, no β-glucan extract
- A15 = Not heated, 15 % β-glucan extract
- D0 = Heated, no β-glucan extract
- D15 = Heated, 15 % β-glucan

3.2.3 Experimental diets

The diets used for this experiment were synthetic diets based on cornstarch and casein, with the presence or absence of 15 % of a barley extract of β-glucan (Table 3.1). Refer to Appendix I for β-glucan extract characteristics. Adding the β-glucan to a synthetic diet would allow any physiological effects observed to be attributable directly to the β-glucan. The decision to add 15 % of the extract was made because in several *in vivo* and *in vitro* preliminary trials performed to evaluate the effects of this specific β-glucan on

Table 3.1 Composition of experimental diets (as is basis)

INGREDIENTS	DIET A0 (g/kg)	DIET A15 (g/kg)	DIET D0 (g/kg)	DIET D15 (g/kg)
Cornstarch	540.6	390.6	540.6	390.6
Sugar	100.0	100.0	100.0	100.0
Casein	232.6	232.6	232.6	232.6
Hydrolysed cellulose	30.0	30.0	30.0	30.0
Soya bean oil	38.8	38.8	38.8	38.8
Limestone	13.5	13.5	13.5	13.5
Potassium carbonate	24.4	24.4	24.4	24.4
Dicalcium phosphate	11.5	11.5	11.5	11.5
Salt	3.6	3.6	3.6	3.6
Minerals premix*	1.5	1.5	1.5	1.5
Vitamins premix*	0.5	0.5	0.5	0.5
Chromic oxide	3.0	3.0	3.0	3.0
β-glucan extract	0.0	150.0	0.0	150.0
Heat	NO	NO	YES	YES

*Refer to Appendix II for mineral and vitamin composition of premix.

digesta characteristics of chicks (especially viscosity), it was found that when the extract was added to the diet at levels lower than 15%, no viscosity changes were observed (Appendix III).

The diets also contained chromium oxide (0.3%), which was used as an indigestible marker, and its concentration was used as a reference value from which digestibility coefficients were calculated. Nutrient levels recommended by the NRC (1994) were used as the basis for diet formulation. Diets D0 and D15 were mixed with 10% water and cooked for 3 minutes in a "National" microwave oven model NE 6790 at "High" temperature. This procedure was performed to simulate the humidity and heat that occurs during the pelleting process, which in turn is likely to increase the solubility of the β-glucan, thereby increasing its anti-nutritive effects. Diets A0 and A15 were also mixed with 10% water but were not cooked. In this way, all diets had similar water contents.

3.2.4 Experimental procedures

Once the birds were allocated to the experimental diets, they were housed in individual cages in a controlled-temperature shed, with an average ambient temperature of 24° C. The animals were fed *ad libitum* from Day 0 to Day 6 of the study. Water was also available *ad libitum*. Daily feed intake was recorded. From days 3 to 5 total excreta of all birds were collected (days 18 to 20 of age). At 4 PM on Day 6 the food was removed

and the birds were starved for 16 hours until 8:00 AM the next day (Day 7), when food was made available again for four hours before slaughter. This ensured an adequate amount of digesta present in the intestine to obtain a representative sample. Birds were euthanased with an intracardial injection of 1 ml of sodium pentobarbitane (Pentobarb 300 - P.A.R. Class II Chemstock Animal Health, Ltd., Christchurch, New Zealand), and then weighed. The entire gastro intestinal tract was then removed and weighed. The small intestine was divided into two anatomical parts, the first extending from the duodenum to Meckel's diverticulum or *diverticulum vitelli*, which is regarded as the beginning of the ileum. The second part extended from Meckel's diverticulum to the beginning of the caeca. Both sections of the small intestine were weighed before digesta were collected from them. Caeca were weighed full, they were emptied, and then reweighed. Digesta from the second half were flushed with reverse osmosis (RO) water into labelled bags. Care was taken not to handle the intestine. The samples were then frozen at –20°C for subsequent chromium determination and digestibility analysis. Digesta from the first part of the small intestine were collected by squeezing into culture tubes. The tubes were kept on ice before digesta physico-chemical analyses were made. Finally, the empty weights of the two halves of the small intestine were registered.

3.2.5 Analytical procedures

3.2.5.1 Nutrient Digestibility

Digesta samples from the second half of the small intestine were freeze-dried and then, together with the diet samples, they were analysed for Nitrogen and Carbon content using a LECO FP-2000 analyser (LECO Corporation, 3000 Lakeview Av. St. Joseph MI 49085-2396 USA) following the Dumas process (Granger, 1997). Faeces and diets were analysed for gross energy content using a Gallenkamp bomb calorimeter. All samples were analysed for dry matter (Harris, 1970) and for chromium content (Czarnocki *et al.*, 1960; Williams *et al.*, 1962; Fenton and Fenton, 1979) using methods developed at the Nutrition Laboratory, Institute of Food, Nutrition, and Human Health, Massey University, Palmerston North, New Zealand.

From the data obtained from the laboratory, N, C, and GE digestibilities were determined using the following equation:

$$Digestibility\,(\%) = \frac{\frac{x\,in\,diet}{Cr\,in\,diet} - \frac{x\,in\,digesta}{Cr\,in\,digesta}}{\frac{x\,in\,diet}{Cr\,in\,diet}} \quad (1)$$

where x is C, N or GE concentration and Cr is the concentration of the Chromium marker

3.2.5.2 Digesta physico-chemical properties

Samples obtained from the first half of the small intestine were analysed to determine the influence of β-glucan on water holding capacity (WHC) and on the insoluble solids volume (ISV) of the digesta.

Insoluble solids volume (ISV) was measured to obtain a reflection of the water uptake of the food particles, or food swelling, which might be important in terms of nutrient dissolution. The ISV is calculated as a difference in volume before and after centrifuging. The volume (v) was determined from the height of the solids in the tube (h) in Kimax 10 ml culture tubes. To achieve this, a known volume was first added to ten tubes and the heights were registered (Table 3.2).

Table 3.2. Height (cm) of known volumes added to tubes

	Volume added	
Tube no.	5ml	10 ml
1	3.40	6.60
2	3.40	6.50
3	3.45	6.60
4	3.45	6.55
5	3.45	6.55
6	3.45	6.70
7	3.45	6.50
8	3.50	6.70
9	3.50	6.70
10	3.50	6.55
Mean	3.44	6.60
SD	0.06	0.08
CV (%)	1.70	1.20

Then, from the following equation:

$$h = mv + c \quad (2)$$

m was calculated from the height measurements made after the addition of 5 and 10 ml

water: m = (6.60-3.44)/(10-5) = 6.32 and c was calculated after addition of 10 ml. h = 6.6 cm so, 6.6 = (0.632 x 10) + c and c = 6.6 - 6.32 = 0.28. Therefore, the equation to determine volume (v ml) from height (h cm) of material in tube is $h = 0.63v + 0.28$ and $v = (h - 0.28) / 0.63$ml.

Water holding capacity (WHC) was determined as the difference in sample weight before and after drying the digesta overnight at 100°C. It was measured as an indication of the gelling influence of the β-glucan on the diet.

3.2.6 Statistical analysis

Data was analysed by analysis of variance using the GLM procedure of SAS (SAS, 1997), using the following model:

$$Y_{ijk} = \mu + \beta_i + C_j + \beta_i C_j + \varepsilon_{ijk}$$

where:

Y_{ijk} = The observation of the k^{th} individual exposed to the j^{th} and to the i^{th} treatment

μ = The population mean

β_i = The fixed effect of adding β-glucan

C_j = The fixed effect of heating the diet

$\beta_i C_j$ = The effects' interaction

ε_{ijk} = The residual error

This model allowed all individual effects and possible interactions to be assessed. The significance level was set at $\alpha = 0.05$. Where applicable, differences between means were determined using the least significant difference (LSD) method with the GLM procedure of SAS (SAS Institute, 1997).

3.3 Results

3.3.1 Nutrient Digestibility

Digestibility of nutrients was affected by both the presence of β-glucan and the heat treatment of the diet. For the diets containing β-glucan, a lower N digestibility (95.8 vs 97.0%, P<0.01), C digestibility (81.9 vs 90.9, P<0.01), and GE digestibility (71.6 vs 77.4%, P>0.05) were observed, although the effect of the β-glucan was not significant in the case of GE digestibility. Heating the diets lowered N digestibility (95.9 vs 97.0%; P<0.01). Carbon and GE digestibility were also lowered (85.0 vs 87.8% for C and 72.3 vs 76.6% for GE), though not significantly so (P>0.05). No significant interactions were found. The interaction least-square mean N, C, and GE digestibility values are presented in Table 3.3, and Table 3.4 presents the level of significance of the main effects.

Table 3.3 Interaction least-square mean values for N, C, and GE digestibility (%) of the experimental diets

	Diet not heated		Diet Heated		Pooled SE
	- β-glucan	+ β-glucan	- β-glucan	+ β-glucan	
N digestibility	97.5	96.6	96.7	95.1	0.27
C digestibility	92.9	82.8	88.9	81.1	1.2
GE digestibility	78.2	75.1	76.6	68.0	0.04

Table 3.4 Level of significance of β-glucan, heat, and their interaction on N, C, and GE digestibility

	N digestibility	C digestibility	GE digestibility
β-glucan	**	**	NS
Heat	**	NS	NS
β-glucan X Heat	NS	NS	NS

** P<0.01, NS Not Significant.

3.3.2 Weights of Digestive Organs

The weights of the digestive organs were analysed using the body weight of the birds as a covariate, which was significant for all the organs. The body weight diminished the error of the model used when it was incorporated as a covariate.

The analysis showed that heating the diets did not affect the weight of any of the organs. Inclusion of β-glucan in the diets increased the weight of the second half of the small intestine full (9.86 vs 8.81 g for diets with and without β-glucan, respectively, P=0.08). The full weight of the caeca was also increased with the inclusion of β-glucan (4.12 vs 3.64 g for diets with and without β-glucan, respectively, P=0.02). However, in the case of the empty caeca, β-glucan inclusion decreased its weight (2.48 vs 2.68 for diets with and without β-glucan, respectively, P=0.08). The rest of the organs were not affected by the inclusion of β-glucan in the diets. Table 3.5 presents the interaction least-square mean organ weights, and Table 3.6 presents the level of significance of the factors.

Table 3.5. Interaction least-square mean weight (g) of digestive organs of birds fed the experimental diets

	Diet not heated		Diet Heated		Pooled SE
	- β-glucan	+ β-glucan	- β-glucan	+ β-glucan	
Whole gut full	43.04	43.36	39.29	44.18	3.17
Small int 1 full	14.98	14.35	13.08	15.11	1.31
Small int 2 full	8.32	9.31	8.52	11.21	1.01
Caeca full	3.56	4.68	3.22	4.06	0.39
Small int 1emp	10.76	9.63	9.92	11.65	0.83
Small int 2 emp	4.34	4.64	4.35	4.99	0.39
Caeca emp	2.43	2.94	2.21	2.75	0.29
Whole gut emp	17.52	17.20	16.48	19.39	1.28

Table 3.6. Level of significance of β-glucan, heat, and their interaction on the weights of digestive organs

	Whole gut		Small intestine				Caeca	
			1st half		2nd half			
	Full	Empty	Full	Empty	Full	Empty	Full	Empty
β-glucan	NS	NS	NS	NS	*	NS	**	*
Heat	NS	NS	NS	NS	NS	NS	NS	NS
β-gluc X Heat	NS	NS	NS	NS	NS	NS	NS	NS

* P=0.08; ** P=0.02; NS Not Significant.

3.3.3 Digesta physico-chemical properties

For the birds consuming the diets containing β-glucan, the water holding capacity (WHC) mean values were higher (P<0.01) than those for the birds consuming the diets with no β-glucan (3.1 vs 2.1 g/g dry digesta). Heating the diet did not have any significant (P>0.05) effect on the WHC of the digesta. The ISV was significantly increased (P<0.01) in the presence of β-glucan (1.8 vs 0.81 ml). However, heating the diet decreased ISV (0.92 vs 1.7 ml, P<0.01). No significant interactions were found (table 3.8). The mean WHC and ISV of the digesta are shown in Table 3.7.

Table 3.7. Interaction lest-square mean WHC and ISV values of digesta of birds fed the experimental diets

	Diet not heated		Diet Heated		Pooled SE
	- β-glucan	+ β-glucan	- β-glucan	+ β-glucan	
WHC (g/g dry digesta)	1.95	3.16	2.23	2.95	0.17
ISV (ml)	1.03	2.40	0.59	1.30	0.22

Table 3.8. Level of significance of β-glucan, heat, and their interaction on the physical characteristics of the digesta

	WHC	ISV
β-glucan	**	**
Heat	NS	**
β-glucan X Heat	NS	NS

** P<0.01; NS Not Significant.

3.4 Discussion and Conclusions

In this study, the inclusion of β-glucan and heating of the diet (to simulate conditions encountered during processing) decreased digestibility of all nutrients evaluated. Several studies have reported that cooking and processes such as pelleting or extrusion of feedstuffs can induce major changes in the architecture of the cell wall matrix of grains contained in such feeds. These processes, rather than affecting the amount of NSP present in the food, can affect the relative concentrations of soluble and insoluble NSP in the diet, or of compounds such as β-glucans, which are major sources of dietary fibre (Selvendran and Robertson, 1990; cited by Robertson *et al.*, 1997). Factors such as animal species, physical presentation of the feed, variations between methods and within a method depending on different conditions such as different temperatures or humidities, can affect the way cooking, pelleting or extrusion modify the nutritional properties of a feedstuff. However, there is general agreement regarding the fact that these processes increase the amount of soluble fibre in general and soluble NSP in particular. Moreover, Cummings *et al.* (1979) and Nyman and Asp (1982; both authors cited by Bjork *et al.*, 1984) reported that soluble fibre is more easily fermented than insoluble fibre. This might be true for pigs, in which pelleting, extrusion, and cooking of barley-based diets have been demonstrated to increase β-glucan solubility, and improve crude protein, energy, starch, dry matter, and NSP digestibility, presumably due to fermentation of the soluble β-glucan in the upper gut (Fadel *et al.*,1988; Fadel *et al.*,1989; Graham *et al.*,1989).

