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**Nutritive Value for Pigs and Poultry  
of Barley Cultivars Varying in  
Beta-Glucan Content and  
Starch Characteristics**

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

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## *Abstract*

The nutritive value of a hulled conventional barley (NB) cultivar, four hulless barley cultivars (C0, C1, C2 and C3) that varied in fibre and  $\beta$ -glucan contents and starch characteristics (waxiness), as well as a wheat (WT) was determined for pigs and broiler chickens. In the pig trial, the apparent digestible energy (ADE) of NB, C0, C1, C2, C3 and elsewhere WT was determined. In the broiler trial, the apparent metabolizable energy (AME) content and ileal amino acid digestibility of NB, C1, C2, and C3 were examined without or with exogenous  $\beta$ -glucanase supplementation.

The pig trial utilised 15 growing male pigs (average weight, 32.5 kg). The assay diets contained 99.75% of the test ingredient and were fortified with minerals and vitamins. The total faecal collection method was used. Faeces were collected, weighed and sub-sampled daily for 5 days after a week of acclimatisation period. The apparent digestible energy (ADE) of the four hulless barley cultivars ranged from 15.83 to 16.48 MJ/kg DM. The hulless barley cultivar C2 was significantly different ( $P < 0.05$ ) from hulled NB and wheat WT. However, hulless barley cultivars C0, C1, and C3 did not differ ( $P > 0.05$ ) significantly from each other and, even though they were numerically higher than values for NB and WT. In terms of the apparent digestibility coefficient (ADC), hulless barley C1 and C2 had the highest values (0.8795 and 0.8837, respectively), but these were not significantly different ( $P > 0.05$ ) from hulless barley C0 and WT. The lowest ADE and ADC values were determined for hulled barley (15.59 MJ/kg and 0.8257, respectively). It was observed that the hulless barley with high non-starch polysaccharides (NSP) concentrations had the lowest ADE contents.

In the broiler trial, the influence of exogenous  $\beta$ -glucanase (Allzyme BG; Alltech, Inc., Nicholasville, KY) supplementation on the apparent metabolizable energy (AME) and apparent ileal digestibility coefficient (AID) of amino acids in a normal, hulled barley cultivar and three hulless barley cultivars was investigated. The assay diets contained 96.3% barley, and were fortified with

minerals and vitamins. Titanium oxide was included as an inert marker for the estimation of ileal amino acid digestibility. The AME of barley was influenced ( $P < 0.001$ ) by the cultivar type. The AME of the NB was determined to be 12.68 MJ/kg DM, while the values for the three hulless cultivars were 10.87, 12.92 and 10.20 MJ/kg DM, respectively. These data suggest that starch characteristics and  $\beta$ -glucan contents are additional factors that may influence the available energy in barley.  $\beta$ -glucanase supplementation improved ( $P < 0.001$ ) the AME of all barley cultivars, with improvements ranging from 5.4 to 21.9%. The cultivar type had no influence ( $P > 0.05$ ) on the AID of most amino acids. The average AID of 15 amino acids in the hulled barley and the three hulless cultivars were 0.70, 0.68, 0.72 and 0.73, respectively. Enzyme supplementation improved ( $P < 0.001$ ) the AID of all individual amino acids in the four barley cultivars, with increases in individual amino acid digestibility ranging from 18.1% for threonine to 11.4% for arginine. The average AID of 15 amino acids in the un-supplemented and supplemented cereal was 0.66 and 0.75, respectively.

Overall, it was observed that the barley cultivars, which were high in NSP and  $\beta$ -glucan, had lower energy digestibility for pigs and broiler chickens. Hulless barley C2 that is characterized as having normal starch was found to have the highest available energy for both species.

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## *List of Abbreviations*

<b>AA</b>	<b>Amino acid</b>
<b>ADC</b>	<b>Apparent digestibility coefficient</b>
<b>ADE</b>	<b>Apparent digestible energy</b>
<b>ADG</b>	<b>Average daily gain</b>
<b>AID</b>	<b>Apparent ileal digestibility</b>
<b>AOAC</b>	<b>Association of Official Analytical Chemist</b>
<b>Ala</b>	<b>Alanine</b>
<b>Arg</b>	<b>Arginine</b>
<b>Asx</b>	<b>Aspartic Acid</b>
<b>BGU</b>	<b>β-Glucanase Unit</b>
<b>C0</b>	<b>Cultivar 0</b>
<b>C1</b>	<b>Cultivar 1</b>
<b>C2</b>	<b>Cultivar 2</b>
<b>C3</b>	<b>Cultivar 3</b>
<b>CP</b>	<b>Crude protein</b>
<b>DE</b>	<b>Digestible energy</b>
<b>DF</b>	<b>Dietary fibre</b>
<b>DM</b>	<b>Dry matter</b>

<b>EZ</b>	<b>Enzyme</b>
<b>FCR</b>	<b>Feed conversion ratio</b>
<b>FI</b>	<b>Feed intake</b>
<b>GIT</b>	<b>Gastro intestinal tract</b>
<b>Glu</b>	<b>Glutamic Acid</b>
<b>Gly</b>	<b>Glycine</b>
<b>His</b>	<b>Histidine</b>
<b>IDF</b>	<b>Insoluble dietary fibre</b>
<b>Ile</b>	<b>Isoleucine</b>
<b>Leu</b>	<b>Leucine</b>
<b>Lys</b>	<b>Lysine</b>
<b>ME</b>	<b>Metabolisable energy</b>
<b>MJ</b>	<b>Mega joule</b>
<b>NSP</b>	<b>Non-starch polysaccharides</b>
<b>Phe</b>	<b>Phenylalanine</b>
<b>Pro</b>	<b>Proline</b>
<b>Ser</b>	<b>Serine</b>
<b>SCFA</b>	<b>Short chain fatty acid</b>
<b>Thr</b>	<b>Threonine</b>
<b>TiO<sub>3</sub></b>	<b>Titanium oxide</b>
<b>Tyr</b>	<b>Tyrosine</b>
<b>Val</b>	<b>Valine</b>

**WG**

**Weight gains**

**WT**

**Wheat**

## Chapter One

### General Introduction

Barley (*Hordeum vulgare. L*) ranks amongst the top four crops in world grain production after maize, wheat, and sorghum. Barley contributes significantly to the world's food supply, for both human and livestock consumption. The main use of barley is as a malt product for human consumption. As an animal feed, barley is used for the feeding of both ruminant and non-ruminant animals. In non-ruminant animals, however, the use of barley has been limited, particularly in poultry and young pigs. This is due to the limited ability of poultry and young pigs to digest the fibre and non-starch polysaccharides (NSP) in barley (Bach Knudsen, 1997). Therefore, in these diets, the addition of exogenous  $\beta$ -glucanase has been recommended to improve digestibility.

Compared to maize and wheat, conventional hulled barley is nutritionally less preferred due to its high fibre and NSP contents, which lowers energy and nutrient digestibility (Xue *et al.*, 1997) and causes poor performance in monogastric animals. Barley is used extensively in pig and poultry diets, even though the feeding value is lower than that of corn, wheat, and sorghum. Hulless cultivars of barley are now available and these have better nutritive value than the hulled cultivars. In hulless cultivars, the hull is less firmly attached to the kernel and consequently is detached during threshing, resulting in a low fibre content (Thacker *et al.*, 1998). This makes the hulless barley more digestible compared to hulled barley. A number of studies have shown that hulless barley has a better digestibility of nutrients and more available energy than hulled barley (Baidoo & Liu, 1998; Sauer *et al.*, 2002).

The presence of the waxy gene in barley, as in other grains, produces a starch that is predominately amylopectin. In barley, the gene is also associated with an increase in  $\beta$ -glucan and extract viscosity (Wood *et al.*, 2001). The ratio of amylose to amylopectin in the barley endosperm is an important grain characteristic affecting feed quality (Bhatty, 1993). For most barley, the content of

amylose is much lower than the content of amylopectin. Low amylose waxy barley is known to have a lower nutritional value than normal waxy barley due to the lower amylose to amylopectin ratio. Waxy barley with a high amylopectin content is more digestible than both low and normal hulless waxy barley cultivars (Tester *et al.*, 2004).

The nutritional value of the barley and the adverse effects of  $\beta$ -glucans on nutrient digestibility and the performance of poultry can be improved by supplementation with exogenous  $\beta$ -glucanases (Xue *et al.*, 1997) The use of  $\beta$ -glucanase is reported to improve the nutritive value of barley for piglets, but the results are variable depending on the cultivar type (hulled vs. hulless) and the waxiness of the barleys. It is generally reported that older pigs are not affected by  $\beta$ -glucan (Campbell & Bedford, 1992) and quite often  $\beta$ -glucanase addition causes only a small improvements in nutrient digestibility in pigs (Graham *et al.*, 1989).

Results from two trials conducted with pigs and broiler chickens are reported in this thesis. The digestible energy of four hulless barley cultivars, one wheat, and one conventional hulled barley was measured in pigs (Chapter 3) using the total excreta collection method. In the broiler chicken trial (Chapter 4), the apparent metabolisable energy as well as the amino acid digestibility of three hulless barley cultivars and one conventional hulled barley cultivar were measured. The influence of  $\beta$ -glucanase supplementation on these nutrient utilisation parameters in broiler chickens was also examined. The over all discussion and conclusions of these two findings are presented in chapter five.

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## Chapter Two

### Literature Review

#### 2.1. Barley Crop

Archaeological evidence has shown that barley (*Hordeum vulgare. L*) was one of the earliest cultivated cereals. Barley has been used in bread-making and as a feed for livestock for centuries (Nash, 1985). In some areas of the world, barley is a preferred grain for cultivation due to its resistance to drought and ability to mature in severe climates with a short growth season (Svihus & Gullord, 2002). Barley is considered as a “coarse grain” because a major part of the commodity is used for animal feeding (Fischbeck, 2002).

Today, besides its use as an animal feed, barley is also used in human diets particularly in the Western countries such as Canada (Bull & Bradshaw, 1995; Izydorczyk, 2001; Wiebe, 1979). The most important use of barley, however, is in the production of alcoholic beverages (Wiebe, 1979), fermentation, and in the food industry (MacGregor & Fincher, 1993).

In many countries, barley is used as a cereal source in pig and poultry diets but its inclusion levels in young growing animals, especially in poultry, is restricted because of its fibre and non-starch polysaccharide (NSP) content (Bi *et al.*, 1998; Svihus & Gullord, 2002). The fibre and the NSP are known to have a negative impact on the nutritional value of the barley.

#### 2.2. Nutrient composition of barley

A nutrient is defined as any chemical element or compound in the diet that supports the normal reproduction, growth, lactation or maintenance of life processes and includes protein, carbohydrates, and fat, all of which can provide

energy, as well as vitamins and inorganic elements. The nutrient composition of feeds is determined by chemical analysis, whereas the energy content is determined by combusting a sample in a bomb calorimeter.

Starch, dietary fibre and protein are the main components of barley grain. According to work done by Bach Knudsen *et al.* (1987), the average chemical composition of conventional, hulled barley was: dietary fibre 22.3%, starch 58.4%, protein 12.0%, low molecular weight sugar 2.2%, fat 3.4% and ash 2.2% . Barley also contains both water soluble and insoluble polysaccharides which constitute between 2 and 16% of the dry matter (Bhatty *et al.*, 1991).

### 2.2.1. Starch

Cereals are particularly rich sources of starch. Starch is a polysaccharide made up of glucose units linked together to form long chains. The number of glucose molecules in a single starch molecule varies from five hundred to several hundred thousands, depending on the type of starch. It is the storage form of energy for plants, just as glycogen is the storage form of energy for animals (Kent & Evers, 1994).

Starch is the major constituent of the barley kernel and its concentration is inversely related to protein content (Bhatty, 1993). Kernel plumpness and uniformity are important characteristics in barley starches. It is reported that, generally, the denser the kernel, the higher the nutrient content (e.g. starch and protein versus air spaces and fibre) of barley. Plumpness is an indication of endosperm size and starch content. The higher the plump kernel percentage, the higher the starch content (Ullrich, 2002). The content of starch in the barley cultivars has been reported to vary between 50.1 and 61.8% (Table 2.1). Ullrich (2002) also reported that carbohydrates account for around 80% of grain with starch ranging between 40-65% (mean ~55%); protein, 6.9 to 25.0% (mean 14.8%); and lipids, 0.9 to 3.2% (mean 1.6%).

