THE USE OF ENZYME SUPPLEMENTATION FOR WHEAT-BARLEY DIETS IN POULTRY AS A MEANS OF IMPROVING PRODUCTIVE PERFORMANCE

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PREFACE

The experimental work described in this dissertation was carried out in the School of Agricultural Sciences and Agribusiness, University of KwaZulu-Natal, Pietermaritzburg, from January 2007 to October 2009, under the supervision of Dr Mariana Ciacciariello.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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DECLARATION

I, Masefo Josephina Mojoma, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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   i. Their words have been re-written but the general information attributed to them has been referenced
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Signed

Ms M J Mokoma

Dr M Ciaciariello

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ABSTRACT

The objective of the study was to evaluate the effect of an exogenous multi-blend enzyme (β-glucanase and xylanase) on the performance of the broiler chickens and laying hens fed diets based on wheat and barley. Experiments were conducted on a flock of broilers and two flocks of laying hens. In both cases feed and water were provided *ad libitum*.

The enzyme effect of enzyme addition on the broiler performance involved 2080 day-old male and female chicks in 48 pens, allocated one of four dietary treatments (0, 50, 100 or 200 g/ton enzyme supplementation), to 35 days of age. On day 35, ten birds from each treatment were sacrificed for the analysis of the digestive organs weight (gizzards and livers). The trial was divided into two phases: a starter (1 to 21 d) and grower (22 to 35 d). Feed consumption was measured weekly and birds were also weighed weekly.

The investigation of enzyme effect in laying hen diets involved 896 birds for each specific period. Each replicate consisted of four cages (four birds per cage) with a common feeder; 16 hens/pen of 56 pens. Eggs were weighed three times a week, feed consumption weekly and birds every weeks

The addition of a multi-blend enzyme significantly improve body weight, body weight gain, food intake, and feed conversion ratio for both sexes ($P<0.05$) in broiler chickens. There was a significant improvement in egg production in laying hens ($P<0.05$). Egg weight and egg mass were not significantly improved.

Wheat and barley have cell wall components (arabinoxylans and β-glucans respectively) which have a negative effect on the nutritive value of these feeds and therefore performance in poultry fed diets based on these ingredients. Addition of an exogenous multi-blend enzyme (β-glucanase and xylanase) could help reduce these effects and improve performance and digestibility values in poultry. The null hypothesis was there will be no difference between supplemented and un-supplemented diets based on wheat and barley in performance of poultry. The results of this study suggest that the inclusion of 50 g/ton enzyme helps improve poultry performance, especially in young birds.

CHAPTER 1

GENERAL INTRODUCTION

The poultry industry has shown a significant growth over the past four decades. Improved genotypes and market demands have put pressure on the production practices. Nutrition is one of the most important inputs in poultry production. It is widely known that feed costs represent approximately 80% of the total production cost, being therefore the most challenging input to manage. The availability of raw materials for poultry is affected by many factors such as environmental conditions, economy of the country, competition with human demands and prices of ingredients. Drought or heavy rains can lower the harvest quantities and lead to lower availability of raw materials and increase in prices. The choice of energy and protein sources is the most challenging as they are the main constituents of the feed.

Cereal grains are the most common energy sources in poultry feed. In spite of the large variety of grains available, only a few are used due to quality issues or the presence of anti-nutritional factors. Maize is commonly used in South Africa as well as in the USA and Brazil. But lately there has been a decrease in maize production and increase in demand for both human and animal consumption of maize, which has lead to a rise in international prices for maize. Animal nutritionists are faced with the challenges of formulating cheaper, high quality feeds and must also find alternatives that are good energy sources and cheap in other to substitute for maize.

Cereals like wheat, barley oats and rye have become ever more popular because of better understanding of their composition and the development of commercial enzymes. In South Africa the use of these cereals and commercial enzymes is not as popular as it is in Europe or USA. A great deal of research is been conducted to continue improving the quality and utilisation of these raw materials. These cereals are good sources of energy and some cases, protein. However, these are known to have negative effects on the performance of young chickens when they added in high concentrations in the feed. This has been found to be due to their content of anti-nutritional factors (ANFs) or nutrients diluents, depending on their solubility, which are very resistant to the animal digestive enzymes and tend to form a viscous environment in the small intestine. Even though starch is the main carbohydrate in cereals, other carbohydrates in the cereals grains are structural components of the cell wall.

These ANFs are carbohydrates and form part of the grain's cell wall; the fall under a major known as non-starch polysaccharides (NSPs) and they can be soluble or insoluble fibres. Cell walls of the grains of wheat, rye, and barley contain high level of soluble NSPs known as arabinoxylans or Pentosans and β-glucans, respectively. As these NSPs are soluble in water, they dissolve in the intestines and lead to an increase in the viscosity of the contents of gastrointestinal tract (GIT). This, in turn, is responsible for the reduce growth performance and digestibility values observed in poultry. These problems can be avoided by supplementation of wheat-, barley-, rye- and oats- based diets with suitable exogenous enzymes.

The main uses of these exogenous enzymes in animal feeds are: to break down anti-nutritional factors which cannot be broken down by bird's digestive enzymes, to increase the availability of nutrients, to supplement the enzymatic system of animals, and reduce the environmental impact and cost of feed production. There are four types of enzymes that can be used in the poultry and other animal feeds based on cereals. They are known to break down; carbohydrates, lipids, proteins and phytic acids. These enzymes can be administered in the liquid or powdered form depending on the processing and form of feeds.

In the present study the exogenous multi-blend enzyme (β-glucanase and xylanase) was added into wheat and barley diets fed to broiler chickens from day old to 35 days and two groups of laying hens from age of 20 to 32 weeks and 28 to 40 weeks of age. The aim of the study was to evaluate the effect of these enzymes on the performance of broilers and laying hens.

CHAPTER 2

LITERATURE REVIEW

2.1 Gastrointestinal anatomy and digestive physiology of chickens

The digestive system of poultry has a fairly simple structure and possesses some differences compared to that of mammals, which are mostly meant to make birds lighter for flying. Even though broilers and layers do not fly they still maintain these adaptations. Poultry feeds contain highly complex molecules that need to be broken down to smaller/ simple molecules (nutrients) in order to be absorbed and utilised by the body for growth and production. Chemical and mechanical processes of digestion take place in the GIT. Once in the lumen, these nutrients are transferred to the portal blood stream and to the target tissues. The GIT of a bird, like in mammals, has accessory digestive organs which secret substances that are essential in the digestion process.

2.1.1 Beak

Chickens possess a beak instead of lips and teeth as observed in mammals. Feed particles are transferred into the mouth without changing form, and water is swallowed by following the movement of the head. Chickens do not have a soft palate and epiglottis. The mouth and the pharynx form one opening known as buco-pharyngeal cavity (McLelland, 1981; Freeman, 1983; Denbow, 2000). The tongue has an acute angle shape with salivary glands and taste buds. Birds have poor taste ability as compare to mammals, which is thought to be due to fast transit rate of food due to lack of mastication (Klassing, 1998). Mason and Clark (2000) reported that chickens have 24 taste buds. The tongue is attached by the hyoid bone which allows the movement of food particles towards the oesophagus (Gentle, 1973).

2.1.2 Oesophagus and muscular stomachs

The oesophagus is a long dilatable tube found between the pharynx and the proventriculus or glandular stomach. The oesophagus is made up of longitudinal and circular muscle fibres that help transfer of food to the crop and proventriculus by peristalsis (McLelland, 1981; Freeman, 1983; Denbow, 2000). It has a balloon-like structure in the middle known as the

crop, which lies between the cervical and intra-thoracic parts of the oesophagus. These parts also tend to regulate the passage rate of the ingesta into and from the crop. In the crop, the ingesta is stored, moistened, and fermented (Denbow, 1994). The emptying of the crop depends on several factors, for example; the capacity of the crop, composition of the food, how full the gizzard is, or food particle size. Blood supply to the oesophagus is from the aorta branch known as the splenic artey (Freeman, 1983).

2.1.3 Glandular and muscular stomachs

From the crop the food enters the lower part of the oesophagus to be transported to the glandular stomach or proventriculus. The proventriculus is distinguished by a thicker glandular wall and is situated at the top of the gizzard (Figure 2.1). These glands form rows that are visible and their alveoli are surrounded by cells that secret gastrin, hydrochloric acid (HCl) and pepsinogen. Pepsinogen is the precursor of pepsin which is responsible for the digestion of proteins. Both secretions are regulated by the nervous system and chemical stimulation (Denbow, 1994).

Food passes into the muscular stomach, also known as a gizzard, through the isthmus, to be grinded for further digestion in small intestines. The gizzard is the thickened muscular organ found below the proventriculus. The gizzard's inner wall is covered by keratin which is different from other keratinous structures of the body (Aitken, 1958). Further research reported this layer to be a cuticle layer known as the koilin layer (Akester, 1986; Abe *et al.*, 2001; De Voe *et al.*, 2003), which helps with the grinding and reducing food particle size, but most importantly protects the gizzard from damage. Koilin is the protein-polysaccharide secreted by the glands in the mucosa layer and is solidified by the HCl from the proventriculus (Akester, 1986; De Voe *et al.,* 2003).

The muscular stomach is comprised of different thick and thin muscles that contract sequentially to transfer the chime into the duodenum. These two stomachs complement each other, to perform the functions of the true stomach. Solubilisation of mineral salts (calcium carbonate and phosphate) by HCl occurs in the gizzard (Freeman, 1983; Orban *et al.,* 1992; Guinnotte *et al*., 1995). Orban *et al.,* (1992) has also reported that in laying hens, the calcification is dependent on acid-base state due to availability of CO_3^{2-} ions. The gastric artery takes blood to the proventriculus and gizzard from the aorta (Freeman, 1983).

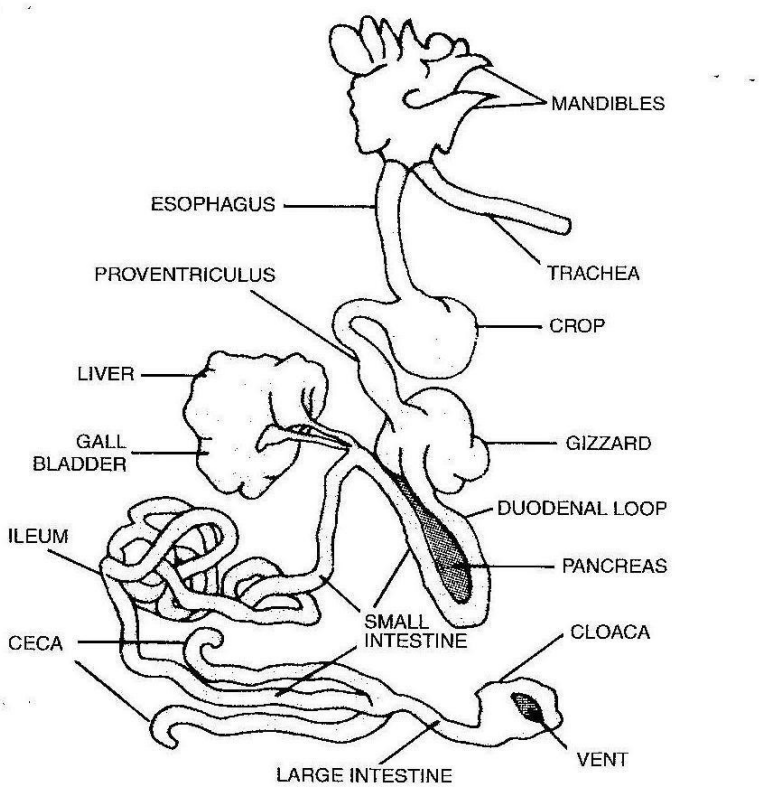


Figure 2.1 The digestive system of the chicken (Bell, 2002).

2.1.4 Small intestines

The small intestines of a chicken are 120 cm long and are divided into three parts namely; the duodenum, jejunum and ileum. The duodenum is the U-shaped section around the pancreas connecting the gizzard to the jejunum. It is in the duodenum where pancreatic fluid and bile are secreted. The junction between the gizzard and the duodenum, also known as the pylorus, acts as a filter that allows only very fine and small particles of chime to enter the duodenum. Panceatic enzymes which are responsible for the hydrolysis of proteins, carbohydrates and fats are secreted into the duodenum (Freeman, 1983; Denbow 2000; Shih and Hsu 2006).

This is where further chemical digestion continues. The duodenum controls the emptying of the gizzard by means of hormonal secretions from the duodenal cells; secretin and cholecystokinin (CCK) which are stimulated by the digesta from the gizzard and its acidity (Freeman, 1983; Denbow, 1994).

Secretin is the hormone produced and secreted by the cells in the crypts of Lieberkuhns, which are invaginations around the villi. Its secretion is stimulated by the low pH of the

chime caused by HCl from the stomach. This hormone stimulates the secretion of sodium bicarbonate from the pancreas to neutralize the acid for the digestive enzymes to function efficiently. It also inhibits the release of gastrin and reduces HCl secretion. Cholecystokinin (CCK) is produced and secreted by I-cells of the small intestine mucosa. Its secretion is also stimulated by fat and protein rich chime that enters the duodenum, inducing the secretion of bile and pancreatic enzymes. Cholecystokinin also inhibits the emptying of the stomach and HCl secretion (Krogdahl, 1985; Denbow, 1994).

The jejunum is the convoluted tube found between the pancreatic loop and Meckel's diverticulum. It is approximately 50 cm long. The chemical processes of digestion and absorption take place in this section. The jejunum is followed by the ileum which is similar in length. The absorption of nutrients occurs in the ileum, whilst undigested materials move through to the caeca and large intestines for further bacterial digestion.

The intestinal walls of poultry are similar to that of mammals but lack Brunner's glands (Aitken, 1958). The mucosa has three layers known as the external layer of smooth muscle which is responsible for the peristaltic contractions. The intermediary is composed of blood vessels and nerves. The inner layer consists of glands and villi. In the ileum, the pH is high to allow for appropriate function of the digestive enzymes and is regulated by the bicarbonate ions from the bile. Nutrients are absorbed through the villi, and carried through the portal blood system to the liver where they are distributed to target organs and tissues (Figure 2.2). The small intestine blood supply is by the mesenteric artery (Larbier and Lecercq, 1994).

2.1.5 Large intestines

Poultry have a very short large intestine (colon, caeca and rectum), but have two large caeca (Denbow, 2000). The undigested food materials (mainly fibre) are fermented in the caeca by the microbial population present in these organs. There is also water, short chain fatty acids, proteins and vitamins absorption that occurs in the caeca, even though most water re-absorption takes place in the colon. There is minimal digestion in the large intestine. The short chain fatty acids are the products of the bacterial fermentation (Vergara *et al.,* 1989; Jozefiak *et al.*, 2004). The movement of chime between the colon and the caeca is controlled by the ileo-caeco-colonic (i.c.c.) junction. This junction helps with the reverse peristalsis where undigested feed material is taken back into the caeca. The anti-peristaltic movement in the large intestines occurs at the same time as peristaltic movement in the ileum exerting

pressure on the i.c.c. junction forcing material into the caeca (Hodgkiss, 1984). The inferior or posterior mesenteric artery takes blood to the large intestines (Larbier and Lecercq, 1994).