However, in the case of chickens, the information reported is rather different from that for pigs. Early studies on the effects of feeding pelleted cereal diets to poultry reported improvements in the feeding value of barley diets, as well as on feed intake, weight gain, and feed conversion ratio, although there were no effects on AME content. Other studies also reported no beneficial effects of pelleting barley on energy digestibility (Bhatty, 1993). Antoniou and Marquardt (1982; cited by Classen and Bedford, 1991) reported that the nutritional quality of rye and wheat was decreased by autoclaving, which might increase soluble arabinoxylans, the major NSP of these grains. This, in turn, would augment the viscosity in the digestive tract, leading to a lower nutrient digestibility. Wood (1984) reported that cooking of barley led to local high viscosity and

inhibition of diffusion, delaying nutrient release. In the present study, N, C, and GE digestibility were decreased by heat treatment of the experimental diets. Although the viscosity of the digesta was not evaluated in this experiment, preliminary *in vitro* studies (Appendix III) showed no significant increase in the viscosity of digesta residue of diets containing high levels (up to 15%) of the β-glucan extract used. However, the detrimental effect of the β-glucan in the digestibility of the diets was clear. This suggests that perhaps high viscosity is not the only factor needed for β-glucan anti-nutritive effects to take place. For example, Choct (1997) suggested that changes in the intestinal flora and in the levels of water and electrolytes in the intestine are other effects that β-glucan has, which might contribute to its anti-nutritive properties.

Heat treatment of the diets decreased N digestibility in a significant way. A possible explanation is that heating of the diets favoured the Maillard reaction, which is characterised by a reaction between reducing sugars and free amino acid groups in proteins, leading to a decrease in both protein digestibility and amino acid availability (Bjorck and Asp, 1983). The Maillard reaction is also known as "non enzymatic browning" because of a brown colour the feed acquires after the reaction takes place. In this study, the heated diets were noticeably darker than the non-heated ones, which strongly suggests that a Maillard reaction occurred.

The fibre hydration properties such as water holding capacity and swelling capacity are of paramount importance because they affect the biological effects of dietary fibre such as induction of colonic fermentation and increase in stool weight in the lower intestine (Auffret *et al.*, 1994), as well as the pattern of nutrient absorption, postprandial satiety, and intestinal motility in the upper intestine (Kay, 1982).

Water holding capacity, which was the only hydration property of fibre evaluated in the present study, is defined as the amount of water that can be taken up per unit of weight of dry fibre to the point at which no free water remains (Southgate *et al.*, 1993). Fibre hydration occurs by absorption of the macromolecules into the surface, and by entrapment within the interstices of the gel matrix of the fibre. The saturation capacity of the fibre is determined by the chemistry and morphology of the macromolecules, as well as by the electrolyte concentration and pH of the surrounding medium (Kay, 1982). Factors such as particle size (Kay, 1982; Kritchevsky, 1988; Gordon, 1989; Auffret *et*

al., 1994; Sungsoo *et al.*, 1997), pH (Robertson and Eastwood, 1981; Kay, 1982; Gordon, 1989; Sungsoo *et al.*, 1997), ion strength of the solution (Sungsoo *et al*, 1997), and method of fibre preparation, i.e., dried vs fresh fibre (Robertson and Eastwood, 1981; Kay, 1982), can affect WHC. However, the effect of cooking on WHC is not broadly documented. McConell *et al.* (1974) evaluated the effect of cooking cauliflower, as well as winter and spring cabbage, on WHC. They found that cooking had no effect on the WHC of these vegetables. This is in agreement with the results of the present work, which showed no significant differences between the WHC of heated and non-heated diets. However, these authors evaluated the WHC of vegetables, which can have different characteristics to those of grains. Rallet *et al.* (1990) evaluated the effect of extrusion-cooking on the physico-chemical properties of wheat bran and found that water uptake was increased by extrusion treatments which had low or intermediate intensity, while severe conditions decreased water uptake. The results of these researchers differ from the results of the present study. This might be due to the fact that extrusion-cooking not only involves water and temperature, but also a shear action towards the ingredients being extruded, which can fragment some of the dietary fibre polymers and, as mentioned before, particle size is one of the most important factors affecting WHC of dietary fibres. On the other hand, as was expected, β-glucan inclusion increased WHC in a significant way. This indicates that the hydration properties of the β-glucan extract were not lost after the extraction procedure took place.

Although ISV was measured to obtain a reflection of the water uptake of the food particles in the diets (section 3.2.5.2), it became instead an indication of the changes that heat and β-glucan produced on the soluble and insoluble fibre fractions. The significant increase in ISV produced after β-glucan inclusion was expected. The fact that ISV values decreased when the diets were heated agrees with reports of Antoniou and Marquardt (1982; cited by Classen and Bedford, 1991), Fadel *et al,* (1988), Fadel *et al* (1989) and Graham *et al.* (1989), in which treatments involving heat increased the amount of the soluble NSP, and decreased the amounts of insoluble NSP.

The weights of the digestive organs were, in part, a reflection of the digesta characteristics. The inclusion of β-glucan in the diets increased only the full weights of the second part of the small intestine, and the caeca. It is possible that the digesta of

those birds had a higher WHC as a consequence of the presence of the β-glucan, causing more water to be trapped within the digesta particles, making it heavier. It could also mean that that there were more undigested nutrients present in the digesta of chickens fed the diets containing β-glucan, which had a bulking effect on the digesta. The empty weight of the caeca was decreased with the inclusion of the β-glucan, and the weights of the rest of the organs were not affected by the β-glucan inclusion. This effect was not expected and does not coincide with reports of different authors such as Bedford (1996), who reported that an increase in intestinal mass could be observed when birds eat a diet with a low digestibility, or Brenes *et al.* (1993), who mentioned that the increase in the size of the pancreas and gastrointestinal tract observed when are fed barley-based diets might be an adaptive response to an increased need for enzymes. In addition, Brown (1979) and Wyatt *et al.* (1988; both authors cited by Pluske *et al.*, 1998) have suggested that feeding viscous fibres increase the "work" needed to propel the digesta across the intestine, which may lead to muscular hypertrophy, increasing the intestine weight. Also, McCullough *et al.* (1998) showed that fibre could have a proliferative effect on the intestinal mucosa, especially of the colon. Probably, although the β-glucan extract conserved some properties such as water holding capacity, those properties were not enough to affect the weight of digestive organs. In addition, heating the diets did not have any significant effect on the weight of digestive organs,

In conclusion, the present study has confirmed the anti-nutritive effects of barley β-glucan through the use of a commercially prepared extract. These anti-nutritive effects were manifested by a decrease in nutrient digestibility, namely nitrogen, carbon, and gross energy, although the decrease in the digestibility of gross energy was not significant. The β-glucan extract also maintained some of its physico-chemical properties, particularly the water holding capacity, which was higher in the digesta of broilers fed the diets containing β-glucan.

Although heat treatment of the diet presumably increased the solubility of the β-glucan, the anti-nutritive effects of this NSP were not increased in a significant way, contrary to what was hypothesised. Finally, these results indicate that the specific barley β-glucan extract used in the present work conserved some of its anti-nutritive and physico-chemical properties. However, it is not known to what extent the extract properties are

similar to those of intact β-glucan. Further research comparing the extract with barley is required to evaluate the extent to which the effects observed in this study are correlated to those observed in barley-based diets.

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CHAPTER 4

THE USE OF β-GLUCANASE IN BARLEY-BASED DIETS CONTAINING THREE DIFFERENT LEVELS OF β-GLUCAN AND FED TO BROILER CHICKENS

4.1 Introduction

It is well established that the β-glucan present in barley causes growth depression, lower nutrient digestibility, and sticky droppings when fed to broiler chickens. The use of enzymes to overcome these problems has become very common. Inclusion of β-glucanase in poultry feeds has found broad acceptance in the poultry industry, allowing barley to be used at higher levels of inclusion in broiler diets (Wood, 1984; Campbell and Bedford, 1992; Bhatty, 1993). Improvements in nutrient digestibility (Choct *et al.*, 1996), decreases in the weights of digestive organs (Svihus *et al.*, 1997 a and b), increases in weight gain (Yu *et al.*, 1998), improvements in feed conversion ratio (FCR) (Choct *et al.*, 1996), reductions in the viscosity of digesta (Yu *et al.*, 1998), and a decrease in the moisture content of the digesta (Choct *et al.*, 1996), which is a reflection of the water holding capacity of the β-glucan, have been reported when β-glucanase is included in barley-based diets fed to broiler chickens. These findings support the use of this enzyme to overcome the anti-nutritive effects caused by the β-glucans contained in barley.

The β-glucanase contained in commercial enzyme preparations may hydrolyse β-glucan, which in turn decreases its molecular weight and thus, its viscosity (Bhatty, 1993). In fact, it is generally accepted that a reduction in the viscosity caused by barley β-glucans through the use of enzymes is the main factor responsible for the improvement in broiler performance (Annison and Choct, 1991).

In the previous experiment, the anti-nutritive effects of barley β-glucan were demonstrated by including a commercially prepared barley β-glucan extract in a synthetic diet. In the present experiment, a more realistic approach was achieved by using a barley-based diet instead of a synthetic diet to evaluate the effects of different levels of β-glucan on nutrient digestibility, weights of digestive organs, phyisico-chemical characteristics of the digesta, intake, weight gain, and feed conversion ratio. In addition, a β-glucanase was added to some of the diets.

The hypotheses of the present experiment were:

1. The anti-nutritive effects of barley β-glucan increase as the level of β-glucan present in the diet increases.

2. The use of a commercial β-glucanase increases nutrient digestibility, decreases weights of digestive organs as well as viscosity and water holding capacity of the digesta, and improves weight gain and feed conversion ratio of broiler chickens fed barley-based diets.

To test these hypotheses, six barley-based diets containing three different levels of β-glucan with the presence or absence of a commercial β-glucanase were fed to 15-day-old broilers. The different levels of β-glucan in the diets were achieved by adding specific amounts of a barley β-glucan extract to the diets.

4.2 Materials and methods

4.2.1 Animals and management

Thirty-six male Golden Coast commercial Ross broilers were used for this experiment. The birds were raised for the first two weeks of life according to standard procedures in practice at the Massey University Poultry Research Unit in Palmerston North, New Zealand, where the experiment was carried out. They were fed a Tegel commercial broiler starter diet manufactured by Tegel Foods Ltd., Levin, New Zealand, until the trial was commenced, and had free access to water.

4.2.2 Experimental design

At the beginning of the experiment (Day 0), 36, 15-day-old male broilers were weighed and randomly allocated in a 3x2 factorial arrangement of treatments. The factors were: "low", "medium" or "high" level of β-glucan in the diet, and the absence or presence of a β-glucanase from *Trichoderma viride* (Allzyme BG Concentrate, Alltech Inc.). The β-glucanase activity as determined by the manufacturer was 1200 BGU/g. Treatments were assigned as follows:

- L - = Low β-glucan, without enzyme
- L + = Low β-glucan, with enzyme
- M - = Medium β-glucan, without enzyme
- M + = Medium β-glucan, with enzyme
- H - = High β-glucan, without enzyme
- H + = High β-glucan, with enzyme

4.2.3 Experimental diets

Six different barley-based diets were used in this experiment. Nutrient levels recommended by the NRC (1994) were used as the basis for diet formulation. The diets

also contained chromium oxide (0.3%), which was used as an undigestible marker, and its concentration was used as a reference value from which digestibility coefficients were calculated. In addition to the barley, a barley β-glucan extract (refer to Appendix I for extract characteristics) substituted cornstarch in four of the diets to increase their β-glucan content from "low" to "medium" and "high". The diets were analysed for β-glucan content at the Crop and Food Research Institute, Christchurch, New Zealand, and the values obtained were:

- Diets L - & L + : 19.8 g per kg of diet
- Diets M - & M + : 50.7 g per kg of diet
- Diets H - & H + : 68.3 g per kg of diet

The composition of the diets is shown in Table 4.1.

Table 4.1 Composition of experimental diets (g/kg air-dry basis)

INGREDIENT	DIET L- (g/kg)	DIET L+ (g/kg)	DIET M- (g/kg)	DIET M+ (g/kg)	DIET H- (g/kg)	DIET H- (g/kg)
Barley	576.4	576.4	576.4	576.4	576.4	576.4
Soyabean meal	204.1	204.1	204.1	204.1	204.1	204.1
Cornstarch	85.0	85.0	41.7	41.7	0	0
Blood meal	10.6	10.6	10.6	10.6	10.6	10.6
Meat&Bone meal	90.3	90.3	90.3	90.3	90.3	90.3
Soyabean oil	27.5	27.5	27.5	27.5	27.5	27.5
Vits&Mins premix*	3.0	3.0	3.0	3.0	3.0	3.0
Salt	1.6	1.6	1.6	1.6	1.6	1.6
Limestone	0.4	0.4	0.4	0.4	0.4	0.4
Methionine	1.1	1.1	1.1	1.1	1.1	1.1
Chromic oxide	3.0	3.0	3.0	3.0	3.0	3.0
β-glucan	0	0	43.3	43.3	85.0	85.0
β-glucanase	0	0.05	0	0.05	0	0.05

*Refer to Appendix II for composition of mineral and vitamin premix.

4.2.4 Experimental Procedures

The housing conditions, as well as all the experimental procedures used in this experiment, were the same as those used in the experiment of Chapter 3, section 3.2.1.

4.2.5 Analytical procedures

4.2.5.1 Nutrient digestibility

The samples obtained were analysed for N, C, and GE digestibility following the same procedures described in Chapter 3, section 3.1.5.1.

4.2.5.2 Digesta physico-chemical properties

Samples obtained from the first part of the small intestine were analysed to determine the influence of β-glucan on insoluble solids volume (ISV) and on the water holding capacity (WHC) of the digesta using the procedures described in Chapter 3, section 3.1.5.2. Viscosity was determined using a Brookfield digital viscometer model DV-II+.