**Table 2.1 Characteristics and chemical composition (% dry matter) of some barley cultivars**

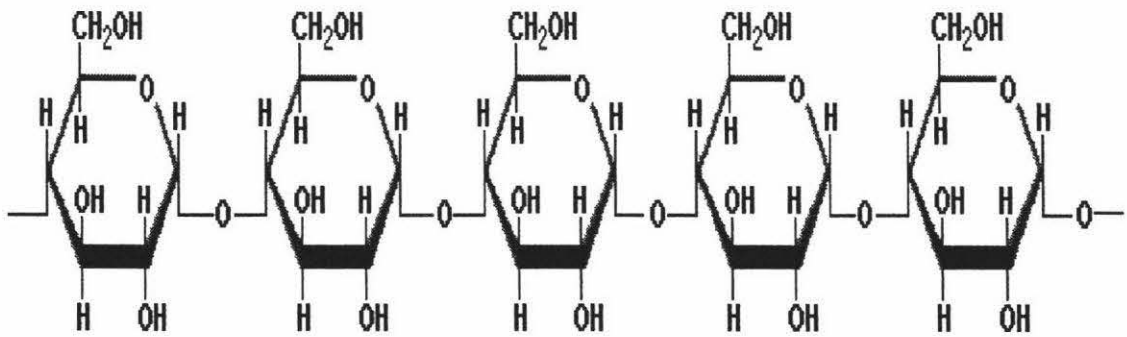
Cultivar Characteristics	Starch	Protein	$\beta$ -glucan	Ash
Conventional hulled; Normal starch	60.7	9.3	4.7	2.3
Hulless; High-amylose starch	50.1	11.3	6.9	2.5
Hulless; High-amylopectin (waxy) starch	53.7	11.4	6.1	2.5
Hulless; Normal starch	61.8	12.0	4.6	2.3
Hulless; High amylopectin (waxy) starch	58.7	14.1	5.6	2.0

**Adapted from: Elfverson *et al.*, (1999)**

### 2.2.2. Amylose and Amylopectin

Starch contains two major components, namely amylose and amylopectin which are held together by hydrogen bonding (Bull & Bradshaw, 1995). Amylose is the minor component of most starches (~ 20 to 30%), and consists of relatively long chains of  $\alpha$ -(1 $\rightarrow$ 4)-linked D-glucose residues (Bull & Bradshaw, 1995; MacGregor & Fincher, 1993). Amylopectin is a branched polymer, and is the most abundant type of starch comprising 70 to 80%. It is composed of chains of  $\alpha$ -(1 $\rightarrow$ 4)-linked glucose residues interconnected through  $\alpha$ -(1 $\rightarrow$ 6) bonds (Bull & Bradshaw, 1995; MacGregor & Fincher, 1993).

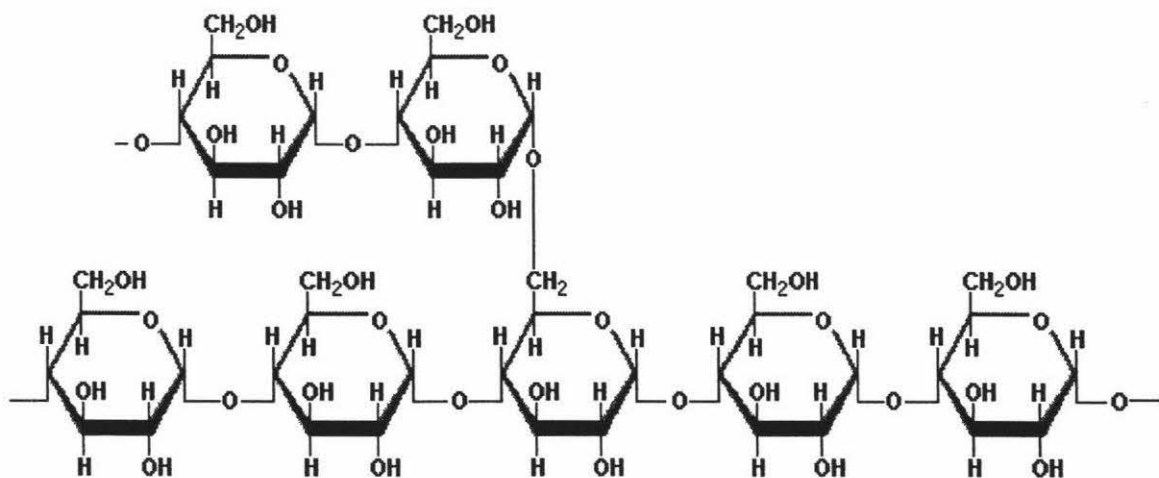
Figure 2.1. Basic structure of amylose



Source: (Anonymous, 2004)

Both amylose and amylopectin are insoluble in cold water (Zhou & Kaplan, 1997). Unlike amylose, the unit chains in amylopectin are relatively small, and the 4 to 5% of inter-chain  $\alpha$ -(1 $\rightarrow$ 6)-linkages in the molecule lead to a highly branched, compact structure (MacGregor & Fincher, 1993). Indigenous waxy barleys have storage starch containing about 2 to 10% amylose and 89 to 90% amylopectin, while so-called waxy strains in other cereals have been reported to have no amylose and 100% amylopectin in storage starch (Domon *et al.*, 2002). Due to a lack of branched structure, amylose is less digestible than amylopectin (Zhou & Kaplan, 1997).

Figure 2.2. Basic structure of amylopectin



Source: (Anonymous, 2004)

### 2.2.3. Protein

The protein content of barley varies depending on cultivar, location, rainfall (Bull & Bradshaw, 1995), and soil type (Bach Knudsen *et al.*, 1987; Valaja *et al.*, 1997). It is reported that the protein content of barley varies between 9.3 and 14.1% (Table 2.1). High levels of soil nitrogen provides for increased protein content. Torp *et al.* (1981) have reported that the protein content of barley varies between 8.1 and 14.7% in the same genotype grown at different locations, even at similar nitrogen fertilisation levels. Reported ranges in the nutrient composition of barley are shown in Table 2.2.

**Table 2.2. Reported ranges in nutrient composition (% DM) of barley**

Component	Content
Starch	60-64
$\beta$ -glucans	3.4-6.1
Cellulose	1.4-5.0
Simple carbohydrates (glucose, fructose, sucrose, maltose), Olygosaccharides (raffinose, fructosans)	0.16-1.8
Protein	8-15
Lipids	2-3
Minerals	2-3

**Source: MacGregor (2003)**

In barley, hordein is the major storage protein which is affected by the hordein composition but it is also positively correlated with the amounts of hydrolytic enzymes such as amylases and  $\beta$ -glucanases (Shewry, 1993). Hordein limits the protein quality of the grain for pigs and poultry, due to its low content of essential amino acids, lysine and threonine (Shewry, 1993). The content of lysine and threonine in conventional barley is known to be low, which is due largely to hordein, and it contains only 0.6 and 2.4 mol% of these two amino acids, respectively (Bhatty, 1993). Whereas albumin and globulin fractions contain 5.0 and 6.0 mol% and the glutelin fraction is 4.6 and 5.7 mol%, respectively (Bhatty & Whitaker, 1987).

### 2.2.4. Fibre and non-starch-polysaccharides

Fibre is the thin thread of plants or seeds that cannot be digested. Non-starch polysaccharides are part of dietary fibre and in this context it represents the chemical portion of polysaccharides and other plant materials in the diet that are not digested by endogenous secretions (Ravindran, 2001; Trowell *et al.*, 1976). The fibre content of barley is reported to range between 5 to 6% (Bi *et al.*, 1998; Bull & Bradshaw, 1995). Fibre levels in barley are normally greater than those of other grains such as sorghum or maize (Boyles *et al.*, 2004), which makes it a less preferred grain in the feeding of monogastric animals. The NSP levels in barley, wheat and maize can be seen on Table 2.3.

**Table 2.3. The NSP levels in maize, wheat and barley**

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

Source: Smith and Annison (1996)

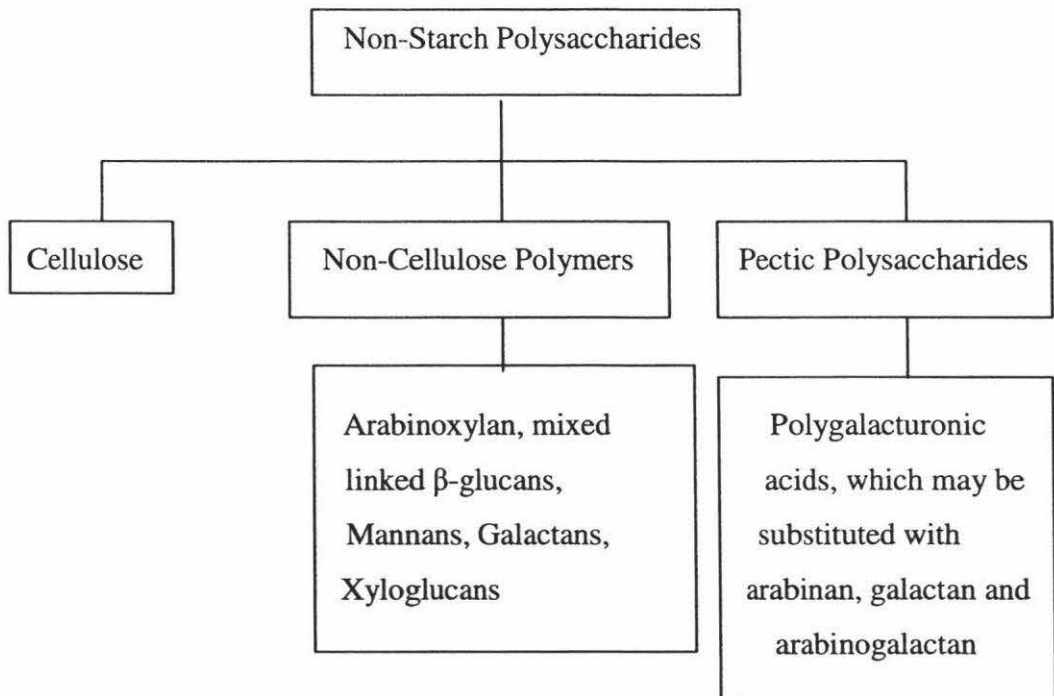
The term non-starch polysaccharides includes a variety of polysaccharide molecules excluding  $\alpha$ -glucans (Choct, 1997). Commonly, NSP is used interchangeably with the term 'fibre' (Johnston *et al.*, 2003). However, the definition and quantification of fibre in non-ruminant diets is difficult due to the complexity and diversity of the polysaccharides involved (Grieshop *et al.*, 2001; Low, 1993).

In most plants, cellulose is present as the structural polysaccharides in the cell wall. In cereals,  $\beta$ -glucans in barley and arabinoxylan in wheat and triticale are the major NSP (Choct, 1997; Smith & Annison, 1996). Cellulose is the major structural and fibrous constituent of plants found in the wall of cellular tissue that is usually combined with xylans and lignin and by far the most abundant molecule in nature (Padilla, 2002). Individual cellulose chains tend to align and aggregate which leads to the formation of crystalline microfibrils, which have been stabilised by extensive intermolecular hydrogen bonding giving considerable tensile strength to the cell wall (Fincher & Stone, 1986). On the other hand, arabinoxylan is particularly abundant in the walls of the aleurone cells, in the starchy endosperm, in the husk and presumably in the wall remnants that make up the other material tissues surrounding the grain. Arabinoxylan consists of a complex chemical structure composed of two sugars, arabinose and xylose in a branch structure. The arabinose originates from pectin and xylose comes from hemicellulose (Fincher & Stone, 1986). The classification of the NSP is shown in Figure 2.3.

The soluble fibre fraction that consists mainly of mixed-linked (1-3)(1-4)- $\beta$ -glucans is associated with increased digesta prior to viscosity which is one of the problems in poultry, which in turn inhibits the digestion and absorption of nutrients (Svihus & Gullord, 2002). Poultry lack the enzyme necessary to digest  $\beta$ -glucans. Therefore, to overcome the adverse effect of  $\beta$ -glucans, the use of exogenous enzymes aimed at breaking down the structure of soluble and viscous fibre is necessary.

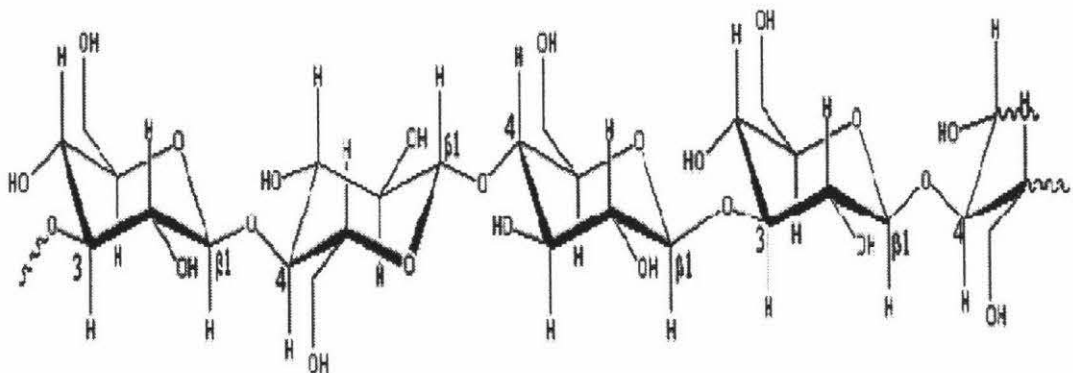
$\beta$ -glucans occur in the bran of grains such as barley, oats, rye and wheat, generally in amounts of about 7%, 5%, 2% and less than 1%, respectively (Chaplin, 2004). The level of (1-3)(1-4)- $\beta$ -glucan in barley may vary considerably, depending on the cultivar, growing conditions and method of determination. A range of about 2 to 10.7% has been reported in published literature (Bhatty, 1993).

