DIETARY INCLUSION OF COMPOSITE ENZYME AS AN ALTERNATIVE TO ANTIBIOTIC ON THE PERFORMANCE OF BROILER CHICKEN

A Thesis

By

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CERTIFICATE

*This is to certify that the thesis entitled "**DIATERY INCLUSION OF COMPOSITE ENZYME AS AN ALTERNATIVE TO ANTIBIOTIC ON THE PERFORMANCE OF BROILER CHICKEN**" submitted to the Department of Poultry Science, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, as partial fulfillment for the requirements of the degree of Master of Science (MS) in Poultry Science, embodies the result of a piece of bona fide research work carried out by **MD. MANIRUZZAMAN**, Registration No.: 14-06225, Semester: **JANUARY-JUNE /2021** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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DEDICATED TO MY LATE FATHER

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The Author

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LIST OF ACRONYMS AND ABBREVIATION

ABBREVIATION	FULL WORD
ADG	Average daily gain
AGPs	Antibiotic growth promoter
ANOVA	Analysis of variance
Avg	Average
BWG	Body weight gain
CFU	Colony forming unit
Cm^2	Square centimeter
CP	Crude protein
DOC	Day old chick
DP	Dressing percentage
e.g.	For example,
et al.	And others/associates
EU	European union
FAO	Food and agricultural organization
FC	Feed consumption
FCR	Feed conversion ratio
FDA	Food and drug administration
FI	Feed intake
G	Gram
GALT	Gut-associated lymphoid tissue
GFI	Global food initiative
GIT	Gastro intestinal tract
i.e.	That is
IB	Infectious bronchitis
K Cal	Kilo calorie
Kg	Kilogram
L	Liter
LSD	Least significant difference
M.S.	Master of science

LIST OF ACRONYMS AND ABBREVIATION

ABBREVIATION	FULL WORD
ME	Metabolizable energy
Mi	Milliliter
Mm	Millimeter
Pg.	Picogram
MT	Metric ton
ND	Newcastle disease
No	Number
NS	Non-Significance
RH	Relative Humidity
SE	Statistical error
SPSS	Statistical package for social science
TLRs	Toll-like receptors
TM	Trade mark
Viz	Such as
Vs.	Versus
WHO	World health organization
Wks.	Weeks

LIST OF SYMBOLES

SYMBOLES	FULL MEANING
^{0}C	Degree Celsius
^{0}F	Degree Fahrenheit
@	At the rate of
:	Ratio
<	Less than
>	Greater than
*	5% level of significance
&	And
/	Per
±	Plus-minus
%	Percentage

DIETARY INCLUSION OF COMPOSITE ENZYME AS AN ALTERNATIVE TO ANTIBIOTIC ON THE PERFORMANCE OF BROILER CHICKEN

ABSTRACT

The present work aimed at studying growth performance, carcass traits and health status of broiler chicken feed enzyme over a period of 4 weeks. A total of 200-day-old Lohmann broiler were randomly assigned to five treatment groups, each with four replicates (10). T_1, T_2 was control and antibiotic. whereas T_3, T_4, T_5 was provided as 0.05%, 0.1% and 0.15% of enzyme respectively. The results revealed significant ($P<0.05$) difference in feed intake (T_2-2197.00±30.20 g and T_5-2324.75±19.76 g) and live weight (T_5-1937.5±17.97 g and T_2-1808.00±23.68 g). There is no significance in FCR value & livability. The flock uniformity percent were significant. In control group T_1 (78.25±2.29) was average and other groups were uniformed. Highest hemoglobin (T_5-9.12±0.18g/dl), RBC (T_2-3.97±0.09 mill/cum), WBC (T_1-14.48±0.74 mill/cum), lymphocytes (T_1-38.50%), Monocytes (T_5-2.25±.25 %), Platelets (T_3-31.25±0.63x10^4 /mm^3), PCV (T_5- 28.48%), MCV (T_5-87.74±0.25 FI), MCH (T_4-28.77 Pg.) were found in the enzyme treated groups, which is an indication of good health. *E. coli* and *salmonella spp*. count was significantly ($P<0.05$) lower in birds fed 0.15% enzyme supplemented diet and with a descending order of 0.1% and 0.05% enzyme level. *Salmonella spp.* and *E. Coli* count was also significantly ($p<0.05$) higher in birds fed control. The results of the study demonstrate the beneficial effects of supplementing enzyme on body weight and dressed yield in the treated groups in broiler chicken. Enzyme is therefore, suggested to be used as an alternative to antibiotics of broiler chicken ration for higher profitability.

Keywords: Broiler, Enzyme, growth performance, Blood parameter, Bacterial count.

CHAPTER I

INTRODUCTION

Broiler farming has emerged as one of the fastest growing agro- industries in the world, even in Bangladesh. Research on broiler meat production globally indicates poultry as a fast-growing sector especially in developing countries. The discovery of the growth promoting property of antibiotics led to their use as antibiotic feed additives (AFAs) in animal feed at sub-therapeutic doses. Although this has been beneficial for animal health and productivity, it has been, essentially, a double-edged sword. The continued and non-judicious use of AFAs has led to the selection and dissemination of antibiotic-resistant strains of poultry pathogens such as *Salmonella spp., Campylobacter spp.* and *Escherichia coli*. The rapid spread of drug-resistant pathogens as well as emergence of antibiotic-related environmental pollutants is of global concern. Hence, the identification and development of new and effective alternatives to antibiotics that do not hinder productivity is imperative. Sub-therapeutic levels of antibiotics given to poultry as growth enhancer may result to the development of antibiotic-resistant bacteria, which are hazardous to animal and human health (Sarica *et al*., 2005).

Meanwhile, the use of organic supplements such as enzymes are generally believed to be safer, healthier, and less subject to hazards. In the developed countries people's enzyme in diet of human, livestock, poultry as beneficial product. Thus, Enzyme could be incorporated in poultry feed instead of antibiotic in order to stimulate or promote effective use of feed nutrients which result in more rapid gain, higher production and better feed efficiency. Moreover, Enzyme contains active substances that can improve digestion and metabolism and possess bacterial and immune-stimulant activities. Therefore, alternatives to AGP need to be proposed to poultry producers in order to maintain animal health, productivity and carcass quality.

Go to combination of feed enzymes like xylanase, amylase and protease been shown in reduction in the amount of undigested nutrients or substrate in the duodenum, jejunum and ileum increasing the digestibility of even so-called simple diets and improving healthy broiler performance. Protease has effects in reducing undigested proteins and can also stimulate the production of mucus and could be associated with better

responses of chickens in response to coccidia challenges. Enzyme like xylanase has been shown to produce Arabino-xylo-Oligosaccharides (AXOX) especially in the AXOX) especially in the cecal phase. These act as prebiotics and selectively stimulate the growth of beneficial bacteria.

It has been reported that many enzymes like β-glucanase, xylanase, amylase, α-galactosidase, lipase, phytase etc., have been in use since a long time (Kim *et al.,* 2015*).* Exogenous enzymes have been used mainly in the diet which are based on corn and soybean meal and they contain different level of anti-nutritive factors e.g. NSP and protease inhibitors and they are main hindrance in the process of normal digestion and absorption in the gut of the bird (Kim *et al.,* 2011). The tendency of reducing cost for the poultry feed by using non-conventional ingredients containing anti-nutritional factors and fiber, is also one of the causes to encourage the use of enzymes as these types of ingredients cannot be completely digested and absorbed by the chicken (Shehab *et al*., 2012**)**. Exogenous enzymes are used to meet the lack of endogenous enzymes which are necessary for the Digestion of certain type of nutrients in various feed stuff or hydrolysis for anti-nutritional factors present in the feed stuffs. (Shehab *et al*., 2012**)** reported the use of exogenous enzymes reduces the pollutant potential of excreta as it is an important environmental issue.

Gut health and enteric disease resistance is often dependent upon the digestibility of feed components and feed formulation. It has been reported that the gut health is compromised when poorly digested protein meals are given to birds. However, with the addition of exogenous enzymes including xylanases, phytases and ß-glucanases the digestibility of wheat, rye and even corn-based diets can be improved. The response to dietary enzyme supplementation is greater when antibiotics are not used than when they are, but the performance responses do not approach the level That is observed when diets contain enzymes and antibiotics together (Tabook *et al.,* 2006). Enzymes are perhaps the most extensively reviewed products that seem to can limit the performance losses associated with removal of antibiotic growth promoters. The supplementation of enzymes enhances feed Digestibility and nutrients availability to the host through their beneficial effects, assuming they also influence the gut microbial ecosystem. The use of exogenous enzymes alters the gut microflora populations in the small intestine and caeca of the birds (Tabook *et al.,* 2006).

It has been reported that with the use of exogenous carbohydrase the proportion of lactic and organic acids and VFAs concentration was increased and ammonia production was decreased. (Osera *et al.,* 2008). The increased VFAs concentration helps the hydrolysis of NSP and supports the growth of beneficial bacteria in the gut of the broilers. Bedford & Coweison (2012) reported that exogenous enzymes modulate the gut microbiota of birds which may affect the health of the birds and the extent of digestion accomplished by the host. Generally speaking the improvement in the nutrients digestibility by the addition of exogenous enzymes is much smaller as compared to the loss of the substrate which must be available to the gut microflora beneficial for the host .Numerous authors have established that by application of enzymes production performances can be improved up to 10% (Bergh *et al*., 1999; Cowieson *et al*., 2000; Cmiljanić *et al*., 2001) whereas in some papers the positive effect of enzymes wasn't registered (McNab and Bernard, 1997; Perić *et al*., 2002). Obviously positive effect of these additives depends on the quantity and quality of feeds included into the mixture, used level of energy and type of enzymes, as well as fattening conditions (Acamovic *et al*., 2001). Objective of this research was to investigate the effect of addition of enzyme complex to diets of different nutritive value on performance of broiler chicken.

Now a day's people are very concern about antibiotics resistance. Most of the framers use antibiotics at high dose for preventing diseases. But it is unethical because antibiotics are used for treating the disease. If we use this in healthy or normal birds, it causes residual effect. Most of the farmer doesn't know that proper management can prevent different types of diseases. And if we use enzyme in farm, it increases the production performance and reduce the risk of disease susceptibility. In a while, the profit will be higher and feed will be safe for the people.

Although lots of work has been done n enzyme in the world but remarkable work has done on the effects and usage of the enzyme as alternative to antibiotic in Bangladesh. The enzyme releases the nutrients contents of food, increase its ability to absorb and neutralize toxic elements in food could justify its significance use in poultry diets. With the application of proper processing techniques, its beneficial value can be comparatively produced with the existing ones in the industry today. Most of the studies determining enzyme inclusion level in animal feeds have been associated with feed ingredients. Based on a few studies on the inclusion in poultry feeds, a maximum of 10

% is recommended. There is need to determine the extent o which dose of enzyme as an alternative to antibiotics can be utilized by broiler chickens and the effects it would have on the growth and biological properties of broiler chicken when sed as a feed additives' accepted levels would also be ensured to provide a readily available source of nutrients in the diets of chickens. Enzyme can be grown and utilizes by industry for both human and animal considering is multi beneficial uses.

Thus, the resent study was conducted to evaluate the effects of enzyme on growth performance, various meat qualities and carcass development of broiler chicks.

Objectives

1. To investigate the enzyme supplemented diets on general performance of broiler chicken.

2. To determine some microbiological and hematological properties of broiler chicken with enzyme supplement.

CHAPTER 2

REVIEW OF LITERATURE

The main purpose of this chapter is to get up-to-date information regarding the research works addressed here. Important information related to the present study was represented below. It is well documented that antibiotics benefit animal growth, performance, and health. However, increasing concerns regarding overuse of antibiotics has prompted extensive investigation into alternatives to use of subtherapeutic antibiotics in production diets. Composite enzyme is important natural growth promoters and it enhance the digestibility of feed content by gut development of chick. Hosamani *et al*. (2001) added multienzyme (beta-D-glucosidasescellulase, protease, amylase, and phytase) to the normal broiler diet (contained 22% crude protein, 6% crude fiber and 2900 Kcal of metabolize energy / kg diet). They found that enzyme supplementation significantly (P> 0.05) improved the body weight of chicks as compared to those receiving normal basal diet.

Yamazaki *et al.* (2002) supplemented commercial enzyme complex (cellulose, phytase and pectinase) to low crude protein diets (19% Crude protein) based on corn and soybean meal. They observed that apparent metabolizable energy (AMEn) content was significantly increased with enzyme supplementation.

2.1 Source of enzyme for broiler

Enzymes are created in each living organism from the simplest unicellular forms of life to the highest developed plants and animals. Most of the enzymes presently used in the beverage and food industry are from Aspergillus, but cellulases and hemicellulases are derived from Trichoderma. Newly, genes encoding has been used in cloning for various enzymes, including phytases, xylanases, and β-glucanases and expressed in various commercial systems (plants and microorganisms). Morales *et al*. (2004); Campeanu *et al*. (2002); Bovill *et al*. (2001); and Coenen, (2000) uses large amounts production of an inexpensive enzyme by permanently selecting suitable microbes, increasing them in systems of modern fermentation and by efficient regulation of the enzyme extraction and purification. Paryad *et. al.* (2008). States that the most important selection criteria

that have been used to enzyme properties are grouped by their strength properties, functions and potentials, highlighting among these

- Tolerance to high acidity
- Improve gut development.
- Increase digestive ability.
- Resistance to bile salts.
- Adhesion capacity to intestinal cells.
- Direct antagonistic effect on enterobacteria.
- Antisecretory effect against the toxins of pathogenic microorganisms.
- Trophic effect on the mucosa through the production of polyamines.

2.2 Impact of combined enzyme on mode of action of broiler production

Fanimo *et al.* (2004) viewed the effects of enzyme supplementation of shrimp waste meal-based diets on the performance and nutrient utilization of starter and finisher broiler. The values of average final weight, daily weight gain, feed conversion ratio was significantly ($p>0.05$) influenced by the dietary treatment. The daily feed intake of the starter broiler was significantly ($p>0.05$) higher than those of other treatment. Birds fed fish meal diet without enzyme had the highest serum uric acid and serum cholesterol at the starter phase. Finisher broilers fed fish meal- based diet without enzyme had a significantly ($P<0.05$) higher serum creatinine value. Carcass characteristics measured were insignificantly influenced by the dietary treatments. On the other hand, Pinheiro *et al.* (2004**)** showed Fanimo *et al.* (2004) viewed the effects of enzyme supplementation of shrimp waste meal-based diets on the performance and nutrient utilization of starter and finisher broiler. The values of average final weight, daily weight gain, feed conversion ratio was significantly ($p>0.05$) influenced by the dietary treatment. The daily feed intake of the starter broiler was significantly ($p>0.05$) higher than those of other treatment. Birds fed fish meal diet without enzyme had the highest serum uric acid and serum cholesterol at the starter phase. Finisher broilers fed fish meal- based diet without enzyme had a significantly ($P<0.05$) higher serum creatinine value. Carcass characteristics measured were insignificantly influenced by the dietary treatments.

