EFFECT OF IPIL IPIL AND BEAN LEAVES SUPPLEMENTATION ON GROWTH PERFORMANCE AND EGG PRODUCTION OF JAPANESE QUAIL

A THESIS

BY

SUMON SARKAR

Registration No.: 1505016

Session: 2015-2016

Semester: July-December, 2016

MASTER OF SCIENCE (MS)

IN

PHYSIOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2016

EFFECT OF IPIL IPIL AND BEAN LEAVES SUPPLEMENTATION ON GROWTH PERFORMANCE AND EGG PRODUCTION OF JAPANESE QUAIL

A THESIS

BY

SUMON SARKAR

Registration No.: 1505016
Session: 2015-2016
Semester: July-December, 2016

Submitted to the Department of Physiology & Pharmacology
Hajee Mohammad Danesh Science and Technology University, Dinajpur,
Bangladesh in partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE (MS)

IN

PHYSIOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2016

EFFECT OF IPIL IPIL AND BEAN LEAVES SUPPLEMENTATION ON GROWTH PERFORMANCE AND EGG PRODUCTION OF JAPANESE QUAIL

A THESIS

BY

SUMON SARKAR

Registration No.: 1505016
Session: 2015-2016
Semester: July-December, 2016

Approved as to style and contents by

____

(Dr. Fahima Binthe Aziz)
Supervisor

____

(Dr. Md. Mahmudul Hasan)
Co-Supervisor

____

(Dr. Rakibul Islam)
Chairman
Examination Committee

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2016

DEDICATED
TO MY
BELOVED PARENTS

ACKNOWLEDGEMENT

The author is ever grateful to his creator Almighty God for his blessings to enable him to carry out this research work and complete the thesis.

The author would like to express heartfelt gratitude to his honorable Supervisor, ***Dr. Fahima Binthe Aziz****, Associate Professor, Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for her supervision, scholastic guidance, innovative suggestions, constructive criticism, helpful comment, inspiration and timely instructions throughout the entire period of the research.*

The author expresses deep indebtedness to his Co-supervisor, ***Dr. Md. Mahmudul Hasan****, Assistant Professor, Department of physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for his scholastic guidance, untiring assistance and advice in preparing the thesis.*

The author owes arrears of gratitude to ***Dr. Rakibul Islam****, Associate Professor and Chairman,* ***Dr. Md. Bazlar Rashid,*** *Assistant Professor and* ***Mst. Misrat Masuma Pervez****, Lecturer, Department of Physiology & Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their helpful advice and co-operation in providing facilities to conduct the experiments.*

The author humbly desires to express profound gratitude and thanks to his all reverend teachers of the Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their kind help, co-operation, encouragement and valuable suggestions.

With due pleasure the author wishes to acknowledge the healthy working relationship of staff of the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

Finally, the author is very much grateful to his beloved parents and brother for their sacrifice, inspiration, encouragement and endless love and continuous blessing for educating himself up to the postgraduate level.

The Author

ABSTRACT

This study was conducted in the Department of Physiology and Pharmacology at Hajee Mohammad Danesh Science and Technology University, Dinajpur to determine the effect of Ipil ipil and bean leaves supplementation on egg production and growth performance of Japanese quail. A total of 40 “Japanese Quail” at the age of 42 days (06 weeks) old were divided into four groups named T_0, T_1, T_2 and T_3 respectively. Group T_0 was considered as control which fed only with commercial layer ration. Group T_1 was supplemented with formulation of 2 gm grinded Ipil Ipil leaves, Group T_2 with 2 gm grinded Bean leaves and Group T_3 with 2 gm grinded Ipil Ipil leaves plus 2 gm grinded Bean leaves per kg feed respectively. Observations were recorded for growth performance, egg production and egg quality of quail. Increased egg production rate was observed in Ipil Ipil supplemented groups (group T_1). Body weights were increased significantly ($p<0.05$) in all treated groups in respect to the control and highest was recorded in combined Ipil Ipil and Bean leaves supplemented groups (Group T_3). In treatment groups, there were significant increase in egg production, but in case of control group no increasement of egg production was observed. There was no significant pathological change in any internal organs of the layer of treated groups. Best result was found in the group T_3. The present study reveals that combined supplementation of Ipil Ipil and Bean leaves gives better result over the other groups in respect to growth performance, egg production and egg quality.

CONTENTS

CONTENTS (Contd.)

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

B. wt.	:	Body weight
Conc.	:	Concentration
Cu. mm	:	Cubic millimeter
d.w.	:	Drinking water
ESR	:	Erythrocyte Sedimentation Rate
et al.	:	Associates
Fig.	:	Figure
gm	:	Gram
Hb	:	Hemoglobin
i.e.	:	That is
J.	:	Journal
Kg	:	Kilogram
Lit	:	Liter
Ltd.	:	Limited
mg	:	Milligram
mm^3	:	Cubic millimeter
No.	:	Number
PBS	:	Phosphate Buffer Solution
PCV	:	Packed Cell Volume
PM	:	Population Mean
SE	:	Standard Error
SM	:	Sample Mean
TEC	:	Total Erythrocyte Count
Vol.	:	Volume
µg	:	Microgram
%	:	Percent
&	:	And
@	:	At the rate of
<	:	Less than
>	:	Greater than
0^0C	:	Degree centigrade
FSH	:	Follicular Stimulating Hormone
LH	:	Luteinizing Hormone
PRL	:	Prolactin
ng	:	Nanogram
±	:	Plus minus
GDP	:	Gross Domestic Product

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Bangladesh is a highly populated country and growth of population is increasing very fast in comparison to its land size, as a result huge pressure is created on people's basic needs. Demand of protein of this booming population is a great threat for us. There are so many sources of protein but it is impossible to fulfill the demand without Poultry meat and egg. Different types of poultry are present. Quail is one of them. Quail meat is popular to all of us and there is no religious restriction to consume. According to our socio-economic situation, the knowledge of our farmer is very little because most of them are not properly trained for quail production.

Characteristics of Quail

Quails are very small sized bird. An adult quail weights between 150 to 200 grams and an egg weights around 7 to 15 grams. Female quails start laying eggs within their 6 to 7 weeks of age and continuously lay one egg daily. They lay about 300 eggs in their first year of life. After that they produce about 150 to 175 eggs in second year. Eggs production gradually decrease after their first year of laying period. Quail egg is very suitable for human health. It contains 2.47 % less fat than chicken egg. Many people believe that 'quail eggs help to prevent blood pressure, diabetic, pant etc'.

Quail meat is very tasty and nutritious. Fat is very low in their meat. So quail meat is very suitable for blood pressure patients. Eggs are very beautiful with multiple color. Quails do not incubate their eggs. So you have to use an incubator or brooder chickens for hatching their eggs.

Benefits of Quail Farming

Quails are smaller sized poultry birds, and it's very easy to handle them. The main benefits of starting quail farming business are listed below.

Quails are smaller sized bird, so they can be raised within small place. Feeding cost of quails are comparatively lower than chickens or other poultry birds. Diseases are less in quails, and they are very hardy. Quails grow very fast and gain maturity faster than any other poultry birds. They start laying eggs within their 6 to 7 weeks of age. It takes about

16 to 18 days to hatch their eggs. Meat and eggs of quail are very tasty, delicious and nutritious. So it's a great source of food and nutrition.

Quail farming needs small capital, and labor cost is very low. Quails can be raised successfully in commercial method. Some people have already started commercial quail farming business. Quails are very strong bird and diseases or other health problems are less. So risks are less in this business. Quail meat contain less fat. So, it is suitable for high blood pressure patients. Their food to meat or eggs converting efficiency is satisfactory. They can produce one kg meat or eggs by consuming three kg food. You can raise 6 to 7 quails in the same place that is required for one chicken. As the quail eggs are smaller in size, so the price is also lower than other bird's egg.

As a result, all types of people can buy quail eggs and you can easily sell the eggs. As the primary costs are less, so you can start this business with a very little investment. You can raise about 6 to 8 quails within 0.91 squire meter area. As it is a lucrative business venture, so commercial quail farming business can be a great source of income and employment for the unemployed educated people. Even, you can start raising a few quails along with your current profession.

Now a day's our unemployed young generation is coming in this business for long return of value and profit. Pharmaceutical companies take this advantage. They are convincing farmers for using synthetic phytase as a growth promoter and egg increaser for chicken as well as quail. As a result, each and every quail is a depot of antibiotics and other inorganic substances. When these quails are consumed by human these antibiotic and other inorganic residue enters into human body and causing serious human health hazards with drug resistance (Kibria *et al.,* 2009).

So, scientists are again concentrating on the use of our ancient medicinal system to find beneficial herbs and plants which can be safely used to increase the production. Such plants, Ipil Ipil (*Leucaena leucocephala*) and Bean (*Dolichos lablab*) are common plant which can be used as an alternative source of phytase.

A phytase (myo-inositol hexakisphosphate phosphohydrolase) is any type of phosphatase enzyme that catalyzes the hydrolysis of phytic acid (myo-inositol hexakisphosphate) – an indigestible, organic form of phosphorus that is found in grains and oil seeds – and releases a usable form of inorganic phosphorus. While phytases have been found to occur in animals, plants, fungi and bacteria, phytases have been most commonly detected and characterized from fungi.

Phytic acid and its metabolites have several important roles in seeds and grains, most notably, phytic acid functions as a phosphorus store, as an energy store, as a source of cations and as a source of myo-inositol (a cell wall precursor). Phytic acid is the principal storage forms of phosphorus in plant seeds and the major source of phosphorus in the grain-based diets used in intensive livestock operations. The organic phosphate found in phytic acid is largely unavailable to the animals that consume it, but the inorganic phosphate that phytases release can be easily absorbed. Ruminant animals can use phytic acid as a source of phosphorus because the bacteria that inhabit their gut are well characterized producers of many types of phytases. However, monogastric animals do not carry bacteria that produce phytase, thus, these animals cannot use phytic acid as a major source of phosphorus and it is excreted in the feces.

Phytic acid and its metabolites have several other important roles in Eukaryotic physiological processes. As such, phytases which hydrolyze phytic acid and its metabolites, also have important roles. Phytic acid and its metabolites have been implicated in DNA repair, clathrin-coated vesicular recycling, control of neurotrans-mission and cell proliferation. The exact roles of phytases in the regulation of phytic acid and its metabolites and the resulting role in the physiological processes described above are still largely unknown and the subject of much research.

Phytase is used as an animal feed supplement often in poultry and swine – to enhance the nutritive value of plant material by liberation of inorganic phosphate from phytic acid (myo-inositol hexakisphosphate).