Figure 2.3. Classification of non-starch polysaccharides



Source: Choct (1997)

Figure 2.4. β-glucan structure



Source: Chaplin (2004)

### 2.3. Hulled vs. Hulless Barley

There are two types of cultivated barley, the hulled and the hulless varieties, with the cultivation of hulled varieties far exceeding that of the hulless varieties (Chen, 1997). Pigs and poultry are unable to digest the fibrous hull in

conventional barleys (Small, 1999). Therefore, it is necessary to reduce the fibre contents in barley diets through the processing and/or the addition of exogenous enzymes. An alternative approach to increase digestibility is to use hulless barleys. Hulless barley differs from normal, conventional barley in that the hull is less firmly attached to the kernel and consequently is detached after threshing, leading to a higher concentrations of nutrients and increased density (Thacker, 1999). In hulless barley cultivars, the grain as harvested consists only of a one-seeded fruit or caryopsis, whereas in hulled barleys, it consists of a caryopsis enclosed in the two flowering glumes that make up the hull or husk (Duffus & Cochrane, 1993). The hull of conventional barley composed of the lemma and palea, and comprises about 13% of the kernel and is the main contributor to the high fibre in the grain. It is also reported that hulless barley is higher in protein and contains more essential amino acids (Table 2.4) than hulled barley (Baidoo & Liu, 1998) and supply more available energy for pigs and poultry (Sauer *et al.*, 2002).

**Table 2.4. Typical analysis (%) of hulled and hulless barley (DM basis)**

	Hulled barley	Hulless barley
Dry matter	89	86.5
Crude protein	11.5	13.5
Lysine	0.40	0.49
Crude fibre	5.0	2.0
Neutral detergent fibre	16.8	13.8
Acid detergent fibre	2.6	2.4

**Source: Kephart (1996)**

Hulless barley has been recommended as a good energy source in pig diets and has been shown to improve growth performance compared to hulled barley (Kephart, 1996; Thacker *et al.*, 1998). Based on work done by Sauer *et al.* (2002), the digestible energy content of hulless barley is 9% higher than hulled barley.

## 2.4. Normal vs. Waxy Barley

Barley cultivars with low (about 2%), normal (about 25%) and high (about 40%) amylose content in the starch are available today (MacGregor & Fincher, 1993). Starches are defined as waxy when the amylose content in starch is low (~15%), normal when amylose represents ~ 16 to 35% and high-amylose when it exceeds ~36% (Tester *et al.*, 2004). The waxy gene produces starch that is about 96 to 100% amylopectin compared to 75% amylopectin in normal barley (Tester *et al.*, 2004). Generally, waxy barley starch contains 97-100% amylopectin while normal starch cultivars consist of 75-85% amylopectin (Ullrich *et al.*, 1986) and high amylose starch consists of only 55% amylopectin (Oscarsson *et al.*, 1997).

It is reported that the size distribution of granules and amylose content are important factors for the technological and nutritional properties of barley starches (Gudmundsson & Eliasson, 1992). High amylose barley contains more small starch granules (29 to 43 wt%) than waxy barley, which has about 14% of the weight in normal barley, which has 4.6 to 15% of the weight (Morrison *et al.*, 1986). Barleys with high-amylose have lower  $\beta$ -glucan (5.6%) than waxy barley which has 6.6% (Czuchajowska *et al.*, 1998). Whereas, high amylose and waxy barley have been observed to have a higher  $\beta$ -glucan content than those from normal starch barley (Newman & Newman, 1987; Oscarsson *et al.*, 1996).

Therefore, in pig and ruminant animals there is no apparent difference in the nutrient value between waxy and normal barley (Xue *et al.*, 1997). However, some reports with broiler chickens indicate that waxy barley is inferior to normal barley as feed grain for poultry. These reports also found that supplemental  $\beta$ -glucanase has improved the performance of the birds equal to those fed maize-based diets (Xue *et al.*, 1997).

## 2.5. Nutritional Value of Barley for Pigs and Poultry

The nutritional value of barley is affected by many factors such as chemical composition and digestibility of nutrients, the physiological status of the animal (Chen, 1997), cultivar, environmental conditions (Valaja *et al.*, 1997), as well as feed processing (Sauer *et al.*, 2002). For a diet to be used optimally by pigs and poultry, its components must be digestible and the nutrients must be available for utilisation (Harrold, 1999). It is known that improvements in energy digestibility can also be dependent on the chemical and physical (particle size) characteristics of the barley (Noblet & van Milgen, 2004).

The hull composition of barley are also major factors affecting the digestible energy (DE) (Kim, 1999). However, among hulled barleys, the digestibility of energy also fluctuates between varieties (Bhatty *et al.*, 1975). The waxiness (starch composition) of barley may also affect energy availability. This is in accordance with earlier discussions that hullless and waxy barleys are known to have better digestibility in comparison to hulled, conventional barley (Bhatty, 1993).

The primary function of cereals in pig and poultry rations is to provide energy for growth and reproduction with energy for supporting body maintenance functions. Thus, the DE or AME content is the major nutritional criterion for the evaluation of feed barley (Chen, 1997). Based on work done by English *et al.* (1988), on average, barley has 12.6 MJ/kg DE, 11.5% crude protein, 1.9% crude fat, 5.0% crude fibre, 0.08 % calcium, 0.42% phosphorus, and 2.5% ash. However, the results on hullless and hulled barleys are markedly different. For instance, the prediction on 0% hull barley was 84% digestible and it had 15.73 MJ DE/kg on DM, whereas barley with 30% hulls was only 64% digestible and it contained 12.01 MJ/kg DE on DM (Chen, 1997).

The nutritive value of the same barley sample for pig and poultry is known to be different. For instance, barley is less digestible and yields less energy when fed to poultry than when fed to pigs (Fincher & Stone, 1986). This statement has

been clarified with the work done by Wiseman (1984) on two different types of barley, 2-row barley and 6-row barley. The ME values for 2-row and 6-row barley for pigs was 12.26 and 11.76 MJ/kg, respectively. Whereas, the ME values were 10.04 and 9.62 MJ/kg, respectively, in broilers and were 11.67 and 11.28 MJ/kg, respectively, in adult roosters.

Determination of the nutritive value of barley can be seen from two different aspects. Firstly, it is related to the digestible energy contents, and secondly, the digestible amino acid contents. The digestibility energy content of barley is significantly correlated with protein and gross energy content. The digestible protein content is largely related to the amino acid digestibility, which is also influenced by both genetic and environmental factors. Of these, the environment has a strong effect on the contents of protein and amino acids (Ullrich, 2002).

Similar to the energy component, the digestibility of the crude protein varies in different species of animals. Wiseman (1984) found that the apparent faecal digestibility of protein in 2-row and 6-row barleys for pigs were higher than for poultry. The values were 78% (2-row) and 76% (6-row), respectively, in pigs and 70% for both varieties in poultry. The digestibility of crude protein is dependent on the fibre and hull contents (Chen, 1997). According to Bell *et al.* (1983), the apparent faecal digestibility of protein decreased from 75.0 to 72.0% as hulls increased from 0 to 30% and from 82.2 to 62.5% as dietary crude protein decreased from 21.9 to 8.0% (DM basis) in barley. The metabolic faecal protein increased from 1.49 to 2.79 g/100g dietary DM as barley hulls increased from 0 to 30%. Furthermore, the apparent faecal digestibility of hull protein was 44% and the corresponding value for non-hull protein was about 75%.

## 2.6. Digestive Physiology of Pigs and Poultry

Though both pigs and poultry are monogastrics, there are distinct differences in their digestive physiology. The digestion in monogastrics involves a combination of mechanical, chemical and microbial activities all contributing to the sequential degradation of food components. The pig's stomach represents about 30% of the volume of the digestive tract where limited mixing of food occurs. The main factors that influence the potential for the response of the enzymes in pigs in comparison to poultry are included the gastrointestinal anatomy, digestive capacity and digesta characteristics (Partridge, 2000). On the other hand, a chicken has a crop and a gizzard (muscular stomach) below the true stomach (proventriculus) in which glands release endogenous secretions. The anatomical difference between pigs and poultry explains the reasons why pigs do not suffer from digestive inefficiencies attributed to dietary  $\beta$ -glucan. In poultry, the crop provides a comparatively balanced environment for the enzyme activities, whereas the stomach of pigs seems to be a more hostile environment with a lower pH than poultry (Choct & Cadagon, 2001), even though it is not hostile to the enzyme that secreted in the stomach.

### 2.6.1. Digestive physiology of pigs

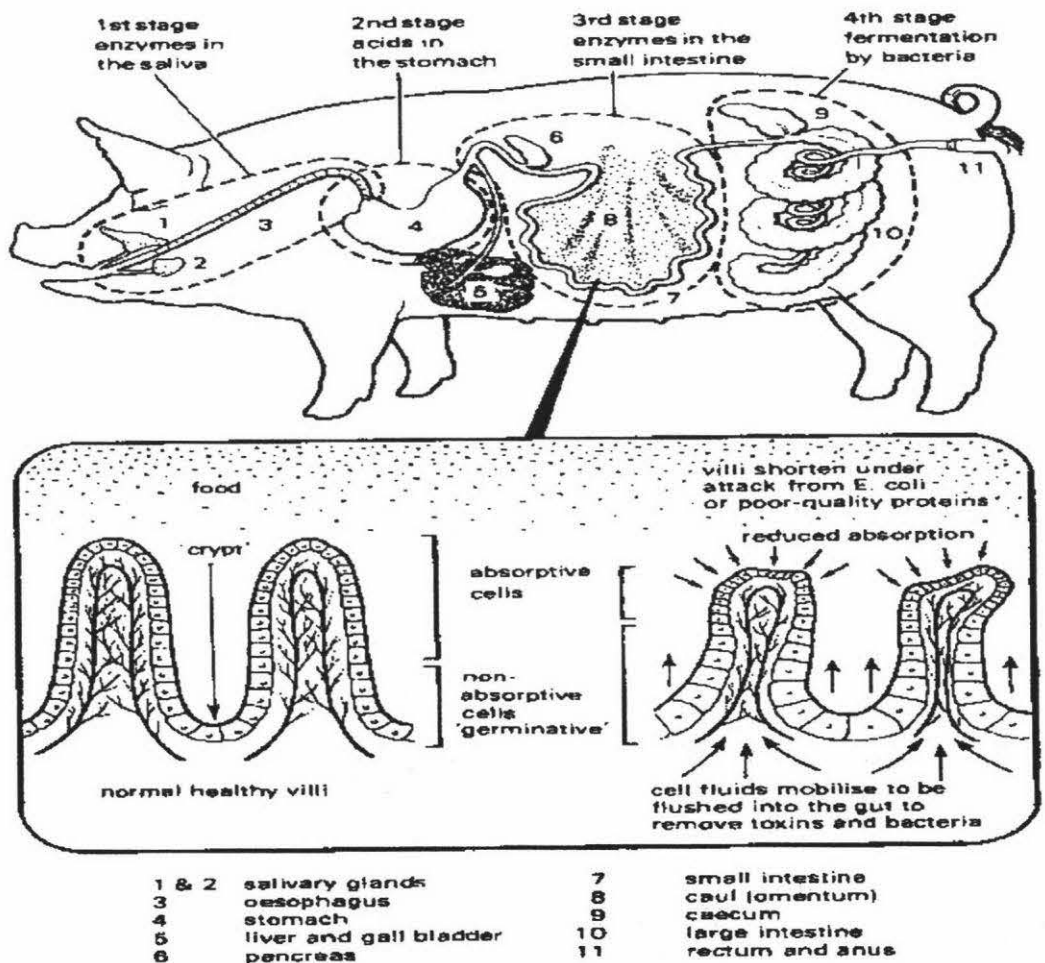
The digestion processes start mechanically from the mouth (Figure 2.5) with the combination of saliva and amylase. The grinding action of the teeth breaks down feed into smaller particles to make it easier to swallow and to increase the surface area that is exposed to enzymes. Chewing also mixes the feed with saliva, which lubricates it and adds saliva and some amount of amylase that initiate to break down the feed. Mastication and alimentary muscular contractions mechanically diminish the size of ingested food particles (Maskell & Johnston, 1993).