4.2.6 Statistical analyses

Data was analysed by analysis of variance using the GLM procedure of SAS (SAS 1997), using the following model:

$$Y_{ijk} = \mu + \beta_i + C_j + \beta_i C_j + \varepsilon_{ijk}$$

where:

Y_{ijk} = The observation of the k^{th} individual exposed to the j^{th} and to the i^{th} treatment

μ = The population mean

β_i = The fixed effect of adding β-glucan

C_j = The fixed effect of the presence or absence of the β-glucanase

$\beta_i C_j$ = The effects' interaction

ε_{ijk} = The residual error

This model allowed all individual effects and possible interactions to be assessed. The significance level was set at α=0.05.

Where applicable, differences between means were determined using the least significant difference (LSD) method with the GLM procedure of SAS (SAS Institute, 1997).

4.3 Results

4.3.1 Nutrient digestibility

The level of β-glucan, regardless of β-glucanase inclusion, affected nutrient digestibility depending upon the nutrient. In the case of N digestibility, no effect of β-glucan level was observed (P=0.121). Carbon and GE were significantly (P<0.05 for C; P<0.01 for GE) affected by the level of β-glucan in the diet. The lowest C digestibility values were obtained with diet "high" followed by diet "low", with diet "medium" having the highest digestibility values (68.6 vs 69.3 vs 72.2 %). However, the C digestibility obtained with diet "low" was not different (P>0.05) from those values obtained with diets "medium" and "high", which were different (P<0.05) amongst each other. Gross energy digestibility followed the same pattern of C digestibility with diet "high" giving the lowest digestibility values followed by diets "low", and then "medium" (65.8 vs 72.2 vs 74.0 %). In this case, the GE digestibility value obtained with diet "high" was different (P<0.01) from those obtained with diets "low" and "medium", which were not different (P>0.05) between each other.

The inclusion of β-glucanase increased N, C (P<0.01), and GE (P<0.05) digestibility (84.2 vs 80.7 % for N; 72.8 vs 67.6 % for C; 71.9 vs 69.7 % for GE). No significant interactions were found for any of the nutrients analysed (Table 4.2). The interactions least-square means for N C, and GE digestibility are presented in Table 4.3.

Table 4.2 Levels of significance of β-glucan, enzyme, and their interaction on N, C, and GE digestibility

	N digestibility	C digestibility	GE digestibility
β-glucan	NS	*	**
Enzyme	**	**	*
β-glucan x enzyme	NS	NS	NS

*P ≤ 0.05; ** P < 0.01; NS Not significant

Table 4.3 Interaction least-square means for N, C, and GE digestibility (%)

	Low β-glucan		Medium β-glucan		High β-glucan		Pooled
	- enzyme	+ enzyme	- enzyme	+ enzyme	- enzyme	+ enzyme	SE
N dig.	79.2	82.8	82.2	85.3	80.7	84.5	1.3
C dig.	67.4	71.1	69.0	76.5	66.3	71.0	1.7
GE dig.	70.6	74.8	72.9	75.1	65.7	65.9	1.1

4.3.2 Weights of digestive organs

To determine if the weight of the birds at slaughter had any effect on the weight of the digestive organs, an ANOVA was conducted using bird weight as a covariate (SAS, 1997). The results of this analysis showed that the covariant helped to explain the weights of the whole intestine full and empty, and the 1st (anterior) part of the small intestine, both full and empty ($P<0.01$).

The β-glucan level in the diet affected the weights of the whole gut full and empty, and the 2nd part of the small intestine empty ($P<0.01$). It also influenced the weights of 1st part of the small intestine full and empty as well as the 2nd part of the small intestine full ($P<0.05$). In the case of the full weight of the whole gut, birds eating diet "low" had the lightest weights followed by diet "medium", with diet "high" giving the highest values (91.30 vs 93.69 vs 105.30 g). However, the value obtained with diet "high" was different ($P<0.01$) to those obtained with diets "low" and "medium", which were not different to each other ($P>0.05$). For the 1st part of the small intestine full and empty, the second part of the small intestine full and empty, and the whole gut empty, birds eating diet "medium" had the lightest weights followed by diet "low", with diet "high" giving the highest organs weights:

- First part of the small intestine full 29.33 vs 31.10 vs 32.85 g
- First part of the small intestine empty 20.35 vs 21.73 vs 22.88 g
- Second part of the small intestine full 19.70 vs 19.73 vs 22.73 g
- Second part of the small intestine empty 9.34 vs 9.72 vs 10.93 g
- Whole gut empty 34.77 vs 36.59 vs 39.27 g

In the case of the 1st part of small intestine full and empty, the means of diets "low" were not different ($P>0.05$) to those of diets "medium" and "high", which were different ($P<0.01$) between each other. For the 2nd half of the small intestine full and empty, as well as for the whole gut empty, the means obtained with diets "high" were different ($P<0.05$) to those obtained with diets "medium" and "low", which were not different between each other ($P>0.05$). The inclusion of the β-glucanase in the diets significantly

decreased the full weight of the whole gut and the 2nd part of the small intestine full (P<0.01):

- Whole gut full 92.80 vs 100.67 g
- Second part of the small intestine full 19.02 vs 22.41 g

No significant (P>0.05) interactions were found for any of the parameter evaluated (Table 4.4). The interaction least-square mean values of the weights of the digestive organs are presented in Table 4.5.

Table 4.4 Levels of significance of β-glucan, enzyme, and their interaction, on weights of digestive organs (using bird weight as a covariate)

	Whole gut full	SI 1 full	SI 2 full	Caeca full	SI 1 empty	SI 2 empty	Caeca empty	Whole gut empty
Bird weight	**	**	NS	NS	**	NS	NS	**
β-glucan	**	*	*	NS	*	**	NS	**
Enzyme	**	NS	**	NS	NS	NS	NS	NS
β-glucan x Enzyme	NS	NS	NS	NS	NS	NS	NS	NS

*P < 0.05; ** P < 0.01; NS Not significant

Table 4.5 Interaction least-square mean weights of digestive organs (g)

	Low β-glucan		Medium β-glucan		High β-glucan		Pooled SE
	- enzyme	+ enzyme	- enzyme	+ enzyme	- enzyme	+ enzyme	
Whole gut full	93.05	89.38	97.30	90.07	111.67	98.94	3.15
SI 1 full	31.76	30.45	29.31	24.36	32.99	32.72	1.18
SI 2 full	20.68	18.78	21.65	17.74	24.90	20.55	1.26
Caeca full	9.40	9.01	9.27	7.66	10.41	9.33	0.97
SI 1 emp	21.60	21.86	20.04	20.66	23.19	22.56	0.76
SI 2 emp	10.06	9.53	9.41	9.27	11.44	10.43	0.50
Caeca emp	4.99	5.15	5.32	4.83	5.83	5.08	0.29
Whole gut empty	36.65	36.54	34.77	34.76	40.46	38.07	1.15

4.3.3 Physico-chemical properties of the digesta

Water holding capacity (WHC), insoluble solids volume (ISV), and viscosity (Visc) of the digesta were significantly (P<0.01) affected by the level of β-glucan in the diet. The lowest WHC value was obtained with the diet "low" followed by diet "high", with diet

"medium" giving the highest values (2.76 vs 3.17 vs 3.28 g/g dry digesta). The mean WHC value obtained with diet "low" was different ($P<0.01$) to the means obtained with diets "high" and "medium", which were not different ($P>0.05$) between each other. The ISV followed a pattern more consistent with the level of β-glucan in the diet. Diet "low" gave the lowest values followed by diets "medium" and "high" (3.92 vs 4.32 vs 5.37 ml). The mean ISV value obtained with diet "low" was different ($P<0.05$) to the values obtained with diets "medium" and "high", which were not different ($P>0.05$) to each other. A significant ($P<0.01$) interaction between β-glucan level and enzyme was found for viscosity (Table 4.6). The mean viscosity value obtained with diet "Low without β-glucanase" (13.9 cP) was significantly higher ($P<0.01$) to the mean viscosity of the rest of the diets, which were not significantly different to each other (Table 4.7). The inclusion of β-glucanase in the diets did not affect WHC and ISV in a significant way ($P>0.05$).

Table 4.6 Level of significance of β-glucan, enzyme, and their interaction on the digesta physico-chemical properties

	WHC	ISV	VISC
β-glucan	**	**	**
Enzyme	NS	NS	**
β-glucan x enzyme	NS	NS	**

** $P < 0.01$; NS Not significant

Table 4.7 Interaction least-square mean values for digesta physico-chemical properties

	Low β-glucan		Medium β-glucan		High β-glucan		Pooled SE
	- enzyme	+ enzyme	- enzyme	+ enzyme	- enzyme	+ enzyme	
WHC (g water/g dry digesta)	2.8	2.8	3.4	3.1	3.2	3.2	0.93
ISV (ml)	4.1	3.8	4.3	4.4	5.8	5.0	0.40
Visc (cP)	13.9b[1]	3.6a	4.3a	3.0a	3.1a	2.6a	1.0

[1] Within rows, means with the same letter were not significantly different ($P > 0.05$).

4.3.4 Performance data

The β-glucan level of the diets did not have any significant ($P>0.05$) effect on weight

gain or FCR (Table 4.8). However, inclusion of β-glucanase improved the total gain of birds (265.1 vs 235 g) and reduced FCR (2.43 vs 2.78 g feed/g gain, $P<0.05$). No interactions were found for any of the indices ($P>0.05$). The interaction least-square mean values of the, total gain and FCR are showed in Table 4.9.

Table 4.8 Interaction least-square mean values for total gain (TG) and feed conversion ratio (FCR)

	Low β-glucan		Medium β-glucan		High β-glucan		Pooled
	- enzyme	+ enzyme	- enzyme	+ enzyme	- enzyme	+ enzyme	SE
TG (g)	257.2	286.7	202.5	266.2	246.0	242.5	16.5
FCR (g feed/g gain)	2.6	2.4	3.1	2.4	2.7	2.6	0.16

Table 4.9 Level of significance of β-glucan, enzyme, and their interaction on total gain (TG) and feed conversion ratio (FCR)

	TG	FCR
β-glucan	NS	NS
Enzyme	*	*
β-glucan x Enzyme	NS	NS

** $P < 0.01$; * $P < 0.05$ NS Not significant

4.4 Discussion and Conclusions

In the present study, the addition of a commercially prepared β-glucanase to barley-based diets with three different levels of β-glucan significantly increased N, C, and GE digestibility. The weights of digestive organs including the whole gut, the 2nd half of the small intestine also decreased. The viscosity of the digesta decreased accompanied by increased weight gain and decreased feed conversion ratio of 15-day-old broiler chickens. Authors such as Hesselman and Aman (1986), Almirall *et al.* (1995, cited by Bedford, 1996), Choct *et al.* (1996) and Svihus *et al.* (1997 a and b) have reported similar improvements in nutrient digestibility with the addition of exogenous β-glucanases to barley-based diets. Brenes *et al.* (1993) and Svihus *et al.* (1997 a and b) have also reported decreases in the weight of digestive organs with the addition of β-glucanase to barley-based diets. Increases in weight gain of broiler chickens fed barley-based diets with the presence of exogenous β-glucanase have been reported by Hesselman and Aman (1986), Classen *et al.* (1988), Brenes *et al.* (1993), Philip *et al.* (1995), Choct *et al.* (1996), Svihus *et al.* (1997 a and b) and Yu *et al.* (1998), whereas improvements in FCR have been reported by Hesselmand and Aman (1986), Classen *et al.* (1988), and Choct *et al.* (1996). The reports of all these authors support the observations of the present study.

The reduction in the viscosity of digesta of chickens fed enzyme-supplemented diets in comparison to chickens fed diets without β-glucanase addition has been reported by authors such as Gohl *et al.* (1978), White *et al.* (1983), Annison and Choct (1994), Choct *et al.* (1996), Svihus *et al.* (1997b), and Yu *et al.* (1998). This topic is very well documented mainly because viscosity has been regarded as one of the main factors responsible for the anti-nutritive effects of NSP (Choct, 1997). In fact, the viscosity reduction achieved with the use of enzymes is considered largely responsible for the majority of the improvements observed in broilers fed diets based on cereals containing viscous NSP (Campbell and Bedford, 1992). However, in the present experiment a significant viscosity reduction with β-glucanase addition was solely obtained in the diet containing only the barley ("low" β-glucan content). There were no significant ($P>0.05$) differences between viscosity values obtained in the rest of the diets. This could have been a reflection of the β-glucan extract capturing the native β-glucan of the barley in

the case of diets "medium" and "high", and of the action of the β-glucanase in diet "low with enzyme". However, the inclusion of the β-glucanase had a positive effect on N, C, and GE digestibility, weights of some digestive organs, viscosity of the digesta, as well as weight gain and FCR of chickens fed all diets regardless of the viscosity of the diet. This has already been reported in a previous study (Campbell and Bedford, 1992) which showed that broiler chickens fed barley with a low viscosity (7.0 cP or less) frequently responded to enzyme addition. Campbell and Bedford (1992) also pointed out that this response is difficult to explain by viscosity reduction alone, and that it may be due to β-glucanase and other cell-wall-degrading enzymes releasing encapsulated intracellular nutrients, particularly starch and protein, which are then exposed to digestive enzymes within the gut lumen. Annison and Choct (1991) mentioned that although the majority of enzyme-induced improvement in the feeding value of barley is due to β-glucanase activity, other enzymes are also likely to be involved in the improvement of the nutritive value of barley. Campbell and Bedford (1992) have reported that the enzyme additives destined for animal feedstuffs generally exhibit activity towards a range of substrate materials. These results can indicate that viscosity is not the only mechanism of action involved in the anti-nutritive effects of β-glucan. Choct (1997) described other factors apart from an increase in the viscosity of digesta that influence the anti-nutritive effects of β-glucan. These factors include modification of endogenous secretions of water, proteins, electrolytes, and lipids, as well as interactions with gut microflora. In addition, NSP can bind ions and small molecules (Smits, 1996) such as bile salts, which in turn can affect lipid absorption and metabolism.