Phytate is the major form of phosphorus found in cereal grains, beans and oilseed meals feed to poultry birds (Ravindran *et al.,* 1995). Approximately 61–70% phosphorus found in poultry diet ingredients is in the form of phytatephosphorus. But the monogastric animals like poultry birds are unable to utilize this phytate phosphorus, as they lack endogenous phytase, which necessitates in the addition of inorganic feed containing phosphates to poultry diets in order to meet the phosphorus requirements of poultry (Yu*et al.,* 2004). It results in relatively large amounts of phosphorus in the manure that contribute to environmental pollution (Guo *et al.,* 2009). Exogenous phytase of microbial origin can be used as an alternative that can help to reduce phosphorus excretion in poultry (Yu *et al.,* 2004). The beneficial effect of exogenous phytase in poultry ration has been supposed to be due to the direct hydrolytic effects on phytate and the subsequent improvement in the availability of minerals, amino acids, and energy (Selle & Ravindran, 2007). It has also been suggested that phytase in poultry diets improves gut health as indicated by reduced secretions from the gastrointestinal tract (GIT) which consequently improves the efficiency of utilization of energy (Oduguwa *et al.,* 2007;Pirgozliev*et al.,* 2008). The main objective of this current review therefore is to determine the effect of dietary phytase feed additives on the growth performance, feed efficiency, protein/ amino acid digestibility, energy utilization, mineral retention, and bone growth and egg production of quail. By realizing all sorts of problem we are planning to rear quail by using herbal medication like Ipil ipil and bean leaves extract instead of any Synthetic agent, to avoid human health hazards as well as economic Quail production in Bangladesh. The general objective was to see the egg and growth performance of quail by providing ipil ipil and bean leaves.

Therefore, the present study was designed with the following objectives:

1. To see the effect of Ipil ipil and Bean leaves supplementation on egg production and egg quality of quail.
2. To see the effect of Ipil ipil and Bean leaves supplementation on body weight gain of quail.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

This chapter presents the review of relevant literatures, which consist of the effects of phytase enzyme on feed conversion, growth performance and egg production of quail. Many researchers have been conducted researches in these topics in chicken. But limited research work has been performed in case of quail.

2.1 Domestication

The earliest records of domesticated Japanese quail populations are from 12th century Japan; however, there is evidence that the species is actually domesticated as early as the 11th century (Hubrecht R, Kirkwood J, 2010). These birds were originally bred as songbirds, and it is thought that they were regularly used in song contests (Hubrecht R, Kirkwood J, 2010, Mills, AD, 1997).

In the early 1900s, Japanese breeders began to selectively breed for increased egg production. By 1940, the industry surrounding quail eggs was flourishing. However, the events of World War II led to the complete loss of quail lines bred for their song type, as well as almost all of those bred for egg production. After the war, the few quails left were used to rebuild the industry, and all current commercial and laboratory lines today are considered to have originated from this population of quails (Hubrecht R, Kirkwood J, 2010, Mills, AD, 1997)

2.2 Distribution and habitat

Populations of the Japanese quail are known to mainly inhabit East Asia and Russia. This includes India, Korea, Japan, and China (Barilani, M *et al*., 2005, Puigcerver *et al.,* 2007). Though several resident populations of this quail have been shown to winter in Japan, most migrate south to areas such as Vietnam, Cambodia, Laos, and southern China (Birdlife International, 2013) This quail has also been found to reside in many parts of Africa, including Tanzania, Malawi, Kenya, Namibia, Madagascar, and the area of the Nile River Valley extending from Kenya to Egypt (Pappas, J 2013).

Breeding sites of the Japanese quail are largely localized to East and Central Asia (Barilani, M *et al*., 2005, Puigcerver *et al.,* 2007), in such areas as Manchuria, southeastern Siberia, northern Japan, and the Korean Peninsula. However, it has also been observed to breed in some regions of Europe, as well as Turkey (Pappas, J 2013).

The Japanese quail is primarily a ground-living species that tends to stay within areas of dense vegetation in order to take cover and evade predation (Buchwalder, T; Wechsler B, 1997). Thus, its natural habitats include grassy fields, bushes along the banks of rivers, and agricultural fields that have been planted with crops such as oats, rice, and barley (Pappas, J 2013, Buchwalder, T; Wechsler B, 1997). It has also been reported to prefer open habitats such as steppes, meadows, and mountain slopes near a water source.

The establishment of quail production assumed importance worldwide in the last decade, not only for being a laboratory animal for poultry and biomedical research, but also because it is commercially exploited for production of meat and eggs. The poultry meat and egg industry have a rich story, which goes through various stages of development since the beginning, from captive birds, now reaching the most advanced and organized segment of agriculture worldwide .The quail production story is more recent and also has the same characteristics that occurred in the broiler and laying hen production.

During the 60's to 80's the quail production was regarded as subsistence activity in Brazil, in the small backyard rearing system. From the investment small backyard rearing system. From the investment of the genetic selection and quality of product, quail breeding producers could see a good deal for the future. Since then, it was seen the growth of large farms producing eggs, housing more than 100,000 birds in automated low-cost production and a regular supply of eggs with good quality, well packed, and safe to the market, and still making room for growth. The production of quail has been derived in meat and egg production. The major meat production countries are Spain, France eggs are China, Japan, Brazil and France, China and the United States of America. Leading the production of quail eggs are China, Japan, Brazil and France. In the Latin America, Brazil leads the production, followed by Venezuela, Peru, Colombia and Bolivia.

Global trends in the rearing of quail follow opposed directions when compared Brazil and the rest of the world. Worldwide there is a decrease in the use of quails in research as animal models, followed by a significant decline in peer reviewed articles. Nonetheless, in Brazil, it has been seen an increase in scientific papers regarding quail production since 2002, with the development of new production technologies giving support for the continued growth of the segment. Following this trend, a research group led by the author, founded the Center for Studies in Poultry Science and Technology (NECTA) at the Federal University of Lavras, Minas Gerais, contributing with advanced studies in poultry science and discussing the quail production. Then, the first forum for discussion of quail production occurred in 2002. Since then, it has been accomplished international symposia every 2 years with abundant discussions and exchange of technical expertise what resulted in improvement in the Brazilian quail egg and meat chain. As result from these meetings, could be ranked the main research lines to be followed by the quail industry led by the feeding studies (nutritional requirements specific to the stages of breeding / rearing, production, performance and internal and external quality of eggs), management practices (housing density, beak trimming, feed management, equipment, environmental comfort), health (diseases and vaccines, biosecurity) and genetics (genotypexenvironment interaction, production, uniformity of egg, viability, selection and breeding).

The current situation in Brazil is a growing demand for processed eggs (pickles), however, there is a limited supply in day-old chicks what will restrict the market. On the other hand, there is a need to develop breeding programs to obtain better defined strains to produce eggs of good size and in the case of quails for meat production, the reduction of the effects of inbreeding. Studies of interaction between factors responsible for changes in production characteristics are critical for the quail egg chain.

The general trend in egg production is the automation in big farms, integrated with the egg processing industries. Advances have also been observed in the production technology of pickled eggs, always seeking to improve the quality of the product to the consumer.

Populations of the Japanese quail are known to mainly inhabit East Asia and Russia. This includes India, Korea, Japan, and China. Though several resident populations of this quail have been shown to winter in Japan, most migrate south to areas such as Vietnam, Cambodia, Laos, and southern China. This quail has also been found to reside in many parts of Africa, including Tanzania, Malawi, Kenya, Namibia, Madagascar, and the area of the Nile River Valley extending from Kenya to Egypt.

Breeding sites of the Japanese quail are largely localized to East and Central Asia, in such areas as Manchuria, southeastern Siberia, northern Japan, and the Korean Peninsula.

However, it has also been observed to breed in some regions of Europe, as well as Turkey.

The Japanese quail is primarily a ground-living species that tends to stay within areas of dense vegetation in order to take cover and evade predation. Thus, its natural habitats include grassy fields, bushes along the banks of rivers, and agricultural fields that have been planted with crops such as oats, rice, and barley. It has also been reported to prefer open habitats such as steppes, meadows, and mountain slopes near a water source.

2.3 Behavior

From studies of captive-bred Japanese quail, seven distinct displays and calls have been recognized in males. Three of the calls were also observed to be uttered by females (Johnsgard 1988). The call of this quail consists of "deep hollow sounds, several times repeated in quick succession" (Finn 1911). The male's call is typically three notes. The female will utter a "long" call which allerts the male to her receptivity to copulate (Johnsgard 1988). In addition, these quail engage in courtship-feeding. The male will hold a small worm in his beak, uttering a soft croaking call. The female approaches the male and takes the small worm to eat. The male then attempts to copulate with the female (Lambert 1970).

This quail and its European counterpart are migratory. *Coturnix japanica* will migrate to India (Finn 1911), northern Japan and Korea for the summer (Hoffmann 1988). They winter in southeast China, Hainan, Taiwan, and southern Japan. Their migration covers 400-1000 km, which is remarkable for a bird not known for its flying capability (Hoffmann 1988). Overall, their migration route follows a north-south pattern (Johnsgard 1988).

2.4 Physical Description

The Japanese quail is similar in appearance to the European Common Quail, Coturnixcoturnix. Overall, they are dark brown with buff mottling above and lighter brown underneath. They have a whitish stripe above the eye on the side of the head. Legs are orangish-gray to pinkish-gray as is the beak (Hoffmann 1988). In contrast to the males, females usually (but not always) lack the rufous coloring on the breast and black flecking or markings on the throat (Johnsgard 1988). There are variations in plumage color. Some birds are whitish to buff with rufous to chestnut mottling above. Others have a very dark brown appearance with little to no mottling. In addition, there have been golden-brown varieties bred in captivity (Hoffmann 1988). Wing sizes in males and females is similar ranging from 92 to 101 mm. Both male and female have similar sized tails ranging from 35-49 mm in length (Johnsgard 1988).

2.5 Reproduction

As with other quail, eggs were laid at a rate of one per day (Lambert 1970), with 7-14 eggs per clutch (Hoffmann 1988). An egg averages 29.8 by 21.5 mm is size and weighs 7.6 g (Johnsgard 1988). Incubation time is 19-20 days (Lambert 1970), although clutch sizes have been associated with latitude and length of photoperiod. In Japan, clutch size is 5-8 eggs, while in Russia, clutch size is 5-9 eggs (Johnsgard 1988). The chicks are considered to be mature and able to mate after four weeks old (Hoffmann 1988). The breeding season varies with location. In Russia, the season starts in late April and continues to early August. In Japan, nesting occurs from late in May and usually ends in August. On the rare occasion, eggs may be found in nests in September (Johnsgard 1988).

2.6 Production performance of Japanese quail

The production of eggs with eggshell quality is an important concern of the egg industry. According to Roland (1988), losses due to low eggshell quality or other reasons may reach 20% before the eggs arrive at retail. Hurwitz (1989) asserted that the nutritional factors that affect eggshell quality depend on the metabolic exchanges, which occur during egg formation. In the uterus, the organic fraction of the eggshell is synthesized by the glands, and calcium – its largest component – is mobilized from the blood. Eggshell is sensitive to calcium availability, and carbonate is influenced by dietary factors that affect acid-base balance. This author also observes that eggshell mineral content is 90%, out of which 98% consist of calcium carbonate. It is well known that, during eggshell formation, the transference of calcium from the plasma to the uterus in layers in very fast, of an average of one minute.