The stomach of pigs is functionally differentiated into four distinct regions: oesophageal, cardiac, gastric gland, and pyloric which are both a temporary storage organ, and the first major centre of digestive activity. Much of

the physical structure of feeds is disrupted in the stomach, which makes the chemical structures more available for enzymatic hydrolysis in the small intestine, apart from the initiation of protein digestion (Chen, 1997).

There are three parts of the small intestine: duodenum, jejunum and ileum. The central jejunum accounts for 80 to 90% of the small intestinal length with the remainder being approximately equally divided between the cranial duodenum and the caudal ileum (Longland, 1991). As in poultry, the majority of digestion by host enzymes and the absorption of the end products occur in the small intestine. Nutrient absorption is facilitated by the greatly increased surface area provided by the villi and microvilli, which occur along the length of the small intestine (Chen, 1997).

Figure 2.5. The structure and function of the digestive tract of the pig



Source: Klasing (1998)

The large intestine begins at the junction of the ileum and caecum. It consists of a short, blind-ended caecum, which continues into the colon at the point of ileal attachment (Chen, 1997). The size of the large intestine of mature pigs is about 4-5m long and it has a much greater diameter than the small intestine (Low & Zebrowska, 1989). The large intestine is separated from the small intestine by the one-way, ileo-caecal valve. The majority of microbial fermentation occurs in the large intestine, the end products of which are absorbed across the mucosa (Chen, 1997).

In pigs there are some sources of endogenous  $\beta$ -glucanase, which have not been identified (they could be of microbial or feed origin), that can breakdown the  $\beta$ -glucan in feed. Some  $\beta$ -glucan degradation also seems to occur in the stomach, even though it has probably not been resolved whether this reflects enzymatic or acid hydrolysis (Choct & Cadagon, 2001).

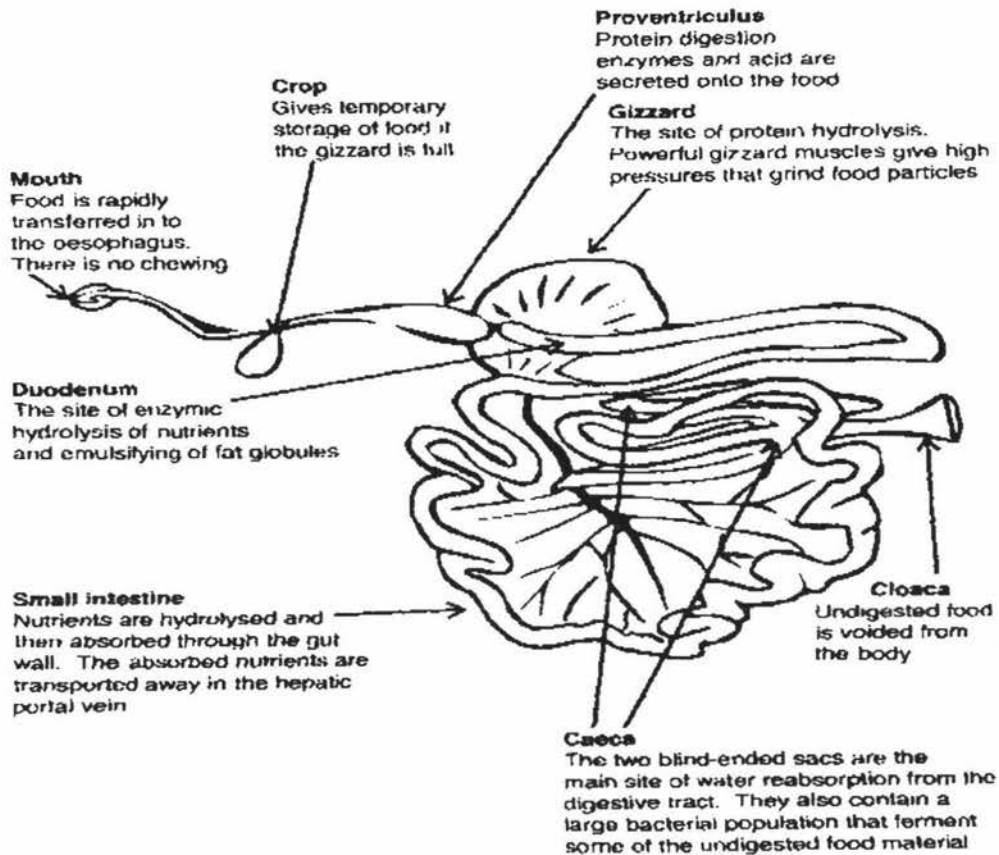
### **2.6.2. Digestive physiology of poultry**

The gizzard in poultry functions as teeth due to the fact that poultry have no teeth. In the gizzard, feeds are massaged and ground to reduce their size and increase their surface area. It also serves as a location for the action of HCl and pepsin, which is added to the food during passage through the proventriculus (Klasing, 1998). The inclusion of exogenous enzymes in the feed can increase the digestibility of nutrients and, therefore, it helps maintain a desirable gut microflora.

The small intestine plays an important role in the enzymatic digestion and absorption of the end products of digestion. The small intestine consists of duodenum, jejunum and ileum. The duodenum originates from the gizzard and forms a loop around the pancreas. The bile and the pancreatic ducts enter the duodenum. Posterior to the duodenal loops is the jejunum, followed by the ileum (Figure 2.6). The digestive enzymes produced in the tubuloacinar glands are collected into one, two, or three ducts that enter the duodenum, usually near the entrance of the bile duct. Klasing (1998) stated that avian pancreatic juice

contains enzymes similar to those of mammals, including amylase, lipase, trypsin, chymotrypsin, carboxypeptidases A, B, and C, deoxyribonucleases, ribonucleases, and elastases.

**Figure 2.6. The structure and function of the digestive tract of chicken**



Source: Klasing (1998)

## 2.7. Anti-nutritive effects of fibre and NSP on pigs and poultry

Anti-nutritive factors found in plants affect the digestion and absorption of dietary proteins by binding with food proteins, thus making them less digestible or by reacting with the gut wall and affecting their secretory or protective function (Kim, 1999). The anti-nutritive factors in barley are poorly digested by animals. Dietary fibre (DF) exhibits a range of physical properties that act in concert with the chemical properties to determine the physiological effects of the feed. In the analysis of DF, it is common to characterise it in accordance with its solubility, as

soluble NSP are believed to be the components that influence digestion and absorption in the foregut. In contrast, the insoluble dietary fibre (IDF) primarily acts in the large intestine where, due to its physical presence, it effectively increases faecal bulk, dilutes colonic contents and decreases transit time (Bach Knudsen, 2001). However, although modern analytical techniques enable quantification and characterisation of the physical and chemical properties of DF in the plant materials, understanding of the nutritional significance of these measurements is still far from complete (Bach Knudsen, 2001). Insoluble dietary fibre is initially digested in the hindgut as a result of fermentation. Pigs do not secrete enzymes in the small intestine that attack components of IDF so they pass through relatively untouched to the large intestine (Shi & Noblet, 1993; Varel & Yen, 1997). Insoluble dietary fibre can negatively affect the total tract digestibility of dietary nitrogen and ether extract (Shi & Noblet, 1993).

Dietary fibre can influence the gut health of the animals through several potential mechanisms (Johnston *et al.*, 2003). The type and levels of fibre in the diet directly influence the structure and function of the gut. Hypertrophy of the gastrointestinal tract (GIT) of pigs is observed when fed diets high in fibre (Pond *et al.*, 1989). While a proportional increase in the size and weight of the GIT may not directly influence the health of the animal, it does increase the energy and amino acid requirements for maintenance because the metabolically active GIT requires an unequal amount of the animal's total consumption of energy and protein (Baldwin *et al.*, 1980).

The general rule is that the dietary fibre (defined as non-starch polysaccharides and lignin) is more than three times the amount indicated by crude fibre measurement (Partridge, 2003). For example, maize contains 2.5% crude fibre, but it actually contains 10% total fibre. Similarly, wheat contains 8-11% crude fibre, but in reality contains 30-40% total fibre (Partridge, 2003). It is known that not only the level of fibre, but also its location in the cell wall matrix creates a protective 'box' effect whereby potentially useful nutrients such as starch, amino acids and minerals are unavailable for digestion in the small intestine. The fibre also acts like a 'sponge' in the gut, absorbing water-soluble nutrients and interfering with their effective digestion and absorption (Dierick & Decuypere,

1994; Hazzledine & Partridge, 1996). The overall effect is that the bacteria in the hindgut get the benefit of many of these nutrients rather than the host animal, and growth and feed conversion suffer.

Lignin, a component of the fibrous cell wall, is not digested by monogastric animals or fermented by the resident microbes in the gut (Graham *et al.*, 1986; Shi & Noblet, 1993). However, it influences the digestibility of other fibrous components of the diet. As a plant is fully grown, cellulose becomes lignified to increase the rigidity of the plant structure. In this process, cellulose becomes less accessible to microbes in the hindgut, which depresses the rate and extent of fermentation. Dietary digestibility is reported to be inversely proportional to lignin concentration (Johnston *et al.*, 2003).

Oligosaccharides that are undigested, such as mannan-oligosaccharides, fructo-oligosaccharides, and galacto-oligosaccharides, can limit the population of pathogenic bacteria in the gut and improve the health of the animal (Pettigrew, 2000). Mannan-oligosaccharides bind to lectins on the cell walls of pathogenic bacteria like *E. coli* and prevent them from binding to and colonizing epithelial tissue (Pettigrew, 2000). The pathogenic bacteria-oligosaccharide complex then safely passes through the digestive tract and is excreted. Other oligosaccharides escape breakdown in the upper digestive tract and arrive in the colon where they are fermented producing short chain fatty acids (SCFA).

Chickens fed diets containing soluble NSP show negative effects on productivity, which is due mainly to an increase in the viscosity of digesta in birds (Choct & Annison, 1992). Viscous digesta reduces the diffusion of digestive enzymes as well as of nutrients (Edwards *et al.*, 1988). Moreover, viscosity also reduce the contact between digestive secretions and substrates (Smith & Annison, 1996).

The animal's enzymes are unable to hydrolyse NSP. Traditionally, fermentation of NSP has been considered to be a post-ileal activity of the endogenous microflora. However, there is an increasing evidence that some NSP are at least partly degraded anterior to the large intestine in a pig (Chen, 1997).

Furthermore, it is known that soluble NSP influences the digestion of nutrients such as starch, protein, and fat and the apparent metabolisable energy content and the litter quality in poultry (Bedford & Schulze, 1998; Choct & Annison, 1992; Classen, 1996).

Pigs fed with high fibre diets produced an increased volume of manure, which results in proportionally heavier gastro-intestinal tracts than pigs fed low fibre diets, and contributes to slight increases in maintenance energy requirements (Yen, 2001). Fermentation of NSP in the hindgut of pigs yields SCFA and lactic acid (Bach Knudsen, 2001). This hindgut fermentation can supply up to 17% of the total digestible energy in growing pigs and up to 25% in sows (Shi & Noblet, 1993). Cunningham *et al.* (1962) stated that total tract digestibility of NSP increases as the pig matures. For most types of NSP, sows have higher digestibility coefficients than growing pigs (Noblet & Shi, 1993). The improvement with age is particularly noticeable with feedstuffs that are high in IDF, which is digested mainly in the hindgut (Noblet & LeGoff, 2001). Improved digestibility of NSP with age results from a more voluminous large intestine and cecum (Pekas, 1991) that contain a more extensive microbial population and fermentation (Yen, 2001). Furthermore, sows generally receive a much smaller quantity of feed relative to their body size compared with growing pigs. This situation allows a slower transit time of digesta and a more contact of endogenous enzymes and microbial populations with feed in the gut, which should improve digestibility (Johnston *et al.*, 2003).

Nutritionally, the level and type of dietary NSP greatly influence the site and degree to which dietary polysaccharides are digested (Johnston *et al.*, 2003). It is not easy to describe with certainty the effects of various fibre components such as SDF or IDF on digestibility because they are not a homogenous substance. Fibrous feeds may contain predominantly one type of fibre or another type but they are not pure. Consequently, it can be concluded that the fibre source is predominantly one type of fibre and this conclusion is used to generalize the effects of that fibre component (Johnston *et al.*, 2003).

## 2.8. The use of $\beta$ -glucanase

In 1925, Clickner and Follwell (1925) first reported the use of feed enzymes in poultry diets. They stated that the use of an enzyme product named Protozyme in pullet diets improved bird performance. After more than four decades, brilliant work from Burnett (1966) found the presence of  $\beta$ -glucan contents in barleys and reported its effect on the viscosity and nutritive digestion in the gut of chickens. Therefore, the feeding value of barley for broiler chickens may be enhanced by dietary supplementation with enzyme sources containing  $\beta$ -glucanase (Burnett, 1966; Hesselman *et al.*, 1982) in order to reduce the influence of high  $\beta$ -glucan in barley diets.

