USE OF COMMERCIAL PHYTASE ENZYME ON GROWTH PERFORMANCE OF BROILER

A Thesis

By

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CERTIFICATE

This is to certify that the thesis entitled "USE OF COMMERCIAL PHYTASE ENZYME ON GROWTH PERFORMANCE OF BROILER" submitted to the Department of Animal Nutrition, Genetics and Breeding, 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 Animal Nutrition, embodies the result of a piece of bona fide research work carried out by Md. Akhtaruzzaman, Registration No.: 12-04788, Semester: Jan-June/2019 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.

Dated:
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DEDICATED TO MY
BELOVED GRANDMOTHER
AND MY PARENTS

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The Author

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ACRONYMS AND ABBREVIATIONS

Abbreviation		Full meaning
A.M	=	Ante meridian
ACTH	=	Adrenal Corticotrophin hormone
ANOVA	=	Analysis of Variance
BANSDOC	=	Bangladesh National Scientific And Technical Documentation Centre
BARC	=	Bangladesh Agricultural Research Council
BBS	=	Bangladesh Bureau of Statistics
BLRI	=	Bangladesh Livestock Research Institute
Ca	=	Calcium
CAT	=	Catalase
CF	=	Crude Fiber
Cm	=	Centimeter
cm^2	=	Square Centimeter
CONTD.	=	Continued
CP	=	Crude Protein
CRD	=	Complete Randomized Design
DMD	=	Dry Matter Digestibility
Dr.	=	Doctor
e.g.	=	For Example
EDTA	=	Ethylene Diethyle Tetraacitic Acid
et al.	=	And others/Associates
FC	=	Feed Consumption
FTU	=	Phytase Unit of Measurement
FCR	=	Feed Conversion Ratio
i.e.	=	That is
IBV	=	Infectious Bronchitis Vaccines
kcal	=	Kilo-calorie
Kg	=	Kilogram
LSD	=	Least Significant Difference

ACRONYMS AND ABBREVIATIONS (CONT'D)

Abbreviation		Full meaning
Ltd.	=	Limited
M.S.	=	Master of Science
ME	=	Metabolizable Energy
mm	=	Millimeter
mmol	=	Millimol
MT	=	Metric ton
N	=	Nitrogen
NDV	=	Newcastle Disease Vaccine
No.	=	Number
NS	=	Non-significant
P	=	Phosphorus
NPP	=	Nonphytate phosphorous
PU	=	Phytase Unit.
PA	=	Phytic acid.
ppm	=	Parts per Million
SAU	=	Sher-E-Bangla Agricultural University
SED	=	Standard Error Difference
SPSS	=	Statistical Package for Social Sciences
viz.	=	Such as
Vs	=	Versus
WPSA	=	World's Poultry Science Association

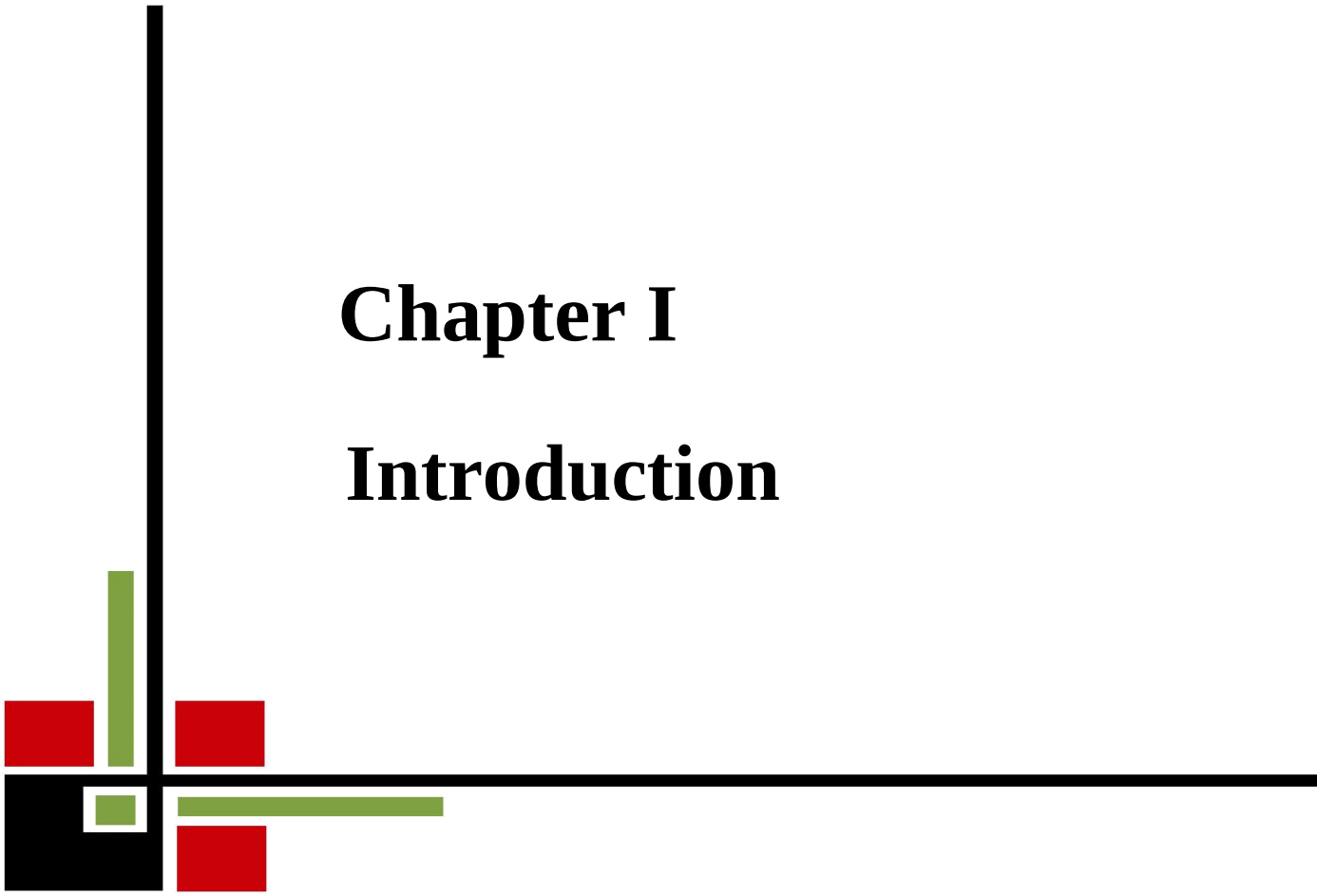
LIST OF SYMBOLS

Symbols		Full meaning
:	=	Ratio
@	=	At the rate of
+	=	Plus
<	=	Less than
>	=	Greater than
°C	=	Degree Celsius
°F	=	Degree Fahrenheit
%	=	Percentage
&	=	And
*	=	5% level of significance
**	=	1% level of significance
/	=	Per

USE OF COMMERCIAL PHYTASE ENZYME ON GROWTH PERFORMANCE OF BROILER

ABSTRACT

A total of 120 day-old Cobb 500 broiler chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. The present study was designed to evaluate the productive performance and health status of commercial broiler chicks fed diet containing phytase enzyme compared to base diet. The aims of the present study were to investigate the influence of a phytase enzyme on the growth performance of broiler, Body weight, Feed intake, FCR, carcass merits and P status of broilers (tibia ash contents) of male and female broiler were compared to control feeding of broiler. A total of 120 day-old Cobb 500 chicks were used in this experiment. Chicks were divided randomly into 4 experimental groups of 3 replicates (10 chicks with each replications). One of the 4 experimental group was fed this diet as control while, the remaining Three groups were fed diet mixed with 3 categories of phytase enzyme (fungal, bacterial mixed fungal and bacterial). Here this study T_1 = (control), T_2 = (Bacterial phytase enzyme), T_3 = (Combied Bacterial and fungal phytase enzyme) and T_4 = (fungal phytase). The duration of experiment work 28 days. Results of the experiment showed that addition of phytase diets improved ($P<0.5$) broilers growth performance and tibia ash content percent with impact starting from the beginning of the fourth week of the feeding trial. However, it has no effect on feed intake. Feed conversion ratio and dressing percent were increased ($P<0.05$) in birds fed diets supplemented with phytase. Phytase supplementation had no significant effect on both male and female carcass cuts compared to control. To investigated the effect microbial phytase supplementation of microbial phytase specially mixed fungal and bacterial phytase in starter and finisher diets of broilers(1.5+0.1) g /100 kg of the feeds proved to be beneficial for increasing the growth performance of broiler, P status of broiler(tibia ash contents).



Chapter I

Introduction

CHAPTER 1

INTRODUCTION

1.1 General Background

Poultry farming has emerged as one of the fastest growing agribusiness industries in the world, even in Bangladesh. Research on meat production globally indicates poultry as the fastest growing livestock sector especially in developing countries. It has triggered the discovery and widespread use of a number of "feed additives". The term feed additive is applied in a broad sense, to all products other than those commonly called feedstuffs, which could be added to the ration with the purpose of obtaining some special effects. The main objective of adding feed additives is to boost animal performance by increasing their growth rate, better-feed conversion efficiency, greater livability and lowered mortality in poultry birds. These feed additives are termed as "growth promoters" and often called as non-nutrient feed additives.

Two distinct phytase products are available in the market: one derived from submerged liquid fermentation that uses genetically manipulated organisms to achieve maximum enzyme production and the other based on solid state fermentation that uses normal organisms for enzyme production. Because of the technology and the nature of organism used, besides phytase product also contains several side enzyme activities, including protease, amylase, cellulase, xylanase, and β-glucanase Potter (1988).

1.2 State of the Problems

Generally, among the grains and by-products used in feeds, phytic acid-type phosphorus accounts for 56-70%, and the use of phosphorus in feed is only about 30%.
The presence of phatic acid not only inhibits phosphorus utilization within the body, but also reduces the availability of other minerals because it forms salts with Ca and Mg.
The cost of feeds ingredients reduces profitability of livestock (especially broiler) business to a marginal level. On the other hand, large fraction of dietary phosphorus which is not available to animal is dumped into manure creating significant hazards to the environment Mondal *et al.* (2007).

To solving this problem, attempts were made to use some feed additives or enzymes. These enzymes had variable effects on animal's general performance and the utilization of feed phosphorus. Phytase enzyme is one of the promising additives to solve part of the stated problem.

1.3 Justification of the Study

Large portion of phosphorus (P) in plant feed ingredients is present in the form of phytate, which is largely unavailable for non-ruminants Rezaei *et al.* (2007). Phytic acid can ironically binds minerals and proteins in aqueous medium Sebastian *et al*. (1997). The interest in the use of microbial feed enzymes such as phytase arises from the need to improve the availability of phytate bound phosphorus and to reduce phosphorus levels in effluent from intensive livestock operations.

