DIETARY EFFECTS OF GARLIC POWDER ON PRODUCTION PERFORMANCE, EGG YOLK CHOLESTEROL CONCENTRATION AND MICROBIAL LOAD IN CLOACA OF LAYING QUAILS

A Thesis

By

MITU BANU
Semester: January -June, 2017
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MASTER OF SCIENCE (M.S.)
IN
POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

NOVEMBER, 2017

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Submitted to the
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Hajee Mohammad Danesh Science and Technology University
Dinajpur-5200

NOVEMBER, 2017

DEDICATED
TO MY
BELOVED PARENTS
AND
FAMILY

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The Author

ABSTRACT

The experiment was conducted from January-April 2017 at the poultry farm under the Dairy and Poultry Science Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur to investigate the effects of dietary garlic powder on production performance, egg yolk cholesterol and microbial load in cloaca of laying quails. In this study, ninety-six 70-days old laying quail were allocated into four dietary treatments with three replication of eight (8) birds in each. Diets were supplied with T_0 (control), T_1 (1.5% garlic powder), T_2 (3% garlic powder) and T_3 (4.5% garlic powder) garlic powder mixed meal for 12 weeks. Eggs were collected and weighted daily. Laying performance, egg quality and feed conversion ratio were evaluated. Results showed that the feed intake, egg mass, feed conversion ratio, body weight and egg qualities were insignificant among treatment groups. The egg production and egg weight were significant among the treatment group. However, the egg yolk cholesterol was significantly decreased with higher level of garlic powder supplementary diet. Egg yolk cholesterol was decreased at 12.20, 12.26 and 11.36% with 1.5, 3 and 4.5% level of garlic powder supplementation, respectively. Supplementation of garlic powder mix in the diet of laying quails significantly ($P < 0.05$) decreased *E. coli* and *Salmonella spp.* at 128.33×10^6, 118.33×10^6, 68×10^6 and 134.66×10^6, 94.66×10^6, 75.33×10^6, with inclusion of 1.5, 3 and 4.5% levels of garlic powder in the diet .

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ABBREVIATIONS AND SYMBOLS

Abbreviations	Full meanings
%	Percentage
° C	Degree celcius
/	Per
:	Ratio
@	At the rate of
<	Less than
>	Greater than
±	Plus minus
ANOVA	Analysis of Variance
AP	Available phosphorus
Av.	Average
Ca	Calcium
CF	Crude Fibre
Cm	Centimeter
Cm^2	Square centimeter
Contd.	Continued
CP	Crude Protein
CRD	Completely Randomized Design
Cu	Copper
DCP	Di Calcium Phosphate
DM	Dry matter
e.g.	For example
et al.	And others
FCR	Feed Conversion Ratio
Fig.	Figure
G	Gram
GP	Garlic powder
HSTU	Hajee Mohammad Danesh Science and Technology University
HU	Haugh Unit
i.e.	That is

K	Potassium
Kcal	Kilo-calorie
LSD	Least Significant Difference
Max	Maximum
ME	Metabolizable Energy
Mg	Milligram
Mg	Magnesium
MJ	Mega Joule
Mm	Millimeter
Mn	Manganese
Na	Sodium
No.	Number
NPN	Non Phytate Phosphorus
NRC	National Research Institute
NS	Non-significant
P	Phosphorus
PBS	Phosphate Buffer Solution
Ppm	Parts per million
R	Replication
RH	Relative Humidity
SED	Standard Error Difference
Sq.	Square
SS	Salmonella Shigella
TP	Total Phosphorus
Zn	Zinc

CHAPTER I

INTRODUCTION

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INTRODUCTION

The Japanese quail, *Coturnix japonica*, is a species of Old World quail found in East Asia. First considered a subspecies of the common quail, it was distinguished as its own species in 1983 (Hubrecht and Kirkwood, 2010). The Japanese quail has played an active role in the lives of humanity since the 12th century, and continues to play major roles in industry and scientific research. In our country, commercial farming of these birds is increasing day by day as the investment and maintenance is very low when compared to other birds. Quail farming is very profitable like other farming ventures, such as chicken, turkey or duck farming business. Meat and eggs of quail are very tasty and nutritious. Quail eggs are very nutritious than other poultry eggs. Because quail eggs contain comparatively more protein, phosphorus, iron, vitamin A, B_1 and B_2. Quail eggs had significantly higher concentration of cholesterol per gram of yolk than chicken and duck egg. Jalaludeen *et al.* (2006) also reported that the eggs of chicken, duck and quail contain 423, 884 and 844 mg of cholesterol per 100 g. The egg has more beneficial effect. It cures cancer, high blood pressure, HIV AIDS, Ageing, allergy, bronchitis, diabetes, digestive disorder, gallstone etc.

Garlic (*Allium sativum*) and garlic supplements are consumed in many cultures for their hypolipidemic, antiplatelet and procirculatory effects (Amagase *et al.,* 2001). In addition to these benefits, some garlic preparations have been reported to possess hepatoprotective, immune-enhancing, anticancer and chemopreventive activities. Some preparations appear to be antioxidative, whereas others may stimulate oxidation (Imai *et al.,* 1994). The cardiovascular-protective effects of garlic have also been evaluated extensively in recent years (Yeh and Liu, 2001). In animal experiments, garlic extracts have been shown to lower plasma lipid and cholesterol in rats (Chi, 1982; Mathew *et al.,* 1996), rabbits (Bordia and Verma, 1980) and chickens (Qureshi *et al.,* 1983a,b). Moreover, a number of intervention studies have similarly shown that garlic and garlic preparations significantly reduced plasma lipids, especially total cholesterol and low density lipoprotein (LDL) cholesterol in humans (Jain *et al.,* 1993; Steiner *et al.,* 1996). However, recent studies suggested that not all garlic preparations may be hypocholesterolaemic (Simon *et al.,* 1995; Isaacsohn *et al.,* 1998; McCrindle *et al.,*

1998). Although the reason for these inconsistencies is not readily apparent, it is worth noting that garlic contains a variety of organosulphur compounds. Some of the sulphur compounds such as allicin, ajoene, S-allylcysteine, diallyl disulphide, S-methylcysteine sulphoxide and S-allylcysteine sulphoxide may be responsible for the therapeutic properties of garlic (Chi *et al.,* 1982). Other contributing factors may include the subject recruitment, duration of experiment, dietary control, lifestyle and methods of lipid analyses (Warshafsky *et al.,* 1993; Silagy and Neil, 1994). Allicin has been proposed as the active compound produced by garlic responsible for health promotion and hypocholesterolaemic benefits (Lawson, 1998). It can reduce the levels of serum cholesterol, triglyceride and LDL (Adler and Holub, 1997). In terms of the mechanism of action, it reduces cholesterol synthesis, inhibits fatty acid synthesis and platelet aggregation and prevents thrombosis. Allicin has also been used for treating and preventing cardiovascular diseases (Tanamai *et al.,* 2004). Although allicin is often emphasised in dehydrated powder, many preparations contained no allicin, possibly reflecting its instability (Yan *et al.,* 1993). Qureshi *et al.* (1983b) reported that significant decreases in hepatic HMG-CoA reductase (79–83%), cholesterol 7α-hydroxylase (43–51%), fatty acid synthetase (17–29%) activities accompanied the feeding of the petroleum ether, methanol and water-soluble fractions of garlic. Garlic powder is thought to retain the same ingredients as raw garlic, however, the proportions and amounts of various constituents differ significantly *(*Iberl *et al.,* 1990). Reuter *et al.* (1996) reported garlic as a plant with antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and cardiovascular- protecting effects. Moreover, garlic is very rich in aromatic oils, which enhance digestion and positively influenced respiratory system being inhaled into air sacs and lungs of birds. Also it was found that garlic has strong antioxidative effects (Gardzielewska *et al.,* 2003).

