EFFECTS OF β-MANNANASE ON NUTRIENT UTILIZATION AND PERFORMANCE OF LAYING CHICKEN

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DECLARATION

The work presented on this document is my own done in collaboration with my advisory committee, except for where reference is made to the work of others.

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LIST OF ABBREVIATIONS

AA amino acid(s)

AID apparent ileal digestibility

AMEn nitrogen corrected apparent metabolizable energy

ANF anti-nutritional factors

ATTD Apparent total tract digestibility

BW Body weight

CP crude protein

CS Corn-SBM diets

CSD Corn-SBM-DDGS diets

DDGS distiller's dried grains with solubles

DM dry matter

DP Degree of polymerization

FCR Feed conversion ratio

GDP Gross domestic product

GE gross energy

ME metabolizable energy

NRC National Research Council

NSP non-starch polysaccharide

SBM Soybean meal

SEM standard error of the mean

ABSTRACT

The study was done at the Department of Animal Science, University of Manitoba. Two experiments were conducted to investigate the effects of β-mannanase on nutrient utilization and performance of laying hens. Experimental diets were based on soybean meal (SBM), corn and distillers dried grains with solubles (DDGS). The first study was designed to evaluate the effect of β-mannanase (CTCZYME, CTC Bio Inc., Seoul, South Korea) on performance of laying hens and egg quality traits. A total of 160 Lohmann hens were allocated to one of 4 diet groups in 2 levels of dietary protein plus energy content [18.5% crude protein with 2,850 kcal/kg metabolizable energy (ME) (Adequate-ME&CP) vs. 17.5% crude protein with 2,700 kcal/kg ME (Low-ME&CP)]. β-mannanase was added at 0 and 0.04% of diet to both diet types. The experiment lasted for 12 weeks and was designed in a 2 × 2 factorial arrangement. Each group had 8 replicates of 5 hens. Eggs were collected daily and egg production was calculated on a hen-day basis. Egg weight, albumen height, egg specific gravity, eggshell breakage and egg shell thickness were measured bi-weekly and Haugh Units was calculated from the egg weight and albumen height. Feed intake was recorded weekly and calculated as g/hen/day. There were no significant differences in overall egg production and feed efficiency among birds fed on the 4 dietary treatments for the 12-week period. However, average feed conversion ratio (FCR) from the second to the last month of hens fed the low-ME&CP diet was higher than that of hens fed the adequate-ME&CP diet irrespective of enzyme supplementation. Feeding the low-ME&CP diet significantly increased ($P<0.05$) overall feed intake and reduced egg shell thickness compared with layers fed the adequate-ME&CP diet independent of β-mannanase supplementation. Neither dietary protein and energy content nor β-mannanase supplementation affected ($P>0.05$) egg shell strength, egg specific gravity and Haugh Units. A significant

interaction ($P<0.05$) was observed in egg weight between dietary protein and energy content and β-mannanase supplementation, suggesting that β-mannanase supplementation increased egg weight only in hens fed the adequate-ME&CP diet. These results, therefore, showed that feeding adequate-ME&CP diets with added β-mannanase supplementation could be considered as a dietary strategy to improve egg weight in laying hens. In the second experiment, seventy two layers were randomly allocated to 4 treatments, each of which had 24 pens of 3 chickens per pen and were used to investigate the effects of β-mannanase on nutrient digestibility and nitrogen corrected apparent metabolizable energy (AMEn). Two diets were formulated to meet the National Research Council (NRC, 1994) nutrient requirements for layers. Diet 1 was based on corn and SBMwhereas Diet 2 was based on corn, SBM and DDGS. Each of the two diets was supplemented with either 0% or 0.04% β-mannanase. Each diet was fed to 6 groups of birds with 3 birds in each group making a total of 18 birds per treatment. For all apparent ileal digestibility (AID) calculations, the interaction between enzyme supplementation and energy levels was not significant for all treatments. Supplementing diets with β-mannanase increased apparent ileal DM digestibility of the experimental diets. Adding DDGS affected the AID of arginine, histidine, threonine, aspartate, glycine and proline, whereasβ-mannanase supplementation had no effect on AID of any of the amino acids. There was a trend towards the improvement of AID of protein with the addition of β-mannanase. Supplementing the diet with β-mannanase also improved the AMEn of DDGS containing diet but reduced the AMEn of the control diets. The results indicate that β-mannanase supplementation of corn/SBM/DDGS diets may improve calcium and energy utilization of layers, whereas it may not affect ileal amino acid digestibility

1.0 INTRODUCTION

Agriculture has, for many years formed the backbone of Kenya's economy. The sector employs more than 75% of the population and contributes approximately 25% to the total GDP (Republic of Kenya, 2005). Livestock production, as one component of agriculture, covers 42% of agricultural output and it also plays an important role in the national economy as it contributes 10% of the total GDP (Ministry of Livestock and Fisheries Development, 2008). The livestock subsector comprises of a mixture of large, medium and small-scale farmers. Kenya's population is growing rapidly and has more than tripled in the past40 years. Combined with the limited arable land, this demographic growth poses critical challenges to food security. Poultry production offers the greatest potential to boost food security in Kenya. This is mainly due to their small size and fast reproduction rate compared to most other livestock and the fact that it fits well with the concept of small-scale agricultural development. Moreover, it is ecofriendly and does not compete for scarce land resources.

Demand for high quality commercial poultry feeds has risen because of the high demand for white meat, which is considered a healthier alternative to red meat (McCarthy *et al*., 2004). In addition, fast returns from poultry farming are attracting more investors in the sector. This high demand, coupled with prevailing competition for cereals and leguminous grains for human food consumption and industrial production, decreasing arable land and unavailability of local sources for essential minerals have negatively affected availability of feed ingredients leading to high costs of feeds. Generally, the main challenges facing the animal feed industry in Kenya include poor quality and high cost of ingredients and feed, inadequate extension services, inadequate flow of research information, uneven distribution of livestock feed and provender millers, unavailability of local sources of vitamins, amino acids, macro- and micro-nutrients and frequent

droughts (Ministry of Livestock and Fisheries Development, 2008). To avoid overdependence on human food such as maize for production of feeds for monogastric animals, millers have resorted to using by-products from a wide range of agro-industrial processes in poultry feed formulation. The most common products include cereal by-products and oil seed cakes that result from either industrial processes or milling activities. The most common industrial processes yielding great amounts of by-products are brewing, baking and oil extrusion from seeds. The bulk of cereal by-products result from grain mills that are processed to produce refined grains (rich in starch) and mainly comprise bran, germ or both. Breweries by-products such as BDG have less starch compared to whole grains because most of it is used in alcohol production during fermentation. On the other hand, oil cakes (by-products from oil extraction) from seeds and copra (from coconut) contain high levels of starch, proteins and some residual oil and most of them have very high fiber content.

These by-products have been used as alternative sources of energy and proteins for animal feeds but their inclusion in diets for monogastric animals is constrained by their high levels of various anti-nutritional factors (ANF) and especially the high non-starch polysacharrides (NSP) content (Bedford, 1995; Meng *et al.*, 2005). Soybean meal, canola meal, DDGS, copra meal and guar gum meal are among the by-products included in monogastric feed formulation. Among the NSPs found in these common feed ingredients is β-mannan, which is the main focus of this study.

1.1 Context of the study

Feed is the most important input in animal production accounting up to 80 per cent of production costs. It is, therefore, important to ensure the effective use of feed in order to maximize production. Various studies have indicated variation in performance of monogastric animals

based on the type of ingredients that constitute their feed due to the presence of ANF in them. The ANF vary in type and concentration among feed ingredients. Their effects are also variable depending on animal species. High levels of various ANF and especially the high NSP content present in potential feed ingredients limit their inclusion in monogastric diets. Among the NSPs found in these ingredients is the insoluble NSP, mannan. Nutritionally significant amounts of β-mannan are found in SBM, canola meal, DDGS, copra meal and guar gum meal among other by-products commonly included in poultry diets (Kok *et al*., 1999; Tucker *et al*. 2004; Hsiao *et al*. 2006).β-mannans present in these feed ingredients are potential anti-nutritional factors, with a negative impact on the availability and utilization of nutrients and performance of laying hens. Mannanase treatment has the potential to improve the nutritive value of lower cost and low quality feed ingredients that have high mannan content. Despite the potential of this enzyme on animal performance, little has been published concerning β-mannanase application in layer feed. This study therefore aims to provide information on the use of exogenous enzymes to target β–mannans so as to improve the nutritive value of these products in laying hen diets.

1.2 Objectives

Overall objective

The overall objective of the study was to determine the efficacy of a commercial β-mannanase in improving performance, nutrient digestibility and energy utilization in layer chickens.

Specific objectives

i) To assess the effects of β-mannanase on feed intake, egg production, and egg quality in laying chickens fed diets based on wheat, soybean meal, and canola meal.

ii) To evaluate the effectiveness of β -mannanase on the nitrogen corrected apparent metabolizable energy content (AMEn) and digestibilites of amino acids, calcium and phosphorus in layer chicken diets.