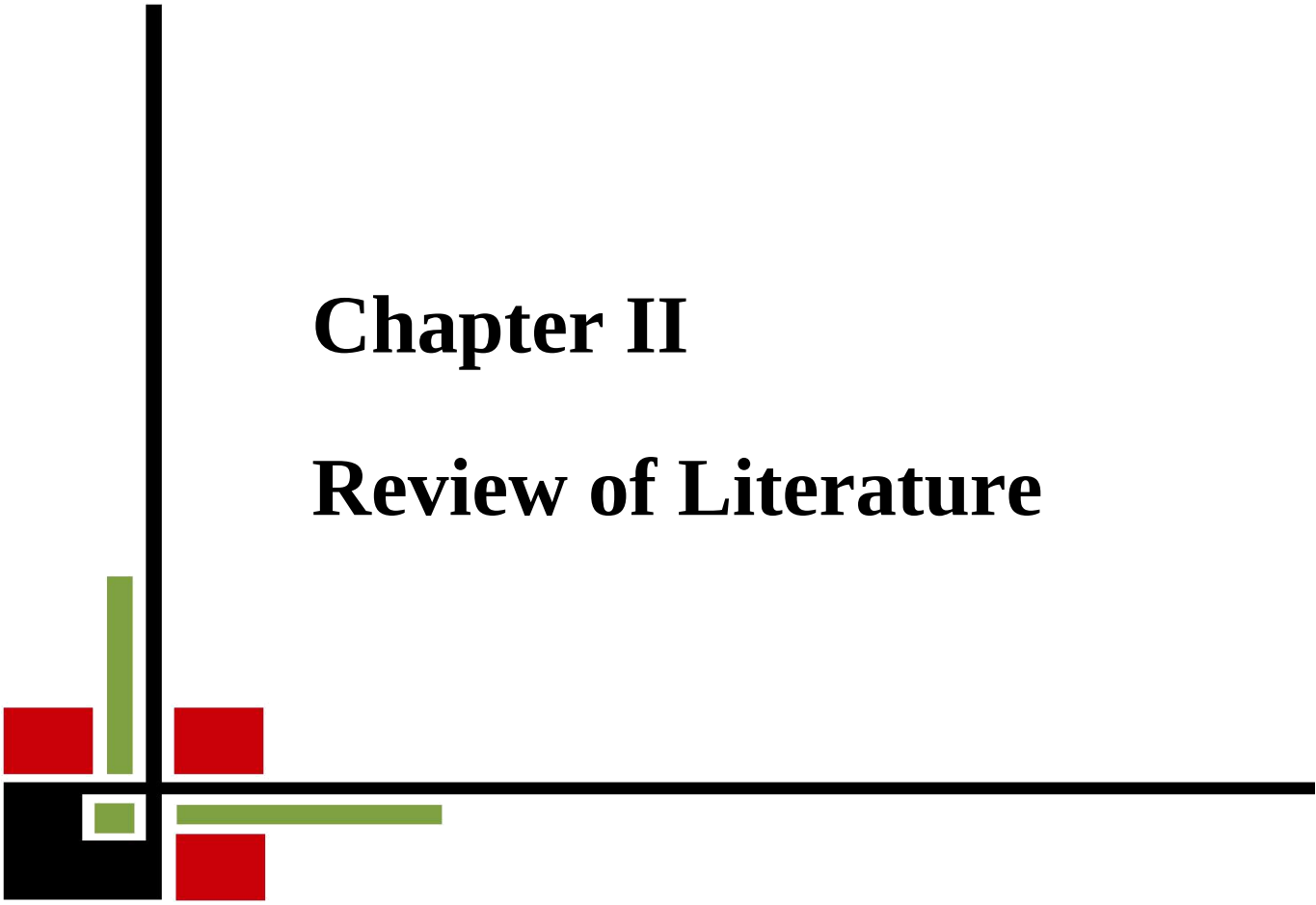
In the studies to reduce excretory P of broilers Nelson (1967) and Kornegay (1999) reported that phytase supplementation improved the utilization of phytate P derived from plant feedstuffs, and decreased excretory P by approximately one-third without depressing performance. However, these studies were conducted at only the starter phase at seven to 14 days of age. Practically the data at the finishing phase are needed, but there are few reports which covered all of the feeding phase in broilers. One such application in poultry phytic acid, that is abundant in all plant seeds, serve as the chief storage form of phosphorus. The phytic acid molecule has a high P content (28.2%), and since a major portion of poultry and pig diets consist of plant derived ingredients, P from the phytic acid assumes considerable nutritional significance. The ability of poultry and pigs to use phytate P is poor Ravindran *et al*. (2006), Wu *et al.* (2003). National Research Council (1994) due to insufficient quantities or lack of intestinal phytase secretion. This inadequacy of poultry and pigs to use phytate P results in the excretion of large amounts of P in the manure, posing an environmental concern especially in areas of intensive animal production. For the phytate-P to be used by monogastric animals, the phytate must be dephosphorylated, and this requires the provision of exogenous sources of phytase. During the past decade, advances in biotechnology and fermentation technology have resulted in the large- scale production of microbial phytases capable of hydrolyzing phytic acid and releasing phytate bound P.

1.4 Objectives

1. To investigate the influence of phytase enzyme on growth performance of broilers
2. To find out the effect of phytase enzyme on carcass merits and P status of broilers (tibia ash contents) of male and female broiler
3. To recommend the best commercial phytase enzyme in Bangladesh

Chapter II

Review of Literature



CHAPTER 2

REVIEW OF LITERATURE

It is very important to review the past research works which are related to the proposed study before conducting any type of survey or experiment. . In literature microbial phytase is the most commonly used exogenous enzyme in the feed for monogastric animals. Phytase can reduce the ant-nutritional effect of phytate and improve the digestibility of phosphorous (P), calcium, amino acids and energy, growth performances well as reduce the negative impact of inorganic P excretion to the environment. The benefits of using phytase in animal feed are well recognized.

2.1 phytase enzyme

Phytase is an enzyme that breaks down the indigestible phytic acid (phytate) portion in grains and oil seeds; thereby, releasing digestible phosphorus and calcium for the non -ruminants Nelson (1967), O'Dell *et al.* (1972), Raboy (1990), Ravindran *et al.* (1999) and Todd *et al.* (2004).

Phosphorus from the phytate molecule can be made more available to poultry by the addition of phytaze enzymes Denbow *et al*.(1995), Qian *et al. (*1997), Zanini and Sazzad (1999) and Ravindran *et al*. (2000).Phytic acid (PA) occurs naturally in plants and serves as the storage form of phosphorus. The primary storage site of PA implants is in seeds, which are the primary ingredients in poultry diets. The P in seed – based ingredients used in poultry diets, 50 to 80% found as phytate phosphorus (PP), phytate phosphorus is poorly available to poultry Taylor and Coleman (1979), Sebastion *et al.* (1996) and Zanini and Sazzad (1999).

Approximately two-third of the phosphorous in plant feedstuffs is present as phytic acid Cromwell (1980).

Phytic acid is highly reactive and readily forms chemical complex with Ca, Fe, Mg, Cu, Zn, carbohydrate and proteins Radcliffe (2002). Phytic acid can also act as an anti-nutrient due to the ability of the complex to bind starch, protein and trace minerals such as Phosphorus, zinc, iron, calcium and magnesium Kornegay (1999), Camden.*et al*. (2001) and Radcliffe (2002). Phytic acid has the ability to bind protein at acidic, alkaline, and neutral PH Anderson (1985). However, the interaction between phytic

acid and protein leads to decreased olubility of protein and eventually reduce its utilization Cheryan (1980). Some workers have found that microbial phytase has a positive influence on the utilization of nutrients other than P, such as amino acids Yi *et al*. (1996), Namkung and Leeson (1999), Ravindram *et al. (*1999) and Ravindram *et al.* (2000).

Furthermore, it was reported that mineral-phytate complexes might prevent lipid utilization, and by preventing the formation of mineral- phytate complex, phytase might reduce the degree of soap formation in the gut and enhance the utilization of energy derived from lipids Ravindran *et a*l. (2001).

Phytate refers to the PA molecule, which is generally chelated to Mg, Ca, Na, and K, and cases to proteins and starch Selle *et al*. (2000).

Phytase activity is expressed as "phytase units" or "FTU" per unit of feed (i.e. - FTU/kg or FTU/lb.) FTU is a worldwide standard unit. One phytase unit is the activity of phytase that generates 1 micromole of inorganic phosphorus per minute from an excess of sodium phytate at pH 5.5 and 37 degrees Celsius Zyla *et al.* (1995).

2.2 Impact of Phytase on Growth Performance of broiler

Phytases are classified by which phosphate they remove first from the phytate molecule. There are two main types; those that remove from the third or sixth carbon on the inositol ring Onyango *et al.* (2004). Without the addition of phytase, diets utilizing inorganic P to supplement the birds nutritional requirement of P needed an average 0.33% increase in nPP compared to diets that had phytase added. With the increase, parameters such as BWG, feed conversion ratio, mortality, and tibia ash were observed to have comparable performance with birds fed diets treated with phytase Yan *et al.* (2001). At an inclusion rate above 400 FTU/kg to P deficient diets, broilers raised from day-of-hatch to 21 days of age fed diets treated with phytase had an improvement in BWG as well as feed intake ($P<0.01$) Denbow *et al*. (1995). At phytase concentrations higher than 1000 FTU/kg, mortalities were on average 5%, closer to normal expectations at 21 days of age. Broilers raised from day 7 to 27 days fed a maize based diet with phytase responded with greater weight gain feed conversion efficiency, feed intake, as well as nutrient utilization (AME, N retention, AMEn) ($P<0.01$) Liu *et al.* (2014) and Huff *et al.* (1998) concluded that P deficient diets with an inclusion of

phytase fed to broilers could reduce the needed total P between 11 -25% with no significant decrease in bird performance or health. Karimi *et al.* (2013) observed broiler chicks raised from day one to almost three weeks of age had no significant decrease in bird performance or health when fed diets supplemented with 500 FTU/kg of phytase. In addition, phytase inclusions higher than commercial levels responded with greater improvements in broiler performance such as toe and tibia ash P retention ($P<0.05$). This could be due to phytase liberating a majority of the available phytate bound P within the diet. Bougouin *et al.* (2014) found with an addition of over 1000 FTU/kg, phytate P retention could be improved by 8.6%. With improved bird growth and performance, the supplementation can lead to better litter quality. Phytate can cause the bird to intake higher levels of sodium, shifting the mineral balance. Aggravating the osmotic balance in the body leads to increased water consumption and excretion. Litter becomes moist, reducing the quality, which increases the probability for litter quality issues. These can lead to flock health issues due to bacterial/fungal growth from the moist litter Selle *et al.* (2009). Phytase can reduce these phytate-nutrient interactions.