The primary objective of adding exogenous enzymes to diets is to improve the utilisation of nutrients in raw materials. Barley contains a relatively high concentration of  $\beta$ -glucans, which decrease the digestibility and absorption of all nutrients, produces sticky droppings, pasty vent, wet litter, and causes a depression in the performance of chicks (Annison & Choct, 1991) and young pigs.  $\beta$ -glucans form gels in the digestive tract of birds and are not degraded because of the lack of appropriate enzymes and the rapid rate of passage in poultry. Inclusion of barley in poultry and pig diets have been associated with reduced levels of available energy (Harrold, 1999). This has changed with the commercial availability of  $\beta$ -glucanase enzyme products.  $\beta$ -glucanases were the first enzymes successfully used in commercial poultry diets (Campbell *et al.*, 1989; Choct & Cadagon, 2001). Usually,  $\beta$ -glucanases are added to the diets in a dry form (0.5%) and act upon hydration within the digestive tract.

The addition of  $\beta$ -glucanase to poultry diets can effectively eliminate the problems with wet droppings while increasing the availability of dietary energy and reducing the effective variation in bird performance (Harrold, 1999) in comparison with diets unsupplemented with exogenous  $\beta$ -glucanases. Mannion (1981) studied the influence of bacterial and fungal enzyme supplementations at 0.15 and 0.3% of barley-based diets on the growth and nutritive utilisation in broiler chickens and reported that body weight gain was improved by 12 to 25%

and food consumption increased by 3 to 21% with enzyme treatment. Also, the metabolisable energy contents of the diets were improved by 1.53 MJ/kg DM. Marquart *et al.* (1994) found that improvements in the weight gain and feed conversion ratio following  $\beta$ -glucanase supplementation in broilers for barley-based diets were 16 and 6%, respectively.

**Table 2.5. Reported influence of  $\beta$ -glucanase supplementation of barley-based diets on the performance and DE of pigs**

Reference	Age	Improvements over unsupplemented control (%)			
		WG	FI	FCR	DE
Yin <i>et al.</i> (2001)	3 weeks	-	-	-	+3.0
WeiFen & DongYou (2004)	Piglets	+5.4	+3.2	+ 11.5	-
Li <i>et al.</i> (1996)	3 weeks	-	-	-	+4.3
Thomke <i>et al.</i> (1980)	Piglets	+5.0	-	+5.0	-
Baidoo <i>et al.</i> (1998)	Piglets	+12 – 17	-	-	-
Taverner & Campbell (1988)	Piglets	-	-	-	+13
Inborr and Graham (1991)	Piglets	+17	-	-	-

Other experiments on the use of  $\beta$ -glucanase have been reported by Hesselman and Aman (1986), using early-yellow (high viscosity barley) and combine ripeness barley (low viscosity barley) with or without  $\beta$ -glucanase supplementation has shown that the supplementation of  $\beta$ -glucanase on both barley-based diets resulted in increased feed consumption, live weight gain and an improved feed conversion ratio over the whole experimental period in chicken (Table 2.6). In the same experiment, Hesselman and Aman (1986) also observed that chickens fed with low viscosity barley were heavier at the end of the

experiment, and chickens fed enzyme supplements have a 10% shorter small intestine. An earlier study done by Hesselman *et al.* (1982), on the inclusion level of  $\beta$ -glucanase in barley diets of both early yellow ripeness (EYR; DM, 55%) and combine ripeness (CR; DM, 70%) for chickens, reported that  $\beta$ -glucanase supplementation resulted in highly significant improvements for all the measured parameters over the entire experiment. Also, it was reported that the response in live weight gain was improved at all levels of  $\beta$ -glucanase inclusion. This finding also reported that a clear improvement was obtained by increasing the levels of  $\beta$ -glucanase supplementation. The live weight gain at three weeks was increased by 10-26%, feed efficiency by 4.9-11% and feed intake by 6.5-15% with 0.05 to 0.5  $\beta$ -glucanase/kg feed averaged over the two stages of ripeness.

Enzyme supplementation on barley-based diets for early weaned pigs has been reported to improve ileal digestibility of starch and NSP (Inbarr *et al.*, 1993). Also, the supplementation of  $\beta$ -glucanase reduced digesta viscosity and increased the concentration of SCFA. Moreover, the bodyweight gain and feed conversion ratio tended to improve in the presence of the enzyme.

According to Svihus & Gullard (2002) (Table 2.6),  $\beta$ -glucanase supplementation improved the weight gain 13.9% and the feed conversion ratio in 14 day-old chickens. However, these improvements decreased as the birds grew with no improvement at 48 days of age. In general, supplementation with  $\beta$ -glucanase improved bird performance and increased the DE in pigs and AME in chickens (Tables 2.5. and 2.6, respectively). Bergh *et al.* (1999) also have reported that enzyme supplementation of diets generally increased the chickens' body weight and feed intake while the feed conversion ratio was decreased in comparison to animals fed diets without enzyme supplementation.

**Table 2.6. Reported influence of  $\beta$ -glucanase supplementation of barley-based diets in chickens**

Reference	Barley Type	Age (days)	Improvements over the unsupplemented control (%)			
			WG	FI	Feed per gain	AME
Mannion (1981)	Normal	1-28	+12-25	+3-21	-	+1.53
Campbell <i>et al.</i> (1984)	Normal	1-42	+10	-	-2.9	-
Hesselman & Aman (1986)	HV* Barley	14-19	+25.46	+14.75	-14.83	-
	LV** Barley	14-19	+27.68	+11.80	-18.33	-
Salih <i>et al.</i> (1990)	Hulless	1-14	+74.6	-	-21	-
		14-24	+25.8	-	-0.5	-
		24-36	+6.4	-	-6	-
		36-48	-3.9	-	-2.7	-
Marquart <i>et al.</i> (1994)	Normal		+6.0	-	+16.0	-
Viveros <i>et al.</i> (1994)	Normal (Beka)	1-28	+25	-	17	-
Fuente <i>et al.</i> (1995)	Normal	1-21	-	-	-	+2.6
Bustany (1996)	Normal	1-21	+4.5	-	-4.3	-
Schutte (1996)	Normal	1-39	+2.4	-1.6	-3.7	-
Yu <i>et al.</i> (1998)	Normal	1-21	+10.6	+2.3	-7.6	-
Svihus & Gullord (2002)	Normal	1-21	+13.9	+4.4	-9.1	+7.3
Wu <i>et al.</i> (2004)	Normal	28-35	-	-	-	+8.8

\* = High viscosity barley; \*\* Low viscosity barley

In conclusion, there are two types of cultivated barley, the hulled and the hulless varieties. Hulless barley is higher in protein and contains more essential amino acids than hulled barley and supply more available energy for pigs and poultry (Baidoo & Liu, 1998; Sauer *et al.*, 2002). Barley is used as a cereal source in pig and poultry diets, however, its inclusion levels in young growing animals, especially in poultry, is restricted due to its fibre and non-starch polysaccharide

(NSP) content, that are known to have a negative impact on its nutritional value (Bi *et al.*, 1998; Svihus & Gullord, 2002). Reducing the fibre contents in barley diets through the processing and/or the additional of exogenous enzymes (glucanases) are alternatives that could be applied.

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## Chapter Three

### Digestible energy content of new barley cultivars in pigs

#### 3.1. Introduction

Barley is one of the major feed grains used as an energy source in pig diets. However, its high fibre content consequently lowers its available energy which results in poor performance in pigs (Hollis & Palmer, 1971). Barley contains beta-glucans in the endosperm cell wall (Aman & Graham, 1987), which may impair its nutritive value. Pigs, like other mammals, do not produce enzymes that are able to degrade the cell wall and store non-starch polysaccharides (Bach Knudsen, 1997).

Compared to chickens, pigs are more adaptable to barley diets (Harrold, 1999). This is due to the longer and larger digestive tract in the pig where high gastrointestinal viscosity is not as likely to be a problem. This is because the depolymerisation of  $\beta$ -glucans has already begun in the stomach (Johansen *et al.*, 1997) and significant quantities of  $\beta$ -glucans can be digested prior to the terminal ileum (Lindberg *et al.*, 2003). According to Thacker *et al.* (1992), any alteration in digestibility as a result of enzyme supplementation may be due to enzymatic degradation of  $\beta$ -glucans present in the feed rather than a reduced intestinal viscosity.

There is a large variation in digestible energy values between barley cultivars. Various types of hulless barley cultivars have been developed recently. It is widely accepted that hulless barleys are more digestible and higher in nutritive value than normal hulled barley, due to the lower crude fibre and NSP (Non-Starch Polysaccharides) contents. (Gill *et al.*, 1966). However, the levels of energy digestibility in these barleys are inconsistent and dependent on the variety of barleys used. Moreover, the proportion of amylose and amylopectin starch appears to influence the digestible energy content. This study was conducted to

determine the digestible energy content of new barley cultivars (hulless and hulled) that varied in NSP contents and starch characteristics (Table 3.1).

### 3.2. Materials and Methods

Digestible energy was calculated using the total collection method in pigs (Boisen & Verstegen, 2000; den Hartog *et al.*, 1988).

#### 3.2.1. Experimental diets and feeding

One wheat, one conventional hulled barley and four hulless barley cultivars varying in their NSP and starch contents (Table 3.1) were tested. Crop and Food Research, Lincoln, New Zealand supplied the cultivars.

**Table 3.1. Properties of barley cultivars and wheat evaluated in pigs and broiler chickens**

Cultivar	Properties
Wheat (WT*) <sup>1</sup>	High yielding feed wheat
Barley Dash (NB*)	Conventional high-yielding barley
Barley 1831 (C0*) <sup>1</sup>	Hulless semi-waxy barley with high $\beta$ -glucan
Barley 1795 (C1*)	Hulless waxy barley with high $\beta$ -glucan
Barley 1822.2 (C2*)	Hulless barley with normal starch
Barley 1978 (C3*)	Hulless semi-waxy barley with higher $\beta$ -glucan

<sup>1</sup>Not been evaluated in chickens \* as referred in both trials

Each experimental diet included 99.75 % of the test ingredient and 0.25% of vitamin and premix. The pigs were fed 10% BW<sup>0.75</sup> per day and this was divided into two equal meals fed at 09.00 and 17.00. Fresh water was available *ad libitum*.

### 3.2.2. Experimental subjects

Fifteen entire Large White x Landrace male pigs with an average weight of  $32.5\text{kg} \pm 3\text{kg}$  were used. The pigs were obtained from a commercial farm. The experimental procedures were approved by Massey University Animal Ethics Committee and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purpose.

### 3.2.3. Experimental design

The trial ran for a total of 28 days (7 day acclimatisation period, 10 day for each of the two experimental periods, and returned to the farm at day 28) and during this time the pigs were housed in metabolic crates. The use of female pigs was deliberately avoided in order to minimise the contamination of faeces with urine when the collection was taking place.

After a one-week acclimatisation period, the experiment was conducted as two ten-day feeding periods. The diet for each pig was different between the two feeding periods. Faeces were collected and weighed daily from day 6 to 10 in each experimental period. Each diets was tested on five different pigs (Table 3.2). The number of ways to arrange six experimental diets ( $k$ ) in pair ( $n$ ) when the order is not important is equal to:

$$\binom{n}{k} = \frac{6!}{2!(6-2)!} = 15$$

Therefore, each of the 15 pigs was fed a different combination of diets.

Table 3.2. Diet allocation of fifteen pigs in two different collection periods

Pig no	Diet (Period 1)	Diet (Period 2)
1	C1	C2
2	C0	C2
3	C0	C1
4	C3	WT
5	C2	WT
6	NB	C1
7	C1	C3
8	NB	C0
9	C0	WT
10	C3	C0
11	C2	NB
12	NB	C3
13	C3	C2
14	WT	NB
15	WT	C1

The faeces from each pig were frozen daily, combined over the five day collection period, and subsequently thawed, thoroughly mixed and sub-sampled. Sub-samples were then freeze-dried and ground up. All faecal samples and diets were analysed for gross energy content. The digestible energy of each diet was calculated as follows:

$$ADE_{diet} = \frac{(FI \times GE_{diet}) - (Excreta\ output \times GE_{excreta})}{FI}$$

Where:

ADE = apparent digestible energy, FI = feed intake, GE = gross energy

### 3.2.4. Chemical analyses

Diet samples were analysed for  $\beta$ -glucan content using the Megazyme mixed linkage  $\beta$ -glucan assay procedure (McCleary *et al.*, 1997) EBC method 8.11.1. Total, soluble and insoluble non-starch polysaccharides in the barley were analysed using an assay kit (Englyst Fiberzym Kit GLC; Englyst Carbohydrate Services Limited, Cambridge, U.K) which is based on the procedures described by Englyst *et al.* (1994).