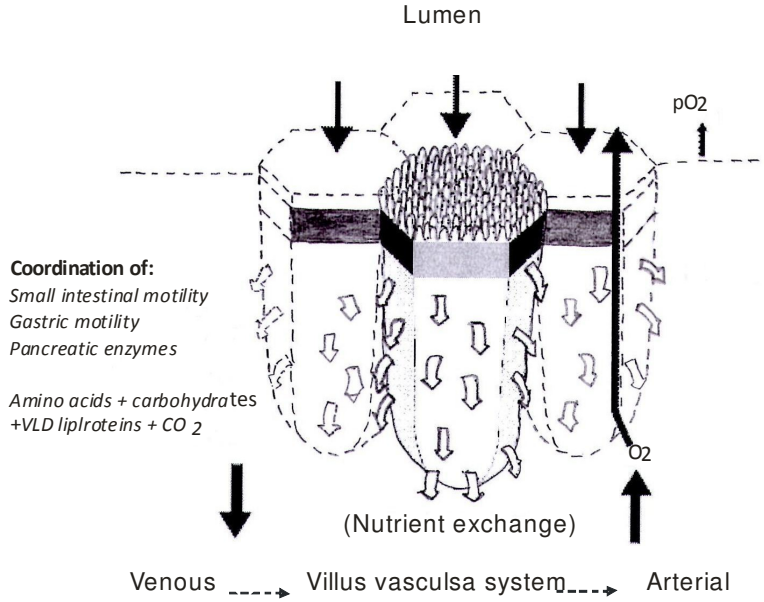


Figure 2.2 The flow of nutrients through the mucosa. (Source: Moran, 2000)

2.1.6 Cloaca

The cloaca is the common posterior opening for the digestive-, urinary- and reproductive-tracts. It is divided into three parts: (1) the coprodeum- which is the dilated part of the rectum where faeces accumulate, (2) the urodeum- where the ureters, the sperm ducts in males and the oviduct in females enter, and (3) the proctodeum- which opens to the outside. Urine from the ureters ascends into the caeca for water electrolytes absorption and gets excreted as white urates paste on the faeces (Denbow, 2000).

2.2 Digestive enzymes

Digestive enzymes are produced in the pancreas and intestinal lumen. Young birds have relatively small reserves for exogenous digestive enzymes (Classen, 1996). It has been reported that pancreatic and intestinal enzyme activity increase with age, although at different rates for specific enzymes (Krogdahl and Sell, 1989; Noy and Sklan, 1995; Jin *et al.,* 1998; Iji

et al., 2001). The secretion of digestive enzymes in the pancreatic tissue and intestinal contents tends to increase with age in turkey poults (Figure 2.3). It is evident that the activity of amylase, protease, trypsin and lipase is influenced by age. Krogdahl and Sell (1989) found that lipase activity was dependent on the fat content of the diet (Figure 2.3). Amylase concentration is reported to increase fast after hatch until eight days of age at which it peaked, and then slowly decreased afterwards (Jin *et al.,* 1998). Even though at hatch the bird has not ingested any food, the intestinal and pancreatic enzymes and nutrient transport abilities are expected because nutrients in the intestines due to absorption of the yolk (Dibner and Richard, 2004).

Due to the composition of cereal grains used in poultry feeds, there are substances or molecules that cannot be digested by the digestive enzymes e.g. cellulose and soluble and insoluble fibre, and can also affect digestion of other molecules e.g. lipids and proteins. Since chickens do not produce enzymes to degrade these molecules the use of exogenous enzymes is crucial in order to increase the digestibility and availability of nutrients to improve poultry performance. There four types of enzymes that are used in poultry and other animal feeds based on cereals and they are known as enzymes that break down. These enzymes can be in the liquid or powder form depending on the processing and form the form of feeds. Silversides and Bedford (1999) also showed that to avoid enzyme inactivation, the enzyme can be in substrate-bound and granulated preparations coated with hydrophobic compounds.

2.3 Nutrient transport in the GIT

In poultry, like in other mammals blood supply to the GIT consists of two systems known the arterial system and venous system. The arterial system comprises of (1) ceolic stem that branches to five arteries which supplies oesophagus and stomach (gizzard and proventriculus), (2) anterior or superior mesenteric artery that supplies small intestine and (3) posterior or inferior mesenteric artery which supplies large intestines (colon, rectum and cloaca). The venous system is comprised of two portal veins. These portal veins branch into a network of capillaries within the liver, from where they emerge and form two hepatic veins that delivers blood into the posterior vena cava (Larbier and Lecercq, 1994).

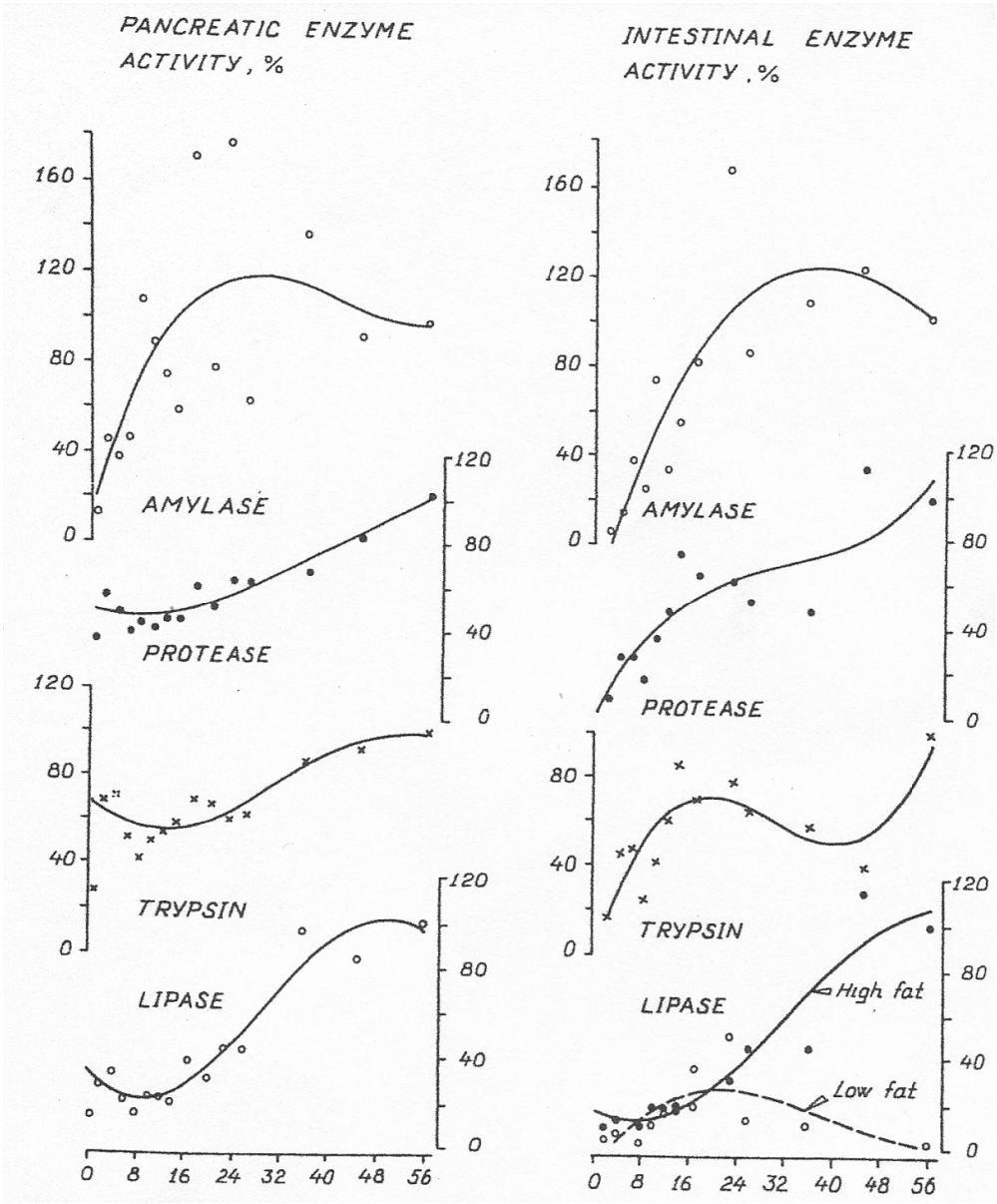


Figure 2.3 Development with age (in days) of digestive enzymes in pancreatic tissue and intestinal contents of poults. The values given as % of that found at 56 d of age, calculated per weight of tissue and dry matter for the pancreas and intestinal contents. (Source: Krogdahl and Sell, 1989)

The motility of the GIT is regulated by the nervous system, endocrine system, composition of the chime and volume of the meal ingested. The peristaltic movements commence from the oesophagus and are transferred down the GIT in slow waves, regulating the passage rate of the digesta (Denbow, 2000).

The nutrients absorption from the intestines is carried out in two routes known as paracellular and transcellular. Three mechanisms of the transcellular route are: (1) passive diffusion-which does not require energy (e.g. monosaccharides, volatile fatty acids); (2) active

transport- which requires energy provided by ATP; (3) facilitated diffusion- which occurs down the concentration gradient, e.g. glucose, amino acids and peptides, electrolytes (calcium and phosphorus).

Pinocytosis also occurs even though is limited to large feed particles. These can be released into the cytoplasm or to the surface of baso-lateral membranes by exocytosis (Larbier and Lecercq, 1994; Denbow, 2000). The endocrine system plays a fundamental role in the digestion process (Table 2.1), as it helps stimulate some secretions that are of importance, e.g. secretin acting on the pancreas to release pancreatic juices which consists of digestive enzymes and ions.

2.4 Composition of alternative cereals used in poultry diets

Cereals are good sources of energy and, to a lesser extent, of protein. However, some cereals seem to have a negative effect on the growth performance of young chickens if they are added at high concentrations in the feeds (Friesen *et al*., 1992). Cereals can be included in different forms; grounded or whole grain, the latter is regarded to be the best method for reducing milling costs (Nahas and Lefrançios, 2001; Yasar, 2003; Wu *et al*., 2004b). It is reported that whole wheat feeds increase the performance in growing birds as compared to ground feeds. Yasar (2003) found that whole wheat, coarse and medium particles significantly increased feed intake, body weight gain and gut size as compared to fine particles up to 21d, and resulted in low ileal viscosity which results in increased passage rate and decreased efficiency of absorption of nutrients.

2.4.1 Anti-nutritional factors in poultry nutrition

The cell wall protects the plant from excessive water loss and maintains the physical integrity of the cell. The structural components of the cell wall are formed by monosaccharides bound together by glycosidic bonds between the hemicetal group of one sugar and the hydroxyl group of the other sugar, β-1→4 or β-1→3 or α-1→6 or α-1→4 or α-1→2 (Figure 2.3a and b). In poultry nutrition there are three categories of NSPs that are of importance; β-glucans found in barley and oats, arabinoxylans or pentosans, found in wheat and rye and raffinose in soya beans (Choct and Annison, 1992a, b; Oscarsson *et al*., 1996; Iji, 1999; Leeson and Summers, 2001). Arabinoxylans and xylans are polymers of arabinose and xylose respectively. Glucans occur as starch in plants, which is a reserve carbohydrate, and is mostly

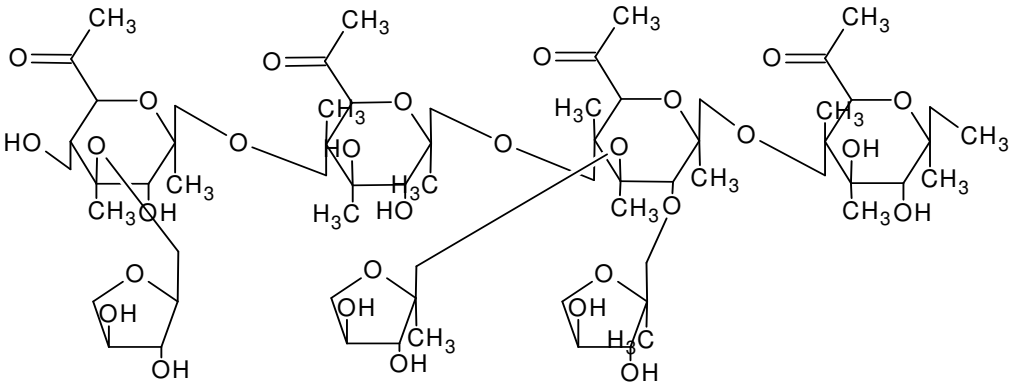
abundant in seeds, fruits, tubers and roots (Choct and Annison, 1992a, b; McDonald *et al*., 2002). Table 2.2 below shows the different NSPs content in cereals commonly used in the poultry diets.

Table 2.1 Gastrointestinal hormones in poultry

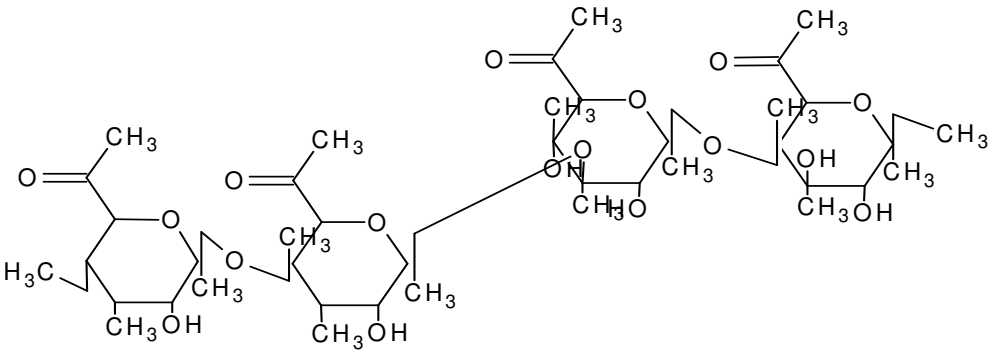
Hormone	**Organ of secretion**	**Stimulation**	**Action**
Gastrin	Proventriculus		Stimulate the secretion of HCL and pepsin
Somatostatin	Proventriculus Gizzard Pancrease Duodenum Ileum	Digesta in the upper tract	Inhibition of secretion of other gut hormones and growth hormone (GH)
Bombesin-like	Proventriculus (as a precursor of por-bombesin)	Nerves of mesenteric plexus	Stimulate gastrin release, pancreatic enzymes and gut motility
Cholecystokinin	Duodenum Jejunum Central Nervous System (CNS)	Digesta in the small intestines	Stimulates contraction of the gall-bladder, pancreatic enzymes, potentiates secretin stimulation, inhibit gastric emptying
Secretin	Duodenum Jejunum	Acid in the duodenum	Stimulates bicarbonate secretion from the pancreas
Vasoactive intestinal peptide (VIP)	Duodenum Jejunum Autonomic nervous system	Vagal stimulation	Same actions as the serectin and glucagon. Act as peripheral hormone and inhibitory neurotransmitter
Pancreatic polypeptide	Pancreas, proventriculus, duodenum	Vagal stimulation	Antagonistic to CCK, regulates carbohydrate and lipid metabolism

Adapted from Freeman, 1983; Denbow, 2000.