2.3 Enzyme effects on nutrient digestibility

Madrid *et al*. (2010) observed the effects of a multi-enzyme complex containing protease and carbohydrase enzymes on the performance and nutrient digestibility of broiler chickens under different rearing conditions from 1 to 42 days of age. They concluded that the multienzyme complex of protease and carbohydrase enzymes might be effective for improving nutrient digestibility in broilers fed with a wheat-soybean meal-based diet under commercial farm conditions. On the other hand, Shirmohammad and Mehri *et al.* (2011) conducted two experiments to determine the effects of dietary supplementation of REAP® enzyme into corn-soybean diet on the performance of broiler chicks. In the first experiment, a total of 16-50 weeks adult roosters (ISA-Brown) were divided into 4 groups with 4 birds per replicate and the experimental diets contained the two levels of energy (2650 and 2759 kcal TMEn/kg diet) with 0 or 0.1% REAP® and were subjected to assay of apparent metabolizable energy (AME). In the second experiment, 360; 3 days old male broiler chicks (Ross) were divided into 4 groups with 3 replicates of 30 birds per replicate and were assigned at random to one of the four experimental diets containing the two levels of energy (3100 and 2980 kcal/kg diet) with 0 or 0.1% REAP®. The body weight gain of the birds fed the low energy diet with 0% REAP was lower significantly than those of the other groups ($p < 0.05$). The breast muscle weights of the low energy diet birds were higher than those of the high ones and those of the lower energy group with 0.1% REAP were the highest ($p < 0.05$). The relative abdominal fat weight was reduced by the dietary REAP ($p< 0.05$). Percentage duodenum weights of high energy group were higher than those of the low energy group. The intestinal lengths (cm/100 g BW) of low energy diet group without REAP were lower than those of the others ($P < 0.05$). The results demonstrated that, dietary REAP improved body weight gain.

Abudabos *et al.* (2012) performed the study to evaluate the effect of feeding Tomoko, a commercial enzyme supplement that contains an acidic protease, α-amylase, pectinase, phytase, glucoamylase, cellulase and *Aspergillus Awamori* cells in a standard corn-soy ration for broiler chicken from 1 to 42 day of age. A total of 960 Cobb 500 chicks were randomly distributed in a randomized complete block design among 16

floor pens with 4 replicate 9 pens/treatment. Two levels of diet density (normal and low) and two levels of enzyme (without and with) in a factorial arrangement resulted in four dietary treatments: T1 = normal density diet; T2 = T1+0.05% enzyme; T3 = low density (low energy, low protein diet); T4 = T3+0.05% enzyme. Body weight was significantly affected by diet density and enzyme at 42 d ($p<0.001$). Enzyme supplementation improved eviscerated, breast and total meat percentages while diet density had a significant effect on all parts yield measured.

2.4 Effects of enzyme supplement diets on growth performance

Meng *et al.* (2005) studied the effect of four enzyme combinations in a 2-wk (5 to 18 d of age) growth performance and nutrient digestibility trial with broiler chickens. All enzyme combinations were effective in improving ($P < 0.05$) weight gain, feed-to-gain ratio. The most complex enzyme combination was found to be superior ($P < 0.05$) to others in improving ileal protein digestibility and feed-to-gain ratio. It is evident from the present studies that the addition of an appropriate combination of carbohydrase enzymes to target cell wall polysaccharide structures could further improve enzyme efficacy in practical wheat, soybean meal (SBM) and peas-based broiler diets. Saleh *et al.* (2005) viewed the effects of a mixture of pure enzymes (cellulase, hemicellulose and pectinase) and a commercial enzyme, Energex, were examined on performance and metabolism abilities in broiler chicks given a maize-soybean meal diet. The mixed enzyme group showed significant improvement in carcass and muscle weight when compared with the control group. It was concluded that a combination of cellulase, hemicellulose and pectinase was effective in improving organic matter and crude protein metabolisabilities and carcass yield of broilers on a maize soybean meal diet. Tabook *et al.* (2006) performed two experiments to evaluate the use of date fiber as a partial replacement of maize as a source of energy for growing broiler chicken. Addition of date fiber or the exogenous enzyme had no significant effect on carcass or meat quality characteristics.

On the other hand,Yu *et al*. (2007**)** found that inclusion commercially available enzyme preparations; either a mixture of protease and carbohydrase (E – 1) or a single protease (E-2), on a low crude protein maize- soybean meal broiler diet. Broiler chickens in the enzyme supplemented groups had better body weight gain as compared to those

without supplementation. Addition of either the single protease or the cocktail of protease and carbohydrase to a maize- soybean meal diet improved chicken growth.

2.5 Use of enzyme instead of antibiotics in broiler production

Nowadays, the efficiency of poultry to convert the feed into meat plays a key role in economics of broiler industry. Therefore, it is highly essential to improve feed efficiency of poultry to produce meat economically and also food safety is more seriously considered than before. On the other hand, economy of food production is also a factor that cannot be ignored. A huge amount of antibiotics has been used to control diseases and improve performances in livestock. However, due to growing concerns about antibiotic resistance and the potential for a ban for antibiotic growth promoters in many countries in the world, there is an increasing interest in finding effective alternatives to antibiotics in poultry production. Poultry feed influences the production cost of chicken. Recently, it is believed that enzyme have beneficial effects to improve the productive performance of poultry. Enzyme especially Exogenous enzyme such as Amylase, Cellulase, Xylanase, Protease, Phytase, B-Glucanase, Pectinase, Mannase, Lipase are most important for chick gut development. (SK+F). Osera *et al.* (2008) reported that research on broiler feeding have showed that the cost with ration represents around 60 to 70 % of the total production cost, leading to the use of additives in rations aiming to improve fowl's performance, being enzymes an important alternative to antibiotic. They concluded that the inclusion of enzymes in diets formulated by taking corn and soy bean as basis did not influence meat yield of noble parts of broilers carcass in none of the levels of addiction to the ration.

However, according to the currently adopted definition by Food and Agriculture Organization and World Health Organization (2001), Enzyme are: live microorganisms which when administered in adequate amounts confer a health benefit on the host. The most important advantage of enzyme is that it neither digestibility of residues in animal production nor exerts any antibiotic resistance by consumption. Therefore, a lot of researchers have partially replaced antibiotics with probiotics as therapeutic and growth promoting agent. The broiler industry is constantly searching for ways to improve its product and quality in order to meet the demands of an increasingly demand of consuming public. In this regard, numerous references exist on increasing poultry meat

yields and improving carcass quality. For this reason, many ingredients have been using in broiler diets, in recent years. Moreover, there is currently a world trend to reduce the use of antibiotics in animal food due to the contamination of meat products with antibiotic residues (Menten, 2001) as well as the concern that some therapeutic treatments for human diseases might be jeopardized due to the appearance of resistant bacteria (Dale, 1992). It is also reported that additional benefits can be gained by supplementing enzyme in broiler diets as feed additives. Enzyme used to get rid of abnormalities in the gastrointestinal tract produced by stress and therefore normalize the gut activity (Kutlu and Görgülü, 2001). Studies on the beneficial impact on poultry performance have indicated that enzyme supplementation can have positive effects. Enzyme are reported to prevent colonization gut by pathogens like *Escherichia coli* and *Salmonella spp*. They also prevent contamination of carcasses by intestinal pathogens during processing and promote higher growth rate and feed conversion efficiency in growing chickens (Hose and Sozzi, 1991; Juven *et al*., 1991). The use of enzyme for meat and carcass quality improvement has been questioned and many unclear results have been shown. Some authors reported advantages of enzyme administration (Jensen and Jensen, 1992; Maruta, 1993; Corrêa *et al*., 2000; Vargas *et al*., 2002), whereas others did not ob-serve improvement when enzyme used (Oscar *et al*., 1990; Quadros *et al*., 2001). There has been others research by scientists to evaluate probiotics on broilers; however, to date, the data is inconclusive.

Therefore, need for research on comparison effect of available enzyme. This study was carried out to evaluate effects of enzyme is better growth performance than antibiotic.

2.6. Effect of enzymes on haemato-biochemical parameters

Meng *et al.* (2004) conducted an experiment on Japanese quail to evaluate the effects of fat type carbohydrase addition and lipase addition on growth performance and nutrient utilization of male broilers fed a wheat-based diet. It was shown that the effects of enzyme supplementation to the maize soybean meal diet on live weight gain, feed intake, feed efficiency and carcass yield of the quails were not statistically significant between groups ($P>0.05$). Enzyme and probiotic supplementation significantly reduced the serum glucose and cholesterol levels among the treated groups ($P<0.01$). Bayram *et al.* (2004) determined the effects of wheat- barley based diets supplemented with enzyme and probiotic on some blood parameters in broilers. Blood total protein, uric

acid, glucose, cholesterol, copper, zinc, aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatinine kinase (CK) values were not affected in the treatment groups of broilers. However, urea, creatine, calcium, phosphorus, magnesium, and iron values were increased, while aminotransferase (ALT) values were lowest in supplemental groups.

On the other hand, Cetin *et al*. (2005) showed the effects of enzyme and probiotic supplementation hematological parameters in turkeys. Probiotic and enzyme supplementation increase in the serum IgG Levels, and significant decreases in the peripheral blood percentage. It was observed that the probiotic supplementation caused significant increases in the erythrocyte count, hemoglobin and hematocrit values, but enzyme supplementation did not have an effect on these parameters. Total leucocytes and differential counts were not affected by dietary enzyme and probiotic were not affected. Enzyme and probiotic supplementation did not affect the performance of quails but decreased serum glucose, cholesterol and protein levels. Moreover, Ahmed *et al*. (2007) carried out an experiment on broilers to study the effect of oral administration of enzymes and vitamins on growth, hematological parameters and biochemical parameters. TEC, PCV and Hb content increased significantly ($p < 0.01$) in the treated groups as compared to that of control group but ESR, SGOT and SGPT values decreased significantly ($p < 0.01$) in all the treated groups as compared to that of control group.Besides,Udeybir *et al.* (2009) conducted an experiment to evaluate growth performance and hematological parameters on a number of 240, day-old broilers. They were randomly divided into 6 groups with 2 replicates of 20 birds each and fed a control maize soya bean diet (T1) ;66:34 maize : pearl millet (T2); 100% pearl millet(T3); T1 + All Enzyme containing proteinase, polygalacturonates, phytase, pentosonase, cellulase, amylase and beta– glucanase (T4); TZ + Allozyme (T5) and T3 + Allozyme (T6) for 6 weeks. It was shown that live weight gain did not differ between treatments. However, higher body weights were recorded in T2 and T3 compared to T1. There were no significant differences in feed intake in diets with and without enzyme. Feed conversion ratio was non-significantly higher inT2 compared to the control. Blood parameters were not significantly different between groups. These results show that pearl millet can be used to replace maize in broiler diets without affecting performance and haemato-biochemical Parameters.

Shehab *et al*. (2012**)** carried out the study to evaluate the effect of dietary enzymes supplementation (Kemzyme plus dry® and phytase) on some serum biochemical and hematological parameters of Japanese quails. Neither tested dietary enzyme or their combination had any significant effect on some biochemical constituents such as total protein, triglycerides, cholesterol, AST, glucose, uric acid, and creatinine. Results showed that blood thyroxine (T4) was not significantly affected by dietary enzymes, while triiodothyronine (T3) was significantly ($p<0.05$) decrease in group supplemented with phytase. Concerning with serum iron, the obtained data showed that there was significant increase ($p<0.05$) in group supplemented with Kemzyme® plus phytase.

Rezaeipour *et al*. (2012) studied the effect of early feed restriction with or without enzyme supplementation on performance, nutrients digestibility and some blood parameters in broiler chickens. The result showed that the feed restriction with enzyme had an increasing effect on crude protein and crude fat digestibility ($p<0.05$). Besides, feed restriction had a significant effect on glucose and cholesterol ($p<0.05$) however, it did not affect blood triglyceride ($p>0.05$).

2.7. Effect of enzyme on microbial test (*Salmonella spp. and E. coli* colony count) of broiler cecal content

There are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistances in pathogenic bacteria in both humans and poultry linked to the therapeutic and subtherapeutic use of antibiotics in livestock (Castanon, 2007; J.I.R., 2007). Current trends in poultry production point to reduction or total elimination of antimicrobial growth promoters (AGPs) use and increase the use of non-antibiotic feed additives that offer similar benefits, such as to improve the growth of broilers and improve the utilization of feed (Mountzouris, *et al.,* 2007).Several groups of these additives are in use such as Enzyme, probiotics, prebiotics, acidifiers, antioxidants and phytogene additives.

Enzyme are a possible alternative to antibiotics in poultry diets. Enzyme usually refers to oligosaccharides which are not digested by the animal enzymes, but can selectively stimulate certain intestinal bacteria species, which have potential beneficial effects on the host health. Enzyme have two advantages relative to probiotics: a technological, because there are no problems with the thermal processing of the feed and the acidic conditions of the digestive system, and a safety, because there is no introduction of any

foreign microbial species into the gut. However, similar to probiotics, results of the effects of enzyme on broiler performance are same. Mannan oligosaccharide (MOS) is derived from the outer layer of enzyme. The effects of MOS on poultry production can be expressed in reduction of diseases by inhibition of pathogenic bacterial colonization to gut lining by binding to them and thus preventing them of proliferating and producing toxins (Benites *et al.*, 2008). Reducing intestinal pathogen counts (Benites *et al.*, 2008 Benites, V. Gilharry, R., Gernat, A.G.and Murillo, J.G. 2008). Improving the immune system (Ferket, 2002 Ferket, P.R. 2002). and exhibit influence on morpho-functional characteristics of intestines (Ferket, 2002 Ferket, P.R. 2002). However, results of the effects of MOS on broiler performance are contradictory. Other reports showed that MOS had no positive influence on the performance of poultry (Waldroup *et al.*, 2003 ; Waldroup, P.W., Fritts, C.A.and Yan, F. 2003).The objective of this study was to further determine the effects of MOS supplementation from SAF-Mannan® (S.I. LeSaffre, Marcq en Baroeul, France) to broiler diets compared to a growth promoting antibiotic (enramycin) on growth performance, histomorphology and bacterial count of small intestinal mucosa in broilers raised in cages under subclinical *C. perfringens* model and to determine the product with the most return and pathogen colonization control.

