EFFECTS OF ENZYME COMPLEX (ALLZYME SSF) AND YEAST METABOLITES (DIAMOND V XPC) ON PERFORMANCE, IMMUNE RESPONSES, GASTROINTESTINAL MORPHOLOGY AND INTESTINAL MICROBIOTA IN BROILER CHICKEN (*Gallus domesticus*)

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

To my lovely son Trevor

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ABSTRACT

A study was done to determine the effects of yeast metabolites (Diamond V XPC) and enzyme complex (Allzyme SSF) on the performance of broiler chickens. 160 Abor Acre broiler chicks were fed on basal diets supplemented with either an enzyme complex of a protease, amylase, xylanase, β-glucanase, pectinase, cellulase and phyatase (0.2g/kg); yeast metabolites (1.25g/kg for broiler starter diet and 0.63g/kg for broiler finisher diet; or both. The basal diet without any feed additive served as a control. Birds were vaccinated with F-strain of Newcastle disease virus vaccine at 28 days of age and later given a booster dose one week later. One bird in each replicate (a total of four birds per dietary treatment) was slaughtered at 21 days of age (before vaccination) and at 42 days of age (after vaccination). During each slaughter, the length of rectum, caeca, ileum, duodenum, jejunum and jejuna villi were taken and recorded. Fresh gizzard, liver and bursa of Fabricius were weighed and recorded. During the last slaughter at 42 days, the abdominal fat pad was removed and weighed while fresh and the weight recorded. Performance parameters such as feed intake, weight gain, body weight, FCR and PER were recorded on 21st and 42nd day of age. Blood was collected during each slaughter and serum obtained by centrifugation to determine the immune response measured by antibody titer. Fecal materials were removed from the rectum for total bacterial counts, coliform counts and coccidian oocysts counts. There were no significant effects ($P<0.05$) of yeast metabolites or enzyme complex on growth performance and weight of internal organs. The treatment diets also had no significant effects ($P<0.05$) on bacteria count, coliforms, oocysts and on antibody titer. Broilers fed diets supplemented with yeast metabolites had longer jejunum and ceacum ($P<0.05$) at 42 days than those fed diets supplemented with enzyme complex. However, no significant effects of yeast metabolites and enzyme complex were found on the length of duodenum, ileum and rectum.

CHAPTER 1

1.0 INTRODUCTION

1.1 Background information

Agricultural sector is the dominant sector in the Kenyan economy accounting for approximately 22% of the country's Gross Domestic Product (GDP). The livestock sector contributes 12% of the GDP and 42% of agricultural GDP (Ministry of Livestock and Fisheries Development, 2008). This mainly comprises of dairy products, meat, eggs, hides, skins and wool from cows, sheep, goats and poultry.

Industrial livestock feeds are generally made for poultry, dairy cattle, beef cattle and pig industries. There are also additional feeds produced for the pet and fish industry. Currently, the poultry industry is the largest consumer of livestock feeds, accounting to 60% of the national feed production comprising of chick and duck mash, growers mash, layers mash, broiler starter and broiler finisher (Ministry of Livestock and Fisheries Development, 2008). Poultry population in 2006 was estimated to be 2.9 million broilers, 3.1 million layers, 28,000,000 indigenous birds and 683,000 other birds (Ministry of Livestock Development, 2006). Egg production in year 2006 was 644 million eggs from hybrid hens and 564 million eggs from indigenous hens (Ministry of Livestock Development, 2006). Chicken meat production was estimated at 4,731,399 broiler chickens slaughtered in year 2006 totaling estimated 7,097 MT of meat, 8 million indigenous birds totaling 11,180 MT of meat and 690,000 culled layers totaling estimated of 897 MT of meat (Ministry of Livestock Development, 2006).

The main objective in formulating poultry feed is to combine different feedstuffs to meet the nutrients requirement of poultry at the least cost. Primary feed ingredients used in poultry diets are cereal grains which are used mostly as a source of energy. These include maize, barley, oats,

wheat, pearl millets and sorghums. Leguminous seeds such as groundnut, soybean, cotton seed, sunflower and rapeseed contain higher crude protein content than cereals, but unfortunately, they have anti-nutritive factors which limit their utilization by non-ruminant animals. Other feed ingredients used in poultry are milling by-products like wheat bran, corn gluten meal, pollard, rice bran, soybean meal, cotton seed cake, sunflower cake and meal, rapeseed meal, and fish meal. Some of the feedstuffs used in poultry such as barley, oats and wheat bran are high fiber feedstuffs and may contain non-starch polysaccharides (NSP) such as cellulose, glucans, pentosans, xylose and arabinose which reduce feed utilization and birds' performance since birds do not possess endogenous enzymes capable of cleaving and digesting the insoluble NSP. For this reason, broilers do not utilize NSPs found in feed in high amounts efficiently (Annison, 1995). This also decreases the digestibilities of any other components found in feeds which in turn decreases their energy value and consequently the growth performance. Enzyme supplementation is recommended with the aim of enhancing the feeding values of such feeds. Other additives that can be used to improve the performance of broilers fed feed containing high amount of NSPs include; botanicals, organic acids, prebiotics and probiotics. Botanical feed additives are plant-derived compounds which are incorporated into diets to improve performance and to improve the quality of feed. Organic acids improve protein and energy digestibilities by reducing microbial competition with the host for nutrients and endogenous nitrogen losses, by lowering the incidence of subclinical infections and secretion of immune mediators, and by reducing production of ammonia and other growth-depressing microbial metabolites (Dibner and Buttin, 2002). Prebiotics are non-digestible food ingredients that affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). They are neither hydrolyzed nor absorbed by mammalian enzymes or

tissues; they selectively enrich one or a limited number of beneficial bacteria. Probiotics on the other hand, are live microbial feed supplements which affect the host animal by balancing intestinal microbes thus enhancing resistance to infection and resulting in reduction of infection and improving and stimulating immunity (Fuller, 1989; Blecha, 2000; Soderholm and Perdue 2001). The most common additives used in our poultry feed today are enzyme complex. However, probiotics (yeast cultures) are currently being introduced in feed industry but mostly in ruminant feed to improve the milk production of dairy cows. Putnam *et al*. (1997) and Robinson and Garrett, (1999) reported an improved milk production in dairy cows fed diets supplemented with yeast metabolites while Cole *et al*. (1982) showed an increased feed intake in beef cattle fed diets supplemented with yeast culture. Feed manufacturers have limited knowledge on use of probiotics to enhance performance in poultry and are unable to discriminate among available commercial feed additives. This study sought to provide information needed to compare the effects of enzymes and probiotics on broiler chicken performance.

1.2 Problem statement

Antibiotics have been used in the past as growth promoters in broilers diets to enhance growth performance. However, increased concerns on their causing resistance to human beings led to complete ban at the beginning of 2006 in Europe. Consequently, the poultry industry has been looking for substances that could replace antibiotics. Several substances and different strategies are under investigation to replace antibiotic growth promoter. Most investigated substances include organic acids, enzymes, probiotics, prebiotics and phytogenic products. In Kenya, poultry diets are commonly supplemented with enzymes to enhance feed utilization and improve growth. However, use of yeast metabolites is currently being used in ruminants to increase milk

production in dairy cattle and to improve weight gain in beef cattle. Little information is available on use of yeast metabolites for feeding non-ruminants. In this light, more information is required to determine the effects of yeast metabolites in poultry and swine. Using poultry as an example of non-ruminant, this study was done to determine the effect of yeast metabolites and enzyme complex in broilers performance.

1.3 Objectives of the study

Broad objective

To assess the effects of supplementing yeast metabolites (Diamond V XPC^{TM}) and enzyme complex (Allzyme SSF) in poultry diets on growth performances, gastro-intstinal morphology and immune response in broiler chickens.

Specific objectives

- To assess the effects of yeast metabolites and enzyme complex on growth performance, gastrointestinal morphology, weight of the liver, bursa and abdominal fat in broiler chicken
- To assess the effect of yeast metabolites and enzyme complex on intestinal morphology and immune response in broiler chicken.
- To determine the synergistic effects of enzyme complex and yeast metabolites in broilers diet

1.4 Hypothesis

H_1: Supplementation of broiler diets with yeast metabolites produces heavier and healthier birds than enzyme complex or control diets.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Use of Feed additives in animal nutrition

Feed additives are products used in animal nutrition for purposes of improving the quality of feed in order to improve the animals' performance and health. They are added after authorization from the relevant regulating agencies following scientific evaluation. There should be sufficient evidence that the products are not harmful to humans and animals and that they do not affect the environment negatively. Additives are compounds added to feed to serve a non-nutritional function (Lyons, 1994).

2.2 Feed additives used to enhance performance in poultry

2.2.1 Antibiotics

These are chemicals produced by living organisms (mold, bacteria) that have bacteriostatic or bactericidal properties. The primary reason for using antibiotics in poultry feeds is for their growth stimulating effect. They are also used to increase egg production, hatchability and shell quality in layers. In higher amounts, they are added to remedy pathological conditions (Richard *et al*., 2006).

2.2.2 Organic acids

Organic acids are any organic carboxylic acid of the general structure R-COOH. However, not all of these acids have effects on gut micro flora. The organic acid associated with anti microbial activity are short chain acids (C1-C7) and are either simple mono-carboxylic acids such as formic, acetic, propionic and butyric acids or are carboxylic acids bearing hydroxyl group such as lactic, malic, tartaric and citric acid. Organic acids improve protein and energy digestibility by

reducing microbial competition with the host for nutrients and endogenous nitrogen losses, by lowering the incidence of subclinical infections and secretion of immune mediators, and by reducing production of ammonia and other growth-depressing microbial metabolites (Dibner and Buttin, 2002).