Phytase supplementation increased the availability of phosphorus and Ca Rezaei *et al.* (2007), Schooner *et al*. (1991), Broze *et a*l. (1994), Kornegay *et al.* (1996) and Sebastian *et al*. (1996). Similarly, phytase increased the availability of nutrient, when was included at rate of 400 FUK /Kg phytase. Toe ash, and toe ash Ca and P percentages were increased with the addition of phytase in both sexes but without significant effect on blood phosphorus concentration Rezaei *et al.* (2007).

In another study when phytase was added to broiler diets it increased body weight gain and feed conversion efficiency, more Ca and P in tibia ash and Ca and phosphorus retention was significantly increased Mondal *et al.* (2007).

When phytase was added to a corn based diet had a significant increase in body weight in the broiler fed for 49 days Huff *et al*. (1998). Serum activity of alkaline phosphates was significantly decreased in the diet supplemented with phytase, while serum cholesterol was significantly decreased Huff *et al*. (1998).

When phytase was added to broiler diets at level of 600 ppm had no effects on broilers growth Hussein (2005). It has been reported that phytase supplementation improved N retention in broiler chickens.

Farrell *et al.* (1993) and Shirley *et al*. (2003) indicated that achieve maximum performance when phytase is supplemented at 1000 PU\Kg diet and that current phytase supplementation levels within the poultry industry may need to be reevaluated.

Mondal *et al.* (2007), Orban *et al*. (1999); Atia *et a*l. (2000) and Ciftci *et al*. (2005) suggested that phytate phosphorous released by phytase is sufficient to meet starter and finisher broiler's growth requirement.

2.3 Phytase effects on nutrients digestibility

Effects of phytase enzyme supplementation on feed nutrients digestibility were investigated by some experiments. Sebastian *et al*. (1997) reported that supplementation of phytase significantly improved of ileal digestibility of crude fat. Phytase may also improve the utilization of protein, amino acid and apparent metabolic energy of the broiler diets supplemented with phytase enzyme Ravindran *et al*. (1999).

Rutherfurd *et al*. (2004) also showed that microbial phytase improved phytate P and total (P digestibility) as well as true ileal amino acid digestibility Onyange *et al*. (2005) indicated that phytase improved broiler retention of P. Ca, N, and a number of amino acids. Microbial phytase on broiler diet has shown reduce phosphorus excretion Simons *et al* (1990), Yan *et al*. (2006) P excretion of broilers could be markedly reduced by phytase supplementation.

Several recent studies have explored the utilization of phytase from 3 to 6 week of age Yan *et al*. (2001).

The negative charge on the phosphate groups bound to phytate may cause it to bind to the amino acids of proteins thereby blocking proper digestion and absorption of amino acids. With the supplementation of phytase, there has been significant increase in amino acid digestibility Cowieson *et al.* (2006). Phytase cleaves the individual phosphate groups off the inositol ring reducing the active sites for forming complexes with other molecules. When differing levels of phytase were applied to individual diets made of various plant protein sources, cereals, and cereal by-products, results indicated significant improvements in most essential amino acids.

Wheat and sorghum had greater improvements in the digestibility of leucine, alanine, and glutamic acid compared to other feedstuffs ($P<0.05$). Phytase increased the amino

acid digestibility of the two feedstuffs by 5.3% to 10.4% (wheat) and 2.7% to 5.5% (sorghum). Digestion in oil seeds such as soybean meal, canola meal, cottonseed meal, and sunflower meal also saw significant increases in amino acid digestibility ($P<0.05$). The type of phytate-protein binding may determine the effectiveness of phytase action on amino acid digestibility in different feedstuffs Ravindran *et al.* (1999). Amongst those amino acids, threonine was found to have consistent and significantly higher digestibility rates across all feedstuffs. This amino acid aids in the formation of glycine that in turn influence neurotransmitters in the central nervous system.

Broilers fed low P diets supplemented with phytase resulted in higher amino acid digestibility in comparison to non-phytase supplemented low-P diets. This increase could be attributed to those phytase diets also demonstrating a significant degradation of phytate in the terminal ileum. This reduces the occurrence of phytate-protein complexes freeing up proteins for digestion by proteases to obtain amino acids for absorption ($P<0.05$) Rutherford *et al.* (2004). Phytase can also affect the protein efficiency ratio (PER), which is defined as the grams of weight gain divided by the grams of protein consumed and can help determine the ability of the bird in utilizing protein from a diet Boling-Frankenbach *et al.* (2001). In birds fed diets comprised of canola meal, an energy source high in protein, there was a significant improvement in PER ($P<0.05$) Kong and Adeola (2011). Ravindran *et al.* (2001) found with broilers raised from hatch to day 28, phytase was able to linearly improve the digestibility of nitrogen and all amino acids in P-adequate, lysine-deficient diets. Highest digestibility responses for nitrogen and amino acids were at a phytase inclusion of 1000 FTU/kg ($P<0.01$). These increases in amino acid digestibility and responses in energy result in the improved performance from when phytase was included in the diet. In addition to phytase hydrolyzing phytate, it could be enhancing the interaction between digestive enzymes and nutrients by disrupting cell walls of diet ingredients thereby improving digestion Ravindran *et al.* (2001).

2.4 Supplementation of Phosphorus and ash content

The inclusion rate of P within the industry has presented issues as to how much to supplement in the diet, whether the bird can efficiently utilize the P within the diet, where the possible excess P ends up, and how it may affect the environment. Efficient absorption of P is dependent on the form the nutrient is supplemented in the birds' diet

and the ability of the gastrointestinal (GI) tract to digest and absorb the mineral. Several factors play into how well a bird's GI tract can carry out the mechanisms to take up P. It begins with the uptake and metabolism of cholecalciferol also commonly known as the inactive form of vitamin D3. Cholecalciferol comes from animal sources which have a greater effect in broilers than plant derived vitamin D3. Cholecalciferol goes through a 2-step hydroxylation process to become active then hydroxylated by 25-hydroxylase in the liver to its pre-active form, 25-hydroxycholecalciferol also known as calcidiol. The molecule then migrates to the kidney where 1-alpha hydroxylase catalyzes the reaction to hydroxylate the molecule to its active form, 1, 25-dihydroxycholecalciferol whose name can be interchangeable with calcitriol. Levels of circulating active vitamin D within the bird are partially influenced by parathyroid hormone levels (PTH) Shanmugasundaram and Selvaraj (2012). The hormone promotes expression of 1- alpha hydroxylase in the kidney, which in turn will increase levels of active vitamin D, which will up regulate the amount of absorbed P (Huber *et al.*, 2015). The transporters that have been found to be involved with P absorption in poultry GI tracts is sodium-dependent phosphate transport protein 2B (NaPi type IIb) and sodium-dependent phosphate transport protein 3 (NaPi type III). The activities of the transporters are influenced by a combined effect of levels of P, vitamin D3, and PTH within the bird. Only a few experiments have been conducted to see how the proteins respond when the bird is exposed to nutritional stresses such as a diet low in P Huber *et al.* (2015). Current knowledge mechanisms of the sodium transporters needs to be further evaluated to thoroughly understand P digestibility in poultry.

It has been proposed that the industry tends to over supplement inorganic P in the diets of broiler chicks Waldroup *et al.* (1974). An experiment was conducted demonstrating that an increase of inorganic P does not improve the performance of the birds raised from four to eight weeks when supplemented 0.12% over the recommended National Research Council (NRC) amount. Following this led to reconsidering the overall 1994 NRC requirements and whether they were in general too high when it came to broilers. Without the supplementation of the enzyme phytase, an enzyme that aids in releasing the bound phosphates from the molecule phytate, P-containing litter from broilers spread across crop fields have the potential to contribute P to nearby waterways leading to larger bodies of water.

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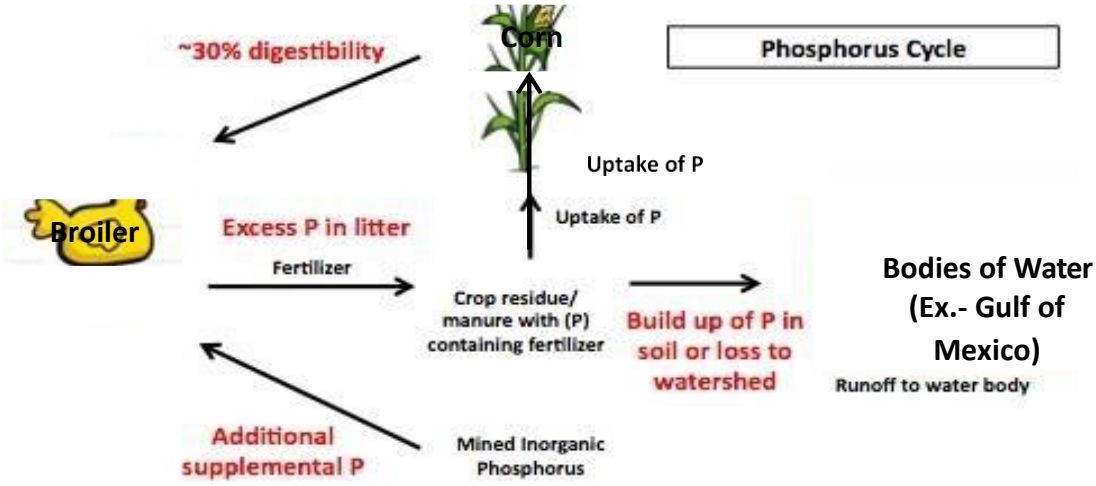


Figure 1. Phosphorus cycle in relation to broilers.