1.3 Hypothesis

Application of β-mannanase to target β-mannans in poultry feeds has positive effects on nutrient digestibility, utilization, and performance of laying chickens.

1.4 Rationale

Mannan and its derivatives (glucomannan and galactomannan) are a group of NSP commonly found in leguminous plants. This group of polysaccharides has been shown to reduce performance of monogastric animals by increasing digesta viscosity and encapsulating important nutrients and therefore preventing their availability to the animal. The need to minimize on the cost of poultry feed has prompted a shift from the use of ingredients used for human consumption such as maize. This can be achieved by finding and using alternative locally available plant protein and energy sources. These ingredients are mainly industrial by-products and are known to have low nutritive value for monogastric animals because they contain high levels of β-mannan which is not digestible by endogenous enzymes of these animals. Therefore, supplementing such diets with β–mannanase has potential to improve poultry production by diversifying on the use of alternative ingredient that are otherwise considered poor quality because of high mannan content.

2.0 LITERATURE REVIEW

2.1 Poultry farming in Kenya

Poultry farming plays an important role in the livelihoods of the populace in Kenya contributing about 7.8% of the total GDP (Omithi and Okuthe, 2008). It employs about three million people and contributes 3.7% of the per capita annual protein consumption. Approximately 65% of all Kenyan households own some form of poultry (Omiti and Okuthe, 2008). Poultry and cattle comprise the bulk of livestock produced in Kenya. Other livestock produced include goats, sheep, pigs, camels, donkeys and rabbits. Pigs and chicken are the two non-ruminant (monogastric) species commonly kept by farmers in Kenya. Poultry farming is mostly done in small scale holdings and consists of a small and vibrant commercial sector dominated by commercial layers and broilers and a large subsistence sector dominated by indigenous birds.

Generally, the high population growth rate in sub-Saharan Africa has resulted in subdivision of land into small holdings to accommodate an increasing number of farmers. This is the case in the densely populated areas of Kenya such as the Central Province, where the area of agricultural land per capita is small and declining and is inadequate for the production of arable and cash crops as well as forage for grazing. Indigenous chickens are preferred for egg and poultry meat because they require low levels of input, are hardy, and adapt well to the rural environments and fluctuations in available feed resources (Kingori *et. al.*, 2010). Their productivity however, is normally low due to poor genotypes, poor feed conversion efficiency and low adoption of modern technologies by farmers (Kingori *et al.*, 2010). In early 2002, hybrid commercial layers were introduced in arid and semi-arid areas and poultry farmers were encouraged to farm poultry as a business enterprise (Nyaga, 2008). Over the years poultry

industry has developed tremendously due to the demand for meat and eggs particularly in the urban areas.

2.2 The livestock feed industry

Feed is the most important input in animal production and it accounts for up to 80% of production costs of non-ruminant animals in Kenya (Nyaga, 2008; Ministry of Livestock and Fisheries Development, 2008). The Kenyan livestock feed industry has two principle components: the pastoral (forage pasture/fodder) and manufactured feeds. The pastoral feeding system provides the principle dietary components for the production of ruminant animals (cattle, goats, sheep, camels), while manufactured feeds are primarily applied to intensive pig and poultry production systems. Conventional feedstuffs are expensive and their prices are unstable and constantly increasing. The main source of protein in poultry rations are either animal proteins or plant proteins. Animal proteins include fish meal, meat meal, blood and bone meal, while plant proteins include groundnut cake, sunflower seed meal, cotton seed meal, sesame meal, and rapeseed among others (Ngari, 2009). The major energy source is maize and its byproducts. In common with many developing countries, the production of cereal grain crops in Kenya is for human consumption. Consequently, only the milling by-products such as maize bran, wheat bran and rice bran are available for livestock feed production. Maize bran and wheat bran are the most commonly used cereal byproducts in Kenya. More refined by-products such as maize germ and wheat pollard are used to a lesser extent. The most widely available oilseed cakes are sunflower and copra. In Kenya, all the feed premixes, the majority of the soya cake, and to a lesser extent cotton cake are imported.

Generally, the main challenges facing the animal feed industry in Kenya include poor quality and high cost of ingredients and feed, inadequate extension services and research information, lack of

appropriate credit facilities, uneven distribution of livestock feed and provender millers, unavailability of local sources of vitamins, amino acids, macro- and micro-nutrients, frequent droughts, lack of appropriate technical know-how in water harvesting, storage and irrigation, high transport costs and lack of market information (Ministry of Livestock and Fisheries Development, 2008).

Due to the high cost of animal feed in Kenya, feed millers have resorted to the use of low quality cereal by-products and vegetables in order to maximize profits (Nyaga, 2008; Ministry of Livestock and Fisheries Development (MOLD), 2008). Industrial by-products have been used as alternative sources of energy and proteins for animal feed but their inclusion in monogastric diets is constrained by their high levels of various anti-nutritional factors (ANF) and especially the high NSP content. For instance, Yang *et al.* (2010) used DDGS as the main protein source in pig diets and reported low ileal digestibilities of protein and amino acids, and increased digesta viscosity. Canola meal, peas and soybean meal have high quality of protein but their use is limited by their high NSP content (Bedford, 1995; Meng *et al.*, 2005).

2.3 The plant cell wall and NSPs

The plant cell wall is composed of many different kinds of polysaccharides, including cellulose, hemicelluloses, pectic substances and galactomannans. The structures of the different plant cell wall components are shown in Figure 1. The NSP components are principally non-α-glucan polysaccharides of the plant cell wall which vary in degrees of water solubility, size, structure and composition in different plant species (Caprita *et al.*, 2010). The concentrations and types of NSP vary between different parts of the plant. In wheat, for instance, water soluble NSP decrease from the inner to the outer layer of the kernel. The NSP content and type also differs among grains (Caprita *et al.*, 2010) for instance, arabinoxylan is predominant in wheat and rye, while β-

glucan is predominant in barley and oats (Bedford, 1995). The NSP content of plants also varies between cultivar of the same species and due to agronomic cultivation conditions, environmental factors prior to harvest and storage conditions after harvest (Caprita *et al.*, 2010).

Non-starch polysaccharides can be classified in terms of solubility or structural function. The water insoluble NSP include cellulose, galactomannans, xylans, and xyloglucans while the water-soluble are the pectins, arabinogalactans, arabinoxylans, and β-glucans. Caprita *et al.* (2010) classified NSP into structural and non-structural polysaccharides because classification by differences in solubility lacks precision with respect to chemical structures and biological functions.

The terms crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) are associated with NSP analysis. Crude fiber refers to the remnants of plant material after extraction with a dilute acid and a dilute alkali and includes variable portions of the insoluble NSP (Caprita *et al.*, 2010). Neutral detergent fiber is the insoluble portion of the NSP plus lignin, whereas ADF refers to a portion of insoluble NSP comprised largely, but not exclusively, of cellulose and lignin (Caprita *et al.*, 2010)

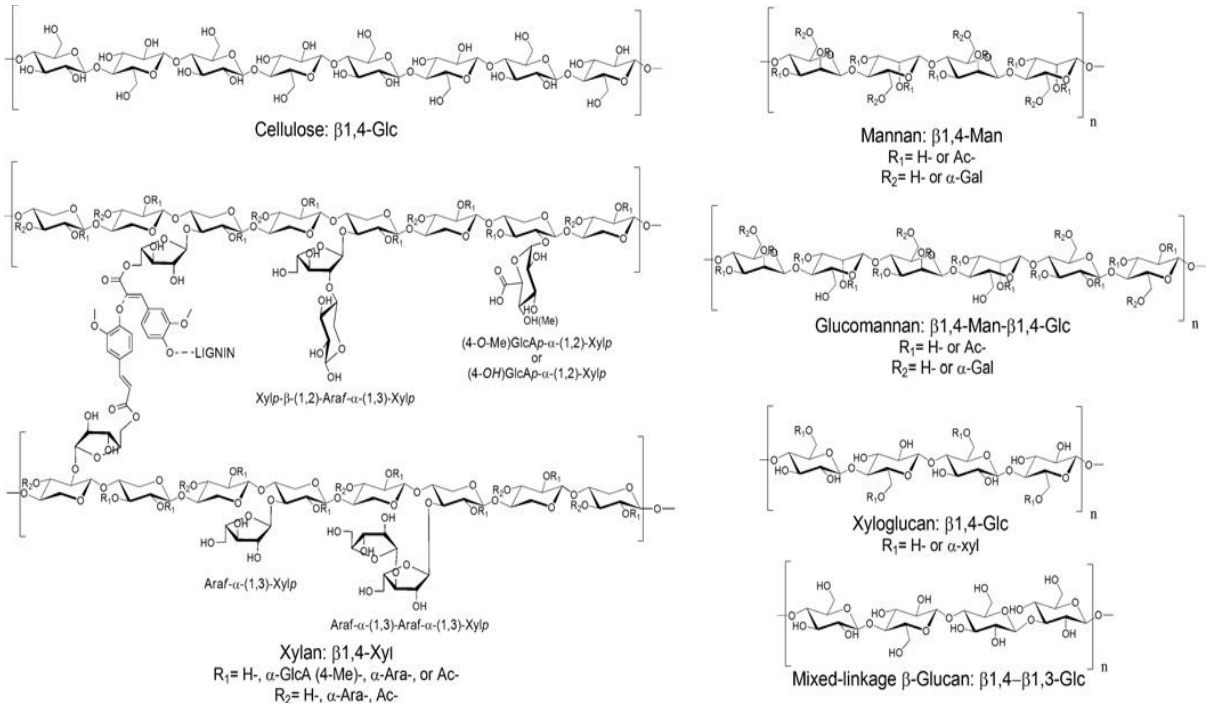

Figure 1: Polymeric structures of cellulose and hemicellulose chains (Gill *et al., 1999*)