2.2.3 Botanicals

Botanicals are plant-derived compounds incorporated into diets to improve performance and to improve the quality of food derived from the animals fed these products. Beneficial effects of botanicals in animals may arise from activation of feed intake and digestive secretions, immune stimulation, anti-bacterial, coccidiostatic, antihelmintic, antiviral or anti-inflammatory activities and particularly antioxidant properties. Most of secondary plant metabolites like flavonoids and glucosinolates have been suggested to act as antibiotics or as antioxidants (Caspar Wenk, 2003).

2.2.4 Enzymes

Ferket (1993) defined enzymes as special proteins that catalyse or accelerate the rate of specific chemical reactions in which the enzyme activity may be dependent on the substrate in a random manner or it may be through very specific sites on substrates such as fat, protein, or carbohydrates. In non-ruminants diets, exogenous enzymes are used to improve digestibility of a wide range of feed components such as fibre, phytate, protein, etc. Fibre-degrading enzymes are used to break down specially non-starch polysaccharides (NSP), which are large polymers, to smaller polymers to alleviate their anti-nutritive activities (Choct and Annison, 1992). This is reflected in better flock performance, better litter quality and improved bird health, which in turn, has a positive influence on total production costs (Saleh *et al*., 2005; Cowieson and Ravindran, 2008). Commercial enzymes have been reported to be effective when added to

poultry diets containing large amounts of Non-Starch Polysaccharides (NSP) such as wheat, barley, sorghum and oats due to well digestion of soluble and insoluble NSP (Hughes *et al*., 2000; Meng *et al*., 2005; Saleh *et al*., 2005; Selle *et al*., 2010).

NSP can be divided into water-soluble and water-insoluble fractions; fractions which have greater relevance to their nutritional values. Birds do not possess endogenous enzymes capable of cleaving and digesting the insoluble NSP. The water-insoluble NSP can be considered practically undigested by poultry and only soluble NSP is potentially digestible (Carré, 1993). However, soluble NSP are known to posses anti-nutritional properties through either encapsulating nutrients and/or depressing overall nutrient digestibility through gastro-intestinal modifications e.g. reducing the absorption in the small intestines. They can also entrap large amounts of water during digestion and form very viscous (thick gel-like) gut contents. Lower viscosity results in improved digestion (more interaction of digestive enzymes with feeds and more complete digestion), absorption (better contact between digested feed nutrients and the absorptive surface of the gut) and health (reducing moisture in manure and nutrients available for harmful gut microflora to proliferate and challenge the birds (Almirall *et al*., 1995).

2.2.5 Mode of action of feed enzymes

Enhance digestibility

The mechanism by which NSP reduces the rate of digestion is by increasing the viscosity of the digesta. Supplementation with exogenous enzymes has been shown to lower viscosity of intestinal contents and to improve digestibility of starch, protein and fat in broiler fed on diets containing wheat (Bedford, 1995). Lower viscosity results in improved digestion by increasing

the interaction of digestive enzymes with feeds, better absorption due to increased contact between digested feed nutrients and the absorptive surface of the gut and also results to better health due to reduced moisture in manure which subsequently reduces nutrients available for harmful gut microflora (Almirall *et al*., 1995).

The rate of digestion of feeds and the absorption of the products of digestion rely on the formation of a complex between the digestive enzyme and its substrate and the subsequent release of the enzyme/substrate complex. The products of digestion must pass through the intestinal lumen to the enterocyte for absorption to occur. Absorption of these products is through the gut wall by diffusion or active uptake of the nutrients. However, as the viscosity of a solution increases, the rate of diffusion decreases (Fengler and Marquardt, 1988). Thus increased intestinal viscosity encountered in chicks fed rye, barley, or oats reduces growth rate, feed efficiency and the apparent ME of the diet substantially. The rate of feed passage is also significantly reduced in birds fed rye- and barley- based diets (Salih *et a.l*, 1991) thus reducing the feed intake. The NSPs may also reduce digestibility by simply encapsulating the material inside the endosperm thus inhibiting the access of digestive enzymes to the starch, fat and protein. The nutritive value of cereals with high levels of soluble NSP can be improved by the use of feed enzymes. Enzymes need to cleave at a few places in the polysaccharide chain to reduce the viscosity of solutions and thus enhance the feed nutritive value.

Improve intestinal microorganism balance

The effects of enzymes on gut microflora were classified by Bedford (2000) into two phases: an ileal phase and a caecal phase. In the ileum, enzymes simply reduce the number of bacteria by increasing the rate of digestion and limiting the amounts of substrates available to the microflora.

In the caecal phase, enzymes produce soluble, poorly absorbed sugars which feed beneficial bacteria. The volatile fatty acids (VFAs) produced by such bacteria may be of benefit not only in controlling populations of Salmonella and Campylobacter species, but also in providing an energy source for the bird (Snel *et al*., 2002).

Also the composition of gut microflora in the proximal small intestine and in the gut wall was shown to be changed by the addition of xylanase (Vahjen *et al*., 1998; Danicke *et al*., 1999; Hubener *et al*., 2002). The authors correlated those effects of xylanase on the gut microflora with its effects on the viscosity of diet, which is well known as one of the major modes of action of enzymes. Inclusion of cereals rich in NSP increases the viscosity of the digesta, reduces apparent nutrient digestibility, alters bacterial profiles and gut physiology. By adding enzymes into a diet, the viscosity of the content is reduced and nutrient uptake and animal performance are improved (Bedford, 2001). High carbohydrate concentration within the ceaca of a bird leads to increased carbohydrate fermentation within the ceaca which supports high levels of Clostridium species. Under stress conditions, from coccidiosis, feed restriction, highly viscous diets, and etc., the Clostridium can move up the intestine by reverse peristalsis where it can proliferate leading to Necrotic Enteritis. The use of enzymes will reduce the concentration of carbohydrates in the gastrointestinal tract, which could reduce fermentative processes in the ceca thus reduce levels of Clostridium.

2.2.6 Prebiotics

Prebiotics are non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon (Gibson and Roberfroid, 1995). Prebiotics include non-digestible oligosaccharides such as fructo-oligosacchardies, soybean oligosaccharides, galacto-oligosaccharides, lactulose, and lactitol, which are dietary carbohydrates and proposed to have good prebiotic properties. Some other products being used as prebiotics include gluco-oligosaccharides, isomalto-oligosaccharides, and xylose-oligosaccharides (Gibson and Fuller, 2000). Although mannan oligosaccharides (MOS) have been used in the same manner as the prebiotics listed above, they do not selectively enrich for beneficial bacterial populations. Instead, they are thought to act by binding and removing pathogens from the intestinal tract and stimulation of the immune system (Spring *et al*., 2000).

2.2.7 Probiotics

Probiotics are live microbial feed supplements which beneficially affect the host animal by improving intestinal balance of microorganisms. Probiotics have a potential to reduce infections in poultry and subsequent contamination of poultry products (Irshad, 2006). In this study, yeast metabolites is used as an example of a probiotic. Yeast metabolites is the dried product composed of *Saccharomyces cerevisiae* and the media it was grown on, dried in such a manner as to preserve the fermenting activity of the yeast. The benefit of the yeast metabolites comes from the metabolites produced during the fermentation process (Coleman, 1996).

2.2.8 Mode of action of probiotics

Immune enhancement

Havenaar and Spanhaak (1994) reported that probiotics stimulate the immunity of the chickens in two ways: a) Flora from probiotic migrate throughout the gut wall and multiply to a limited extent. These flora and beneficial microorganisms in the gut compete for the adhering site in the intestinal wall and this prevents pathogenic bacteria from colonizing the intestinal wall thus preventing disease development (Fuller, 2000); b) Antigens released by the dead organisms are absorbed and thus stimulate an immune response in the system.

Probiotics also improve immunity by enhancing macrophage activity, specifically the ability to phagocytise microorganisms or carbon particles. They have been reported to increase production of antibodies (Havenaar and Spanhaak, 1994).

Competing for adhesion receptors

Most of the bacterial colonies adhere to the intestinal wall and so do the probiotics. This is the reason that the colonies are not swept away due to the peristalsis along the intestinal wall. This effect prevents the pathogenic bacteria from colonizing the intestinal wall thus preventing disease development. The mechanisms of pathogen inhibition by the intestinal microorganisms include competition for nutrient, production of toxic compounds e.g. volatile fatty acids, competition for binding sites on the epithelium and stimulation of the immune system (Fuller, 2000).

2.2.9 Advantage of probiotics over antibiotics

Continuous use of antibiotics results in the intestinal pathogenic micro flora developing resistance and also damage to beneficial micro flora that assist the digestive process. Probiotics

supplementation promotes a balanced intestinal microbiota thus enhancing resistance to infection and reduction to infection. Besides, there is no withdrawal period when using Probiotics as compared to antibiotics and no residual effects (Fuller, 2000).

2.3 Effect of yeast metabolites on broilers on performance

Yeast metabolites are used in poultry diets to enhance growth. Gao *et al*. (2008) reported that supplementation of broiler diet with 2.5 % yeast culture, produced heavier broilers with better feed conversion efficiency. Yalcin *et al*. (2008) reported that yeast metabolites (Saccharomyces cerevisiae) supplementation at the level of 2 g/kg increased body weight gain but did not significantly affect feed intake and feed efficiency in laying hens. Onifade and Babatunde (1996) reported that supplementation of dried yeast (Saccharomyces cerevisiae) to high fibre diets improved body weight gain and feed efficiency of broiler chicks, in broilers fed 0.15% and 0.45% of dried yeast. Studies by Shen *et al.* (2009) reported an increase in average daily gain in nursery pigs supplemented with yeast metabolites compared to the control. Similarly, Shareef and Al-Dabbagh (2009) found that supplementation of broilers with Saccharomyces cerevisiae had significant effects on feed intake and feed conversion efficiency compared to the control. The beneficial effects of Saccharomyces cerevisiae are attributed to the fact that it is a naturally rich source of proteins, minerals and B-complex vitamins. Yeast metabolites contains 1, 3-1, 6 D-glucan and Mannanoligosaccharides which are natural growth promoters for modern livestock and poultry production (van Leeuwen, 2005).