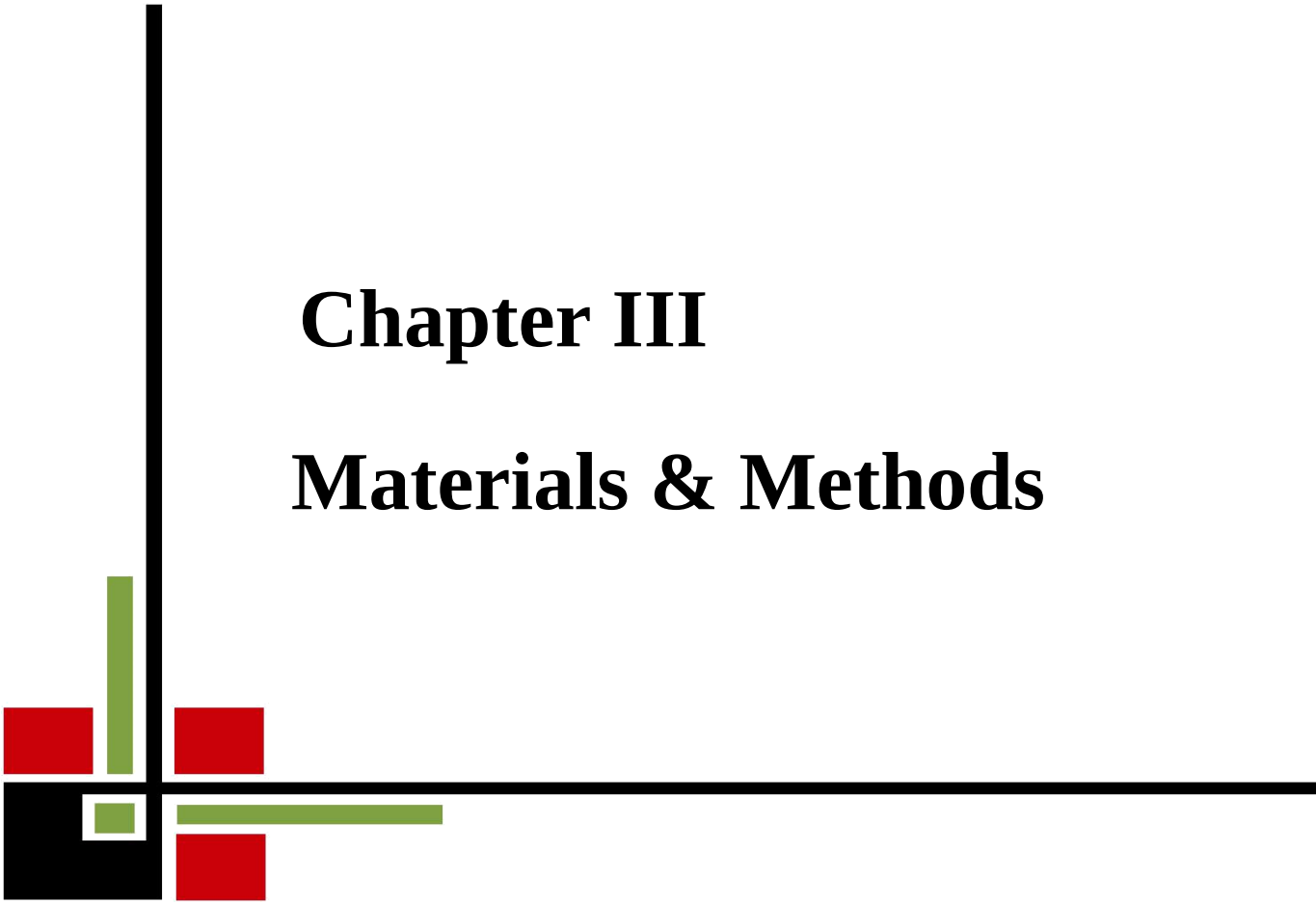
According to Powell *et al.* (2008), there is a large debate over the amount of P being released into the environment from the poultry litter that may be used as fertilizer for crops. The excess richness of nutrients such as P in large bodies of water may lead to plant overgrowth which may decrease oxygen availability for the marine life, which may then cause to an imbalance in marine life. In culmination with the negative impact on marine life, Withers *et al.* (2001) indicated it is a form of pollution that isinhibiting the use of those waters. McGrath *et al.* (2005) found that the P leaking into the surrounding areas near the croplands are polluting the surface and ground waters creating issues for surrounding towns needing the water for use. In addition to environmental issues, P is a costly ingredient. Augspurger (2004) indicated that within the past several years, the price for feed-grade P foranimals has risen four-fold. Out of the P supplements being used in the livestock industry, there are three dominant types: dicalcium phosphate (22% Ca, 18.5% P), defluorinated rock phosphate (33% Ca, 18% P), and mono-dicalcium phosphate (16% Ca, 21% P). Of the three, the first two are the main supplements used within the poultry industry for birds such as layers, broilers, and turkeys.

According to Augspurger (2004), poultry account for 50% of the P supplement usage. The prices of P supplements have risen the past years due to change in the direction of

agriculture and how crops are utilized.. In addition to reducing the amount of P in the diets, another solution is adding different feed additives to potentially release the already available P that is bound to the ingredients in the diet. In Figure1 the cycle characterizes how phytase has been one such solution in alleviating the reliance on inorganic P thereby reducing feed costs and reducing the impact broiler litter may have on the environment. Besides when phytase was added to broiler diets at level of 600 ppm, it decreased the ash of tibia and toe bones compared to the control Hussein (2005).

Chapter III

Materials & Methods



CHAPTER 3

MATERIALS AND 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 covered from 08th May 2018 to 05th June 2018, to investigate the effect of exogenous phytase on growth and meat yield characteristics of broilers.

3.2 Collection of experimental broilers

A total of 120 day-old Cobb 500 broiler chicks were collected from Kazi hatchery, 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 equally for 2 days by maintaining standard brooding protocol. During brooding time only basal diet was given no phytase enzyme was used as treatment. After two days 120 chicks were selected from brooders and distributed randomly in three (3) dietary treatments of 90; another 30 chicks were distributed randomly in one treatment for control. Each treatment had three (3) replications with 10 birds per replication. The total numbers of treatments were four (4) and their replications were twelve (12).

3.4 Experimental treatment

Diet 1: Basal diet

Diet 2: Fungal phytase enzyme 1.5g/kg of feed

Diet 3: Bacterial Phytase enzyme 0.1g/kg of feed

Diet 4: Fungal and bacterial phytase enzyme combined (1.5+0.1)g/kg of feed

Table 1. Layout of the experiment

Treatment groups	No. of replications			Total
	R_1	R_2	R3	
T_1	10	10	10	30
T_2	10	10	10	30
T_3	10	10	10	30
T_4	10	10	10	30
Total	40	40	40	120

3.5 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 Iodophor disinfectant solution (3 ml/liter water). After proper drying, the house was divided into 12pens 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

3.6 Experimental diets

Starter and grower commercial Kazi broiler feed were purchased from the market. Starter diet was enriched with minimum 4 times daily by following Cobb 500 Manual and *ad libitum* drinking water 2 times daily.

Table 2. Name and minimum percentage of ingredients present in Starter ration

Name of ingredients in Starter ration	Minimum percentage Present
protein	21.0 %
fat	6.0%
fiber	5.0%
ash	8.0%
lysine	1.20%
methionine	0.49%
cystine	0.40%
tryptophan	0.19%
threonine	0.79%

Table 3. Name and minimum percentage of ingredients Grower ration

Name of ingredients in Grower ration	Minimum percentage Present
protein	19.0 %
fat	6.0%
fiber,	5.0%
ash	8.0%
lysine	1.10%
methionine	0.47%
cystine	0.39%
tryptophan	0.18%
threonine	0.75%
arginine	1.18%

3.6.1 Collection of phytase enzyme

1. Fungal phytase enzyme collection from Renata Company Limited

2. Bacterial phytase enzyme collection from Argil Company Limited

Table 4. Nutritional composition of phytase enzyme

Phytase enzyme composition	Broilers	
	One kg Phytase – 400 Release	Matrix value (%)
Available Phosphorus	920g	92.00
Calcium	800g	80.00
Lysine	10g	0.96
Methionine	8g	0.80
Cystine	24g	2.40
Threonine	104g	10.40
tryptophan	24g	2.40
Isoleucine	96g	9.60
Arginine	104g	10.40
Histidine	40g	4.00
Leucine	160g	16.00
Phenylalanine	104g	10.40
Valine	120g	12.00
Crude Protein	1800g	180.00
ME/kg	42400 Kcal	42400 Kcal

Source: Renata Company limited

3.7 Management procedures

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

3.7.1 Brooding of baby chicks

The experiment was conducted during 8th May to 5th June, 2018. The average temperature was 31.5^{0}C and the RH was 80% in the poultry house. Common brooding was done for one week. After one week 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^{0}C) 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 (^{0}C) and humidity % were recorded every six hours with a thermometer and a wet and dry bulb 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 6cm. 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 for necessity 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 4 birds. Feeders were cleaned at the end

of each week and 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 was scheduled up to 28 days.

3.7.6 Bio security measures

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

3.7.7 Vaccination

The vaccines collected from medicine shop (Ceva Company) and applied to the experimental birds according to the vaccination schedule.

Table 5. The vaccination schedule

Age of birds	Name of Disease	Name of vaccine	Route of administration
3 days	IB + ND	MA-5 + Clone-30	One drop in each eye
9 days	Gambaro	G-228E (inactivated)	Drinking Water
17days	Gambaro	G-228E(inactivated) booster dose	Drinking Water
21 days	IB + ND	MA-5 + Clone-30	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. Besides ventilation was regulated as per requirement by folding polythene screen.

3.7.9 Sanitation

Strict sanitary measures were taken during the experimental period. Disinfectant (Virkon) was used to disinfect the feeders and waterers and the house also.

3.8 Study Parameters

3.8.1 Recorded parameters

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

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 f 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 by cutting them loose and then the gall bladder was removed from the liver. Cutting it loose in front of the proventiculus and then cutting with both incoming and outgoing tracts removed the gizzard. 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

The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds.

Body weight gain = Final weight – Initial weight

3.10.2 Feed intake

Feed intake was calculated as the total feed consumption in a replication divided by number of birds in each replication.