Restrictions of in-feed antibiotics use in poultry has pushed research toward finding appropriate alternatives such as Direct-Fed Microbials (DFM). In this study, previously tested Bacillus isolates (B. subtilis and B. amyloliquefaciens) were used to evaluate their therapeutic and prophylactic effects against Salmonella enterica serovar Enteritidis (S. Enteritidis) in broiler chickens. For this purpose, initial antibacterial activity of Bacillus-DFM (104 spores/g or 106 spores/g) against S. Enteritidis colonization in crop, proventriculus and intestine was investigated using an in vitro digestive model. Furthermore, to evaluate therapeutic and prophylactic effects of Bacillus-DFM (104 spores/g) against S. Enteritidis colonization, altogether 60 (n = 30/group) and 30 (n = 15/group) 1-day-old broiler chickens were randomly allocated to either DFM or control group (without Bacillus-DFM), respectively. Chickens were orally gavaged with 104 cfu of S. Enteritidis per chicken at 1-day old, and cecal tonsils (CT) and crop were collected 3 and 10 days later during the therapeutic study, whereas they were orally gavaged with 107 cfu of S. Enteritidis per chicken at 6-day-old, and CT and crop were collected 24 h later from two independent trials during the

prophylactic study. Serum superoxide dismutase (SOD), FITC-d and intestinal IgA levels were reported for both chicken studies, in addition cecal microbiota analysis was performed during the therapeutic study. DFM significantly reduced S. Enteritidis concentration in the intestine compartment and both proventriclus and intestine compartments as compared to the control when used at 104 spores/g and 106 spores/g, respectively ($p < 0.05$). DFM significantly reduced FITC-d and IgA as well as SOD and IgA levels ($p < 0.05$) compared to the control in therapeutic and prophylactic studies, respectively. Interestingly, in the therapeutic study, there were significant differences in bacterial community structure and predicted metabolic pathways between DFM and control. Likewise, phylum Actinobacteria and the genera Bifidobacterium, Roseburia, Proteus, and cc_115 were decreased, while the genus Streptococcus was enriched significantly in the DFM group as compared to the control (Metagenomes, $p < 0$).

2.8. The beneficial role of exogenous enzyme

Poultry industry is becoming increasingly receptive to the use of exogenous enzymes supplementation. Enzyme supplementation to the poultry rations has a positive effect on feeds digestibility and leads to better productivity and performance. Moreover, supplementation of commercial enzymes can increase the nutritive value of feed ingredients and diets as well as allow greater flexibility in diet formulation. Reducing intestinal pathogen counts (Benites *et al.*, 2008; Benites, V. Gilharry, R., Gernat, A.G.and Murillo, J.G., 2008). Improving the immune system (Ferket, 2002; Ferket, P.R., 2002). and exhibit influence on morpho-functional characteristics of intestines. It has also a potential effect on the mitigation of environmental pollution by reducing the excretion of some elements such as nitrogen and phosphorus in poultry manure. Enzyme is a functional protein that stimulates or accelerates the rate of specific chemical reactions. Enzymes activity is reliant on the substrate in a random way or at a very particular site on the substrate. The high content of fiber limits usage of sunflower meal (SFM) in poultry diets. The solution for this problem may be using exogenous enzymes and hydrolyze the NSPs, which could be used by avian and increase energy utilization.

CHATER III

MATERIALS & METHODS

3.1 Statement of the experiment

The research work was conducted at Sher-e Bangla Agricultural University Poultry Farm, Dhaka, for a period of 28 days during the period from 30th August 2020 to 26 th September 2020; to investigate the effect of Enzyme as an alternative to antibiotics on the growth performance of broiler in Bangladesh.

3.2 Collection of experimental broilers

A total of 300-day-old Lohmann Meat (Indian River) broiler chicks were collected from Kazi Farm Group, Gazipur, Dhaka.

3.3 Experimental materials

The collected chicks were carried to the university poultry farm early in the morning. They were kept in electric brooders separated 200 bird for enzyme treatment & 100 birds were not used enzyme. It's equally for 7 days by maintaining standard brooding protocol. During brooding time, only basal diet was given, 0.05% enzyme was used as treatment for 200 bird. After 7 days 120 chicks among 200 birds were selected from brooders for enzyme treatment three dietary treatment & 80 birds were selected among 100 birds for control and antibiotic treatment. Each treatment had four replications with 10 birds per replication. The total numbers of treatments were five and their replications were twenty.

3.4 Experimental treatment

T1: Basal Diets / Control

T2: Basal diets +Antibiotics (2gm doxyvet/kg)

T3: Basal Diets + 0.05% Enzyme

T4: Basal Diets + 0.1 % Enzyme

T5: Basal Diets + 0.15% Enzyme

Table 1: Layout of the treatment.

Distribution of treatments and birds			No. of birds
T_2R_3 (10)	T_3R_2 (10)	T_1R_1 (10)	30
T_1R_2 (10)	T_2R_1 (10)	T_3R_3 (10)	30
T_3R_1 (10)	T_1R_3 (10)	T_2R_2 (10)	30
T_5R_2 (10)	T_3R_4 (10)	T_1R_4 (10)	30
T_4R_4 (10)	T_5R_3 (10)	T_2R_4(10)	30
T_5R_4 (10)	T_4R_3 (10)	T_4R_2 (10)	30
T_4R_1 (10)	T_5R_1 (10)		20
Total birds			200

3.5 Experimental diets

Starter and grower commercial Kazi broiler feed were purchased from the local market. Starter diet was enriched with minimum 4 times daily by following Lohmann Meat (Indian River) Manual and *ad libitum* drinking water 2 times daily. Detail composition of feed are presented in table 2, 3, 4 & 5.

Table 2. Nutrient composition of broiler starter ration

Nutrient	Minimum percentage present
Protein	21.0 %
Fat	6.0%
Fiber	5.0%
Ash	8.0%
Lysine	1.20%
Methionine	0.49%
Cysteine	0.40%
Tryptophan	0.19%
Threonine	0.79%

Source: Kazi starter feed (50 kg packet).

Table 3. Nutrient composition of broiler grower ration

Nutrient	Minimum percentage present
Protein	20.0 %
Fat	6.0%
Fiber	5.0%
Ash	8.0%
Lysine	1.10%
Methionine	0.47%
Cysteine	0.39%
Tryptophan	0.18%
Threonine	0.75%
Arginine	1.18%

Source: Kazi grower feed (50 kg packet).

3.5.1 Collection of enzymes

Enzymes are created in each living organism from the simplest unicellular forms of life to the highest developed plants and animals. Most of the enzymes presently used in the beverage and food industry are from Aspergillus, but cellulases and hemicellulases are derived from Trichoderma. Newly, genes encoding has been used in cloning for various enzymes, including phytases, xylanases, and β-glucanases and expressed in various commercial systems (plants and microorganisms). Probably, large amounts production of an inexpensive enzyme by permanently selecting suitable microbes, increasing them in systems of modern fermentation and by efficient regulation of the enzyme extraction and purification. Composite enzyme collection from SK+F company limited. Brand name was Eskazyme Plus WS, A novel blend water soluble enzyme for poultry. I used for my research in broiler rearing.

Table 4. Each gram Eskazyme(R) plus WS contains-

Name of the ingredients	Amount per gram
Amylase	40,000 Unit
Cellulase	28,000 Unit
Xylanase	6,000 Unit
Protease	3,000 Unit
Phytase	750 Unit
B-Glcanase	700 Unit
Pectinase	70 Unit
Mannanase	60 Unit
Lipase	5 Unit

Source: SK +F Company Limited,2020.

Figure1: Enzyme composition

Table 5: Amount of enzyme used in treatment

	Composite enzyme & water requirement	T3 (0.05% Enzyme) (40 bird)		T4 (0.1% Enzyme) (40 bird)		T5 (0.15% Enzyme) (40 bird)	
		Supply	Intake	Supply	Intake	Supply	Intake
First Week	Water (L)	16	14.8	16	14.8	16	14.8
	Enzyme(g)	8	7.4	8	7.4	8	7.4
Second Week	Water (L)	40	34	40	33.6	40	36
	Enzyme(g)	20	17	40	33.6	60	54
Third Week	Water (L)	64	59	64	56.8	64	60
	Enzyme(g)	32	29.5	64	56.8	96	90
Fourth Week	Water (L)	88	76	88	76	88	80
	Enzyme(g)	44	38	88	76	132	120
Total		104	91.9	200	173.8	296	271.6

3.6 Preparation of experimental house

The experimental room was properly cleaned and washed by using tap water. Ceiling walls and floor were thoroughly cleaned and disinfected by spraying diluted timsen solution disinfectant solution (2 gm /liter water). After proper drying, the house was divided into 20 pens of equal size using wood materials and wire net. The height of wire net was 36 cm. A group of 10 birds were randomly allocated to each pen. The stocking density was $1m^2$/10 birds.

3.7 Management procedure

Body weight and feed intake were recorded every week and survivability was observed for each replication up to 28 days of age. The following management procedures were followed by whole experimental period.

3.7.1 Brooding of baby chicks

The experiment was conducted during 30 th August 2020 to 26 th September 2019. The average temperature was 31^0C and the RH was 80% in the poultry house. Common

brooding was done for seven days. After seven days the chicks were distributed in the pen randomly. There were 10 chicks in each pen and the pen space was $1m^2$. Due to hot climate brooding temperature was maintained as per requirement. Brooding temperature was adjusted (below 35^0C) with house temperature. So, when the environmental temperature was above the recommendation, then no extra heat was provided. At day time only an electric bulb was used to stimulate the chicks to eat and drink. Electric fans were used as per necessity to save the birds from the heat stress.

3.7.2 Room temperature and relative humidity

Daily room temperature (^0C) and humidity (%) were recorded every six hours with a thermometer and a digital thermometer respectively. Averages of room temperature and percent relative humidity for the experimental period were recorded.

3.7.3 Litter management

Rice husk was used as litter at a depth of 6 cm. At the end of each day, litter was stirred to prevent accumulation of harmful gases and to reduce parasite infestation. At 3 weeks of age, droppings on the upper layer of the litter were cleaned and fresh litter was added.

3.7.4 Feeding and watering

Feed and clean fresh water was offered to the birds *ad libitum.* One feeder and one round drinker were provided in each pen for 10 birds. Feeders were cleaned at the end of each week and drink drinkers were washed daily. All mash dry feed was fed to all birds *ad libitum* throughout the experimental period.

3.7.5 Lighting

At night, there was provision of light in the broiler farm to stimulate feed intake and body growth. For first 2, weeks 24 hours' light was used. Thereafter, 22 hours light and 2 hours' dark were scheduled up to 28 days.

Fig: Housing preparation

Fig: Rearing of chicken after brooding

Fig: Brooding of day-old chick

Fig: Rearing of chick.

Fig 2: Managemental procedure

3.7.6 Bio security measures

To keep disease away from the broiler farm, recommended vaccination and sanitation program was undertaken in the farm and its premises.

3.7.7 Vaccination

The vaccines collected from medicine shop (Cava Company) and applied to the experimental birds according to the vaccination schedule, given in (table 6).

Table 6. The vaccination schedules

Age of birds (Days)	Name of Disease	Name of vaccine	Route of administration
3	IB + ND	MA-5 + Clone-30	One drop in each eye
11	Gumboro	Hipragumboro (GM97)	Drinking Water
19	Gumboro	Hipragumboro (GM97)	Drinking Water

3.7.8 Ventilation

The broiler shed was south facing and open-sided. Due to wire-net cross ventilation, it was easy to remove polluted gases from the farm. Ventilation was regulated as per requirement by folding polythene screen.

3.7.9 Biosecurity and sanitation

Strict sanitary measures were taken during the experimental period. Disinfectant (timsen) was used to disinfect the feeders and waterers and the house also. The following measures were taken during the experimental period to prevent diseases.

- Entrance of personnel was restricted except researcher, supervisor and co-supervisor who visited the farm following special care.
- Before entrance, hands and feet were washed with soap and clean clothes were wore while working.

- Footbath containing disinfectant (Iosan®, Novartis (Bangladesh) Ltd.) was used before entering the experimental area.
- Adequate precautions were taken for vaccine storage, liquification and different methods of administration.
- New litter materials were dried and disinfected by using Virkon-s®, (Dupont, UK) and mixed with lime powder before use.
- The experimental areas were kept free from rats, rodents and wild birds.
- Dead birds were removed instantly.

Medication

Table 7: Medication schedule

Day	Medication	Dose
0-1	Sugar solution	1gm/L
2-5	Vitamin B complex Vitamin AD 3E	1ml/3L 1ml/4L
6-8	Cocci cure	1.5gm/L
9-10	Vitamin B complex Vitamin AD3E	1ml/3L 1ml/4L
12-15	Calmac Vitamin AD3E	1gm/L 1ml/3L
16-18	Antibiotic Multivitamin	onlyT2 sector,2gm/L 1ml/3L
20-22	Elecromin	1gm/2L
23-28	Electromin Vitamin AD3E	1gm/2L 1ml/3L

3.8 Study parameters

3.8.1 Recorded parameters

Weekly live-weight, weekly feed consumption and death of chicks to calculate mortality percent were recorded. FCR was calculated from final live weight and total feed consumption per bird in each replication. After slaughter of broiler chicken gizzard, liver, spleen, heart, proventriculus and bursa were measured from each bird. Dressing yield was calculated for each replication to find out dressing percentage.

3.9 Data collection

3.9.1 Live weight: The initial day-old live weight and weekly live weight of each replication was kept to get final live weight record per bird.

3.9.2 Dressing yield = Live weight- (blood + feathers + head + shank+ digestive system + Liver+ Heart)

3.9.3 Feed consumption

Daily feed consumption record of each replication was kept to get weekly and total feed consumption record per bird.

3.9.4 Mortality of chicks

Daily death record for each replication was counted up to 28 days of age to calculate mortality.

3.9.5 Dressing procedures of broiler chicken:

Three birds were picked up at random from each replicate at the 28th day of age and sacrificed to estimate dressing percent of broiler chicken. All birds to be slaughtered were weighed and fasted by halal method or overnight (12 hours) but drinking water was provided *ad-libitum* during fasting to facilitate proper bleeding. All the live birds were weighed again prior to slaughter. Birds were slaughtered by severing jugular vein, carotid artery and the trachea by a single incision with a sharp knife and allowed to complete bleed out at least for 2 minutes.

Outer skin was removed by sharp scissor and hand. Then the carcasses were washed manually to remove loose singed feathers and other foreign materials from the surface of the carcass. Afterward the carcasses were eviscerated and dissected according to the methods by Jones (1982). Heart and liver were removed from the remaining viscera. The proventricular was cut and then the gizzard was cut from both incoming and outgoing tract. Dressing yield was found by subtracting blood, feathers, head, shank, liver, heart and digestive system from live weight.