Hemicelluloses are a heterogeneous group of polysaccharides abundant in higher plant cell walls and can be extracted by alkali (Ebringerova *et al*., 2000 and Bemiller 2007). Their composition and structural characteristics vary with plant species and tissue type. Generally, hemicelluloses have a complex chemical structure and comprise of mannans, xylans and galactans on the basis of the predominant sugar type in the main chain (Mohnen *et al*., 2008). One of the most common mannans is *O* acetyl-galactoglucomannan which comprises up to 25 % of the dry weight in softwood (Timell, 1967).However, a range of other mannan-type polysaccharides are synthesized by a wide variety of plants, and are found in different types of plant tissue (Meier *et al*., 1982). Their main role is often to function as structural polysaccharides and/or as reserve energy.

2.4Anti-nutritional role of NSP

To improve poultry production, availability of high quality and reasonably priced feeds is a prerequisite. This can be achieved through identifying and reducing dietary anti-nutritional factors present in feed ingredients available for poultry. The feeding cost in poultry production can be lowered while still maintaining high performance through the use of alternative ingredients, mainly industrial by-products that are more readily available and there is less competition with humans. Inclusion of these products in poultry diets is, however, constrained by their high levels of ANF, especially soluble non-starch polysaccharide (NSP) and phytate. The NSP form the cell walls of cereals, legumes and oil seeds. Generally, by- products contain considerable amounts of NSP (Bharathidhasan *et al.*, 2008). The anti-nutritional role of soluble NSP in chicken diets can be attributed to the NSP coat encapsulation which inhibits access of digestive enzymes to the protein, starch and fat and/ or the fact that the presence of NSP in the intestinal lumen increase viscosity of the intestinal contents. Elimination of these anti-nutritive effects has been reported to have a positive impact on the productivity of poultry (Bedford, 1995).

A combination of feed processing techniques including pelleting, extruding, and development of hulless varieties of barley and oats containing less crude fiber has been used to solve NSP problems especially in monogastrics (Bedford, 1995). Despite application of these strategies, research findings show that the effective utilization of high fiber feedstuff in diets of non-ruminants is still a major challenge. One effective way known to overcome this challenge is the use of feed enzymes to enhance the breakdown of NSP (Bharathidhasan *et al.*, 2008). Bedford (2000) reported that the use of enzymes increased the rate of nutrient digestibility regardless of the types of by-products used as feed ingredients and the mechanism of action of

the enzymes. The main purpose for supplementing feed with enzymes is to aid digestion of substances that an animal is intrinsically incapable of digesting. The NSP hydrolyzing enzymes increase nutrient availability by helping open up the complex feed cell walls thus allowing the animal's own enzymes to access and digest the enclosed nutrients (Nadeem *et al.*, 2005). In that way, nutrient utilization is enhanced.

2.5 Mannans: Structure and occurrence

Mannan polysaccharides are widely distributed in nature and are part of the hemicellulose fraction of leguminous plant seeds, various types of wood, and beans (Ademark *et al*. 1998). The backbones may consist entirely of mannose, as in mannans and galactomannans, or with mannose and glucose in a non-repeating pattern as in glucomannans and galacto-glucomannans. Mannans are a complex group of polysaccharides that consist of mannose molecules linked together to form a polymer and usually closely associate with cellulose and lignin (de Vries, 2003). Mannan polysaccharides are commonly found in various plant seeds where they either serve as energy reserve carbon source mobilized in the process of germination (Dey, 1980) or as structural carbohydrates that cross-link cellulose micro fibrils in plant cell walls (Puls and Schuseil, 1993).Homo- and heteromannans are based on variations of a β-mannan backbone (unsubstituted mannans), which might be interrupted with D-glucose (glucomannans) and/or branched with α-1,6-linked galactose (galactomannans), while some have a backbone containing β-1,4-linked D-mannose and D-glucose residues which are branched by α-1,6-linked D-galactose (galacto-glucomannan). Mannan-based polysaccharides are ubiquitous in nature and occur in different forms in plant cell walls. Depending on their structure and branching degree, these cell wall polysaccharides may play distinct roles in plants. They are insoluble in water, self-interactive and to some extent crystalline in the cell wall (Mulimani and Prashanth, 2002). Since

pure mannans impart hardness to the seeds of monocotyledons and dicotyledons, mannan-containing seeds are very hard and resistant to mechanical change (Buckeridge *et al*., 2000).

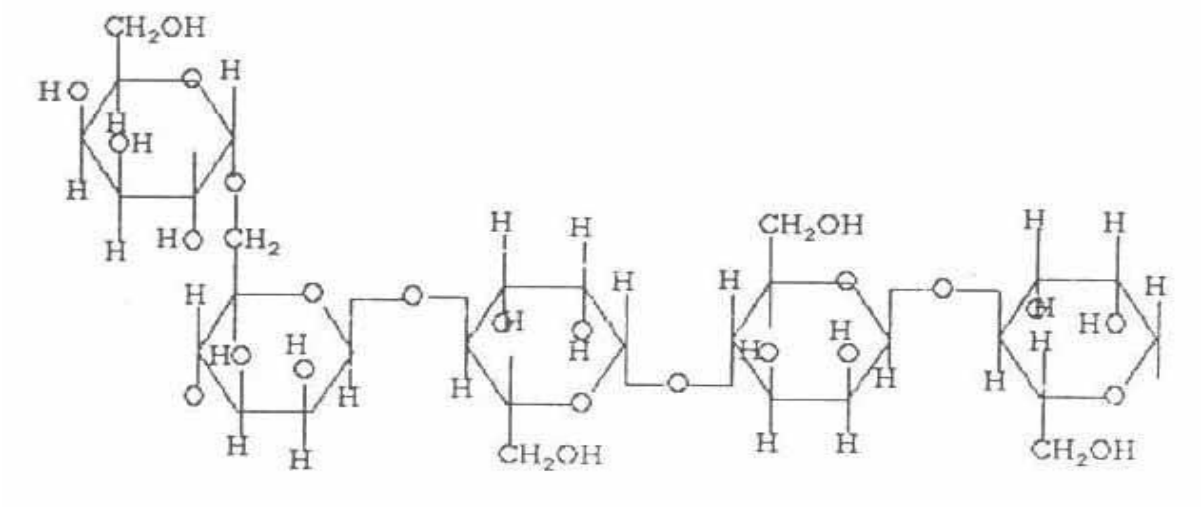

Figure 2: Diagram of a mannan chain. Mannans are 6-carbon sugars which form long Polysaccharide chains (Bewley, 1997)

The degree of galactose substitutions in galactomannans varies widely in nature and has direct influence on the solubility of the polymer in water (*Buckeridge et al*., 2000). For example, pure unsubstituted mannans form insoluble polymers, as observed in ivory nuts (de Vries and Visser, 2001; Capek *et al*., 2000), while substituted galactomannans such as locust bean gum have the ability to form viscous solutions with water (Marraccini *et al*., 2005). This property depends on the molecular size, Gal:Man ratio and the degree of branching of the polysaccharide (de Vries and Visser, 2001).

2.6 Mannan-based storage polysaccharides

Besides amylose and amylopectin, which are the most widespread storage polysaccharides in plants, there is a diverse group of mannan-based storage polysaccharides found in the seeds, roots, bulbs and tubers of various plants (Meier and Reid, 1982). These include the mannans,

galactomannans and glucomannans. Table 1 gives a summary of the chemical structures and characteristics of these polysaccharides.

2.6.1 Mannan

Linear chains of β-(1→4) mannan are found in the plant seed endosperms of certain plant species (Aspinall *et al*., 1953; Meier, 1958). In most cases, these polysaccharides are highly insoluble in water and very dense. Accordingly, it has been suggested that the mannan forms the molecular basis for the hardness which is characteristic for palm kernels, such as the ivory nut. Based on their solubility in alkali, two different fractions of mannan have been isolated from the ivory nut. These fractions differ mainly in their DP and morphology (Aspinall *et al*., 1953; Meier, 1958).