According to Etches (1996), eggshell is formed mostly during the night, when birds do not eat, and this may increase calcium deficiency for egg formation. Therefore, calcium is mobilized from the bones. Leeson *et al.* (1991) verified that calcium requirements are generally very low, except at the time eggshell is deposited. Faria *et al.* (2000) observed that commercial layers lay more frequently during the morning, after a period of fasting during the night, when eggshell is formed.

Aiming at improving eggshell quality in commercial layers, Joly *et al.* (2003) mentions some techniques, such as feeding calcium-rich feeds in the afternoon, high particle size calcium dietary addition, short lighting period during the night, etc. However, in order to successfully apply these techniques in quails, feed intake behavior and lay times must be similar between these two bird species.

Therefore, this study aimed at investigating performance, internal egg quality, and eggshell quality parameters of quails during the day, and the possible influence of the lighting program on these parameters.

2.7 Present status of Japanese quail

Within Japan, the Japanese quail *Coturnix japanica* is bird species familiar to many people. It was first designated as a game species in 1918, and has been captive-bred and released into the wild since the early 1970s. An examination of the annual numbers of quails hunted, based on Wildlife Statistics data and other literature sources, indicates that the population level of Japanese quail started to decline in the 1930s, and has subsequently shown a dramatic decrease. Japanese quail is thought to have no harmful effects on agriculture, and has retained its status as a game species solely owing to its value as a hunting target. In 1998 the Japanese quail was listed as DD (Data Deficient) on the Japanese Red List, and its designation as a game species should therefore be reconsidered as soon as possible. For the Japanese quail population to recover from itsfrom endangered status a combination of stricter hunting regulations and the active restoration of suitable habitat is urgently required quail.

2.8 Effect of phytase on egg production and egg quality

Lucky *et al.* (2014) worked on the effect of dietary exogenous phytase on laying performance of chicken at older ages. A total of 48 Shaver-579 chicken layers aged between 85 to 94 weeks were reared in individual cages and given a basal diet amounting to 115g feed/bird/day. The basal diet fortified with 0.05, 0.10 or 0.15% RenaPhytase-400 constituted of 3 experimental diets to see the effects of exogenous phytase on egg production and egg quality. Results indicated that increasing level of exogenous phytase in diet almost linearly ($P<0.05$) increased egg production and feed conversion but did not affect egg quality. Providing phytase in the diet at 0.05, 0.10 and 0.15% increased egg production by 11.86, 22.2 and 24.58%, respectively. It was shown that highest egg production was found at 0.15% phytase levels in diet. They concluded that egg production of aged hen is increased by adding said amount of exogenous phytase in the diet.

Abeyrathna *et al.* (2014) determine the maximum inclusion levels of dietary RB with or without exogenous phytase, for laying Japanese quail. In a completely randomized design with a 3 x 2 factorial arrangement, 108 quails in 36 cages received six experimental diets ad libitum from 8 to 15 week. Experimental factors were three dietary RB inclusion levels (20, 30 and 40%) and two phytase levels (0 and 1000 FTU/kg). The level of dietary RB, phytase supplementation and their interaction had no significant effects on live weight or feed intake. The total egg production of the quail fed 40% RB was significantly lower than that of quail fed 20 and 30% RB. Egg laying rate of the quail fed 40% RB was significantly lower than those of the quail fed 20 or 30% RB from 6th week onwards. By eighth week, 30% RB resulted in significantly lower egg laying rate compared to the quail fed 20% RB. Feed conversion ratio (FCR) of the quail fed 40% RB was significantly higher than those of 20 or 30 % RB fed. Adverse effects of phytate in 30 or 40% RB on egg number, egg mass and FCR were not mitigated by the supplemental phytase. They concluded that inclusion of more than 20% RB in the diets of laying Japanese quail reduces the production performances.

Ademola *et al.* (2013) effects of microbial phytase and native wheat bran phytase on laying performance, egg quality and shell phosphorus of hens fed two forms of diets. Five experimental diets were formulated for the study. Control and basal diets contained similar levels of nutrients. However, basal diet (T1) containing 15% wheat bran (WB) had lower available phosphorus (AVP). Diet forms (mash and pelleted) and microbial phytase supplementation (0 and 900 phytase unit (FYT) were arranged to examine their interaction effects. The 0 FYT microbial phytase represented the native wheat bran phytase activity in the mash diet only. T1 and T2 were mash and pelleted unsupplemented diets respectively. Diets in T3 and T4 were microbial phytase supplemented in mash and pelleted forms respectively. Laying hens fed unsupplemented mash basal diet (T1) had the highest hen day production (HDP) ($P<0.024$), and the best feed conversion ($P<0.012$). However, those fed mash supplemented diet (T3) had the lowest HDP and worst feed conversion. Microbial phytase supplementation to mash diet

(T3) resulted in lowest egg mass of 45.35 gram daily (P<0.025). Pelleting the unsupplemented diet (T2) yielded poorer feed conversion than those fed unsuplemented mash diet (T1). Hens fed pelleted supplemented diet (T4) had slightly reduced HDP and significantly lower egg mass when compared to the control group. These hens had significantly highest yolk index (P<0.036) and egg shell with the most concentrated phosphorus content (P<0.002). It is concluded that native wheat bran phytase in mash diet containing 15% WB was effective for improved laying performance.

Ahmadi *et al.* (2012) added of nonphytate P (NPP) in diets for laying hens without negatively affecting their productivity and heath is crucial for sustainable egg production. A meta-analytical approach using a full quadratic model was applied to quantify relationships between dietary NPP and phytase levels and performance of laying hens. Except for the quadratic effect of dietary phytase on FCR were significant (P < 0.05). There was a relatively strong relationship between observed and predicted and FCR. Analyses of the model revealed that corn-soybean meal-based diets containing 0.22% of NPP without supplemental phytase resulted in high EP, EM, and feed efficiency in layers In the presence of phytase in feed.

Mohebbifar *et al.* (2011) investigated the effects of phytase supplementation of low phosphorous diets included graded levels of rice bran on productive performance of laying hens and egg quality characteristics. They experimented on Lohmann LSL-Lite hens after production peak were randomly divided in 48 cages and experimental diets including three levels of rice bran with or without phytase and two levels of dietary nonphytate phosphorus were fed to hens with 4 replicates per diet during 7-week trial period. The results indicated that dietary inclusion of rice bran decreased. Phytase supplementation increased egg production. Dietary inclusion of rice bran increased feed intake and feed conversion ratio comparing with control diet. Phytase did not affect on feed intake; however improved feed conversion ratio. Dietary non-phytate phosphorus level did not affect on feed intake, egg production and feed conversion ratio. From the results of this study, it can be concluded that rice bran can be included in laying hens' diets up to 7.5% with no adverse effects on performance. Decreasing non-phytate phosphorus level of Lohmann LSLLite hens' diet up to 0.029% would be beneficial way to minimize environmental pollution and decrease dietary phosphorous expenses with no adverse effect on productive performance and egg quality characteristics.

Mika *et al.* (2011) conducted a study to evaluate the effects of dietary supplementation of phytase B (product of the *Aspergillus niger phy* B gene expressed in *Trichoderma reesei*) on feed intake, laying performance, eggshell quality, and on phosphorus and calcium balance in laying hens. Seventy two, 40 weeks old HyLine brown hens were fed for 14 weeks the following four phosphorus-deficient (0.12% nonphytate phosphorus, NPP), maize-soybean meal-based diets: (1) calcium-deficient (2.8% Ca) control diet; (2) diet 1 + phytase B at the activity of 2.5 acid phosphatase units (AcPU/kg); (3) control diet (3.8% Ca); (4) diet 3 + phytase B at the activity of 2.5 AcPU/ kg. Each dietary treatment was fed to 18 cages of hens, 1 hen/cage kept in individual cages. Hens fed the NPP- and Ca-deficient diets consumed more feed ($P < 0.01$) and excreted less calcium ($P < 0.01$) than those receiving P-deficient diets with the standard calcium level. There were no effects of calcium level on feed utilization, egg mass, egg weight, and eggshell breaking strength. Egg production, although numerically higher in hens fed low Ca diets with no enzyme added, failed to be significantly different due to the low number of hens investigated and therefore the measurement should be considered as preliminary and supplementary. Phytase B increased mean egg weight by about 7% in layers fed the NPP- and Ca-deficient diet (Ca × phytase B interaction, $P < 0.05$), increased shell breaking strength, particularly at the standard calcium level, significantly enhanced amounts of calcium retained by layers and amounts of phosphorus retained by hens fed the Ca-deficient diets. Additionally, phytase B improved Ca retention at both dietary Ca levels and phosphorus retention in hens fed the Ca-deficient diets. Results of the study indicate that the efficacy of phytase B in NPP-deficient diets is strongly influenced by the dietary calcium level and the enzyme may modulate egg weight, eggshell quality, phosphorus and calcium retention in laying hens fed low-NPP, maize-soybean meal-based diets.

Mohammed *et al.* (2010) a total number of three hundred and fifteen 22-weeks old, commercial Hy-line White-36 hens were randomly assigned into five groups, each group contains nine replicate (seven birds per each) to study the effect of phytase supplementation to diets containing rice bran on performance and egg quality of laying hens. The first group was fed basal diet without phytase supplementation (control) and the 2, 3, 4 and 5 groups, were fed on basal diet supplemented with phytase at levels 0.1, 0.15, 0.20 and 0.25 % respectively. The present results indicated that phytase supplementation significantly ($P<0.05$) or ($P<0.01$) increased henday production, accumulative eggs number, egg mass and also improved significantly ($P<0.05$) feed conversion ratio. While, egg weight was significantly decreased. However, feed consumption and egg quality measurements expressed as shell thickness, yolk percentage, albumen percentage did not affected by phytase enzyme supplementation. On the other hand, the overall mean of shell percentage was significantly ($P<0.05$) increased. In conclusion, It is concluded that the best level of phytase supplementation was (2 kg phytse / ton feed) in diets contains rice bran for laying hens performance and egg shell percentage.

Jubarah *et al.* (2010) phosphorus is an indispensable mineral, crucial to growth and development both structurally and metabolically but phytate (a major storage form of phosphorus in seeds of plants) decreases mineral bioavailability and nutrient digestibility. Subsequently, in determining the influence of phytate with/without phytase or citrate on laying performance, 5 soybean-maize based diets of similar nutrient density were formulated: a control diet with 0.21% phytate-P and 0.36 % nonphytate-P but no added phytate, and a second similar diet except that the inorganic phosphorus source was replaced by added phytate-P. The third, fourth and fifth diets were similar to the second diet but they contained 0.3% phytase, 1.5% citrate, 0.3% phytase plus 1.5% citrate, respectively. Each treatment was randomly assigned to 35-week-old laying hens. Hens fed the phytase diet showed a pronounced increase in egg production ($P<0.05$), followed by the citrate treatment, while the phytate-P based diet depressed egg production compared to the rest of the treatments. Though the differences in eggshell thickness were insignificant ($P>0.05$), egg weights were affected ($P<0.05$) by the various dietary treatments; the phytase plus citrate diet yielded the highest egg weight improvement. Carbonic anhydrase in the shell gland mucosal extracts depicted significant variation in its specific activity ($P<0.05$), with no observed differences between the control and the phytate-P, phytase or citrate treatments, but phytase combined with citrate had significantly the highest activity of carbonic anhydrase. Serum zinc and yolk iron were significantly improved ($P<0.05$) by phytase and/or citrate treatments, but serum copper or yolk total lipid and mineral concentrations were not affected by the dietary treatments ($P>0.05$). Both phytase and phytase plus citrate treatments yielded high serum and yolk total cholesterol, followed by the citrate and phytate-P, and the least for the control diet. Supplementary phytase and/or citrate seems to increase nutrients availability. Whether citrate increases susceptibility of phytate to phytase by removing cations bound to phytate or creating digesta pH conducive for phytate hydrolysis is yet to be determined.