Now a days, many people of the world are suffering from various heart disesases there is a high relationship between cholesterol and atherosclerosis. Plasma total cholesterol and low- density lipoprotein (LDL) are closely related to atherosclerosis and excessive concentration of these two materials may lead to coronary artery disease or death. Ordinary quail eggs provide protein, vitamins and lipid that contain high level of cholesterol. Thus, eggs are considered to be a high-cholesterol food. The American Heart Association recommended that cholesterol consumption for each person should be limited up to 300 mg per day and the whole egg yolk consumption should be limited to

three to four per week. In recent days, consumers pay more attention to health and are thus lowering their consumption of high-cholesterol food. But, the consumers have to intake eggs at regular interval which contain cholesterol that risk for health. Therefore, low- cholesterol eggs would not only be beneficial to public's health but also bear business advantage. Egg cholesterol is first biosynthesized in the liver of laying quail and secreted into the plasma in the form of very low density lipoprotein (VLDL) which transfer to the overy. Egg cholesterol has been shown to vary with species of bird, breed or strain as well as age of fowl. The mechanism by which garlic or garlic preparations reduce plasma lipids has not been fully investigated. Animal studies, however, have shown that garlic supplementation in the diet depressed the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase (Qureshi *et al.,* 1983a) and 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase (Qureshi *et al.,* 1983a,b; Youn *et al.,* 1996) . Garlic also has been shown to have strong antimicrobial action (Iwalokun *et al.,* 2004*;* Gbenga *et al.,* 2009). Allicin and its derivatives have been shown to be a larvicidal and bacteriostatic, active against both Gram positive or Gram negative organisms as well as fungi such as Candida albicans and viruses including influenza viruses (Chang and Cheong, 2008). Allium sativum taken at a low dose may have some therapeutics potentials against gastric ulcers associated with H. pylori infection (Adeniyi *et al.,* 2006). Thus the garlic powder could be supplemented in laying quail diet at different level to investigate the efficiency of this feed ingredient for the reduction of egg yolk cholesterol and cloacal microbial load. Therefore, present piece of research work was undertaken with the following objectives:

1. To observe the effect of garlic powder on egg yolk cholesterol and cloacal microbial load of laying quail.
2. To observe the dietary supplementation of garlic powder on production performance and egg quality characteristics of laying quail.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Garlic

Garlic (scientific name *Allium sativum*) is a species in the onion genus, Allium. Its close relatives include the onion, shallot, leek, chive, and Chinese onion. With a history of several thousand years of human consumption and use, garlic is native to Central Asia and northeastern Iran, and has long been a common seasoning worldwide. It was known to Ancient Egyptians, and has been used both as a food flavoring and as a traditional medicine. *Allium sativum* is a bulbous plant. *Allium sativum* grows in the wild in areas where it has become naturalized. Single clove garlic (also called pearl or solo garlic) originated in the Yunnan province of China. Garlic is easy to grow and can be grown year-round in mild climates. While sexual propagation of garlic is possible, nearly all of the garlic in cultivation is propagated asexually, by planting individual cloves in the ground. The garlic plant's bulb is the most commonly used part of the plant. With the exception of the single clove types, garlic bulbs are normally divided into numerous fleshy sections called cloves. Garlic cloves are used for consumption (raw or cooked) or for medicinal purposes. They have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking.

A great deal of low quality clinical research has been conducted to determine the effect of garlic on preventing cardiovascular diseases and on various biomarkers of cardiovascular health, but as of 2015, the results were contradictory and it was not known if there are any effects. A 2016 meta-analysis indicated there was no effect of garlic consumption on blood levels of lipoprotein (a), a marker of atherosclerosis. Because garlic might reduce platelet aggregation, people taking anticoagulant medication are cautioned about consuming garlic. A 2016 meta-analysis of case-control and cohort studies found a moderate inverse association between garlic intake and some cancers of the upper digestive tract. Another meta-analysis found decreased rates of gastric cancer associated with garlic intake, but cited confounding factors as limitations for interpreting these studies. Further meta-analyses found similar results on the incidence of gastric cancer by consuming allium vegetables including garlic. A 2014 meta-analysis of observational epidemiological studies found that garlic consumption was associated with

a lower risk of stomach cancer in Korean people. A 2016 meta-analysis found no effect of garlic on colorectal cancer. A 2014 meta-analysis found garlic supplements or allium vegetables to have no effect on colorectal cancers.

2.2 Nutritive value of garlic powder

Marina Sajid *et al.* (2014) showed that, garlic is commonly used as flavoring, culinary and herbal remedies. It is an essential vegetable throughout the world not only as a spice but also a traditional medicine. In the present research garlic as a raw material was explored to its chemical composition as well as its mineral analysis. The current study proved that it contains 64.58± 2.06% moisture, 7.87±0.32 protein, 0.52±0.01 ether extract, 2.3±0.08 fiber, 2.46±0.09 ash and 22.27±0.95% NFE, whilst, the mineral analysis suggested its composition as calcium, phosphorous, iron, sodium and magnesium as 54.65±1.74, 19.83±0.83, 9.54±0.34, 4.21±0.15, 4.1±0.18 and 3.97±0.13 mg/100 g however, zinc, manganese and copper were in traces.

Mariam and Devi (2016) Garlic is commonly used as flavoring, culinary and herbal remedies. The current study proved that it contains 3.91±0.03% moisture, 19.75±0.12 g protein, 0.49±0.02 g Fat, 1.73±0.01 g/100g crude fiber, 0.49±0.01 Volatile oil, 66.36±0.11 g of Carbohydrate, 348.85±2.11 K. Calais, 3.39±0.02 g total ash and 0.09±0.00 g acid insoluble ash, whilst, the antioxidant analysis revealed its high content of Vitamin C, 41.79±0.21 mg/100g, Selenium 12.1±0.02 mg/100g and zinc 0.9±0.0 mg/100g. However, Heavy metals like Lead, Mercury, Arsenic, Cadmium, Selenium, and Pesticide residue DDT were less than 0.1ppm, 7.23±0.01 mg/g of Alliin and 2.21±0.01 mg/g of γ-Glutamyl-(S)-allyl-L-cysteine estimated by HPCL method. Whereas Microbiological test showed, total bacterial count/g was 6900. Staphylococcus aureas, Total coliform count and total yeast and mold count/g were less than 10 CFU. *E. coli*, Salmonella and Shigella/g were absent. The current study showed that dry garlic powder contains good amount of vitamin C which is a powerful antioxidant, good amount of sulfur compound which has cholesterol lowering activities.

Table 2.1 Chemical composition of Garlic (Mariam and Devi 2016)

Sl. No.	Nutrient content	
1	Moisture, g/100g	3.91±0.03
2	Protein, g/100g	19.75±0.12
3	Fat, g/100g	0.49
4	Crude fibre, g/100g	1.73
5	Volatile oil content on dry basis,% By mass	0.49
6	Carbohydrate g/100g	66.36
7	Energy, K Cals/100g	348.85
8	Total ash, g/100g	3.39
9	Acid insoluble ash, g/100g	0.09
10	pH value	5.49
	Antioxidants	
11	Vitamin C	41.79
12	Selenium	12.1
13	Zinc	0.9
	Sulfur compounds	
14	Alliin mg/g	7.23
15	γ glutamyl-(*S*)-allyl-L-cysteine mg/g	0.221

Leyla Bayan *et al.* (2014) were investigated the effects of garlic and its extracts in a wide range of applications. These studies raised the possibility of revival of garlic therapeutic values in different diseases. Different compounds in garlic are thought to reduce the risk for cardiovascular diseases, have anti-tumor and anti-microbial effects, and show benefit on high blood glucose concentration.