2.6.2 Galactomannan

Galactomannans are reserve polysaccharides in the seed endosperm of leguminous plants (*Leguminosae*) (Reid,1985). In contrast to unsubstituted mannans, the galactomannans are water soluble and can imbibe water, thus providing a water-holding function for the seed (Reid,1985). They are composed of β-(1→4)-linked mannan chains with α-(1→6)-linked galactosyl side groups (McCleary, 1985).Both the solubility and the viscosity of the galactomannans are influenced by the mannose:galactose ratio, which can vary from 1 to 5 (Reid,1985).Furthermore, the distribution of the substituents can vary considerably, which also affects the physical properties of galactomannans (Dea *et al*., 1986, Daas *et al*., 2000). Two of the most well characterized galactomannans are those found in locust bean gum and guar gum, isolated from the seeds of *Ceratonia siliqua* and *Cyanaposistetra gonolobus*, respectively (Goldstein *et al*., 1973, Rol, 1973). In comparison with other polysaccharides, these galactomannans have strong gelling properties.

2.6.3 Glucomannan

Some glucomannans are found as storage polysaccharides in the bulbs, roots and tubers of several types of plants (Meier and Reid, 1982).Many of these glucomannans are water soluble and are composed of a β-(1→4)-linked mannan chain with interspersed glucose residues in the main chain. They are often acetylated and the mannose:glucose ratio ranges from 4:1 to below 1:1 (Meier and Reid, 1982).One of the most thoroughly characterized of these glucomannans is konjac mannan which is isolated from the tubers of *Amorphophallus konjac* (Nishinari*et al*., 1992). This polysaccharide has a mannose to glucose ratio of 1.6:1 and a degree of polymerization above 6000 (Nishinari*et al*., 1992).

2.7 β-mannans in poultry feed

A wide variety of feedstuffs including SBM, DDGS, palm kernel meal, guar gum meal, copra meal, canola meal, and sesame meal contain considerable amounts of β-mannan (Rogel and Vohra, 1983; Dierick, 1989). β-mannans present in these products are potential anti-nutritional factors. Research done with swine has reported that β-galactomannan interferes with glucose metabolism and secretion rates of insulin (Leeds *et al*., 1980; Sambrook and Rainbird, 1985). Also, the high viscosity of β-mannans decrease gain:feed ratio and carbohydrate utilization efficiency of non-ruminants by limiting the access of digestive enzymes to substrate (Dale, 1997).

Soybean meal is a major vegetable protein source in most parts of the world for poultry production. Nutritionally significant amounts of β-mannan are found in SBM; up to 1.3 or 1.6% in the dehulled or non-dehulled forms, respectively (Hsiao *et al*., 2006). Distillers' dried grain with solubles, a co-product from the ethanol industry may be fed to livestock. It is a valuable feed ingredient, which may be included in poultry diets because the ME of DDGS is similar to

that of corn (Stein and Shurson, 2009). The cost of DDGS is often attractive compared with corn and soybean meal and many swine and poultry producers prefer to include greater amounts of DDGS in diets. However, inclusion of DDGS in diets fed to poultry is limited mainly because of poor lysine digestibility but also the presence of NSPs among which are the β-mannans. Soybean meal and DDGS are largely found in North America with SBM being the most popular vegetable protein source for livestock in the world. However, these ingredients are not commonly found in Kenya and millers often have to import SBM. This is quite costly hence there is need to find affordable, locally available alternative ingredients for poultry production. Copra meal is one of the industrial by-products found in abundance in Kenya (EPZA, 2005). It is an agricultural by-product of the coconut industry. Its use in feeds for monogastric animals is limited due to high NSP content (Mendoza *et al.,* 1994; Purwadaria *et al*., 1995). Analysis of copra meal samples found that 60-70% of the carbohydrate portion was composed of β-mannan. In a study conducted with broiler chicken, inclusion of copra meal in the diet resulted in lower growth rate and reduced feed intake and body weight gain (Panigrahi *et al*., 1987)

Table 1: Chemical structure and function of mannans in plants

Polysaccharide	Backbone	Backbone substitution	Function	Features
Pure mannan	(1-4)beta-D-mannose residues	none	Storage polysaccharide Structural in some algae	DP*100-2500 Soluble in boiling water; gradually precipitates on cooling. In thickened endosperm wall.
Galactomannan	(1-4)b-D-mannose residues	Single (1-6)-a-D-galactose residues; non-regular distribution	Storage polysaccharide in legume seeds	Viscous Water soluble DP 1000-10000
Glucomannan	(1-4)-b-D-glucose and mannose residues; non-regular distribution; partially acetylated	none	Storage polysaccharide in monocot seeds, and in bulbs and tubers. Structural mostly in wood	DP 100-5000 Viscous Water-soluble In idioblasts
Galacto-glucomannan	(1-4)-b-D-glucose and mannose residues, non-regular distribution	Single or double (1-6)-a-D-galactose residues on mannose or glucose residues; non-regular distribution	Storage polysaccharide in, for example Asparagus seeds. Structural in dicot primary cell walls	DP 100-200. Can contain small amounts of xylose and arabinose.

*** DP = degree of polymerization, an indication of polysaccharide chain length.**
Matheson, (1990).

2.8 Anti-nutritional effects of β-mannans

Various studies have demonstrated the effects of β-mannan in monogastric animals. Research done with swine has reported that β -galacto-mannan interferes with glucose metabolism and secretion rates of insulin (Leeds *et al*., 1980; Sambrook and Rainbird, 1985). Also, the high viscosity of β-mannans decrease gain:feed ratio and carbohydrate utilization efficiency of non-ruminants by limiting the access of digestive enzymes to substrate (Dale, 1997).

Studies on the effects of mannan degrading enzymes have also been useful in demonstrating the negative effects of β-mannan to monogastric animals by comparing growth and nutrient utilization between groups fed diets with or without exogenous β -mannanase. Daskiran *et al* (2004) using different levels of guar gum demonstrated a positive response to dietary enzyme addition. Addition of β–mannanase to poultry ration resulted in an improved feed conversion ratio and body weight gain at each guar gum level, including negative control.

Studies conducted by Lee *et al*. (2003 a, b) indicate that inclusion of guar gum in poultry rations increases the viscosity of digesta, thereby decreasing growth and feed efficiency. The study showed that inclusion of β–mannanase reduced intestinal viscosity and increased growth and feed efficiency. Other studies using turkeys (Odetallah *et al*., 2002) and swine (Pettey *et al*., 1999) indicate that replacing 48% CP soybean meal with 44% CP soybean meal in turkey diets had negative impacts on body weight and feed conversion of market-age turkeys.

2.9 Reducing anti-nutritive factors in feedstuff.

In order to reduce the negative effects of ANF in animal diets a number of strategies have been employed. Heat treatment, genetic improvement and enzyme supplementation are some of the methods that have been used to reduce the levels of ANF in feed. A combination of techniques is needed to combat issues associated with NSP especially in monogastric animals. Feed processing

techniques such as pelleting, extruding, and development of hulless varieties of barley and oats containing less crude fiber have been used (Bedford, 1995). Despite application of these strategies, research findings show that the effective utilization of high fiber feedstuffs in diets of non-ruminants is still a major challenge. Despite these improvements, fiber digestibility continues to limit the intake of available energy by non-ruminants, and correspondingly, contributes to excessive nutrient excretion. The use of exogenous fibrolytic enzymes has been shown to be an effective means of enhancing the breakdown of NSP and improving the productive efficiency of non-ruminants (Bharathidhasan *et al*., 2008). Regardless of the type of by-products used as feed ingredients and the mechanism of action of the enzymes, Bedford (2000) noted that the use of enzymes increased the rate of nutrient digestibility. The main purpose for supplementing feed with enzymes is to aid digestion of substances that an animal is intrinsically incapable of digesting. Thus, incase NSP hydrolyzing enzymes can help open up the complex feed cell walls, there would be an increase in nutrient availability that allows the animal's own enzymes to access and digest the encapsulated nutrients (Nadeem *et al*., 2005). In that way, nutrient utilization is enhanced.

2.10 Potential of feed enzymes in the poultry feed industry

Research has demonstrated that supplementing poultry diets with fiber-degrading enzymes has significant potential to improve feed utilization and animal performance. With advances in biotechnology and fermentation processes, cost of production of feed enzymes has dramatically been reduced and the use of feed enzymes in poultry diets has become popular. These enzymes are mainly obtained from bacteria or fungi; hence they are referred to as exogenous enzymes. Some of the genes which express them have already been cloned for large-scale industrial production. The increased use of feed enzymes is also due to a better understanding of the

structure of substrates and the mode of action of the enzymes (Bedford and Schulze, 1998). Some of the enzymes commonly used in the poultry feed industry include cellulose (β-glucanases), xylanases, Iphytases, amylases, proteases, lipases, and galactosidases. Improvements in poultry performance due to the use of exogenous enzymes can be attributed mainly to:

1) Reduced viscosity in the diet and digesta
2) Release of available phosphorus from phytate hydrolysis,
3) Release of encapsulated nutrients such as starch, fat, protein and minerals as a result of solubilization of cell wall, NSP.
4) Hydrolysis of certain types of carbohydrate-protein linkages and therefore improved availability of amino acids, and
5) Elimination of the anti-nutritive properties of certain dietary components, including NSP, by their enzymatic hydrolysis to the prebiotic type components which, in turn, may facilitate gut development and health in young chickens (Meng *et al*., 2005).