2.4 Effects of yeast metabolites on gastrointestinal morphology

Long villi are generally associated with superior gut health as well as improved nutrient absorption (Sims *et al*., 2004). The morphology of the gastrointestinal tract is greatly influenced by the diet of the animal (Santin *et al*., 2001). Due to the high cell turnover rate, the intestinal lining only needs to be exposed to a specific dietary factor for a short period of time for any changes to occur in the structure of the mucosa (Iji *et al*., 2001). Zhang *et al*. (2005) observed increased villi height and superior ileal mucosa development at 21 days in chickens supplemented with a yeast cell wall product prepared from Saccharomyces cerevisiae. Intestinal villi are tiny, finger-like projections that are approximately 0.5-1mm in length and project from the wall of the small intestine and have additional extensions called micro-villi which protrude from epithelial cells lining of the villi. Micro-villi increase the absorptive area and the surface area of the intestinal wall. Longer villi are associated with more mature epithelia and enhanced absorptive function due to increased absorptive area of the villus. This greater length increases the activities of enzymes secreted from the tips of the villi, Hampson, (1986), resulting in improved digestibility. Santin *et al.* (2001) observed an increase in jejuna villi height in the first week when broilers were supplemented with Saccharomyces cerevisiae. Yeast metabolites is used in poultry diet to increase the length of intestinal microvilli.

2.5 Effects of yeast metabolites on immune response

Mannanoligosaccharide is **a** naturally derived extract from the cell wall of Saccharomyces cerevisiae and their components include proteins, glucans and phosphate radicals as well as mannose (Klis *et al*., 2002). Mannanoligosaccharides contain protein which has relatively high proportions of serine, threonine, aspartic and glutamic acids, and a small quantity of methionine

(Song and Li, 2001). They promote intestinal micro-flora balance and also have immune-modulation properties. In a study done by Oliveira *et al*. (2009), broilers fed diet supplemented with MOS had higher titers against Newcastle Disease than those from birds fed diets without additives or only with enzymes. The reason for the positive effects of MOS on antibody titers against NDV was that it is derived from microorganisms, just like the yeast cells. Thus, this substance may be considered as a stranger compound to the host and result in increased immune response. The immune response is mediated by activation of macrophages which have mannans binding receptors, as demonstrated by McKenzie *et al*. (1998).

2.6 Effects of yeast metabolites on intestinal microbiota

The development of favorable microflora in the poultry gut can be enhanced by using probiotics especially during periods of stress (Krehbiel *et al*., 2003). Stress in poultry can be due to consumption of contaminated feed, bad weather, poor-management, transportation, poor housing conditions, changes in feed, presence of mycotoxins in feed, prolonged antibiotic therapy and disease. Kabir *et al*. (2004) reported that probiotic microorganisms produce a bactericidal or bacteriostatic substance. These substances inhibit harmful bacteria, by lowering of the gut pH making it impossible for pathogenic microorganisms to survive. In addition, competition for energy and nutrients between probiotic microorganisms and other bacteria may result in a suppression of pathogenic species.

CHAPTER 3

3.0 EFFECT OF YEAST METABOLITES AND ENZYME COMPLEX ON GROWTH PERFORMANCE AND GASTROINTESTINAL MORPHOLOGY IN BROILER CHICKEN.

3.1 ABSTRACT

A study was done to investigate the effects of yeast metabolites (Diamond V XPC) and enzyme complex (Allzyme SSF) on the performance and gastrointestinal morphology in broiler chicken. One hundred and sixty broiler chicks were fed on diets formulated according to KeBS specifications supplemented with either enzyme complex (0.02%) or yeast culture. Diet 1 was supplemented with yeast culture, Diet 2 with both yeast metabolites and enzyme complex, Diet 3 enzyme complex only and Diet 4 was the control diet without yeast metabolites or enzyme complex. One bird per replicate (a total of 16 birds) was slaughtered when they were 21 and 42 days old. Length of rectum, caeca, ileum, duodenum and jejunum and the weight of gizzard, liver and bursa of Fabricius and the height of intestinal villi recorded. Performance parameters such as feed intake, weight gain, body weight, FCR and PER were recorded on 21st and 42nd day of age. There were no significant effects ($P>0.05$) of yeast metabolites on growth performance and on carcass characteristics across treatments. However, birds fed diet supplemented with yeast metabolites had a trend of increased performance compared to the control diet. Enzyme complex had no effects ($P>0.05$) on broiler performance or gastrointestinal morphology.

3.2 INTRODUCTION

One of the major challenges faced by the poultry industry in the developing world is about improving efficiency of production. To meet this challenge and maintain the efficiency of feed utilization, series of attempts have been made by researchers. These include incorporation of

antimicrobials and other natural products, such as yeasts to animal feeds (Kung, 1992; Muihead, 1992). Live yeast addition to animal feed has been known to improve the nutritive quality of feed and performance of animals. Available literature suggest that use of microbial preparations have some beneficial effects in poultry production such as improvements in growth rate and feed efficiency, prevention of intestinal infections and improved nitrogen utilization (Mohan *et al*., 1996). Kanat and Calialar (1996) reported that active dry yeast effectively increases body weight gains without affecting feed/gain ratio in broiler chicks. Mateova *et al*. (2008) showed that the application of probiotics and prebiotics significantly improved the weight gain of broiler chicken. Paryad and Mahmoudi (2008) showed that the inclusion of 1.5% Saccharomyces cerevisiae yeast in broilers ration improved bodyweight gain, feed intake and feed conversion ratio.

3.2.1 Objective

The objectives of this study were to assess the effects of yeast metabolites and enzyme complex on body weight, weight gain, feed intake, feed conversion ratio, protein efficiency ratio, abdominal fat pad and gastrointestinal morphology in broiler chicken.

3.3. MATERIALS AND METHODS

3.3.1 Study site

The research work was done at the University of Nairobi, College of Agriculture and Veterinary Sciences (CAVS) in the poultry unit, Animal Production Department.

3.3.2 Experimental diets

Two basal diets consisting of broiler starter and broiler finisher diets were formulated according to Kenya Bureau of Standard (KeBS). Maize grain and pollard were used as the main sources of energy while soybean meal, fishmeal and corn gluten meal were used as the protein sources.

The broiler starter was formulated to contain approximately 3000 kcal of metabolizable energy (ME) per kilogram of feed, protein content of 220 grams per kilogram (DM basis), calcium and available phosphorus levels in the diets was 1% and 0.4% respectively while lysine and methionine levels was 5 % and 2 % of protein respectively. Broiler finisher was formulated to contain 2900 Kcal of metabolizable energy (ME) per kilogram of feed, protein content of 180 grams per kilogram feed, calcium and available phosphorous level of 1% and 0.4% respectively while lysine and methionine levels was 5 % and 2 % of protein respectively (NRC 1994) as shown in Table 1

3.3.3 Additives used

The additives used in this experiment were enzyme complex and yeast metabolites (Diamond V XPC). Enzyme complex is an enzyme complex containing protease, amylase, xylanase, beta-glucanase, pectinase, cellulase and phytase that is used in broiler diets to improve performance. Diamond V XPC (Yeast culture) is the dried product composed of Saccharomyces cerevisiae and the media of processed grain by-products, roughage products, cane molasses, malt, and corn syrup and dried in such a manner as to preserve the fermenting activity of the yeast.

Treatment diet 1 was prepared by mixing the formulated feed (broiler starter or broiler finisher) with yeast metabolites (Diamond V XPC), treatment diet 2 was the formulated diet supplemented with both yeast metabolites and enzyme complex, treatment diet 3 was the formulated diet supplemented with only enzyme complex and treatment 4 was the control diet with no yeast metabolites or enzyme complex.

The inclusion rate for the yeast metabolites was 1.25g/kg for broiler starter diet and 0.63g/kg for broiler finisher diet while that of enzyme complex was 0.2g/kg in both broiler starter and broiler finisher. The reason for using this inclusion rate is because some researchers had used a higher level and some a lower level and reported a significant benefit on performance. For example, studies done by Paryad and Mahmoudi (2008) at an inclusion rate of 1.5% and Onifade and Babatunde (1996) at an inclusion rate of 0.001%. This study used the above inclusion rate which was at a middle level to access the efficacy of yeast metabolites.

Table 3: Ingredient composition (g/kg) of the basal diets

	Broiler starter	Broiler finisher
Maize grain	480	350
Pollard	150	200
Wheat bran	0	230
Corn oil	30	60
Soybean meal	170	10
Fishmeal	100	90
Corn gluten meal (60%)	50	50
Dicalcium Phosphate	1	0
Limestone	10	0
Broiler premix	2.5	2.5
Coccidiostat	1	1
Iodized salt	5	5
Totals	**999.5**	**998.5**
Composition of formulated diets		
Metabolizable energy (ME) (Kcal/kg)	3000	2900
Crude protein (%)	22	18
Calcium (%)	1	1
Available phosphorous (%)	0.4	0.4
Crude fiber (%)	6	6
Lysine (% of protein)	1.1	0.9
Methionine (% of protein)	0.5	0.4

3.3.4 Experimental procedure

One hundred and sixty Arbor Acres day-old chicks were obtained from a commercial hatchery. The chicks were grouped into four batches of forty chicks and randomly assigned to the four treatment diets in a complete randomized design (CRD) experiment.