Mary McNally *et al.* (2011) found that 1 teaspoon serving of raw garlic contains 4 calories (ME), 0.93 grams of carbohydrates (CHO) 0.18 gram CP, 0.10 gram CF, 5 mg calcium, 2mg phosphorous, 11 mg K, 0.9 mg vitamin C, 0.035 milligram vitamin B-6 Choline content for 1 teaspoon of raw garlic is 0.6 milligram; choline content for 1/8 teaspoon of garlic powder is 0.2625. While 1/8 teaspoon serving of garlic powder contains 1.25 calories (ME), 0.28125 grams carbohydrates, 0.06375 gm CP, 0.0375 gm CF, 0.25 mg calcium, 0.25 mg phosphorous, 4.625 mg K, 0.006375 mg vitamin B-6, 0.2625 mg choline.

2.3 Dietary effect of garlic powder of chicken

Tim Daniels *et al.* (2013) showed that supplemented the raw garlic to their hens for decades, possibly longer, to help them treat infection and respiratory problems but also to improve their appetite and the size and quality of the eggs they lay. After a few weeks of use, the sulphur from chicken's droppings is also reduced which can make your chicken coop and run smell better. When freshly crushed, garlic releases allicin and allicetoins that have antibacterial properties. Louis Pasteur discovered this as far back as 1858. It is known to kill only the bad bacteria or 'pathogens' and not the 'good' bacteria but allicin is also thought to be a deterrent to the ectoparasite red mite due to the taste of the allicins in the blood. Garlic is used in some red mite treatments such as 'Breck-a-Sol', which is an acaricide that has been approved for use in the U.K.

2.4 Dietary effect of garlic powder in ruminants feeding

Afshar Mirzaei-Aghsaghali *et al.* (2012) were reported that garlic (*Allium sativum*) have many biological activities, such as protective roles in cardiovascular function, as antihypertensive. Garlic can have positive effects on the performance of different animals. Garlic has various properties including improve nutrient digestibility, antimicrobial, anti-inflammatory, anti-oxidant and immunostimulant in animal's nutrition. Thus, this review has discussed the effects of garlic in ruminants.

2.5 Dietary effect of garlic powder in rabbit

Md. Jinnat Hossian *et al.* (2015) were investigated the feeding effect of garlic powder supplementation on growth performance, digestibility of nutrients and carcass characteristics of growing rabbit. For this purpose he used, 12 male New Zealand White growing rabbits were distributed randomly in three treatment groups, *i.e.* control (T_0), adding 0.25% garlic powder (T_1) and adding 0.50% garlic powder (T_2). Body weight at 35 days of age was higher (376.75 kg) in T_1 group. The digestibility of crude protein, crude fiber and digestible crude fiber values were higher in T_1 diet than the others. Carcass protein content was higher and fat content was lower in T_1 diet. Therefore, it was concluded that adding 0.25% garlic powder may be practiced for economic rabbit production.

2.6 Effect of dietary garlic on cholesterol

Sobhani Keyvan *et al.* (2015) were investigated the effect of feeding garlic supplemented diet on performance and egg quality in laying japanese quail. He used three hundred quails aged nine weeks and allocated to 3 dietary treatments. Each treatment comprised 5 replicates of 20 quails. The diets were supplemented with 0, 5 and 10 g/kg garlic powder. The addition of garlic powder did not significantly affect body weight, egg production, feed consumption, feed efficiency, egg shell thickness, egg albumen index, egg yolk index and egg Haugh unit. Adding 5 and 10 g/kg garlic powder to the laying quail diets increased egg weight ($p<0.01$. The results of this study demonstrated that garlic powder addition had a significant cholesterol-reducing effect in serum and egg yolk without adverse effects on performance and egg traits of laying quails.

Hatice Kaya *et al.* (2012) were investigated that the effects of inclusion of oven dried garlic powder (*Allium sativum*) at different levels and copper into diets of hens on performance, egg quality traits, yolk and serum cholesterol content. He used 240 Lohmann white layers, 38 wks of age, were allocated randomly eight groups, each formed 6 replicate cages .He supplemented 200 ppm copper (CuSO4.5H2O), 2% garlic powder, 2% garlic powder + 200 ppm copper, 4% garlic powder, 4% garlic powder + 200 ppm copper, 6% garlic powder and 6% garlic powder + 200 ppm copper from week 38th to 50th. The result of the study showed that Egg weight, egg production and feed consumption decreased with garlic powder and copper supplementation, no differences in the egg quality traits, Egg yolk cholesterol concentration decreased linearly with increased levels of garlic powder but serum cholesterol concentration increased. The supplementation of 200 ppm copper and combinations of garlic powder and copper did not have a significant effect on cholesterol and triglyceride concentrations of egg and serum. Consequently, without having a significant effect on laying performance and egg quality characteristics, oven dried garlic powder can be used up to 6% as a hipocholesterolemic agent in practical layer diets without copper.

Canogullari *et al.* (2010) showed that the effect of dietary garlic powder on performance, egg yolk and serum cholesterol concentration in laying quails to reduced the plasma and egg yolk cholesterol concentration. He took one hundred and twenty 10-weeks-old quails were allocated to four dietary treatments and supplemented with 0 (control), 1, 2, 4% garlic powder for 12 weeks. The result of this study showed that egg yolk index, egg

shell weight and egg shell thickness were significantly affect. There was a significant ($P < 0.05$) reduction in the egg yolk cholesterol concentration when the dietary level of garlic powder was increased from 0 to 4 g/kg. Plasma high density lipoprotein (HDL) cholesterol concentrations increased ($P < 0.05$) with increasing levels of dietary garlic powder. Plasma cholesterol ($P < 0.05$) and triglyceride ($P < 0.05$) concentration decreased with garlic powder supplementation.

Sibel Canogullari *et al.* (2009) were evaluated that the effect of garlic powder on egg yolk and serum cholesterol and performance of laying hens. He examined one hundred and forty, 50-week-old, Hy-line white layers with four dietary groups. Each group comprised seven replicates of five layers in groups of four. He supplemented 0% (control), 0.5%, 1%, and 2% garlic powder for 12 weeks. Egg production increased in the 0.5 and 1% garlic powder supplemented groups compared with the control group and in the 2% garlic powder supplemented group ($P<0.05$). The supplementation of garlic powder had no significant effects ($P>0.05$) on egg yolk index and egg yolk weight. The results of this study demonstrate that garlic powder addition decreased egg yolk cholesterol and plasma LDL cholesterol concentrations.

Khan *et al.* (2008) were conducted to evaluate the potential for local dietary garlic to influence egg yolk and blood cholesterol concentration and overall performance of native deshi layers. He used forty 30-week-old desi layer supplemented with 0 (control), 2, 6 and 8% oven dried garlic powder for 6 week. The result of the study showed that differences among diet in weight gain and egg production were found significant as averaged over 6 week. No differences were observed among diets in feed intake, feed efficiency, egg weight and egg mass with increasing levels of dietary garlic. Serum and egg yolk cholesterol concentration decreased with increasing level of dietary garlic.

Yalçın *et al.* (2007) were conducted to study the effects of dietary garlic powder on laying performance, egg traits and blood serum cholesterol level of quails. He took three hundred quails aged nine weeks allocated to 3 dietary treatments. Each treatment comprised 5 replicates of 20 quails. The diets were supplemented with 0, 5 and 10 g/kg garlic powder. The results of this study demonstrated that garlic powder addition had a significant cholesterol-reducing effect in serum and egg yolk without adverse effects on performance and egg traits of laying quails.