Lim *et al.* (2003) employing a factorial arrangement of two levels (3.0 and 4.0%) of Ca, two levels (0.15 and 0.25%) of non-phytate phosphorus (NPP) and two levels (0 and 300 U/kg diet) of microbial phytase was carried out with 960 ISA-brown layers from 21 to 41 wk of age. There was a significant interaction between NPP level and phytase for egg production. High NPP level and phytase supplementation increased egg production only in the second 10-wk period (31 to 41 wk). High NPP and low Ca increased feed intake and a significant interaction between level of NPP and Ca was observed in the first 10 wk. High NPP improved feed efficiency only in the second 10-wk period. Low NPP improved egg specific gravity and eggshell thickness but decreased Haugh units in the first 10-wk period; high NPP decreased the percentage of broken and soft-shell eggs in the second period. Low Ca decreased egg specific gravity, egg shell strength and egg shell thickness in both periods and increased Haugh units in the second 10-wk period. Phytase supplementation decreased the percentage of broken and soft-shell eggs. High NPP increased fiber availability but decreased Ca availability. High Ca decreased Ca availability, whereas phytase increased availability of dry matter, fiber, and P. High NPP increased retention of P and Fe but also increased excretion of P. High Ca decreased retention of Zn and Fe. Phytase supplementation increased P retention, resulting in decrease of P excretion. In conclusion, supplementation of microbial phytase at a level of 300 U per kg diet of laying hens can improve egg production, decrease broken and soft egg production rate, and P excretion. The effects of phytase supplementation are significantly modified by the level of Ca and NPP.

Jalal *et al.* (2000) hens were fed corn-soybean meal diets containing nonphytate phosphorus (NPP). Phytases A and B were added at 0.25, 0.15, and 0.10% at 250 to 300 units of phytase (FTU)/kg feed in a 3 × 3 factorial; 0.35% was a control diet. Phytase supplementation had a significant effect on several production parameters: feed intake, feed conversion, and egg mass. Results showed non-significant effects ($P < 0.06$) on feed intake when hens were supplemented with phytase A or B and consumed more feed compared to the basal diet at 0.10% NPP. The feed conversion of birds fed 0.10% NPP without phytase was the least efficient compared to the other nine treatments ($P < 0.05$). Egg mass was significantly greater for hens supplemented with phytases A and B than for hens fed the basal diet at low (0.10%) NPP ($P < 0.05$). There were no significant differences in egg production, egg weight, specific gravity, Haugh units, wet shell, or dry yolk percentages. Phytase supplementation improved Ca and P digestibility's to varying degrees. Supplementation of phytase in normal, corn-soybean meal diet improves feed intake, feed conversion and egg mass and elicited a response in shell quality and egg components at the low (0.10%) NPP.

2.9 Effects of phytase on growth performance

Saima *et al.* (2014) a 28 days trial was conducted to evaluate efficacy of microbial phytase in diets for Japanese quails. For this purpose, 900 experimental birds were divided into six groups, each group containing 150 chicks and further sub-divided into10 replicates. Diet A (positive control) was formulated according to NRC (1994) requirements set for the Japanese quail (CP 24% and ME 2900 Kcal/Kg). Diet B differed from diet A in Ca (Calcium) and P (Phosphorus) i.e. 0.15% Ca and 0.20% P less to Diet A, respectively. Four different levels of phytase enzyme (250, 500, 750, 1000 FTU/kg of feed) were added to diet B to formulate diets C, D, E and F treatments respectively. Results revealed that body weight gain, feed consumption, FCR, keel /shank length, dressing percentage of birds in groups consuming 750 and 1000 FTU/kg phytase were significantly higher ($P<0.05$) than those of B, C and D. The growth performance of group E and F was comparable with those of group A (+ve control). Maximum leg weakness, swollen joints and crippled legs were observed in group B (39.30%) followed by C, D (21.33%, and 16.0%). Keeping in view, performance and mortality rate, it is recommended that microbial phytase may be used with greater confidence in Japanese quail ration.

Narasimha *et al.* (2013) conducted a trial to evaluate pure NSP enzyme combination derived from in vitro studies and commercially available phytase to corn-soybean meal based low energy diets singly and combination of both. The experiment was conducted by using completely randomized design on one hundred and fifty layer birds (40 weeks) of uniform body weight and production with five treatments, six replicates and five hens in each replicate for three laying periods with twenty eight days in each laying period. The performance was measured in terms of egg production, feed intake, weight changes, feed efficiency, egg quality, nutrient retention, and gut health. Egg production improved ($P<0.05$) with supplementation of phytase alone or in combination of phytase and NSP (non-starch polysaccharides) enzymes. No effect of supplementing NSP enzymes, phytase alone or in combination was observed on feed intake, FCR, egg quality traits and retentions of DM, OM and NFE. Significantly (P<0.05) higher retentions of CP, CF, EE, GE and phosphorus was observed with supplementation of NSP enzymes and similar trend was observed with both NSP and phytase to BD except for phosphorus indicating no associative effect of phytase and NSP enzymes on above nutrient retentions. Intestinal pH, viscosity and *E. coli* count significantly (P<0.05) reduced with supplementation of NSP enzymes and no further improvement was observed on these variables with supplementation of phytase with NSP enzymes. Gut histology revealed broad and disrupted villi with little goblet cell activity. No significant (P<0.05) effect on feed cost due to addition of phytase and/or NSP enzymes to BD was observed. The cost of feed to produce dozen eggs was comparable among SD, BD and BD supplemented with NSP enzymes and phytase.

Gao *et al.* (2013) compared the efficacy of a novel transgenic corn-derived phytase (TCDP) and two other commercial microbial phytases (PA and PB) in the long-term feeding study of laying hens. The treatments consisted of a positive control (PC) diet adequate in phosphorus (P); a negative control diet low in P (0.10% NPP. Eight diets were fed to Hy-line hens (n = 576) from 50 to 66 weeks of age. They found that with a reduction in dietary P in the NC diet, egg production, egg mass, feed intake, final BW, BW gain, eggshell thickness, and eggshell strength of laying hens decreased (P<0.05). In addition, the number of soft-shelled, cracked and broken eggs increased (P<0.05) in the NC group. The addition of TCDP, PA or PB significantly increased laying production and egg quality (P<0.05), and performed similarly in hens fed the PC diet. Hens fed each source of phytase had greater apparent ileal P digestibility, tibia ash and bone breaking strength than hens fed the NC diet (P<0.05). Results from this study indicate that the addition of TCDP to a P-deficient diet improves laying performance, egg quality, ileal P utilization, and bone mineralization, and TCDP is as efficacious as two commercial microbial phytases when P-deficient diets for laying hens were supplemented with it.

Tang *et al.* (2012) conducted to assess the effects of a novel thermostable phytase in male broiler chicks (Ross 308) fed available P (AP)-deficient diets on growth performance and bone mineralization. The treatments consisted of 8 experimental diets: 1 positive control diet containing an adequate level of AP, 1 negative control diet deficient in AP and 6 diets with the same level of AP as in the negative control but supplemented with different levels phytase. The addition of phytase significantly improved (P < 0.05) BW gain, feed intake, FCR, toe ash, tibia ash and tibia P of broilers compared with those fed the negative control diet. No significant differences (P > 0.05) were found in FCR and bone mineralization among the broilers fed different levels of phytase and those fed the positive control diet. In conclusion, normal growth performance and bone mineralization were maintained in broilers fed AP-deficient diets supplemented with thermostable phytase.

Abou-Elezz *et al.* (2011) this study consisted of two experiments, aimed at determining the effect of the dietary inclusion of either Leucaena Leucocephala (LLM) or Moringa Oleifera Leaf Meals (MOLM) on Rhode Island Red (RIR) hens' egg production and quality. In the first experiment, thirty six RIR hens, at 36 weeks of age, were randomly divided into four groups each of nine birds and were allocated in individual cages. The four groups corresponded to four dietary treatments containing 0 (control), 5, 10, and 15 % of LLM, respectively. Simultaneously, the second experiment was carried out following the same design but using MOLM instead of LLM. The egg production and quality traits were monitored for five weeks, preceded by one week of adaptation. The results showed a quadratic effect on the egg laying rate (57.10, 57.46, 53.25, and 47.46 %), egg mass (g/hen/d) and feed conversion due to the LLM treatments (0, 5, 10, and 15 %, respectively). The MOLM treatments decreased linearly the egg laying rate (60.00, 59.72, 56.13, and 51.87 %) and the egg mass, and had a quadratic effect on the feed intake (111.15, 111.93, 107.08, and 100.47g/hen/d) when including 0, 5, 10, and 15 % of MOLM, respectively. The yolk color increased linearly by the rise in both the MOLM and the LLM levels. Other results were obtained in the albumen and yolk proportions (%) and in the yolk coefficient, while no adverse effects were found on the other egg quality traits due to the LLM or MOLM treatments. The MOLM or the LLM could be acceptable as sustainable feed resource up to 10 % in laying hen diets.

Yan *et al.* (2009) experimented on 68-week-old Hy-Line brown laying in a 6-week feeding trial to compare the efficacy of phytases Optiphos (OPT) and Natuphos (NAT), which were isolated from Escherichia coli and Aspergillus niger, respectively. Feed intake, egg production, egg quality, apparent nutrient digestibility and serum P and Ca concentration were evaluated to compare the effect of the two phytases. Feed intake and eggshell thickness were not affected by the treatments. Superior effects (P<0.05) of OPT were only observed in egg production and egg weight compared with NAT. Characteristics such as eggshell breaking strength, apparent digestibility of N, Ca and P and serum P concentration were equally increased with the supplementation of both phytases (P<0.05), where no significant difference was observed in those characteristics between PC and phytase supplementation at 500 FTU/kg. Equally effective improvements (P<0.05) were also observed in egg production and DM digestibility, where no improvements were observed (P<0.05) between the PC group and the groups with phytase supplementation at 500 FTU/kg. Equal increases in the serum Ca level were observed when the groups with phytase supplementation were compared to the PC group. Overall, the results of this study suggest that NAT and OPT are equally effective at liberating phytate-bound complexes when included in 0.2% available phosphorus diets for 68-week laying hens; either source of phytase can be fed to commercial 68-week laying hens at 500 FTU/kg to correct the negative effects associated with a 0.2% available phosphorus diet. In conclusion, either source of phytase can be fed to commercial first cycle laying hens at 500 FTU/kg to effectively replace inorganic phosphorus when economically justified. (Key Words: Laying Hens, Phytase, Egg Quality, Egg Production, Serum).