2.10.1 Reduction of viscosity in the diet and digesta

When soluble NSPs dissolve in the digestive tract, they form high molecular weight viscous aggregates (White *et al*. 1981; Bedford and Classen, 1992). This results in increased digesta viscosity which presents a major problem especially to poultry. The complications associated with increased digesta viscosity include reduced gastrointestinal passage rate (Almirall and Esteve-Garcia, 1994), reduced diffusion of digestive enzymes, their substrates and their products (Johnson *etal.* 1984; Bedford and Morgan, 1996) and stimulation of bacterial proliferation, particularly in the small intestine (Choct *etal.* 1996; Hock *etal.* 1997). Increased digesta viscosity

also reduces fat emulsification thereby impairing fat digestion particularly that of saturated fats (Pasquier *et al.,*1996**),**

Intestinal viscosity and the deleterious effects associated with increased viscosity of the intestinal contents can be counteracted by enzyme supplementation (Bedford *et al*., 1991; Almirall *et al*., 1995). Research has demonstrated that incorporation of hydrolytic enzymes such as cellulases and hemicellulases (xylanase or mannanase) in poultry rations enhances feed efficiency and nutrient digestibility (Chesson, 1987; Campbell and Bedford, 1992; Jackson *et al*., 1999).

2.10.2 Health and environmental benefits of enzymes

With increasing consumer concerns about the use of growth promoters and antibiotics in livestock production, enzymes have become popular because they are natural products of fermentation and therefore pose no threat to the animal or the consumer.A study conducted by Morgan and Bedford (1995) showed that coccidiosis can be prevented by using enzymes. Birds fed a wheat-based diet with and without glycanase supplementation showed vastly different responses to coccidiosis challenge. Growth was depressed by 52.5% in the control group but by only 30.5% in the enzyme group, which also had a much better lesion score.

Approximately 50-80% of the phosphorous contained in vegetable feeds for poultry is bound in phytates. Adding phytase to poultry diets augments the animals' own digestive enzymes, enabling chicken to break down and use more of the bound phosphorous in certain feed ingredients. Because phytase releases the bound phosphorous in feed, more phosphorous is available to the chicken and less phosphorous is excreted. In this way, phytase supplementation in poultry feed provides not only a nutritional benefit but also an environmental benefit.

2.11 Mannan degrading enzymes

Complete degradation of β-mannans requires a concerted action of several enzymes; for instance, a study conducted by Reid and Edward (1995) suggested that at least three enzymes, including α-1,6-β-galactosidase, β-1,4-mannanase and β-1,4-mannosidase, are required for the digestion of β-galactomannans an NSP found in soybean hulls. β-Mannanase catalyzes the random hydrolysis of β-1,4-mannosidic linkages in the main chain of mannan polymers producing oligosaccharides of various lengths. Further hydrolysis by α-galactosidase removes the side groups while β-mannosidase and β-glucosidase catalyze the removal of terminal mannose and glucose residues, respectively (Franco *et al.,* 2004; Stalbrand *et al.,* 1993). Several fungi and bacteria secrete these enzymes simultaneously when growing on mannan-based polysaccharides. β-mannanase from these microorganism has been widely studied focusing mainly on enzyme systems from fungal species such as *Trichoderma* and *Aspergillus*, *Sclerotium* (Puchart *et al*., 2004; Ademark *et al*., 1998; Gübitz *et al.,* 1996).

2.12 Mode of action of β-mannanase

β-mannanase (Endo-1,4-β-mannanase, EC.3.2.1.78) are hydrolytic enzymes which catalyze randomly the breaking of β-1,4 mannosidase linkages within the backbones of mannan, galactomannan, glucomannan and galactoglucomannan (Stoll *et al.,* 1999).The mode of action of β-1,4-mannanase on a substrate often depends upon the source of the enzyme as well as the type of mannan (de Vries and Visser, 2001).Its action causes a rapid decrease in the viscosity of polysaccharide solutions, thus increasing accessibility of the polymer to other enzymes. β-1,4-mannanase releases linear and branched manno-oligosaccharides of various lengths. These are then further hydrolyzed into monomers by β-mannosidase and α-galactosidase (Kremnický and Biely, 1997). Hydrolysis of substituted or branched polysaccharides by β-1,4-mannanases is

hindered by the degree and pattern of galactose substitution on the mannan backbone and the distribution of glucose within the main chain (de Vries, 2003).

2.13 Benefits of β-mannanase on animal performance

Research done with swine and broilers (Jackson *et al*., 1999; Teves *et al*., 1988) proposed that the benefits of β-mannanase come from the release of entrapped nutrients by disrupting the cereal cell walls and allowing a greater digestion of encapsulated nutrients, which in turn improves the nutrient and energy utilization values. β-mannanase can reverse the negative impact caused by β-galactomannan which interferes with glucose metabolism and insulin secretion rates in swine (Jackson *et al*.,1999). The suppression of insulin secretion can impair the intestinal uptake and utilization of glucose and amino acids in peripheral tissues by monogastric animals. Daskiran *et al*. (2004) attempted to use β-mannanase supplementation of broiler diets containing varying levels of β-mannan. They used guar gum to alter the dietary β-mannanlevels. They reported that body weight was reduced with the addition of guar gum and the addition of β-mannanase restored body weight to control values. Studies conducted by Jackson *et al*. (2004) also indicated that mannanase treatment of SBM improved the feed efficiency and BW of broilers. This improvement was attributed to increased availability of nutrients and also apparent metabolizable energy in the mannanase treated SBM.

Among the products of β-D-mannan hydrolysis by β-mannanase are mannoligosaccharides which are expected to beneficially affect the host. There are studies demonstrating that dietary mannanoligosaccharides could improve chicken health, either by interfering with bacterial attachment to the epithelial cell (Spring *et al*.,2000) or by enhancing the immune system (Newman and Newman 2001; O'Quinn *et al*., 2001), which may partially contribute to the improvement in BW gain in diets with β-mannanase (Sundu *et al*., 2006).

3.0 EXPERIMENT ONE:

PERFORMANCE AND EGG QUALITY TRAITS OF HENS FED DIETS SUPPLEMENTED WITH β-MANNANASE IN RESPONSE TO DIETARY PROTEIN AND ENERGY CONTENT.

3.1 Introduction and objectives

Kingori (2012) noted that approximately 30% of eggs produced are downgraded due to both external defects and internal defects. External defects include shell quality, cleanliness, shape, texture and soundness while internal defects are in the yolk and albumen and include blood and meatspots, double yolks, mottled and discolored yolks, rotten eggs, watery whites, discolored whites and round worms. A variety of factors including genetics, hen age and body weight and diet influence the egg quality (Silversides and Budgell, 2004; van den Brand *et al.*, 2004). Measures of egg quality include Haugh units, and shell strength and thickness. The Haugh unit is a major indicator of albumen quality in eggs. It is the relationship between the weight of the egg and the thickness of the albumen (Stadelman and Cotterill, 1995). The higher the Haugh unit value, the firmer the egg, and the higher the quality. In the egg industry, the eggshell strength and thickness acts as a packaging material and its quality is essential to consumer safety and selection. Jackson *et al.* (1999) demonstrated that β- mannanase increased egg size in a study conducted using a corn-soybean meal diet for laying chickens. Several other studies have shown improvement in feed conversion with the addition of β- mannanase to layer diets (Wu *et al.*, 2005).

A study was done to evaluate the effects of β-mannanase on the performance of laying chickens and on egg quality. Specific objectives of the study were as follows:

i) Evaluate the effects of dietary energy and protein levels on performance of laying chickens and egg quality.

ii) Determine the effects of adding β-mannanase enzyme to layinghen diets based on corn and soybean meal on layer performance and egg quality.

iii) Assess the interaction between β-mannanase enzyme and dietary energy and protein levels on laying performance and egg quality in laying chickens fed corn-SBM-based diets.

3.2 Materials and Methods

3.2.1 Experimental diets

Four standard corn-SBM-based diets (CS) were formulated to meet the National Research Council (NRC, 1994) nutrient requirements for laying hens more than 18 weeks old as shown in Table 2 below. Diet 1 and 2 contained approximately 2,865 kcal of ME and 18.5% crude protein per kilogram to form the adequate ME&CP diet group. Diet 3 and 4 were low energy and low protein diets containing 2,715 kcal ME and 17.5% crude protein per kilogram of diet (low ME&CP). β- mannanase was added to the diets at 0% and 0.04 % of the diets as shown in Table 2.

Each diet constituted an experimental treatment and was fed to a group of 5 birds, replicated 8 times making a total of 40 birds per treatment. Feed and fresh water was provided for *ad libitum* consumption.