The way the different levels of β-glucan affected nutrient digestibility, weights of digestive organs, and digesta physico-chemical properties were unexpected. For nutrient digestibility, a decreased digestibility, with increasing β-glucan level was hypothesised. However, this did not occur. The highest C and GE digestibility were obtained with diets "medium" while diets "high" gave the lowest digestibility, and the digestibility obtained with diet "low" was not different ($P>0.05$) to that of the other diets in the case of C, and it was not different ($P>0.05$) to diet "medium" in the case of GE. These variations in the digestibility could be a reflection of a reaction between the β-glucan present in the barley and the β-glucan extract. As mentioned in Appendix I, when dispersed in water, the β-glucan extract forms a soft gel, not a viscous solution, making

the extract behave differently to solutions of the same concentration. It is possible that when barley and the β-glucan extract were mixed, the extract captured some of the native β-glucan from the barley, decreasing its anti-nutritive effects (G.D. Coles, pers. comm.). This could have caused the variations in digestibility previously mentioned, which did not reflect what was hypothesised. However, N digestibility was not affected ($P>0.05$) by the amount of β-glucan present in the diets. Maybe, the β-glucan levels in the diets were not sufficient to affect N digestibility. Probably, for N digestibility to be affected, the detrimental β-glucan threshold level or concentration was not achieved in this experiment.

The weights of the small intestine (1st and 2nd parts, full and empty), and the whole gut full and empty, were affected by the β-glucan level in the diet ($P<0.05$). For the 1st part of the small intestine full and empty, the weights obtained with diets "low" and "high" were higher than those obtained with diets "medium", while for the second part of the small intestine the weights obtained with diets "high" were significantly ($P<0.05$) higher than those with diets "low" and "medium", which had no differences between them. This differences between the 1st and the 2nd part of the small intestine could be due to the action of micro-organisms in the 2nd part of the small intestine which could have fermented some of the β-glucan contained in diets "low" and "medium". Maybe, the fermentation was not enough to decrease the anti-nutritive effects of β-glucans of diet "high", which was reflected as higher weights of 2nd half of small intestine and whole gut for birds on diets "high" compared to diets "low" and "medium". Increases in the weights of digestive organs, i.e. caeca, with increasing levels of barley (and thus β-glucan) have also been reported by Yu *et al.* (1998). Brenes *et al.* (1993) concluded that the increased size of the gastrointestinal tract observed when wheat and barley-based diets were fed to broilers might be an adaptive response to an increased need for enzymes. In addition, Bedford (1996) reported that when nutrient digestibility decreases and chickens perceive a lower nutrient density diet, the response is an increase in intestinal mass.

The WHC values obtained in diets "low" were significantly lower ($P<0.01$) than those obtained in the other two diets. The values obtained in diets "medium" and "high" were not different ($P>0.05$). This suggests that only the β-glucan of the extract had an effect

on the WHC of the digesta, while the β-glucan of the barley had very little or no effect. Probably, the fact that the barley β-glucan was encapsulated by the cell wall of the grain made some of its properties less noticeable than in the case of the extract, where the β-glucan of the extract was totally exposed to the digesta.

Although the ISV was originally intended to be an indication of the water uptake of the food particles in the digesta (Chapter 3 section 3.2.5.2), it was instead an indication of the amount of insoluble compounds present in the digesta. Thus, it increased as the amount of β-glucan either from the barley or from the extract increased.

Although the β-glucan level had an effect on C and GE digestibility, it did not affect the weight gain and the FCR of the chickens ($P<0.05$). This could probably be due to the short time of the experiment.

In conclusion, the inclusion of an exogenous β-glucanase to barley-based diets fed to 15-day-old birds improved N, C, and GE digestibility, decreased the viscosity of the digesta as well as the weight of the whole gut and the 2nd half of the small intestine, improved weight gain and FCR.

The animals' responses to the three different β-glucan levels in the diets were variable. In some cases, like GE digestibility, and the weights of the whole gut empty and the second part of the small intestine full and empty, the anti-nutritive effects of diets "low" and "medium" were similar between them, but significantly lower than the effects of diet "high". In other cases like viscosity, diets "high" and "medium" had similar effects, which were significantly lower than the effects of diets "low". In the case of WHC and ISV, the values obtained with diets "medium" and "high" were similar, but significantly higher than the values obtained with diets "low". These variations can most likely be explained by the different behaviour of the β-glucan extract used in this experiment in contrast to other extracts or to the intact (native) β-glucan from barley. The β-glucan extract used in these experiments forms a soft gel when dispersed in water rather than a viscous solution. The behaviour of these gels in a rheometer is quite different to solutions of the same concentration. It is believed that the β-glucan extract captured the native β-glucan of the barley, making it unextractable, and hindering any anti-nutritional effect it could have. Another explanation to these unexpected results could be

attributable to a threshold level of β-glucan. The β-glucan contained in some of the diets may not have been enough to elicit anti-nutritive effects compared to other diets. However, a number of questions remain unanswered in this regard, and more studies combining the β-glucan extract with barley need to be done.

It was also demonstrated that although some of the diets used in this experiment had no significant viscosity decreases with the inclusion of a commercial β-glucanase, there were still improvements in nutrient digestibility, weights of digestive organs, weight gain and FCR when the enzyme was included. This was probably due to the action of other enzymes contained in the β-glucanase commercial preparation, or to the β-glucanase acting on other ingredients of the diet in addition to the β-glucan. This could also suggest that viscosity is not the only factor responsible for the anti-nutritive effects of β-glucan and that other effects might also be involved. It is possible that the gelling capacity of the β-glucan extract was one of the main factors influencing the anti-nutritive effects of the extract.

4.5 References

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CHAPTER 5

DEVELOPMENT OF AN *IN VITRO* DIGESTIBILITY ASSAY TO SIMULATE *IN VIVO* DIGESTA PROPERTIES, AND NITROGEN, AND CARBON DIGESTIBILITY IN CHICKENS

5.1 Introduction

Interest in the development of *in vitro* methods to assess the digestibility of feedstuffs for poultry has increased in recent years due to the fact that the determination of digestibility by conventional methods requires larger quantities of feed than *in vitro* analyses, a number of animals, and considerable expenditure on equipment and labour (Graham and Lowgren, 1991). Furthermore, many of the *in vivo* techniques are criticised on ethical grounds, and this pressure is becoming more intense.

The feed industry imposes a continuing demand to have rapid *in vitro* methods capable of assessing the nutritional quality of both the raw ingredients and diets (McNab, 1991). The evaluation of the digestibility of diets based on ingredients that contain anti-nutritional factors, such as β-glucans in barley, is an important tool which can be used in diet formulation.

In the present work, an *in vitro* digestibility method was developed to assess the effects of barley β-glucans on the digestibility of N and C, as well as on the physico-chemical characteristics of the digesta, such as insoluble solids volume, water holding capacity and viscosity. Prior to this experiment, a number of pre-trials were carried out to evaluate the effects a β-glucan extract and different heat and humidity treatments applied to the extract and/or to the diet had on digesta physico-chemical properties (Appendix III). However, the *in vitro* digestibility method used in those pre-trials was designed to measure digesta physico-chemical properties and not nutrient digestibility. A modification of this method was done to allow the recovery of undigested protein, to accurately measure protein digestion.

The objective of the present work was to evaluate the accuracy of the *in vitro* digestibility method to predict *in vivo* N and C digestibility and digesta physico-chemical properties. This was done by digesting the diets used in Chapters 3 and 4 with the *in vitro* method, and correlating these *in vitro* values to those obtained *in vivo* in Chapter 3 and Chapter 4.

5.2 Material and Methods

5.2.1 Experimental diets

The diets used in Chapters 3 and 4 were also used for these *in vitro* experiments. Refer to sections 3.2.1 and 4.2.1 for composition of the experimental diets.

5.2.2 Experimental procedures

The *in vitro* digestibility method used was developed by Dr. J. Monro at the Crop and Food Research Institute, Palmerston North, New Zealand. The method is described in Table 5.1. In addition, during the different steps of the digestion process, measurements of insoluble solids volume (ISV), viscosity (Visc), and water holding capacity (WHC) were registered. These are also indicated in Table 5.1.

Table 5.1 *In vitro* simulation of chicken digestion process for testing the effects of β-glucan on digesta properties and nutrient digestibility

STEPS	GUT REGION SIMULATED	DIGESTA PROPERTY
1. Weigh 2 g feed into 10 ml culture tubes in quadruplicate.		
2. Add 5 ml water and allow to stand 30 min.	Crop	
3. Add 1.0 ml NaOH/1.0% pepsin. Mix. Check that pH 2.5 ±0.1. Incubate 42° C 1h.	Proventriculus	
4. Add 1.0 ml NaOH to give pH 7 ±0.1 (about 1 ml) and mix. Add 1 ml 5% pancreatin (centrifuged supernatant at 2500 g for 20 min. at pH 7.0) and mix. Incubate 2 hrs.	Duodenum	Visc
5. Centrifuge 2500 g, 20 min. Mark residue and digesta heights. Divide tubes into two sets of duplicates.		ISV
6. Add 2 ml 20% sulphosalicylic acid. Mix, and after 30 min centrifuge at 2500 g for 20 min..		
7. Discard supernatant, resuspend in 4% sulphosalicylic acid. Centrifuge at 2500 g for 20 min.		WHC
8. Fill tube with ethanol, resuspend residue, and centrifuge 2x. Acetone wash.		
9. Dry residue overnight and weigh.		

5.2.3 Analytical procedures

5.2.3.1 Digesta properties

The methodologies used to measure ISV, Visc, and WHC were the same as those used in Chapters 3 and 4, and are described in Chapter 3 section 3.2.5.2.

5.2.3.2 *In vitro* nutrient digestibility

Once the *in vitro* digestion process was finished, the dried digesta residues were analysed for nitrogen and carbon following the procedures described in Chapter 3 section 3.2.5.1.

From the data obtained in the laboratory, N and C digestibility were calculated as follows:

The g of N or C in the diet were calculated from the following equations:

$$g\ nutrient\ in\ diet = \frac{2 \times \%\ nutrient}{100} \quad (1)$$

$$g\ nutrient\ in\ residue = \frac{residue\ weight \times \%\ nutrient\ in\ residue}{100} \quad (2)$$

Then, from the data obtained, digestibility was calculated using the following equation:

$$nutrient\ \ digestibility\ \% = \frac{g\ of\ nutrient\ in\ diet - g\ of\ nutrient\ in\ residue}{g\ of\ nutrient\ in\ diet} \quad (3)$$

5.2.4 Statistical analysis

The means for each variable (*in vitro* and *in vivo* N and C digestibility, ISV, WHC, and visc) were calculated, and correlation and regression were calculated between the *in vitro* and *in vivo* data using Jandel Sigmaplot (Jandel, 1995). This was done first for each experiment in a separate way and then, the data of both experiments were analysed together to gain advantage of a larger number of observations. In addition, the *in vivo* and *in vitro* pooled values for overall N and C digestibility were plotted using Jandel Sigmaplot (Jandel, 1995).

5.3 Results

The *in vitro* and *in vivo* mean values for N and C digestibility, ISV, WHC and Visc are presented in Table 5.2.

Table 5.2 Mean values for *in vitro* and *in vivo* N and C digestibility, ISV, WHC and Visc of the digesta

	N digestibility (%)		C digestibility (%)		ISV (ml)		WHC (g water/g dry digesta)		Visc (cP)	
Diet	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*
					Experiment Chapter 3					
A0[1]	97.4	97.5	68.2	92.9	2.3	1.0	0.95	2.0	NM[2]	NM
A15	96.6	96.6	59.8	82.8	3.6	2.4	2.2	3.2	NM	NM
D0	97.9	96.7	90.0	88.8	3.2	0.5	1.7	2.2	NM	NM
D15	96.0	95.1	78.0	81.1	5.6	1.3	3.6	3.0	NM	NM
					Experiment Chapter 4					
L-	90.2	79.2	90.0	67.1	4.0	4.1	0.09	2.8	2.0	13.9
L+	91.7	82.8	90.1	70.6	3.8	3.8	0.09	2.8	1.9	3.6
M-	92.0	82.2	91.1	68.4	3.9	4.3	0.08	3.4	2.1	4.3
M+	93.9	85.3	92.1	76.2	4.3	4.4	0.08	3.2	1.7	3.0
H-	93.8	80.6	92.2	66.5	4.6	5.8	0.08	3.2	2.1	3.1
H+	92.6	84.5	91.8	71.0	4.4	5.0	0.08	3.2	2.0	2.5

[1]Refer to Appendix IV for abbreviations meanings; [2]Not measured

When the data of both experiments were analysed independently, the only parameters having high correlation values which were also significant were ISV and WHC of Chapter 4 (r=0.91, P=0.01; r=0.76, P=0.08, for ISV and WHC respectively). No significant correlations between *in vitro* and *in vivo* data were found for the rest of the parameters (Table 5.3). When the pooled data of both experiments were analysed, a high and significant correlation value (r=0.93, P<0.01) was obtained between the *in vitro* and *in vivo* N digestibility. The *in vivo* and *in vitro* C digestibility had a higher correlation when the data was analysed together, however it was negative (r = -0.64, P<0.05). The correlation values between *in vitro* and *in vivo* ISV and WHC did not improve when the means of both experiments were analysed together (Table 5.4).

Table 5.3 Correlation (r) values coefficient of determination (R^2) values, the standard error of the estimate (SEE), and their significance (P-value) for *in vitro* and *in vivo* N and C digestibility, ISV, WHC, and Visc.

	Experiment Chapter 3				Experiment Chapter 4				
	N dig	C dig	ISV	WHC	N dig	C dig	ISV	WHC	Visc
r	0.77	0.15	0.18	0.77	0.56	0.32	0.91	0.76	0.11
R^2	0.59	0.02	0.03	0.59	0.31	0.10	0.82	0.57	0.01
SEE	0.79	6.63	0.97	0.45	2.11	3.78	0.34	0.19	4.85
P-value	0.24	0.85	0.82	0.23	0.25	0.54	0.01	0.08	0.84

Table 5.4 Correlation (r) and coefficient of determination (R^2) values, the standard error of the estimate (SEE), and their significance (P-value) for *in vitro* and *in vivo* N and C digestibility, ISV, and WHC, of pooled data

	N dig	C dig	ISV	WHC
r	0.93	-0.64	0.38	0.037
R^2	0.87	0.41	0.14	0.19
SEE	2.7	7.6	1.79	0.49
P-value	<0.01	<0.05	0.28	0.59

The plotted mean *in vitro* and *in vivo* values for N and C digestibility are presented in Figures 5.1 and 5.2.