As these NSPs are soluble in water, they dissolve in the intestines and they are not affected by digestive enzymes of monogastric animal and young ruminants. This leads to an increase in digesta viscosity, which results in a reduction in growth performance due to a lower digestibility of nutrients (Choct and Annison, 1990; McNab and Smithard, 1992; Smits and Choct, 1996; Leeson and Summers, 2001; Nahas and Lefrançios, 2001; Yu *et al*., 2002; Jozefiak *et al*., 2004). Increased digesta viscosity restricts activity of digestive enzymes and disrupts the nutrient absorptive mucosal surface (Choct et al., 1995; Iji, 1999; Nahas and Lefrançios, 2001).



(a) Arabinoxylans



(b) β-(1→3), (1→4)-D-glucan

Figure 2.4 Major soluble non-starch polysaccharides: (a) arabinoxylans found in rye and wheat. (b) β-(1→3), (1→4)-D-glucan found in barley. Source: Smits and Annison (1996)

Table 2.2 NSPs content (arabinose, arabinoxylans, pentosans, xylose and β-glucans) of barley, rye and wheat

In grain	**NSP**	**Barley**					**Rye**	**wheat**	
Choct and Annison, 1992b	Arabinoxylans							Water extractable Pentosans (WEP (g/kg)	Alkali extractable Pentosans (AEP) (g/kg)
								0.52	0.66
	β-glucans							184.7	40.3
	Pentosans							515	665
Chesson, 2001	Arabinoxylans	**Total grain**: Endosperm- 8.7% Aleurone- 7.9% Bran- 56.5%		**Soluble**: Endosperm- 35.5% Aleurone- 4.4% Bran- 28.2%				**Total grain**: Endosperm- 13.4%, Aleurone-1.1%, Bran-85.5%)	**Soluble**: Endosperm- 64.2%, Aleurone- 2.5%, Bran- 33.3%)
	β-glucans	**Total grain**: Endosperm- 29.5%, Aleurone- 1.8%, Bran-11.6%		**Soluble**: Endosperm- 33.5%, Aleurone- 2.7%, Bran- 52.2%				**Total grain** Endosperm- 39.6%, Aleurone-1.7%, Bran-58.7%	**Soluble:** Endosperm- 53.9%, Aleurone- 2.4%, Bran- 43.7%
Fuente *et al*., 1998	Arabinoxylans	**0 wks**	**3 wks**	**6 wks**	**16 wks**	**32 wks**			
		5.25%	5.69%	5.92%	4.92%	5.55%			
	β-glucan	6.23%	6.60%	6.77%	5.26%	4.73%			
Mathlouthi *et al.,* 2002	Arabinose						5.7 g/kg		
	Xylose						8.7 g/kg		
	Arabinoxylans						14.4 g/kg		
	β-glucans						7.6 g/kg		
Mathlouthi *et al.,* 2003	Arabinose			1.4 g/kg					2.4 g/kg
	Xylose			1.6 g/kg					2.8 g/kg
	Arabinoxylans			3.0 g/kg					7.85 g/kg
Jozefiak *et al.,* 2007	Arabinoxylans						75.79 g/kg		57.57 g/kg
	β-glucans						21.6 g/kg		7.7 g/kg
In diet									
Iji, 1999	Arabinose			14.6 mg/g					9.42 mg/g
									11.4 mg/g
	Xylose			21.2 mg/g					

2.4.2 Wheat

Wheat is commonly used as a source of energy in poultry diets in many countries (Rogel *et al.,* 1987; Garnsworthy *et al.*, 2000; McCracken *et al.*, 2001; Pirgozliev *et al.,* 2003; Kan and Hartnell, 2004). There are different types of wheat depending on the season (winter or spring), seed coat, colour (white or red), and wheat can be classified as being hard or soft. The varieties also determine the nutrient composition, especially the protein content (Rogel *et al.,* 1987; Leeson and Summers, 2001; Carré *et al.,* 2005). As developments in plant breeding occur, colour and time of planting are now changeable. The composition of wheat is found to be more variable than that of any other cereal (McCracken *et al.,* 2001; 2002; Pirgozliev *et al.,* 2003). For example, hard wheat has a protein level varying from 10 -18% CP. This may be due to different growing conditions (McDonald *et al*., 2002; Woolfolk *et al*., 2002). The growing conditions seem to have a large impact on its composition, especially nitrogen content (Woolfolk *et al*., 2002). High temperatures can also be problematic, as they may reduce both the starch and lysine content (Donovan *et al*., 1983; Triboï *et al.,* 2003). Very cold temperatures (e.g. frost) inhibit starch synthesis resulting in small and shrunken kernels (Leeson and Summers, 2001). Hard wheat is ideal to improve pellet quality and durability, but due to this characteristic, it is also preferred for human consumption for the enhancement of baking characteristics. *Durum* wheat is another type of hard wheat that is used in manufacturing of pasta (Leeson and Summers, 2001; Carré *et al.,* 2005).

Structurally, wheat gets its physical hardness from the strong bonds between starch molecules and higher protein content (Rogel *et al.,* 1987; Carré *et al.,* 2005). Carré *et al.* (2002) also showed that soft wheat in pelleted diets for broiler chickens had more digestible starch than hard wheat, which is thought to be due to the interaction between starch and protein molecules in hard wheat.

2.4.3 Rye

Rye is a hardy grain, similar to wheat but more tolerant to frost and drought. It is regarded as a winter cereal (McDonald *et al.,* 2002). It has a highly developed root system that can use less than 20-30 % of water than wheat. Rye is not widely used in feeding animals due to the poor resultant growth rate and feed conversion efficiency, but it is mostly used for human consumption (Bedford and Classen, 1992). The composition of rye is similar to that of wheat, though it has a higher lysine and lower tryptophan content (Annison and Choct, 1991;

Mathlouthi *et al*.,, 2002; McDonald *et al.,* 2002). It has a lesser feeding value, due to the highest concentration of NSPs as compared to wheat and triticale, especially when fed to younger chickens (Mathlouthi *et al*., 2002; Jòzefiak *et al*., 2007).

2.4.4 Barley

Barley is one of the most popular grains used in malting and feeding of farm animals especially in Europe (McNab and Smithard, 1992; Oscarsson *et al.,* 1996; Von Wettstein *et al*., 2003). It has a metabolisable energy of 13.2MJ/kg DM for poultry and its protein content ranges between 11-12% CP (McDonald *et al*., 2002). It also has a very low lipid content, of less than 25g/kg DM (McDonald et al., 2002). Fibre in barley consists of 4-11% arabinoxylans, 3-7% β-glucans and small quantities of cellulose. β-glucans are reported to be the major ANFs in the aluerone and endosperm layers (Wang *et al*., 1992; Almirall *et al*., 1995; Classen, 1996; Oscarssson *et al*., 1996; Yu *et al*., 2002; Von Wettstein *et al*., 2003; Ravindran *et al*., 2007).

Barley can be used hulled or de-hulled. Dehulled barley is reported to have high water soluble dietary fibre content compared to that of hulled barley (Francesch *et al*., 1994; Yu *et al*., 2002). Conventional hulled barley has a high content of insoluble fibre and NSPs compared dehulled barley and other cereals (Ravindran *et al*., 2007). Birds cannot digest β-glucans in barley as their digestive enzyme system does not produce β-glucanase (Yu *et al*., 2002). Barley also contains trypsin inhibitor, which is related to impounding of arginine. However the biggest problem with barley in poultry is the β-glucans content (Almirall *et al*., 1995; Leeson and Summers, 2001), which diminishes the nutritive value (Choct Annison, 1990, 1992; Naha sans Lefrançios, 2001). β-glucans are glucose polymers which contain a mixture of β-1→3 and β-1→4 bonds (Figure 2.3), that makes their structure different from that of cellulose, which has β-1→4 bonds, which makes β-glucans to be more problematic to poultry (Wang *et al*., 1992; Von Wettstein *et al*., 2003).

2.5 Effects of cereals on poultry performance

2.5.1 Wheat

The inclusion of wheat in poultry feeds is not recommended at high levels due to content of water soluble NSPs- arabinoxylans or pentosans (Choct and Annison, 1992a, b; Smits and Annisson, 1996; Iji, 1999; Ravindran *et al.,* 1999; Silversides and Bedford, 1999; Ouhida *et al*., 2000; Cheeson 2001). They have a negative effect on the performance of birds by increasing digesta viscosity which results in the reduced digestibility and absorption of nutrients (Choct and Annison, 1992a, b; Smits and Annisson, 1996; Iji, 1999; Ravindran *et al.,* 1999; Silversides and Bedford, 1999; Ouhida *et al*., 2000; Cheeson 2001; Preston *et al*., 2001).

Increased digesta viscosity also leads to reduced body weight, feed conversion ratio, feed intake, egg production, egg weight and mass and increase incidences of wet litter due to sticky droppings which results in dirty eggs in laying hens (Francesch *et al*., 1995; Pan *et al*., 1998; Mathlouthi *et al*., 2003; Costa *et al*., 2008). However, Hetland *et al*., (2004), reported that if birds are fed low energy feed regardless of increased viscosity they will increase their consumption in an attempt to meet their energy requirements.

Arabinoxylans or pentosans inhibit the digestion of starch, protein and fat (Choct and Annison, 1992b). These effects can be improved by the addition of exogenous enzymes (xylanase or β-glucanase) or water treatment of cereals whereby NSPs are extracted or by antibiotic supplementation, as monogastric animals don't posses enzymes that can digest NSPs (Annison and Choct, 1991; Choct and Annison 1992a, b). Antibiotic are reported to reduce the proliferation of pathogenic bacteria and increased fermentation in the hint gut which is associated with increased NSPs (Choct and Annison 1992a, b; Yang *et al*., 2009).

2.5.2 Rye

Rye is regarded as the least palatable of all cereals and so reduces feed intake and can also cause digestive problems. Pentosans in rye are reported to not only depress growth, but also digestibility of fat and amino acids (Annison and Choct, 1991). Annison and Choct, (1991) reported that due to high pentosans content, rye should be fed at low quantities as it can be less digestible. Like wheat, rye should be crushed or grounded coarsely when feeding animals. It is not commonly used in poultry diets due to the depressed appetite and, therefore, slow growth found in chicks (Bedford and Classen, 1992), increase intestinal viscoty

(Mathlouthi *et al*., 2002). It can also be very dangerous if contaminated with a fungus known as ergot, which is the common effect on rye, though it can be found in other cereals such as wheat, barley or oats. Ergot produces alkaloids and can reduce blood flow, which results in coldness and insensitivity of the extremities (McDonald *et al*., 2002). As a grain or its by-products, rye is mostly used in feeding ruminants and pigs. For poultry feeding, the awns need to be removed, as they tend to decrease nutrient digestibility (McDonald *et al*., 2002).

2.5.3 Barley

Barley is not commonly used in poultry diets due to its lower metabolisable energy (ME) and high fibre content, which results in limited nutrient uptake, slow initial broiler growth and sticky droppings on the vent (Von Wettstein et al.,2003). This is reported to be due to high content of β-glucans (Francesch *et al*., 1994; 1995; Almirall *et al*., 1995; Scott *et al.,* 1998a; Yu *et al*., 2002; Von Wettstein *et al*., 2003; Ravindran *et al*., 2007).the genetic factors and growth condtions of the barley determine the concentration of β-glucans (Friesen *et al*., 1992). The inability of birds to digest β-glucans leads to increase in digesta viscosity (Choct and Annison, 1990; Nahas and Lefraçios, 2001), which disrupts the nutrient absorption in the GIT (Choct *et al*., 1995; Iji, 1999; Nahas and Lefraçios, 2001). The rate of diffusion to the intestinal microvilli increases, with the decrease in digesta viscosity, and this is a function of the thickness of the unstirred boundary layer. This layer can be indirectly affected by the motility of the digesta, which also affect the rate of absorption of all nutrients (Choct *et al*., 1995; Iji, 1999; Hetland *et al*., 2004).

The nutritive value of barley can be improved by dehulling and or the addition of exogenous enzymes in barley-based diets (Francesch *et al.,* 1994; Almirall *et al*., 1995; Scott *et al*., 1998a; Yu *et al*., 2002; Ravindran *et al*., 2007). Several authors reported that removing hulls reduced the fibre content and significantly reduced viscosity which is the major problem with barley diets (Scott *et al*., 1998a; Yu *et al*., 2002; Ravindran *et al*., 2007). The use of exogenous β-glucanase in barley diets increased the digestibility and improved the performance in chickens (Francesch *et al.,* 1994; Almirall *et al*., 1995; Scott *et al*., 1998a; Yu *et al*., 2002; Ravindran *et al*., 2007).

2.6 Use of enzymes in poultry feeds

Enzymes that can be added to feeds are known as exogenous enzyme while ones manufactured by animals are endogenous enzymes (Bedford, 2008). In the past decades there have been numerous studies on the use of exogenous enzymes in poultry feeds. Exogenous enzymes increase the digestibility of complex molecules especially in young animals, which do not have a well developed enzyme profile. These enzymes are also useful to lower the cost of feed production by increasing nutrient availability in the feed and to reduce the potential environmental impact of animal manure used in the fields (Leeson and summers, 2001; Silversides *et al.* 2006).

These enzymes should be active at the pH level of the specific portion of the GIT of the animal where the substrates become available. During feed preparation, these enzymes can be post-processing to avoid being destroyed by the conditions used e.g. high temperatures during pelleting. The inclusion of various exogenous enzymes improves the availability of plant polysaccharides, oils and proteins which are protected by the cell wall structures or bound up in chemical form (e.g. phosphorus as phytic acid) and cannot be broken down by the endogenous enzymes (Freeman, 1983; Bedford and Schulze, 1998). They may also destroy or inhibit materials that can interfere with the absorption and utilization of nutrients (Bedford and Schulze, 1998; Iji 1999). These enzymes are not only beneficial to the animal's health but also to humans and plants by reducing excretion of harmful substances e.g. nitrogen and phosphorus that may pollute the environment (Freeman, 1983; Silversides et al., 2006).

The undigested NSPs bypass the small intestines and undergo fermentation in the caeca and colon to produce short chain fatty acids (Choct *et al.,* 1999; Silva and Smithard, 2002; Engberg *et al*., 2004; Wang *et al*., 2005; Moran, 2006). These volatile fatty acids provide energy and control development of the gut flora (Bedford, 2008). The use of enzymes is reported to increase the fermentative bacteria count in the caeca and reduce the build-up of viscous digesta in the small intestines (Choct *et al*., 1999). This restores the normal function of small intestines and improves efficiency utilization of nutrients by the bird (Choct *et al*., 1999; Engberg *et al*., 2004; Wang *et al*., 2005).

Several authors have shown that the use of exogenous enzymes in NSPs-rich diets is very beneficial as they can help hydrolyse NSPs, reduce digesta viscosity, and most importantly improve nutrient absorption, growth and performance of the birds (Smits and Annison, 1996; Iji, 1999, Bedford, 2000; Hetland et al., 2004; Wang et al., 2005). Bedford (2000) reported

that exogenous enzymes also reduce the variability in nutritive values amongst feed ingredients, which improve the precision in feed formulations. These enzymes are produce from a variety of bacteria and fungi including aerobes, anaerobes, mesophiles, thermophiles and extremophiles. The most commonly used carbohydrases in poultry are xylanase and β-glucanase.