3.2.2 Management of birds and experimental design.

One hundred and sixty birds, 16 week old Lohmann hens were housed in cages where light was provided for 16 hours. Room temperature was set and maintained at 29°C. Feed and water were provided *ad libitum.* The birds were weighed and assigned to 4 treatments, each replicated 8 times with five birds per replicate making a total of 40 birds per treatment. Birds were housed in cages measuring 30.5x40.6 cm, providing a floor space of 1239 square centimeters per bird.

3.2.3 Data collection methods

Egg production was recorded daily while feed consumption was computed on a weekly basis. Egg quality parameters were determined on a bi-weekly basis. Body weight gain was obtained by weighing the hens at the beginning and end of the experiment.

Egg mass (g of egg/ hen per d) and feed conversion (g of feed/g of egg) were calculated from egg production, egg weight and feed consumption. The experiment was divided into three 4-week phases.

3.2.4 Assessment of egg quality parameters

3.2.4.1 Egg specific gravity

This was determined on a bi-weekly basis using 4 gradient saline solutions; 1.070, 1.075, 1.080, and 1.085 according to the method of Holder and Bradford (1979).

3.2.4.2 Egg weight

This was measured using all eggs produced during 2 consecutive days by weighing the eggs individually.

3.2.4.3 Albumen heights

These were measured using a micrometer and Haugh Units calculated using the formula described by Roush (1981).

3.2.4.4 Eggshell thickness

This was determined using a caliper after washing and drying the shells at room temperature.

TABLE 2: Composition of experimental diets used in Experiment 1

	Experimental diets	
Ingredient (%)	**Adequate ME&CP**	**Low ME&CP**
Corn	55.95	62.66
Soybean meal (SBM)(46.4% CP)	27.20	23.65
Soy oil (9200)	3.25	0.00
Limestone (38)	8.45	8.51
Shell/Bone builder (38)	1.55	1.49
Dicalcium phosphate (21/17)	1.79	1.81
Lysine (79%)	0.090	0.160
DL-Met	0.130	0.130
Mineral premix[1]	0.50	0.50
Threonine	0.09	0.09
Vitamin premix[2]	1.00	1.00
Total	100.00	100.00
Calculated nutrient composition		
CP (%)	18.52	17.51
ME (kcal kg^{-1})	2,866	2,716
Calcium(%)	4.20	4.20
Available phosphorus (%)	0.45	0.45
Lysine (%)	0.98	0.74
Methionine (%)	0.46	0.45
Met+Cys(%)	0.76	0.95
Analysed nutrient composition		
CP (%)	17.27	16.52
Gross energy(kcal kg^{-1})	3,554	3,466

[1]Provided the following per kilogram of diet: Mn, 137.9 mg; Cu, 18.3 mg; Fe, 212.8 mg; Zn, 123.5 mg; Se, 0.3 ppm; I, 0.6 mg;

[2]Provided the following per kilogram of diet:vitamin A, 9,000IU; vitamin D3, 3,000 IU; vitamin E, 27.0 IU; riboflavin, 6.3 mg; pantothenic acid, 17.1 mg; niacin, 93.3 mg; folic acid, 1.4 mg; vitamin B12, 0.02mg; menadione, 2.2 mg.

3.2.5 Chemical analysis

Samples of the diets were analyzed for their contents of nitrogen, calcium, phosphorus, dry matter and gross energy.

3.2.6 Statistical analysis

Data were subjected to ANOVA by using the GLM procedure of SAS, Inst. Inc., Cary, NC, with energy/protein level and enzyme as the main effects. If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. Data were presented as least square means with respective standard errors of the mean. Significance was defined as $P < 0.05$.

3.3 RESULTS: EXPRIMENT 1

3.3.1 Effects of energy and protein levels and β-mannanase on feed intake and egg production

No significant differences were observed in hen day egg production. The effects of energy and protein levels and β-mannanase on the above parameters above are shown in Table 3 below. Dietary energy and protein regimes had a significant effect ($P<0.05$) on feed intake in the second and third phases andon overall feed intake. Feeding the birds with low-ME&CP diets significantly increased ($P<0.05$) their feed intake (Table 3). An interaction between enzyme and diet was observed in the third month of the study. Enzyme supplementation of the low-ME&CP diet resulted in lower feed consumption ($P<0.05$) compared to the low-ME&CP diets without enzyme but similar with feed consumption of birds fed adequate-ME&CP diets with or without enzyme.

The effect of diet and enzyme on feed conversion ratios is presented in Table 5. During the second phase and over the course of the experiment, birds given adequate-ME&CP diets had significantly better feed conversions (P<0.05) than those on the low-ME&CP diets.

3.3.2 Effects of energy and protein levels and β-mannanase on egg quality

No significant differences were observed in Haugh Units, and eggshell breaking strength among the 4 dietary treatments for the 12-week period. The effects of β-mannanase and ME&CP level on egg quality performance are presented in table 4 below. There was a significant interaction between enzyme and ME&CP level where egg weights of hens fed adequate-ME&CP diets were increased with addition of mannanase (P<0.05).

TABLE 3: Effects of β-mannanase on feed intake and egg production

Dietary treatment		feed intake (g feed/hen/day)				Egg Production (%)			
ME&CP Level	**Enzyme**	**Month 1**	**Month 2**	**Month 3**	**Overall**	**Month 1**	**Month 2**	**Month 3**	**Overall**
Adequate	without	106.4	115.2	109.8 [a]	110.7	87.6	95.8	92.9	92.1
Adequate	with	105.5	117.2	112.8 [a]	111.8	83.8	96.6	96.0	92.2
Low	without	109.4	119.8	122.1 [b]	116.7	88.8	95.0	93.9	92.6
Low	with	110.6	119.8	113.5 [a]	114.9	93.5	97.0	96.9	95.8
SEM		2.42	1.42	1.60	1.11	3.80	1.63	1.82	1.88
P-value									
Diet		0.106	0.018	0.001	0.001	0.164	0.892	0.610	0.284
Enzyme		0.946	0.493	0.096	0.750	0.908	0.402	0.111	0.392
Diet*Enzyme		0.668	0.492	0.001	0.190	0.279	0.724	0.981	0.409

a,b Values within the same column with no common superscripts differ (P < 0.05).

TABLE 4: ***Effects of β-mannanase on egg weight and Eggshell Thickness***

Dietary treatment		Egg Weight (g)				Eggshell Thickness (µm)			
ME&CP Level	**Enzyme**	**Month 1**	**Month 2**	**Month 3**	**Overall**	**Month 1**	**Month 2**	**Month 3**	**Overall**
Adequate	without	63.1[b]	63.7	63.1	63.3[ab]	15.35	15.11	14.80	15.09
Adequate	with	65.0[a]	64.8	64.6	64.8[a]	15.31	15.19	15.44	15.31
Low	without	63.3[b]	63.9	63.2	63.5[ab]	15.09	14.87	14.47	14.81
Low	with	62.3[b]	62.5	60.1	61.6[b]	15.17	14.71	14.41	14.76
SEM		0.71	0.67	1.22	0.67	0.137	0.138	0.286	0.152
P-value									
Diet		0.104	0.134	0.081	0.036	0.153	0.014	0.025	0.011
Enzyme		0.534	0.787	0.503	0.781	0.899	0.771	0.32	0.571
Diet*Enzyme		0.048	0.070	0.069	0.019	0.652	0.39	0.232	0.375

a,b Values within the same column with no common superscripts differ (P < 0.05).

TABLE 5: Main effects of β-mannanase on FCR

	FCR (g of feed/g of egg)			
	Month 1	**Month 2**	**Month 3**	**Overall**
ME&CP level				
Adequate	1.99	1.88	1.86	1.91
Low	1.93	1.98	2.04	1.99
β-mannanase				
Without	1.96	1.94	1.99	1.96
With	1.97	1.93	1.91	1.94
SEM	0.071	0.042	0.086	0.048
P-value				
Diet	0.466	0.023	0.042	0.117
Enzyme	0.848	0.742	0.394	0.606
Diet*Enzyme	0.371	0.912	0.941	0.644

a,b Values within the same column with no common superscripts differ (P < 0.05)

4.0 EXPERIMENT TWO:

EVALUATION OF *IN VIVO* NUTRIENT DIGESTIBILITY AND AMEn IN LAYER CHICKENS FED DIETS SUPPLEMENTED WITH β –MANNANASE

4.1 Introduction

Mannan and its derivatives are potential anti-nutritive factors commonly found in poultry feed ingredients such as soybean meal, sesame meal and guar gum (Dierick, 1989; Rogel and Vohra, 1983). Corn distillers' dried grainswith solubles (DDGS), a by-product of the ethanol production industry, also contains substantial amounts of β-mannans (Tucker *et al*., 2004). Due to their highly viscous properties, mannans are intensely anti-nutritional. It has been shown that this group of NSP reduces feed conversion and decreases the efficiency of carbohydrate utilization inmonogastric animals (Dale, 1997). Research done with broilers has demonstrated that β-mannanase improved dry matter digestibility and the AME of corn-SBM-based diets (Jackson *et al*., 2004; Kong *et al*., 2011). Research investigating the effect of β-mannanase on nutrient digestibility and AMEn in layers is inadequate. Therefore, the objective of this study was to evaluate the effects of adding β-mannanase to layer chicken diets on digestibilities of protein, amino acids, calcium and phosphorus, and the nitrogen-corrected apparent metabolizable energy (AMEn).