Rapid estimation of the amylose/amylopectin ratio in the diet samples were measured using the Hovenkamp-Hermelink *et al.* (1988) method. Standards were prepared using stock solutions of starch components as 25 mg of amylose or amylopectin and 10 ml 45% (w/v) aqueous  $\text{HClO}_4$  made up to a final volume of 100 ml by adding distilled water. Starch solutions were mixed to create standard solutions of 10, 20, and 60% amylose. Standard solutions were diluted with water to final starch concentrations of 6.25, 3.13, and 1.56 mg/100 ml.

Starch content was measured using an assay kit (Megazyme, Boronia, VIC, Australia) based on the use of thermostable- $\alpha$ -amylase and amyloglucosidase (McCleary *et al.*, 1997). Starch extraction from the ground cultivars was performed by adding 0.5 ml of 45%  $\text{HClO}_4$  and leaving it for 4 minutes. 8 mls of distilled water was then added and the tube mixed thoroughly. After mixing, the solutions were centrifuged for 60 seconds at  $650 \times 2$ .  $\text{I}_2$ -KI staining was achieved by mixing 4 ml of starch solution or extract and 5 ml of a diluted (1:2, v/v) Lugol's solution (2 g KI plus 1g  $\text{I}_2$  in 300 ml  $\text{H}_2\text{O}$ ). Sample extracts were diluted 1:10 before use. Absorbances were measured immediately afterward at two wavelengths (550 nm and 618 nm). Blanks consisted of 4 ml appropriately diluted  $\text{HClO}_4$  and 5 ml diluted Lugol's solution.

Feed and faecal samples were analysed in duplicate for dry matter (930.15; AOAC, 1990), organic matter (942.05; AOAC, 1990), and gross energy (adiabatic bomb calorimeter, Gallenkamp and Co. Ltd., London). The feed samples were also analysed for total nitrogen (macro Kjeldahl procedure, Kjeltec Auto 1030 analyser, Tecator, Sweden; 955.04; AOAC, 1990), crude fat (soxhlet extraction,

hexane extract; 963.15; AOAC 1990), neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin. NDF was determined by the method of Robertson & Van Soest (1981), without sodium sulphite, with alpha amylase and is expressed exclusive of residual ash. ADF and lignin were expressed exclusive of residual ash (973.18; AOAC, 1990). Crude protein was calculated as total N x 6.25 (954.01; AOAC, 1990).

### **3.2.5. Statistical analyses**

A linear model, with period as a random effect and diet as a linear effect, were fitted to the data. Differences between treatments were tested using Fisher's Least Significant Different test (LSD) where appropriate.

### 3.3. Results

The gross chemical composition of the wheat and hulless barley cultivars is presented in Table 3.3. It is recorded that the crude protein of four hulless barley cultivars ranged from 10.35 to 13.73 %, whereas, the gross energy content ranged from 18.05 to 18.32 MJ/kg and starch content between 58.60 and 65.50%. The gross energy and starch content for both hulled barley and wheat were 59.65 and 67.83%, respectively, and 18.02 and 18.12 MJ/kg, respectively. The percentages of NSP and  $\beta$ -glucans for hulless barley cultivars ranged between 12.34 and 16.51%; and 4.00 and 7.03% respectively. The NSP and  $\beta$ -glucan contents for hulled barley and wheat were 18.87 and 11.81%, and 5.02 and 0.75%, respectively. The amount of amylose ranged from 5 to 38% for hulless barley cultivars and for NB, WT was 47 and 55%, respectively.

The ADE content of four hulless barley cultivars, wheat and hulled, normal barley cultivars is shown in Table 3.4. The ADE content of these four barley cultivars ranged from 15.83 to 16.48 MJ/kg/DM. Hulless barley C2 had a superior ADE with 16.48 MJ/kg/DM compared to other hulless barley cultivars. The ADE of hulless barleys C3, C0 and C1 were 15.83, 15.95 and 16.03 MJ/kg/DM, respectively (Table 3.4). The ADE of wheat and hulled NB were 15.65 and 15.59 MJ/kg/DM, respectively.

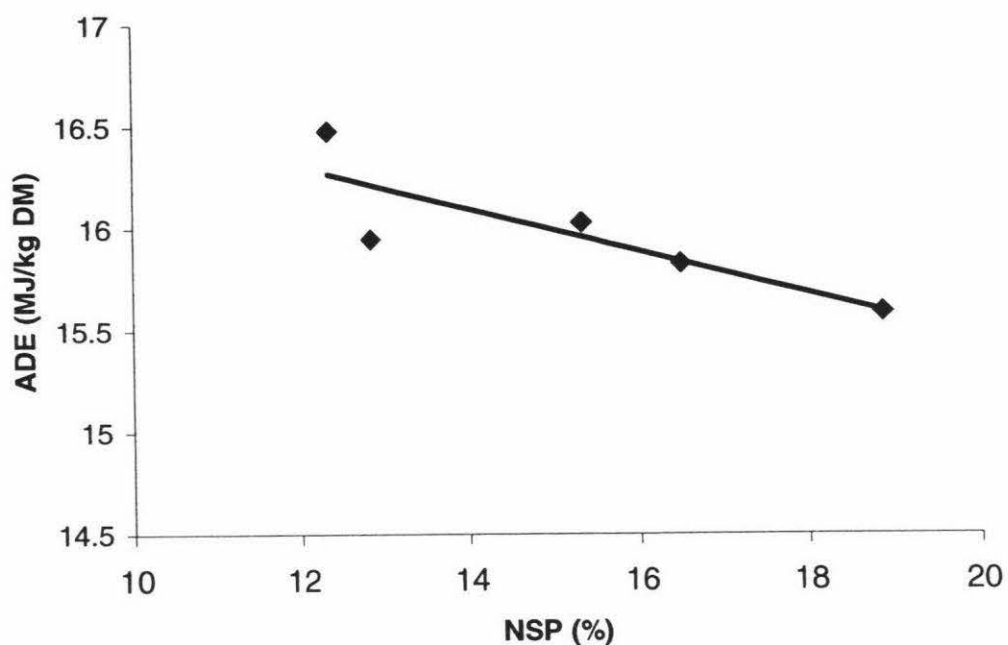
**Table 3.3. Gross composition of the wheat, normal, hulled and hulless barley cultivars on DM basis (g/100g)**

	Wheat	Hulled	Hulless	Hulless	Hulless	Hulless
	WT	NB	C0	C1	C2	C3
Dry Matter	88.9	89.02	88.68	89.37	89.92	90.27
Ash	1.78	2.70	1.96	1.96	1.92	1.82
Crude Protein (N x 6.25)	13.37	11.62	12.26	10.50	10.35	13.73
Gross Energy (MJ/kg)	18.07	18.88	18.25	18.23	18.65	18.57
Total Fat	1.99	2.47	3.39	3.16	2.61	3.39
Neutral Detergent Fibre	11.23	15.76	8.14	9.81	11.61	10.26
Acid Detergent Fibre	2.86	4.70	1.47	2.17	1.81	2.26
Lignin	1.03	0.86	0.50	0.72	0.79	0.93
Starch	67.83	59.65	63.60	64.23	65.50	58.60
Non-starch						
Polysaccharides	11.81	18.87	12.86	15.33	12.34	16.51
Arabinose	2.70	2.70	2.14	2.35	2.34	2.66
Xylose	4.50	5.39	2.82	3.47	3.23	3.66
Manose	0.22	0.34	0.34	0.45	0.33	0.44
Galactose	0.45	0.45	0.34	0.34	0.33	0.33
Glucose	3.49	9.44	6.65	8.17	5.67	8.97
Uronic	0.45	0.56	0.34	0.45	0.33	0.33
Amylose	55	47	9	13	38	5
B-glucan	0.75	5.02	5.11	5.46	4.00	7.03

Similar to ADE, the energy digestibility coefficient (ADC) of the hulless barley cultivars investigated in this trial (Table 3.4) was also highest in barley C2 (0.8837), followed by hulless barley C1 (0.8795), hulless barley C3 (0.8523) and hulled NB (0.8257). The ADC in wheat was not different from hulless barley

cultivars ( $P > 0.05$ ) but it was different from hulled NB ( $P < 0.05$ ). These differences in both ADE and ADC show that as the total NSP in the diet increased the ADE and ADC decreased.

**Figure 3.1 Relationship between non-starch polysaccharides and apparent digestible energy contents in four hullless barley cultivars and hulled conventional barley.**



$$ADE (MJ / kg) = 17.54(\pm 0.572) - 0.103(\pm 0.037) \times NSP(\%)$$

$$R^2 = 0.720$$

$$MSE = 0.200$$

**Table 3.4. Apparent digestibility coefficient (ADC) and apparent digestible energy (ADE) contents of hulless barley cultivars, conventional barley and wheat in pig**

Cultivars	ADC	ADE (MJ/kg DM)
Wheat WT	0.8664 <sup>bc</sup>	15.65 <sup>a</sup>
Hulled NB	0.8257 <sup>a</sup>	15.59 <sup>a</sup>
Hulless C0	0.8742 <sup>bc</sup>	15.95 <sup>a</sup>
Hulless C1	0.8795 <sup>c</sup>	16.03 <sup>ab</sup>
Hulless C2	0.8837 <sup>c</sup>	16.48 <sup>b</sup>
Hulless C3	0.8523 <sup>b</sup>	15.83 <sup>a</sup>
Pooled SE	0.0088	0.16

*Means within columns with different superscripts are different (P < 0.05).*

### 3.4. Discussion

In this experiment, the nutritive values of hulled and hulless barleys were strongly related to the variation in NSP,  $\beta$ -glucan and starch, similar to that reported by other authors (Kim, 1999; Shi & Noblet, 1993). It was shown that high levels of NSP and  $\beta$ -glucan content decreased the ADE and ADC of barley cultivars. This experiment found that the cultivars having waxy and normal starch, low in  $\beta$ -glucan contents showed superior ADE and ADC than those with higher NSP and  $\beta$ -glucan contents. This is in concordance with the statement from the Svihus & Gullord (2002) that NSP, particularly  $\beta$ -glucans, limited the digestion and absorption of the barley diets. Hulless normal barley C2 that was lower in  $\beta$ -glucan contents had the highest ADE (16.48 MJ/kg/DM) of cultivars tested. The overall digestible energy content of hulless barleys varied between 15.83 and 16.48 MJ/kg/DM and 0.24 to 0.89 MJ/kg/DM and was greater than the hulled NB.

Compared to the finding reported by Sauer *et al.* (2002) on both hulless and hulled barley energy digestibility, this experiment was lower by about 8.11%. This lower digestibility is assumed to be associated to proportion of hulls in the hulless barley cultivars. It is reported that the higher the percentage of hulls in the hulless barley, the lower the energy digestibility (Zijlstra *et al.*, 2004). According to work done by Fairbairn *et al.* (1999) using 20 barley samples, the ADE ranged from 11.25 to 13.11 MJ/kg (90% DM) with an overall mean of 12.28 MJ/kg (90% DM). The maximal difference in ADE between hulled barley (NB) and hulless barley (C2) was 0.89 MJ/kg DM, which is less than the 1.67 MJ / kg DM reported by Zijlstra *et al.* (2004). Furthermore, due to the variability of the nutritive value of conventional, hulled barley cultivars (Villamide *et al.*, 1997), the differences are comparable with other reports.

Hulless barley C1 and C2 had slightly higher ADC's (0.8795 and 0.8837 respectively), hulless barley C0 and wheat WT (0.8742 and 0.8664, respectively). The small difference in ADC between wheat and hulless barley cultivars was in agreement with the previous finding from Zijlstra *et al.* (2004) which stated that the ADE content of cleaned hulless barley was equal to that of wheat. The other factor that needs to be taken into consideration is the percentage of hulls in the hulless barley cultivars used, as there was no such information provided in that experiment. The range of differential of the hulless barley cultivars and the hulled NB in this present experiment were from 0.02 to 0.05%. It was also observed that the hulled NB had the lowest ADC with 0.8257. This finding is in concordance with reports from various authors stating that hulled barleys have lower DE because of anti-nutritive effects (NSP, fibre and  $\beta$ -glucan) in the hulls which lowers the nutritive value (Burnett, 1966; Johnston *et al.*, 2003; Kim, 1999; Shi & Noblet, 1993).

It was found that the levels of  $\beta$ -glucans had an impact on the digestible energy of the wheat and barley. The variation in NSP content also contributed to the variation in digestible energy contents. As  $\beta$ -glucan and NSP increased, the digestible energy content decreased. Therefore, the variation in the chemical composition of barley cultivars explains the variation in the ADE content of barleys (Bhatty *et al.*, 1975; Fairbairn *et al.*, 1999; Henry, 1988).