Liu *et al.* (2007) the effects of phytases on the performance of layers and the ileal nutrient digestibility of corn, soybean, and by-product meal-based diets were assessed with 320 Hy-Line brown layers from 23 to 28 wk of age. Layers were grouped randomly into 5 treatments with 8 replicates per treatment and 8 layers per replicate. The 5 diets consisted of a positive control diet with adequate Ca (3.30%), total P (0.50%), and nonphytate P (NPP; 0.28%) and a negative control diet and 3 phytases (phytase A derived from Aspergillus niger and phytases B and C derived from Escherichia coli) supplemented at 300 phytase units/kg of feed respectively. Phytase supplementation in the negative control diet improved the digestibility of P and Ca by 11.08 and 9.81% (P < 0.05), respectively, whereas it improved the digestibility of amino acids by 2 to 8% (P<0.05). Supplementing phytases in the negative control diet improved the rate of lay, egg mass, and eggshell quality to the levels of birds fed the positive control diet. These results suggest that supplementing phytases can improve the digestibility not only of Ca and P, but also of amino acids in layers. The use of phytase reduces phosphorus excretion in poultry manure by allowing the birds to utilize more of the phytate phosphorus. Phytate phosphorus has the ability to complex with cations such as calcium, magnesium, zinc, copper, and nitrogen and certain gastrointestinal proteases, thus reducing the availability of these cations and of amino acids. The use of phytase may free these cations and proteases bound in phytate phosphorus complexes and improve many production parameters and body structure characteristics in broilers and laying hens, such as body weight, bone ash content, feed consumption, egg weight and egg shell quality.

Francesch *et al.* (2005) performed a 24-week trial to evaluate the efficacy of an experimental phytase on performance, egg quality, tibia ash content and phosphorus excretion in laying hens fed on either maize or a barley-based diet. At the end of the trial, an ileal absorption assay was conducted in order to determine the influence of phytase supplementation on the apparent absorption of calcium and total phosphorus (P). Each experimental diet was formulated either as a positive control containing 3.2 g/kg non-phytate phosphorus (NPP), with the addition of dicalcium phosphate (DCP), or as a low P one, without DCP addition. Both low P diets (containing 1.3 or 1.1 g/kg NPP) were supplemented with microbial phytase at 0, 150, 300 and 450 U/kg. Low dietary NPP (below 1.3 g/kg) was not able to support optimum performance of hens during the laying cycle (from 22 to 46 weeks of age), either in maize or barley diets. Rate of lay, daily egg mass output, feed consumption, tibia ash percentage and weight gain were reduced in hens fed low NPP diets. The adverse effects of a low P diet were more severe in hens on a maize diet than in those on a barley diet. Low dietary NPP reduced egg production, weight gain, feed consumption and tibia ash content and microbial phytase supplementation improved these parameters. Hens given low NPP diets supplemented with phytase performed as well as the hens on positive control diets containing 3.2 g/kg of NPP. A 49% reduction of excreta P content was achieved by feeding hens on low NPP diets supplemented with phytase, without compromising performance. Phytase addition to low NPP diets increased total phosphorus absorption at the ileal level, from 0.25 to 0.51 in the maize diet and from 0.34 to 0.58 in the barley diet. Phosphorus absorption increased linearly with increasing levels of dietary phytase

Musapuor *et al.* (2005) conducted an experiment to study the effects of different levels of phytase, vitamin D3, calcium and available phosphorus on phytate phosphorus utilization in laying hens. Dietary phytase caused a significant ($P<0.05$) increase in feed consumption, feed conversion ratio, tibia ash weight, tibia ash percentage, tibia phosphorus plasma phosphorus and phosphorus digestibility. However, dietary phytase caused a significant ($P<0.05$) decrease in plasma alkaline phosphatase activity and excreta phosphorus percentage. Also phytase had no beneficial effect on egg shell quality traits. Interaction between phytase and calcium on tibia phosphorus, plasma calcium and excreta phosphorus were significant (P<0.05). Interaction between phytase and available phosphorus on tibia phosphorus was significant (P<0.05). Overall, they concluded that in low phosphorus diet which food consumption is low, phytase would increase food consumption as well as retention of phosphorus in bones. Also, the lower excreta of phosphorus by using phytase could decrease pollution.

Musapuor *et al.* (2005) conducted to study the effects of different levels of phytase, calcium and available phosphorus on phytate phosphorus utilization in laying hens. One hundred ninety two 30-week ages White Leghorn (Hy-line W-36) laying hens were randomly allocated in cages for 12 dietary treatments with arranged of 3*2*2 factorial experiment with four replicates and four hens per replicate. The experimental period lasted 90 days, when the age of hen was 42 weeks. Dietary phytase caused a significant (P<0.05) increase in feed consumption, feed conversion ratio, tibia ash weight, tibia ash percentage, tibia phosphorus, plasma phosphorus and phosphorus digestibility. However, dietary phytase caused a significantly (P<0.05) decrease in plasma alkaline phosphatase activity and excreta phosphorus percentage. Also phytase had no beneficial effect on egg shell quality traits. Available phosphorus levels had significant effect (P<0.05) on tibia ash weight and tibia ash percentage. Reduction dietary available phosphorus caused a significant (P<0.05) decrease in feed consumption. Effect of dietary calcium were significant (P<0.05) on tibia ash weight, feed consumption and plasma phosphorus. Interaction between phytase and calcium on tibia phosphorus, plasma calcium and excreta phosphorus were significant (P<0.05). Interaction between phytase and available phosphorus on tibia phosphorus were significant (P<0.05). Overall, it could be concluded that in low phosphorus diet which food consumption is low, phytase would increase food consumption as well as retention of phosphorus in bones. Also, the lower excreta of phosphorus by using phytase could decrease pollution.

Casartelli *et al.* (2004) conducted an experiment to evaluate the effects of the enzyme phytase in diets formulated with different phosphorus sources on performance, eggshell quality and excretion of commercial laying hens. Two hundred and eighty-eight commercial Hysex brown laying hens were evaluated during two production phases, which included eight twenty-eight-day cycles, using a completely randomized design in a 3 × 2 factorial with six replicates of eight birds per treatment. Three phosphorus sources (calcium and sodium phosphate, micro-granulated dicalcium phosphate and triple super phosphate) and two phytase levels (0 or 1000 FTU/kg diet) were tested in the composition of the diets. After the post-peak period, triple super phosphate decreased bird performance and eggshell quality. It was possible to reduce the levels of phosphorus supplementation when phytase was added to the diet. Besides, phytase supplementation reduced phosphorus, calcium and nitrogen excretions but affected mean egg weight at production peak.

Berry *et al.* (1980) forty eight laying hens were used in a 6 x 8 randomized block design to compare the efficiency of Leucaena leaf meal and grass meal as sources of yolk pigments. Leaf meals were added to a low pigment diet (LP) to supply 10 or 20 mg dihydroxyxanthophyll (DHX) / kg. Leucaena from Malawi was added to supply both 10mg (L10) and 20mg DHX/kg (L20) that from Bangkok was added to supply 10mg DHX/kg (B10) and the grass to supply 20mg DHX/kg. A fifth diet (L20c) was the same as the L20 diet except that coconut oil replaced the groundnut oil in the basal diet. There were no significant differences between treatments with respect to egg production, egg yield, mean daily food intake and live weight change during the 28-day experimental period. After 7 days, significant differences ($P<0.001$) in Roche fan score (RFS) and Beta- carotene equivalent (BCE) values were evident between the 3 DHX levels (0, 10 and 20 DHX/kg). Further changes during the second week were followed by stabilization in yolk colour during the last two weeks. No differences were detected within DHX level for visual yolk colour measurement (RFS) except that on day 28 the RFS for the L20c diet was detected as significantly greater ($P < 0.05$) than the other two 20mg DHX treatments (L20 and G20). BCE measurements gave significant differences ($P < 0.001$) within the 20mg DHX treatments (L20 vs. L20c +G20) from day 7 onwards. In addition on day 28 the BCE values for diets L20c and G20 were detected as significantly different ($P < 0.001$). The absence of any deleterious effects on egg production in this short term study suggests that at inclusion rates of 10-25 g/kg leucaena is an effective yolk pigmenter.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted in the poultry research unit under the Department of Physiology & Pharmacology at Hajee Mohammad Danesh Science and Technology University, Dinajpur. The duration of experiment was about 28 days.

The total number of 40 female quails were randomly selected and divided into 4 groups (T_0, T_1, T_2, T_3) at completely randomized design for assessing the effect of Ipil ipil and Bean leaves supplementation on egg production, egg quality and growth performance of quail.

Group T_0 were kept in control. Group T_1, T_2 and T_3 were fed 2g ipil ipil, 2g bean leaves and 2g ipil ipil plus 2g bean leaves supplementation, respectively.