2.7 Effects of fibre degrading enzymes on poultry performance

2.7.1 β-glucanase

This is carbohydrases that degrade β-glucans found in the cereals grains of barley and oats into monosaccharides. Because these cereals have high levels of β-glucans, they are not commonly used in poultry feed (Von Wettstein *et al*., 2003). β-glucanase can be endo-acting fungal cellulases or bacterial β-glucanase (Francesch and Perez-Vendrell, 1997; Von Wettstein *et al*., 2003). It is used in poultry feeds to reduce digesta viscosity and improve bird performance, which is more pronounced in young chickens (Friesen *et al*., 1992; Esteve-Garcia *et al*., 1997; Ouhida *et al*., 2000; Mathlouthi *et al.,* 2003). Sieo *et al.* (2005) reported β-glucanase to have caused improved feed passage rate and intestinal characteristics such as intestinal viscosity, and reduced weight and length of intestinal parts.

2.7.1.1 β-glucanase studies in broilers

In order to overcome the problems observed when feeding barley-based diets to young birds, exogenous enzymes have been developed. Exogenous enzymes in poultry diets have been researched over decades and seem to have given positive results in regard to the growth performances of broiler chickens (Choct and Annison 1992a, b; Francesch *et al.* 1994; Choct *et al.,* 1995; Esteve-Garcia *et al*. 1997; Choct *et al*., 1999; Wu *et al.,* 2004a, b; Wu and Ravindran 2004; Cowieson *et al.,* 2005, 2006; Wang *et al*., 2005).

β-glucanase can be added to barley diets fed to poultry to alleviate problems caused by the β-glucans, especially in young birds (Francesch *et al.,* 1994; Almirall *et al.,* 1995; Fuente *et al*., 1998). Almirall *et al.,* (1995) found that β-glucanase had more effect in young broiler chicks as compare to mature birds (1 y old roosters). This is thought to be due to the fact that the GIT of mature birds can withstand the negative effects caused by the increased viscosity. Francesch *et al*., (1994) reported that when β-glucanase was added at 150mg/kg to barley

diets (three different cultivars: Beka, Barbarrosa and Albacete) fed to broilers (0 to 42 d) viscosity was reduced and nutrient digestibility increased. This significantly improved feed conversion ratio and weight gain (Francesch *et al*., 1994; Almirall *et al*., 1995; Esteve-Garcia *et al*., 1997). Almirall et al. (1995) showed that feed intake in young chicks was also increased when barley diets were supplemented with β-glucanase, this was thought to be due to reduced digesta passage rate.

Different cereal cultivars have different content levels of NSPs. This could also determine the amount of enzyme to be added to specific diets and also the amount of cereal to be used in the diet. Scott *et al*. (1998b) reported that both hulled and de-hullled barley had negative effect on the growth performance of broiler as expected, but these effects were improved by the addition of exogenous β-glucanase in the diets (enzyme was included at 0.15% of the total ration). On the contrary, Nahas and Lefrançios (2001) and Yu *et al*. (2002) did not find any positive results with the addition of β-glucanase when using diets based on whole and de-hulled barley, respectively. Nahas and Lefrançios (2001) reported that enzyme supplementation in whole barley diets increased feed intake (FI) while there was no effect on feed conversion ratio (FCR) in growing birds. This was thought to be due to the rainy conditions under which barley was grown and maturing time, which are known to lower the β-glucans content. According to Yu *et al.* (2002) although de-hulled barley contains high levels of β-glucans content, it has lower crude fibre which reduce the effects of in digest viscosity in the GIT and increase FI and weight gain. However, in this trail, supplementation with β-glucanase did not have an effect on the overall broiler performance.

Although there were contradictory results with regards to β-glucanase effects on FI, nutrient utilization of barley diets was found to be affected by different factors, e. g the age of the animal, barley cultivars, storage time of barley, inclusion levels and feed processing. All these factors are reported to have an interaction with β-glucanase action (Choct and Annison, 1992a, b; Francesch *et al*., 1994; Choct *et al*., 1995; Esteve-Garcia *et al*., 1997; Villamide *et al*., 1997; Choct *et al*., 1999; Wu *et al*., 2004a, b; Wu and Ravindran, 2004; Cowieson *et al*., 2005; Wang *et al*., 2005).

2.7.1.2 β-glucanase studies in laying hens

Dunstan, (1973) reported an increase in egg weight, FI and FCR when unsupplemented barley replaced wheat and oats in laying hens diets. Though this was the case, barley was

known to have low metabolizable energy as compare to wheat (11.5 vs. 13.4 MJ/kg). Increased FI might have been due to the birds trying to increase the energy intake from barley diets. Brufau *et al*. (1994) and Francesch *et al.* (1995) found an increase in egg weight and improved FCR when laying hens were fed increasing doses of β-glucanase to barley diets during the start of the laying period. Roberts and Choct (2006) did not find any effect on egg weight when laying hens were fed barley diets supplemented with four different commercial enzymes (Activity (U/g)); Treatment 1; amylase 100, β-glucanase 430, xylanase 500; Treatment 2; amylase 1400, β-glucanase 6000, cellulase 3500, protease 450; Treatment 3; B-glucanase 21 400, xylanase 37 700, cellulase 10 800, protease 177; Treatment 4; β-glucanse 210; xylanase 260. The lack of response might have been due to the age of the hens (37 to 42 wk), as in most studies egg weight was only affected at the start of the laying period (Brufau *et al*., 1994; Francesch *et al*., 1995).Wyatt and Goodman (1993) also showed that enzyme supplementation might be more efficient at peak production as the hens' requirements are at the highest to maintain body growth and high production. There were no enzyme effects observed on egg production and FI (Brufau *et al*., 1994; Francesch *et al*., 1995). Wyatt and Goodman (1993) reported significant improvement of 4% in egg production with addition of β-glucanase to barley diets. Lázaro *et al.* (2003) also observed significant increase in egg production at the enzyme dose of 250 mg/kg in barley diets.

As shown in previous sections, barley is reported to increase water consumption which leads to an increase in the incident of wet litter that result in dirty eggs. Brufau *et al.* (1994) reported a significant reduction in water: feed consumption ratio (10-15.7%) with increasing doses of enzyme. Francesch *et al.* (1995) also reported an improved effect of enzyme supplementation on water: feed consumption ratio and reduction in the percentage dirty eggs. In agreement, when Lázaro *et al*. (2003) compared maize diets with wheat, rye and barley diets, rye produced the highest digesta viscosity followed by barley then wheat (111.9, 33.2 and 11.1 centipoise) respectively. Supplementation with an enzyme complex (β-glucanase and xylanase) reduced viscosity more effectively in barley diets than in wheat and rye diets and thus significantly reduced incidence of dirty eggs. Even though laying hens have been reported to survive the negative effect of NSPs (Dustan, 1973; Wyatt and Goodman, 1993), reports have shown that enzyme supplementation can help improve egg production (Wyatt and Goodman, 1993; Lázaro *et al*., 2003), egg weights (Brufau *et al*., 1994; Francesch *et al*., 1995), and quality of eggs (egg cleanliness) (Wyatt and Goodman, 1993; Brufau *et al*., 1994; Francesch *et* al., 1995; Lázaro *et al*., 2003).

2.7.2 Xylanase

Xylanase is an enzyme that digests xylans and xylose, which are the NSPs found in wheat and rye grain cell walls. As shown in the previous sections, plant cell walls tend to be a problem for birds to digest due to their structural components, especially for monogastrics and young animals (Annison and Choct, 1991). To overcome this problem when using wheat or rye, exogenous xylanase can be added to the diets. Xylanase addition increases AME of wheat when broilers are fed on low metabolisable wheat diets (Choct *et al*., 1995; Steenfeldt *et al*., 1998a; Wu *et al*., 2004a). This also increased the digestibility of proteins, fat and starch as their digestibility is associated with metabolisable energy (Choct *et al*., 1995; Bedford and Schulze 1998; Steenfeldt *et al*., 1998a; Wu *et al*., 2004a).

Xylanase help deconstruct the plant's structural material by breaking down hemicellulose, which is a major component of the plants' cell wall. This increases the exposure of nutrients to the endogenous enzymes and increases digestion and absorption in the small intestines (Annison and Choct, 1991; Choct *et al*., 1995; Wu *et al*., 2004a). Most herbivores produce and use xylanase to digest these compounds (Leeson and Summers, 2001). Xylanase reduced the digesta viscosity of birds fed on wheat-based diets and so improved growth rate and FCR (Steenfeldt *et al*., 1998a; Silversides and Bedford, 1999; Cowieson *et al*., 2005). Choct et al. (1999) reported that the addition of xylanase also reduced microbial proliferation and activity in the ileal digesta by modifying the microflora, competition for nutrients and reducing fermentation.

2.7.2.1 Xylanase studies in broiler chickens

Xylanase was reported to significantly improve broiler performance by reducing digesta viscosity, improve AME by 24. 3 %, FCR improved by 34 % (Choct *et al*., 1995), and 6%-8% (Steenfeldt *et al*., 1998a). In contrast, Wang et al. (2005) did not observe any improvements in AME with the increasing levels of enzyme from 0 to 1000 mg/kg feed. But the overall growth performance was improved. Choct and Annison (1992a, b) reported that pentosans in wheat and rye inhibit digestion and absorption of nutrients, and so negatively affect the performance. Body weight gain was improved by addition of exogenous xylanase in wheat- (Engberg *et al*., 2004; Wu and Ravindran 2004; Wu *et al*., 2004b; Wang *et al*., 2005) or rye- based diets (Silva and Smithard, 2002). Cowieson et al. (2005) investigated the effects of pelleting temperature and addition of xylanase in wheat-based diets. The

researchers found that exogenous xylanase improved BWG and FCR in birds fed on diets pelleted at 85 or 90^0C as compared to diets pelleted at 80^0C.

Pentosans increase intestinal digesta viscosity, lower the passage rate of the digesta and increase the microbial count (Choct and Annison, 1992a; b; Choct *et al*., 1995; Steenfeldt *et al.*, 1998a; Wang *et al*., 2005). Exogenous xylanase supplementation alleviated this problem and increased nutrient digestibility (Choct *et al*., 1995; Esteve-Gaarcia *et al*., 1997; Steenfeldt *et al*., 1998a; Marron *et al*., 2001; Wang *et al*., 2005). Wu et al. (2004b) reported a significant reduction in viscosity with enzyme supplementation irrespective of the wheat form used (whole wheat versus ground wheat), with improvements being more pronounced in post-pelleting inclusion of whole wheat. Engberg *et al*. (2004) showed that digesta viscosity was highly reduced in whole wheat diets with xylanase supplemented than in pelleted diets (P = 0.042).

2.7.2.2 Xylanase studies in laying hens

It has been reported that NSPs have lesser effects in mature birds than in young birds (Choct and Annison 1992b); Wyatt and Goodman, 1993). Campbell and Campbell (1989) showed that rye can be substituted for wheat in laying hens diets as long as the inclusion level in the diets are kept below 39%, as higher levels depress performance. Pan *et al.* (1998) suggested that mature birds might still have a limited capacity to tolerate the effect of NSPs as the addition of an enzyme combination (Cellulase: 11.080 m/g; Glucanase: 27.170m/g; Xylanase: 36. 940m/g) improved egg performance (to 90 % and 87%) for wheat and rye, respectively. There was also significant improvement in BWG (98 to 126 g), FCR (2.20 to 2.14) and AME_n (2.880 to 3.059 Kcal/kg) in hens fed on wheat and rye diets. This was thought to be due to more energy being available to the birds for production. In agreement, Lázaro *et al*., (2003) reported an improvement in egg production and FCR when an enzyme was added to wheat-, barley-, and rye-based diets. The enzyme was added at (0, 250, 1250 and 2500 mg/kg) to 500 g/kg of wheat or barley and 350 g/kg of rye. Mathlouthi *et al.* (2003) also observed and improvement in BWG (86 g) and FCR with addition of enzyme in wheat/barley- based diets ($P \leq 0.05$). Costa *et al*. (2008) observed improved egg production, FCR and egg mass with addition of enzyme. Jaroni *et al.* (1999) did not find any effect on egg production, BWG or FCR. However, egg weight and mass were significantly improved when enzyme was supplemented to wheat diets. In agreement, Silversides *et al*., (2006) did

not observe any enzyme effect on the performance of two strain of laying hens (ISA- Brown and ISA-White hens).

Non starch polysaccharides are known for increased digesta viscosity in poultry. In laying hens this affect the quality of eggs by increasing the incidence of dirty eggs (Wyatt and Goodman, 1993). Lázaro *et al.* (2003) observed a significant reduction in digesta viscosity in wheat, barley and rye diets with enzyme supplementation. Reduction in viscosity was achieved with lowest level of enzyme addition (250 mg/kg) in wheat- and barley- based diets while for the rye diets it was at the highest level (2500 mg/kg). This response was supported by improved nutrient digestibility, with AME_n being increased by 2.5 %. Exogenous enzyme is reported to increase AME_n of feeds and so iprove the performance in poultry. Even though there are specific levels of enzyme supplementation and inclusion level of cereals, as different researchers use different levels and methods enzyme application.

2.8 DISCUSSION

Non starch polysaccharides in the feed have been shown to affect performance and the nutritive value of cereals in poultry. The addition of exogenous enzyme has been reported to improve the negative effects observed when feeding certain cereals. Even though enzyme addition in cereals diets improves efficiency utilization, there are still many factors that need to be taken into consideration when formulating poultry feeds. The age of the bird, the type of the cultivar, the time of harvesting, storage type, inclusion levels of cereals in the diets, supplemental levels of exogenous enzymes in the diets and also the form of feeds. The health of the digestive system is one of the crucial aspects when feeding animals. Any feed ingredient that can damage or change the morphology of the digestive system should be avoided or improved by processing. The composition of the raw materials used in animal feed in this case chickens should be studied carefully to avoid any disturbances or damage. This can also affect the cost of production severely.

Different researchers have used different levels of addition of exogenous enzymes, however, reports in the literature do not agree on a specific dose for the optimum inclusion of these enzymes. This might be due to wide number of factors, such as type of the cereal used, feed processing, heating after enzyme addition and the development of new enzymes. The present study was designed to test a new enzyme preparation for broilers and laying hens fed wheat-, barley- and rye- based diets.

CHAPTER 3

The Use of Exogenous Enzymes on Wheat-Barley Based Diets to Improve Performance Broiler Birds

3.1 Introduction

Although maize is the most commonly used cereal in poultry feed around the world (Wang *et al*., 2005), wheat, barley and rye are also used in some parts of the world. However, their inclusion in the diets for young chickens has shown a negative effect on performance. These negative effects are reported to be due to the content on ANFs or nutrient diluents or soluble fibre present in the grains (Chesson, 2001; Lázaro *et al*., 2003; Hetland *et al*., 2004).