A factor that may contribute to the lower ADE and ADC in hulled NB is the percentage of neutral detergent fibre (NDF). The NDF of hulled NB was the highest of all the barley cultivars in this trial with a value of 15.76%, whereas the NDF of four hulless cultivars ranged from 8.14 (C0) to 11.61% (C2) (Table 3.3). This is also in agreement with the previous report by Le Goff and Noblet (2001) which stated that the energy digestibility in growing pigs was reduced by approximately 1 percentage point for each 1% additional NDF in the diet.

In conclusion, hulless barley cultivars low in fibre, NSP and  $\beta$ -glucan content had a better digestible energy content than hulled NB. The negative correlation between NSP content and ADE is well demonstrated in pigs (King & Taverner, 1975). Other factors that contributed to lower digestibility of energy in hulless barley cultivars were the waxiness and starch characteristics.

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## Chapter Four

### Nutritional value of barley cultivars for broiler chickens as influenced by enzyme supplementation

#### 4.1. Introduction

Barley is an important energy source in poultry diets, but its use is limited due to the presence of  $\beta$ -glucans. These compounds reduce the nutritive value of barley by increasing digesta viscosity and, thus, reducing the availability of nutrients for digestion and absorption (Perttila *et al.*, 2001). In addition, the performance of broilers fed barley-based diets is adversely affected. Wet and sticky excreta is another problem associated with the feeding of barley (Svihus *et al.*, 1995).

The use of exogenous  $\beta$ -glucanase supplementation in broiler diets is known to increase the nutritive value of barley. Adding enzyme increases nutrient digestibility and apparent metabolisable energy (AME) contents, improves feed conversion efficiency and lowers the incidence of wet droppings (Hesselman *et al.*, 1981; Svihus *et al.*, 1995; Yu *et al.*, 1998). Due to these reasons, the use of  $\beta$ -glucanases is recommended in barley based broiler diets. However, improvements in broiler performance with  $\beta$ -glucanase supplementation have been inconsistent. The varying responses to enzyme addition may be due to differences in the contents and solubility of  $\beta$ -glucan in the different cultivars of barley (Yu *et al.*, 1998) as well as the hulled and hullless characteristics, and the waxiness, of the barley cultivars.

This experiment was designed to determine the influence of  $\beta$ -glucanase supplementation on the AME and apparent ileal amino acid digestibility of the four barley cultivars that vary in their fibre and  $\beta$ -glucan contents and starch (waxiness) properties.

## **4.2. Materials and Methods**

### **4.2.1. Enzyme**

The  $\beta$ -glucanase (Allzyme BG) was supplied by Alltech, Inc, Nicholasville, KY, USA. The enzyme product was produced as an extract from the fermentation of the fungal organism *Trichoderma longibrachiatum* and contained 6000  $\beta$ -glucanase units (BGU)/g. One unit of glucanase (BGU) is defined as that quantity of enzyme that liberates 1  $\mu$ mol of reducing sugars (expressed as glucose) in one minute at pH 5.0 at 30°C. The enzyme was added at the level of 50 g per tonne, as recommended by the manufacturer. The granular formulation of the enzyme was used.

### **4.2.2. Barley cultivars**

Four barley cultivars were evaluated. One was a conventional, hulled barley and the other three were hulless cultivars that differed in starch characteristics (waxiness) and  $\beta$ -glucans contents (Table 3.1). Crop & Food Research, Lincoln, New Zealand, provided the barley cultivars. Upon receipt, the grains were ground to pass through a 3-mm sieve.

### **4.2.3. Experimental diets**

The assay diets contained 96.3% barley, which was used as the only source of protein and energy, and fortified with minerals and vitamins (Table 4.1). Titanium oxide was included as an inert marker for the estimation of amino acid digestibility. The assay diets were offered for seven days and the total excreta collection was carried out during the last four days.

**Table 4.1. Composition of the basal diet used in the apparent metabolisable energy and amino acid digestibility assays**

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	g/kg
Barley cultivars <sup>1</sup>	96.3
Titanium oxide	3.0
Dicalcium phosphate	17.0
Limestone	13.0
Salt	2.0
Vitamin-trace mineral premix	2.0

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<sup>1</sup>Each of the four cultivars to be fed without or with enzyme

#### **4.2.4. Experimental procedures**

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

A total of 200 male broiler (Ross) chicks were obtained as day-olds from a commercial hatchery. The chicks were raised on floor pens in an environmentally controlled room and received a commercial starter diet from day 1 to 21. On day 18, the birds were transferred to grower cages and given a 3-day adjustment period. On day 21, the birds were weighed individually and those with relatively high or low body weights were discarded. A total of 160 birds were selected and

distributed into 32 groups (pens) of five birds each so that average weights per pen were similar. The eight dietary treatments (four barley cultivars, without or with  $\beta$ -glucanase supplementation) were then randomly assigned to four pens each. The diets were fed from day 21 to 28. Feed and water were available *ad libitum*.

From day 25 to 28, feed intake and excreta output were measured quantitatively per pen over four consecutive days. On day 28, all birds were sacrificed by an intravenous injection of sodium pentobarbitone. The small intestine was immediately exposed and contents of the lower ileum were expressed by gentle flushing with distilled water into plastic containers. The ileum was defined as that portion of the small intestine extending from the vitelline diverticulum to a point 40 mm proximal to the ileo-caecal junction. The ileum was divided into two halves and the digesta was collected from the lower half towards the ileo-caecal junction. Digesta were pooled within a pen, lyophilised and ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at  $-4^{\circ}\text{C}$  for chemical analyses. Samples of diets and ileal digesta were assayed for titanium and amino acids.

Excreta were pooled within a pen, mixed well into slurry in a blender and representative samples were obtained and lyophilised. Dried excreta samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at  $-4^{\circ}\text{C}$  for chemical analyses. The gross energy and dry matter contents of diets and excreta were determined.

#### **4.2.5. Chemical analyses**

The dry matter content was determined using the AOAC standard procedures (AOAC, 1990). Gross energy was determined using the procedure outlined in chapter 3 (section 3.2.4). Amino acids were determined by hydrolysing the samples with HCl (containing phenol) for 24 h at  $110 \pm 2^{\circ}\text{C}$  in glass tubes sealed under vacuum. Amino acids were detected on a Waters ion exchange

HPLC system, and the chromatograms were integrated using dedicated software (Millenium, Version 3.05.01, Waters, Millipore, Milford, MA) with the amino acids identified and quantified using a standard amino acid solution (Sigma, St. Louis, MO). Sulphur-containing amino acids (cysteine and methionine) and tryptophan were not determined. Titanium content was measured on a UV spectrophotometer following the method of Short *et al.* (1996).

#### 4.2.6. Calculations

The AME of the diets was calculated using the following formula:

$$AME_{diet} = \frac{(FI \times GE_{diet}) - (Excreta\ output \times GE_{excreta})}{FI}$$

The apparent ileal AA digestibility were calculated using the following formula:

$$Apparent\ AA\ digestibility = \frac{(AA/T_i)_d - (AA/T_i)_i}{(AA/T_i)_d}$$

Where:

FI = feed intake, GE = gross energy,  $AA/T_i_d$  = the ratio of amino acids to titanium in the diet,  $AA/T_i_i$  = the ratio of amino acids to titanium in the ileal digesta

#### 4.2.7 Statistical analyses

Two-way analysis of variance was employed to determine the main effects (barley cultivars and  $\beta$ -glucanase) and their interaction by using the GLM procedure of SAS (1997) using the pen as the experimental unit. Differences were considered significant at  $P < 0.05$ , although P values up to  $P \leq 0.10$  are shown in the text if the data suggested a trend.

### 4.3. Results

The proximate and fibre compositions of the barley cultivars are presented in Chapter 3 (Table 3.4). The amino acid composition of the barley cultivars is presented in Table 4.2. It can be seen that amino acid concentrations in hulless barley C3 was highest compared to those of other cultivars. For instance, the content of lysine was highest in hulless C3 (0.523 g/100g) followed by the hulled NB (0.406 g/100g). The observed differences in amino acid concentrations between the four cultivars are largely a reflection of differences in protein contents. The amino acid profile suggests that barley protein is deficient in most essential amino acids, especially lysine.

**Table 4.2. Amino acid composition (g/100g dry matter) of barley cultivars**

Amino Acids	Hulled	Hulless	Hulless	Hulless
	NB	C1	C2	C3
Dry Matter	89.02	89.37	89.92	90.27
Crude Protein	11.62	10.50	10.35	13.73
Aspartic Acid	0.773	0.637	0.672	1.086
Threonine	0.377	0.310	0.355	0.468
Serine	0.453	0.363	0.426	0.525
Glutamic Acid	3.176	2.416	2.753	3.793
Proline	1.415	1.037	1.135	1.832
Glycine	0.462	0.356	0.402	0.554
Alanine	0.454	0.369	0.412	0.579
Valine	0.595	0.488	0.546	0.708
Isoleucine	0.418	0.354	0.389	0.524
Leucine	0.815	0.647	0.724	1.007
Tyrosine	0.380	0.251	0.338	0.438
Phenylalanine	0.656	0.451	0.513	0.816
Histidine	0.311	0.230	0.258	0.345
Lysine	0.406	0.307	0.343	0.523

The influence of exogenous  $\beta$ -glucanase supplementation on the AME is shown in Table 4.3. The AME was influenced ( $P < 0.001$ ) by the cultivar's type. The average AME content of the normal, hulled barley was determined to be 13.13 MJ/kg DM, while the values for the three hulless cultivars were 11.85, 13.37 and 11.31 MJ/kg DM, respectively.  $\beta$ -glucanase supplementation improved ( $P < 0.001$ ) the AME of all barley cultivars, with improvements ranging from 5.4 to 21.9%. The response tended to be greater when the initial AME of the cereal was lower, as indicated by the cultivar x enzyme interaction ( $P = 0.07$ ).

The cultivar type had no influence on the apparent ileal digestibility of most amino acids (Table 4.4). The exceptions were the digestibility of aspartic acid ( $P < 0.05$ ), glycine ( $P < 0.05$ ), alanine ( $P < 0.05$ ) and phenylalanine ( $P < 0.05$ ) and tyrosine ( $P < 0.01$ ), which differed between cultivars. It was also observed that the interaction of cultivars and enzymes on apparent ileal amino acid digestibility was not significant ( $P > 0.10$ ). The average apparent ileal digestibility coefficients of the 15 amino acids in the hulled NB and the three hulless cultivars were 0.70, 0.68, 0.72 and 0.73, respectively. Enzyme supplementation improved ( $P < 0.001$ ) the AID of all amino acids in the four barley cultivars, with increases in individual amino acid digestibility ranging from 18.1% for threonine to 11.4% for arginine. The average AID of 15 amino acids in the unsupplemented and supplemented cereal was 0.66 and 0.75, respectively.

**Table 4.3. Apparent metabolisable energy of barley cultivars as influenced by exogenous  $\beta$ -glucanase supplementation**

Cultivar	Enzyme	AME, MJ/kg DM
Hulled NB	-	12.68
	+	13.58
Hulless C1	-	10.87
	+	12.83
Hulless C2	-	12.92
	+	13.82
Hulless C3	-	10.20
	+	12.43
Pooled SEM		0.26
Main effects:		
Barley type:		
-	Hulled NB	13.13 <sup>a</sup>
-	Hulless C1	11.85 <sup>b</sup>
-	Hulless C2	13.37 <sup>a</sup>
-	Hulless C3	11.31 <sup>b</sup>
Enzyme effect:		
-		11.67 <sup>b</sup>
+		13.16 <sup>a</sup>
Significance, $P \leq$		
-	Cultivar	< 0.0001
-	Enzyme	< 0.0001
-	Cultivar*Enzyme	0.07

*Means within column with different superscripts are different ( $P < 0.0001$ )*

Table 4.4. Apparent ileal amino acid digestibility of barley cultivars as influenced by exogenous  $\beta$ -glucanase supplementation