LAYOUT OF THE EXPERIMENT

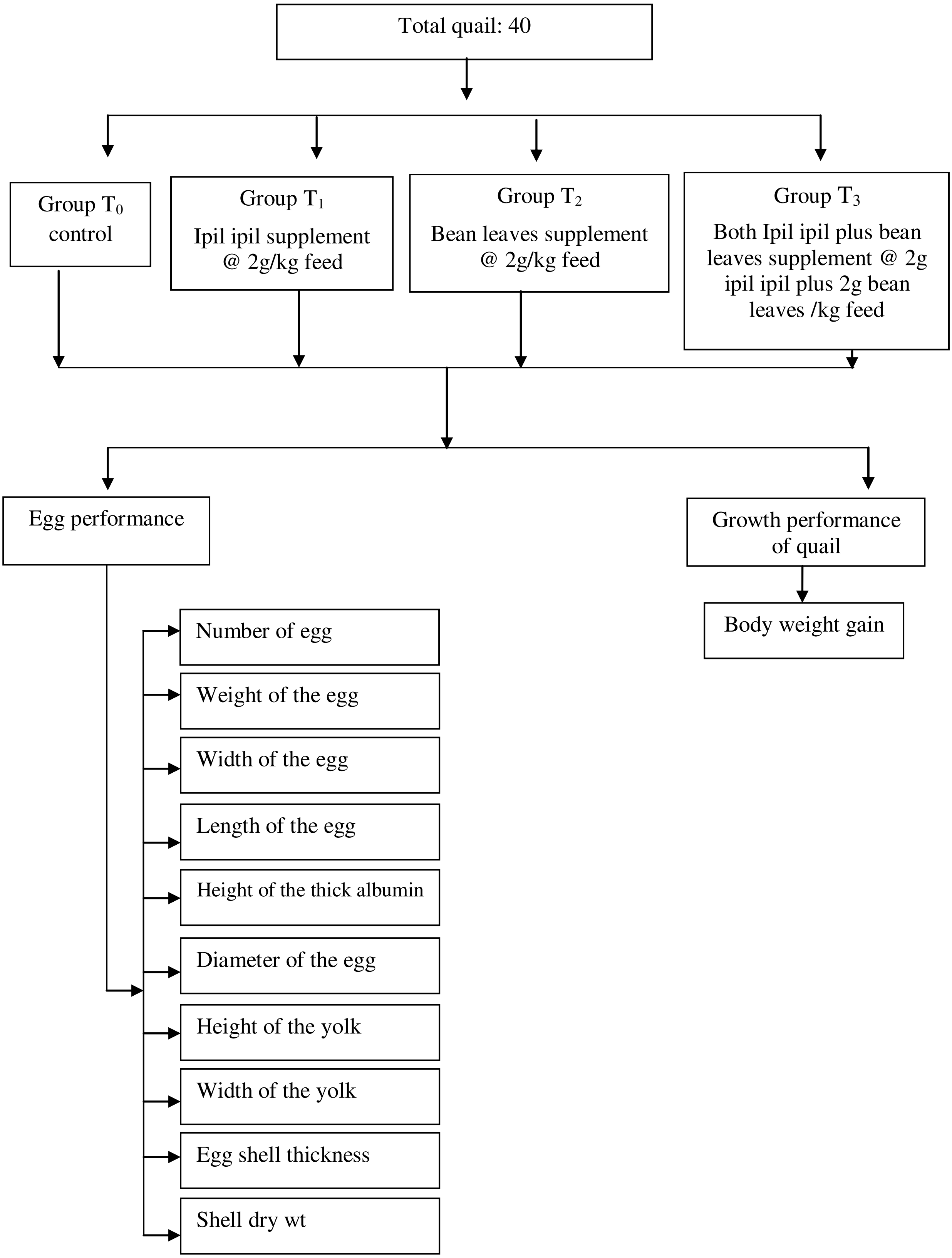


Fig 1: Layout of the experimental design (each group consisting of ten quails)

3.1 Collection and management of quails

At 42 days of age, Japanese layer quails were collected from A. R. Enterprise (quail hatchery and farm), Bogra. The body weights of assigned quails were taken with digital weight balance and the data were recorded. The finally selected 40 quails were housed under normal husbandry condition and reared quail in quail cage. All of them were fed with commercial crumbled plus mesh feed at the rate of 30 g per bird per day and fresh water *ad-libitum.*

Plate 1: Japanese quail in experimental shed

3.2 Measurement of Body weight

The body weight of each quail measured with the help of digital balance.

Plate 2: Measurement of Body weight

3.3 Collection of Ipil Ipil and bean leaves

Ipil Ipil and bean leaves were collected from the BRAC nursery, Dinajpur.

Plate 3. Ipil Ipil and Bean leaves

Plate 4. Grinded Ipil ipil

Plate 5. Grinded Bean leaves

Plate 6: Feed supply to the Japanese Quail

3.4 Experimental diets

The Ipil Ipil and bean leaves were dried and grinded. The grinded leaves were added with commercial quail ration and served to different groups.

3.5 Measurement of egg production and body weight gain

Egg production, body weight and egg quality were recorded.

3.6 No. of egg record

Egg production was recorded for each quail at the same time each day during laying period. The incidence of broken eggs and soft-shelled eggs were identified and recorded. The number of eggs laid on successive days by a particular quail determined the length of each sequence and the number of pauses in each quail's oviposition determined the number of sequences. For each quail, the length of laying sequence was determined on the day the last egg of the current clutch was laid.

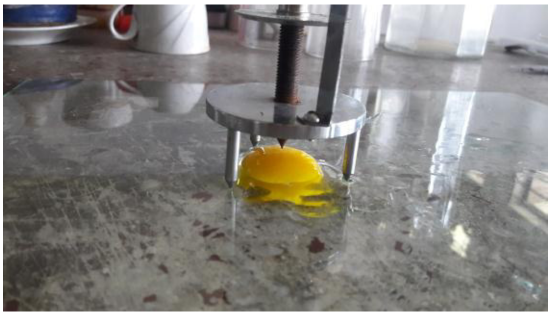
3.7 Observation of internal and external egg quality

Egg qualities were measured from those eggs laid by quails of different treatment group. Measured egg qualities were egg weight, shell dry weight, fresh albumin weight, fresh yolk weight, egg shell thickness, height of the thick albumin, height of the yolk, width of the yolk, width of the egg and diameter of the egg albumin. For quality determination egg weight was recorded by an electric weighing balance. The length of egg was measured by a slide calipers. The width was also estimated by slide calipers. The eggs were then carefully broken down on a glass plate (40 x 20cm) to determine the internal egg qualities.

Eggs of quail

Weighing of egg

Egg width determination



Diameter of egg albumin determination

Egg yolk width determination

Egg yolks height determination

Plate 7: Observation of internal and external egg quality of quail

3.8 Weight of different egg component

The method outlined by Chowdhuri (1988) was followed for partition in different egg components. At first, egg was broken on glass plate. Then the yolk was separated carefully from albumin with the help of a spatula and transferred to a previously weighed petridish by a spatula and weighed. Precautions were taken at all stages to avoid rupture of yolk.

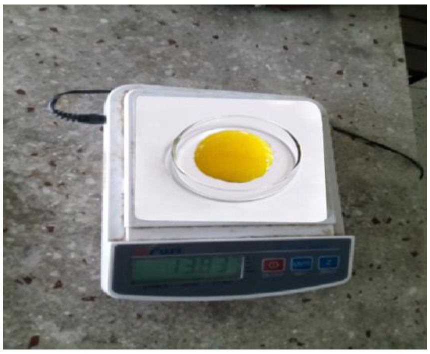


Plate 8: Measurement of fresh yolk weight

Plate 9: Measurement of egg shell weight

The shell of the broken eggs were rinsed and washed thoroughly in tap water by keeping membranes in intake. Then shell membrane were immersed in a beaker of water for removal of the shell membranes. The shell and shell membranes were oven dried separately at 105 cover night keeping them in a glass petridish. On the following day, oven dried shell and shell membranes were taken. Finally the following calculations were made for different components suggested by Chowdhuri (1988).

1. Fresh yolk weight:

(Weight of yolk + weight of petridish)-weight of petridish.

2. Fresh albumin weight:

(Weight of wet albumin + weight of petridish)-weight of petridish.

3.9 Shell thickness

After removing of shell membrane, shell thickness (mm) was measured by screw gauge.

Plate 10: Measurement of shell thickness

3.10 Statistical analyses

Data were analyzed by analysis of variance using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986). All analyses were performed by SPSS program version 22.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSSION

This study investigated the effect of Ipil ipil and Bean leaves supplementation on growth performance, egg production and egg quality of quail. This experiment was conducted in the Department of Physiology and Pharmacology, Faculty of Veterinary and Animal Science. The results of these study are discussed under following headings.

4.1 Egg Production

Egg production of different groups of quail were recorded from 6 weeks to 10 weeks quail treated with ipil ipil, bean leaves and combined ipil ipil plus bean leaves. The average egg production of different groups of quail were recorded. Quails treated with ipil ipil leaves showed average egg production 21.8± 0.32 within 06-10 weeks, bean leaves treated groups showed average egg production 22.5 ± 0.50 and combined treatment supplementation showed average egg production 23.3 ±.47 within 6 weeks to 10 weeks. (Table 1). Control group showed average egg production 17.5 ± 0.34. Result showed highest egg production in combined treatment group (23.3 ± 0.47) and lowest in control group (17.5 ± 0.34). Moreover, ipil ipil and bean leaves supplementation increase egg production (Kurtoglu *et al.,* 2004 and Panda *et al*., 2008). Our study express the same results.

Table 1: Effect of ipil ipil, bean leaves and combined treatment on egg production parameters of quail

	Group			
	T0	T1	T2	T3
No. of Egg production	$17.5^{c} \pm 0.34$ (45.7%)	$21.8^{b} \pm 0.33$ (55.4%)	$22.5^{ab} \pm 0.5$ (57.3%)	$23.3^{a} \pm 0.47$ (59.2%)
Weight of the egg (g)	$09.7^{b} \pm 0.6$	$10.2^{b} \pm 0.51$	$09.8^{b} \pm 0.66$	$10.5^{b} \pm 0.34$
Width of the egg (mm)	$25.67^{a} \pm 0.06$	$25.54^{a} \pm 0.06$	$25.14^{a} \pm 0.06$	$24.74^{a} \pm 0.18$
Length of the egg (mm)	$32.7^{a} \pm 0.14$	$31.21^{b} \pm 0.30$	$30.98^{b} \pm 0.23$	$32.06^{a} \pm 0.24$
Height of the thick albumin (mm)	$3.14^{b} \pm 0.07$	$3.39^{a} \pm 0.04$	$3.60^{a} \pm 0.09$	$3.54^{a} \pm 0.06$
Diameter of the egg (mm)	$45.59^{a} \pm 0.11$	$42.79^{c} \pm 0.25$	$43.76^{b} \pm 0.29$	$41.34^{d} \pm 0.24$
Height of the yolk (mm)	$6.61^{b} \pm 0.11$	$6.84^{b} \pm 0.10$	$6.12^{b} \pm 0.08$	$6.92^{b} \pm 0.10$
Width of the yolk (mm)	$29.52^{b} \pm 0.12$	$29.63^{b} \pm 0.10$	$28.15^{b} \pm 0.38$	$30.60^{b} \pm 0.28$
Egg shell thickness (mm)	$0.17^{b} \pm 0.01$	$0.16^{c} \pm 0.01$	$0.12^{d} \pm 0.04$	$0.19^{a} \pm 0.01$
Shell dry wt (g)	$3.19^{b} \pm 0.06$	$3.05^{b} \pm 0.05$	$3.13^{b} \pm 0.11$	$3.38^{b} \pm 0.09$

Note: Values followed by same superscripts in the same column are not statistically significant ($p>0.05$), different superscripts indicate that difference is significant ($P<0.05$).