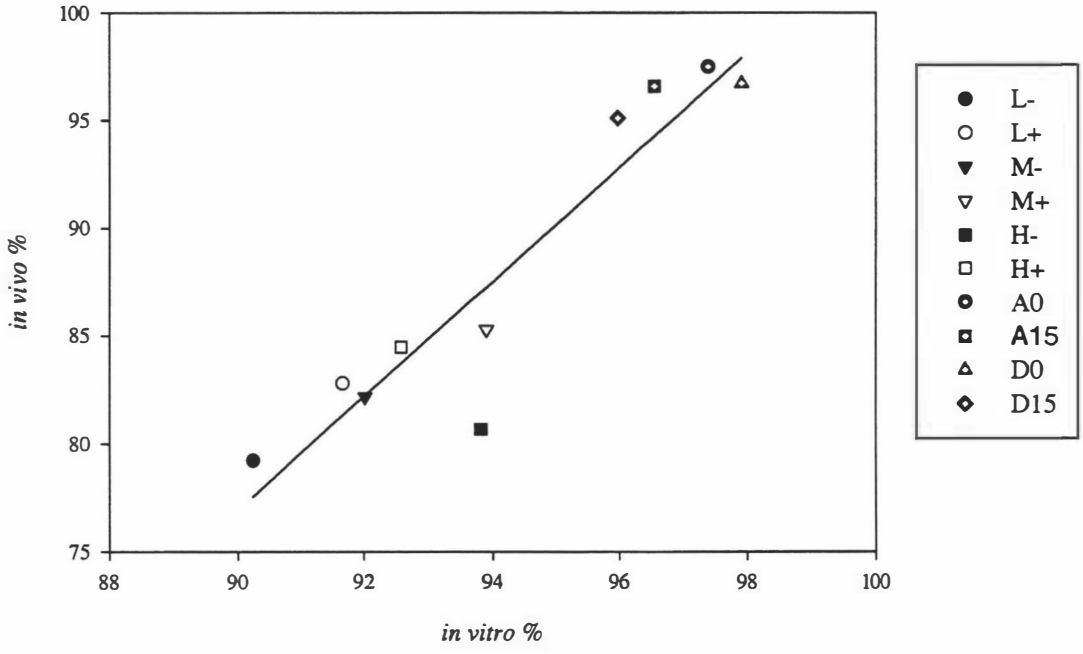

Figure 5.1 Comparison of *in vitro* and *in vivo* N digestibility (%)

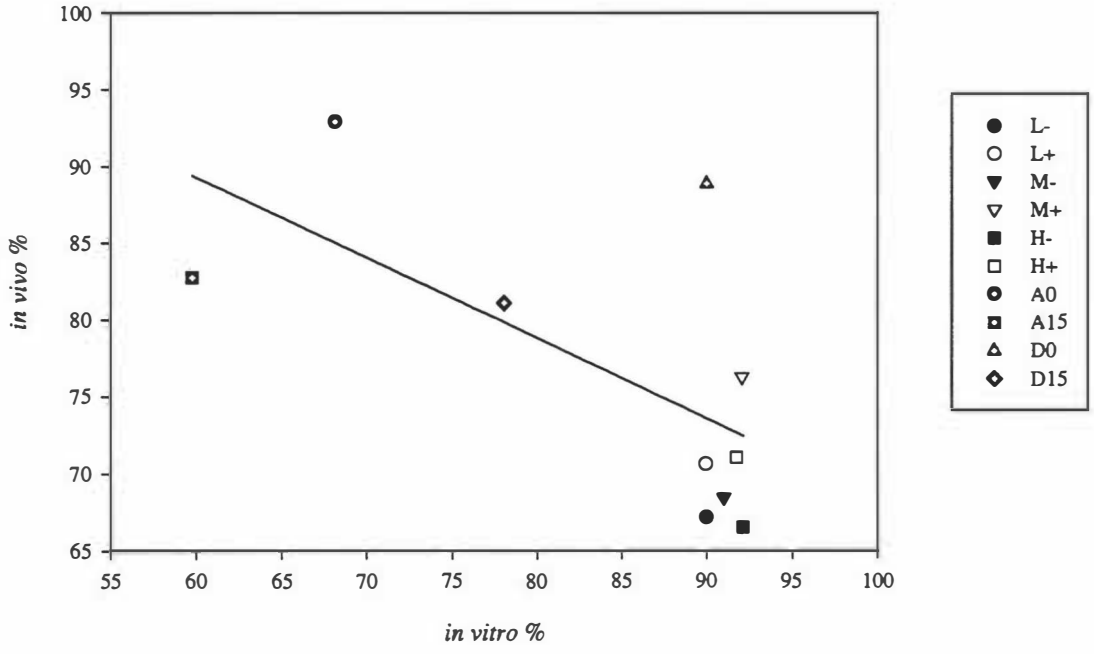

Figure 5.2 Comparison of *in vitro* and *in vivo* C digestibility (%)

5.4 Discussion and Conclusions

Although there were no significant correlations between *in vitro* and *in vivo* N and C digestibility when the data of the experiments were analysed in an independent way, analysing the data together gave high and significant correlation values between *in vitro* and *in vivo* N digestibility ($r=0.93$, $P<0.01$; $r = -0.64$, $P<0.05$ for N and C digestibility, respectively).

Buchmann (1979) developed an *in vitro* method very similar to the one used in the present experiment to predict the digestibility of N of several barley samples. Although he did not give regression or correlation values, the *in vitro* and *in vitro* data obtained apparently fitted a straight line. He pointed out that the accuracy of his *in vitro* method could have been demonstrated throughout a range of different barley samples which had considerable variations in protein digestibility and chemical composition due to the fact that they were all grown in different locations, different years, and in different conditions (field vs pot-grown).

This supports the results of the present experiment, in which the N digestibility could be predicted by the *in vitro* method, regardless of the composition of the diets (a synthetic diet based on cornstarch and casein vs a barley-based diet), and regardless of the treatment applied to them (heat or the inclusion of a β-glucanase).

The correlation value obtained for the digestibility of C when the data of both experiments were analysed together ($r = -0.64$) was significant ($P<0.05$), in comparison to the correlation values obtained when the experiments were analysed in a separate way ($P>0.05$). However, a low correlation between the *in vivo* and *in vitro* C digestibility was expected because in the *in vitro* method no specific enzymes to hydrolyse carbohydrates such as amylase were used. Although the pancreatin used in the *in vitro* method could have contained some pancreatic amylase, it was most likely insufficient to completely degrade the carbohydrates present in the diets, because other enzymes such as disaccharidases assist also in the degradation of carbohydrates. As well as endogenous enzymes, the microflora present in the intestine, which is hard to simulate in an *in vitro* digestion method, plays an important role in the degradation of carbohydrates. The fact that the correlation value for the C digestibility was negative was probably a reflection of the effect of heating the diet. The *in vivo* digestibility was

lower when heat was applied to the diet, in contrast to the *in vitro* digestibility which was higher when heat treatment was applied to the diet (refer to Fig. 5.2). Thus, the *in vitro* digestion did not really reflect what happened during the *in vivo* digestion. This could have been due to the assay not being specifically designed to analyse C digestibility, and maybe the relationship did not reflect what really happened, with the result obtained in fact being an artifact of the data set.

Although the correlation values between *in vitro* and *in vivo* N and C digestibility increased when the data of both experiments were analysed together, the correlation values of the physico-chemical properties of the digesta did not improve and, in the case of ISV, it decreased from r=0.91, P=0.01 in the experiment of Chapter 4, to r=0.38, P=0.28 when both experiments were analysed together. The lack of correlation between the *in vitro* and *in vivo* physicochemical properties of the digesta could be explained by the fact that such properties are greatly affected by the churning action that takes place in the gastrointestinal tract of the chicken, mainly in the gizzard, as well as by the action of the micro-organisms present in the intestine of the bird, which were not simulated by the *in vitro* method.

In conclusion, the *in vitro* digestibility method used in the present experiment predicted, in an acceptable way, the digestibility of N (R^2=0.87, SEE=2.7, P<0.01) in diets of different composition (a synthetic diet based on cornstarch and casein, and a barley-based diet), which had different treatments applied to them (heat or the inclusion of a β-glucanase). This suggests that the *in vitro* method may have some practical use across a broad range of diets as a screening method for digestibility of N in chickens. However, the *in vitro* method did not predict in an accurate way the digestibility of C (even though a significant correlation was found), nor the changes in the physico-chemical properties of the digesta (ISV, WHC, and viscosity). Although statistically the in vitro method predicted in vivo C digestibility in an acceptable way (r=-0.64, P<0.05), biologically it did not because no enzymes to degrade carbohydrates were included in the method, and the results obtained might had been an artifact of the data set. The physico-chemical properties of the digesta were not predicted by the in vitro method because some of the conditions that most affect them like the churning action of the gizzard and the effect of the micro-organisms that inhabit the gastrointestinal tract of the chickens were not replicated in the method. If the accuracy of the method to predict C digestibility and the

changes in physical properties of the digesta is to be improved, the factors previously mentioned should be taken into account and incorporated into the development of a new method.

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CHAPTER 6

EFFECTS OF A β-GLUCAN EXTRACT IN COMBINATION WITH FLAX OR COCONUT OIL ON SERUM CHOLESTEROL AND TRIGLYCERIDE LEVELS IN RATS

6.1 Introduction

High levels of plasma cholesterol are recognised as a significant risk factor for cardiovascular disease (Newman *et al.*, 1989; Schneeman and Lefevre, 1986), which is one of the leading causes of death in industrialised countries, accounting for huge costs for treatment and care (Newman *et al.*, 1989). Because of this, considerable efforts have been made on researching the dietary factors that could be associated with lower levels of cholesterol in plasma (Schneeman and Lefevre, 1986). Experimental human and animal studies have demonstrated the ability of several dietary fibres to lower plasma cholesterol. From the fibres evaluated, the water-soluble fibres appear to be the most effective in lowering plasma cholesterol levels (Schneeman and Lefevre, 1986). Amongst the soluble fibres, pectins, gums, and β-glucan have been extensively studied. The β-glucan is found predominantly in barley and oats (Newman *et al.*, 1989). Oat bran has been demonstrated to selectively lower plasma cholesterol, increase the high density lipoprotein (HDL) fraction of cholesterol, and decrease the low density lipoprotein (LDL) cholesterol (Bhatty, 1993). The hypocholesterolemic effects of barley have been demonstrated in several studies. Klopfenstein and Hoseney (1987; cited by Bhatty, 1993) reported lower cholesterol levels in rats fed barley bread compared to control rats. Newman *et al.* (1987b; cited by Bhatty, 1993), and Fadel *et al.* (1987; cited by Bhatty, 1993) reported reductions in serum cholesterol and LDL-cholesterol with different kinds of barley cultivars. In the study of Fadel *et al.* (1987), the inclusion of β-glucanase reversed the hypocholesterolemic effects of barley, establishing that β-glucan is the component responsible for these properties of barley.

In addition to fibre being a dietary factor which can affect the levels of cholesterol in blood, the ability of dietary fats to affect cholesterol levels have been studied in an extensive way since the 1950's. In general, studies have concluded that saturated fatty acids can raise cholesterol levels and polyunsaturated fatty acids can reduce cholesterol levels, while monounsaturated fatty acids have been demonstrated to lower LDL-cholesterol specifically. Polyunsaturated fatty acids have been demonstrated to mainly lower HDL-cholesterol (Khosla and Sundram, 1996).

In the present experiment, the hypocholesterolemic effects of barley β-glucan were

evaluated through the inclusion of a barley β-glucan extract in a synthetic diet based on cornstarch and casein, compared to diets without the presence of the extract. In addition, the effect of coconut oil, a rich source of saturated fatty acids, and flax oil, a source rich in polyunsaturated fatty acids, on plasma cholesterol levels were also evaluated individually, and in combination with the β-glucan extract.

The hypotheses of the present experiment were:

1. Animals consuming the diets containing β-glucan will have lower cholesterol levels compared to the animals consuming diets without β-glucan.

2. The cholesterol levels of rats consuming diets containing coconut oil will be higher to those of rats fed the diets containing flax oil.

6.2 Materials and Methods

6.2.1 Animals and management

A total of 36 male Sprague Dawley rats was used for this experiment. The rats were raised according to standard procedures in practice at the Massey University Small Animal Physiology Unit in Palmerston North, New Zealand, where the experiment was carried out. After weaning (at 3 weeks of age), the rats were fed a commercial rat diet manufactured by UniFeeds Ltd, Palmerston North, New Zealand (refer to Appendix V for diet composition) for one week, until the trial commenced.

6.2.2 Experimental design

At the beginning of the experiment (Day 0), 36 male Sprague-Dawley rats were randomly selected, weighed, and randomly allocated in a 2 X 2 factorial arrangement of treatments with the respective factors being "coconut oil" or "flax oil" as the source of lipid in the diet, and the absence or presence of a barley β-glucan extract added at 10% to the diet. Treatments were assigned as follows:

F0 = Flax oil, no β-glucan

C0 = Coconut oil, no β-glucan

F10 = Flax oil, 10% β-glucan

C10 = Coconut oil, 10% β-glucan

6.2.3 Experimental diets

The diets used for this experiment were synthetic diets based on cornstarch and casein, with the absence or presence of a barley β-glucan extract (refer to Chapter 3 for extract characteristics). The diets also contained chromic oxide (0.3%) which was used as an indigestible marker, and its concentration was used as a reference value from which digestibility coefficients were calculated. Nutrient levels recommended by the NRC (1995) were used as the basis for diet formulation. Table 6.1 shows the ingredients

composition of the experimental diets, and Table 6.2 shows the chemical composition of the diets.