Each group was further subdivided into four replicates of ten chicks per replicate. Chicks were weighed and put into the experimental metal cages. Each cage measured 1.0 m by 1.0 m therefore providing a floor space of $0.1m^2$ per chick. The floor was covered with wood shaving about 10 cm deep. For the first four weeks, the cages were heated using infrared bulbs. Temperatures were maintained at 32°C in the first week and this was reduced by 2°C every week by adjusting the height of the bulb to 26°C by the end of the third week. Feed wastage was monitored by carefully collecting sieving and weighing all the uneaten feed on the floor which was then subtracted from the initial feed offered. Feed and water was provided *ad-libitum.* The first experimental phase was growing phase (1^{st} day to 21^{st} day) followed by finishing phase (22^{nd} to 42^{nd} day).The feeding trial lasted for six weeks. Broiler chicks were fed on broiler starter mash for the first three weeks and later fed on the broiler finisher for the last three weeks.

3.4 Data collection

3.4.1 Chemical analyses

The basal diets were subjected to proximate analysis using AOAC, (2000) procedures. Calcium and phosphorus contents were determined using atomic absorption spectrophotometry and calorimetry respectively.

3.4.2 Growth performance

Body weight gain and feed intake measurements were determined weekly. Body weight gain was the difference in weight between two consecutive weighing. Feed intake was the difference in feed offered and feed left over at the end of every week. Feed conversion ratio was determined as the ratio between feed intake and body weight gain while the Protein efficiency ratio (PER) was calculated as the ratio of the body weight gain and protein intake. Mortality was assessed daily by counting the number of birds that died throughout the experimental period.

3.4.3 Gut morphology

One bird per replicate (a total of four birds per dietary treatment) was slaughtered when they were at 21 and 42 days of age. Rectum, caeca, ileum, duodenum and jejunum were removed and their length measured using a one-meter ruler and the weight of the fresh gizzard taken. Villi height and mucosa thickness were measured from the jejunal section of the intestines and the length of different sections of the intestines measured and weight of internal organs taken. On 42^{nd} day, the abdominal fat pad was removed and weighed. The abdominal fat was used as an indicator of carcass fat content and was calculated as a percentage of body weight. Longitudinal and transverse samples of mid-portions of jejunum were fixed in 10% formalin. They were processed routinely, embedded in paraffin wax and sectioned on microtome at a thickness of 5 μm. The sections were stained with haematoxyline and eosin (H&E). The heights of the villi and the mucosa thickness, in μm, were then measured using a Leica DM 500 light microscope (Leica Microsystem Cambridge Limited) with Leica Application Suit system and a magnification of X4. The height of villi and the mucosa thickness were then converted into millimeters (mm).

3.4.4 Weight of the liver, bursa of fabricius and abdominal fat pad

During each slaughter, liver and bursa of fabricius were removed and their weight taken using a weighing balance while still fresh and recorded. At 42 days of age, the abdominal fat pad was removed, fresh weight taken and recorded.

3.5 Statistical analysis

All data were analyzed using one way analysis of variance (ANOVA) procedures (Steel and Torrie, 1980) appropriate for a completely randomized design by the GENSTAT package (2000). The level of statistical significance was preset at $P \leq 0.05$. Significant treatment means were separated using orthogonal linear contrasts.

3.6 RESULTS AND DISCUSSION

3.6.1 Raw material and experimental diets

Table 2 below shows the chemical compositions of the basal diets used which was within the NRC (1994) specifications. The gross energy contents were 3200 Kcal/kg of diet in the broiler starter diet and 3000 Kcal/kg of the diet in the broiler finisher diet. It was not possible to analyze for metabolizable energy and therefore used gross energy for this study. The NRC specifications for energy in broiler starter and broiler finisher diets are 3000 Kcal/kg ME and 2900Kcal/kg ME respectively. It was not possible to determine the ME values in this study. The protein content was 26% in the broiler starter and 25% in the finisher diet. This was within the NRC specification of 22% for broiler starter diet and 18% for broiler finisher. Calcium and total phosphorus were 2.0% and 0.8% respectively in both starter diet and finisher diet.

Table 4: Chemical composition of the basal diet on DM Basis

	Broiler starter	**Broiler finisher**
Gross energy (Kcal/kg)	3125	3762
Moisture (%)	12.3	15.5
Dry matter (%)	87.7	84.5
Crude protein (%)	25.2	26.1
Crude fiber (%)	4.7	6.0
Ash (%)	9.7	5.7
Ether extract (%)	8.2	4.6
NFE (%)	40.1	42.1
Calcium (%)	2.1	2.0
Total phosphorous (%)	0.8	0.8

3.6.2 Broiler performance at 21 and 42 days of age

The effects of yeast metabolites and enzyme complex on feed intake, FCR, body weight gain and protein efficiency ratio are shown in Table 3.

3.6.3 Results on feed intake

There were no significant effects of yeast metabolites and enzyme complex on feed intake. The mean feed intake per bird was 974g/chick at 21 days and 1709g/chick at 42 days as shown on Table 3 which was close to NRC (1994) specification of 880g/chick and 1920g/chick at 21 days and 42 days respectively. Higher feed intake was noted for birds fed on diets supplemented with enzyme complex than those fed on the control diet during the starter phase (21 days), but the differences were not statistically significant. These results agreed with those of Nadeem *et al.* (2005) who reported an increase in feed intake with addition of enzyme from day 1 to day 28. Feed intake at 42 days in birds fed diet supplemented with enzyme complex was lower than in birds fed control diet though the difference was insignificant; this finding was in agreement with Hajati *et al*. (2009) and Hana Zakaria *et al*. (2010). Reduction in feed intake at 42 days in broilers fed diets supplemented with enzyme might be attributed to birds fulfilling their nutrients requirement by taking less amount of feed due to changes in digestibility of energy and amino acids rather than improved digestible nutrient intake (Hana Zakari *et al*., 2010, Samarasinghe *et al.,* 2000). The results of this study agreed with Zhang *et al*. (2005), who reported insignificant differences on growth performance of broilers supplemented with yeast metabolites for the first three weeks

Table 5: Effects of yeast metabolites and enzyme complex supplementation on body weight, feed intake, weight gain, feed efficiency, protein efficiency ratio and mortality

a. Day 21

	Diets					
	Feed Additive					
Performance at 21 days	Yeast Culture	Yeast Culture/ Enzyme complex	Enzyme complex	Control	Mean	SE
Body weight (g/chick)	576	522	564	556	555	20.1
Weight gain (g/broiler)	528	472	516	507	506	20.1
Feed intake (g/chick)	983	941	990	983	974	34.5
FCR (gain/feed)	1.9	2.0	1.9	1.9	1.9	0.1
PER (gain/protein)	2.4	2.2	2.3	2.3	2.3	0.1
Mortality (%)	2.5	5.0	2.5	2.5	3.1	2.6

b. Day 42 of Experiment

Body weight (g/chick)	1795	1648	1665	1728	1709	68.1
Weight gain (g/ broiler)	1747	1598	1618	1679	1660	68.1
Feed intake (g/ chick)	4177	4142	3913	4219	4114	159.5
FCR (gain/feed)	2.4	2.6	2.4	2.5	2.5	0.1
PER (gain/protein)	2.1	1.8	2.1	2.0	2.0	0.1
Mortality (%)	2.5	7.5	2.5	2.5	3.8	3.2

3.6.4 Growth and feed conversion ratio

The results of the effects of diet on body weight gain, feed conversion ratio, protein efficiency ratio and mortality in broiler chickens is shown in Table 3. Enzyme complex and yeast metabolites supplementation did not affect body weight, weight gain, FCR, PER and mortality rate ($P>0.05$). The mean body weight was 555g per broiler for the first three weeks and1709g per chick at the end of six week feeding period. The result showed that supplementing the broilers with yeast culture, enzyme complex or the combination of yeast metabolites and enzyme complex had no significant effect on weight gain. These results were in agreement with Vahjen *et al*. (2005) who found no effect with multi-enzyme supplementation on weight gain. Nadeem *et al*. (2005) also observed no significant response on average weight gain and FCR in broilers supplemented with Rovabio enzyme (a NSP degrading enzyme containing β-glucanase, xylanase, cellulase, pecetinase and protease). Rabie *et al.* (2010) fed broilers with probiotics (Avian Plus) and enzyme (Sicozyme) or their combination and found no significant effects on growth performance in all treatment diets. Similarly Midili *et al*. (2008), Gunal *et al,* (2004) and Sayyazadeh *et al*. (2006) showed that dietary probiotics supplementation did not significantly affect live body weight, weight gain or feed intake of broiler chicks. Gheisari and Kholeghipour (2006) found no effect on body weight, daily gain, feed-intake and feed conversion ratio on broilers supplemented with live yeast culture.

However, other studies done differ with the findings in this study. Paryad and Mahmoudi (2008) found that yeast metabolites supplementation resulted in a significant improvement of weight gain and feed conversion ratio at 42 days of age. The researchers supplemented the basal diet with different levels of yeast (0%, 0.5%, 1.5% and2%) and noted the highest weight gain ($P<0.05$) and improved feed conversion ratio ($P<0.05$) in birds fed at 1.5% yeast level compared

to other dietary treatments. Alam *et al*. (2003) found significant responses in liveweight, feed intake and feed efficiency in broilers supplemented with exogenous enzyme ($P<0.01$). He reported that broilers fed on diets supplemented with enzymes had a tendency to convert feed to liveweight more efficiently than those on the control diet (without enzyme). Wang *et al*. (2005) found that supplementation of birds with xylanase and β-glucanase improved the body weight, average daily feed intake, average daily gain , and feed conversion ratio ($P < 0.01$) during the 7- to 21 day phase. For the whole experimental period of 42 days, FCR decreased with increase in enzyme levels in the diet and the average daily gain increased linearly with increased enzyme supplementation. Shareef and Al-Dabbagh (2009) found significant effects on body weight gain, feed intake and feed conversion ratio on broiler chicks supplemented with yeast metabolites for the first three weeks. Similarly Gao *et al*, (2008), in a study with different levels of yeast metabolites (2.5g/kg, 5.0g/kg , 7.5g/kg and the control), found significant effects on performance at a level of 2.5g/kg of yeast metabolites in the diet. There were no dietary effects at yeast metabolites levels of 5.0g and 7.5g per kilogram of diet.