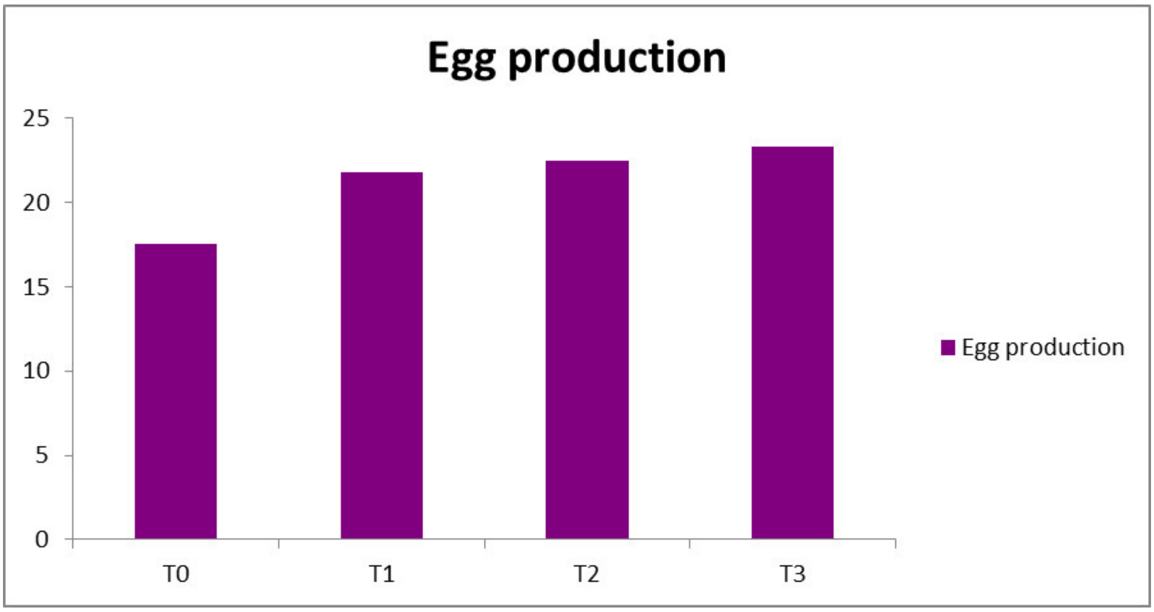


Fig. 2: Effect of ipil ipil and bean leaves supplementation on number of egg

4.2 Body weight

Body weight of different groups of quails were recorded from 06 to 10 weeks quail treated with ipil ipil leaves, bean leaves and combined ipil ipil plus bean leaves. The average body weight of different group of quails were recorded. Quails treated with ipil ipil leaves showed average Body weight gain 123.5 $^{a}\pm$ 3.30 to 149.2 $^{a}\pm$ 5.14 g within 06-10 weeks, bean leaves treated groups showed body weight gain 123.8 $^{a}\pm$ 3.42 to 149.0 $^{a}\pm$ 6.49 g and combined treatment supplementation showed body weight 126.8 $^{a}\pm$ 2.78 to 155.1 $^{a}\pm$ 7.15 g within 06-10 weeks (Table 2). Control group showed average body weight gain 128.5 $^{a}\pm$ 4.00 to 151.9 $^{a}\pm$ 5.48 g. Result showed that the body weight of different groups more or less same with the control group. Results are contrary to that observed by Saima *et al.* (2014) A 28 days' trial was conducted to evaluate efficacy of microbial phytase in diets for Japanese quails. For this purpose, 900 experimental birds were divided into six groups, each group containing 150 chicks and further sub-divided into10 replicates. Diet A (positive control) was formulated according to NRC (1994) requirements set for the Japanese quail (CP 24% and ME 2900 Kcal/Kg). Diet B differed from diet A in Ca (Calcium) and P (Phosphorus) i.e. 0.15% Ca and 0.20% P less to Diet A, respectively. Four different levels of phytase enzyme (250, 500, 750, 1000 FTU/kg of feed) were added to diet B to formulate diets C, D, E and F treatments respectively. Results revealed that body weight gain, feed consumption, FCR, keel /shank length, dressing percentage of birds in groups consuming 750 and 1000 FTU/kg phytase were significantly higher ($P<0.05$) than those of B, C and D. The growth performance of group E and F was comparable with those of group A (+ve control). Maximum leg weakness, swollen joints and crippled legs were observed in group B (39.30%) followed by C, D (21.33% and 16.0%).

Table 2: Body weight of quail at the age of 70 days

Group	Body weight (g) at the age of 70 days (end of the experiment)
T_0	$151.9^a \pm 5.48$
T_1	$149.2^a \pm 5.14$
T_2	$149.0^a \pm 6.49$
T_3	$155.1^a \pm 7.15$

N. B. Values followed by same superscripts in the same column are not statistically significant ($p>0.05$).

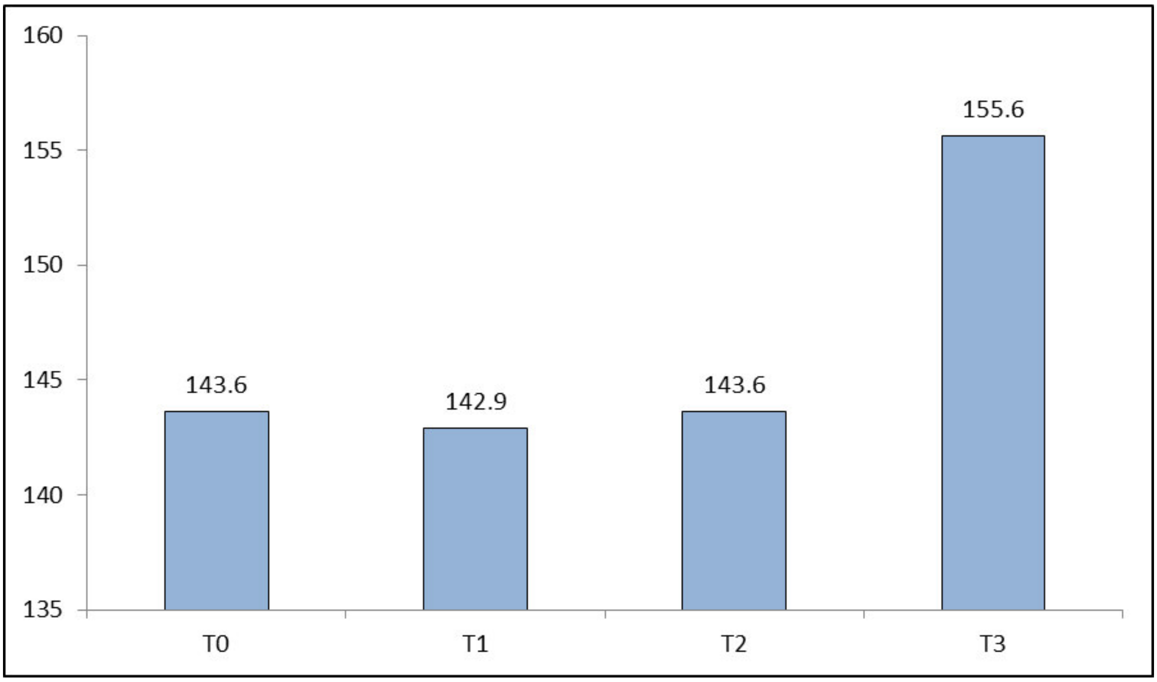


Fig. 3: Effect of ipil ipil and bean leaves supplementation on growth performance

4.3 External and internal egg quality

Table 1 demonstrate that there exist a significant ($P<0.05$) difference among the mean values like Length of the egg (mm), height of the thick albumin (mm), diameter of the albumin (mm), shell thickness (mm) corresponding to the different level of ipil ipil and bean leaves treatment. But no significant ($P>0.05$) difference among the mean values like weight of the egg (g), width of the egg (mm), height of the yolk (mm), width of the yolk (mm), shell dry wt (g) corresponding to the different level of ipil ipil and bean leaves treatment. These results indicate that treated with ipil ipil and bean leaves extract had no adverse effect on external and internal qualities of eggs.

CHAPTER V

CONCLUSION AND RECOMMENDATION

CHAPTER V

CONCLUSION AND RECOMMENDATION

This research work was conducted to study the effect of Ipil ipil and bean leaves supplementation on egg production, growth performance and egg quality of quail. The treatment group T_1, T_2, T_3 recorded statistically significant ($P<0.01$) increase for egg production than that of control group T_0. Net egg production was increased in Ipil Ipil and bean leaves supplemented group than control group T_0. Body weights were increased significantly ($P<0.05$) in all treated groups in respect to the control group and highest was recorded in combined Ipil Ipil and bean leaves supplemented groups (Group T_3). It is concluded that supplementation of 2 g grinded Ipil Ipil leaves plus 2 g grinded Bean leaves per kg feed (T_3) of treatment groups caused significant increase in egg production ($P<0.01$) and as compared to that of other groups of quails. From this experiment we found that, between the control group and the treatment group of birds, the combined group is more suitable than any other groups. That there exist a significant ($P<0.05$) difference among the mean values like Length of the egg (mm), Height of the thick albumin (mm), Diameter of the albumin (mm), Shell thickness (mm) corresponding to the different treatment. But no significant ($P>0.05$) difference among the mean values like Weight of the egg (g), Width of the egg (mm), Height of the yolk (mm), Width of the yolk (mm), Shell dry wt (g) corresponding to the different treatment. From the present field and laboratory trial, it can be concluded that combined supplementation of 2 g grinded Ipil Ipil leaves plus 2 g grinded Bean leaves per kg feed is highly beneficial for enhancing egg production and our formulations could be used as an egg enhancer and growth promoter for quail.

In fact, only few trials had been performed to evaluate the effects of Ipil Ipil and Bean leaves supplementation on growth performance, egg production and egg quality of quail. I did the work in short term basis (only 4 weeks) and modern equipments were not available. Before field application as an egg enhancer of quail, further trial on a large scale basis is needed and also to make the findings more accurate and effective. Further study is essential to see any adverse effect in relation to histopathology before making a definite conclusion.

This was a preliminary work and the technology was very simple. Farmers could adopt that technology without any specialized technical knowledge and medicinal ingredients. As a result by using grinded Ipil ipil and Bean leaves with normal commercial ration, small scale quail farmers would be able to sustain in their farming business and fulfill our protein demand. As well as a positive contribution in our national GDP of Bangladesh, these can helps in alleviating poverty through creating employment opportunity especially for rural population.

Further study can be concluded Ipil ipil and Bean leave may be used as a feed supplement to increase the number of eggs of quail which may encourage the quail farmers to rear quail commercially and earn more profit as well as can contribute to meet the national protein demand.

Further study can be recommended to see bio digestibility of nutrients, blood characteristics and biochemical constituents of egg.

REFERENCES

REFERENCES

Abeyrathna, H.M.W.N., Atapattu, N.S.B.M. and Gunawardane, W.W.D.A. (2014). Effects of the Level of Dietary Rice Bran with or without Phytase, on Performance and Egg Parameters of Laying Japanese quail. Tropical Agricultural Research, 26(1): 39–47.

Abou-Elezz, F.M.K., Sarmiento-Franco, L., Santos-Ricalde, R. and Solorio-Sanchez, F. (2011). Nutritional effects of dietary inclusion of Leucaena leucocephala and Moringa oleifera leaf meal on Rhode Island Red hens' performance. Cuban Journal of Agricultural Science, Vol. 45.

Adelson, P. A., Claude P. and Jean J. D. (2008). Phosphatase and phytase activities in nodules of common bean genotypes at different levels of phosphorus supply. Plant Soil, 312: 129–138.

Ademola, S.G., Lawal, T.E., Odu, O., Shittu, M.D., Oladiran, H.O. and Adeshina, M.T. (2013). Effects of native and microbial phytase on laying performance, shell ash and phosphorus content of hens fed mash and pelleted diets. Online Journal of Animal and Feed Research, 3(6): 230-234.