Table 6.1 Ingredient composition of experimental diets (g/kg as-is basis)

INGREDIENT	DIET F0	DIET CO	DIET F10	DIET C10
Casein	160.0	160.0	160.0	160.0
Cornstarch	477.0	477.0	377.0	377.0
Flax oil	150.0	0	150.0	0
Coconut oil	0	150.0	0	150.0
Vitamin premix[1]	50.0	50.0	50.0	50.0
Mineral premix[1]	50.0	50.0	50.0	50.0
Sugar	70.0	70.0	70.0	70.0
Cellulose	30.0	30.0	30.0	30.0
Chromic oxide	5	5.0	5.0	5.0
β-glucan	0	0	100.0	100.0
DL-methionine	6.0	6.0	6.0	6.0
Tryptophan	2.0	2.0	2.0	2.0

[1]Refer to Appendix VI for composition of vitamin and mineral premixes.

Table 6.2 Nutrient composition of experimental diets

NUTRIENT	DIET F0	DIET C0	DIET F1O	DIET C10
Dry matter (%)	94.9	96.3	94.4	95.4
Nitrogen (% DM)	2.44	2.57	2.52	2.87
Fat (% DM)	17.5	14.1	14.1	15.8
Fatty acids (mg/g diet as is basis):				
C8	-	11	-	8
C10	6	8	1	16
C12	9	60	4	48
C14	6	21	3	27
C16	12	12	9	16
18:0	6	5	5	7
18:1	21	8	17.5	11
18:2	20	5	19	7
18:3	68	1	68	2

6.2.4 Experimental procedures

The day before the experiment started (Day –1), the rats were fasted for 16 hours from 5 PM until 9 AM the next day (Day 0), when they were anaesthetised with Forane (Isofluorane, inhalation anaesthetic, Abbot Laboratories). Blood samples were collected from the tail vein into ice-cold tubes. The tubes were kept on ice before analyses were made. After each rat was bled, it was placed under a desk lamp to raise body temperature until observed to be awake (about 5 min.).

The rats were then housed in individual stainless steel wire mesh cages in a controlled-temperature room having an average ambient temperature of 22 ± 2°C, with a 12 hour light/dark reverse cycle.

The animals were fed the experimental diets in stainless steel feeders for four weeks. In the first days the rats were offered between 10 and 13 g of food per day. The amount was then gradually increased until they were fed 17 ± 1 g of food per day during the last week of the experiment. The rats had access to their food 24 hours a day from Days 0 to 17 of the trial. From Days 18 to 28 they were kept on an hourly feeding regime in which they were presented with their food once an hour, for 10 minutes, eight times daily from 9:00 AM to 4:00 PM. The hourly feeding regime was adopted to ensure an even flow of digesta throughout the gut, which would minimise the effect of time when sampling the digesta after slaughter. Water was available on an *ad libitum* basis. Feed refusals were recorded daily. The rats were weighed weekly, and daily feed intake (DFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated every week.

On Day 25 of the trial the rats were fasted from 5:00 PM to 9:00 AM the next day (Day 26), when the bleeding procedure performed on Day 1 was repeated.

On Day 29 of the study the rats were weighed and hourly fed 6 or 7 times from 8:00 AM. At 1:00 PM, euthanasia commenced. For euthanasia, rats were anaesthetised with CO_2, killed by cervical dislocation and weighed. The small intestine of the rats was removed immediately. Hair and blood were washed off the small intestine with reverse osmosis (RO) water, and then the small intestine was dried with a paper towel. Digesta of the distal ileum were then flushed into labelled bags with RO water using a plastic syringe, taking care not to handle the intestine.

6.2.5 Analytical procedures

6.2.5.1 Blood lipids

After collecting blood samples, plasma and serum were prepared by centrifugation (20 min at 2500 g) in a Heraeus Minifuge T centrifuge and assayed for total cholesterol and triglycerides using a cholesterol kit (UNI-KIT) and a triglycerides kit from Roche

Diagnostic Systems (New Zealand) Limited. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were also assayed using a Boehringer-Mannheim Kit (kit numbers 1661442 and 1985604 for HDL-cholesterol and LDL-cholesterol, respectively). However, and because the HDL-cholesterol values obtained with the commercial kit were higher than the total cholesterol values obtained, HDL-cholesterol was calculated using the following formula by Friedewald *et al.* (1972):

$C_{HDL} = C_{plasma} - C_{LDL} - TG/5$

Where,

C_{HDL} = High density lipoprotein cholesterol

C_{plasma} = Plasma total cholesterol

C_{LDL} = Low density lipoprotein cholesterol

TG = Triglycerides

6.2.5.2 Nutrient digestibility

Digesta samples from the distal ileum were freeze-dried and analysed for Nitrogen and Carbon following the same procedures practised in Chapter 3, section 3.1.5.1. From the data obtained in the laboratory, N digestibility was calculated. Refer to Chapter 3 section 3.2.5.1 for the formulas used to calculate digestibility.

6.2.6 Statistical analysis

Data was analysed by analysis of variance using the GLM procedure of SAS (SAS, 1997), using the following model:

$Y_{ijk} = \mu + \beta_i + C_j + \beta_i C_j + \varepsilon_{ijk}$

where:

Y_{ijk} = The observation of the k^{th} individual exposed to the j^{th} and to the i^{th} treatment

μ = The population mean

β_i = The fixed effect of adding β-glucan

C_j = The fixed effect of the kind of oil present in the diet

$\beta_i C_j$ = The effects´ interaction

ε_{ijk} = The residual error

This model allowed all individual effects and possible interactions to be assessed. The significance level was set at α=0.05. Where convenient, differences between means were determined using the least significant difference (LSD) method with the GLM procedure of SAS (SAS Institute, 1997).

6.3 Results

6.3.1 Blood lipids

First, the blood lipid values obtained on Day 0 of the experiment were analysed to evaluate if there were significant differences between treatment groups. Results from that analysis showed no significant differences ($P>0.05$). Then, the lipid values obtained on Day 26 of the experiment were analysed using the first values as a covariate. This analysis showed no significant effects of the covariate on any of the final values obtained ($P>0.05$). Thus, blood lipid values obtained on Day 26 of the experiment were analysed using the model previously described (Section 6.2.6), and results obtained are presented in Tables 6.3 and 6.4.

Total cholesterol levels were decreased by the inclusion of β-glucan in the diets (1.58 vs 1.76 mmol/l for diets with and without β-glucan respectively, $P=0.07$). The type of oil used did not have any significant effect on the plasma levels of total cholesterol in rats ($P>0.05$).

The calculated HDL-cholesterol levels of rats fed the diets containing coconut oil were significantly lower ($P<0.05$) than the HDL-cholesterol values of rats fed the diets containing flax oil (0.97 vs 1.18 mmol/l for rats on diets containing coconut and flax oil respectively). The inclusion of the β-glucan in the diets did not have any significant effect ($P>0.05$) on the levels of the calculated HDL-cholesterol in blood.

The LDL-cholesterol levels of rats fed the diets containing coconut oil were lower ($P<0.01$) than those of rats fed diets containing flax oil (0.22 vs 0.28 mmol/l for diets with coconut oil and flax oil, respectively). The inclusion of β-glucan in the diets did not have any significant effect on the levels of LDL-cholesterol ($P>0.05$). However, as the kits used to measure HDL and LDL-cholesterol levels, as well as the formula used to calculate HDL were designed for human use, the results obtained may not reflect the real values.

In the case of triglycerides, a significant interaction ($P<0.05$) was found. The interaction shows that in the diets containing coconut oil, the inclusion of β-glucan decreased the

triglycerides values (0.75 vs 1.25 mmol/l, P<0.01 for diets C10 and C0, respectively). However, in the diets containing flax oil, the β-glucan inclusion did not have any significant effect (P>0.05). Also, it was observed that animals on the diet containing flax oil and no β-glucan had lower triglyceride levels than animals on the diet containing coconut oil and no β-glucan (0.53 vs 1.25 mmol/l, P<0.01 for diets F0 and C0, respectively). The same was observed for coconut and flax oil diets containing β-glucan (0.46 vs 0.53 mmo/l for diet F10 and C10, respectively, P<0.01), although the reduction was more important in diets not having any β-glucan. The TG results should be considered carefully as the method used to measure them is designed for human use.

Table 6.3 Interaction least-square means of TC, HDL (observed and calculated) and LDL-cholesterol, and TG (mmol/l) of rats fed the experimental diets

	Coconut oil		Flax oil		Pooled SE
	No β-glucan	10% β-glucan	No β-glucan	10% β-glucan	
TC	1.80	1.50	1.71	1.65	0.094
HDL-chol (observed)	1.91	1.73	1.94	1.84	0.095
HDL-chol (calculated)	1.0	0.95	1.19	1.17	0.089
LDL-chol	0.24	0.21	0.28	0.28	0.018
TG	1.25c[1]	0.75b	0.53ab	0.46a	0.10

[1] Within rows, means with the same letter were not significantly different (P<0.05)

Table 6.4 Level of significance of β-glucan, oil, and their interaction on TC, HDL and LDL-cholesterol, and TG

	TC	HDL-chol (observed)	HDL-chol (calculated)	LDL-chol	TG
Oil	NS	NS	NS	**	**
β-glucan	ξ	NS	*	NS	**
Oil X β-glucan	NS	NS	NS	NS	*

ξP=0.07; *P<0.05; ** P < 0.01; NS Not significant

6.3.2 Nutrient digestibility

A significant (P<0.01) interaction between type of oil used and the inclusion of β-glucan was found for N digestibility (Table 6.6). The interaction showed that the inclusion of β-glucan to the diets containing coconut and flax oil decreased N digestibility (84.0 vs 92.2 %, P<0.01 for diets C10 and C0, respectively; 70.5 vs 90.4 %, P<0.01 for diets F10 and F0, respectively). It also showed that the diet containing coconut oil and β-glucan

had a higher N digestibility than the diet containing flax oil and β-glucan (84.0 vs 70.5 % for diets C10 and F10, respectively). However, when the diets did not have β-glucan, the N digestibility was the same with both oils. Table 6.5 presents the interaction least-square means for N digestibility.

Table 6.5 Interaction least-square mean digestibility for Nitrogen (%)

	Coconut oil		Flax oil		Pooled SE
	No β-glucan	10% β-glucan	No β-glucan	10% β-glucan	
N digestibility	92.2a[1]	84.0b	90.4a	70.5c	0.99

[1] Within rows, means with the same letter were not significantly different (P<0.05)

Table 6.6 Level of significance of β-glucan, oil, and their interaction on N digestibility

	N digestibility
Oil	**
β-glucan	**
Oil X β-glucan	**

** P < 0.01; NS Not significant

6.3.3 Performance data

Performance data (DFI, ADG, and FCR) tended to be lower (or higher in the case of FCR) in rats consuming diet C10. The intake of rats fed diets C0, F0, and F10 was similar (P>0.05), but almost twice as high as the intake of rats fed diet C10 (14.06, 14.26, and 14.19 g for diets C0, F0, and F10, respectively, vs 8.55 g for diet C10, P<0.01). Regarding ADG, rats fed diet C10 had the lowest ADG of all (0.93 g) followed by rats fed diets C0 and F10, which had similar values (5.09 vs 5.06 g for diets C0 and F10, respectively, P>0.05), while diet F 10 gave the highest ADG value (5.65g), which was significantly different to values obtained with diets C0 and F10 (P<0.05). Although the ADG of rats fed the different diets was variable, the FCR was only affected by diet C10, with that of diets C0, F0, and F10 being lower to C10 (P<0.05), but similar to each other (P>0.05) (2.91, 2.67, and 3.75 g for diets C0 F0, and F10, respectively, vs 8.93 for diet C10). Table 6.7 shows interaction least-square mean DFI, ADG, and FCR values, and Table 6.8 shows the significance of the effects.

Table 6.7 Interaction least-square mean daily feed intake (DFI), average daily gain (ADG), and feed conversion ratio (FCR)

	Coconut oil		Flax oil		Pooled
	No β-glucan	10% β-glucan	No β-glucan	10% β-glucan	SE
DFI (g)	14.06a[1]	8.55b	14.26a	14.19a	0.35
ADG (g)	5.09a	0.93c	5.65b	5.06a	0.17
FCR (g feed / g gain)	2.91a	8.93b	2.67a	3.75a	1.21

[1] Within rows, means with the same letter were not significantly different (P<0.05)

Table 6.8 Level of significance of β-glucan, oils and their interaction on DFI, ADG, and FCR

	DFI	ADG	FCR
Oil	**	**	*
β-glucan	**	**	**
Oil X β-glucan	**	**	*

*P<0.05; ** P < 0.01; NS Not significant

6.4 Discussion and Conclusions

In the present study, the addition of a barley β-glucan extract to a synthetic diet based on cornstarch and casein decreased the levels of total cholesterol (TC) in the plasma of rats after 28 days of feeding (1.58 vs 1.76 mmol/l, P=0.07). The hypocholesterolemic effect of β-glucan and other soluble fibres have been demonstrated by a number of studies using different types of species such as humans, rats, and chickens. In a review on the role of cereal β-glucans in nutrition and health, Klopffenstein (1988) mentioned that rats fed glucan-rich breads had lower TC levels in blood after 35 days of feeding, compared to rats fed the control bread. Newman *et al.* (1992) also reported lower TC levels in the serum of rats and chickens fed barley-based diets with a high content of barley (30 to 60%). Mazur *et al.* (1990) reported cholesterol levels in serum of rats to be decreased by the intake of diets rich in fermentable carbohydrates, and McIntosh *et al.* (1991) reported that barley foods containing β-glucan are capable of lowering serum TC in hypercholesterolemic men in comparison to similar wheat foods. Also, in a review of the effects of soluble fibre on serum cholesterol, Topping (1991) reported that soluble NSP isolates of foods high in NSP have produced reductions between 6 and 30 % of serum TC in subjects with varying degrees of hypercholesterolemia. Peterson and Qureshi (1997) also reported decreases in the serum TC levels of chickens fed barley-based diets with respect to the control diets. Kritchevsky and Story (1993) mentioned that gelling fibres could reduce serum cholesterol levels in rats, regardless of whether the diets contain cholesterol or not.