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

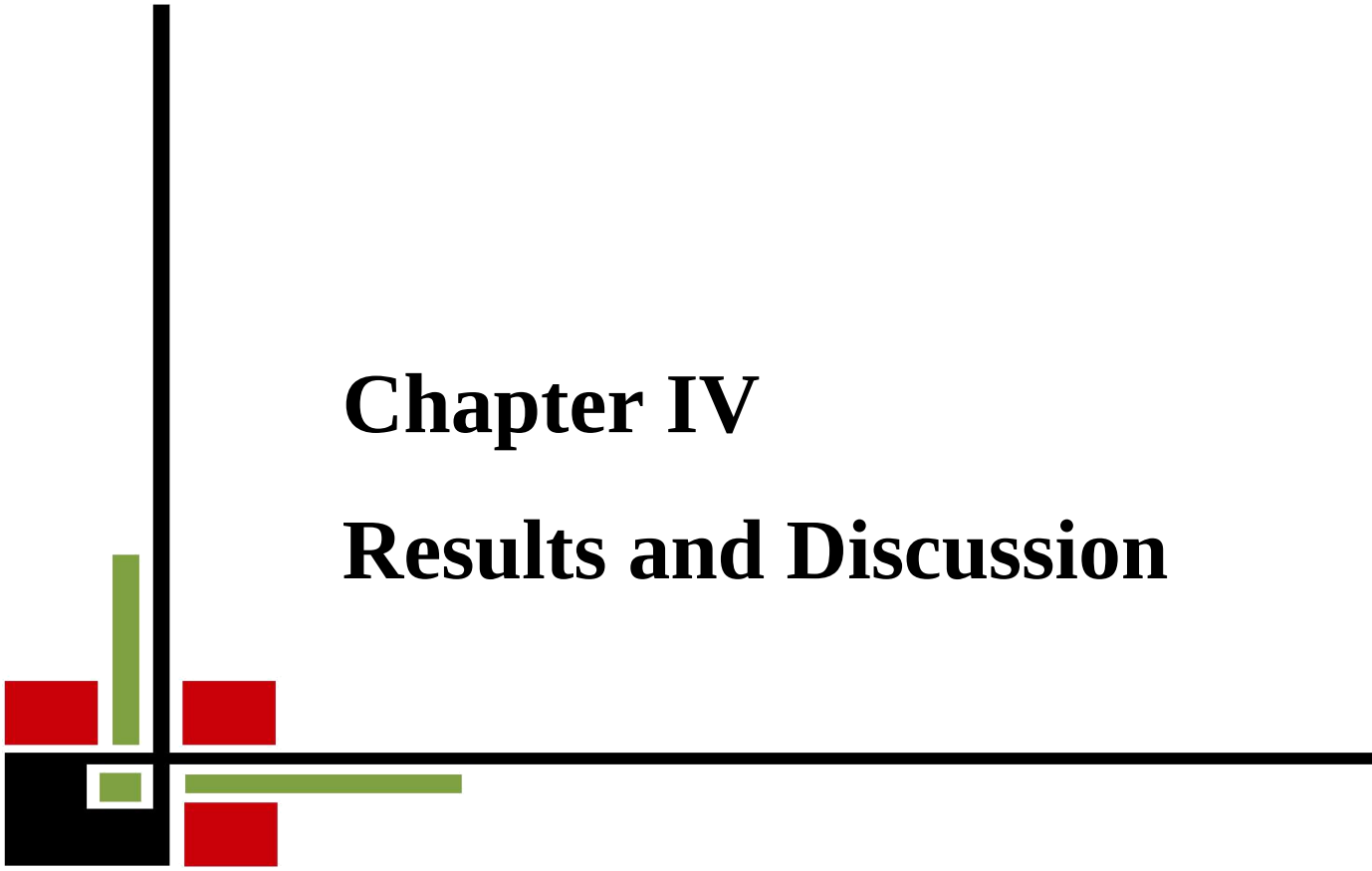
3.10.3 Feed conversion ratio

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

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

3.10.4 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. Experiment was laid out in Randomized Complete Block Design (RCBD)



Chapter IV

Results and Discussion

CHAPTER 4

RESULT AND DISCUSSION

4.1 Production performances of broiler chicken

4.1.1 Final Live weight

Data presented in Table 8 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, and T_4 were $1578.67^{\mathbf{b}} \pm 6.3$, $1579.03^{ab} \pm 58.574$, $1613.00^{a} \pm 24.269$ and $1496.33^{b} \pm 58.574$ respectively. The highest result was found in T_3 ($1613.00^{a} \pm 24.269$) and lowest result was in T_4 ($1496.33^{b} \pm 58.574$) group (Table 6).

These results are in agreement with those obtained by the effect of phytase was also reported in broilers Sohail and Roland (1999), Fernandez *et al.* (1999), Bozkurt *et al.* (2006), Mondal *et al*. (2007) and ducks Orban *et al.* (1999).

Phytase supplementation at levels of 1000 PU/Kg) (Bacterial), (1000 and 1000 PU/Kg) Mixed Bacterial and fungal phytase and 1000PU/Kg (fungal phytase) in both starter and finisher diets solved the problem of P deficiency and resulted in birds average body weights similar to control. However, levels of phytase lower than 500 PU/kg had no effect on improving broilers body weights Mondal *et al.* (2007). Results of this experiment also is in agreement with those of Qian *et al.* (1997), Huff *et al.* (1998), Namkung and Leeson. (1999), Zyla *et al*. (2000) and Bozkurt *et al*. (2006) which reported that the growth rate and feed conversion ratio of broilers fed low P diets containing phytase were comparable or even better than those obtained for broilers fed the standard P diets. These results supported the concept that phytase was improving P availability and growth rate.

4.1.2 Feed Consumption (FC)

The mean body FCR of broiler chicks at the end of 4^{th} week in different groups were 1782.30 ± 59.743, 1785.83 ± 28.047, 1875.23 ± 25.266, 1872.90 ± 22.592 respectively.
The overall mean FCR of different groups showed that there was no significant ($P>0.05$) increase in groups T_1, T_2, and T_3 compared to control (Table 6).

Similar findings were reported by Monndal *et al.* (2007) when broilers fed with P-deficient diets supplemented with phytase at levels higher than 500 PU/kg.

4.1.3 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) was significantly (P<0.05) lower for birds supplemented with Mixed Bacterial and fungal phytase enzyme ($1.37^{b} \pm 0.02$) than control birds ($1.47^{a} \pm 0.05$). However, Feed conversion ratio (FCR) was significantly (P<0.05) higher in T_1 group ($1.47^{a} \pm 0.05$) (control) compared to T_2 ($1.43^{a} \pm 0.01$), T_3 ($1.37^{b} \pm 0.02$) and T_4 ($1.35^{b} \pm 0.04$) groups respectively (Table 6).

The current study supports the observations of Huff *et al.* (1998), Ravindan *et al.* (2001), Bozkurt *et al*. (2006) and Mondal *et al.* (2007) who reported that phytase supplementation to broiler diets caused numerical improvement in feed .

4.1.4 Dressing Percentage (DP)

Bacterial and fungal mixed phytase enzyme T_3 supplemented group had a greater ($P < 0.05$) carcass percentage T_3 ($73.05^{a} \pm 1.357$) compared with the control group T_1 ($66.30^{b} \pm 1.642$), T_2 ($68.60^{ab} \pm 0.288$), and T_4 ($69.95^{ab} \pm 2.630$) DP % (Table 9). At the end of Experiment, evaluation of dressing percentage on slaughtered representative birds revealed that T_3 group had significantly higher dressed percentage followed by T_3, T_2, T_1 and lower in T_1 groups (Table 6 and figure 2).

These results agreed with previous findings of Bougouin *et al.* (2014) and Pillai *et al.* (2006) who showed that phytase supplementation significantly increased percentages of most of carcass merits compared to control diets.

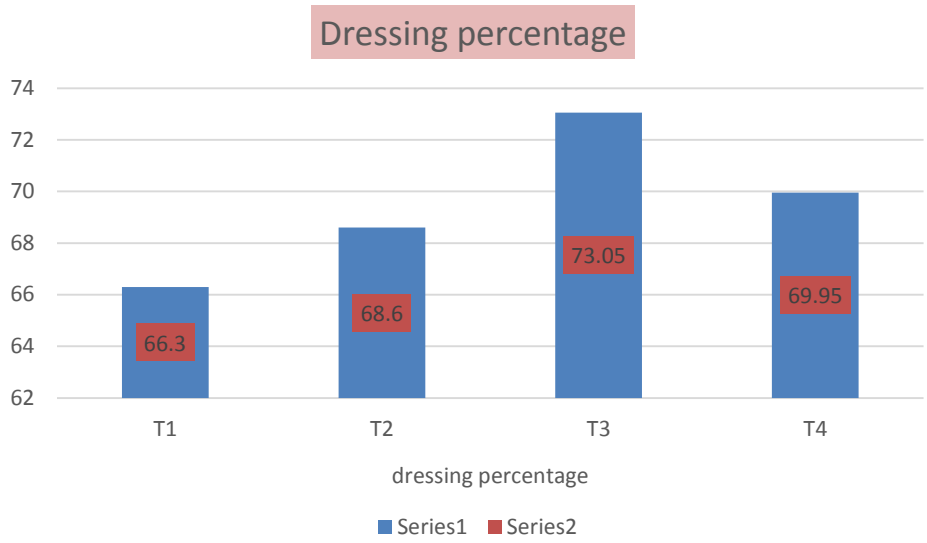


Figure 2. Effects of feeding different phytase enzyme on dressing percentage of broiler at different weeks.

Table 6: Production performance of broiler chicken treated with Phytase enzyme

Treatment	T_1	T_2	T_3	T_4	Mean ±SE
Final live Weight (g/broile)	1578.67^{b}± 6.386	1579.03^{ab}± 58.574	1613.00^{a}± 24.269	1496.33^{b}± 58.574	1496.33± 19.180
FC (g)	1782.30± 59.743	1785.83± 28.047	1875.23± 25.266	1872.90± 22.592	1829.07± 20.845
FCR	1.47^{a}±0.05	1.43^{a}±0.01	1.37^{b}±0.02	1.35^{b}±0.04	1.36± 0.032
DP%(Skinless)	66.30^{b}±1.642	68.60^{ab}± 0.288	73.05^{a}± 1.357	69.95^{ab} ± 2.630	69.47±1.033

Here, T_1 = (control), T_2 = (Bacterial phytase enzyme), T_3 = (Mixed Bacterial and fungal phytase enzyme) and T_4 = (fungal phytase) Values are Mean ± S.E (n = 15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different(P<0.05)
- ✓ Mean within same superscripts don‟t differ (P>0.05)significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference

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

4.1.5 Weekly Body weight gain

The mean body weight gains (g) of broiler chicks at the end of 4th week in different groups $420.00^{a} \pm 30.551$, $506.67^{ab} \pm 46.308$, $530.00^{a} \pm 5.774$, $446.67^{ab} \pm 27.285$, 475.83 ± 18.808 were respectively. The overall mean body weight gain of different groups showed that there was significant ($P<0.05$). The highest result was found in T_3 ($530.00^{a} \pm 5.774$) mixed fungal and bacterial phytase enzyme and lowest result was in T1 control (Table 7 and Figure 3).

These results are in agreement with those of previous researchers that Similar findings were reported by Monndal *et al.* (2007) when broilers fed diets supplemented with phytase at levels higher than 500 PU/kg significantly ($P<0.05$) improved weight gain of chickens compared to the control groups.

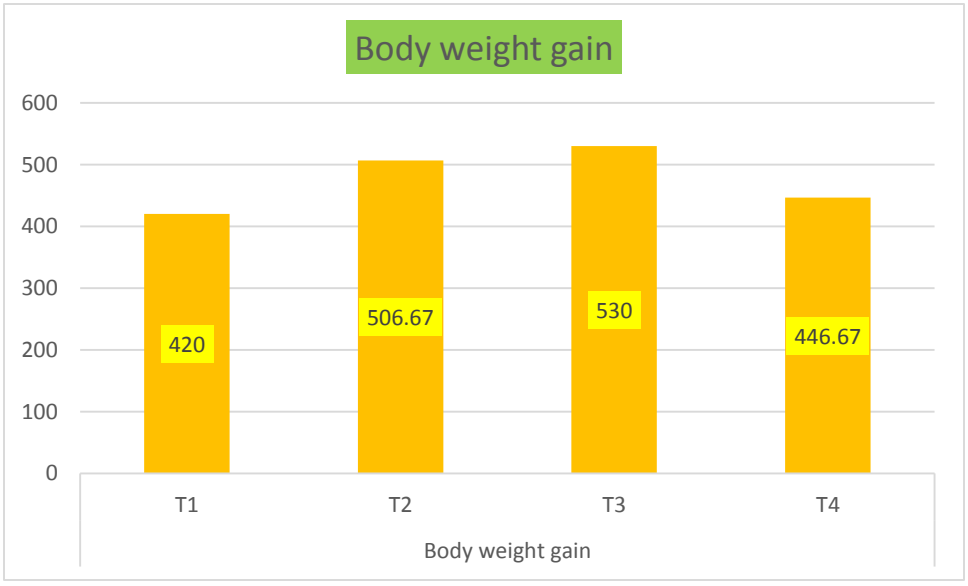


Figure 3. Effect of phytase enzyme on body weight gain

Table 7. Effects of feeding different Phytase enzyme on body weight gain (BWG) (g/bird) of broiler chickens at different weeks.