4.2 Materials and methods

4.2.1 Experimental diets

Two diets were formulated to meet the National Research Council (NRC, 1994) nutrient requirements for layers. Diet 1 was corn-SBM based whereas Diet 2 was corn-SBM-DDGS

based. Each of the two diets was supplemented with either 0% or 0.04% β-mannanase to make 4 dietary treatments as shown in Table 6 below:

TABLE 6: Ingredient and nutrient composition of experimental diets: Experiment 2

	Experimental diets	
Ingredient (%)	**CS**	**CSD**
Corn	56.5	51.82
Soybean meal (SBM)(46.4% CP)	27.76	21.95
DDGS	0	10
Soy oil (9200)	2.77	3.25
Limestone (38)	8.53	8.7
Shell/Bone builder (38)	1.56	1.56
Dicalcium phosphate (21/17)	1.35	1.07
Lysine (79%)	0.07	0.19
DL-Met	0.156	0.156
Mineral premix[1]	0.5	0.5
Threonine	0	0
Vitamin premix[2]	0.5	0.5
Chromic Oxide	0.3	0.3
Total	56.5	51.82
Calculated nutrient composition		
CP (%)	18.03	18.03
ME kcal kg^{-1}	2,851	2,851
Calcium	4.20	4.21
Available phosphorous	0.44	0.44
Lysine	0.98	0.97
Methionine	0.46	0.48
Met+Cys	0.77	0.77
Analysed nutrient composition		
CP (%)	17.27	16.52
Gross energy(kcal kg^{-1})	3,554	3,467

[1]Provided the following per kilogram of diet: Mn, 137.9 mg; Cu, 18.3 mg; Fe, 212.8 mg; Zn, 123.5 mg; Se, 0.3 ppm; I, 0.6 mg;
[2]vitamin A, 9,000IU; vitamin D3, 3,000 IU; vitamin E, 27.0 IU; riboflavin, 6.3 mg; pantothenic acid, 17.1 mg; niacin, 93.3 mg; folic acid, 1.4 mg; vitamin B12, 0.02mg; menadione, 2.2 mg.

Each diet was fed to 6 groups of birds with 3 birds in each group. Birds were acclimated to the experimental diets for 9 days before the onset of the experiment. Thereafter, excreta were collected from each cage for the subsequent 3 days.

4.2.2 Management of experimental birds

Seventy two 41-week old Lohmann layer chickens were kept in confined cages with a 16-h light regimen. Room temperature was set and maintained at 29°C. Feed and water was available for *ad libitum* consumption. The birds were weighed and randomly allocated into 4 treatments. Each diet was fed to 6 groups of birds with 3 birds in each group. The cage dimensions were 30.5x40.6 cm, providing 413 cm^2 per bird.

4.2.3 Data collection

The excreta was collected 3 times a day and immediately frozen at -20°C. The excreta samples from each hen were pooled for the 3-day collection period. Thereafter, it was thawed, weighed, homogenized then freeze-dried. Freeze-dried excreta and feed were ground and stored at room temperature until analysis.

On the last day of the experiment, all birds were killed by cervical dislocation. The contents of jejunum (from the end of the duodenum to Meckel's diverticulum) were obtained for the determination of digesta viscosity. Contents of the lower part of the ileum were collected and digesta samples were pooled. The samples were frozen immediately at −20°C pending determination of apparent ileal nutrient digestibility.

4.2.4 Sample preparation and chemical analysis

Samples of the diets and excreta were analyzed for their contents of nitrogen, calcium, phosphorus, dry matter, gross energy and chromic oxide. Samples of the diets and digesta were also analyzed for amino acids.

Dry matter was determined according to the AOAC procedures (Procedure 4.1.06; AOAC, 1998), and gross energy was determined using the Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL). Nitrogen (N) was determined using the Leco Nitrogen analyzer and crude protein calculated as % *N X 6.25*. Samples for calcium and phosphorus analyses were ashed and acid digested according to AOAC (1990) procedures (method 990.08) and read on a Varian inductively coupled plasma mass spectrometer. Samples for amino acid analysis were prepared by acid hydrolysis according to AOAC procedures (Procedure 4.1.11 alternative 3; 1998). Samples for analysis of sulphur-containing AA (methionine and cysteine) were subjected to performic acid oxidation prior to acid hydrolysis. Feed, digesta, and excreta samples were analyzed for chromic oxide using the procedures described by Williams *et al*. (1962).

4.2.5 Calculations and statistical analysis

Apparent total tract digestibility of nutrients and digestibility of nitrogen was calculated using the following equation:

% Apparent nutrient digestibility = $100 - \{[(Cd/Cf) \times (Nf/Nd)] \times 100\}$

Where Cd = chromium oxide (CrO_2) concentration in the diet, Cf = CrO_2 concentration in the excreta; Nf =nutrient concentration in the excreta. Nitrogen-corrected apparent metabolizable energy (AMEn) content of experimental diets was calculated using the following equation:

AMEn (kcal/kg of diet) = GE kcal/kg diet − [GE kcal/kg excreta × (CrO_2% diet/ CrO_2% excreta)] − 8.22 × {N% diet − [N% excreta × (CrO_2% diet/ CrO_2% excreta)]},

Where GE is gross energy, N is nitrogen, CrO_2 is chromic oxide, and 8.22 is the energy equivalent of uric acid nitrogen, that is, 8.22 kcal/kg of uric acid nitrogen.

Data were subjected to ANOVA by using the GLM procedure of SAS, Inst. Inc., Cary, NC, with energy/protein level and enzyme as the main effects. If differences in treatment means were

detected by ANOVA, Duncan's multiple range test was applied to separate means. Data were presented as least square means with respective standard errors of the mean. Significance was defined as $P < 0.05$.

4.3 RESULTS: EXPERIMENT 2

Results for the analysis of AID of amino acids, dry matter and crude protein of the layer chickens are summarized in Table 7. Only the main effects are presented for AID since there were no significant interactions of enzyme and diet on the digestibility. Neither diet nor enzyme had a significant effect on the viscosity of the ileal contents. The enzyme improved ileal DM digestibility in both diet types as shown in Table 8. There was a trend for an improvement ($P=0.07$) of ileal digestibility of CP with addition of enzyme to the diets. Diet type caused significant differences ($P < 0.05$) on AID of arginine, histidine, threonine, aspartate, glycine and proline, where birds fed DDGS containing diets had lower ($P < 0.05$) amino acid digestibility (Table 7). Addition of enzyme on either diet had no effect on AID of any amino acid.

There was a significant interaction of diet and enzyme on calcium digestibility. β-mannanase significantly increased ($P < 0.05$) the ATTD of calcium in birds fed DDGS containing diets but had no effect on the CS group (Table 8). Inclusion of DDGS in the diets resulted in a reduced ($P < 0.05$) total tract DM digestibility. Compared to the birds fed on CS diets, birds fed on DDGS containing diets had much higher ATTD of phosphorus ($P< 0.05$).There was significant ($P < 0.05$) diet*enzyme interaction on the AMEn where enzyme had no effect on the AMEn of DDGS containing diets but depressed AMEn of the CS group.

Table 7: Main effects of β-mannanase and DDGS on viscosity and apparent ileal digestibilities of DM, N and amino acids for layers fed the experimental diets

	DDGS		Enzyme			P Value		
	without	with	without	with	SEM[2]	DDGS	Enzyme	Interaction
Viscosity	1.99	1.88	1.97	1.90	0.1	0.324	0.504	0.412
Digestibility (%)								
DM	69.63	70.17	67.90	71.90	1.73	0.757	0.032	0.641
CP	78.90	77.13	76.02	80.00	2.14	0.417	0.078	0.458
Essenial amino acids								
Arg	87.94	84.55	87.29	85.20	1.58	0.045	0.201	0.804
His	61.79	55.27	61.16	55.90	3.42	0.071	0.140	0.444
Ile	81.86	78.46	81.18	79.15	2.08	0.118	0.340	0.643
Leu	84.95	83.26	84.99	83.23	1.43	0.250	0.233	0.964
Lys	84.68	80.68	83.56	81.80	2.43	0.116	0.477	0.614
Met	90.44	87.11	89.70	87.85	1.77	0.075	0.310	0.225
Phe	84.89	82.42	84.65	82.66	1.73	0.168	0.263	0.698
Thr	74.85	69.02	73.93	69.94	2.41	0.025	0.113	0.623
Val	79.73	76.85	79.08	77.49	2.09	0.183	0.456	0.800
Nonessential amino acids								
Ala	83.21	80.20	81.36	82.06	1.83	0.117	0.189	0.710
Asp	81.39	76.93	80.67	77.65	1.69	0.016	0.088	0.843
Glu	87.37	84.87	87.09	85.15	1.30	0.070	0.152	0.943
Gly	78.64	73.14	77.59	74.19	2.09	0.016	0.120	0.639
Pro	84.52	81.72	84.20	82.04	1.20	0.030	0.088	0.769
Ser	80.77	65.92	80.11	66.58	11.80	0.223	0.265	0.335
Tyr	81.27	80.89	80.92	81.25	1.99	0.849	0.871	0.124
Total AA	83.06	79.56	82.54	80.08	1.73	0.057	0.170	0.632