All these studies confirm the finding of the present study. However, in an experiment similar to the present study in which tortillas were enriched with a β-glucan extract and fed to rats for 25 days, Hecker *et al.* (1998) reported that the β-glucan-enriched tortillas did not have any significant effect in the levels of TC in serum when compared to the control group.

The TC concentrations in blood were not affected by the type of oil present in the diet. The fact that flax oil, a rich source of n-3 PUFA, did not have a significant effect on the blood TC levels coincides with reports of Herold and Kinsella (1986), Harris (1989), Kinsella (1990), and Nestel (1990) (all cited by McNamara, 1992). They demonstrated

that the major response to n-3 PUFA intake is a reduction in plasma TG levels, which was also demonstrated in our study, but not TC levels. Harris (1989; cited by McNamara, 1992) also mentioned that n-3 PUFA intake reduces plasma TG levels, and that LDL and HDL levels are either not changed or modestly increased, which is reflected as no changes in the levels of TC in blood. Regarding coconut oil, early studies by Hegsted *et al.* (1965; cited by Khosla and Sundram, 1996) in men fed different kind of SFA showed apparently no effect of lauric acid, the main fatty acid present in coconut oil, on TC levels in blood. However, these authors also mentioned that myristic acid, the second most important acid present in coconut oil, was the most potent SFA increasing TC levels in blood, and Khosla and Sundram (1996) have pointed out that myristic acid has a substantial hypercholesterolemic role. When Denke and Grundy (1992; cited by Khosla and Sundram, 1996) fed cholesterol-free diets to men, they found that lauric acid raised TC but TG were unaffected. In a review of dietary fatty acids, lipoproteins, and cardiovascular disease, McNamara (1992) mentioned that although coconut oil appears to have a substantial hypercholesterolemic effect in humans and animals, the data available is limited and not always consistent. Some studies on the effects of lauric acid have reported it as has potent hypercholesterolemic effects, while others reported it has small effects on plasma TC. McNamara (1992) also mentioned that data from animal studies have shown a pronounced hypercholesterolemic effect of coconut oil when compared with PUFA (as is the case of the present experiment), but it is unclear whether the hypercholesterolemic effects are due to the lauric or the myristic content of coconut oil.

Regarding the levels of TG in blood, the data obtained showed that animals fed coconut oil had significantly higher levels of TG than animals fed the diets containing flax oil, regardless of the presence or absence of β-glucan (1.25 vs 0.53 mmol/l for diets C0 and F0, respectively, $P<0.01$; 0.75 vs 0.46 mmol/l for diets C10 and F10, respectively, $P<0.01$). These results coincide with reports of Meijer *et al.* (1987), in which the TG concentrations in the blood of rats fed coconut oil were significantly higher than those of rats fed corn oil. However, when Katan *et al.* (1994) applied two predictive formulas to rank commercially available oils by their effects on plasma lipids and lipoproteins, their calculations showed that coconut oil decreases the levels of TG in blood by 0.2 mmol/l or 15 mg/dl. Coconut oil is a rich source of lauric acid and, according to Khosla

and Sundram (1996), lauric acid did not affect the TG levels of men fed cholesterol-free diets.

Flax oil is a rich source of α-linoleic acid, which is a n-3 polyunsaturated fatty acid (PUFA). In numerous metabolic studies, n-3 PUFAS have been demonstrated to possess significant hypolipidemic effects, whith the major response being a reduction in plasma TG levels (McNamara, 1992). Harris (1989; cited by McNamara, 1992) demonstrated that the primary effect of n-3 PUFA intake is a reduction in plasma TG levels. He also reported that the decrease in plasma TG levels in response to n-3 fatty acids intake is positively related to the extent of hypertriglyceridemia exhibited by the studied subjects, although in normolipidemic men a 25% reduction of TG plasma levels was observed.

The inclusion of β-glucan in the diets did not have a similar effect in diets containing coconut oil and flax oil. For the diets containing coconut oil, β-glucan inclusion decreased TG levels in blood (0.75 vs 1.25 mmol/l for diets C10 and C0, respectively, $P<0.01$). However, for diets containing flax oil, β-glucan inclusion did not have any significant effect. This coincides with reports of Abbey *et al.* (1993) who evaluated the effects of NSP and fish oil on plasma lipids of rats. He found that in rats consuming the diets containing fish oil, which like flax oil is a rich source of n-3 PUFA, the inclusion of NSP (pectin, methylcellulose, and guar gum) in the diet did not have any significant effect on TG levels in blood. Authors such as Klopfenstein (1988), Mazur *et al.* (1990) and Peterson and Qureshi (1997) have reported reductions in serum TG levels of animals (rats and chickens) fed fermentable carbohydrates or barley-based diets. However, these authors analysed the effects of NSP inclusion alone, and not in combination with dietary fat, as in the present study and as in the study of Abbey *et al.* (1993).

The TG levels should be considered carefully due to the fact that the methodology used to measure TG in the present experiment was designed for human use. The method involves the use of a lipase to break down the triglycerides in free fatty acids and glycerol, and the levels of glycerol are measured as an indicative of triglycerol levels. In humans, the levels of free glycerol in blood are very small, and do not affect the accuracy of the method. However, it is not known if the amount on free glycerol in rats

is higher than in humans. If it is, this method overestimates the amount of triglycerides found in blood, affecting the results previously presented (Phil Pearce, pers. comm.).

Regarding HDL- and LDL-cholesterol, the values obtained may not reflect the actual levels of HDL and LDL, as the kit tests used in the present experiment are designed specifically for human use. The calculated HDL-cholesterol also may not reflect the real values due to the fact that the formula by Friedewald *et al.* (1972) was not designed for use in rats (Phil Pearce, pers. comm.). Therefore, the HDL-cholesterol and LDL-cholesterol values obtained in the present study will not be discussed.

Although it was beyond the scope of the present work to discuss the reasons for the variations in N digestibility, DFI, ADG, and FCR, the effects that the β-glucan inclusion had on some of these parameters may help to explain at least one of the proposed mechanisms for the hypocholesterolemic effects of β-glucan.

Several mechanisms have been proposed for the hypocholesterolemic effects of β-glucan, the main ones being bile acid binding, and alterations in the absorption of lipids in the intestine. The final consequence of both mechanisms is an alteration of cholesterol metabolism. The increased viscosity produced by soluble fibres has been regarded as the factor responsible for the alterations in lipid absorption (Vahouny, 1982; Newman *et al.*, 1989; Topping, 1991). However, Topping (1991) pointed out that not all studies had given the same results. Some of the studies he reviewed concluded that viscous NSP have marked hypocholesterolemic effects. However, in other studies where NSP of different viscosities were fed to rats, no effects on plasma TC were found, and another study reported that from the NSP evaluated, the one having the lowest viscosity gave the lowest plasma TC values. Topping (1991) concluded that while viscosity may be involved in cholesterol reduction and alterations of fat absorption, the relationship is not a simple one. In addition to viscosity, gel formation has also been proposed as a mechanism which may delay lipid and other nutrient absorption (Anderson and Chen, 1979; Cassidy and Calvert, 1993). Cassidy and Calvert (1993) suggested that gelling fibres may modify the resistance of the surface-associated unstirred water layer of the small intestine, which in turn can influence nutrient flux and absorption. From other studies reviewed by Cassidy and Calvert (1993), these authors suggested that gelling fibres also interfere with the diffusion of lipid-containing micelles within the small

intestine. The results of the present experiment coincide with the suggestions made by Anderson and Chen (1979) and Cassidy and Calvert (1993). As mentioned in Appendix I, the barley β-glucan extract used in the present experiment tends to form a soft gel rather than a viscous solution when mixed with water. Thus, it is believed that the formation of this gel, rather than an increase in viscosity, was one factor responsible for the reductions in TC and TG of rats fed the diets containing β-glucan. It is possible that these reductions were due to alterations in the absorption of lipids, as a decreased N digestibility with β-glucan inclusion, probably due to a lower N absorption, was demonstrated.

In conclusion, the present study showed lower TC and TG levels in the blood of rats fed diets containing a β-glucan extract, in contrast to rats fed diets without β-glucan. It was also demonstrated that the addition of coconut oil, a rich source of SFA, and flax oil, a source rich in n-3 PUFA, had no effects on the serum TC levels of rats. However, rats fed coconut oil had higher TG levels in blood than rats fed flax oil. The inclusion of β-glucan decreased the TG levels in rats fed coconut oil but not in rats fed flax oil. In addition, it is proposed that the hypocholesterolemic effects of the β-glucan extract used in the present experiment were due to a decrease in lipid absorption in the small intestine, which was caused by the β-glucan inducing a gel formation which delayed nutrient absorption. In addition, this gel formation was considered to be one of the main factors responsible for the hypocholesterolemic effects of the β-glucan extract.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The present work evaluated the anti-nutritive effects of barley β-glucans in broiler chickens as well as its hypocholesterolemic effects in rats. In order to do this, a commercially prepared barley β-glucan extract was used.

The inclusion of the β-glucan extract in a synthetic diet based on cornstarch and casein confirmed the anti-nutritive effects of β-glucan in broiler chickens. Decreases ($P<0.01$) in N, and C digestibility were found in birds consuming the diets containing β-glucan. In addition, the WHC of the digesta of chickens fed the diets containing β-glucan was higher ($P<0.01$) than that of the chickens fed the control diet, indicating that the β-glucan extract conserved some of its physico-chemical properties. The weights of the second part of the intestine full, and the full caeca were higher ($P<0.01$) in birds fed the diets with β-glucan, which could possibly be a reflection of the increase in the water holding capacity (WHC) of the digesta of those birds. However, the β-glucan addition did not increase ($P>0.05$) the empty weight of any of the digestive organs evaluated, contradicting the findings of Brenes *et al.* (1993), and Bedford (1996) who reported increases in the size of the gastrointestinal tract of birds fed barley-based diets.

Further, heat treatment of the synthetic diets showed that the anti-nutritive effects of β-glucan were not increased ($P>0.05$) by heat. Although the insoluble solids volume (ISV) of the digesta was decreased ($P<0.01$), which probably indicated an increase in the solubility of the β-glucan, the anti-nutritive effects of the β-glucan were not increased by heat treatment, as proposed by Antoniou and Marquardt (1982; cited by Classen and Bedford, 1991), and Classen and Bedford (1991).

In a further experiment, different amounts of β-glucan were included in barley-based diets to provide three different levels of dietary β-glucan (low (19.8 g β-glucan/kg diet), medium (50.7 g β-glucan/kg diet), and high (68.3 g β-glucan/kg diet)). The responses to the different levels of β-glucan were diverse. In some cases such as GE digestibility and the weights of the whole gut and the second part of the small intestine, the values of diets low and medium were similar, but significantly lower ($P<0.05$) than the values of the diet high. For other measurements such as viscosity, diets medium and high gave similar values ($P>0.05$), which were significantly lower ($P<0.01$) than the values given by diet low. The variable responses obtained with the

different levels of β-glucan are attributed to the different behaviour the β-glucan extract has in contrast to the native or intact β-glucan in barley. When the β-glucan extract is mixed with water, it forms a soft gel rather than a viscous solution. It is believed that the intact β-glucan from barley was captured by the β-glucan extract, hindering some, or maybe all the anti-nutritive effects it could have. In addition, this gel formation is considered one of the main factors influencing the anti-nutritive effects of the β-glucan extract. Another explanation for the variable responses obtained is attributed to a threshold level of β-glucan, which in some diets was not enough to elicit its anti-nutritive effects.

Including a β-glucanase in all the barley-based diets (low, medium, and high) improved N. C ($P<0.01$), and GE ($P<0.05$) digestibility, decreased the viscosity of the digesta ($P<0.05$) and the weights of the whole gut and the second part of the small intestine ($P<0.01$), and improved ($P<0.01$) weight gain and FCR of the birds.

This experiment also demonstrated that the anti-nutritive effects of β-glucan were not only due to increases in the viscosity of the digesta because although the viscosity of the digesta of birds fed diets medium and high was low, the diets still had anti-nutritive effects, and that the gelling capacity of the β-glucan extract might be equally important.

The anti-nutritive effects of the β-glucan extract were further evaluated using an *in vitro* digestibility method, which simulated the gastrointestinal tract of the chicken. The method demonstrated to have a good accuracy ($r=0.93$, $P<0.01$) in predicting N digestibility of all the diets used in the chickens' experiments, regardless of the composition of the diet or the treatments they had. Although a significant correlation between the *in vitro* and *in vivo* C digestibility ($r=-0.64$, $P<0.05$) was found, the *in vitro* digestion did not reflect what happened during the *in vivo* digestion. This was possibly due to the fact that the *in vitro* assay was not designed to analyse C digestibility, and the results obtained were an artifact of the data set. Digesta physico-chemical properties (ISV, WHC and viscosity) were not accurately predicted by the *in vitro* method, due presumably to the fact that the physical action of the digestive tract of the chicken as well as the action of the microorganisms that inhabit the chickens'

gut (which greatly influence the physico-chemical properties of the digesta) were not simulated in the *in vitro* method.

Finally, the hypocholesterolemic effects of barley β-glucan in rats examined by feeding male rats a synthetic diet based on cornstarch and casein which had β-glucan extract added. In addition, coconut oil (rich in SFA), and flax oil (rich in PUFA) were also added to the diets to evaluate their effects on blood lipids. Rats consuming diets containing the β-glucan extract not only had lower total cholesterol (TC) levels in blood, but also lower triglycerides (TG) levels compared to rats fed diets with no β-glucan extract inclusion. Serum TC levels were not affected by the kind of oil present in the diet. However, rats fed coconut oil had higher ($P<0.01$) TG levels in blood than rats fed flax oil. The inclusion of β-glucan decreased ($P<0.01$) the TG levels of rats fed the diets containig coconut oil, but not in rats fed flax oil. In addition, it is suggested that the decreases in TC and TG levels in the blood of rats fed β-glucan were exerted through a decrease in the absorption of lipids due to a gel formation in the digesta induced by the β-glucan extract. In addition, this gel formation was considered to be one of the main factors responsible for the hypocholesterolemic effects of the β-glucan extract.