Khan *et al.* (2007) reported that Effects of dietary garlic on performance and serum and egg yolk cholesterol concentration in laying hens reduced serum and egg cholesterol concentration. He used total Forty 30-week-old white leghorn layers (ten hens per diet) were caged individually and fed diets supplemented with 0 (control), 2, 6 and 8% oven dried garlic powder (at low temperature i.e., 55°C) for 6 week. The result showed that dried garlic powder in the diets of commercial laying hens reduced serum and yolk cholesterol concentrations. It was also concluded that dietary garlic powder had better effects on layer performance.

Yalcin *et al.* (2006) have showed that, Effect of garlic powder on the performance, egg traits and blood parameters of laying hens. This study demonstrated that garlic powder addition increased egg weight and decreased egg yolk cholesterol concentration (mg g^{-1} yolk) and serum triglyceride and cholesterol concentrations without adverse effects on performance and egg traits.

Lim *et al.* (2006) reported that, Effects of dietary garlic powder and copper on cholesterol content and quality characteristics of chicken eggs reduced the content of egg yolk cholesterol in laying hens. He used total of one hundred and eighty, 50-wk-old, Hy-Line Brown layers were divided into 6 groups with 3 replicates per group (10 layers per replicate) and fed one of six diets containing GP 0%, GP 1%, GP 3%, GP 5%, Cu 200 ppm, or GP 3%-Cu 200 ppm for 5 wks. There were no differences in the laying performances and feed intakes Eggshell strength, eggshell thickness and yolk color were also not affected by feeding of GP and Cu. With increasing dietary GP, Haugh unit was linearly increased after 2 wks. of storage ($P < 0.05$). The levels of serum total cholesterol in hens fed diets containing GP or Cu were lower than that of the control ($P < 0.05$), but high density lipoprotein cholesterol was not influenced by dietary GP or Cu. The content of egg yolk cholesterol from hens fed diets containing GP or Cu was significantly decreased from that of the control, except for the GP 1% group.

Chowdhury *et al.* (2002) have showed that, Effects of dietary garlic on cholesterol metabolism in laying hens that reduce the serum and yolk cholesterol concentration. He examined on thirty-six, 28-wk-old, Hisex Brown, Isa Brown, Lohmann, Starcross, Babcock, and Starcross-579 strains (six hens per strain) were fed diets supplemented with 0 (control), 2, 4, 6, 8, or 10% sun-dried garlic paste for 6 wk. The result showed that, there were no differences ($P > 0.05$) among diets or strains in egg weight, egg mass,

feed consumption, feed efficiency, and BW gain as averaged over 6 wk. Egg production and yolk weights were significantly higher in the Babcock strain in comparison with other strains. Serum and egg yolk cholesterol concentrations decreased linearly ($P < 0.05$) with increasing levels of dietary garlic. Serum and egg yolk cholesterol concentrations also differed among different strains ($P < 0.05$).

Mottaghitalab *et al.* (2002) reported that, Effects of garlic (*Allium sativum*) on egg yolk and blood serum cholesterol in Aryan breed laying hens reduced egg and serum cholesterol level.

Konjufca *et al.* (1997) reported that, Modulation of cholesterol levels in broiler meat by dietary garlic and copper reduced cholesterol levels of broiler meat without altering growth of the chickens or feed efficiency. He used male Ross ´ Ross 208 chickens were fed from hatching to 21 d of age either a control diet (based on corn and soybean meal) or the control diet supplemented with 0, 1.5, 3.0, and 4.5% of a commercial garlic powder in Experiments 1 and 2. Once the dose response relationship was established, 3% garlic powder or 63 or 180 mg/kg copper as cupric citrate or cupric sulfate pentahydrate were supplemented to the diet (Experiments 3, 4, 5 and 6). The results of the study is that garlic and copper alter lipid and cholesterol metabolism. However, they do not work by the same mechanism. Feeding dietary garlic or copper for 21 d reduced cholesterol levels of broiler meat without altering growth of the chickens or feed efficiency.

Qureshi *et al.* (1983) reported that, Suppression of avian hepatic lipid metabolism by solvent extracts of garlic: Impact on serum lipids lowered the cholesterol level. The effects of ginseng root powder and of serially extracted solvent fractions of ginseng on avian hepatic cholesterol metabolism and lipogenesis and on avian serum lipoprotein cholesterol levels were examined.

Reddy *et al.* (1991) reported that, Effect of feeding garlic oil on performance and egg yolk cholesterol concentration .He used twenty 26-wk-old Single Comb White Leghorn pullets were divided into two groups of 10 birds. The birds were individually caged in a naturally ventilated poultry house and fed a corn and soybean meal diet with or without .02% garlic oil for two 28-day periods. The result showed that, dietary garlic oil did not affect egg production, egg weight, and feed efficiency. Total plasma lipids, plasma cholesterol, and yolk cholesterol were not affected by the dietary treatment.

Simon *et al.* (1995) on the effect of garlic on plasma lipids and lipoproteins in mild hypercholesterolemia. This study found no demonstrable effect of garlic ingestion on lipids and lipoproteins. The ingestion of garlic has been reported to have many cardiovascular effects, including a reduction in plasma cholesterol concentration and the susceptibility of LDL to oxidation. A double-blind, placebo-controlled, randomised crossover study was conducted in subjects with mild to moderate hypercholesterolemia who were subject to strict dietary supervision and assessment.

2.7 Microbial effect of dietary garlic powder

Eid *et al.* (2014) Investigated the effect of garlic powder on performance of broilers (e.g. growth rate, cumulative feed intake and feed conversion rate) and immune response for Newcastle virus (NVD) disease at 7, 14 and 21 days post vaccination and avian influenza virus (AIV) disease at 22, 29 and 36 days post vaccination. He examined the effect on One hundred and sixty Hubbard chicks at one-day-old were chosen randomly and divided into four groups (40 birds in each group). The chicks in the first group were fed on control diet free of garlic, (GP0), but the 2nd, 3rd and 4th groups received diet supplemented with 100 (GP100), 150 (GP150) and 200 (GP200) g garlic powder/tonne, respectively. The results showed that the diets containing feed additives of garlic powder had a highly significant effect on broilers' performance ($P<0.0001$)and antibodies titers for NVD and AIV diseases. It was improved live body weights, LBW and increased feed conversation rate (FCR); decreased cumulative feed intake (CFI); and mortality rate.

Jimoh *et al.* (2013) were investigated the in vivo antimicrobial potential of garlic against Clostridium perferinges and resultant promotant effects on performance of the broiler chickens. Garlic powder was used as an alternative to GPAs (Growth Promotant Antibiotics) to prevent subclinical Necrotic Enteritis (NE) due to C. perferinges.He used 120 day-old broiler chicks were randomly distributed to six treatment groups of 20 chicks each (2 replicates 10 chicks). Six isonutrient diets supplemented with garlic at graded levels of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 g kg^{-1} were fed to the birds for seven weeks. The results showed that garlic significantly ($p>0.05$) depressed feed intake (3310 g feed/bird at 1.0 g kg-1 supplementation) but improved FCR. The supplement has no significant effect on weight gain but C. perfringens colony counts in the treated groups, were numerically reduced (lowest count, 0.93x105 cfu g^{-1} at 1.0 gkg^{-1}.

Olobatoke *et al.* (2011) were investigated the potential of garlic powder (GP) in improving production efficiency, egg quality, and gut health of laying hens. He used seventy-two 30-wk-old Dekalb white strain hens. Hens were randomly allotted into 3 dietary treatment groups in a complete randomized design experiment. The 3 dietary treatments were control (no garlic addition) and 3 and 5% GP additions to a basal diet on weight ratio basis. The results from this study revealed significant ($P < 0.05$) increases of 0.81 mm in albumen height and 2.71 Haugh units of fresh eggs at 3% GP addition. Egg and albumen weights increased significantly ($P < 0.05$) by 2.06 and 1.84 g, respectively, at 5% GP over the control treatment. Egg production decreased significantly at 5% GP following a decrease in feed consumption. Similarly, log bacterial count in feces showed a dose-dependent reduction as dietary GP increased. Organoleptic evaluation of eggs from treatment birds revealed a strong garlic flavor in eggs from 5% GP group compared with the control and 3% GP groups.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Statement of the experiment

The experiment was conducted for a period of 12 weeks from 1st March to 23rd May, 2017 to investigate the dietary effect of garlic powder on production performance, egg yolk cholesterol and microbial load of laying quail.