These ANFs are carbohydrates and structural parts of grain's cell wall. The cell walls of grains of wheat and barley contain high levels of arabinoxylans and β-glucans, respectively (Choct and Annison, 1990; Annison and Choct, 1991).They are known to reduce growth performance and digestibility of nutrients in poultry (Choct and Annison, 1990; Annison and Choct, 1991; Smits and Annison, 1996; Iji, 1999; Chesson, 200; Leeson ans Summers, 2001; Lázaro *et al*., 2004; Hetland *et al*., 2004; Wang *et al*., 2005). These ANFs cannot be digested by bird's digestive enzymes and tend to form a viscous environment in the small intestines, decreasing the contact between the digestive enzymes and the substrates. As a consequence, the energy value of the diet is reduced especially in chicks (Choct and Annison, 1990; Annison and Choct 1991; Lázaro *et al*., 2004); Nutrient digestion, absorption (Smits and Annisonm 1996) and growth performance (Smits and Annisonm 1996; Iji, 1999; Chesson, 200; Leeson ans Summers, 2001; Lázaro *et al*., 2004; Hetland *et al*., 2004; Wang *et al*., 2005) are also depressed.

In addition to these effects, Choct *et al*., (1999) reported that NSPs reduce digesta passage rate which leads to reduced feed intake, an increase in proliferation of bacteria in the GIT (especially fermentative microflora) which change the environment within the GIT as well as its morphology, which may negatively affect digestive enzyme activity (Choct *et al*., 1999; Iji, 1999; Wu and Ravindran, 2004; Wu *et al*., 2004 a, b; Wang *et al*. 2005).

There have been numerous studies on the effects associated with the use of exogenous enzymes such as β-glucanase and xylanase in wheat- and barley- based diets in poultry feeds. The enzymes are said to hydrolyse NSPs, reduce digesta viscosity and improve nutrient absorption (Cheeson, 2001; Wang *et al*., 2005). The feed companies have been improving the efficacy of enzymes used to hydrolyse these NSPs in the past decade, and with advances in

molecular technology these continue to improve constantly. Due to the high specificity of enzymes for substrates, these newly developed enzymes need to be tested before they can be released to the market.

The objective of this study was to evaluate a novel combination of xylanase-glucanase for wheat- , barley- and rye- based broiler diets offered to 35 days of age. Additionally this trial was used to investigate the potential of enzyme addition on the morphology of the small intestine mucosa and digestive organs (liver and gizzard).

3.2 Materials and methods

A total of 2880 day old broilers (Ross 788; Ross Breeders, South Africa) were sexed and placed in an environmentally controlled house. Sixty birds were allocated in each of 48 pens, containing soft wood shavings, two feeders and nipple drinkers. For the first four days, chick bell drinkers were made available. Food and water were provided *ad libitum*. Temperature, ventilation and lighting programmes (uniform light of 48 hours, and then were given 16L; 8D until age 35days) followed the instructions of the primary breeder. The experiment was divided into two phases: a starter (1 to 21d) and grower phase (22 to 35 d), where birds were provided different feeds (Table 3.1).

Birds were randomly assigned to one of four experimental treatments: a control diet (wheat-, barley- and rye diet) with no addition of enzyme product, and diets containing 50, 100 and 200 g of the enzyme preparation /tonne of feed (multi enzyme blend of xylanase and β-glucanase, as T1 to T4 respectively. There were 12 replicates per treatment and 60 birds per replicate. Diets were free of antibiotic growth promoters and were provided in a mash form.

Birds were weighed at 1 day old and thereafter 32 birds per pen were weighed weekly (full crops). Food intake was recorded weekly as the difference of food offered and the amount that remained in the troughs at end of the week. Mortalities were recorded daily and weighed for the adjustment of feed conversion ratio.

At 35 d of age, 10 birds per treatment were killed by cervical dislocation. The abdominal cavity was opened and the GIT from proventriculus to caeca was carefully excised for further evaluation of morphological changes.

Table 3.1 Ingredients and nutrient composition of the diets as analysed

Ingredient (% as fed)	Starter (0-20 days)	Grower (22-42 days)
Wheat	36.2	37.7
Barley	20.0	20.0
Rye	8.00	8.00
Soyabean meal 50	27.7	24.2
Soyabean oil	4.00	6.06
L-Lysine	0.29	0.20
DL- Methionine	0.30	0.24
L-Threonine	0.05	0.06
Salt	0.36	0.34
Limestone	0.81	0.56
Di-calcium Phosphate	1.93	2.14
Vitamin/Mineral premix	0.50	0.50
Calculated provision (% as fed)		
ME (MJ/kg)	12.4	13.0
Crude Protein	21.1	19.5
Crude Fat	5.47	7.54
Crude Fibre	2.69	2.60
Lysine	1.30	1.13
Methionine	0.59	0.51
Methionine + Cystine	0.95	0.85
Tryptophan	0.80	0.75
Calcium	0.90	0.85
Total Phosphorus	0.75	0.77
Digestible Phosphorus	0.38	0.40
Na	0.18	0.17
K	0.88	0.82

Premix Composition for different growth stages (inclusion 5kg/ ton): Starter (0-21days): Vit. A 12.0 MIU; Vit. D3 5.0 MIU; Vit E75.0 g; Cu 20.0 g; Zn 100.0 g; Mn 100.0 g; Choline Chloride 250.0 g; Ca 29.10% and Ash 89.88%. **Grower (22-42 days):** Vit. A 12.0 MIU; Vit. D3 5.0 MIU; Vit. E 75.0 g; Cu 20.0g; Zn 80.0g; Mn 80.0g; Choline Chloride 200.0 g; Ca 30.50% and Ash 91.70%.

Furthermore, the gizzards and livers were collected and weighed. Intestinal samples were taken from duodenum (pancreatic loop), jejunum (from pancreatic loop to Meckels's diverticulum) and ileum (from Meckel's diverticulum to ileocaecal junction). Five 1 cm

samples were cut from each section and embedded using the standard procedure for resin embedding using Epon/ Araldite at the Electron Microscope Centre, University of KwaZulu-Natal.

The experiment had a factorial design with main effects of sex and enzyme addition. Data were analysed by a two way analysis of variance. Standard curve regression analyses were performed where appropriate using Genstat 11th edition.

3.3 Results

Enzyme addition had a significant effect on both male and female BW at each age measured (Table 3.2). There was no enzyme × sex interaction, but sex had a significant effect on BW at each age measured except at 21 d. Feed conversion ratio was significantly reduced from 21 to 35 days by addition of enzyme (P<0.001), with a large improvement in FCR being observed with addition of enzyme at 100 g/ton. Whilst increasing the enzyme dosage did not produce further improvement in this parameter (Figure 3.1). There was no due to sex on FCR to 35 days of age (P>0.05).

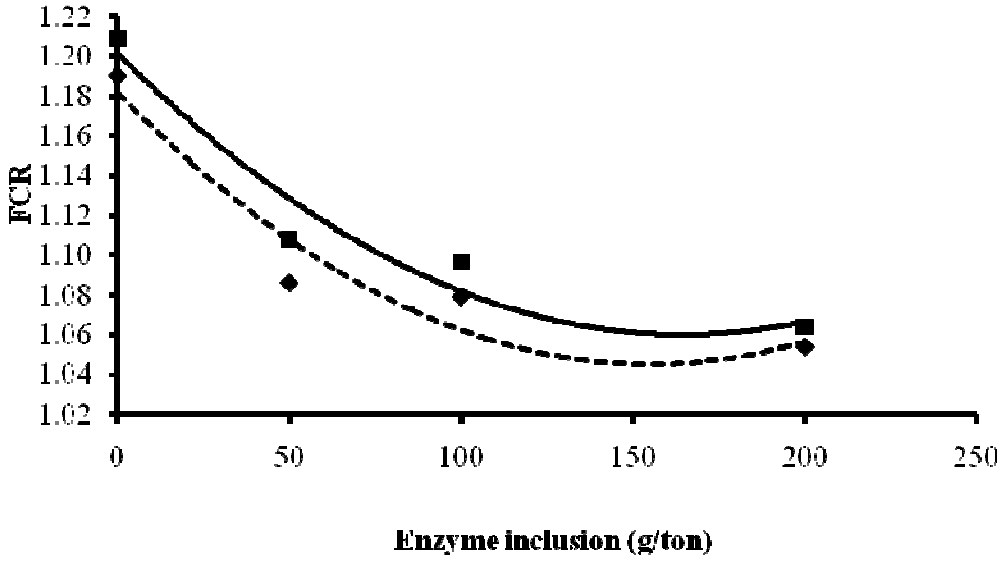


Figure 3.1 The effect of exogenous enzyme addition on feed conversion ratio (FCR) up to 35 d of age in male (■) and female (♦) broilers fed on wheat-barley based diets. Males = $0.0132x^2 - 0.001x + 1.201$, $R^2 = 0.788$. Females = $0.009x^2 - 0.00066x + 1.181$, $R^2 = 0.823$

Table 3.2 The effect of enzyme addition to wheat-barley diets on mean body weight (BW), food intake (FI), feed conversion ratio (FCR) on both male (M) and female (F) from 7 to 35 days of age.

Age (d) body weight										
	7		14		21		28		35	
	F	M	F	M	F	M	F	M	F	M
0	129	128	322	318	637	652	1070	1090	1565	1649
50	138	127	356	338	724	733	1204	1260	1757	1873
100	141	131	363	350	737	743	1226	1299	1741	1917
200	145	132	379	359	763	766	1290	1346	1823	1943
p										
Enzyme	<.001		<.001		<.001		<.001		< 0.001	
Sex	<.001		<.001		0.15		<.001		< 0.001	
E*S	0.03		0.30		0.88		0.25		0.03	
SED	2.87		6.51		11.0		18.4		21.30	
LSD	5.79		13.2		22.3		37.1		43.1	
Age (d) Feed intake										
	7		14		21		28		35	
	F	M	F	M	F	M	F	M	F	M
0	18.8	18.7	48.1	47.1	75.8	79.0	108	117	144	155
50	19.8	18.4	51.1	48.1	82.2	83.5	117	124	147	161
100	19.9	18.3	48.9	49.3	81.6	83.7	118	128	145	164
200	19.5	18.2	50.5	49.6	83.9	85.4	123	132	148	164
p										
Enzyme	0.787		0.058		< .001		< .001		0.008	
Sex	< .001		0.093		0.043		< .001		< .001	
E*S	0.36		0.36		0.90		0.83		0.27	
SED	0.63		1.30		1.94		2.44		2.74	
LSD	1.27		2.63		3.91		4.92		5.54	
Age (d) Feed conversion ratio										
	7		14		21		28		35	
	F	M	F	M	F	M	F	M	F	M
0	1.92	1.90	1.94	1.92	1.55	1.56	1.29	1.37	1.19	1.21
50	1.89	1.89	1.87	1.83	1.48	1.48	1.26	1.27	1.09	1.11
100	1.85	1.84	1.75	1.84	1.44	1.46	1.25	1.25	1.08	1.10
200	1.76	1.80	1.74	1.77	1.42	1.43	1.23	1.23	1.05	1.06
P										
Enzyme	0.012		0.002		< .001		< .001		< .001	
Sex	0.90		0.66		0.76		0.11		0.15	
E*S	0.92		0.48		0.97		0.17		0.99	
SED	0.06		0.06		0.04		0.03		0.02	
LSD	0.12		0.13		0.09		0.06		0.05	

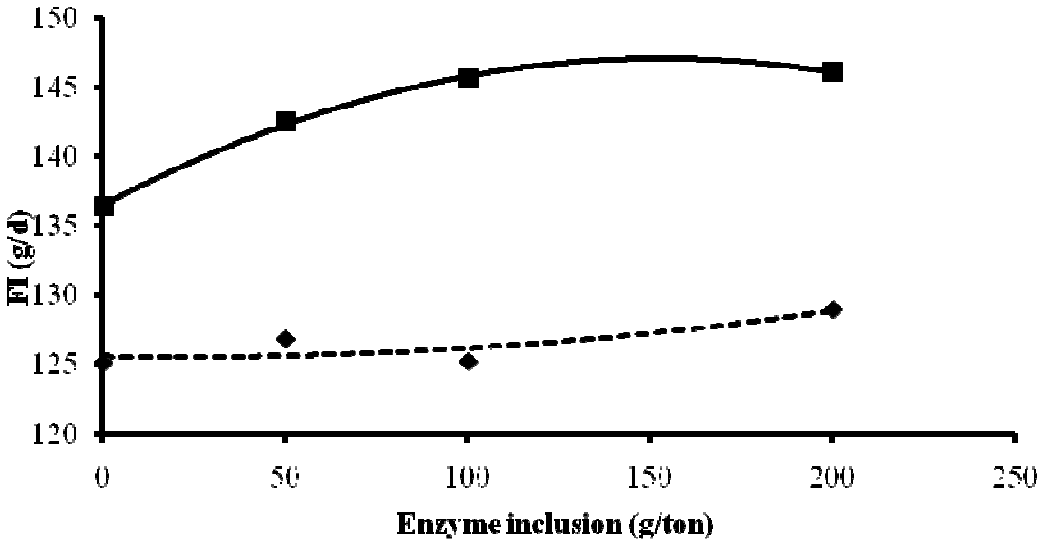


Figure 3.2 The effect of enzyme addition on body weight gain (BWG) to 35 days of age in male (■) and female (♦) broilers fed wheat and barley based diets**.** Males = $-0.00012x^2 + 0.389x + 138.7$, $R^2=0.768$. Females = $0.00026x^2 - 0.0881x + 125.5$, $R^2=0.742$.

Feed intake to 35 d of age followed a similar shape (Figure 3.2), with a sharp improvement from 0 to 100 g/ton in male broilers. In the case of female broilers there was a tendency for linear improvement in FI due to the addition of enzyme ($P>0.05$). The addition of this particular combination of exogenous enzymes had a significant ($P<0.05$) interaction between enzyme and sex for BWG to 35 d of age (Table 3.3). This trend was observed in both males and females. Birds fed diets supplemented at the level of 200g/ton were the heaviest in both sexes at 35 d of age (Figure 3.3).

Table 3.3 The effect of exogenous enzyme addition to wheat- barley based diets on body weight gain (BWG) and food intake (FI) for male (M) and female (F) broilers to 35 days of age.

	BWG to 35 d		FI to 35 d	
Enzyme	F	M	F	M
0	1436	1522	862	931
50	1620	1746	879	985
100	1599	1786	862	994
200	1678	1811	888	980
p				
Enzyme	<.001		0.06	
Sex	<.001		<.001	
E*S	<.010		0.24	
SED	20.4		21.6	

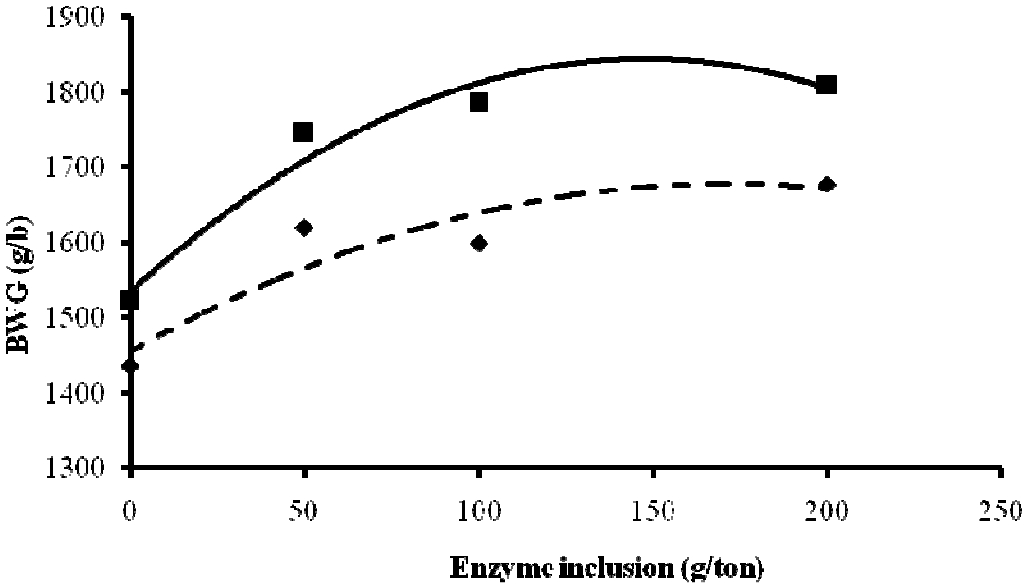


Figure 3.3 The effect of enzyme addition on body weight gain (BWG) to 35 days of age in male (■) and female (♦) broilers fed wheat-barley based diets.
Males = $-0.014x^2+4.196x+1535$, $R^2=0.957$ and females = $-0.007x^2+2.585x+1456$, $R^2=0.847$.