Throughout all the animal experiments performed in the present study, the anti-nutritive effects, as well as the hypocholestrolemic effects in rats elicited by barley β-glucans, were confirmed through the use of a commercially extracted barley β-glucan. This did not only confirm the findings of authors such as White *et al.* (1983), Klopfenstein (1988), Annison and Choct (1991), Annison and Choct (1994), and Hecker *et al.* (1998) regarding the anti-nutritive and hypocholesterolemic effects of barley β-glucans, but also demonstrated that the β-glucan extract conserved its anti-nutritive and hypocholesterolemic properties after the extraction method was performed. One of the main findings of the present study was that an increase in the viscosity of the digesta of animals fed β-glucan is not the main factor responsible for the effects this NSP has on nutrient digestibility as well as its influence on TC and TG metabolism. It is known that the extraction method modified the capacity of the β-glucan extract to form viscous solutions, and that it forms a soft gel when dispersed in water. The formation of this gel, rather than an increase in the viscosity is believed to be one of the main factors contributing to the anti-nutritive and hypocholesterolemic

effects of the β-glucan extract. This is supported by results of the experiment where the effects of three different levels of β-glucan were evaluated (Chapter 4), in which the anti-nutritive effects of β-glucan were confirmed, even though the diets fortified with the β-glucan extract did not show high viscosities.

In conclusion, the anti-nutritive, and hypocholesterolemic effects of barley β-glucans were demonstrated through the use of a commercially prepared barley β-glucan extract. Broiler chickens fed synthetic diets containing the β-glucan extract had lower ($P<0.01$) N and C digestibility, and a higher ($P<0.01$) WHC of the digesta, which was reflected as a heavier gastrointestinal tract. When the β-glucan extract was combined with barley and fed to broiler chickens, variable results in nutrient digestibility and physico-chemical characteristics of the digesta were obtained. This was attributed to an encapsulation of the native β-glucan from barley by the β-glucan extract, or to the fact that a threshold level of β-glucan was needed for the anti-nutritive effects to be noticeable, which probably was not reached in some of the diets. In addition, an *in vitro* method to evaluate the anti-nutritive effects of β-glucan in broiler chickens was developed. The method accurately predicted N digestibility, but not the digestibility of C nor the changes in the physico-chemical properties of the digesta like ISV, WHC or viscosity induced by β-glucan.

In rats, β-glucan proved to decrease the levels of TC and TG in blood. These effects could have probably been elicited by a decrease in the digestion and absorption of lipids, as a consequence of the β-glucan inducing a gel formation in the digesta. In addition, coconut oil, a rich source of SFAS increased the levels of serum TG in rats, while flax oil, a rich source of PUFAS decreased serum TG levels. Neither of the oils had effects on TC levels in blood when they were fed alone, but when coconut oil was combined with the β-glucan extract, serum TC levels were decreased. The TC levels induced by feeding flax oil were not changed by the inclusion of β-glucan.

It is suggested that an increase in the viscosity of the digesta of animals fed β-glucan is not the main mechanism responsible for the anti-nutritive effects of this NSP because the β-glucan extract used in the present work does not induce increases in viscosity when dispersed in water, but the formation of a soft gel. If increases in the viscosity were the main mechanism for the anti-nutritive and hypocholesterolemic

effects of β-glucan, the extract used would have not been able to induce these effects the way it did. However, it is not known to what extent the effects of the β-glucan extract are similar to the effects of intact β-glucan. Further studies comparing the β-glucan extract used in the present experiment and barley are required.

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APPENDICES

Appendix I Characteristics of barley β-glucan extract

Prepared at: New Zealand Institute for Crop and Food Research, Christchurch, New Zealand

Commercial name: Glucagel

Purity: 70%

Physical appearance: White, odourless powder

Chemical composition:

Dry Matter %	Organic Matter %	Nitrogen %	Carbon %
89.04	98.04	0.553	38.28

Chemical features: Low molecular weight, partially depolymerised, when dispersed in water forms a soft gel, not a viscous solution

Appendix II Composition of mineral and vitamin premix used in chicken's diets

Nutrient	Amount
Vit A (IU)	11.1
Vit D (IU)	2.4
Vit E (IU)	60.0
Vit K (IU)	4.0
Vit B1 (IU)	3.0
Vit B2 (IU)	12.0
Vit B3 (IU)	35.0
Vit B5 (IU)	12.8
Vit B6 (IU)	10.0
Vit B12 (IU)	0.017
Folic acid (IU)	5.2
Biotin (IU)	0.20
Mn (ppm)	125.0
Zn (ppm)	60.0
Cu (ppm)	5.0
Co (ppm)	0.30
Fe (ppm)	25.0
I (ppm)	1.0
Mo (ppm)	0.5
Se (ppm)	0.2

These series of *in vitro* experiments were designed to evaluate how different concentrations and treatments of β-glucan would affect digesta physical properties (insoluble solids volume (ISV), water holding capacity (WHC), and viscosity (visc)). The studies were done at the Crop and Food Research Institute, Palmerston North, New Zealand.

The diet used in these preliminary trials was the same synthetic diet used in experiment of Chapter 3. Refer to section 3.2.3 for diets composition. A description of the β-glucan concentrations and pretreatments is given in Table III.1.

Table III.1 β-glucan concentrations and pre-treatments

Experiment no.	Treatment name	β-glucan conc. (%)	β-glucan treatment
Experiment 1	A1	3 & 5	None.
	B1	3 & 5	Dissolved in hot water, added hot.
	C1	3 & 5	Mixed with cold water, added cold.
	D1	3 & 5	Dissolved in hot water, gelled, cooled.
Experiment 2	E2	0,5,10,15& 20	5 ml water were added to the β-glucan, mixture heated in a boiling water bath for 10 min with frequent inversion to dissolve the β-glucan. Tubes were cooled. Then the diet was added and mixed into the β-glucan solution.
Experiment 3	A3	0, 5 & 15	None.
	B3	0, 5 & 15	Gelatinised, mixed with the diet and dried before digesting.
	C3	0, 5 & 15	Gelatinised, mixed with the diet, digested immediately.
	D3	0, 5 & 15	Mixed dry with the diet, then the mixture was combined with 5 ml water, heated at 100°C for 10 min and digested immediately.
Experiment 4	A4	0 &15	None.
	B4	0 &15	Dissolved in 5 ml hot water (100°C) for 10 min, mixed with diet, and digested immediately.
	D4	0 &15	Mixed dry with the diet, mixture combined with 5 ml water, heated at 100°C for 10 min, and digested immediately.

The *in vitro* digestibility method used was developed by Dr. J. Monro at the Crop and Food Research Institute, Palmerston North, New Zealand. The method is described in Table III.2.

Table III.2 In vitro digestibility method to evaluate the effects of β-glucan concentration and treatment in the physical characteristics of chicken's digesta

STEPS	GUT REGION SIMULATED	VOLUME (ml)	MEASUREMENT
1. Weigh 2 g feed into 10 ml culture tube			
2. Add 5 ml water. Allow to stand 30 min. Centrifuge, mark residue height, resuspend.	Crop	5	
3. Add 1.0 ml of 1M HCl containing 0.5% pepsin. Mix. Incubate 42° C 1 h. Centrifuge, mark residue height, resuspend.	Proventriculus	6	
4. Add 1.0 ml 1M NaOH and mix thoroughly.		7	
5. Add 1 ml 5% pancreatin (centrifuged supernatant adjusted to pH 7.0).	Duodenum	8	
6. Incubate 1.5 hrs 42 C			
7. Allow to stand 30 min. Remove 0.5 ml from top of liquid for viscosity measurement.			
8. Centrifuge 2500 g. 20 min. Remove 0.5 ml supernatant for viscosity measurement. Centrifuge, mark residue and digesta heights.			VISC ISV
9. Drain the tubes by inverting 20 min. Weigh.			
10. Dry tubes overnight in vacuum oven. Reweigh.			WR

The ISV, WHC, and viscosity measurements were done according to the methodologies described in Chapter 3, section 3.2.5.2 Data was analysed by analysis of variance using the GLM procedure of SAS (SAS, 1997). In addition, analysis of variance was performed and differences between means were determined using the least significance difference method (LSD) with the GLM procedure of SAS. The interaction least-square mean results as well as the significance of the factors of each experiment are presented from Table III.3 to Table III.10

Table III.3 Interaction least-square mean ISV and WHC of *in vitro* experiment one

	Treatment A1		Treatment B1		Treatment C1		Treatment D1		Pooled SE
	3% β-gluc	5% β-gluc	3% β-gluc	5% β-gluc	3% β-gluc	5% β-gluc	3% β-gluc	5% β-gluc	
ISV	0.93cf*	1.05g	0.87bcd	0.89bce	0.81abc	0.75a	0.85adef	0.97edfg	0.03
WHC	1.41a	1.52b	1.59c	1.73e	1.57bc	1.68d	1.45a	1.85f	0.015

*Within columns, means with the same letter were not significantly different (P<0.05).

Table III.4 Significance of β-glucan treatment, concentration, and their interaction on the digesta physico-chemical characteristics of *in vitro* experiment one

	ISV	WHC
β-gluc treatment	**	**
β-gluc conc.	ξ	**
Treatment X Conc.	*	**

**P<0.01, * P<0.05; ξ=0.08

Table III.5 Interaction least-square mean ISV, WHC and VISC of *in vitro* experiment two

	No β-gluc	5% β-gluc	10% β-gluc	15% β-gluc	20% β-gluc	Pooled SE
ISV	1.67a*	2.07b	3.21c	3.97d	4.92e	0.078
WHC	1.16a	1.25a	1.94b	2.09b	2.31c	0.061
VISC	1.09c	1.43ab	1.49b	1.36a	1.38a	0.026

*Within columns, means with the same letter were not significantly different (P<0.05).

Table III.6 Significance of β-glucan treatment, concentration, and their interaction on the digesta physico-chemical characteristics of *in vitro* experiment two

	ISV	WHC	VISC
β-gluc treatment	**	**	**

** P<0.01

Table III.7 Interaction least-square mean ISV, WHC and VISC of *in vitro* experiment three

	Treatment A3			Treatment B3			Treatment C3			Treatment D3			Pooled SE
Gluc (%)	0 % gluc	5 % gluc	15 % gluc	0 % gluc	5 % gluc	15 % gluc	0 % gluc	5 % gluc	15 % gluc	0 % gluc	5 % gluc	15 % gluc	
ISV	1.73b c*	2.25d	3.18e f	1.70a c	2.14d	3.10a f	1.70b	2.02d	3.63g	3.02a e	4.08h	5.06i	0.075
WHC	1.21a	1.49b	1.80d	1.22a	1.48b	1.87e	1.22a	1.38f	1.13g	3.25c	3.26c	3.07h	1.85
VISC	1.06a b	1.05a b	1.18c d	1.04b	1.13a bc	1.23b d	1.03a	1.27d e	1.32e f	1.24d	1.39g	1.41g	1.26

*Within columns, means with the same letter were not significantly different (P<0.05).

Table III.8 Significance of β-glucan treatment, concentration, and their interaction on the digesta physico-chemical characteristics of *in vitro* experiment three

	ISV	WHC	VISC
β-gluc treatment	**	**	**
β-gluc conc.	**	**	**
Treatment X Conc.	**	**	**

** P<0.01

Table III.7 Interaction least-square mean ISV, WHC and VISC of *in vitro* experiment four

	Treatment A4		Treatment B4		Treatment C4		Pooled SE
	No β-gluc	15% β-gluc	No β-gluc	15% β-gluc	No β-gluc	15% β-gluc	
ISV	1.61a*	1.87b	1.61a	20.4b	2.65c	3.10d	0.034
WHC	1.33a	3.03ab	1.33a	3.30ac	3.92ad	4.40bcd	0.793

*Within columns, means with the same letter were not significantly different (P<0.05).

Table III.4 Significance of β-glucan treatment, concentration, and their interaction on the digesta physico-chemical characteristics of *in vitro* experiment one

	ISV	WHC
β-gluc treatment	**	∂
β-gluc conc.	**	∂
Treatment X Conc.	ξ	NS

** P<0.01; ξ=0.06; ∂=0.08; NS Not significant

Appendix IV Diets' abbreviations used in Chapter 5

A0	No β-glucan, no heat
A15	15% β-glucan, no heat
D0	No β-glucan, heated
D15	15% β-glucan, heated
L-	Low β-glucan, no β-glucanase
L+	Low β-glucan, plus β-glucanase
M-	Medium β-glucan, no β-glucanase
M+	Medium β-glucan, plus β-glucanase
H-	High β-glucan, no β-glucanase
H+	High β-glucan, plus β-glucanase

Appendix V Composition of diet given to rats between weaning and beginning of the experiment

Ingredient	g/kg (dry matter basis)
Wheat	401.0
Barley	300
Broll	50
Lucerne	50
Meat & bone meal	60
Fish meal	70
Skim milk powder	50
Soya bean oil	10
Salt	5
Vit & min premix	4

Appendix VI Composition of mineral and vitamin premixes used in rats' experiment

Vitamin composition

Vitamin	Concentration in diet (mg/kg)
A	5.0
D_2	25.0
E	200.0
K_3	3.0
B_1	5.0
B_2	7.0
B_6	8.0
D-Panthotenic acid	20.0
Folic acid	2.0
Nicotinic acid	20.0
B_{12}	50.0*
d-Biotin	1.0
Myo-inositol	200.0
Choline chloride	1500.0

*µg/kg

Mineral composition

Mineral	Concentration in diet (units/kg)
Ca (g)	6.29
Cl (g)	7.79
Mg (g)	1.06
P (g)	4.86
K (g)	5.24
Na (g)	1.97
Cr (mg)	1.97
Cu (mg)	10.7
Fe (mg)	424.0
Mn (mg)	78.0
Zn (mg)	48.2
Co (µg)	29.0
I (µg)	151
Mo (µg)	152
Se (µg)	151