In the current study, broilers fed on diets supplemented with yeast metabolites had greater weight gain than in other groups though insignificant. The mean FCR was 1.936 and the mean PER was 2.303 for the first three weeks and on the sixth week the FCR mean was 2.489 and PER mean was 1.992 as shown on Table 3. There was no significant effects of the dietary treatments on FCR ($P>0.05$) and PER. However, the FCR was higher in broilers fed diets supplemented with yeast culture. This implies that the yeast metabolites additive was capable of modifying the gastrointestinal environment to improve efficiency of feed utilization. PER is the ratio of weight gain and protein consumed by the bird, it shows the efficiency of a bird to convert protein

consumed in feed to meat. Birds fed on diets supplemented with yeast metabolites (Diet 1) and enzyme complex (Diet 3) had higher PER, but the effect was not significant.

A total of six birds died during the experimental period and the post mortem results showed that two chicks died of aflatoxicosis while the other four died of handling and transportation stress. Lack of significantly response to yeast metabolites and enzyme complex supplementation may be due to various reasons. According to Banerjee (1992), benefits may be from the use of enzyme preparation as feed additives are observed mainly in feeds containing high amounts of barley, wheat, sunflower, rice bran or oat grains. These cereals contain high non-starch polysaccharide (NSP) levels. The feed used in this study basically contained corn and soybean meal which does not contain large amount of NSP. The lack of response might also be attributed to the possibility that the diets fed were of extremely good quality and allowed the birds to perform close to their genetic potential (Acamovic, 2001). The inconsistency between results might also be due to differences in microbial species in birds, or the insufficiency of supplement activity and dosage, type of yeast culture, quality of diet or experimental conditions.

3.6.5 Gastrointestinal morphology

Non Starch Polysaccharides (NSP) alter the morphology of the gut by shortening and thickening the mucosa causing atrophy of the villi (Jaroni *et al.,* 1999), hyperplasia and hypertrophy of goblet cells (Viveros *et al.,* 1994). These changes affect the absorption of nutrients. Chickens being a non-ruminant are unable to secrete the enzymes needed to breakdown some of the compounds such as non-starch polysaccharides and phytate present in the feed ingredients. These components are indigestible and interfere with the utilization of other nutrients. Supplementation of NSP hydrolyzing enzymes and phytase improves the nutritive value of feedstuffs and reduce

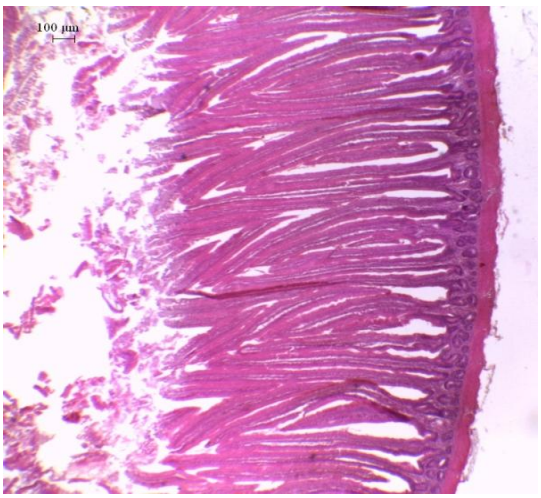
the negative effects such as atrophy of the intestinal villi, enlarged digestive organs and increased size of gastro intestinal tract (Viveros *et al.,* 1994).

The results of length of the sections of the intestines and weight of various organs as affected by yeast culture, enzyme complex or their combination are shown on Table 3. There were no significant effects of enzyme complex and yeast metabolites on internal organ weights both at 21 days and 42 days. Hanumantha *et al*. (2003) observed that supplementation of multi- enzyme to corn-soybean based diet did not significantly affect the intestinal length and weight compared to the control group. The results of this study also agreed with those of Alam *et al*. (2003), Bharathidhasan *et al*. (2009), Abudabos (2010), Nadeem *et al*. (2005) and Hajati (2009) who reported no significant responses on the weights of the liver, gizzard and bursa of fabricius with enzyme addition. Hana Zakaria *et al*. (2010) also found no significant effects on the percent of heart, gizzard and abdominal fat pad in birds fed diet supplemented with enzyme.

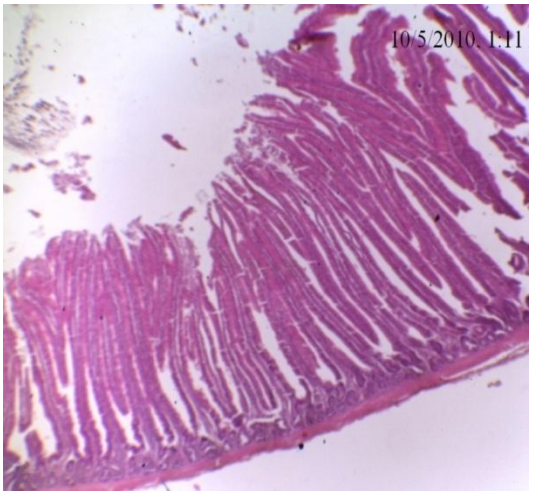
The results of this study showed no significant effects of dietary treatments on gut morphology on the 21st day. However, there was a significant effect on length of jejunum and of ceacum at 42th day ($P<0.05$). The study showed shorter jejunum and ceacum in broilers fed diet supplemented with enzymes and control diet compared to the broilers fed diet supplemented with yeast metabolites diet. These findings do agree with the study done by Viveros *et al.* (1994) who reported reduction in length in gastrointestinal tract segments in broilers fed diet supplemented with enzymes. The findings of this study agreed with that of Shareef and Al-Dabbagh (2009) and Karaoglu and Durdag (2005). Shareef and Al-Dabbagh (2009) used different percentages of bakers yeast (0%, 0.5%, 1%, 1.5% and 2.0%) and found no significant response on the weight of liver, gizzard and bursa of fabricius. Collazos *et al.* (2008) also reported insignificant effect on organ weight and abdominal fat in broilers supplemented with probiotics.

Table 6: Effects of yeast metabolites and enzyme complex supplementation on gastro intestinal morphology

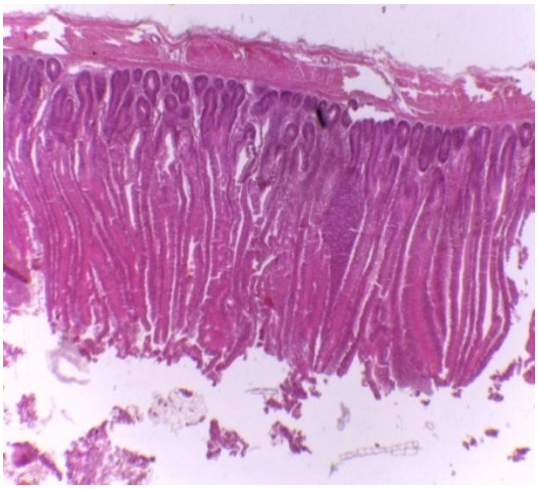
	Yeast Culture	Yeast Culture/Enzyme complex	Enzyme complex	Control	Mean	S.e.
a. Carcass measurement at day 21 of experiment						
Live weight (g)	650	668	625	622	614	24.7
Gizzard (% body wt)	4.9	4.6	4.7	4.9	4.8	0.229
Duodenum (cm)	23.8	21.1	26.1	21.8	23.2	2.08
Jejunum (cm)	49.8	45.9	44	42.9	45.6	3.86
Ileum (cm)	40.5	36.5	40.6	34.9	38.1	2.93
Rectum (cm)	6.4	5.9	5.9	5.7	6.0	0.381
Caeca (cm)	11.4	10.7	11.8	12.6	11.6	0.679
Liver (% body wt)	2.9	2.9	3.4	3.7	3.2	0.286
Villi height (mm)	1.5	1.1	1.3	1.2	1.3	0.155
Mucosa thickness (mm)	1.6	1.6	1.5	1.4	1.5	0.192
Bursa of fabricius (% body wt)	0.4	0.4	0.5	0.4	0.5	0.048
b. Carcass measurement at day 42 of experiment						
Live weight (g)	2175	1925	1850	1975	1981	113
Gizzard (% body wt)	3.3	3.7	3.4	3.1	3.4	0.2
Duodenum (cm)	33.9	30.9	33.1	31.5	32.8	2.0
Jejunum (cm)	77.8^{b}	80.6^{b}	71.2^{a}	63.4^{a}	73.2	29.2
Ileum (cm)	64.2	65.4	67.0	60.3	64.2	3.7
Rectum (cm)	19.6^{b}	19.6^{b}	16.9^{a}	16.9^{a}	18.3	0.6
Caeca (cm)	8.6	9.1	8.5	8.7	8.7	1.1
Liver (% body wt)	2.1	2.3	2.3	2.4	2.3	0.1
Villi height (mm)	1.5	1.2	1.2	1.1	1.3	0.2
Mucosa thickness (mm)	1.6	1.4	1.4	1.3	1.4	0.2
Bursa of fabricius (% body wt)	0.3	0.3	0.2	0.2	0.3	0.1
Abdominal fat (% body wt)	2.1	2.1	2.7	2.1	2.2	0.2



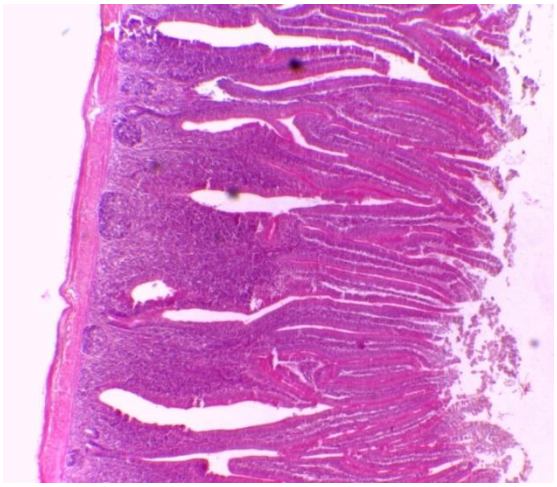
Villi as observed from a jejunal section of small intestines in broilers fed diet supplemented with enzyme complex at 21 days of age