There was a significant reduction ($P<0.001$) in liver weight with increasing dosage of enzyme product, whilst there was no significant effect observed for gizzard weight (Table 3.4). The results of the intestinal samples were not presented as they were not clear for analysis. This might have been due to the orientation of the samples in the gel at fixing and also the cutting technique.

Table 3.4 The effect of enzyme addition to wheat-barley diets on the liver and gizzard weight (g) for broilers at 35 days of age.

	Liver Weight	**Gizzard Weight**
0	66.3	61.3
50	48.9	61.5
100	33.7	62.9
200	53.4	63.4
P		
Enzyme	<.001	0.96
SED	3.58	4.43

3.4 Discussion

The objective of this trial was determined the effect of supplementation of wheat- barley based diets with a multi-blend of exogenous enzymes (β-glucanase and xylanase) on the performance of broiler chickens (both males and females). The addition of these enzymes to wheat-barley based diets significantly improved BWG and FCR. This suggests that the addition of exogenous enzymes increased the digestibility of nutrients. The improvement observed in the performance of the birds is consistent with previous studies (Almirall *et al*., 1995; Esteve-Garcia *et al*., 1997; Ouhida *et al*., 2000; Silva an Smithard, 2002; Wu *et al*., 2004a; b; Cowieson *et al*., 2005; Wang *et al*., 2005).

Several authors (Esteve- Garcia *et al*., 1997; Silvers and Bedford, 1999; Ouhida *et al*., 2000; Wu *et al*., 2004a; b; Cowesien *et al.,* 2005) showed that even though the addition of xylanase and β-glucanase in wheat and barley based diets improved FCR irrespective of the feed used, FI was not significantly affected. These reports are in contradiction with the results of present study, where the addition of exogenous enzymes increased FI. Although due to the increased BWG, FCR was still improved. Overall, this suggests that there was an improvement in nutrient utilization. This is supported by previous studies where Van der Klis *et al.* (1995); Ouhida *et al*. (2000); Svihus and Gullord (2002) and Wu *et al*. (2004b) reported that these improvements can be associated with the increase in nutrient digestibility, reduction of digesta viscosity and increased apparent metabolisable energy (AME). Steenfeldt *et al*. (1998b) also reported a significant improvement AME when an enzyme was added to wheat-based diets. Preston *et al*. (2000) did not find improvements in any of these performance parameters when enzymes were added to wheat diets, but observed a significant improvement in AME by 2% ($P<0.05$).

There was no significant enzyme effect on gizzard weight in this trial. This was in agreement with Wu and Ravindran (2004), when xylanase was added to both ground wheat and whole wheat diets. Wu *et al*. (2004a) reported a lack of significant effect of xylanase supplementation on the gizzard weight. Contrarily, Engberg *et al.* (2004) found a significant increase in relative gizzard weight when xylanase was added to airtight stored whole wheat compared to supplemented pelleted diets, which could have been due to the increased mechanical digestion and retention time of the whole wheat for grounding.

The liver weight was reduced significantly with enzyme supplementation (100 g/ton). This is consistent with previous studies. Wang *et al*. (2005) reported that enzyme supplementation

decreased liver weights linearly when added to wheat-based diets. The NSPs are thought to increase digestive organs' weight, as an adaptation to the changes caused by the NSPs effects. Enzyme supplementation may reverse this by degrading NSPs to simple molecules.

The results on the intestinal morphology in the present study were not presented.Wu *et al*. (2004b) reported that xylanase supplementation in wheat diets significantly increased the ileal villus height. This explains why nutrient utilization improved, bigger surface area for absorption. Wu *et al*. (2004a) reported that phytase supplementation increased duodenal villus height while xylanase had no effect. It was also reported that unsupplemented diets shortened and thickened the duodenal villus.

It is therefore concluded that the inclusion of this enzyme combination had a positive effect in BW, BWG, FI and FCR on male birds and, to a lesser extent on female birds. The reduction on liver weight also showed the benefits of supplementing wheat-barley feed with these enzymes, supporting the potential beneficial effects of using this type of products to improve nutrient utilization when added at 100 g/ton in wheat-barley based diets for broiler chickens to 35 days of age.

CHAPTER 4

The Use of Exogenous Enzymes in Wheat-Barley Based Diets to Improve Performance of Laying Hens

4.1 Introduction

It has been reported that the addition of exogenous enzymes in wheat, barley or rye diets improved the performance, especially in young birds (Pan *et al.,* 1998; Mathlouthi *et al*., 2003; Wu *et al.,* 2004a). However, there are conflicting reports in the literature with regards to these benefits in older birds or laying hens. Choct and Annison (1992a; b) and Wyatt and Goodman (1993) reported a lack of beneficial effect of exogenous enzymes in older birds (broilers or laying hens), which might be due to the fact that the digestive tract of mature birds can tolerate the negative effects of NSPs better. On the contrary, Lázaro *et al.,* (2003) found that the addition of a fungal β-glucanase/xylanase enzyme complex to laying hen diets increased performance. Pan *et al*. (1998) and Jaroni *et al*. (1999) reported that the use of exogenous enzymes reduced the number of dirty eggs in laying hens and improved FCE. The supplementation of feed with exogenous enzymes was reported to increase the AME content of these grains (Wyatt and Goodman 1993; Pan *et al*., 1998; Lázaro *et al*., 2003; Wu *et al*., 2004a).

The cell walls of cereal grains contain high content of complex carbohydrates (NSPs) which interfere with the digestion and absorption of certain nutrients in the GIT, particularly in young birds (Jaroni et al., 1999; Chesson, 2001; Wang et al., 2005; Mourão et al., 2006; Costa et al., 2008). Arabinoxylans or pentosans and β-glucans, which are found in wheat, rye, oats and barley, are of particular interest. In laying hens the use of wheat, barley and rye instead of maize results in high water consumption, high digesta viscosity, poor feed efficiency, large volumes of wet and sticky droppings which increase the number of dirty eggs, affect egg production, and quality of eggs (Francesch *et al*., 1995; Pan *et al.* 1998; Jaroni *et al.,* 1999; MacIsaac *et al.,* 2002; Mathlouthi *et al.,* 2003; Mourão *et al.,* 2006; Roberts and Choct, 2006).

The objective of this study was to evaluate a novel combination of xylanase and β-glucanase for wheat and barley -based diets on the performance of laying hens.

4.2 Materials and methods

Lohmann brown classic hens were housed in 40 x 50 cm cages, with four hens per cage and sixteen hens per pen (four cages). Each replicate consisted of one pen with one common feeder and three nipple drinkers per pen. There were 56 pens in total. Food and water were supplied *ad libitum* by means of one nipple drinkers for two cages and one line feeder in front of the cage. The house was open sided with no temperature control. Lighting was supplemented in the morning and evening to provide a cooonstant photoperiod of 16L: 8D. Four dietary treatments with the same nutrient composition (Table 4.1) were used in each of two experiments (4 treatments x 14 replicates x 16 birds = 896 birds). Experiment 1 was conducted with birds from 20 to 32 weeks of age and experiment 2 with a different group from 28 to 40 weeks of age.

Table 4.1 Ingredients and nutrient composition of the diets as calculated

Ingredient (%, as fed)	Intake 120g/day
Wheat	40.8
Barley	20.0
Rye	8.00
Soyabean meal 50	17.56
Soyabean oil	2.57
DL- Methionine	0.14
Salt	0.29
Limestone	8.33
Dicalcium Phosphate	1.78
Vitamin/Mineral premix	0.50
Calculated provision (%, as fed)	
Poultry ME (MJ/kg)	11.3
Crude Protein	16.3
Crude Fat	4.03
Crude Fibre	2.43
Lysine	0.78
Methionine	0.38
Methionine + Cystine	0.68
Thrionine	0.57
Calcium	3.70
Total Phosphate	0.67
Digestible Phosphate	0.34
Sodium	0.15

Birds were randomly assigned to one of four experimental treatments: a control diet (wheat-barley based diet) with no addition of the enzyme product, or diets containing 50, 100, 200 g of enzyme preparation/tonne of fed (multi enzyme blend of Xylanase and β-glucanase) as T1 to T4 respectively. Diets were free of antibiotic growth promoters and were provided in a mash form. Birds were weighted at the beginning of each experiment and monthly afterwards (full crops). Food intake was determined weekly by the difference of food offered and the amount that remained in the troughs at the end of the week. Mortalities were recorded daily and their weights were recorded for the adjustment of FCR. Eggs were recorded daily for the calculation of egg production and classified into normal and abnormal groups and weighed three times a week. The abnormal group consisted of dirty, soft, small and broken (cracked) eggs.

Data were analysed using one- way analysis of variance to determine if there were any differences between mean BWG in birds on different dietary treatments. Linear regression analysis was also used for each set of data using Genstat 11th Edition (2008).

4.3 Results

4.3.1 Experiment 1

Enzyme addition did not affect the mean FI except at 25 weeks of age, and FCR laying hens fed on wheat and barley based diets from 20 to 32 weeks of age (Tables 4.2 and 4.3). However, there was a significant effect on FCR at 25th and 26th week ($P<0.001$) and an overall significant ($P<0.05$) response (Figure 4.2).

Table 4.2 The effect of enzyme addition wheat-barley diets on mean body weight gain in laying hens from 20 to 32 weeks of age.

	BWG (g/day) at different age in days		
Enzyme (g/ton)	**28**	**56**	**84**
0	87.7	102.9	48.4
50	71.8	112.3	68.2
100	73.5	109.7	50.2
200	73.2	97.1	63.9
p			
Enzyme	0.77	0.886	0.326
SED	17.21	21.01	12.77

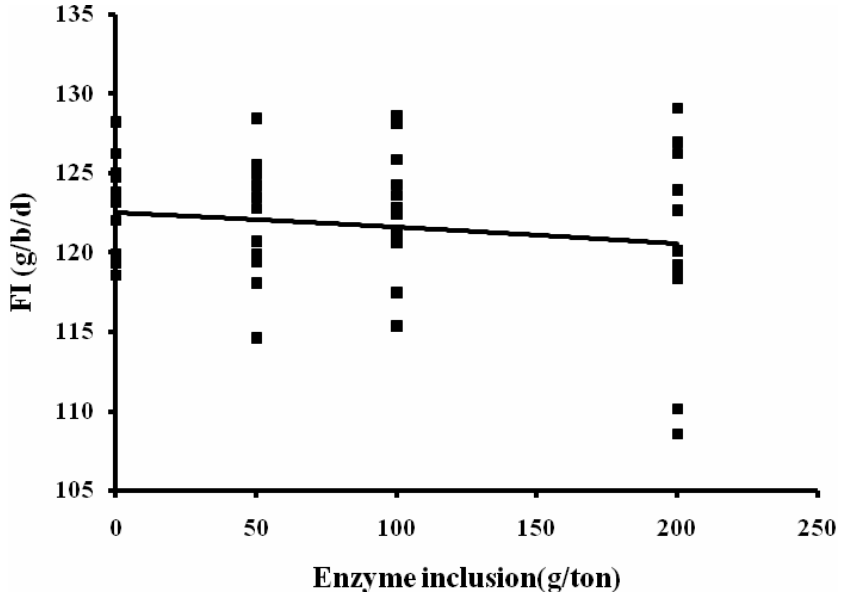


Figure 4.1 The effect of exogenous enzyme supplementation on feed intake (FI) in laying hens fed on wheat-barley diets from the age of 20 to 32 weeks. FI = $-3x^2$ - 0.00989x + 126.16, R^2= 0.306

There was no significant effect of enzyme supplementation on mean rate of lay (ROL), egg mass (EM) or egg weight (EW) (Table 4.4). Enzyme supplementation had a significant (P<0.047) effect on ROL (Figure 4.3). There was no difference between supplemented diets and control diet for EW and EM (Table 4.4).

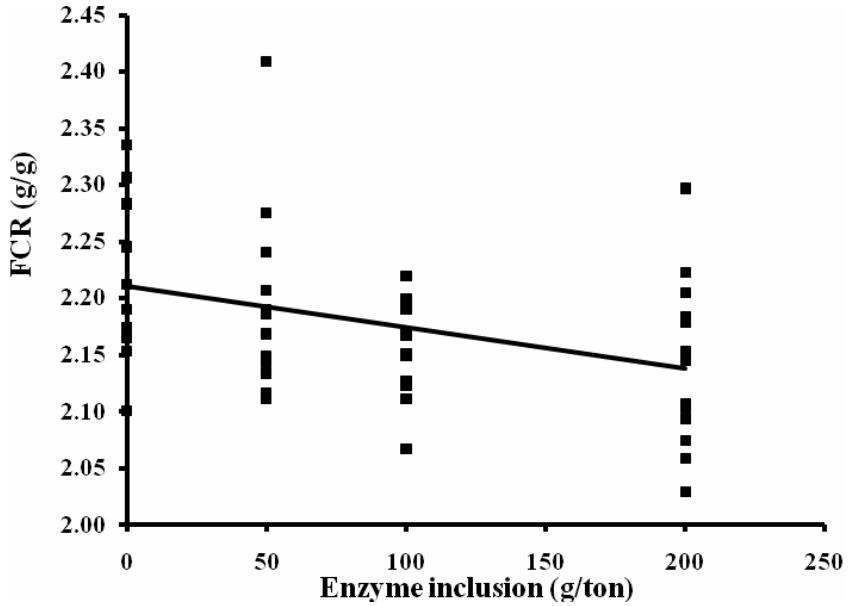


Figure 4.2 The effect of exogenous enzyme supplementation on feed conversion ratio (FCR) in laying hens fed on wheat-barley diets from the age of 20 to 32 weeks. FCR = $0.086x^2$ - 0.000401x + 2.159, R^2= 0.26

Table 4.3 The effect of enzyme addition to wheat-barley diets on mean food intake (FI) and feed conversion ratio (FCR) on laying hens from the age of 20 to 32 weeks.