Treatment	1st week BWG	2nd week BWG	3nd week BWG	4nd week BWG
T_1	164.67±3.383	294.00±3.215	444.67±2.667	420.00^{a}±30.551
T_2	168.17±6.953	300.50±4.782	448.00±8.145	506.67^{ab}±46.308
T_3	171.50±1.803	301.83±8.428	450.00±5.774	530.00^{a}±5.774
T_4	166.67±5.608	304.67±15.059	448.67±4.667	446.67^{ab}±27.285
Mean±SE	167.75±2.205	300.25±4.054	447.83±2.489	475.83±18.808

Here, T_1 = (control), T_2= (Bacterial phytase enzyme), T_3 = (Combined Bacterial and fungal phytase enzyme) and T_4 = (fungal phytase) Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different($P<0.05$)
- ✓ Mean within same superscripts don‟t differ ($P>0.05$)significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference

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

4.1.6 Weekly Feed consumption (WFC)

The mean body FC (g) of broiler chicks at the end of 4th week in different groups were $801.67^{a} \pm 18.836$, $686.00^{b} \pm 13.650$. $719.67^{b} \pm 13.860$, $794.00^{a} \pm 8.185$ respectively. The overall mean FC of different groups showed that there was significant ($P<0.05$) increase in groups T_3, T_4, and T_5 compared to T_1 (Table 8). Similar findings were reported by Monndal *et al.* (2007) when broilers fed diets supplemented with phytase at levels higher than 500 PU/kg.

Table 8. Feed consumption (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments

Treatment	1st Week Feed Consumption/ Bird (g)	2nd Week Feed Consumption/ Bird(g)	3rd Week Feed Consumption/ Bird (g)	4th Week Feed Consumption/ Bird (g)
T1	131.57±2.298	356.67±7.881	585.33±6.227	801.67^{a}±18.836
T2	138.50±3.163	367.67±3.528	593.67±14.495	686.00^{b}±13.650
T3	137.97±3.968	328.00±50.083	596.67±5.783	719.67^{b}±13.860
T4	131.23±3.509	361.67±3.844	586.00±8.386	794.00^{a}±8.185
Means ± SE	134.82±1.742	353.50±11.797	590.42±4.265	750.33±15.970

Here, T_1 = (control), T_2= (Bacterial phytase enzyme), T_3 = (Mixed Bacterial and fungal phytase enzyme) and T_4 = (fungal phytase) Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different($P<0.05$)
- ✓ Mean within same superscripts don't differ ($P>0.05$) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference

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

4.1.7 Weekly Feed Conversion Ratio (WFCR)

The mean body FCR of broiler chicks at the end of 4th week in different groups were 1.73^{ab} ± 0.147, 1.38^{b} ± 0.153, 1.51^{ab} ± 0.023, and 1.79a ± 0.089 respectively. The overall mean FCR of different groups showed that there was significant ($P<0.05$) increase in groups T_1, T_2, and T_3 compared to control (Table 9). The current study supports the observations of Huff *et al.* (1998) and Ravindan *et al.* (2001). Bozkurt *et al*. (2006) and Mondal *et al.* (2007) who reported that phytase supplementation to broiler diets caused numerical improvement in feed efficiency compared to control.

Table 9. Effects of feeding different Phytase enzyme on FCR of broiler chickens at different weeks.

Treatment	1st Week FCR	2nd Week FCR	3rdWeek FCR	4thWeek FCR
T_1	0.84±0.036	1.34±.007	1.56±.017	1.73^{ab}±.147
T_2	0.83±0.022	1.33±.030	1.56±.016	1.38^{b}±.153
T_3	0.77±0.009	1.30±.010	1.57±.022	1.51^{ab} ±.023
T_4	0.79±0.023	31±.005	1.55±.045	1.79^{a}±.089
Mean±SE	0.80±0.014	1.32±.009	1.56±.012	1.60±.070

Here, T_1 = (control), T_2= (Bacterial phytase enzyme), T_3 = (Mixed Bacterial and fungal phytase enzyme) and T_4 = (fungal phytase) Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different($P<0.05$)
- ✓ Mean within same superscripts don't differ ($P>0.05$)significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference

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

4.1.8 Relative giblet weight (liver, heart and gizzard)

The relative weight of liver (g) of broiler chicks in the dietary group T_1, T_2, T_3 and T_4 were 37.13 ± 1.305, 37.22 ± 1.696, 38.49 ± 3.663, 35.33 ± 3.177 respectively. The highest results were obtain in T_3 and lowest was in T_4 group. However, there was no significant ($P>0.05$) difference in the relative weight of liver between the groups. (Table 10).

The qualified weight of heart of different groups showed that there was no significant ($P>0.05$) difference between the groups and the values were ranged from 7.21 ±.766, 7.45 ± .277, 6.52 ± 0.368. The highest results were obtain in T_3 and lowest was in T_2 group. However, there was no significant ($P>0.05$) difference in the relative weight of liver between the groups. (Table 9). The comparative weight of gizzard of different groups did not show any significant ($P>0.05$) difference in groups T_1 (42.61 ± 2.017), T_2 (41.38± 3.236), T_3 (33.56 ± 3.387), and T_4 (35.83 ± 2.038). The highest results were obtain in $T_1$and lowest was in T_3 group. However, there was no significant ($P>0.05$) difference in the relative weight of liver between the groups. (Table 10).

The relative weight of intestine (g) of broiler chicks in the dietary group T_1, T_2, T_3 and T_4 were 94.33^{b} ± 3.099, 103.50^{ab} ± 4.842, 116.72^{a} ± 6.436 and 108.56^{ab} ± 4.46 respectively. The highest results were obtain in T_3 and lowest was in T_1 group. However, there was significant ($P<0.05$) difference in the relative weight of intestine.

The parts concerned are gizzard, liver and heart. Although it was reported by Moharrery and Mohammadpour (2005) that phytase supplementation had no impact on gizzard and liver weights.

Table 10. Effect of dietary supplementation of Phytase enzyme on Liver, Gizzard, Intestine and heart weight of different Treatment

Treatment	Liver weight (g)	Gizzard weight (g)	Intestine weight (g)	Heart weight (g)
T_1	37.13±1.305	42.61±2.017	94.33^{b}±3.099	7.21±.766
T_2	37.22±1.696	41.38±3.236	103.50^{ab}±4.842	6.25±.628
T_3	38.49±3.663	33.56±3.387	116.72^{a}±6.436	7.45±.277
T_4	35.33±3.177	35.83±2.038	108.56^{ab}±4.463	6.52±.368
Mean±SE	4.086±1.180	38.35±1.631	105.78±11.109	6.86±.275

Here, T_1 = (control), T_2= (Bacterial phytase enzyme), T_3 = (Mixed Bacterial and fungal phytase enzyme) and T_4 = (fungal phytase) Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different(P<0.05)
- ✓ Mean within same superscripts don't differ (P>0.05)significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference

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

4.1.9 Immune organs

Effect of Phytase enzyme supplementation on immune organs of Cobb 500 strain broiler chicks during the period from 0 to 28 days of age are summarized in Table 11 and Figure 5. The comparative weight of spleen (g) of broiler chicks in the dietary group T1, T2, T3 and T4 were 2.04±.303, 1.70±.186, 1.78±.365, 1.78±.147 respectively. The highest value was T1 (2.04±.303) and lowest value was (T2 1.70±.186) On the other hand, the relative weight of spleen of different groups showed that there were no significant (P>0.05) difference among the groups and the values were ranged from 1.78±.147 to 2.04±.303. The weight of bursa was higher in T3 group (2.42±.159) compared to the other group which values were T1 (1.89±.402), T2 (1.50±.348) and T4 (2.22±.682) correspondingly. But these values are not significantly differing among the treatments (Table 11). In accordance with the present results, Moharrery and Mohammadpour (2005) showed that the relative and absolute weights of thymus and bursa were induced for the groups fed diet containing phytase enzyme compared to the control group. These results may be considered as good

indicator of healthy status of chicks fed dietary phytase enzyme.

Table 11. Effects of Phytase enzyme on immune organs

Treatment	Spleen weight (g)	Bursa weight (g)
T1	2.04±.303	1.89±.402
T2	1.70±.186	1.50±.348
T3	1.78±.365	2.42±.159
T4	1.78±.147	2.22±.682
Mean ± SE	1.82±.120	2.01±.215

Here, T_1 = (control), T_2 = (Bacterial phytase enzyme), T_3 = (Mixed Bacterial and fungal phytase enzyme) and T_4 = (fungal phytase) Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different($P<0.05$)
- ✓ Mean within same superscripts don't differ ($P>0.05$) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference

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

4.1.10 Ash percent

Ash was significantly ($P<0.05$) higher for birds supplemented with mixed Bacterial and Fungal phytase enzyme (22.29^{a}±.455) than control birds ($18.92^{\mathbf{b}}$ ±1.076). However, ash percent was significantly ($P<0.05$) higher in T_3 group (22.29^{a}±.455) compared to T_2 ($19.43^{\mathbf{ab}}$ ± 0.918) and T_4 ($19.33^{\mathbf{ab}}$ ± 1.203) and T_1 ($18.92^{\mathbf{b}}$ ± 1.076) groups respectively (Table 12 and Figure 4)

In the present study, the effects of phytase enzyme on broiler performance parameters including average ash percent was in agreement with previous studies

The percentage of male and female broilers tibia crude ash was significantly increased by the addition of dietary phytase. This agrees with the several studies on broilers Sebastian *et al*. (1996), Zyla *et al.* (2000), Mondal *et al.* (2007), pekin ducks Orban *et al*. (1999) and turkeys Atia *et al.* (2000). However, it disagrees with others Fernandez *et al.* (1999)

Harter-Dennis and Sterling (1999) and Bozkurt *et al*. (2006).