Villi as observed from a jejunal section of small intestines in broilers fed diet supplemented with yeast metabolites at 21 days of age

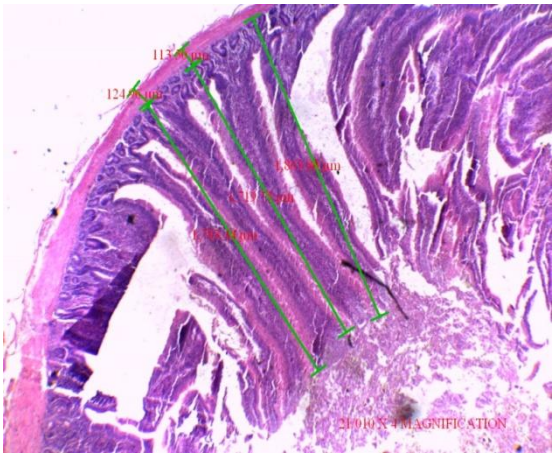


Villi as observed from a jejunal section of small intestines in broilers fed control diet at 21 days of age

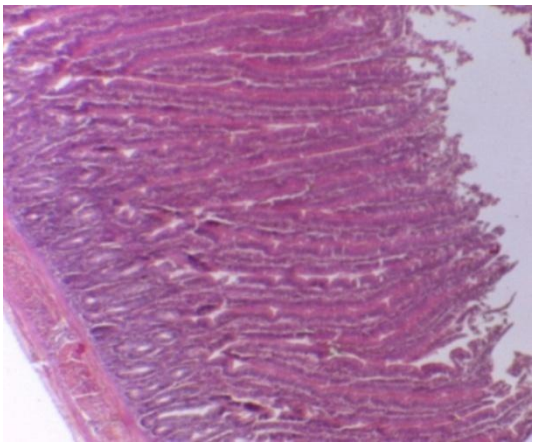


Villi as observed from a jejunal section of small intestines in broilers fed diet supplemented with both enzyme complex and yeast metabolites at 21 days of age

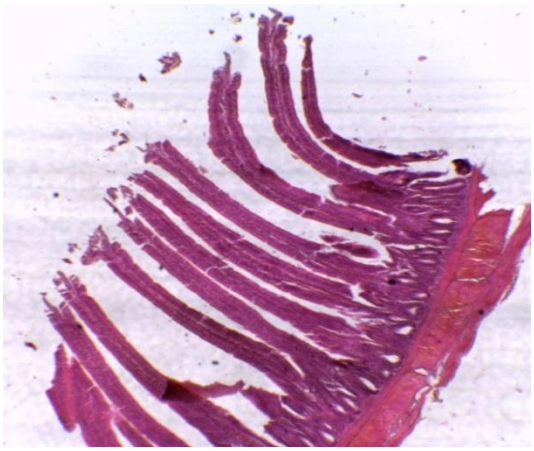
Figure 1: Photographs of jejunal villi at 21 days of age.



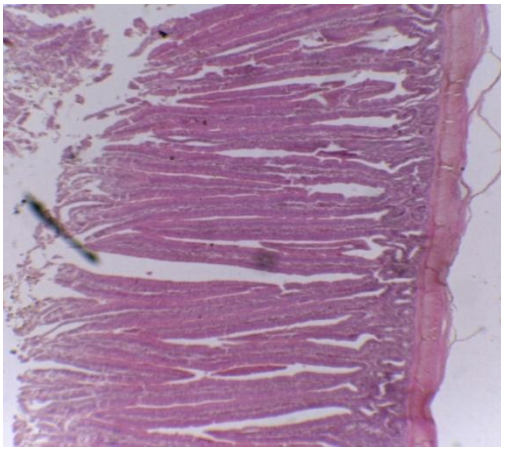
Villi as observed from a jejunal section of small intestines in broilers fed diet supplemented with yeast metabolites at 42 days of age



Villi as observed from a jejunal section of small intestines in broilers fed diet supplemented with both enzyme complex and yeast metabolites at 42 days of age



Villi as observed from a jejunal section of small intestines in broilers fed control diet at 42 days of age



Villi as observed from a jejunal section of small intestines in broilers fed diet supplemented with enzyme complex at 42 days of age

Figure 2: Photographs of jejunal villi at 42 days of age

The findings of this study showed no significant effect on villi height and mucosa thickness both at 21st day and 42nd day. However, table 4 shows that birds fed on yeast metabolites had longer villi and thicker mucosa than other groups though non-significant. The yeast metabolites contains Mannan Oligosaccharide**,** a naturally derived extract from the cell wall of Saccharomyces cerevisiae which has a trophic effect on intestinal wall by increasing the villi height in broiler chicken, Santin *et al*. (2001). Taller villi indicate more mature epithelia and enhanced absorptive function due to increased absorptive area of the villus. Greater villus height increases the activities of enzymes secreted from the tips of the villi resulting in improved digestibility, Hampson, (1986). This explains the better weight gain in broilers supplemented with yeast metabolites compared to other group. Thick mucosa as observed in yeast metabolites supplemented broilers increases the surface area of absorption. This finding agrees with Brummer *et al*. (2010) who found that Bio-MOS had no effect on villi height and width, crypt depth of mucosa thickness. In a study done by Santin *et al*. (2001), he found that supplementation of birds with 0.2% Saccharomyces cerevisiae cell wall increased the duodenal, jejunal and illeal villi significantly during the first week. However, at day 28 and day 42, supplementation of feed with yeast cell wall had no significant effects on villi height. The findings of Gao *et al.* (2008), did not agree with this results, where they concluded that yeast metabolites significantly increased villi height in the duodenum and jejunum at day 21 linearly with increasing level of yeast culture. At d 42, though duodenal villi increased linearly with increase in yeast metabolites level, the jejuna villi were variable with the level of yeast culture. Zhang *et al*. (2005) also reported greater villi height in birds supplemented with whole yeast or yeast cell wall.

3.7 Conclusions

The study done showed that supplementation of broiler's diet with yeast metabolites or enzyme complex had no effect on body weight, weight gain, feed intake and feed conversion ratio. Supplementing broiler diet with yeast metabolites also had no effect on gastrointestinal length both at 21 days of age and 42 days of age. However, supplementation of broilers diet with alloenymes resulted into shorter jejunum and ceacum at 42 days of age.

3.8 Recommendations

Further studies are necessary to determine the appropriate rate of inclusion of yeast metabolites that will produce healthy broilers in term of growth, immunity and gut health. Also studies using feed with high fiber contents are recommended.

CHAPTER 4

4.0 EFFECTS OF YEAST METABOLITES AND ENZYME COMPLEX ON IMMUNE RESPONSE AND INTESTINAL MICROBIOTA IN BROILER CHICKEN.

4.1 ABSTRACT

To evaluate the effect of yeast metabolites (Diamond V XPC), enzyme complex (Allzyme SSF), or their combination on the immune response and gut health in broiler chickens, one hundred and sixty broiler chicks were fed on diet formulated according to KeBS specifications supplemented with either enzyme complex (0.02%) or yeast culture. Diet 1 was supplemented with yeast culture, Diet 2 with both yeast metabolites and enzyme complex, Diet 3 enzyme complex only and Diet 4 was the control diet without yeast metabolites or enzyme complex. Birds were vaccinated with F-strain of Newcastle disease virus vaccine at 28 days of age and later given a booster dose one week later. One bird per replica (sixteen broilers) were slaughtered at 21 days of age (before vaccination) and at 42 days of age (after vaccination). During slaughter, blood was collected and serum obtained by centrifugation and used to study immune response measured by antibody titer. During each slaughter, fecal materials were removed from the rectum for total bacterial counts, coliform counts and coccidian oocysts counts. The results showed that the dietary treatments had no significant effects on bacterial count, antibody titer against NDV and on coccidian count. However, there was an increase in antibody titer after the birds were vaccinated.

4.2 INTRODUCTION

The immune system of the newly hatched chick is prepared to fight pathogens in a non-specific manner through innate immunity, and can develop specific modes of defense through cellular and humoral immunity, which requires contact with antigens. Acquired immunity differs from the innate responses by possessing specificity in recognition of foreign invaders (antigens) and the development of memory; thus resulting in a more rapid response than was elicited during the primary exposure. Broilers have lymphoid structures spread throughout the intestinal tract, and these are made up of diffuse and aggregated components. Diffuse components include intraepithelial lymphocytes as well as lymphocytes of the mucosa and *lamina propria*. Aggregated components include Peyer's patches, cecal tonsils, and the bursa of Fabricius. These tissues capture antigens available in the digestive tract, which stimulate B lymphocyte-derived plasma cell precursors of IgM, IgG, and IgA, which activate Peyer's patches to enhance general and specific immunity.

The immunological stimulation of the mucosa favors the production of IgA antibodies Bengmark, (2000), mainly in Peyer's patches (Gebert *et al.*, 1999), which block receptors and reduce the number of pathogenic bacteria in the intestinal lumen, Jin *et al.* (1997). The constant production of IgA secreted in large amounts on the surface of the intestinal mucosa occurs because of the continuous stimulation of normal gut microflora.