	Number of weeks											
Enzyme	**FI 1**	**FI 2**	**FI 3**	**FI 4**	**FI 5**	**FI 6**	**FI 7**	**FI 8**	**FI 9**	**FI 10**	**FI 11**	**FI 12**
0	110	120	120	122	120	130	124	127	125	123	121	125
50	112	122	122	119	122	132	120	127	124	121	118	122
100	117	115	124	123	123	120	120	125	124	123	123	124
200	113	119	123	123	120	122	119	121	123	119	120	123
p-value	0.23	0.25	0.77	0.56	0.84	<0.05	0.08	0.15	0.85	0.08	0.66	0.50
SED	3.24	3.42	4.21	3.42	3.48	4.52	2.34	2.76	1.95	1.82	4.01	2.29
	FCR 1	**FCR 2**	**FCR 3**	**FCR 4**	**FCR 5**	**FCR 6**	**FCR 7**	**FCR 8**	**FCR 9**	**FCR 10**	**FCR 11**	**FCR 12**
0	1.98	2.13	2.12	2.13	2.09	2.22	2.08	2.12	2.07	2.06	1.99	2.06
50	2.01	2.13	2.13	2.06	2.11	2.23	2.01	2.12	2.07	2.01	1.95	2.00
100	2.07	2.02	2.15	2.00	2.11	2.02	1.99	2.07	2.07	2.03	2.04	2.09
200	2.03	2.10	2.12	2.11	2.07	2.06	1.96	2.03	2.05	1.99	1.98	3.01
p-value	0.45	0.19	0.97	0.51	0.92	<0.01	<0.01	0.14	0.90	0.12	0.60	0.35
SED	0.06	0.06	0.08	0.10	0.06	0.07	0.03	0.05	0.03	0.03	0.07	0.06

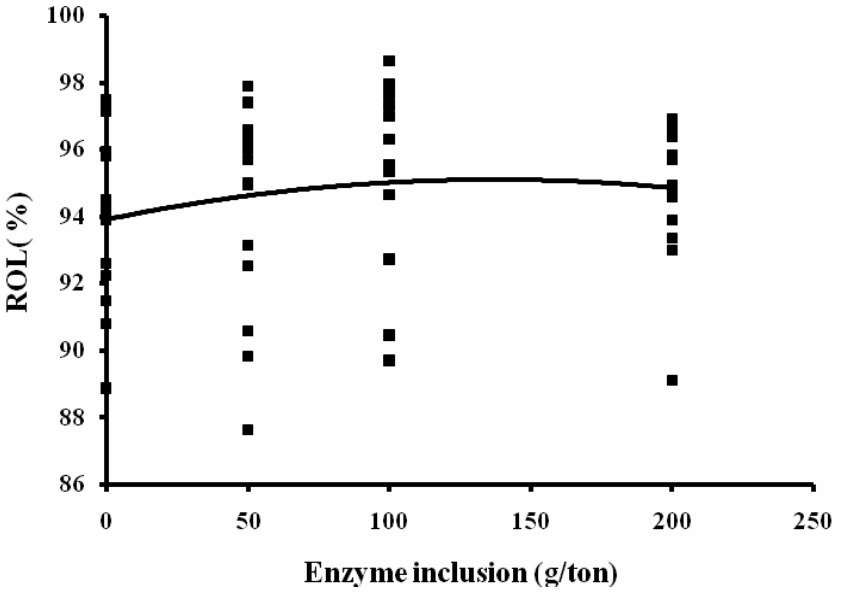


Figure 4.3 The effect of exogenous enzyme supplementation on mean overall rate of lay (ROL) in laying hens fed on wheat-barley diets from the age of 20 to 32 weeks. ROL = $-2.07x^2 + 0.0055x + 95.98$, $R^2 = 0.12$

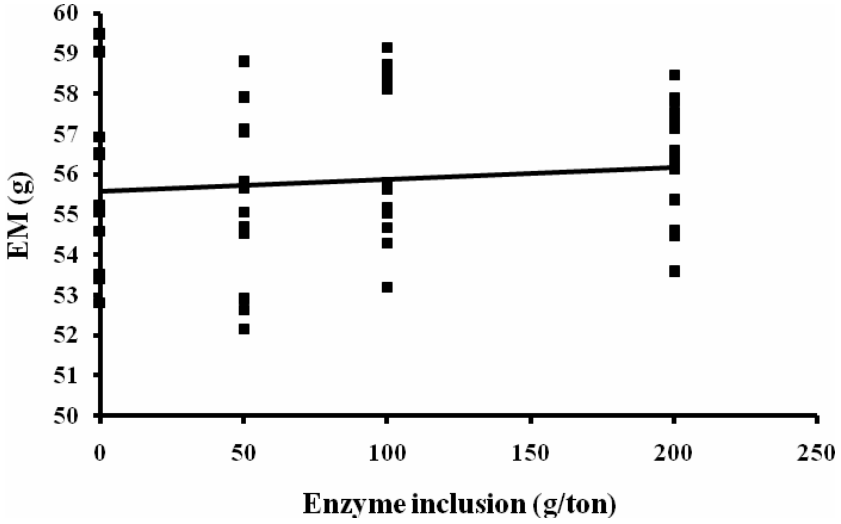


Figure 4.4 The effect of exogenous enzyme supplementation on eggs mass (EM) in laying hens fed on wheat-barley diets from the age of 20 t 32 weeks. EM = $-2.18x^2 + 0.00625x + 57$, $R^2=0.186$

Table 4.4 The effect of enzyme addition to wheat-barley diets on mean rate of lay (ROL), egg mass (EM) and egg weight (EW) for each week in laying hens from 20 to 32 weeks

	Number of weeks											
Enzyme	**ROL 1**	**ROL 2**	**ROL 3**	**ROL 4**	**ROL 5**	**ROL 6**	**ROL 7**	**ROL 8**	**ROL 9**	**ROL 10**	**ROL 11**	**ROL 12**
0	94.7	95.2	94.7	94.3	94.4	94.3	94.1	94.3	94.3	94.2	93.7	93.4
50	93.9	94.8	94.5	94.8	96.0	94.3	93.8	94.0	94.0	94.4	94.1	93.6
100	96.1	95.9	97.2	96.0	96.2	95.1	95.0	94.9	94.9	94.5	94.7	95.7
200	95.1	96.0	95.3	95.5	95.0	94.1	95.0	94.4	94.4	93.3	92.4	93.4
p- value	0.36	0.77	0.15	0.58	0.69	0.86	0.68	0.92	0.87	0.86	0.35	0.26
SED	1.26	1.28	1.28	1.33	1.36	1.22	1.29	1.30	1.37	1.50	1.30	1.33
	EM 1	**EM 2**	**EM 3**	**EM 4**	**EM 5**	**EM 6**	**EM 7**	**EM 8**	**EM 9**	**EM 10**	**EM 11**	**EM 12**
0	52.6	53.6	53.8	54.0	54.7	55.3	56.4	56.5	57.1	56.4	57.0	56.7
50	52.0	53.9	54.1	54.8	55.5	55.6	55.7	56.2	56.8	56.8	56.7	57.1
100	54.0	54.5	56.3	67.3	56.0	56.2	57.0	57.2	57.2	57.2	57.0	57.4
200	53.2	54.6	55.1	55.7	55.3	55.9	57.5	56.6	56.6	55.8	55.9	57.2
p- value	0.18	0.61	0.04	0.31	0.61	0.72	0.32	0.80	0.94	0.65	0.67	0.96
SED	0.92	0.85	0.93	8.01	1.03	0.86	1.00	0.98	0.98	1.13	0.95	1.29
	EW 1	**EW 2**	**EW 3**	**EW 4**	**EW 5**	**EW 6**	**EW 7**	**EW 8**	**EW 9**	**EW 10**	**EW 11**	**EW 12**
0	55.6	56.4	56.8	57.3	57.6	58.6	59.9	59.9	60.4	59.9	60.8	60.7
50	55.4	56.8	57.3	57.8	57.9	59.0	59.4	59.8	59.9	60.2	60.3	61.0
100	56.2	56.8	57.9	58.3	58.3	59.1	60.0	60.2	60.0	60.6	60.2	60.1
200	56.0	59.9	57.8	58.3	58.1	59.3	60.5	60.0	60.1	59.8	60.5	61.2
p- value	0.32	0.74	0.07	0.09	0.76	0.50	0.25	0.85	0.81	0.58	0.76	0.72
SED	0.45	0.54	0.48	0.46	0.63	0.49	0.37	0.38	0.53	0.62	0.61	1.05

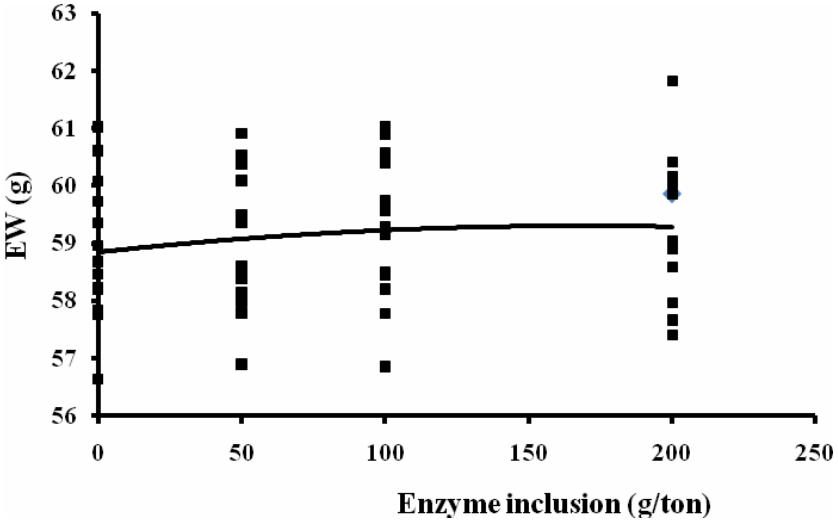


Figure 4.5 The effect of exogenous enzyme supplementation on egg weight (EW) in laying hens fed on wheat-barley diets from 20 to 32 weeks of age

4.3.2 Experiment 2

There was no significant effect of enzyme addition on the weekly average FI, FCR or BWG in laying hens fed wheat and barley diets from 28 to 40 weeks of age (Tables 4.5 and 4.6). Though there was significant response to enzyme addition on FI ($P<0.01$) (Figure 4.6). Mortality in this trial was less than 3% and not associated with dietary effects. There was no significant effect on the FCR (Figure 4.7).

Table 4.5 The effect of enzyme addition to wheat-barley diets on mean body weight gain in Laying hens from the age of 28 to 40 weeks.

Enzyme	BWG (g/d) at different ages in days		
	28	**56**	**84**
0	69.6	55.9	41.1
50	42.7	52.1	42
100	67.1	53.2	37.7
200	55.4	54.3	43.4
P-value	0.306	0.991	0.911
SED	15.73	12.3	8.14

Table 4.6 The effect of enzyme addition to wheat-barley diets on mean weekly food intake (FI) and feed conversion ratio (FCR) on laying hens from the age of 28 to 40 weeks.

	Number of weeks											
	FI 1	**FI 2**	**FI 3**	**FI 4**	**FI 5**	**FI 6**	**FI 7**	**FI 8**	**FI 9**	**FI 10**	**FI 11**	**FI 12**
0	103	119	113	119	122	128	125	127	125	126	123	124
50	105	121	116	129	122	121	133	127	125	124	125	123
100	103	118	111	126	121	126	125	127	127	127	125	124
200	105	128	113	128	122	127	127	130	128	125	128	126
p												
Enzyme	0.44	0.17	0.88	0.17	0.82	0.45	0.42	0.44	0.26	0.77	0.25	0.65
SED	1.74	5.08	6.12	4.92	2.11	4.74	5.34	1.77	1.54	2.76	2.14	1.71
	FCR 1	**FCR 2**	**FCR 3**	**FCR 4**	**FCR 5**	**FCR 6**	**FCR 7**	**FCR 8**	**FCR 9**	**FCR 10**	**FCR 11**	**FCR 12**
0	1.68	1.95	1.83	1.94	1.94	2.05	2.00	2.02	1.97	1.98	1.92	1.93
50	1.70	1.94	1.84	2.05	1.93	1.91	2.13	2.00	1.95	1.92	1.91	1.89
100	1.68	1.91	1.78	2.05	1.95	2.04	2.02	2.02	1.99	1.98	1.93	1.95
200	1.70	2.08	1.84	2.09	1.95	2.03	2.03	2.04	2.00	1.92	1.97	1.97
p												
Enzyme	0.92	0.19	0.93	0.61	0.93	0.25	0.42	0.64	0.39	0.46	0.59	0.11
SED	0.03	0.08	0.01	0.11	0.04	0.08	0.09	0.03	0.03	0.05	0.04	0.03

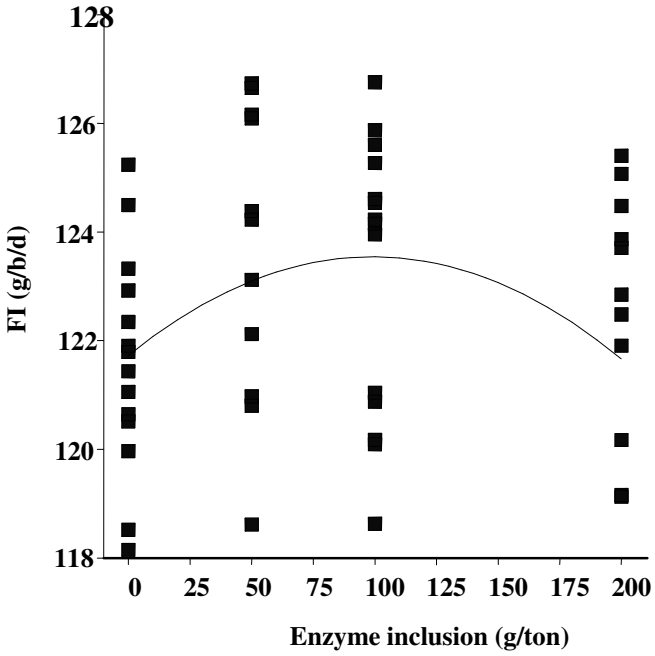


Figure 4.7 The effect of exogenous enzyme supplementation on the mean feed intake (FI) in laying hens fed on wheat-barley diets from 28 to 40 weeks of age. $FI = 20.55x^2 + 0.000321x + 103.65$, $R^2 = 0.84$

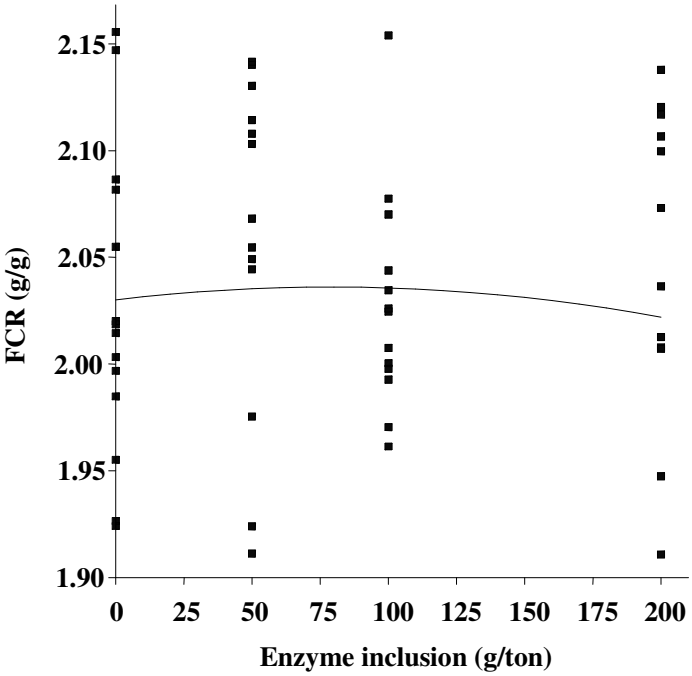


Figure 4.8 The effect of exogenous enzyme supplementation on the mean feed conversion ratio (FCR) in laying hens fed wheat- barley diets from 38 to 40 weeks of age. $FCR = 0.305x^2 - 0.0001253x + 1.7693$, $R^2 = 0.16$