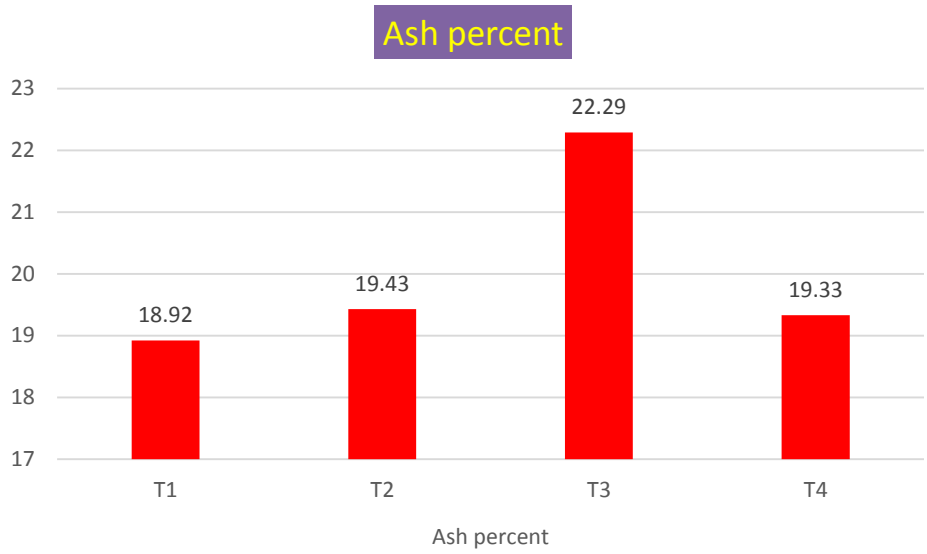


Figure 4. Effect of phytase enzyme on Ash Percent

Table 12. Effects of supplementation of Phytase enzyme to broiler diets on ash percentage.

Treatment	T_1	T_2	T_3	T_4	Mean ±SE
Ash	18.92 ^{b}± 1.076	19.43^{ab}± 0.918	22.29± 0.455	19.33^{a}± 1.203	19.99±0.57

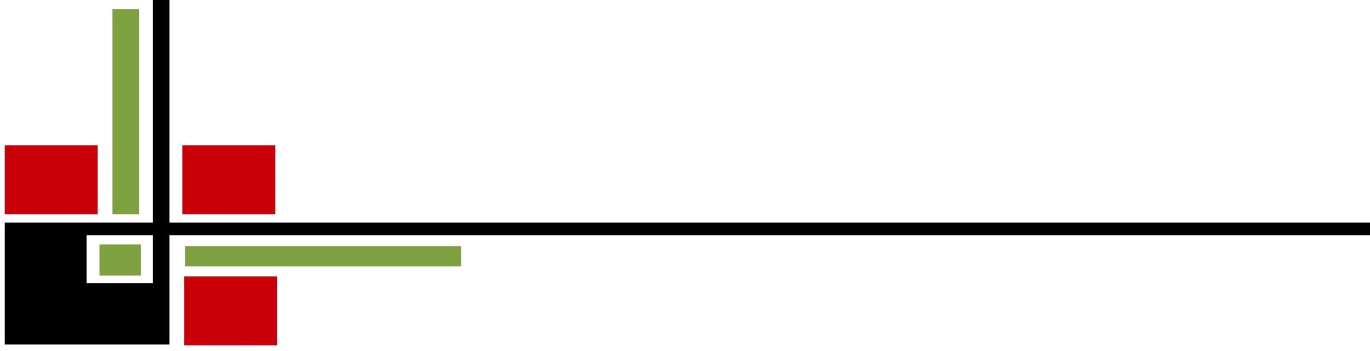
Here, T_1 = (control), T_2 = (Bacterial phytase enzyme), T_3 = (Mixed Bacterial and fungal phytase enzyme) and T_4 = (fungal phytase) Values are Mean ± S.E (n=15) one way ANOVA (SPSS, Duncan method).

- ✓ Mean with different superscripts are significantly different(P<0.05)
- ✓ Mean within same superscripts don't differ (P>0.05) significantly
- ✓ SE= Standard Error
- ✓ LSD= Least Significant Difference

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

Chapter V

Summary and Conclusion



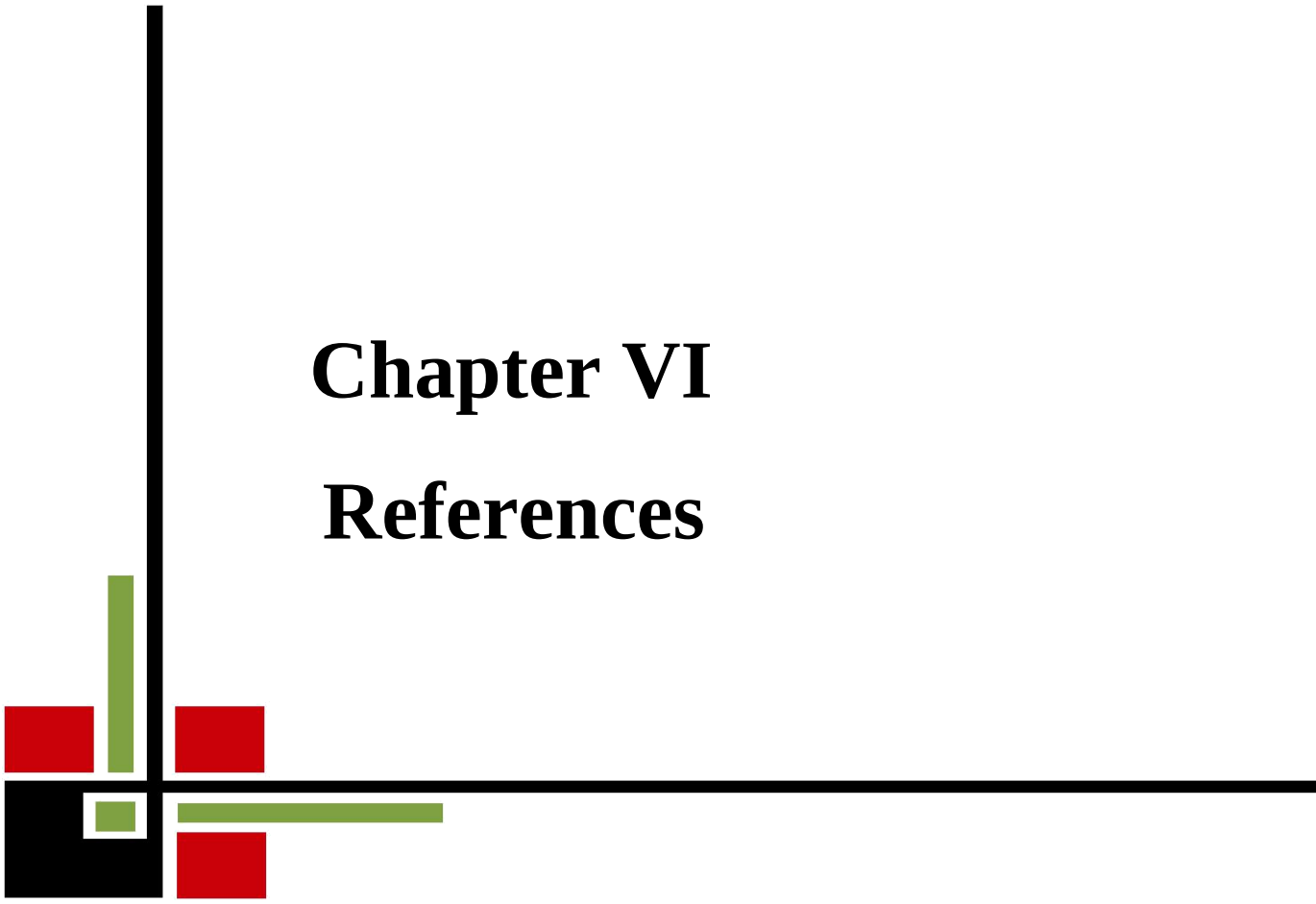
CHAPTER 5
SUMMARY AND CONCLUSION

A total of 120 day-old Cobb-500 broiler chicks were reared in Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. Chicks were divided randomly into 4 experimental groups of 3 replicates (10 chicks with each replications). One of the 4 experimental group was fed this diet as control while, the remaining three groups were fed diet with different type of phytase enzyme, one is Bacterial phytase enzyme another is Fungal phytase enzyme three type use of combined fungal and bacterial phytase enzyme in the experiment.

The effects of supplementation of Bacterial, fungal, Combined Bacterial and fungal phytase were measured. The performance traits *viz.* body weight, weight gain, feed consumption, FCR, dressed bird weight, relative giblet weight, survivability and meat yield of broiler on different replication of the treatments was recorded and compared in each group. At 28 days of age, 36 broilers were dissected to compare meat yield characteristics among different treatments. The group T_3 showed higher body weight compared to any other group T_1, group T_2 and group T_4. The weight gain, Feed conversion ratio (FCR) was significantly ($P<0.05$) lower for birds supplemented with Combined Bacterial and fungal phytase enzyme ($1.37^{b}\pm0.02$) than control birds ($1.47^{a}\pm0.05$).Bacterial and fungal combined phytasee enzyme T_3 supplemented group had a greater ($P < 0.05$) dressing percentage T_3 ($73.05^{a}\pm1.357$) compared with the control group T_1 ($66.30^{b}\pm1.642$).The percentage of male and female broilers tibia ash was significantly increased by the addition of Bacterial and fungal combined phytase enzyme on broiler ration.

Analyzing the above research findings on the growth performance of broilers, P status of broiler (tibia ash contents) by using dietary phytase enzyme was very effective.

It can be recommended by the study that incorporation of microbial phytase specially mixed fungal and bacterial phytase in starter and finisher diets of broilers(1.5+0.1) g /100 kg of the feeds proved to be beneficial for increasing the growth performance of broiler, P status of broiler(tibia ash contents).