Cultivar	EZ	AA (%)															
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg	Average
Hulled NB	-	0.59	0.52	0.60	0.80	0.80	0.58	0.61	0.68	0.67	0.70	0.66	0.75	0.57	0.62	0.73	0.66
	+	0.69	0.61	0.70	0.88	0.88	0.68	0.72	0.77	0.77	0.80	0.76	0.83	0.68	0.72	0.80	0.75
Hulled NB	-	0.59	0.52	0.60	0.80	0.80	0.58	0.61	0.68	0.67	0.70	0.66	0.75	0.57	0.62	0.73	0.66
	+	0.69	0.61	0.70	0.88	0.88	0.68	0.72	0.77	0.77	0.80	0.76	0.83	0.68	0.72	0.80	0.75
Hulless C1	-	0.59	0.53	0.60	0.76	0.75	0.55	0.59	0.65	0.65	0.66	0.55	0.68	0.52	0.56	0.65	0.62
	+	0.69	0.62	0.70	0.86	0.86	0.64	0.69	0.76	0.76	0.77	0.70	0.79	0.65	0.68	0.76	0.73
Hulless C2	-	0.59	0.57	0.65	0.78	0.75	0.60	0.63	0.68	0.68	0.70	0.67	0.71	0.55	0.61	0.69	0.66
	+	0.72	0.68	0.75	0.88	0.86	0.70	0.74	0.79	0.80	0.81	0.79	0.83	0.69	0.73	0.79	0.77
Hulless C3	-	0.70	0.60	0.65	0.79	0.79	0.65	0.70	0.71	0.71	0.73	0.67	0.76	0.59	0.70	0.74	0.70
	+	0.75	0.59	0.72	0.84	0.84	0.71	0.74	0.76	0.77	0.79	0.74	0.81	0.67	0.75	0.78	0.75
Pooled SEM		0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02
Main effects:																	
Barley type:																	
- Hulled NB		0.64 <sup>a</sup>	0.57 <sup>a</sup>	0.65 <sup>a</sup>	0.84 <sup>b</sup>	0.84 <sup>b</sup>	0.63 <sup>b</sup>	0.66 <sup>b</sup>	0.72 <sup>a</sup>	0.72 <sup>a</sup>	0.75 <sup>b</sup>	0.71 <sup>b</sup>	0.79 <sup>b</sup>	0.63 <sup>b</sup>	0.67 <sup>b</sup>	0.76 <sup>b</sup>	0.70 <sup>b</sup>
- Hulless C1		0.64 <sup>a</sup>	0.58 <sup>a</sup>	0.65 <sup>a</sup>	0.81 <sup>a</sup>	0.81 <sup>a</sup>	0.60 <sup>a</sup>	0.64 <sup>a</sup>	0.70 <sup>a</sup>	0.70 <sup>a</sup>	0.72 <sup>a</sup>	0.62 <sup>a</sup>	0.73 <sup>a</sup>	0.58 <sup>a</sup>	0.62 <sup>a</sup>	0.70 <sup>a</sup>	0.67 <sup>a</sup>
- Hulless C2		0.65 <sup>a</sup>	0.62 <sup>b</sup>	0.70 <sup>b</sup>	0.83 <sup>b</sup>	0.81 <sup>a</sup>	0.66 <sup>c</sup>	0.67 <sup>b</sup>	0.73 <sup>b</sup>	0.74 <sup>b</sup>	0.75 <sup>b</sup>	0.73 <sup>b</sup>	0.77 <sup>b</sup>	0.62 <sup>b</sup>	0.67 <sup>b</sup>	0.74 <sup>b</sup>	0.71 <sup>b</sup>
- Hulless C3		0.72 <sup>b</sup>	0.64 <sup>b</sup>	0.69 <sup>b</sup>	0.82 <sup>a</sup>	0.82 <sup>a</sup>	0.68 <sup>c</sup>	0.72 <sup>c</sup>	0.74 <sup>b</sup>	0.74 <sup>b</sup>	0.76 <sup>b</sup>	0.71 <sup>b</sup>	0.79 <sup>b</sup>	0.63 <sup>b</sup>	0.72 <sup>c</sup>	0.76 <sup>b</sup>	0.73 <sup>c</sup>
Enzyme effects:																	
-		0.62 <sup>a</sup>	0.55 <sup>a</sup>	0.62 <sup>a</sup>	0.78 <sup>a</sup>	0.77 <sup>a</sup>	0.59 <sup>a</sup>	0.63 <sup>a</sup>	0.68 <sup>a</sup>	0.67 <sup>a</sup>	0.70 <sup>a</sup>	0.64 <sup>a</sup>	0.81 <sup>a</sup>	0.56 <sup>a</sup>	0.62 <sup>a</sup>	0.70 <sup>a</sup>	0.66 <sup>a</sup>
+		0.72 <sup>b</sup>	0.65 <sup>b</sup>	0.72 <sup>b</sup>	0.86 <sup>b</sup>	0.86 <sup>b</sup>	0.69 <sup>b</sup>	0.72 <sup>b</sup>	0.77 <sup>b</sup>	0.77 <sup>b</sup>	0.79 <sup>b</sup>	0.75 <sup>b</sup>	0.72 <sup>b</sup>	0.67 <sup>b</sup>	0.72 <sup>b</sup>	0.78 <sup>b</sup>	0.75 <sup>b</sup>
Significance, $P \leq$																	
- Cultivar		0.02	0.07	0.08	0.32	0.46	0.03	0.04	0.44	0.40	0.23	0.002	0.04	0.45	0.23	0.08	0.19
- Enzyme		<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
- Cultivar*Enzyme		0.54	0.94	0.91	0.40	0.34	0.77	0.60	0.61	0.58	0.58	0.58	0.40	0.79	0.60	0.46	0.61

Means within column with different superscripts are different ( $P < 0.0001$ )

## 4.4. Discussion

### 4.4.1. Apparent metabolisable energy

The AME value significantly differed between barley cultivars. Interestingly, the AME of conventional hulled barley was greater than two of the hulless cultivars (C1 and C3). Hulless barley C2 was determined to have a higher AME content than the other two hulless cultivars, but its AME was not different from that of conventional hulled barley. It is known that the AME content of barley cultivars is related to chemical composition (Chen, 1997) and, in particular, to the fibre and  $\beta$ -glucan contents (Johnston *et al.*, 2003). The differences observed in the AME values were, in part, consistent with the  $\beta$ -glucan and NSP levels of the barley cultivars, as it has been reported that there is a negative correlation between NSP levels and AME for poultry (Annison, 1991; Choct & Annison, 1990). Hulless cultivars C1 and C3 had higher contents of  $\beta$ -glucan than hulless cultivar C2 and this was reflected in AME differences. The finding that the hulled barley cultivar had AME contents higher than these two cultivars is in contrast to previous reports (Yu *et al.*, 1998). However, it is known that the nutritive value of conventional, hulled barley cultivars can be variable (Villamide *et al.*, 1997) and this may explain the higher AME of the hulled cultivar compared to two of the hulless cultivars. Overall, the results from this experiment suggest that  $\beta$ -glucan contents are more important than fibre contents in determining the AME in barley. Another factor that may be important is the starch characteristics (waxiness) of barley cultivars.

The supplementation of  $\beta$ -glucanase resulted in significant improvements in the AME of all barley cultivars, with an average increase of 12.8%. This finding is in agreement with previous reports (Bergh *et al.*, 1999; Svihus & Gullord, 2002; Viveros *et al.*, 1994). The magnitude of improvement with the added enzyme, however, varied with cultivar type. Improvements were greater in hulless cultivars C1 and C3, with values of 18.0 and 21.9%, respectively. It must be noted that these two cultivars had high  $\beta$ -glucan contents and their initial AME values were lower.

The ratio of amylose to amylopectin in both hulless barley C1 and C3 were also lower compared to hulled NB and hulless barley C2 (Table 3.4). Whether the differences in waxiness contributed to the greater AME responses in hulless C1 and C2 is difficult to ascertain. On the other hand, the degree of improvement was relatively lower in hulless barley C3 and conventional hulled barley (7.0 and 7.1%, respectively).

The present results showed that exogenous  $\beta$ -glucanase would have the greatest benefit in barleys that have high levels of the substrate,  $\beta$ -glucans. It was also observed that the higher the waxiness of the barley (lower amylose and amylopectin) the better the  $\beta$ -glucanase improvements. Scott *et al.* (1998) reported that enzyme supplementation improved the AME of both hulled and hulless barleys by about 11.3%. The  $\beta$ -glucan contents of the barleys used in their study were not presented. Svihus & Gullord (2002) found that the improvements *due to  $\beta$ -glucanase supplementation in normal barley were 7.3%, whereas Fuente et al. (1995) and Mannion (1981) have observed improvements of 2.6 and 1.5%, respectively.*

#### **4.4.2. Amino acid digestibility**

Although the average AID of hulless barley C1 was numerically lower than those of the other three cultivars, the differences were not significant. The average AID coefficients of hulless barley C3, hulless barley C2, conventional hulled NB, and hulless barley C1 was 0.73, 0.72, 0.70 and 0.68, respectively. The similar AID values observed for barley cultivars are in contrast to the differences determined for the AME contents.

The improvements in the average AID due to enzyme supplementation of hulled NB, hulless barley C1, hulless barley C2, and hulless barley C3 were 13.6, 17.7, 16.6 and 7.14%, respectively. Enzyme supplementation improved the AID of all individual amino acids in the four barley cultivars, with increases in individual amino acid digestibility ranging from 11.4% for arginine to 18.1% for threonine. The

improvement in average AID with  $\beta$ -glucanase supplementation was 13.6%. These results are consistent with previous reports by Bedford (1995), which stated that  $\beta$ -glucanase supplementation improved the AID of amino acids in barley-based diets by 10 to 15% for 3-week old broilers. Bergh *et al.* (1999) reported that the ileal digestibility of total  $\beta$ -glucan was improved by enzyme supplementation and it was concluded that the  $\beta$ -glucanase used in the experiment have reduced the intestinal viscosity or nutrient encapsulation due to the presence of intact cell walls and, thus, facilitated nutrient uptake. In their study, enzyme supplementation significantly improved the ileal digestibility of all nutrients. Wu *et al.* (2004) reported that  $\beta$ -glucanase supplementation improved the AID of protein by 9.8%.

In conclusion, it is showed that the supplementation of exogenous  $\beta$ -glucanase was positively influenced the AME and AID of amino acids in all barley cultivars in broiler chickens. The effects of exogenous  $\beta$ -glucanase were more significant on the hullless barley cultivars that have higher  $\beta$ -glucan substrates.

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## Chapter Five

### General Discussion and Conclusions

The present study evaluated the nutritional value of hulled and hullless barley cultivars that differed in fibre and NSP contents for pigs and broiler chickens. In the pig experiment; one wheat, one hulled barley and four hullless cultivars were assessed to obtain information on the available energy content (ADE). In the broiler trial, two of these dietary treatments (one wheat and one hullless barley cultivar) were omitted; and one hulled conventional barley and three hullless barley cultivars were used in order to examine their nutritive value without or with exogenous  $\beta$ -glucanase supplementation. To evaluate the influence of soluble NSP and  $\beta$ -glucan contents of these barley cultivars in broiler chicken, a commercially prepared barley  $\beta$ -glucan extract (Glucagel<sup>TM</sup>) was used.

In the pig trial, the influence of fibre and NSP as well as the  $\beta$ -glucan contents of the dietary treatments was examined. Total collection method was used to determine the digestible energy contents. The results suggested that more NSP and fibre as well as  $\beta$ -glucans in barley diets, the lower will be the digestible energy contents. The hullless barley cultivars tested had higher ADE contents than the hulled barley cultivar. Wheat had the highest ADE, whereas the hulled conventional barley had the lowest. Among the hullless barley cultivars, hullless barley C2 was determined to have the highest ADE. Hullless barley C2 had similar characteristics as normal hullless barley and low levels of  $\beta$ -glucans. These data demonstrate the negative correlation between the NSP, fibre and  $\beta$ -glucans contents and the ADE contents,

which is in agreement with those of King and Taverner (1975). The higher ADE contents of hulless barley cultivars are consistent with earlier reports stating that hulless barley cultivars, in some cases, may have similar or better nutritional value than wheat (Zijlstra *et al.*, 2004). Therefore, the use of hulless barleys may be advantageous over the use of hulled conventional barleys to overcome the negative effects on pig performance. However, the use of hulled barley cultivars that are low in fibre and  $\beta$ -glucan contents could be considered, since hulled barleys are known to vary in their nutritional value (Villamide *et al.*, 1997).

In the broiler trial, the AME value of hulled normal starch barley was not different from hulless, normal starch barley cultivar. It was also observed that AME value of hulled conventional barley was higher than those of two other hulless barleys (C1 and C3). It was determined that these differences between hulled and hulless cultivars in the AME and AID of amino acids were due to the  $\beta$ -glucan content rather than the levels of NSP and fibre. The use of  $\beta$ -glucanase supplementation positively influenced the AME and AID of amino acids in all barley cultivars.

These data showed that the anti-nutritive effects of NSP and fibre appear to be more important in affecting the nutritive value of barleys for pigs, whereas in broiler chickens, the  $\beta$ -glucan content appears to be the major contributory factor.

The results from pig and broiler chicken trials have a unique similarity. Hulless barley C2 was observed to have higher ADE and ADC in pigs. Similarly, in broiler chickens, the same hulless barley cultivar was found to have higher AME and AID contents. Therefore, it is concluded that hulless barley C2, which is characterized

as hulless normal starch barley with low  $\beta$ -glucan content, has better nutritive value in both pigs and broiler chickens than hulless waxy barleys, which are high in  $\beta$ -glucan contents.

In conclusion, the present data demonstrated that hulless barleys low in fibre, NSP and  $\beta$ -glucan contents showed a better ADE and ADC in pigs. A similar principle is applicable for broiler chickens. This information is useful in selecting suitable barley types for use in pig and poultry diets.

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