3.2 Venue of the experiment

The experiment was conducted at Hajee Mohammad Danesh Science and Technology University (HSTU) Poultry Farm, egg quality& cholesterol determination was performed at the Dairy & Poultry Science Laboratory of the Department of Dairy and Poultry Science. The Microbial test was performed at Microbiology Laboratory of the Department of Microbiology, HSTU, Dinajpur.

3.3 Preparation of birds

A total of 96 laying quails of 10 weeks of age were selected and the birds were divided into four groups having 3 replications containing 8 birds in each replication. Eight hens kept in each replication were considered as an experimental unit (Replication). Quails were randomly distributed in every replication.

3.4 Layout of the experiment

Layout of the experiment is shown in table 3.1. There were three replications in each dietary phase treatment. Thus total number of replicates was twelve.

Table 3.1 Table showing the distribution of quails to different dietary garlic powder levels in cage.

Replication (R)	Treatment (T)			
	T_0	T_1	T_2	T_3
R_1	8	8	8	8
R_2	8	8	8	8
R_3	8	8	8	8

* **T_0 =** Control (basal diet)
* **T_1 =** (Control +1.5% garlic powder)
* **T_2 =** (Control +3% garlic powder)
* **T_3 =** (Control +4.5% garlic powder)

3.5 Collection of experimental materials and feed

Garlic powder was collected from dealer of Pran Company, Uttara, Dhaka. Loose feed were used for ration formulation for feeding experimental hens and it was purchased from the local market of Dinajpur. The ration was formulated to meet all nutrient requirements as specified by the 9th revised edition of National Research Council (NRC, 1994) for quail and was designated as the control diet. Diets were supplied with 0 (control), 1.5, 3 & 4.5% garlic powder. Feed and water were provided adlibitum. The chemical composition of experimental diets is shown in the table 3.2.

Fig. 3.1: Feed sample of experimental diet

Table 3.2 Ingredient amount and Chemical composition of experimental diet.

Ingredient (kg)	Treatment			
	T_0	T_1	T_2	T_3
Maize	51.2	49.7	48.2	46.7
Rice polish	6	6	7	8.5
Soybean meal	22	22	22	22
Protein concentrate (Propec)	8	8	8	8
Meat and bone meal	3.5	3.5	3.5	3.5
DCP	0.800	0.800	0.800	0.800
Limestone	8	8	7	6.5
Salt	0.500	0.500	0.500	0.500
Vitamin-mineral premix*	0.250	0.250	0.250	0.250
Lysine	0.060	0.060	0.060	0.060
Methionine	0.170	0.170	0.170	0.170
Toxin binder	0.060	0.060	0.060	0.060
Garlic powder	0	1.5	3	4.5
Chemical composition of control diet.				
Metabolizable Energy, ME (KCal/kg)	2704.12	2659.43	2645.46	2617.71
Crude Protein (%)	22.29	22.44	22.59	22.23
Crude Fibre (%)	3.64	3.67	3.69	3.76
Ether Extract (%)	5.11	5.110	5.13	5.12
Calcium (%)	3.76	3.76	3.38	3.38
Phosphorus (%)	0.78	0.75	0.74	0.71
Lysine (%)	1.38	1.37	1.39	1.36
Methionine (%)	0.36	0.37	0.39	0.38

* Added vitamin-mineral premix (Rena-Layer, Renata Animal Health Ltd) @ 250 g per 100 kg which contained: Vitamin A : 4800 IU; Vitamin D : 960 IU; Vitamin E : 9.2 mg; Vitamin k_3 : 800 mg; Vitamin B_1 : 600 mg; Vitamin B_2 : 2 mg; Vitamin B_3 : 12 mg; Vitamin B_5 : 3.2 mg; Vitamin B_6 : 1.8 mg; Vitamin B_9 : 2 mg; Vitamin B_{12} : 0.004 mg; Co: 0.3 mg; Cu : 2.6 mg; Fe : 9.6 mg; I : 0.6 mg ; Mn : 19.2 mg; Zn : 16 mg; Se : 0.48 mg; DL-Methionine : 20 mg; L-lysine : 12 mg.

3.6 Lighting

During the whole experimental period, all hens were exposed to a 16 hours continuous photoperiod (natural light + artificial light) in an open sided house. Electrical bulbs were used for additional light at night.

3.7 Routine management

Hens were provided to similar care and management in all replications throughout the study period. Adequate hygiene and sanitation were maintained properly.

3.8 Data collection and record keeping

The following data were collected per replication throughout the experimental period

3.8.1 Body weight change

The quails were weighed at start (initial body weight) and then at the end of experiment (final body weight). Body weight gain/loss was calculated by the differences between initial body weight and final body weight.

Body weight change = Final body weight - initial body weight

The following records were kept during the whole experimental period:

3.8.2 Hen day egg production percent

The bird day egg production percent was determined replication wise by the following formula.

$$\text{Hen day egg production (HDEP) (\%)} = \frac{\text{No.of eggs laid}}{\text{Total no.of days}} \times 100$$

3.8.3 Egg mass output

Egg mass output was also determined replication wise by the following formula.

$$\text{Egg mass output (g/b/d)} = \frac{\text{Weight of the total egg laid}}{\text{No.of days in production}}$$

3.8.4 Determination of egg quality

One egg from each replication of four treatments was considered during the first and last week of experimental period to determine the egg quality characteristics. Each egg for quality determination was cleaned by wet cloth and then numbered by 48 wooden pencil immediately after collection according to garlic powder level.

3.8.4.1 Egg weight

Egg weight was recorded before quality determination by using a digital balance.

3.8.4.2 Yolk index

The yolk index was determined by the formula developed by Wesley and Stadelman (1959).

$$\text{Yolk index} = \frac{\text{Average height of yolk}}{\text{Average diameter of yolk}}$$

The height of the yolk was measured by a spherometer and the diameter by slide callipers. In each parameter, three measurements were taken and the mean value was taken for final calculation.

3.8.4.3 Albumen index

The albumen index was determined according to the formula developed by Heiman and Carven (1936).

$$\text{Albumen index} = \frac{\text{Average height of thick albumen}}{\text{Average diameter of thick albumen}}$$

Average height of thick albumen was determined as the mean of three measurements taken by a spherometer in three different locations of the albumen avoiding the location of chalaza. Average diameter of the thick albumen was recorded as the mean value of three measurements taken by slide callipers.

3.8.4.4 Haugh unit

Haugh unit (HU) of egg was calculated according to Haugh (1937),

$$HU=100\log (H+7.57-1.7W^{0.17})$$

Where, HU=Haugh unit

H= Height of thick albumen

W= Egg weight (g)

Average height of thick albumen was determined as the mean of three measurements taken by a spherometer in three different locations of the albumen avoiding the location of chalazae. Egg weight was determined by digital electrical balance.

3.8.4.5 Shell thickness

Immediately after breaking the eggs, the egg shell was soaked in luke warm water for about 2 hours and then egg shell membranes were separated and egg shell thickness (mm) was measured by screw gauze. Three measurements were taken from three different locations of each shell; two reading from the waist region and one reading from each end of egg.

3.8.4.6 Percent shell and yolk

After breaking the egg, albumen was separated from yolk carefully with the help of a spatula. Then, the yolk was placed in blotting paper for the purpose of removing any white portion in the yolk. Finally, it was weighed with an electric balance. After weighing, percentage of shell and yolk were calculated as total egg weight basis.