Amino acids: Ala = Alanine, Arg = Arginine, Asp = Aspartic acid, Cys = Cysteine, Glu = Glutamic Acid, Gly = Glycine, His = Histidine, Ile = Isoleucine, Leu = Leucine, Lys = Lysine, Met = Methionine, Phe = Phenylalanine, Pro = Proline, Ser = Serine, Thr = Threonine, Tyr = Tyrosine, and Val = Valine

[2]Standard error of mean

Table 8: Effects of β-mannanase and DDGS on total tract DM, N and energy utilization, and AMEnfor layers fed the experimental diets

DDGS	**without**	**without**	**with**	**with**		**P Value**		
Enzyme	**-**	**+**	**-**	**+**	**SEM[2]**	**DDGS**	**Enzyme**	**Interraction**
***ATTD* (%)**								
DM	66.83	65.64	63.57	65.17	0.91	0.054	0.825	0.143
N	42.58	38.84	40.11	41.86	2.02	0.892	0.630	0.190
Ca	51.91[b]	51.58[b]	50.12[b]	66.59[a]	3.12	0.047	0.018	0.014
P	24.45[b]	11.28[c]	40.64[a]	41.40[a]	4.06	0.001	0.143	0.103
Metabolizable energy (Kcal kg^{-1})								
AMEn	3,109.85[a]	2,961.76[b]	3,034.47[ab]	3,065.98[a]	27.39	0.604	0.046	0.004

a,b Values within the same row with no common superscripts differ (P < 0.05).

[2]Standard error of mean

5.0 DISCUSSION

Energy and protein level significantly affected FCR, feed intake, egg weight and eggshell thickness. Generally, birds fed the low-ME&CP diet exhibited higher feed consumption and produced lighter eggs than birds on the adequate-ME&CP diet. Overall egg production was higher than 90% in all groups and no significant differences were observed among the four dietary treatments. The Haugh Unit values recorded for the four treatment groups were within the range of freshly laid eggs (Essien, 1990).

During the first month, layers fed adequate ME&CP diets supplemented with enzyme laid heavier eggs compared to the rest (Table 6). This could be due to degradation of cell wall mannan, which led to the release of encapsulated starch, fat, protein and minerals, hence improving the egg weight. The analyses of month 1 and the overall 12 weeks (Table 6) show that improvement in egg weight was significant ($P < 0.05$) but only for birds fed on adequate ME&CP diets supplemented with enzyme. In the Low ME&CP group however, the enzyme decreased the overall egg weight.

Supplementation of low-ME&CP diets with the enzyme resulted in lower feed consumption and this was similar to the feed intake of birds fed adequate-ME&CP diets. Similarly, Jackson *et al* (1999) observed a decrease in feed intake when they supplemented layer diets based on corn and SBM with β-mannanase. These results may be indicative of improved nutrient utilization. Reduced feed intake may be as a result of increase in available energy due to the degradation of β-mannan to simple sugars. The study of Wu *et al*. (2005) suggested that *β*-mannanase is capable of increasing apparent energy digestibility of corn/SBM based diets in laying hens.

Several studies have shown the beneficial effects of β-mannanase on chicken performance. Reduction of digesta viscosity has been clearly demonstrated with an increase in mannan

digestion. Lee *et al* (2003) observed that *β*-mannanase supplementation resulted in a decrease in ileal viscosity caused by guar hull. Increase in intestinal viscosity impairs digestion by reducing digestive enzyme activities, depressing the absorption of nutrients and slowing gastric emptying (Read, 1986; Smits *et al*., 1997). In his study with swine, Rainbird *et al*. (1984) observed a 35-40% reduction in glucose and water absorption due to increased digesta viscosity associated with addition of guar gum to the diet. Corresponding with the above studies, Maisonnier *et al*. (2003) reported that 0.5% of guar gum supplementation significantly increased intestinal supernatant viscosity and decreased BW gain of broilers from day 7 to day 21.

Mannan hydrolysis by β-mannanase also leads to the release of various mannanoligosaccharides including mannotetraose, mannotriose and mannobiose. The production of mannanoligosaccharides may contribute to improved chicken performance because these oligosaccharides are known to have prebiotic properties which contribute to the maintenance of a healthy gut flora (Wallace and Chesson, 1995). The reduction of innate immune stimulation (Ross *et al*., 2002) and improved energy utilization (Radcliffe *et al*., 1999) would also result from β-mannan degradation by the enzyme.

In experiment II, the type of diet caused significant differences ($P < 0.05$) in the AID of arginine, histidine, threonine, aspartate, glycine and proline (Table 9). Overall, these amino acids had lower ($P < 0.05$) AID among birds fed diets containing DDGS than those fed diets without DDGS. The manufacturing process for corn DDGS may involve high temperatures which can result in damaged protein. These processes may affect the digestibility of lysine and other nutrients through the Maillard reaction (Parsons *et al*., 2006). This could be the reason for the observed differences in AID between the two dietary treatments. The high ATTD of phosphorus among birds fed on the DDGS-containing diets as compared to those fed on CS diets could be

due to the fact that the digestibility of phosphorous in DDGS is higher than in corn or SBM. The increase in dry matter utilization was in agreement with the findings of Kong *et al*. (2011) who also observed an increase in apparent ileal DM digestibility and apparent total tract DM digestibility in diets with β-mannanase supplementation.

The improvement in CP digestibility could explain the findings of the results of experiment 1 where enzyme supplementation in adequate-ME&CP diets caused differences in the egg weight. Previous studies have shown that increasing the CP content of the diet will increase egg production, egg weight and bird's weight (Larbier *et al*., 1993). However, it is well accepted that laying hens do not have specific need for protein but for amino acids, hence the level of protein is not expected to affect performance as long as the amino acid requirements are met (NRC, 1994). In addition, crude protein content has been shown to only affect egg size during the early stages of egg production. It is not clear as to how the enzyme improved utilization of calcium and crude protein since there was no significant effect of either diet or enzyme on ileal viscosity.

In this study, the enzyme reduced the AMEn of the corn/SBM (CS) diets and did not have any significant effect on the corn/SBM/DDGS (CSD) group. These results contradict with those of Kong *et al.* (2011), where β-mannanase supplementation significantly increased the AMEn of corn/SBM diets in broiler chickens. The results obtained in Experiment II also do not support the assumption that reduced feed intake observed in the enzyme-supplemented group in Experiment 1 was due to improved energy availability. A more plausible explanation could be that β-mannanase may have elicited improvements in glucose absorption and utilization therefore making available more energy for production.β-galactomannan has been shown to inhibit secretion of insulin in humans (Morgan *et al*. 1985) and swine (Leeds *et al*., 1980; Sambrook and

Rainbird, 1985). It hasalso been observed that β-mannans reduce glucose and water absorption in swine (Rainbird *et al*., 1984).

Smits *et al*. (1997) reported that β-mannans increase intestinal viscosity thereby slowing down the diffusion rate of substrates, digestive enzymes, and digestion end products and consequently affecting nutrient digestion and utilization. One of the major modes of action through which β-mannanase and most NSP degrading enzymes improve performance is through the reduction of viscosity. This is achieved through the partial degradation of the soluble NSP in question into smaller molecular weight polymers. However, results obtained in Experiment II clearly indicate that β-mannanase had no effect on the viscosity of the diets. According to many studies, viscosity has never been an issue when using corn/SBM diets.

Almirall *et al*. (1995) observed that deleterious effects associated with increased digesta viscosity are more pronounced in chicks as opposed to older chickens. Hence the timing and duration of the inclusion of enzyme could play a big role in the performance of laying hens. In addition, the increase in egg weight with enzyme supplementation could benefit the producers during the early stages of egg production when egg size is often an important factor affecting revenue. Animal responses to β-mannanase supplementation would be greater when using SBM with hulls (i.e. 44% CP) as opposed to SBM without hulls (i.e. 48% CP). This is because mannan is more concentrated in the hull fraction of SBM. James *et al*. (1998) showed that turkeys, fed 44% SBM had lower body weight(BW) and a higher average feed conversion ratio (FCR) than turkeys fed the 48%CP SBM.

6.0 CONCLUSION

This study shows that the addition of β-mannanase improved egg weight, apparent ileal DM and crude protein digestibility and apparent total tract digestibility of calcium in layers fed corn/SBM diets. Further research is needed to explain these positive effects by examining potential mechanisms of β-mannanase, which include reducing digesta viscosity and innate immune stimulation.

The effect of β-mannanase on the corn/SBM diets was not as pronounced. This is probably because of the low concentrations of mannan in SBM. This study can, however, be used as a platform to study β-mannanase effects on locally available ingredients such as copra meal whose mannan content has been shown to be much greater.

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