Yeast metabolites has been used in broilers to improve and stimulate the immune system of the broilers (Shareef and Al-Dabbagh, 2009). The micro-flora from yeast metabolites migrate throughout the gut wall and multiply to a limited extent and the antigen released by the dead organisms are absorbed thus stimulating the immune system of chicken, Havenaar and Spanhaak, (1994). Fuller, (2000) explained that bacteria that colonize the GIT of the chicken, as well as the

microorganisms from the probiotics, adhere firmly to the intestinal wall to avoid being swept away during peristalsis. This prevents pathogenic colonization along the intestinal wall and thus reducing disease development. The proposed mechanisms of pathogen inhibition by the intestinal microbiota includes competition for nutrients, production of toxic conditions (low pH) and compounds (volatile fatty acids), competition for binding sites on the intestinal epithelium and stimulation of immunity (Fuller, 1989; Gibson and Fuller, 2000). Several studies have demonstrated that Yeast metabolites or yeast cell wall components can affect the composition of intestinal microflora (Firon *et al*., 1987; Naughton *et al*., 2001; White et al., 2002). Gao *et al*. (2008) reported that yeast metabolites improve growth performance and immune system. However, in the study done by Shen *et al*. (2009), dietary supplementation of 2.5g/kg yeast metabolites did not affect microbial populations in the gut. The results showed that only the number of E. coli in the cecum of yeast metabolites was decreased compared with the diet without yeast culture. Similarly, Mathew *et al*. (1998), reported yeast metabolites did not change microflora in the duodenum, ileum, cecum, or colon. Van der Peet-Schwering *et al.* (2007) also reported that yeast metabolites did not show any effect on microbial composition in the ileum. The effects of feeding yeast metabolites on the immune response and intestinal microbiota and also the effective level of supplementation in broiler chickens are still controversial. Therefore this study was conducted to evaluate the effects yeast metabolites (Diamond V XPC) and enzyme complex supplementation to the diets on the immune response and intestinal microbiota in broiler chicken.

4.2.1 Objective

The objective of the study is to assess the effect of yeast metabolites and enzyme complex on immune response and intestinal microbiota in broiler chicken.

4.3 MATERIAL AND METHODS

4.3.1 Antibody titer against New Castle Disease Virus

Broilers were vaccinated with New castle vaccine on 28th day and later given New castle vaccine booster one week later. Blood was collected on 21st and 42nd day from jugular vein of 16 birds (1 bird/replicate) and serum was collected by centrifugation. The F-strain of Newcastle disease virus vaccine (Kenya veterinary vaccines production institute) was used as the haemagglutinating virus. 25µl of phosphate buffered saline solution was dispensed into each well of round- bottomed microtitre plate. 25µl of the serum was placed into the first well of the plate and two-fold dilutions of 25µl volumes of the serum were made across the plates. 4 HAU (Haemagglutination Unit) antigens was added in 25µl to each well and left for 30 minutes at room temperature. 25µl of 1% of chicken red blood cells (RBC) was added to each well and allowed to settle for 30 minutes. For controls, the serum samples were replaced with phosphate buffered saline and RBC remains

. The end point was observed when RBC in the control well was agglutinated. The haemagglutination inhibition titers were read as the $\log_{10}$ of the reciprocal value of the highest dilution of serum that completely inhibited the agglutination of the RBC.

4.3.2 Bacteriological Analysis

Fecal materials of one broiler per replicate were collected from the rectum during slaughter on 21st day and 42nd day of the experimental period. One gram of fecal sample was placed in a

sterile bottle thoroughly mixed with 9ml of buffer. This was then diluted in sterile saline solution ranging from 10^{-2} to 10^{-7} for viable count of total bacteria and coliform count. Mackonkey agar and plate count agar were prepared and put into petri dishes. 1 ml of the suspension was sucked from bottle containing dilutions 10^{-5}, 10^{-6} and 10^{-7} using a pipette and a placed on the petri dish containing agar whereby each dilution was duplicated three times. This was spread thoroughly and evenly then incubated for 24 hours. Coliforms were cultured using Mackonkey agar while total bacterial count was cultured using plate count agar. After incubation period the number of colonies in the petri dishes was counted, recorded and the total number of colonies in the three dishes averaged. This was later converted into $\log_{10.}$

4.3.3 Enumeration of Coccidial oocysts

This was done using McMaster technique where a vial is filled with 28ml of saturated salt solution (NaCl). 2g of fecal sample from the rectum was added to the solution and mixed thoroughly with a spatula. After thorough mixing, the solution was sieved to remove excess fecal debris. While the mixing continued, the counting chamber of the McMaster slide was quickly filled with the solution. Each side of the chamber was filled using different droppers. The slide was left to stand for a few minutes to allow the coccidian eggs to rise to the top of the slide. The chamber was placed on the microscope using a magnification power of X 100 and the eggs counted in the two chambers. Each chamber holds a volume of 0.15ml of the sample. The calculation was done to get eggs per gram of fecal material. Dimension of one grid of McMaster slide was 1cm*1cm while the distance between two slides (height) was 0.15 cm, therefore the volume under 1 grid was 0.15cm*1cm*1cm = 0.15ml. 2g of fecal material was suspended in 30 ml so 1g was in 15 ml and the multiplication factor was found to be 100 i.e. volume in which 1g

was suspended/ volume examined (15ml/0.15ml). To get number of eggs per gram, the number of eggs in the chamber was multiplied by 100.

4.4 RESULTS AND DISCUSSION

4.4.1 Immune response

Antibody titer responses have been used as measures of humoral immune status of birds (Sklan *et al*., 1994). The immune system guards the body against foreign substances and protects from invasion by pathogenic organisms. It can be divided into the innate (nonspecific) immune system and the acquired (specific) immune system. The results on effect of yeast metabolites and enzyme complex on immune response in broiler chicken are shown in Table 5 and 6. From the study, there was no significant effect of dietary treatments on antibody titers against NDV. The results showed increase in antibody titers after the birds were vaccinated though the increase was not significant (Figure 3). The findings of this study agreed with Ozsoy and Yalcin, (2011), who found insignificant effect of dietary treatment on antibody titer against NDV on broiler turkey. However, Gao *et al*. (2008) reported that antibody titers to NDV increased linearly ($P<0.05$) when the level of dietary yeast metabolites increased. Gao *et al*. (2008) also reported that with increasing concentration of dietary yeast metabolites (Diamiond VXPC), IgA content increased linearly. This implies that yeast metabolites may stimulate the humoral immune system to produce more antibodies. Newman, (1994) proposed that oligosaccharides in the yeast cell wall could bind to viruses and work as adjuvants of vaccines to increase the titers of antibody in yeast metabolites treated birds. Oliveira *et al*. (2009) vaccinated broilers at the end of third week and found the peak of antibodies produced in the fifth week. He stated that ingestion of

Mannanoligosaccharidess resulted in more titers in comparison to birds fed diet without additives or with only enzymes. Kabir *et al*. (2004) evaluated the dynamics of probiotics on immune response of broilers and they reported significantly higher antibody production ($P<0.01$) in experimental birds as compared to control ones.

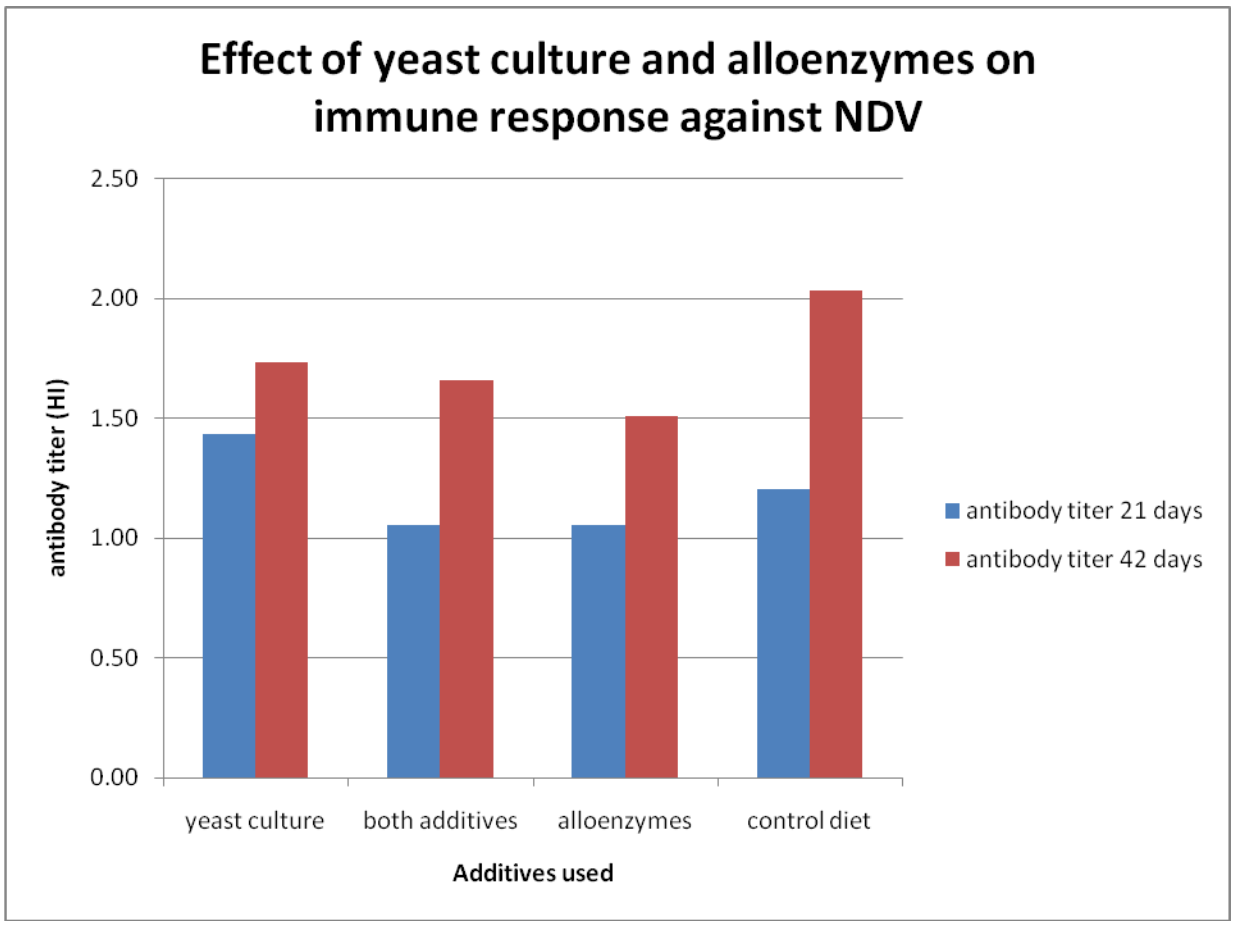


Figure 3: Effect of yeast metabolites and enzyme complex on antibody titer against NDV (HI).