3.9 Determination of cholesterol of egg yolk

Cholesterol of egg yolk was determined in accordance with the method suggested by Lieberman-Burchard (1952) with little modification.

3.9.1 Preparation of solution and reagent

a) Chloroform-methanol solution Chloroform was mixed with methanol at ratio of 2:1 (v/v).

b) Potassium hydroxide (KOH)-33% Ten grams of potassium hydroxide (KOH) pellets were dissolved in twenty milliliter of distilled water to make 33% solution.

c) Petroleum ether (Prepared)

d) Modified Liebermann-Burchard reagent

Twenty volumes of acetic anhydride was chilled at temperature below 5°C in a stoppered glass container and one volume of concentrated sulfuric acid was added. The well shaken mixture was kept at 0°C. Finally ten milliliters (10 ml) of glacial acetic acid was added and then was shaken properly. The mixture was kept at 0°C for 9 minutes. The reagent was allowed to warm at room temperature and thereafter used in the experiment within 1 hour.

e) Standard cholesterol (0.4 mg/ml)-Stock solution

One hundred milligrams (100 mgs) of cholesterol was dissolved in two hundred fifty milliliters (250 ml) of ethanol to make standard solution.

Fig. 3.2: Yolk sample for cholesterol determination

3.9.2 Procedure for cholesterol determination

The eggs were hard cooked to facilitate the separation of yolk and albumen. Cooked eggs were broken and the yolks were separated gently and weighted one gram (1 gm) of yolk sample. The weighted yolk sample was taken in a centrifuge tube and sonnicated with fifteen milliliters (15 ml) of chloroform: methanol solution (2:1v/v) solvent mixture and it was kept overnight for complete extraction of lipid. The extracted was filtered into a forty milliliters centrifuge tube and the residue was re-extracted with chloroform: methanol solution (2:1 v/v). The two filtrates were combined and evaporated under vacuum. Five milliliters ethanol was added to the solid portion contained in the tube and mixed well and 0.3 ml of 33% KOH was added to it. The tube was shaken well and then incubated in a water bath at 37°C-40°C for 55 minutes. After cooling to room temperature, ten milliliters (10 ml) of petroleum ether was added followed by five milliliters (5 ml) deionized water and the contents of the tube were mixed thoroughly.

Petroleum ether aliquot (one milliliter) in duplicate was collected from the clear supernatant petroleum ether layer and was taken in glass tube. Other steps were similar to that of preparation of standard. Result was calculated from standard curve (Fig. 3.4).

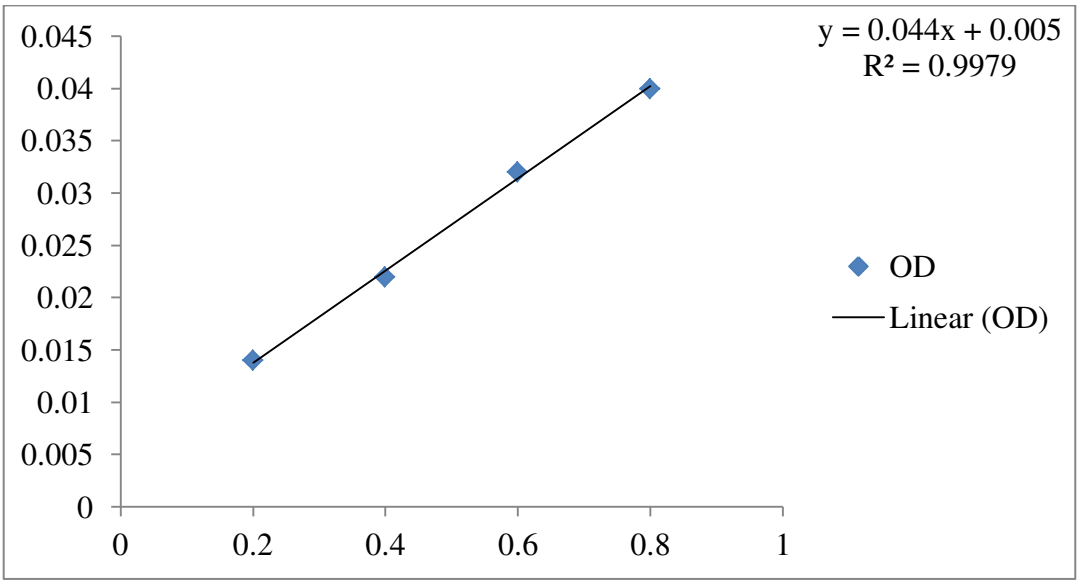

Fig. 3.3: Standard curve of cholesterol

3.9.3 Preparation of standards

Standards were prepared for inclusion with series of determination. This was most conveniently done alone with samples. Five milliliters (5 ml) standard cholesterol solutions (0.4 mg/ml) were taken in a centrifuge tube and 0.30 ml of 33% KOH was added to it. The tube was then incubated for 55 minutes at 37°C-40°C. Ten milliliters (10 ml) of petroleum ether was added followed by five milliliters deionized water and mixed thoroughly. Aliquots of 1, 2, 3 and 4 milliliters from the petroleum ether layer taken into tubes and evaporated to dryness to provide standard equivalent to 0.2, 0.4, 0.6 and 0.8 mg of cholesterol respectively. The tubes containing the dry cholesterol residue of sample and standards were arranged in such a way that one set of standard tubes appeared at the beginning and another set at the end the series. Clear empty tube was kept in the beginning as the blank. The tubes were kept in a water bath at 25°C. Six milliliters (6 ml) of Liebermann-Burchard reagent was added to the blank tube first and then at regular intervals of 1 minute to the sample's and standards tubes. The entire surface of the tubes was washed down with the Liebermann-Burchard reagent while pippeting and the tubes were shaken and returned to the water bath maintained at 25°C in a dark chamber. The reading was taken at 30 minutes after the addition of Liebermann-

Burchard reagent. The intensity of the colour in each tube was read at regular interval of one minute against the blank in a spectrophotometer set at 620 nm.

3.10 Identification of *E. coli* and *Salmonella spp.*

3.10.1 Sample collection

The samples were collected from cloacae of laying quail through pre-sterilized cotton swab and immediately transferred into screw capped test tubes containing buffered peptone water. A total 36 sample were collected, randomly 3 samples from each replication. Thermo flask containing ice was used to transport the samples from the collection site to Microbiology Laboratory, HSTU for analysis. Collected samples were preserved in a refrigerator at 4ºC until screening out the bacteria.

Fig. 3.4: MacConcy agar media

Fig. 3.5: SS agar media

3.10.2 Bacteriological analysis

The samples were analyzed within 2-6 hours of collection. SS Agar (*Salmonella*-Shigella Agar) & MacConkey were used to identify *Salmonella spp.* and *E. coli*. respectively, These are called selective media. The above media were prepared separately by the following method:

3.10.3 Preparation of SS Agar media

The SS Agar media were prepared by suspending 31.5 gm SS Agar in 500 ml distilled water. The media were heated to boiling with frequent agitation to dissolve completely but not autoclaved or overheated, because overheating may destroy the selectivity of the medium. The media were cooled to about 50°C. The media were mixed well and poured into sterile Petridis sterilizing by laminar air flow.

Fig. 3.6: During agar media preparation

3.10.4 Preparation of MacConkey media

The media were prepared by suspending 27.75 gm MacConkey Agar in 500ml distilled water. The media were heated to boiling with gentle swirling to dissolve completely. The media were sterilized by autoclaving at 121°C for 20 minutes at 15 lbs pressure. Overheating was avoided. Then media were cooled to 45-50°C and poured into sterile Petridis. Surface of the medium was dried when inoculated.