Ahmad, M., Mehran, T. and Hossein A. G. (2015). Effect of phytase supplementation of diets with different levels of rice bran and non-phytate phosphorus on productive performance, egg quality traits, leukocytes profile and serum lipids of laying hens reared indoor under high environmental temperatures. AFST, pp. 222–233.

Ahmadi, H. and Rodehutscord, M. (2012). A meta analysis of responses to dietary nonphytate phosphorus and phytase in laying hens. Poultry Science, 91: 2072–2078.

Ahmadi, H. and Rodehutscord, M. (2012). Ameta-analysis of responses to dietary nonphytate phosphorus and phytase in laying hens.

Ahmed, E.S., Nasser E.K., Kamelia M.Z., Tahia E.A. and Fathy A.E. (2012). Effect of Dietary Enzyme supplementation on Egg Laying Performance and Nutrient Digestibility of Japanese Quails. IJAVMS, 6(5): 377-384.

Anna, M., Martina L. and Alena P. (2014). Effect of exogenous phytase on egg quality in laying hens. Acta fytotechn. zootechn. 17(3): 79–83.

Attia, Y.A., El-Tahawy, W.S., Abd El-Hamid E., Abd El-Hamid, S.S., Hassan, A.N., and El-Kelaway M.I. (2012). Effect of phytase with or without multienzyme supplementation on performance and nutrient digestibility of young broiler chicks fed mash or crumble diets. Italian Journal of Animal Science volume 11:e56.

Barilani, M; Deregnaucourt S; Gellego S; Galli L; Mucci N; Piomobo R; Puigcerver M; Rimondi S; Rodriguez-Teijeiro JD; Spano S; Randi E (2005). "Detecting hybridization in wild (Coturnix c. coturnix) and domesticated (Coturnix c. japanica) quail populations". Biological Conservation 126: 445–455.

Berry, S. and D'Mello, J.P.F. (1980). A of leucaena leucocephala and grass meals as sources of yolk pigments in diets for laying hens.

Birdlife International. Retrieved 20 October 2013.

Buchwalder, T; Wechsler B (1997). "The effect of cover on the behavior of Japanese quail (Coturnix japanica)".Applied Animal Behaviour Science 54: 335–343.

Casartelli, E.M., Junqueira, O.M., Laurentiz A.C., Filardi R.S., Lucas Júnior J. and Araujo L.F. (2004). Effect of phytase in laying hen diets with different phosphorus sources. Rev. Bras. Cienc. Avic. 7(2).

Etches, Robert J. (1996). Reproduction in poultry. Wallingford : CAB international, X, 318 p. ill.

Faria, J.C.; Bezerra, I.C.; Zerbini, F.M.; Ribeiro, S.G.; Lima, M.F. (2000). SituaÁ„oatual das geminiviroses no Brasil. Fitopatologia Brasileira, BrasÌlia, 25: 125-137.

Francesch, M., Broz, J. and Brufau, J. (2005). Effects of an experimental phytase on performance, egg quality, tibia ash content and phosphorus bioavailability in laying hens fed on maize- or barley-based diets. Br. Poult. Sci. 46(3): 340-8.

Gao, C.Q., Ji, C., Zhang, J.Y., Zhao, L.H. and Ma, Q.G. (2013). Effect of a novel plant phytase on performance, egg quality, apparent ileal nutrient digestibility and bone mineralization of laying hens fed corn–soybean diets. Animal feed Science & Technology, 186(1-2): 101–105.

George, M., Lolas and Pericles M. (2006). The phytase of navy beans (*phaseolus vulgaris*) Journal of Food Science, 42(4): 1094–1097.

Hoffmann, E. (1988). Coturnix Quail. Taipei: Yi Hisien Publishing Co.

Hubrecht, R. and Kirkwood, J. (2010). The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals.John Wiley & Sons. pp. 655–674.

Hughes, A.L., Dahiya, J.P., Wyatt, C.L. and Classen, H. L. (2009). Effect of Quantum phytase on nutrient digestibility and bone ash in White Leghorn laying hens fed corn-soybean meal-based diets. Poultry Science, 88: 1191–1198.

Jalal, M. A. and S. E. Scheideler (2000). Effect of Supplementation of Two Different Sources of Phytase on Egg Production Parameters in Laying Hens and Nutrient Digestibility.

Johnsgard, P. (1988). The Quails, Partridges, and Francolins of the World. Oxford: Oxford University Press.

Joly, P., Cadi, A., Delmas, V., Prévot-Julliard, A.-C., Pieau, C., and Girondot, M. (2003). Successful reproduction of the introduced slider turtle (Trachemys scripta elegans) in the south of France. Aquat. Conserv. In press.

Jubarah, S. K., R. H. Davis and P. F. Dodds (2010). Effect of phytate on laying hen performance, carbonic anhydrase activity and total cholesterol level. Department of Animal Science, CNRES, University of Juba.

Kh, A., Mohammed, M. A., Toson, H.H.M., Hassanien, M.A.H., Soliman, El-Nagar, S.H.M. (2010). Effects of phytase supplementation on performance and egg quality of laying hens fed diets containing rice bran. Egypt. Poultry Science, 30(3): 649-659.

Lambert, R. (1970). Notes on the breeding and behaviour of Japanese Quails.Avicultural Magazine, 76(5): 177-179.

Lazali, M., Louadj, L., Ounane, G., Abadie, J., Amenc, L., Bargaz, A., Valérie L.P. and Jean J.D. (2014). Localization of phytase transcripts in germinating seeds of the common bean (*Phaseolus vulgaris* L.) Planta. 240: 471–47.

Leeson, S., Summers J.D. and L.J. Caston, (1991). Diet dilution and compensatory growth in broilers. Poult. Sci., 70: 867-873.

Lim, H.S., Namkung, H. and Paik, I.K. (2003). Effects of Phytase Supplementation on the Performance, Egg Quality, and Phosphorous Excretion of Laying Hens Fed Different Levels of Dietary Calcium and Nonphytate Phosphorous. Poultry Science, 82: 92–99.

Liu, N., Liu, G.H., Li, F.D., Sands, J. S., Zhang, S., Zheng A.J. and Ru, Y.J. (2007). Efficacy of Phytases on Egg Production and Nutrient Digestibility in Layers Fed Reduced Phosphorus Diets. Poultry Science, 86: 2337–2342.

Lucky, NJ., Howlider, M.A.R., Alam, M.A. and Ahmed, M.F. (2014). Effect of dietary exogenous phytase on laying performance of chicken at older ages. Bangladesh Journal of Animal Science. 43(1): 52-55.

Mansoori, B. and Modirsanei, M. (2015). Effect of dietary available phosphorus and phytase on production performance of old laying hens and tibia bone quality. IJVM, 9(2): 125-134.

Mills, AD; Crawford LL; Domjan M and Faure JM. (1997). "The Behavior of the Japanese or Domestic Quail Coturnix japanica". Neuroscience and Biobehavioral Reviews. 21(3): 261–281.

Mohebbifar, A. and Torki, M. (2010). Phytase supplementation of low phosphorous diets included graded levels of rice bran on productive performance of laying hens. International Conference on Biology, Environment and Chemistry vol. 1.

Musapuor, A., Pourreza, J., Samie, A. and Moradi S.H. (2005). The Effects of Phytase and Different Level of Dietary Calcium and Phosphorous on Phytate Phosphorus Utilization in Laying Hens. International Journal of Poultry Science, 4(8): 560-562.

Narasimha, J., Nagalakshmi, D., Viroji Rao S.T. and Y. Ramana R. (2013). Additive Effect of NSP Enzymes and Phytase on Performance, Egg Quality, Nutrient Retention and Gut Health of Laying Hens Fed Corn-Soybean Meal Based Low Energy Diets. IOSR Journal of Agriculture and Veterinary Science, 6(5): 22-28.

Pace, S.C. (2014). The Effect of High Phytase Inclusion in Laying Hen Diets. PhD thesis, University of Leeds.

Pappas, J. (2013). "Coturnix japanica". Animal Diversity Web. Retrieved 20 October 2013.

Puigcerver, Manel; Vinyoles, Dolors; Rodríguez-Teijeiro, José Domingo (2007). "Does restocking with Japanese quail or hybrids affect native populations of common quail Coturnixcoturnix?". Biological Conservation 136(4): 628–635.

Ravindran, V., Ravindran, G. and Sivalogan, S. (1994). Total and phytate phosphorus contents of various foods and feedstuffs of plant origin.

Roland, D.A. (1988). Research note: egg shell problems estimates of incidence and economic impact. Poult. Sci. 67: 801-1803.

Roland, P.E., Zilles, K., (1989). Functions and structures of the motor cortices in humans. Curr. Opin. Neurobiol. 6: 773–781.

Saber, K., Louche, J., Debez, A., Plassard, C., Drevon, J.J. and Abdelly, C. (2009). Effect of phosphorus deficiency on acid phosphatase and phytase activities in common bean (*Phaseolus vulgaris* L.) under symbiotic nitrogen fixation. Symbiosis, 47: 141-149.

Saima, M.A. Shad, Pasha, T. N., Akram, M., Ditta, Y. A. and Khan, M.Z.U. (2014). Effect of microbial phytase supplementation on growth performance of Japanese quails. The Journal of Animal & Plant Sciences, 24(1): 19-23.

Sharifi, M.R., Shams, M., Shargh, S., Senobar H. and Jenabi, S. (2011). The effects of dietary nonphytate phosphorus levels and phytase on laying performance and egg quality parameters of Japanese quails (*Coturnix japonica*). Arch. Geflügelk, 76(1): 13-19.

Tang, H.O., Gao, X.H., Ji, F., Tong S. and Li, X.J. (2012). Effects of a thermostable phytase on the growth performance and bone mineralization of broilers. Journal of Applied Poultry Research, 21: 476–483.

Tischler, A., Halas, V. and Tossenberger, J. (2015). Effect of dietary NPP level and phytase supplementation on the laying performance over one year period. Poljo Privreda, 21: (1): 68-72.

Yan, L., Zhou, T.X., Jang, H. D., Hyun1, Y., Kim, H.S. and Kim, I.H. (2009). Comparative Effects of Phytase Derived from Escherichia coli and Aspergillus niger in Sixty Eight-week-old . Asian-Australian Journal of Animal Science, 22(10): 1391–1399.

Ziaei, N., Shivazad, M., Mirhadi, S.A. and Gerami, A. (2009). Effects of Reduced Calcium and Phosphorous Diets Supplemented with Phytase on Laying Performance of Hens. Pakistan Journal of Biological Sciences, 12: 792-797.

Żyła, K., Mika, M. Świątkiewicz, S., Koreleski, J., Piironen, J. (2011). Effects of phytase B on laying performance, eggshell quality and on phosphorus and calcium balance in laying hens fed phosphorus-deficient maize-soybean meal. Czech Journal of Animal Science, 56, 2011(9): 406–413.