3.10 Calculations

3.10.1 Live weight gain

Average body weight gain in a replication was calculated by deducting initial Feed intake in a replication. Body weight gain = Final weight – Initial weight

3.10.1.1. Feed intake

$$\text{Feed intake } \left(\frac{\text{g}}{\text{bird}}\right) = \frac{Feed\ intake\ in\ a\ replication}{No.of\ birds\ in\ a\ replication}$$

3.10.2 Feed conversion ratio

Feed conversion ratio (FCR) was calculated as the total feed consumption divided by weight gain in each replication.

$$\text{FCR} = \frac{Feed\ intake(kg)}{Weight\ gain\ (kg)} \text{x } 100$$

3.10.3 Flock uniformity

Uniformity can be calculated by individually weighing at least 100 birds. Individual bird weights are necessary to measure how much each bird's body weight differs from the flock average weight. This calculation is called the deviation. A good quality sample is a selection of birds which represents the entire population.

$$\text{S} = \sqrt{\frac{\sum\ (x-x^{-})}{n-1}}$$

X= The value in the data distribution

X^{--} = The sample mean

N= Total number of observations.

$$\text{C.V.} = \frac{Standard\ deviation}{Average\ body\ weight(kg)} \text{x } 100$$

C.V.= Coefficiency of variation

3.11. Chemicals used for hematological studies

Anticoagulant (4% trisodium citrate) (E Merck, dramstdt) used to prevent coagulation of blood. Normal physiological saline was composed of 8.5 gm sodium chloride (NaCl) and 1000ml distilled water. Hayem's solution was prepared to use in TEC by mixing the following constituents: 1.0 g sodium chloride (NaCl), 5.0 gm sodium sulfate (Na2so4), 0.5 gm mercuric chloride (HgCl2) and 200 ml distilled water. 1% hydrochloric acid (HCl) was used for estimation of hemoglobin.

3.11.1 Blood collection

A series of sterile test tubes containing anticoagulant (4% sodium citrate) at a ratio of 1:10 were taken. Blood was collected from each group through slaughtering. The hematological measurements were performed within two hours of blood collection.

3.11.2 Instruments and appliances for hematological studies

A Centrifuge machine (Laboratory Centrifuge CMC, Model-YJ03-4000, USA) was used for determination of PCV. A compound microscope (Spencer, USA) was used both in total cellular count of blood. The blood was stored in a refrigerator for two days and later was used for analysis. Scientific balance (Scaltec Instrument, Heiligenstadt-Germany) was used for measuring ingredients of chemicals. Disposable syringe (JMI Syringes and Medical Devices LTD. a joint venture enterprise with South Korea) made in Bangladesh was used for collection of blood from the brids. Hellige Hemometer was used for hemoglobin estimation. Hemocytometer was used for RBC count. Wintrobe hematocrit tube was used for determination of PCV. Special loading pipette was used for loading the hematocrit tube with blood for PCV. Sterile cotton with antiseptic solution was used for maintaining aseptic condition.

Methods

Following hematological parameters were analyzed by Rainbow Diagnostc center.

3.11.3 Total erythrocytes count (TEC)

a) The tip of the dry clean red pipette was dipped into the blood sample and blood was sucked up to 0.5 mark of the pipette.

b) The tip of the pipette was wiped with cotton. Then the tip immediately placed into the red cell diluting fluid and the pipette

was filled with the fluid up to 101 marks.

c) The contents of the pipette were mixed thoroughly by using electric shaker for 5 minutes.

d) The counting chamber was placed with cover glass under microscope using low power (10x) objectives.

e) After discarding 2 or 3 drops of fluid from the pipette a small drop was placed to the edge of the cover glass placed on the counting chamber and the area under the cover glass was filled by the fluid introduced.

f) One-minute time was allowed to settle the cells uniformly into the chamber.

g) The cells were counted from the recognized 80 small squares under high power objective (45x) and was calculated accordingly.

The result was expressed in million/mm3.

3.11.4 Determination of hemoglobin (acid-hematin method)

a) N/10 HCI solution was taken in a graduated diluting tube up to 2 gm mark with the help of a dropper.

b) Citrated well-homogenized blood was then drawn into the Sahli pipette up to 20cmm mark.

c) The tip of the pipette was wiped with sterile cotton to get rid of unwanted blood and the blood of the pipette was immediately Transfer into the dialating containg HCL solution.

d) The pipette was rinsed 2-3 times by sucking fluid from the top of the tube. This blood and acid were thoroughly mixed by a glass

stirrer into the diluting tube. There was the formation of acid hematin in the tube by the hemolysed RBC and HCI.

e) This tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop.

f) The solution was mixed well with a stirrer until the color of the mixture resembled the standard color of the comparator.

g) The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in gm%.

3.12 Microbial examination

3.12.1 Sample collection

Fecal content from dressed bird & preserve it normal freezing temperature (0-4^0 C)

3.12.2 Composition of salmonella shigella (SS) agar media

Table 8: Composition of salmonella shigella (SS) agar media

Ingredients	Grams
Lactose	10.0
Bile salt no.3	8.5
Sodium citrate	8.5
Sodium thiosulfate	8.5
Beef extract	5.0
Proteose peptone	5.0
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	13.5

3.12.3 Preparation of salmonella shigella (SS) agar media

- Suspend 60g of the medium in one liter of deionize or distilled water.
- Mix well by digital mixing machine.
- Heat with frequent agitation and boil for one minute.
- Do not autoclave the media.
- Pour into plates.
- Let the agar solidify and store in the refrigerator (avoid freezing). Prepared culture media can be kept for at least a week in refrigeration.

3.12.4 Composition of eosin methylene blue (EMB) agar media

Table 9: Composition of eosin methylene blue (EMB) agar media

Ingredients	Grams/liter
Peptic digest of animal tissue	10.00
Dipotassium phosphate	2.00
Lactose	5.00
Sucrose	5.00
Eosin-Y	0.40
Methylene blue	0.065
Agar	13.50

Final p^H (25^0 C); 7.2±0.2

3.12.5. Preparation of EMB agar

- Suspend 35.96 grams in 1000 ml distilled water
- Mix unite the suspension uniform. Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 1 Ibs pressure (121^0 C) for 15 minutes. Avoid overheating.
- Cool to 45-50^0 C and shake the medium in order to oxidize the methylene blue (i.e. to store in blue in color) and to suspend the flocculent precipitate.
- Pour into sterile Petri dish plate.
- Allow plates to warm to room temperature.

- The agar surface should be dry before inoculating.
- Inoculate and streak the specimen as soon as possible after collection
- If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface and streak for isolation with a sterile loop.
- Incubate plates aerobically at 35-37^0 C for 18-24 hours and protect from light.
- Examine plates for colonial morphology, if negative after 24 hours incubated again.

3.12.6 Dilution

- If the count is expected to be more than 2.5 x 10^3 per ml or g, prepare decimal dilution as follows.
- Shake each dilution 25 times in 30 cm area.
- For each 10-fold dilution use fresh sterile pipette.
- Pipette 0.1g of fecal content homogenate into a tube containing 900 micro ml of the PBS diluent.
- From the first dilution transfer 100 micro ml to second dilution tube containing 900 micro ml of the diluent.
- Repeat using a third, fourth or more tubes until the desired dilution is obtained.

3.12.7 Pour plating

Label all Petri plates with the sample number, dilution, date and another desired information. Pipette 0.1 g of 10-fold dilution of the fecal content of homogenate and of such dilution which have been selected for plating into a Petri dish. Pour into 10-12 ml of SS & EMB agar media separate Petri dish before prepared. Mix the media and dilutions by swirling gently clockwise, anti-clockwise, to and fro trice and taking care that contents do not touch the lid. Allow to set.

3.12.8 Incubation

Incubate the prepared dishes, inverted at 35^0 C for 24±2 hours. (Or the desired temperature as per fecal regulation).

3.12.9 Counting colonies

Following incubation count all colonies on dishes containing 30-300 colonies and recorder the result per dilution counted.

3.12.10 Calculation

In dishes which contain 30-300 colonies count the actual number in both plates of a dilution as per the formula given below:

$$Tcc = \frac{Number\ of\ colony}{0.025}\ x\ 10^{n}$$

Tcc = Total colony count

n = Which stage colony found.

3.13 Statistical analysis

The data was subjected to statistical analysis by applying one-way ANOVA (Duncan method-1955) using statistical package for social sciences (SPSS) version 16.

CHAPTER IV

RESULTS AND DISCUSSION

Production performances of broiler chicken was evaluated by average live weight, average feed consumption (FC), weekly feed consumption, feed conversion ratio (FCR), average body weight gain, weekly body weight gain, survivability and flock uniformity. And carcass characteristics were taken by dressing percentage (DP) and relative weight of giblet organs.

The parameters research data analysis is given and discussed below:

4.1 Production performances of broiler chicken

4.1.1 Average live weight

Data presented in figure 3 and table 10 showed that the effect of treatments on final live weight (gram per broiler chicken) was significant ($P<0.05$). The relative final live weight (g) of broiler chickens in the dietary group T_1, T_2, T_3, T_4 and T_5 were 1830.50±1.39,1808.00±23.68,1870.00±27.08,1826.00±31.72 and 1937.5±17.9 respectively. The highest live weight was found in T_5 (1937.50±17.97) and lowest result was in T_2 (1808.00±23.68) g.

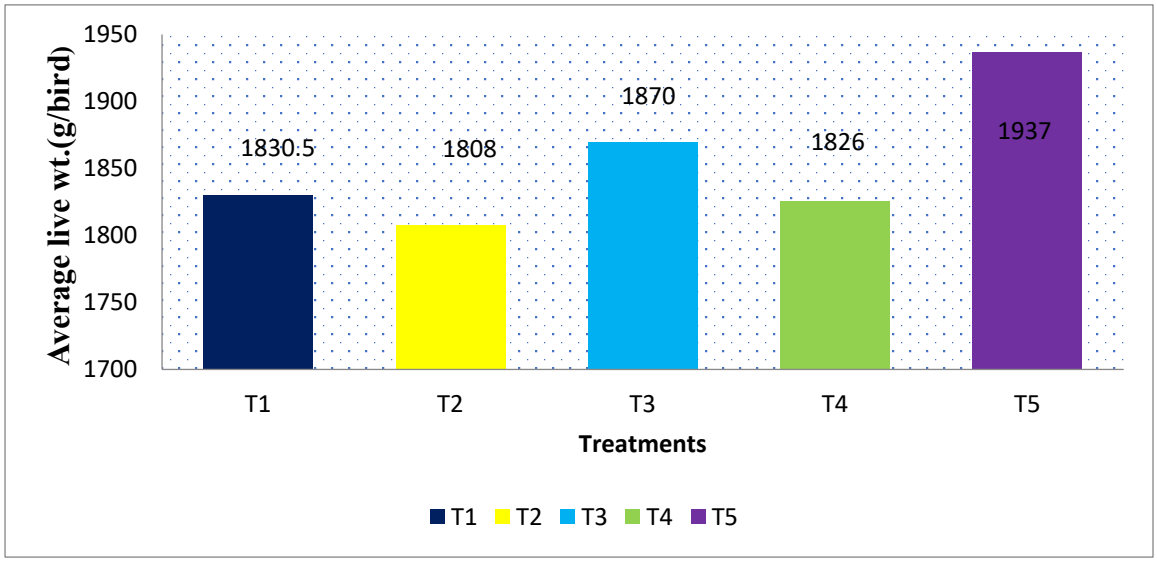

Fig 3: Average live weight of different treatment

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

These results are in agreement with the previous findings of Hosamani *et al*. (2001); Merge *et al*. (2005); Fanimo *et al.* (2004); Saleh et al. (2005) who reported that dietary inclusion of enzyme in the diets of broilers showed improved body weight gain. Therefore, improvement in body weight gain of the birds in this study may be due to better utilization of enzyme, which may have contributed in better growth of the birds

4.1.2 Average feed consumption (FC)

Data presented in table 10 and figure 4 showed that the effect of treatments on final feed consumption (gram per broiler chicken) was significant (P<0.05)

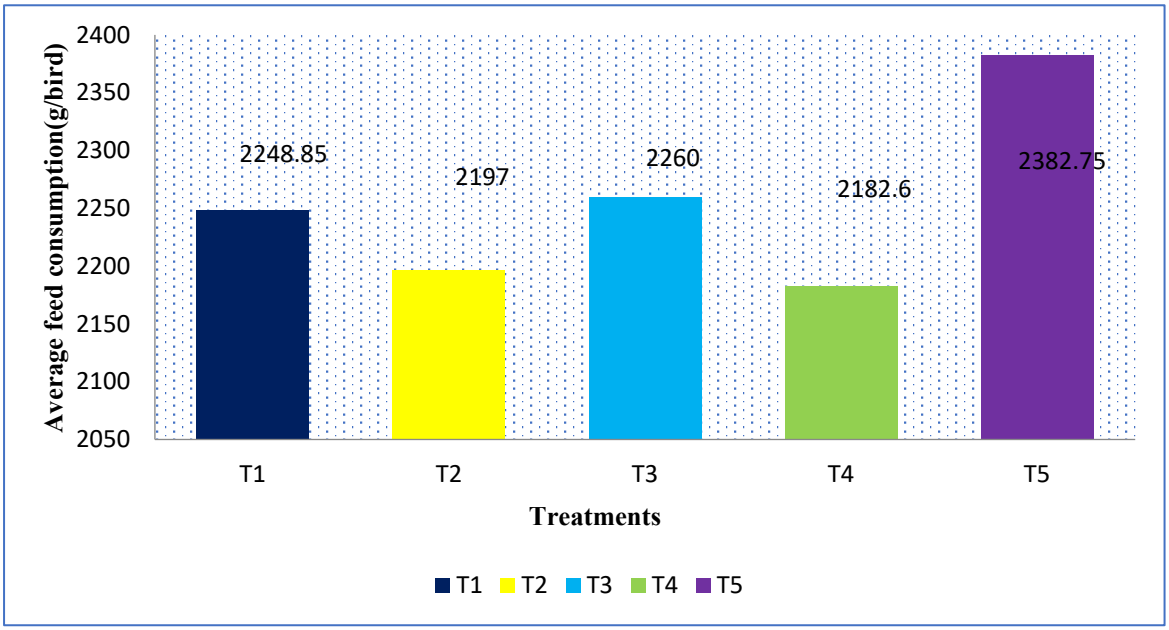

Fig 4: Average feed consumption (g/bird)

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

The mean of total feed consumption of broiler chicks at the end of 4^{th} week in the dietary group T_1, T_2, T_3, T_4 and T_5 were 2248.85±1.65, 2197.00±0.20, 2260.98±18.73, 2182.6±0.34 and 2324.75±19.76 respectively. The highest feed consumption was found in T_5 (2324.75±19.76) and lowest result was in T_2 (2197.00±0.20) group.

Results of the present study supported the findings of Fanimo (2004) and Abudabos (2012) who reported increased feed intake in broilers fed diet supplemented with different levels of Enzyme. Results were also in accordance with those of Rezaeipour *et al.* (2012) who used early feed restriction with or without enzyme supplement in broiler diet and found a significant increase in feed intake.