3.10.5 Culture procedures for both *E. coli* and *salmonella spp.*

1ml of swab suspension was inoculated in a screw cap test tube containing 10ml of nutrient broth and incubated at 37ºC for 24 hours. Then for *E. coli*, samples were streaked on MacConkey agar and incubated overnight. The growth of *E. coli* produces large, pink colonies on an agar plate. For *Salmonella spp*., after inoculation of the sample on nutrient broth, one loop-full of the colony from broth was streaked on XLD agar plate and incubated at 37ºC for 24 hours. Then the positive samples were further inoculated on SS agar and incubated overnight at 37ºC for 24 hours. After incubation, colonies were observed. The colony with a black center in XLD and blackish growth in SS agar were considered as presumptive *Salmonella spp.* positive.

3.11 Statistical analyses

Data were analyzed by analysis of variance using Completely Randomized Design with factorial arrangement of time and treatments (Steel and Torrie, 1986). The significance differences between the treatment means were calculated by the Duncan's Multiple Range Test (Duncan, 1955). All analyses were performed by SPSS Program.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

In this experiment, all the treatments supplied with various levels of garlic powder on laying performance, egg quality, egg cholesterol and microbial load in cloaca which are discussed below.

4.1 Laying performances

The performances of laying quail fed different amount of garlic powder are discussed under the following subheading

4.1.1 Egg production

The Hen-day-egg production observed in different dietary treatments were statistically significant (P> 0.01). Result indicates (Table 4.1) that the feeding of garlic powder mixed meal in the diet of laying quail has significant effect on egg production from first to third month. Feeding of garlic powder up to 4.5 percent levels showed slightly higher egg production whereas the production was slightly decreased when the birds received 3 percent garlic powder in the diet. These result closed with the previous report of Khan *et al.* (2007), who found the egg production was affected during the six weeks in which 0, 2, 6 or 8% garlic powder was fed to the laying hens. In contrast, Chowdhury *et al.* (2002) and Reddy *et al.* (1991) reported that feed consumption, feed efficiency and egg production were not affected by supplements of 0, 2, 4, 6, 8 or 10% garlic paste (P > 0.05) of layer.

Table 4.1 Effect of garlic mix supplementation on Egg production (%) of laying quails

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4th week	61±2.08	68±3.21	59.33±0.88	67.00±2.30	NS
8th week	$71.33±1.45^{c}$	$77.33±1.20^{b}$	$80±1.73^{ab}$	$84.66±1.45^{a}$	*
12th week	$78.66±0.88^{c}$	$84.00±2.00^{bc}$	$86.33±3.28^{b}$	$88.66±0.88^{a}$	*

Diets were supplemented with garlic powder (%) was fed for 12 weeks; values are expressed as mean ± standard error of means. a, b, c Means within row with different superscripts are statistically different (P <0.05), NS= Not significant, *= Significant at 5% level of significance

4.1.2 Egg weight

The egg weights in different dietary treatments during experimental periods were statistically significant (P< 0.01). These results indicate (Table 4.2) that inclusion level of garlic powder up to 4.5 percent in the diet of laying quail has significant effect on egg size. The results are consistent with the report of Yalcin *et al.* (2006), who found that egg weight increased (P < 0.01) when laying hens were fed 5 and 10 g/kg garlic powder supplementation. In contrast, Khan *et al.* (2008) and Chowdhury *et al.* (2002) reported that egg weight was not affected by 0, 2, 6 or 8% garlic powder (P > 0.05) or by 0, 2, 4, 6, 8 or 10% garlic paste (P > 0.05) as averaged over the six-week period respectively.

Table 4.2 Effect of garlic mix supplementation on egg weight (gm/egg) of laying quails

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4th week	7.78±0.28^b	8.98±0.18^a	8.01±0.29^b	9.53±0.119^a	*
8th week	8.82±0.14^c	9.44±0.04^a	9.07±0.12^c	10.39±0.05^a	*
12th week	9.19±0.04^c	9.67±0.05^b	9.40±0.07^c	10.22±0.11^a	*

Diets were supplemented with garlic powder (%) was fed for 12 weeks; values are expressed as mean ± standard error of means. a, b, c Means within row with different superscripts are statistically different (P <0.05), NS= Not significant, *= Significant at 5% level of significance

4.1.3 Egg mass output

The results of the present study showed (Table 4.3) that the egg mass output (gm/quail/day) in different dietary treatments during experimental periods were statistically insignificant (P>0.01) from initial stage to end of third month. The results are agreement with the report of Khan *et al.* (2008), who showed , feed consumption, feed efficiency, egg weight and egg mass were not affected by garlic powder mixed diet (p>0.05) as averaged over the 6 week period.

Table 4.3 Effect of garlic mix supplementation on egg mass output of laying quails

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4st week	7.28 ±1.15	8.98±0.47	8.01±0.84	9.53±0.27	NS
8th week	9.13±0.27	9.75±0.22	9.37±0.38	10.73±0.22	NS
12th week	8.88±0.11	9.37±0.13	8.95± 0.41	9.87±0.30	NS

Diets were supplemented with garlic powder (%) was fed for 12 weeks; values are expressed as mean ± standard error of means. NS= Not significant

4.1.4 Body weight

Body weight of different dietary treatments during experimental periods was almost similar and the differences were non-significant (P>0.05). These results indicate (Table 4.4) that the inclusion level of garlic powder up to 4.5 percent had no effect on body weight. The results are agreement with the report of Canogullari *et al.* (2010), showed body weight gain in laying quail was not affected by garlic powder, Reddy *et al.* (1991), showed body weight gain and egg production in Babcock layer with 0.02% garlic oil was not affected during the 8 weeks trial and Yalcin *et al.* (27) also observed that the body weight and egg production were not significantly affected by dietary treatments (0, 5, and 10 g kg^{-1} of garlic powder) over the 22-week period . In contrast, Samanta and Dey (1991), reported that Japanese quails gained more weight and egg production ($p<0.05$) without affect on feed consumption and feed efficiency with garlic powder. Khan *et al.* (2008) showed that the diets containing garlic gained more weight ($p<0.01$) than those of birds fed diets without garlic in native desi hen.

Table 4.4 Effect of garlic mix supplementation on Body weight (g) of laying quails

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4st week	147.48±1.89	154.8± 3.12	151.0± 7.16	154.8± 5.58	NS
12st week	152.4±0.58	155.9± 1.72	148.1±6.12	148.1±6.24	NS

Diets were supplemented with garlic powder (%) was fed for 12 weeks; values are expressed as mean ± standard error of means. NS= Not significant

4.1.5 Feed intake

Feed intake of laying hens in different dietary treatments during experimental periods was almost statistically similar and the differences were non-significant (P>0.05). So, the result clearly showed (Table 4.5) that the dietary garlic powder up to 4.5 percent in the diet decreased feed intake in the last month of the laying quail due to odour of garlic. The results also closely related with the report of Chowdhury *et al.* (2002) and Reddy *et al.* (1991), showed feed consumption, feed efficiency and egg production were not affected by supplements of 0, 2, 4, 6, 8 or 10% garlic paste, Lim *et al.* (2006) and Yalcin *et al.* (2006), who found no significant changes in layer performance and feed intake when layer diets were supplemented with GP. Similarly, Ologhobo *et al.* (2008) reported no significant effect of dietary sun-dried GP on feed intake, weight gain, and feed conversion ratio of broilers, Reddy *et al.* (1991) showed the body weight gain and egg production in Babcock layer with 0.02% garlic oil was not affected during the 8 weeks trial. In contrast, Khan *et al.* (2010) showed that the inclusion of 0%, 2%, 6%, and 8% garlic powder significantly (P<0.01) increased the feed consumption with increasing levels of dietary garlic in laying hens, Samanta and Dey (1991), who reported that Japanese quails gained more weight and egg production (p<0.05) without affect on feed consumption and feed efficiency with garlic powder.