Table 4.7 The effect of exogenous enzyme addition to wheat –barley diets on mean egg mass (EM), egg weight (EW) and rate of lay ROL in laying hens from 28 to 40 weeks of age

	ROL 1	**ROL 2**	**ROL 3**	**ROL 4**	**ROL 5**	**ROL 6**	**ROL 7**	**ROL 8**	**ROL 9**	**ROL 10**	**ROL 11**	**ROL 12**
0	95.7	96.5	95.2	96.0	96.3	96.0	95.8	96.4	96.1	95.1	95.6	93.7
50	97.1	97.2	95.9	95.7	96.1	95.5	95.5	95.3	94.7	94.7	94.7	93.2
100	95.2	96.1	95.2	95.7	96.4	96.1	95.8	94.9	94.7	95.0	95.4	94.0
200	97.1	96.5	95.3	96.3	97.0	96.4	97.9	97.6	96.9	96.6	97.0	95.2
P-value	0.14	0.80	0.91	0.89	0.74	0.86	0.16	0.19	0.21	0.41	0.26	0.53
SED	1.00	1.09	1.15	0.88	0.89	1.04	1.16	1.32	1.25	1.24	1.16	1.47
	EW 1	**EW 2**	**EW 3**	**EW 4**	**EW 5**	**EW 6**	**EW 7**	**EW 8**	**EW 9**	**EW 10**	**EW 11**	**EW 12**
0	61.2	61.2	61.7	61.6	63.1	62.6	62.6	63.1	63.7	63.9	64.2	64.3
50	61.8	62.4	62.8	62.8	63.3	63.3	62.4	63.7	64.2	64.5	64.9	65.1
100	61.2	61.6	62.1	61.7	61.8	62.0	61.9	63.2	63.7	63.9	64.8	63.6
200	61.8	61.7	61.7	61.7	62.6	62.6	62.7	63.6	64.3	65.0	64.9	64.0
P-value	0.43	0.13	0.17	0.90	0.05	0.15	0.59	0.69	0.22	0.23	0.70	0.07
SED	0.51	0.51	0.57	1.77	0.56	0.57	0.66	0.57	0.38	0.59	0.60	0.40
	EM 1	**EM 2**	**EM 3**	**EM 4**	**EM 5**	**EM 6**	**EM 7**	**EM 8**	**EM 9**	**EM 10**	**EM 11**	**EM 12**
0	58.5	58.4	58.6	58.9	60.8	60.1	59.5	60.5	60.9	60.4	60.4	59.8
50	59.7	59.6	59.2	60.1	60.9	61.0	60.1	61.5	61.4	61.3	61.8	61.4
100	59.2	60.0	59.4	59.5	59.7	59.1	59.9	60.7	61.0	61.2	62.8	60.3
200	59.3	60.4	59.5	59.4	60.4	60.1	60.8	60.9	61.2	62.5	62.6	60.2
P-value	0.41	0.03	0.75	0.92	0.36	0.14	0.66	0.76	0.94	0.20	0.08	0.45
SED	0.71	0.68	0.85	1.72	0.72	0.79	1.05	1.04	0.99	0.95	0.97	1.02

There was no significant effect of enzyme addition in overall on mean EM, EW and ROL in laying hens fed on wheat-barley diets from 28 to 40 weeks of age (Table 4.7). Though there was a significant effect (P<0.05) on EM on the 29th week of age and on EW on the 32nd week of age. The response to enzyme addition showed a significant effect on ROL (P<0.05) but no effect on EW, and EM (Figures 4.9, 4.10 and 4.11). Egg production improved for hens fed diets containing the highest enzyme dosage as compared to hens fed diets without enzyme supplementation.

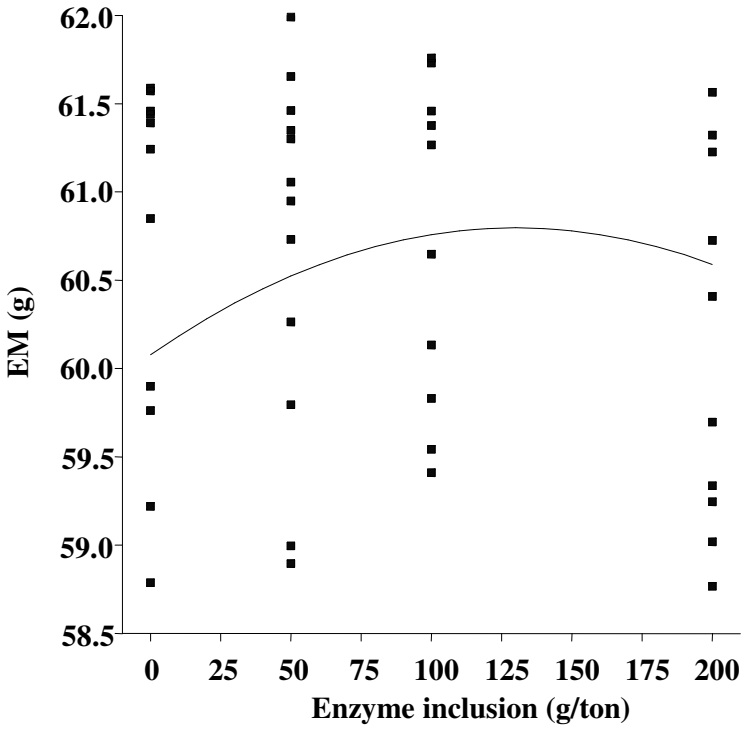


Figure 4.9 The effect of exogenous enzyme supplementation on egg mass (EM) in laying hens fed on wheat-barley diets from 28 to 40 weeks of age. EM = $1.04x^2 - 0.00019x + 59.195$, $R^2 = 0.89$

4.4 Discussion

The aim of this study was to evaluate the effect of β-glucanase and xylanase enzyme multi-blend on the performance of laying hens fed on wheat and barley- based diets. There was no significant effect of enzyme addition on mean FI, FCR, BWG, EM, EW and ROL. There was a significant response in performance due to enzyme supplementation though

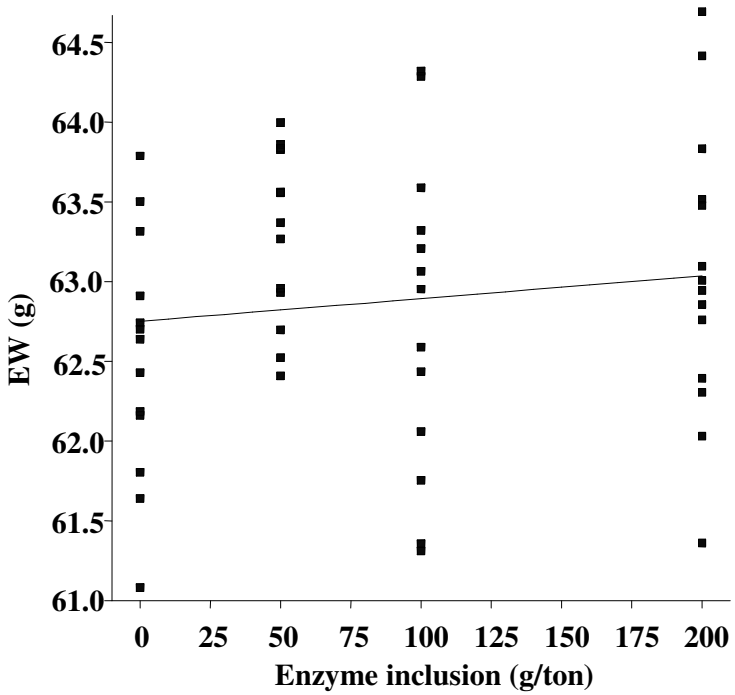


Figure 4.10 The effect of exogenous enzyme supplementation on mean egg weight (EW) in laying hens fed on wheat-barley diets from 28 to 40 weeks of age
EW= $2.767x^2$ +0.000314x + 61.462, R^2= 0.82

In experiment 1, FI decreased but not significantly with an increase in enzyme dosage which was not expected. Francesch *et al*., (1995); Jaroni *et al.,* (1999) and Lázato *et al.,* 2003) reported no significant effect of enzyme addition on FI when birds were fed wheat, barley, and rye diets. Conversely, there was a significant response on FI in experiment 2 with the increasing level of enzyme supplementation up to 100g/ton (P<0.010). This improvement was thought to be due to increased digestibility of nutrients due to enzyme addition. Wyatt and Goodman (1993) reported that enzyme addition to Wanabet barley did not have an effect on BW and FI as compared to other barley types, suggesting that the breed/cultivar of cereals play a huge role in the availability of nutrients from that particular ingredient. Even though there were no significant effects on mean FCR, there was a significant response in FCR in experiment 1 (P<0.05). This showed FCR to improve with increasing levels of enzyme supplementation. This is in agreement with Pan *et al*., (1998) and Mathlouthi *et al*., (2003) who reported the significant effct on FI, FCE, and BWG for birds fed on wheat and rye- diets (Pan *et al*., 1998) and wheat and barley diets (Mathlouthi *et al*., 2003) supplemented with enzyme. Lázaro *et al*. (2003) also observed improvement in FCR when enzyme was supplemented into wheat, barley and rye- diets (P<0.05).

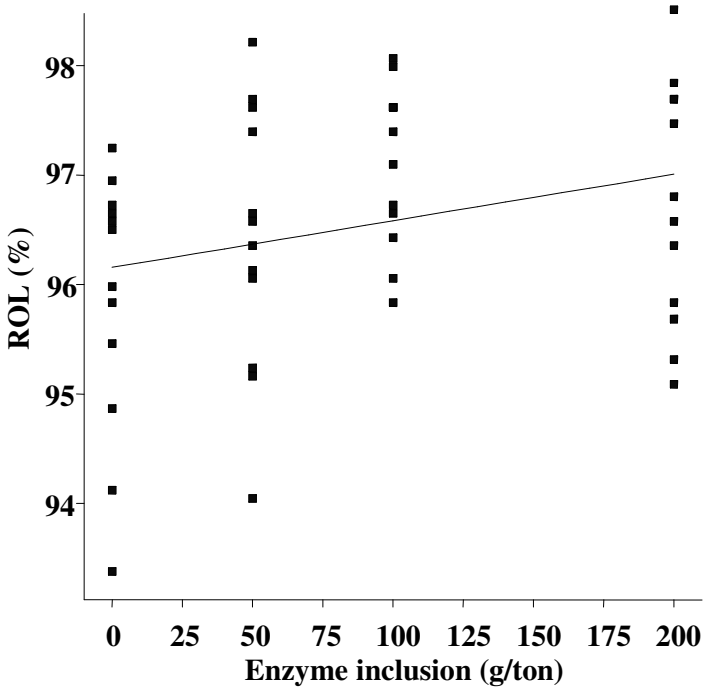


Figure 4.11 The effect of exogenous enzyme supplementation on mean rate of lay (ROL) in laying hens fed on wheat-barley diets from 28 to 40 weeks of age. ROL= $-2.232x^2+ 0.00376x +95.924$, $R^2=.685$

There was no significant response on EW and EM in either experiment 1 or 2, with the addition of exogenous enzyme. In contrast, Brufau *et al*., (1994) Francesch *et al*., (1995) reported an improvement in EW in the early stages of production with enzyme supplementation. Jaroni *et al*. (1999) also reported an improvement in EW with increasing doses of enzyme. But Lázaro *et al*., (2003) observed heavier EW in hens fed on control diets as compared to supplemented diets. In experiment 2, ROL increased with addition of enzyme up to 100 g/ton with no further improvement with addition of 200g/ton. These results are in accordance with the previous studies. Pan *et al*. (1998) observed an improvement in hen-day production with addition of enzyme to wheat and rye-based diets. Wyatt and Goodman (1993) reported a significant 4% improvement in ROL.

The results were not consistent in both experiments. The age of the birds in experiment 2 (28 to 40 weeks) is thought to be the reason for increased FI and ROL. Wyatt and Goodman (1993) suggested that enzyme addition might be advantageous during peak production as there is a high demand for nutrients to compensate for high requirements for production and growth. This might have been the case with hens in experiment 2, thus increased FI. It has been shown in the literature that laying hens do not struggle with NSPs effect as much as

broiler chickens due to their maturity. Though the results were variable it could be concluded that the addition of exogenous enzyme products (β-glucanase and xylanase) did have a positive effect on the performance of laying hens fed on wheat and barley- based diets.

CHAPTER 5

GENERAL DISCUSSION

The work presented in this thesis has shown that supplementation of exogenous enzymes (β-glucanase and xylanase) had a significant positive effect on the performance of male and female broiler chickens fed on wheat and barley based diets. There were also significant effects on the performance in laying hens at two different ages.

In broilers, the inclusion of 100g/ton of this particular enzyme combination did have a positive effect on the body weight to 35 days of age, BWG, FI and FCR of male chickens and, to a lesser extend of female chickens. The addition of further inclusion levels did not produce further improvements in these productive parameters. Liver weights also showed an improvement as a result of enzyme supplementation, supporting the potential beneficial effects of using this type of enzyme products to improve the nutrient utilization in wheat and barley based diets in very young birds.

In laying hens, there were significant improvements on ROL, EM and EW. The addition of enzyme improved egg production in experiment 2 ($P<0.05$). It is therefore thought that the older the birds the less sensitive to the detrimental effects of NSPs and so the response to the supplementation with exogenous enzymes is of less importance in laying hens than in broilers. This might have been the case in the present study. Lázaro *et al*. (2003) reported that wheat, barley and rye- based diets have the same effect as maize diets in laying hens, though the enzyme supplementation improves availability of nutrients to the bird.

The use of exogenous enzyme in poultry feeds is beneficial not only to increase the digestibility of nutrients in cereals but also to reduce total feed costs. These enzymes could be the best solution for countries using wheat, barley and rye in poultry feeds. In the present study, there were no measurements on the effect of the enzyme product on feed costs, egg quality, eggshell quality and composition, intestinal morphology in laying hens and broiler chickens, meat quality in broiler chickens. As in other countries like SA, Brazil and the USA maize is commonly used in poultry diets, it will be good idea to compare maize diets with supplemented wheat and barley diets to compare the effects. Also it will be useful to find out more about the costs of these enzymes to determine if the inclusion is economically viable/ worth it. Further studies will be interesting to look into the above mentioned measurements to evaluate further the effects and benefits of this enzyme combination for broilers and laying hens.

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