Table 10: Effects of enzyme on production performances of broiler chicken

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ± SE	Level of significance
Final Live weight (g)/bird	1830.50 ± 15.39^{b}	1808.00 ± 23.68^{b}	1870.00 ± 27.08^{ab}	1826.00 ± 31.72^{b}	1937.50 ± 17.97^{a}	1854.40 ± 14.24	*
Feed consumption (g)/bird	2248.85± 15.65^{ab}	2197.00 ± 30.52^{b}	2260.98 ± 18.73^{ab}	2182.65 ± 60.34^{b}	2324.75 ± 19.76^{a}	2242.85 ± 17.61	*
Total body weight Gain (G/Bird)	1788.50 ± 15.39^{b}	1764.75 ± 23.46^{b}	1828.00 ± 27.08^{ab}	1784.00 ± 31.72^{b}	1895.50 ± 17.97^{a}	1812.15 ± 14.27	*
FCR	1.25 ± 0.01	1.25 ± 0.03	1.24 ± 0.01	1.22 ± 0.01	1.23 ± 0.01	1.24 ± 0.01	NS
Livability (%)	100.00 ± 0.00	97.50 ± 2.50	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.50 ± 0.50	NS
Uniformity (%)	78.25 ± 2.29^{b}	80.75 ± 2.06^{b}	82.00 ± 2.16^{b}	81.50 ± 5.01^{b}	95.25 ± 1.25^{a}	83.55 ± 1.78	*

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5 = 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

4.1.3 Weekly feed consumption:

The mean feed consumption (g) of broiler chicks at the end of first week in different groupT_1(160.00±0.00),T_2(160.00±.0.00),T_3(155.00±0.00),T_4(155.00±0.00).T_5(155.00 ±0.00). Overall mean feed consumption of different groups showed that there was significant (P<0.05) effects. The higher feed consumption was found in T_1 T_1(160.00±.0.00), T_2(160.00±.0.00) and comparatively lower in T_3(155.00±.0.00), T_4(155.00±.0.00) T_5(155.00±.0.00).

The mean feed consumption (g) of broiler chicks at the end of 2rd week in different groups 391.75±3.52, 376.0±8.62, 392.00±3.14, 376.00±5.26 and 395.25±9.61 were respectively. The overall mean body weight gain of different groups showed that there was significant (P<0.05) effects. The higher feed consumption was in T_5, T_3 & T_1 and comparatively lower in T_2 & T_4.

The mean feed consumption (g) of broiler chicks at the end of 3rd week in different groups 710.50±6.01, 691.25±8.78, 693.25±9.48, 682.75±13.62and 727.00±10.43 were respectively. The overall mean body weight gain of different groups showed that there was significant (P<0.05) effects. The higher feed consumption was in T_5 &T_1 and comparatively lower in T_2, T_3 &T_4.

The mean feed consumption (g) of broiler chicks at the end of 4th week in different groups 986.42±9.90, 969.25±21.74, 1021.18±10.53, 968.90±43.83 and 1047.50±0.29 were respectively. The overall mean feed consumption of different groups showed that there was significant (P<0.05) effects. The higher feed consumption was in T_5, T_3 and comparatively lower in T_2 &T_4.

Results of the present study supported the findings of of Fanimo (2004) and Abudabos (2012) who reported increased feed intake in broilers fed diet supplemented with different levels of composite enzyme.

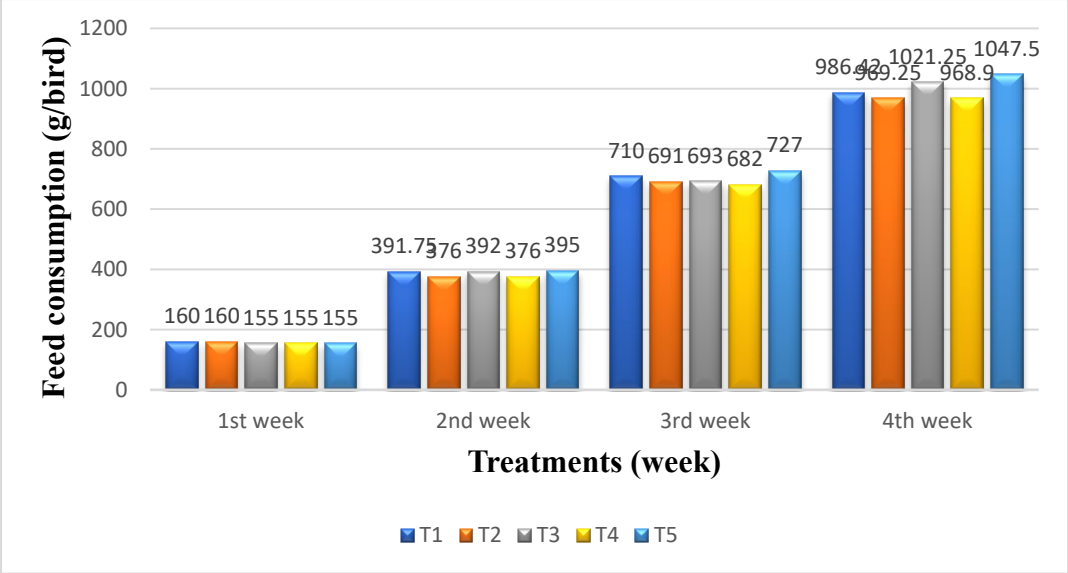

Fig. 5: Effects of enzyme on feed consumption (FC) (g/bird) of broiler chickens at different week

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

Table 11: Effects of enzyme on weekly feed consumption (FC) (g/bird) of broiler chickens at different weeks (g/bird)

Treatment	1st Week FC (g)	2nd Week FC (g)	3rd Week FC (g)	4th Week FC (g)
T_1	160.00 ± 0.00	391.75 ± 3.52	710.50 ± 6.01^{ab}	986.42 ± 9.90^{ab}
T_2	160.00 ± 0.00	376.50 ± 8.62	691.25 ± 8.78^{b}	969.25 ± 21.74^{b}
T_3	155.00 ± 0.00	392.00 ± 3.14	693.25 ± 9.48^{b}	1021.18 ± 10.53^{ab}
T_4	155.00 ± 0.00	376.00 ± 5.26	682.75 ± 13.62^{b}	968.90 ± 43.83^{b}
T_5	155.00 ± 0.00	395.25 ± 9.61	727.00 ± 10.43^{a}	1047.50 ± 0.29^{a}
Mean ± SE	157.00 ± 0.56	386.30 ± 3.24	700.95 ± 5.38	998.65 ± 11.52
Level of Significance	NS	NS	*	*

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

4.1.4 Feed conversion ratio (FCR)

Data presented in table 12 and figure 6 showed that feed conversion ratio (FCR) was not significant (P>0.05). Feed supplemented with enzyme 1gm/L water at T_4 is better (1.31).

However, Feed conversion ratio (FCR) was higher in T_4 group (1.22 ±.01) compared to T_1 (1.25 ±.01), T_2 (1.25 ±.03), T_3 (1.24 ±.01) and T_5 (1.23±.01) groups respectively. These results are in agreement with the findings of Yu et al (2007) who used composite enzyme on broiler and observed FCR than those control chicks. Madrid e al (2010) observed better FCR due to dietary inclusion of enzyme @ 0.05%/kg of diet. Better FCR of the birds using the multi-enzyme may be attributed to the digestion of crude protein, which enhanced growth of the birds due to efficient conversion of feed to meat.

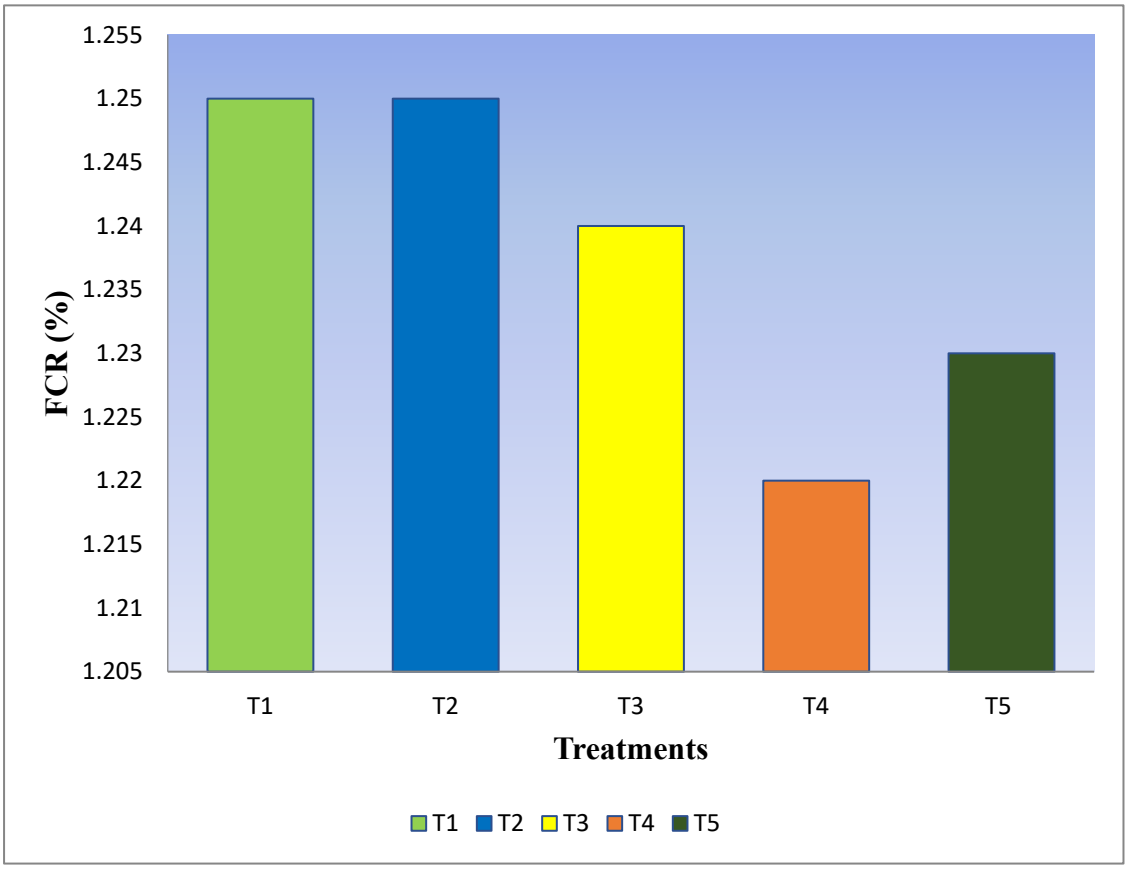

Fig 6: Effects of enzyme on total FCR of broiler chicken at different treatment

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

Table 12: Effects of enzyme on FCR of broiler chicken at different weeks (g/bird)

Treatment	1st week FCR	2nd week FCR	3rd week FCR	4th week FCR
T_1	0.87 ± 0.01	1.14 ± 0.01[b]	1.19 ± 0.01	1.40 ± 0.02
T_2	0.86 ± 0.01	1.12 ± 0.01[b]	1.17 ± 0.03	1.40 ± 0.09
T_3	0.87 ± 0.01	1.14 ± 0.02[b]	1.16 ± 0.00	1.36 ± 0.02
T_4	0.86 ± 0.02	1.30 ± 0.10[a]	1.12 ± 0.04	1.31 ± 0.04
T_5	0.82 ± 0.02	1.10 ± 0.01[b]	1.18 ± 0.02	1.36 ± 0.02
Mean ± SE	0.86 ± 0.01	1.16 ± 0.03	1.17 ± 0.01	1.37 ± 0.02
Level of Significance	NS	*	NS	NS

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

4.1.5 Average body weight gain

Data presented in table 10 and figure 7 showed that the effect of treatments on total body weight gain (gram per broiler chicken) was significant (P<0.05). The relative total body weight gain (g) of broiler chickens in the dietary group T_1, T_2, T_3, T_4 and T_5 were 1788.50±15.39, 1764.75±23.46, 1828.00±27.08, 1784.00±31.72 and 1895.50±17.97 respectively. The highest result was found in T_5 (1895.50±17.97) and lowest result was in T_2 (1764.75±23.46) group.

These results are in agreement with the previous findings of Yu *et al*. (2007); Madrid *et al*. (2010); who reported that dietary inclusion of multi enzyme in the diets of broilers showed improved body weight gain.

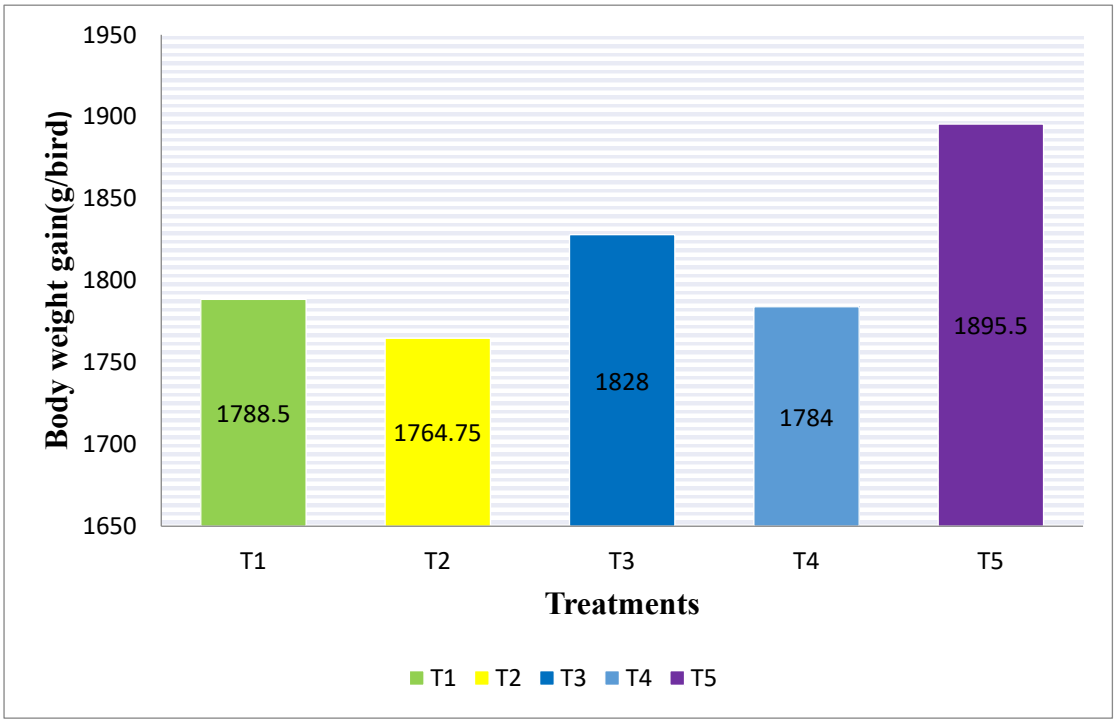

Fig 7: Average body weight gain (g/bird)

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

4.1.6 Weekly body weight gain

Data regarding presented in table 13 and figure 8 showed that the mean body weight gains (g) of broiler chicks at the end of 2nd week in different groups 343.75±2.78, 335.50±5.78, 343.25±2.75, 294.00±20.22, 360.25±9.05 were respectively. The overall mean body weight gains of different

groups showed that there was significant (P<0.05) effects. The highest result was found in T_5 (360.25±9.05) enzyme 1.5gm/kg feed and lowest in control T_4(294.00±20.22).