Chapter VI

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Chapter VII
Appendices

APPENDICES

Appendix 1. Recommended level of nutrients for broiler

Components	Starter	Grower
ME (kcal/kg)	3000	3100
% CP	22	20
% Ca	1.0	0.85
% P (Available)	0.5	0.4
% Lysine	1.2	1.0
% Methionine	0.5	0.45
% Tryptophane	0.21	0.18

Source: Cobb500 Broiler Management Guide, 2016

Appendix 2. Nutrient composition of the ingredients used to formulate experimental diets

Ingredients	DM (%)	ME (K.Cal/kg)	CP (%)	CF (%)	C (%)	P (%)	Lys (%)	Meth (%)	Tryp (%)
Soybean meal	90	2710	44.50	7.5	0.26	0.23	2.57	0.76	0.57
Maize	89.5	3309	9.2	2.4	0.25	0.40	0.18	0.15	0.09
DCP					22	17.21			
Soybean oil	100	8800							
Protein concentate	91.64	2860	63.30	8.1	6.37	3.24	3.87	1.78	.53
Meat and Bone meal	95.5	1044	14.6	2.5	7.0	12.11	.66	0.24	0.12

Source: Cobb500 Broiler Management Guide, 2016

Appendix 3. Recorded temperature (^{0}C) during experiment

Age in weeks	Period	Room temperature (^{0}C)						
		8 A.M	12 A.M	4 P.M.	8 P.M.	12 P.M.	4 A.M	Average
1st	14.05.09-20.05.09	28.9	29.5	31.6	31.5	30.0	29	30.08
2nd	21.05.09-27.05.09	28.3	28.5	32.1	31.6	30.2	28.5	29.87
3rd	28.05.09-03.06.09	27.0	27.2	28.8	27.2	26.0	25.8	27.00
4th	04.06.09-10.06.09	26.8	27.0	28.6	28.5	27.4	27.2	27.58

Appendix 4. Relative humidity (%) during experiment

Age in weeks	Period (day)	Relative humidity (%)						
		8 A.M	12A.M	4 P.M.	8 P.M.	12 P.M.	4 A.M	Average
1st	14.05.09-20.05.09	85	82	73	74	78	80	78.67
2nd	21.05.09-27.05.09	85	83	71	72	77	79	77.83
3rd	28.05.09-03.06.09	86	85	74	75	81	83	80.67
4th	04.06.09-10.06.09	87	86	83	77	84	86	83.83

Appendix 5. Average Live weight, Eviscerated Weight and Dressing Percentage of different replication of broiler chicken under different treatment.

	Live weight (g)	Eviscerated Weight(g)	Dressing Percentage (%)	AVERAGE
T1R1	1567	1063	67.8366305	
TIR2	1580	1075	68.03797468	66.29657
T1R3	1589	1001.31	63.01510384	
T2R1	1600	1095.25	68.453125	
T2R2	1660	1132	68.19277108	68.601197
T2R3	1579	1092	69.15769474	
T3R1	1470	1100.25	74.84693878	
T3R2	1596.45	1180	73.91399668	73.0509
T3R3	1670.65	1176	70.39176368	
T4R1	1480	1100	74.32432432	
T4R2	1533	1000	65.23157208	69.945461
T4R3	1476	1037.34	70.2804878	

Appendix 6. Weight of internal organs of broiler chicken under different treatment groups (g/bird).

Treatment	Replication	Liver weight (g)	Spleen Weight(g)	Gizzard Weight(g)	Bursa Weight(g)	Intestine Weight(g)	Heart weight (g)
T_1	R1(1)	35	2.5	45	2	90	5
	R1(2)	38	2	46	1.5	108	6
	R1(3)	46	3	40.5	1.5	90	7
	R2(1)	37.7	1.5	47	2	124	6.9
	R2(2)	37	2	50	4	72	7
	R2(3)	34.5	3	39	2	100	12
	R3(1)	37	1.I	47	1	87	7
	R3(2)	30	1.5	20	1	108	7.5
	R3(3)	39	1.8	49	2	70	6.5
T_2	R1(1)	53	3	47.5	4	115	7
	R1(2)	36.5	1.5	43	1.5	104	6.5
	R1(3)	32	1.5	53	1	107.5	7
	R2(1)	33	2	33	0.5	85.5	7
	R2(2)	37	1.7	40	1.5	109	6.8
	R2(3)	34.5	1.5	43	1	87	7
	R3(1)	46	1.6	40	1.5	94	5
	R3(2)	34	1.5	37	1.5	120	6
	R3(3)	29	1	36	1	109.5	4
T_3	R1(1)	42.5	3.5	29.5	3	136	9
	R1(2)	37.9	2	34.5	3	80	6.5
	R1(3)	47	2	57	1	122	8.5
	R2(1)	39.5	1.5	32	2.5	136	6
	R2(2)	38	1	28.5	2.5	134	7
	R2(3)	48	2	30	1.6	118	8.5
	R3(1)	40	1.5	29	3	129.5	9
	R3(2)	23.5	1.3	37.8	2.7	95	6
	R3(3)	30	1.2	47	2.5	100	6.5
T_4	R1(1)	42	1.5	46	2.5	120	7
	R1(2)	48	3	33.5	4	131	8
	R1(3)	32	1.5	32	4	96	6.7
	R2(1)	32	2.5	38.5	1	84	6.5
	R2(2)	29	1	42	1.5	130	6.5
	R2(3)	28	1	35	1	115	5
	R3(1)	32	1.5	37	2	86	6
	R3(2)	39	2	22.5	1.5	108	6.5
	R3(3)	36	2	36	2.5	107	6.5

Appendix 7. Total Ash Percent of Tibia.

Treatment	Replication	Ash percent
T_1	R1	20.899
	R2	17.199
	R3	18.669
T_2	R1	21.203
	R2	18.137
	R3	18.936
T_3	R1	22.527
	R2	22.93
	R3	21.41
T_4	R1	20.099
	R2	20.917
	R3	16.968

Appendix 8. Ash percent under different treatments

Replication	Crucibe weight	Crucible +sample weight	Sample wt	After drying crucible + sample weight	After sample weight	Ash percent	Average
T1R1	19.24	24.16	4.92	20.22	0.98	19.91869919	
T1R1	21.18	27.35	6.17	22.53	1.35	21.88006483	
T1R2	22.52	28.39	5.87	23.6	1.08	18.39863714	18.92272
T1R2	22.1	28.1	6	23.06	0.96	16	
T1R3	23.4	28.81	5.41	24.39	0.99	18.29944547	
T1R3	17.13	22.96	5.83	18.24	1.11	19.03945111	
T2R1	19.18	25.66	6.48	20.61	1.43	22.06790123	
T2R1	20.79	28.46	7.67	22.35	1.56	20.33898305	
T2R2	15.67	23.67	8	17.21	1.54	19.25	19.42555
T2R2	22.51	29.5	6.99	23.7	1.19	17.02432046	
T2R3	21.04	28.04	7	22.5	1.46	20.85714286	
T2R3	21.19	27.89	6.7	22.33	1.14	17.01492537	
T3R1	19.4	26.2	6.8	21	1.6	23.52941176	
T3R1	22.55	29.89	7.34	24.13	1.58	21.52588556	
T3R2	20.54	27.03	6.49	21.99	1.45	22.34206471	
T3R2	19.37	26.3	6.93	21	1.63	23.52092352	22.32592
T3R3	23.47	29.93	6.46	24.76	1.29	19.96904025	
T3R3	21.12	30	8.8	23.15	2.03	23.06818182	
T4R1	19.22	25.71	6.49	20.67	1.45	22.34206471	
T4R1	15.63	21.23	5.6	16.63	1	17.85714286	19.32861
T4R2	20.5	26.63	6.13	21.73	1.23	20.06525285	
T4R2	20.79	27.91	7.12	22.34	1.55	21.76966292	
T4R3	19.8	25.96	6.16	20.9	1.1	17.85714286	
T4R3	21.04	27.01	5.97	22	0.96	16.08040201	

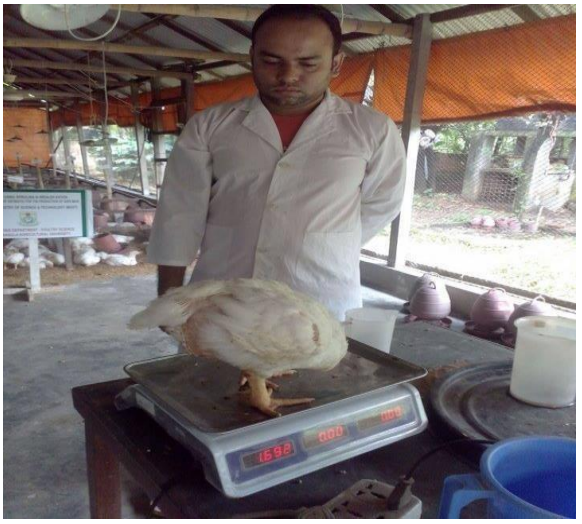
Appendix 9. Feed consumption (g/bird) of 1st, 2nd, 3rd and 4th week under different treatments.

Treatment	Replication	1st Week Feed Consumption / Bird (g)	2nd Week Feed Consumption / Bird(g)	3rd Week Feed Consumption / Bird (g)	4th Week Feed Consumption / Bird (g)
T_1	R1	141	228	596	702
	R2	142.8	373	587	710
	R3	130.1	383	607	747
T_2	R1	140.1	369	620	711
	R2	132.4	361	570	683
	R3	143	373	591	664
T_3	R1	127	363	573	764
	R2	134.3	341	590	821
	R3	133.4	366	593	820
T_4	R1	137.9	369	600	810
	R2	129.8	360	587	783
	R3	126	356	571	789

Appendix 10. Body weight (g/bird) of 1st ,2nd , 3rd. and 4th week under different treatments

Treatment	Replication	1st WeekBody Weight /Bird(g)	2nd WeekBody Weight /Bird(g)	3rdWeek Body Weight /Bird(g)	4thWeek Body Weight /Bird(g)
T_1	R1	169	458	900	1380
	R2	158	458	900	1300
	R3	167	460	910	1290
T_2	R1	179	489	950	1380
	R2	155.2	450	900	1400
	R3	170.3	467	900	1490
T_3	R1	169	480	920	1440
	R2	175	460	920	1450
	R3	170.5	480	930	1470
T_4	R1	169	484	940	1440
	R2	175	450	900	1330
	R3	156	480	920	1330

Appendix 11: Some photograph of phytase enzyme experiment conducted at SAU poultry farm



Farm activities in poultry farm

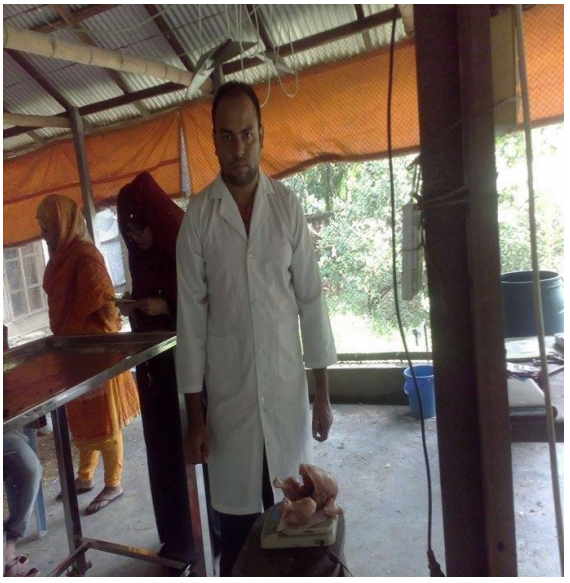
Appendix 11. Cont'd

Fungal phytase enzyme

Bacterial phytase enzyme

Use of dietary phytase enzyme on broiler ration

Appendix 11. Cont'd



Monitoring and weighing of dressed broiler chicken with internal organs.

Appendix 11. Cont'd

Different types of Medication and vaccine used in experiment.