Table 7: Effects of yeast metabolites and enzyme complex supplementation on immune response, gut health and intestinal morphology at 21 days of age

	Diets					
Item	1	2	3	4	Mean	SE
Antibody titer against NDV (HI) at 21 days	1.4	1.1	1.1	1.2	1.2	0.2
Total bacterial count (C.F.U) at 21 days	6.6	7.2	7.1	6.5	6.9	0.4
Coliform count (C.F.U) at 21 days	6.3	7.1	6.8	6.5	6.6	0.4

Table 8: Effects of yeast metabolites and enzyme complex supplementation on immune response, gut health and intestinal morphology at 42 days of age

	Diets					
Item	1	2	3	4	Mean	SE
Antibody titer against NDV (HI) at 42 days	1.7	1.7	1.5	2.0	1.7	0.1
Total bacterial count (C.F.U) at 42 days	7.8	7.6	7.9	8.0	7.8	0.4
Coliform count (C.F.U) at 42 days	7.0	7.0	7.2	7.5	7.2	0.4

4.4.2 Intestinal microbiota

Kabir *et al*. (2004) reported that probiotic microorganisms, once established in the gut, may produce substances with bactericidal or bacteriostatic properties (bacteriocins) such as lactoferrin, lysozyme, hydrogen peroxide as well as several organic acids. These substances have a detrimental impact on harmful bacteria, which is primarily due to a lowering of the gut pH. In addition, competition for energy and nutrients between probiotic and other bacteria may result in a suppression of pathogenic species. The results of the effect of yeast metabolites and enzyme complex on intestinal microbiota are shown on Table 5 and 6. The results of the study showed that the dietary treatment had no significant effect on both the total bacterial count and on the coliform count both at 21st day and 42nd day. This findings agrees with Shane (2006), who found that the concentrations of coliforms, lactobacilli and anaerobes were unaffected by inclusion of MOS in diets. These results did not agree with Stanley *et al*. (2004) who found significantly low bacterial count and low total coliform population in yeast metabolites supplemented chicks. A study done by Shen *et al*. (2009) on nursery pigs showed that the number of E.coli in the ceacum decreased with the yeast metabolites supplementation whilethe number of E.coli in the rectum and colon were not significantly affected by yeast metabolites supplementation. No study was found on intestinal microbiota in broilers' rectum, many studies done are on the effect of yeast metabolites on the intestinal microbiota in ceacum. At 21 days, no coccidian oocysts were found in the fecal material from broilers in all dietary treatments. At 42 days, few coccidian oocysts were found ranging from 0 to 4. This number was too low to be analyzed statistically.

Conclusion

The study done showed that the supplementation of broilers diet with either yeast metabolites or enzyme complex did not have significant effects on gut health and immune response. Therefore, use of yeast metabolites and enzyme complex in broilers feed will be an extra cost to the farmer and to the feed manufacturer with no significant benefit on gut health and immune response in broilers.

Recommendation

Further study is recommended to validate the level of yeast metabolites inclusion in the manufacturer's label.

5.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The aim of this section is to summarize the results of the two experiments where the effects of enzyme complex and yeast metabolites in broiler diets were studied. Over the decades, antibiotics have been used as growth promoters in poultry until they were banned in the European Union in 2006 and later in Kenya. Several studies have been done to find a substitute for antibiotics in poultry feeds to increase production. Some of the substances used in the industry are enzyme complex and yeast metabolites. Two experiments were done in this study to evaluate the effects of including enzyme complex and yeast metabolites in broilers diets. In experiment one, one hundred and sixty day- old chicks were grouped into four groups of forty chicks each and fed four different experimental diets. These diets were basal diet with yeast culture, basal diet with both enzyme complex and yeast culture, basal diet with enzyme complex and basal diet only. The yeast metabolites was incorporated at a level of 1.25g/kg in both broiler starter and broiler finisher diet while enzyme complex was at a level of 0.63g/kg for broiler finisher and 0.2g/kg for broiler starter. The chicks were fed for six weeks and growth, feed intake and gut morphology were monitored. In experiment two, the effects of enzyme complex and yeast metabolites on immune response and intestinal microbiota was demonstrated.

The expected results were that broilers fed diet supplemented with yeast metabolites would produce healthier and heavier birds with less abdominal fat than birds fed control diet. This is because microorganisms in the probiotics compete for the adhering sites in the intestinal wall thus prevent pathogenic bacteria from colonizing the intestinal wall (Fuller, 2000). This competition of adhering site also reduces the competition of food between harmful bacteria and normal bacteria in gut producing a healthier and heavier bird. Also from the study it was

expected that broilers supplemented with enzyme complex would perform better in form of weight gain and produce birds with shorter gastrointestinal morphology as demonstrated in a study done by Viveros *et al.* (1994).

Results of the proximate analysis indicated that the formulated diets were within the NRC requirements for broilers. There were no significant effects of enzyme complex and yeast metabolites on broiler performance. The results on gastrointestinal morphology indicated significant effects on the length of jejunum and ceacum at the end of six weeks. The results indicated shorter jejunum and ceacum in broilers fed diet supplemented with enzyme complex compared to those fed diets supplemented with yeast culture. This can be explained by the fact that supplementation of NSP hydrolyzing enzymes reduce the negative effects such as atrophy of the intestinal villi, enlarged digestive organs and increased size of gastro intestinal tract (Viveros *et al.*, 1994). The results of the study indicated that no significant effects on immune response and intestinal microbiota on the broilers fed diets supplemented with either yeast metabolites or enzyme complex. The results of the study did not meet the expectation of the study. A study done by Paryad and Mahmoudi, 2008 with a higher level of yeast metabolites (1.5%) showed significant effect of yeast metabolites on growth performance. The level of inclusion of yeast metabolites in the broiler diet used in this study could have not been sufficient enough to produce the expected results. Also according to a study done by Banerjee (1992), the effect of enzyme complex on growth performance could only be realized with broilers fed high crude fibre diet. The diet formulated for the study did not have high fiber content with corm and soyabean being the main ingredients.

Conclusion

Based on the findings of the present study, it was concluded that:

- No significant effects were found on broilers fed on diets supplemented with either enzyme complex or yeast metabolites on performance, immune response and intestinal microbiota.
- It is not necessary to supplement broilers diets with yeast metabolites and enzyme complex as it will be a wastage and expensive to both the feed manufacturer and the farmer.

Recommendation

Based on the findings of the present study, the following have been recommended:

- Further studies are needed using different (higher) levels of yeast metabolites and enzymes to determine the optimal levels which promote performance of chicken broilers.
- Further studies are recommended using diets high in Non-starch polysaccharides.

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6.0 APPENDICES

Appendix 1.1: Analysis of variance for body weight, weight gain, feed intake, feed conversion ratio and protein efficiency ratio at 21 days of age.

Source of variation	d.f	Mean of Squares	P value
Body weight	3	2425	0.315
Weight gain	3	2288	0.287
Feed intake	3	1967	0.749
Feed conversion ratio	3	0.01	0.751
Protein efficiency ratio	3	0.02	0.806

Appendix 1.2: Analysis of variance for body weight, weight gain, feed intake, feed conversion ratio and protein efficiency ratio at 42 days of age.

Source of variation	d.f	Means of square	P- value
Body weight	3	17944	0.440
Weight gain	3	10933	0.420
Feed intake	3	84168	0.359
Feed conversion ratio	3	0.03	0.690
Protein efficiency ratio	3	0.02	0.734

Appendix 1.3: Analysis of variance for gastrointestinal morphology at 21 days of age

Source of variation	d.f	Mean of Squares	P - value
Gizzard weight	3	0.1	0.780
Liver weight	3	0.6	0.206
Duodenum	3	20.5	0.357
Jejunum	3	36.8	0.618
Ileum	3	33.3	0.439
Rectum	3	0.4	0.631
Ceacum	3	2.7	0.270
Villi	3	0.1	0.533
Mucosa	3	0.04	0.847
Bursa of fabricius	3	0.006	0.575

Appendix 1.4: Analysis of variance for gastrointestinal morphology at 42 days of age

Source of variation	d.f	Means of Squares	P value
Gizzard weight	3	0.2	0.189
Liver weight	3	0.05	0.482
Duodenum	3	7.77	0.705
Jejunum	3	36.8	0.618
Ileum	3	3.25	0.637
Rectum	3	0.3	0.982
Ceacum	3	9.8	0.008
Villi	3	0.07	0.689
Mucosa	3	0.58	0.745
Bursa of fabricius	3	0.001	0.869

Appendix 1.5: Average weight gain (grammes per bird) 1-6 weeks

	Treatment diets			
Ages in weeks	1	2	3	4
1	82	70	67	68
2	254	223	254	247
3	526	469	515	506
4	817	704	743	733
5	1330	1151	1174	1203
6	1740	1587	1611	1671

Appendix 1.6: Average feed intake (grammes per bird) 1-6 weeks of age

	Treatment diets			
Ages in weeks	1	2	3	4
1	155	138	148	138
2	303	302	325	314
3	516	490	511	528
4	798	807	746	809
5	798	1011	919	1073
6	1256	1283	1164	1256