The mean body weight gains (g) of broiler chicks at the end of 4th week in different groups 705.25± 8.52, 696.75±32.99, 752.75±16.04, 739.00±13.96 and 771.00 ±13.89 were respectively. The overall mean body weight gains of different groups showed that there was significant (P<0.05) effects. The highest result was found in T_5 (771.00 ±13.89) enzyme 1.5gm/kg feed and lowest in control T_2 (696.75±32.99).

These results are in agreement with those obtained by Effect of enzyme on performance of broiler chicks A.M. Shareef and u et al, Madrid et al (2007). Body weight gain for the entire period (3 weeks) were significantly (P<0.05) increased in the treatments 3, 4 and 5, when enzyme was added at a rate of 0.5%,1%, 1.5%, as compared with the other treatments. Best results were seen in treatments 4 and 5. Moreover, these birds also had significantly higher feed intake and feed conversion ratio than others (P<0.05). In all treatments no mortality was recorded.

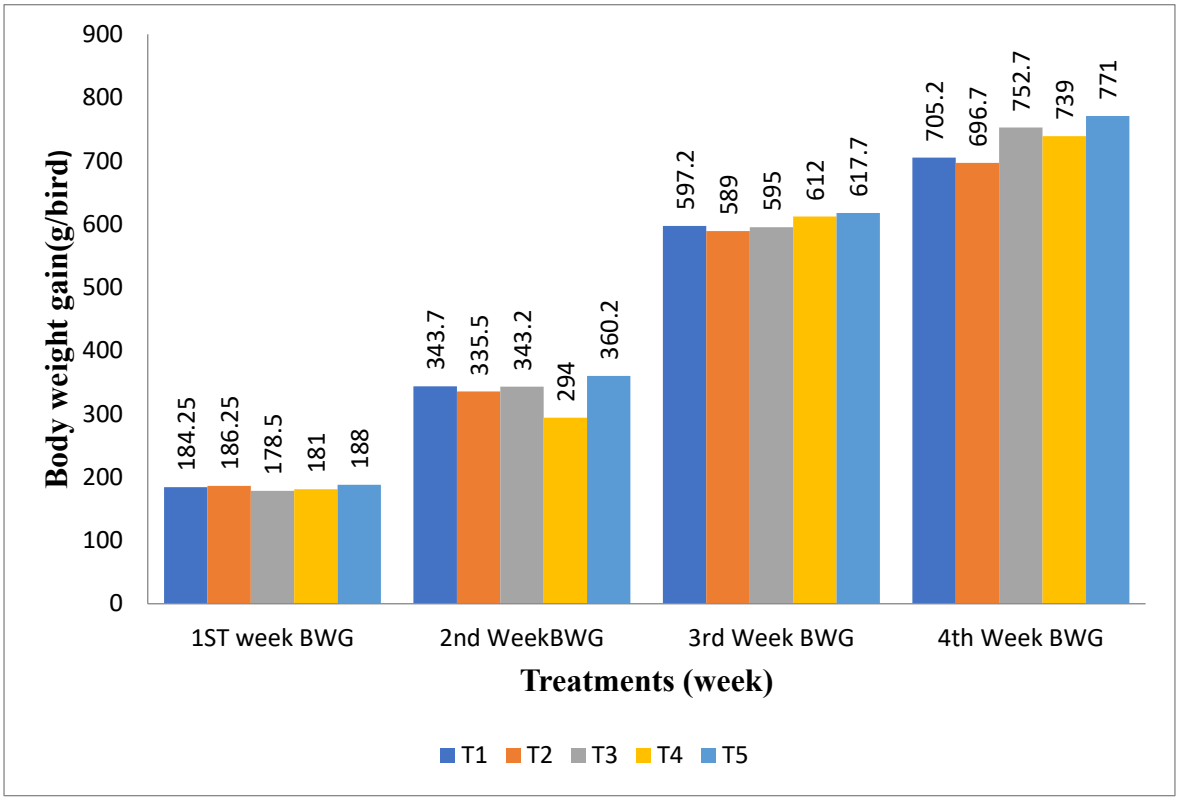

Fig 8: Effects of enzyme on body weight gain (BWG) (g/bird) of broiler

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

Table 13: Effects of enzyme on body weight gain (BWG) (g/bird) of broiler chicken at different weeks (g/bird)

Treatment	1st Week BWG (g)	2nd Week BWG (g)	3rd Week BWG (g)	4th Week BWG (g)
T_1	184.25 ± 2.53	343.75 ± 2.78^{a}	597.25 ± 8.13	705.25 ± 8.52^{b}
T_2	186.25 ± 2.87	335.50 ± 5.78^{a}	589.50 ± 11.19	696.75 ± 32.99^{b}
T_3	178.50 ± 2.06	343.25 ± 2.75^{a}	595.50 ± 8.10	752.75 ± 16.04^{ab}
T_4	181.00 ± 4.30	294.00 ± 20.22^{b}	612.00 ± 24.41	739.00 ± 13.96^{ab}
T_5	188.50 ± 5.69	360.25 ± 9.05^{a}	617.75 ± 5.36	771.00 ± 13.89^{a}
Mean ± SE	183.70 ± 1.68	335.35 ± 6.55	602.40 ± 5.86	732.95 ± 9.93^{a}
Level of Significance	NS	*	NS	*

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

4.1.7 Livability

Data presented in table 10 showed that no birds died at the research period. It showed that 99.5% percent livability of birds. Good management practice, vaccination and quality feed supply with probiotics make the birds healthier and reduced flock mortality.

With similar trials with broilers given different enzyme(s) preparations, the effects on mortality were inconsistent (Udybir *et al*., 2012, Ahmed *et al*., 2010). Shirmohammad and Mehri (2011) reported that there were no significant differences in broiler mortality between the probiotic treatment groups in any of the trials.

4.1.8 Flock uniformity

Data presented in table 10 and figure 9 showed that the flock uniformity of broilers fed diet containing enzyme, antibiotic and control group showed a non-significant (P>0.05) difference among the groups. The flock uniformity is better in 0.15% enzyme group T_5(95.25 ± 1.25) and comparatively lower in Control group T_1 (78.25 ±2.29). Other treatment group is more or less similar.

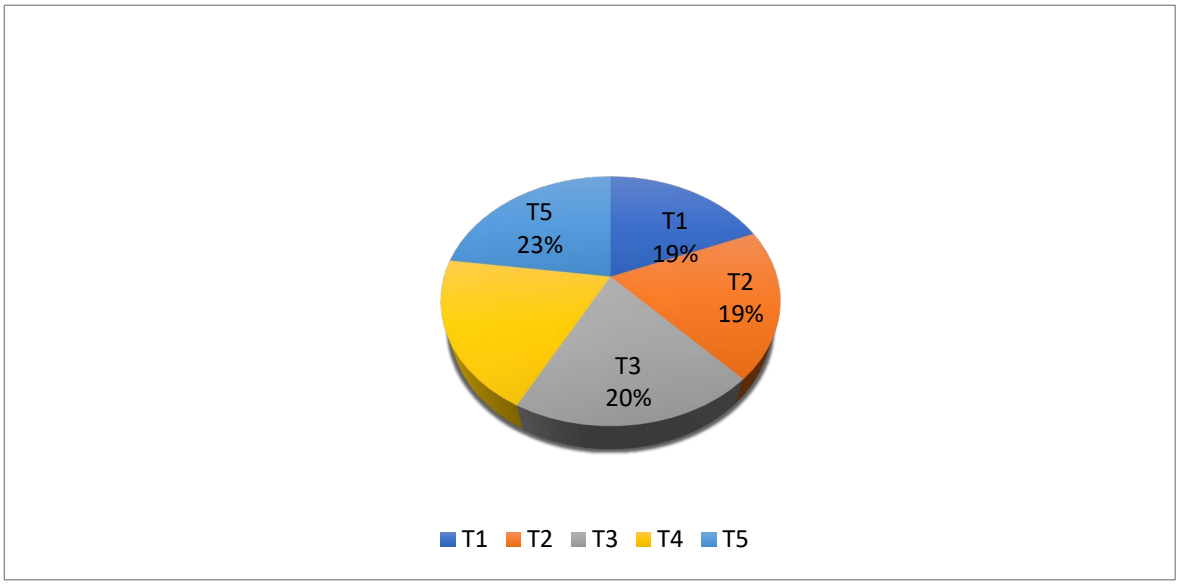

Fig 9: Flock uniformity of different treatment.

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

Carcass characteristics

4.2.1 Dressing percentage (DP)

Data presented in table 14 and figure 10 showed that the dressing percentage at T_5(0.15% enzyme) group was significant ($p<0.05$) carcass percentage (77.75±0.48) compared with the other treatment group T_1 (75.00 ± 0.71), T_2 (76.00 ± 0.41), T_3 (74.50 ± 0.29) and T_4 (76.00±0.41).Experiment, evaluation of dressing percentage on slaughtered representative birds revealed that T_5 group had significantly higher dressed percentage followed by T_1, T_4, T_3 and lower in T_2 groups. This result disagreed with These findings are compatible with those observed by Abudabos (2012) who observed better dressing percentage in broilers by using dried enzyme. The higher dressing percentage in birds fed diet containing 0.15% enzyme may be due to higher body weight gain in the birds of this group compared to other treatment groups.

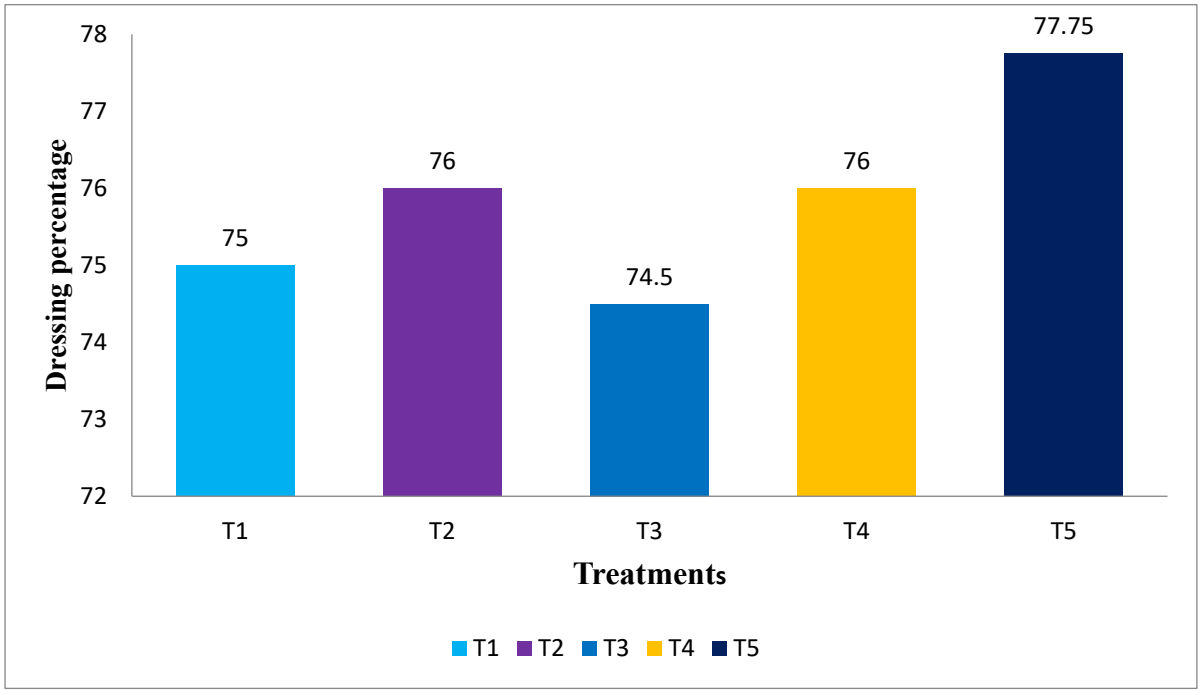

Fig 10: Dressing percentage of different enzyme treatment.

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ ($P>0.05$) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance ($p<0.05$).

4.2.3 Relative weight of giblet organs

Data regarding presented in table 13 and figure 10 showed that relative weight of giblet organs (liver, heart, gizzard and spleen) of broilers fed diet containing enzyme, antibiotic and control group showed a non-significant difference among the groups. In enzyme treatment group the weight of giblet organ is less than in antibiotic and control group.

The glandular abdominal fat showed significant (0.05) effect that in T_5(21.25±0.75) treatment is less than T_1 (40.25±4.07) in control group.

The present findings were not agreement with previous findings (Pinheiro *et al.* (2004) reported more improvements in liver, gizzard and heart of broilers, mules and ducklings by supplementing diets with enzyme.

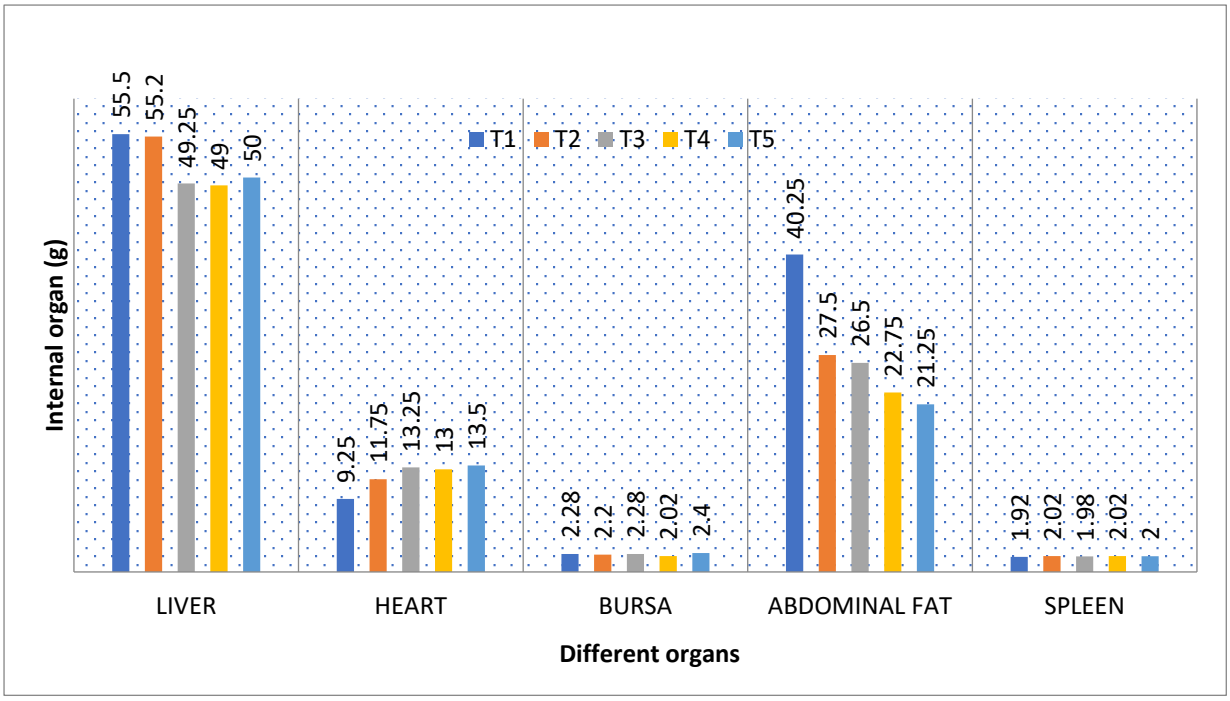

Fig 11: Effects of enzyme on internal organs of broiler chicken under different treatment group

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

Table 14. Effects of enzyme on internal organs of broiler chicken under different treatment (g/bird)

Treatment	Dressing percentage (%)	Liver wt. (g)	Heart wt. (g)	Spleen wt. (g)	Bursa wt. (g)	Abdominal fat wt. (g)
T_1	75.00 ± 0.71^b	55.50 ± 3.28	9.25 ± 0.85^b	1.92 ± 0.05	2.28 ± 0.29	40.25 ± 4.07^a
T_2	76.00 ± 0.41^b	55.25 ± 3.43	11.75 ± 0.75^a	2.02 ± 0.03	2.20 ± 0.11	27.50 ± 0.87^b
T_3	74.50 ± 0.29^b	49.25 ± 3.52	13.25 ± 0.25^a	1.98 ± 0.06	2.28 ± 0.24	26.50 ± 2.50^b
T_4	76.00 ± 0.41^b	49.00 ± 1.83	13.00 ± 0.41^a	2.02 ± 0.03	2.02 ± 0.06	22.75 ± 0.48^b
T_5	77.75 ± 0.48^a	50.75 ± 3.52	13.50 ± 0.65^a	2.00 ± 0.00	2.40 ± 0.37	21.25 ± 0.75^b
Mean ± SE	75.85 ± 0.32	51.95 ± 1.42	12.15 ± 0.44	1.99 ± 0.02	2.24 ± 0.10	27.65 ± 1.77
Level of Significance	*	**NS**	*	**NS**	**NS**	*

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

4.2.4 Effects of enzyme on hematological change of broiler chicken under different treatment

Data regarding table 14 show the hematological change in different treatment. The hemoglobin level of enzyme treatment is comparatively higher than control or antibiotic treatment. The highest hemoglobin level in group T_5((9.12 ± 0.18) and low level of hemoglobin is in group T_1 (8.30 ± 0.07). White Blood cell count are significantly different in different treatment. The highest WBC level is found in T_1 (Control) group (14.48 ± 0.74) and lower level of WBC in group T_2 (5.38 ± 0.28) and T_5 (5.72 ± 0.13).This statistical is supported Cent et.al.(2005),Ahmed et.al.(2007),Meng et.al.(2004),This reported are agree the TEC,Hb level is higher than control

group. Other parameter is not differentiated and its similar opinion Hehab et.al. (2012), Buyrem et al (2004).

Table 15: Effects of enzyme on hematological change of broiler chicken under different treatment

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ± SE	Level of significance
Hb (g/dl)	8.30 ± 0.07^{c}	8.98 ± 0.25^{ab}	8.48 ± 0.10^{c}	8.58 ± 0.03^{bc}	9.12 ± 0.18^{a}	8.69 ± 0.09	*
RBC (million/mm^3)	3.80 ± 0.05^{b}	3.97 ± 0.09^{a}	3.53 ± 0.02^{c}	3.49 ± 0.03^{c}	3.88 ± 0.05^{ab}	3.73 ± 0.05	*
WBC (thousand/ mm^3)	14.48 ± 0.74^{a}	5.38 ± 0.28^{d}	11.70 ± 0.09^{b}	8.58 ± 0.15^{c}	5.72 ± 0.13^{d}	9.17 ± 0.82	*
Neutrophils (%)	55.50 ± 3.33	58.25 ± 0.75	58.75 ± 2.96	60.25 ± 0.85	60.50 ± 0.87	58.65 ± 0.93	NS
Lymphocytes (%)	38.50 ± 1.76	33.25 ± 1.49	34.75 ± 3.47	34.50 ± 1.04	33.00 ± 0.91	34.80 ± 0.90	NS
Monocytes (%)	2.00 ± 0.00^{ab}	1.50 ± 0.29^{b}	1.75 ± 0.25^{ab}	2.00 ± 0.00^{ab}	2.25 ± 0.25^{a}	1.90 ± 0.10	*
Eosinophils (%)	4.00 ± 0.41	4.00 ± 0.00	4.00 ± 0.00	4.50 ± 0.50	4.50 ± 0.29	4.20 ± 0.14	NS
Platelets ($\times 10^4/mm^3$)	29.75 ± 1.20^{ab}	26.12 ± 1.53^{b}	31.25 ± 0.63^{a}	29.95 ± 1.08^{ab}	30.75 ± 1.69^{a}	29.56 ± 0.66	*
PCV (%)	28.38 ± 0.87	27.57 ± 0.95	26.50 ± 0.11	26.52 ± 0.21	28.48 ± 0.65	27.49 ± 0.33	NS
MCV (FI)	86.13 ± 0.24^{a}	87.35 ± 0.53^{a}	83.65 ± 0.30^{b}	83.28 ± 1.16^{b}	87.74 ± 0.25^{a}	85.63 ± 0.49	*
MCH (pg)	27.02 ± 0.27^{b}	28.27 ± 0.38^{a}	27.16 ± 0.09^{b}	26.98 ± 0.26^{b}	28.77 ± 0.19^{a}	27.64 ± 0.20	*

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

4.2.5 Effect of enzyme on microbial test (*Salmonella spp.& E. coli* colony count) of Broiler fecal content.

Data regarding presented in table 15, *Salmonella spp*. number is relatively higher in Control group T_1 (9.20 ± 0.31) & *E.coli* also higher in group T_5(7.65 ± 0.29),T_4 (7.32 ± 0.11), T_1 (6.92 ± 0.39) broilers fed diet containing enzyme, antibiotic group showed a non-significant difference among the groups. It is same recommending in the effects of MOS on poultry production can be expressed in reduction of diseases by inhibition of pathogenic bacterial colonization to gut lining by binding to them and thus preventing them of proliferating and producing toxins (Benites *et al.*, 2008). Reducing intestinal pathogen counts (Benites *et al.*, 2008; Benites, V. Gilharry, R., Gernat, A.G.and Murillo, J.G. 2008). Improving the immune system (Ferket, 2002; Ferket, P.R., 2002). and exhibit influence on morpho-functional characteristics of intestines (Ferket, 2002; Ferket, P.R., 2002). However, results of the effects of MOS on broiler performance are contradictory. Other reports showed that MOS had no positive influence on the performance of poultry (Waldroup *et al.*, 2003; Waldroup, P.W., Fritts, C.A.and Yan, F., 2003).

Table 16: Effect of enzyme on microbial test (*Salmonella spp.& E. coli* colony count) of Broiler fecal content.

Treatment	*Salmonella spp.* (SS) $\times 10^6$ (CFU/ml)	*E. coli* (EMB) $\times 10^6$ (CFU/ ml)
T_1	9.20 ± 0.31^a	6.92 ± 0.39^a
T_2	4.52 ± 0.23^c	4.58 ± 0.14^b
T_3	4.58 ± 0.13^c	5.62 ± 0.75^b
T_4	7.40 ± 0.53^b	7.32 ± 0.11^a
T_5	4.93 ± 0.16^c	7.65 ± 0.29^a
Mean ±SE	6.12 ± 0.45	6.42 ± 0.31
Level of significance	*	*

Here, T_1 = Control, T_2 = Antibiotic, T_3 = 0.05% enzyme, T_4 = 0.1% enzyme and T_5= 0.15% enzyme. Values are Mean ± S.E (n=15) one-way ANOVA (SPSS, Duncan method).

a, b bars with different superscripts differ (P>0.05) significantly

SE= Standard Error

NS= Non significant

* means significant at 5% level of significance (p<0.05).

CHAPTER V

SUMMARY & CONCLUSION

A total of 200-day-old Lohmann meat (Indian river) chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. Chicks were divided randomly into 5 experimental groups of 4 replicates (10 chicks in each replications). One of the five experimental group one was fed with basal diet which was control group. Another group was fed with antibiotic mixed feed. The remaining three groups were fed diet with different dose of composite enzyme: 1st one was 0.5g enzyme/L of water, 2nd one was 1g enzyme/L of water, and the 3rd one was 1.5g enzyme/L of water. The effects of supplementation of antibiotic, enzyme and control on broiler performance were measured. The performance traits *viz.* body weight, weight gain, feed consumption, FCR, dressed bird weight, relative giblet weight, livability, flock uniformity and meat yield of broiler on different replication of the treatments was recorded and compared in each group. At 28 days of age, broilers were dissected to compare meat yield characteristics among different treatments. Final live weight was significantly higher in group T_5 (1937.50±17.97) and lowest result was in T_2 (1808.00±23.68) group. Body weight gain was also significantly higher in group T_5 (1895.50±17.97) and lowest result was in T_2 (1764.75±23.46) group. There is non- significance in FCR value. Feed consumption was higher in enzyme treatment group because we know that enzyme increase the feed consumption. The feed consumption at found in T_5 (2324.75±19.76) and lowest result was in T_2 (2197.00±0.20) group. The livability mean was 99.5 percentage. The flock uniformity was significant. In control group T_1((78.25 ±2.29) was average & other groups were uniformed. The highest FU value was in T_5(95.25 ±1.25) & lowest FU value in group T_1(78.25 ±2.29). In experiment, evaluation of dressing percentage on slaughtered representative birds revealed that T_5 (77.75±0.48) group had significantly higher dressing percentage followed by T_1, T_4, T_3 and lower in T_2 groups. In enzyme treatment group the weight of giblet organ is higher than in antibiotic and control group. The abdominal fat showed significant ($P<0.05$) effect that in control group T_1 (40.25±4.07) treatment is higher than T_5(21.25±0.75).

Blood parameter like TEC, Hb, RBC, WBC, increase significantly ($p<0.05$) in the treated T_1 compared other. Highest hemoglobin (T_5-9.12±0.18 g/dl), RBC (T_2-

3.97±0.09 mill/cum), WBC (T_1-14.48±0.74 mill/cum), lymphocytes (T_1-38.50%), Monocytes (T_5-2.25±.25 %), Platelets (T_3-31.25±0.63 x10^4 /mm^3), PCV (T_5- 28.48%), MCV (T_5-87.74±0.25 FI), MCH (T_4-28.77 Pg.) were found in the enzyme treated groups, which is an indication of good health. *E. coli* and *salmonella spp*. count was significantly ($P<0.05$) lower in birds fed 0.15% enzyme supplemented diet and with a descending order of 0.1% and 0.05% enzyme level. *Salmonella spp.* and *E. Coli* count was also significantly ($p<0.05$) higher in birds fed control.

It can be concluded above experimental data indicates that the inclusion of up to 0.15% enzyme in the basal diets of young broiler chicks might improve the development of the growth performances and improves BW, immune characteristics of broiler chickens. Moreover, non-beneficial microbes are also found less in highest enzyme supplemented diet. So, enzyme may be used in broiler ration in absence of antibiotic. Therefore, it is strongly suggested that enzyme can be used in our country for quality poultry production, diminishes the risk of antibiotic resistance in body and leads a healthier life with safe food consumption. However, commercial application is recommended.

CHAPTER VI

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CHAPTER VII

APPENDICES

Appendix 1. Effects of enzyme on production performances of broiler chicken

Treatment	Replication	Final live weight(g/Bird)	Total feed consumption (g/Bird	Total Body Weight Gain (g/Bird	Final FCR	Survivability
T_1	R_1	1792.0	2202.4	1750	1.254	100
	R_2	1850.0	2259.0	1808	1.249	100
	R_3	1820.0	2270.0	1778	1.271	100
	R_4	1860.0	2264.0	1818	1.245	100
T_2	R_1	1820.0	2137.0	1778	1.212	100
	R_2	1850.0	2171.0	1808	1.214	100
	R_3	1740.0	2280.0	1698	1.342	100
	R_4	1822.0	2114.0	1780	1.180	90
T_3	R_1	1910.0	2269.0	1868	1.215	100
	R_2	1890.0	2296.0	1848	1.251	100
	R_3	1790.0	2207.9	1748	1.263	100
	R_4	1890.0	2271.0	1848	1.229	100
T_4	R_1	1740.0	2018.4	1698	1.189	100
	R_2	1824.0	2198.2	1782	1.234	100
	R_3	1850.0	2205.0	1808	1.220	100
	R_4	1890.0	2309.0	1848	1.249	100
T_5	R_1	1920.0	2266.0	1878	1.213	100
	R_2	1910.0	2347.0	1868	1.256	100
	R_3	1930.0	2349.0	1888	1.244	100
	R_4	1990.0	2337.0	1948	1.199	100

Appendix 2: Recorded temperature & relative humidity% during experimental period

Age in weeks	**Period**	**Average temperature** ^{0}**C**	**Average humidity** **%**
1st	30.08.20-05.09.20	31.1	79.0
2nd	06.09.20-12.09.20	30.0	78.5

3rd	13.09.20-19. 09.20	29.6	78.0
4th	20.09.20-26.09.20	30.9	76.87

Appendix 3. Effects of enzyme dressing percentage of broiler chicken (g/bird)

Treatment	Replication	Live wt.(g)	Dressed wt.(g)	Dressing %
T_1	R_1	1900	1450	76
	R_2	1850	1450	76
	R_3	1875	1412	75
	R_4	1705	1250	73
T_2	R_1	1852	1464	77
	R_2	1825	1335	76
	R_3	1950	1450	76
	R_4	1980	1385	75
T_3	R_1	1925	1420	75
	R_2	1785	1345	74
	R_3	1825	1376	74
	R_4	1812	1350	75
T_4	R_1	1795	1292	76
	R_2	1860	1340	75
	R_3	1803	1345	76
	R_4	2070	1510	77
T_5	R_1	2060	1604	77
	R_2	1985	1540	77
	R_3	2060	1586	78
	R_4	2130	1725	79

Appendix 4. Effects of enzyme on internal organs of broiler chicken under different treatment (g/bird)

Treatment	Replication	Liver(g)	Heart. (g)	Spleen	Bursa	Abdominal fat
T_1	R_1	53	11	2	3.1	33
	R_2	54	10	2	2.2	35
	R_3	50	9	1.9	2	51
	R_4	65	7	1.8	1.8	42
T_2	R_1	51	10	2	2.1	30
	R_2	50	11	2	2.2	27
	R_3	65	13	2.1	2	26

	R_4	55	13	2	2.5	27
T_3	R_1	51	14	2.1	2	33
	R_2	57	13	1.8	2	25
	R_3	40	13	2	2.1	27
	R_4	49	13	2	3	21
T_4	R_1	51	12	2	2.2	24
	R_2	53	13	2	2	22
	R_3	47	13	2.1	2	23
	R_4	45	14	2	1.9	22
T_5	R_1	45	13	2	2	20
	R_2	49	12	2	2.1	22
	R_3	48	14	2	3.5	23
	R_4	61	15	2	2	20

Appendix 5. Effects of enzyme on uniformity of broiler chicken

Treatment	Replication	Uniformity (%)	Average uniformity (%)
T_1	R_1	75.0	78.25
	R_2	77	
	R_3	85	
	R_4	76	
T_2	R_1	82	80.75
	R_2	86	

	R_3	78	
	R_4	77	
T_3	R_1	78	82
	R_2	88	
	R_3	80	
	R_4	82	
T_4	R_1	69	81.5
	R_2	78	
	R_3	88	
	R_4	91	
T_5	R_1	95	95.25
	R_2	92	

Appendix 6. Effects of enzyme on body weight gain (BWG) (g/bird) of broiler chicken at different weeks (g/bird)

Treatment	Replication	1st week	2nd week	3rd Week	4rd Week
T_1	R_1	180	526	1101	1792
	R_2	180	529	1130	1850
	R_3	187	531	1130	1820
	R_4	190	526	1140	1860
T_2	R_1	183	516	1130	1820

	R_2	194	531	1101	1850
	R_3	181	531	1134	1740
	R_4	187	509	1080	1640
T_3	R_1	178	526	1128	1910
	R_2	184	531	1143	1890
	R_3	174	510	1080	1790
	R_4	178	520	1118	1890
T_4	R_1	170	493	1040	1740
	R_2	191	475	1082	1824
	R_3	181	422	1084	1850
	R_4	182	510	1142	1890
T_5	R_1	180	515	1132	1920
	R_2	187	550	1170	1910
	R_3	205	570	1174	1930
	R_4	182	560	1190	1990

Appendix 7: Effects of enzyme on feed consumption (FC) (g/bird) of broiler chickens at different weeks (g/bird)

Treatment	Replication	1st week	2nd week	3rd Week	4rd Week	Total
T_1	R_1	160	390	695	957.4	2202.4
	R_2	160	401	707	990.3	2259
	R_3	160	392	720	998	2270
	R_4	160	384	720	1000	2264

T_2	R_1	160	379	673	925	2137
	R_2	160	375	697	939	2171
	R_3	160	397	713	1010	2280
	R_4	160	355	682	917	2114
T_3	R_1	155	384	696	1034	2269
	R_2	155	397	714	1030	2296
	R_3	155	397	668	989.7	2209.7
	R_4	155	390	695	1031	2271
T_4	R_1	155	361	659	843.4	2018.4
	R_2	155	381	676	986.2	2198.2
	R_3	155	377	674	999	2205
	R_4	155	385	722	1047	2309
T_5	R_1	155	367	697	1047	2266
	R_2	155	410	735	1047	2347
	R_3	155	401	745	1048	2349
	R_4	155	403	731	1048	2337

Appendix 8: Hematological properties of broiler chicken under enzyme treatment

Relication	Hbgm/dl	RBC Mi/cum	WBC Mi/cum	Platelet Mi/cum	Nutrofil S%	Lymhocytes%	Monocytes%	Esonophils%	Basophils%	HCT/PC%	MCVF1	MCHpg	MCHCg/dl

T1R1	8.2	3.87	12300	2650000	55	36	02	04	00	29.8	86.54	27.3	32.19
T1R2	8.5	3.6	15500	2900000	52	42	02	04	00	28.9	86.32	27.3	32.22
T1R3	8.2	3.79	14900	3100000	65	35	02	03	00	25.8	85.43	26.2	32.02
T1R4	8.3	3.89	15200	3200000	50	41	02	05	00	28.7	86.22	27.2	32.10
T2R1	9.3	3.99	6100	2700000	57	30	02	04	00	28.9	85.89	28.6	32.90
T2R2	8.6	3.83	5000	3000000	59	32	01	04	00	25.9	87.54	28.3	3.02
T2R3	8.5	3.82	4900	3100000	57	37	02	04	00	25.8	88.43	27.2	32.01
T2R4	8.6	4.2	5500	2450000	60	32	01	04	00	29.4	87.54	28.9	32.19
T3R1	8.2	3.57	11800	3100000	61	35	02	04	00	26.5	83.17	27.4	32.01
T3R2	8.5	3.50	11900	3000000	51	43	02	04	00	26.6	84.12	27.1	32.03
T3R3	8.5	3.50	11500	3100000	58	35	02	04	00	26.7	84.20	27.0	32.10
T3R4	8.7	3.56	11600	3300000	65	26	01	04	00	26.2	83.11	27.2	32.00
T4R1	8.5	3.5	8500	2800000	60	35	02	04	00	26.1	82.22	26.1	31.00
T4R2	8.6	3.56	8300	2900000	61	34	02	06	00	27.1	85.19	27.5	31.97

T4R3	8.6	3.4	8500	2980000	56	32	02	04	00	26.5	80.51	27.1	31.51
T4R4	8.6	3.5	9000	3300000	65	37	02	04	00	26.3	85.22	26.9	31.95
T5R1	9	3.75	5500	2950000	60	35	03	05	00	28.7	87.43	28.2	32.17
T5R2	9.5	3.87	6000	2700000	63	31	02	05	00	28.8	88.50	28.9	32.32
T5R3	8.7	3.88	5500	3150000	60	32	02	04	00	26.6	87.54	228.9	32.19
T5R4	9.3	4.0	5900	3500000	59	34	02	04	00	29.7	87.50	28.9	32.66

Appendix 9: Effect of enzyme on microbial test (*Salmonella* and E. *coli* colony count) of broiler fecal content.

Treatment	Replication	***Salmonella* spp. (SS) $\times 10^6$ (CFU/ml)**	***E. coli* (EMB) $\times 10^6$ (CFU/ ml)**
T_1	R_1	9.2	6.4
	R_2	8.5	7.2
	R_3	9.5	7.3
	R_4	9.1	7.01
T_2	R_1	3.9	4.5
	R_2	4.8	4.2
	R_3	4.9	4.8
	R_4	4.5	4.8
T_3	R_1	4.5	5.9
	R_2	4.6	4.2
	R_3	4.9	4.8
	R_4	4.3	7.6
T_4	R_1	8.5	7.2
	R_2	8.1	7.1
	R_3	6.7	7.6
	R_4	6.3	7.4
T_5	R_1	5.2	7.8
	R_2	4.9	8.4
	R_3	5.1	7.1
	R_4	4.5	7.3

Some pictorial view of my experiment

Plate 01: Preparation of brooding & receiving

Plate 02: Chick observation, preparation of stall and chick distribution

Plate 03: Vaccination programme

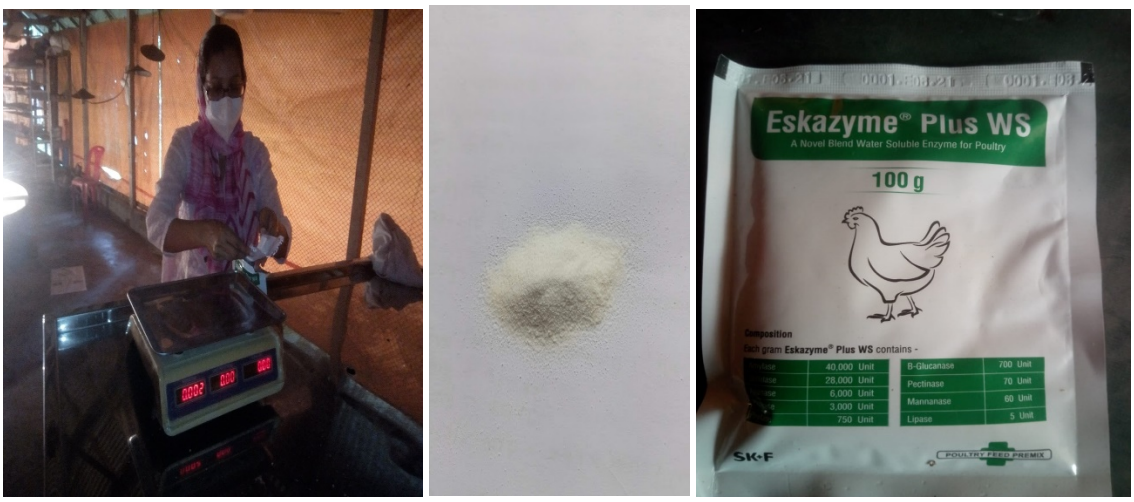


Plate04: Measurement of enzyme

Fig:Measurement of day old chick. Fig: weighting of seven days chick. Fig:Feed weighting

Plate 05 : Measurement of chick & feed at different weeks

Plate 06: Data collection

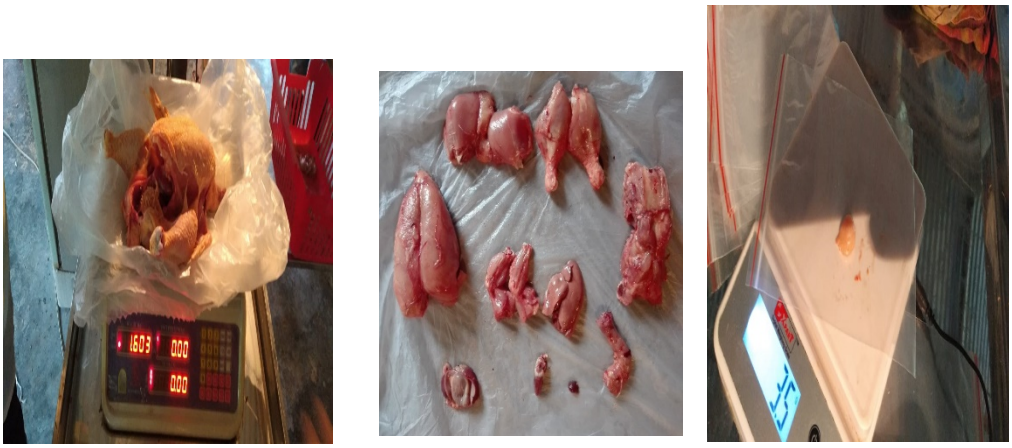
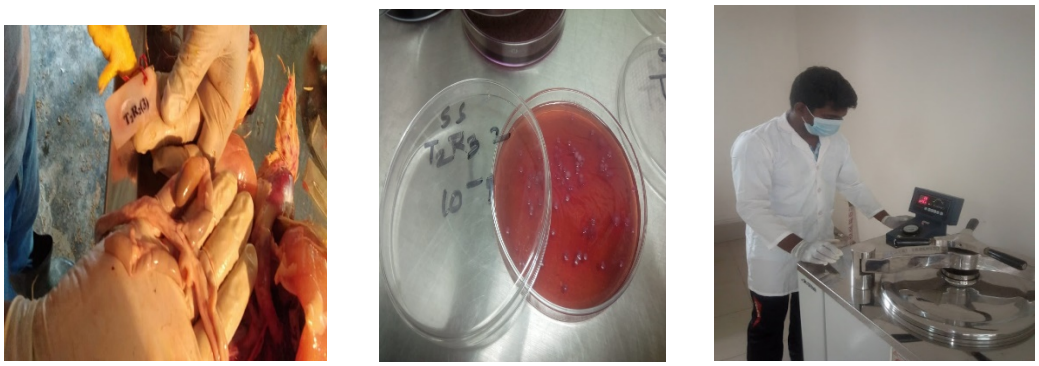


Plate 07: Data collection



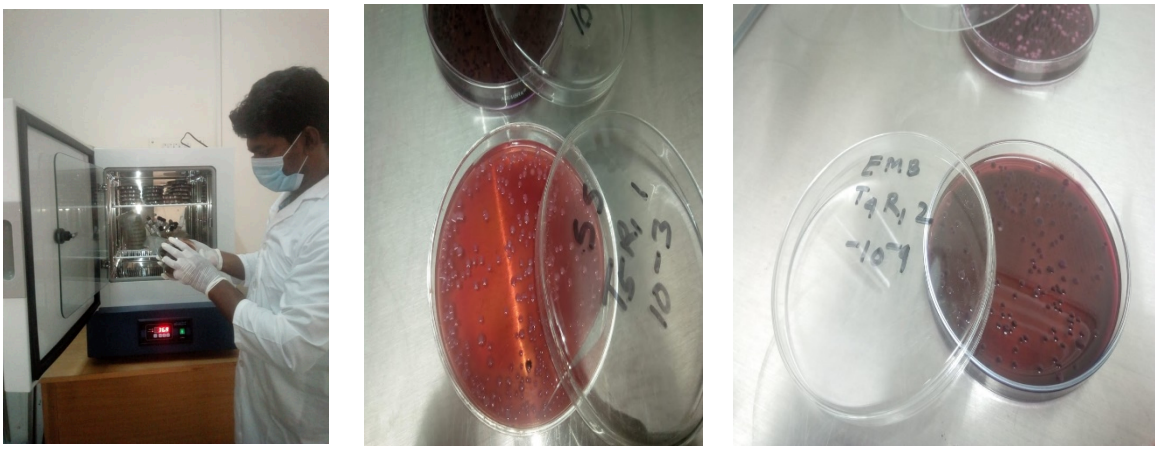


Plate 08: Microbiological test of cecal content (*E.* coli & Salmonella sp colony counting)

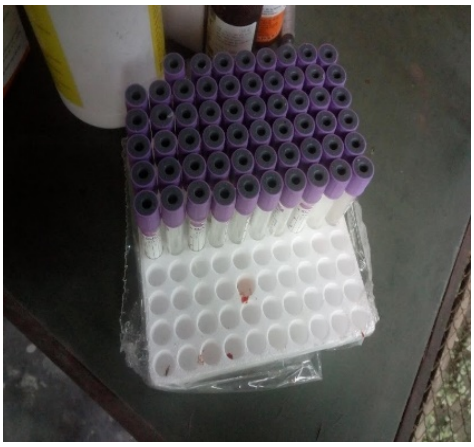


Plate 09: Blood collection & CBC Test.

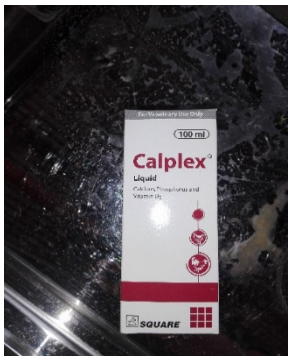


Plate 10: Medication of broiler chick