Table 4.5 Effect of garlic mix supplementation on feed intake (g) of laying quails

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4th week	222±4.16	219±10.50	236.6±12.0	270.0±17.32	NS
8^{th} week	203.3±12.0	192±9.07	206.6±8.81	217±6.50	NS
12^{th} week	179.3±0.66	172.0±8.73	193.6±8.95	180.±5.77	NS

Diets were supplemented with garlic powder (%) was fed for 12 weeks; values are expressed as mean ± standard error of means. NS= Not significant

4.1.6 Feed efficiency

Feed conversion ratio in different dietary treatment at 1.5, 3, 4.5 percent level had no significant effect (P>0.05) on feed efficiency. The results indicate (Table 4.6) that there was no effect on feed efficiency after feeding up to 4.5 percent level of garlic powder. This is in agreement with the result of Chowdhury *et al.* (2002) and Reddy *et al.* (1991) reported that feed efficiency was not affected by supplements of 0, 2, 4, 6, 8 or 10% garlic paste (P > 0.05) as averaged over the 6-week period or by supplements of 0.02% garlic oil over eight weeks. In contrast, Canogullari *et al.* (2010) supplementation of diets with garlic powder had significant (P < 0.05) effects on feed consumption, feed efficiency.

Table 4.6 Effect of garlic mix supplementation on feed conversion ratio of laying quails

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4st week	3.03± 0.02	3.17± 0.05	3.07± 0.20	3.18± 0.044	NS
8st week	3.04±.05	3.02±0.04	3.14±0.03	2.99±0.07	NS
12st week	2.48± 0.09	2.40± 0.08	2.80± 0.12	2.84± 0.21	NS

Diets were supplemented with garlic powder (%) was fed for 12 weeks; values are expressed as mean ± standard error of means. NS= Not significant

4.2 External and internal egg quality

It was observed that the shape index, shell thickness, albumin weight, albumin index, yolk weight, yolk index and Haugh unit of the eggs laid by quails fed different diet were almost similar during experimental period and differences were non-significant. These results indicate (Table 4.7, 4.8, 4.9, 4.10 and 4.11) that feeding dietary garlic powder mixed meal up to 4.5 percent level had no effect on external and internal qualities of egg. However, egg shell thickness decreased slightly after supplementation of 1.5%, 3%, 4.5% garlic powder mixed feed. Egg shell weight slightly improved at the level of 3 percent garlic powder mixed feed. Haugh unit slightly improve at the level of 1.5 percent and 4.5 percent in first month but decreased gradually in last month. Egg yolk index

decreased slightly after supplementation of garlic powder up to 4.5 percent level. Egg albumin index slightly improved at the level of 3 percent and decreased gradually at the level 1.5% and 4.5% (P>0.05). Similar results have been obtained by Yalcin *et al.* (2006) reported that the supplementation of garlic powder had no significant effect (P > 0.05) on egg albumen index, egg shell index and egg Haugh unit values when laying hens were fed 5 and 10 g/kg garlic powder for 22 weeks.

Yalcin *et al.* (2006) yolk weight did not differ significantly (P > 0.05) among dietary treatments. However, Mottaghitalab and Taraz (2002) showed that the inclusion of 0, 5, 10 and 15 g/kg garlic powder significantly (P < 0.01) decreased yolk weight. Chowdhury *et al.* (2002) also reported that yolk weight responded quadratically (P < 0.05) in weeks three and four to increasing levels of sun-dried dietary garlic paste.

Table 4.7 Effect of garlic mix supplementation on external internal quality of egg

Parameter	Period	Treatment				Level of Significance
		T_0	T_1	T_2	T_3	
Egg shell thickness (mm)	4th Week	0.212±0.001	0.211±0.0003	0.211±.0005	0.210±0.0005	NS
	12th week	0.212±0.0003	0.212±0.0003	0.211±0.0008	0.206±0.001	NS
Egg shell weight	4th Week	1.69±0.01	1.703±0.02	1.76±0.008	1.71±0.012	NS
	12th week	1.69±0.01	1.70±0.02	1.74±0.02	1.65±0.038	NS
Haugh unit	4th Week	78±1.52	80±0.57	77.66±1.45	81±0.57	NS
	12th week	75±2.88	75.33±0.33	70.33±0.33	73.66±1.20	NS
Albumin Index	4th Week	8.50±0.24	8.88±0.17	8.46±0.25	8.72±0.12	NS
	12th week	7.97±0.14	8.49±0.04	8.63±0.08	8.49±0.24	NS
Yolk Index	4th Week	38.42±1.26	37.39±0.87	38.09±1.48	38.83±0.91	NS
	12th week	31.12±0.58	32.51±1.21	33.70±1.81	36.07±1.48	NS
Yolk weight	4th Week	4.78±0.15	4.83±0.11	4.86±0.15	4.91±0.08	NS
	12th week	4.66±0.18	4.77±0.10	4.94±0.68	4.53±0.09	NS

Diets were supplemented with garlic powder (%) was fed for 12 weeks; values are expressed as mean ± standard error of means. NS= Not significant

Sakine Yalçın *et al.* (2007) also reported that the addition of garlic powder had no significant effect ($p>0.05$) on the egg shell thickness, egg albumen index, egg yolk index and egg Haugh unit. Canogullari *et al.* (2010), also showed that supplementation of garlic powder had no significant effect ($P > 0.05$) on egg yolk index, egg shell weight and egg shell thickness. In contrast, Lim *et al.* (2006) reported that with increasing dietary garlic powder, the Haugh unit linearly increased after two weeks of storage. Canogullari *et al.* (2010), showed that addition of garlic powder had significant differences ($P < 0.05$) in egg albumen index, egg shell index and Haugh unit.

4.3 Egg Yolk cholesterol

This study showed that egg-yolk cholesterol was decreased significantly by supplementation of dietary garlic powder mixed feed in quail-ration ($P < 0.05$). It is evident from Table 4.12 that a tendency of reduced egg yolk cholesterol was observed in the dietary treatments with inclusion of 1.5 percent garlic powder meal. However, the highest level of cholesterol was 14.2 mg/gm at 1.5 % level and lowest level was 11.36 mg/gm at 4.5% level of garlic powder meal whereas cholesterol of "control egg" ranged from as low as 10 mg/gm of yolk to as high as 18 mg/gm of yolk (USDA, 2008). Thus, the result of current study clearly showed that garlic powder meal at 1.5, 3, 4.5 percent dietary level had beneficial effect in reduction of egg yolk cholesterol. The similar result obtained from Canogullari *et al.* (2010) who found egg yolk cholesterol concentrations per gram of yolk decreased linearly ($P < 0.05$) with increasing levels of garlic powder as 1, 2, 4 percent. Chowdhury *et al.* (2002) reported that cholesterol concentration per gram of yolk decreased linearly ($P < 0.01$) with increasing levels of sun-dried dietary garlic paste. Khan *et al.* (2007) also reported that dietary garlic at 2, 6 or 8% reduced egg yolk cholesterol, on average over six weeks, by 5.70, 14.28 and 23.57%, respectively, as compared to the control diet. Sharma *et al.* (1979) observed that egg yolk cholesterol was reduced by 4.1 and 5.5% when laying hens were fed 10 and 30 g/kg garlic powder for 3 weeks, respectively. Supplementation of 5, 10 or 15 g/kg of garlic powder (Mottaghitalab and Taraz, 2004) and 30 g/kg garlic powder (Lim *et al.*, 2006) reduced serum and egg yolk cholesterol concentration. Yalçın *et al.* (2006) also reported that the levels of serum cholesterol in laying hens were significantly ($P < 0.01$) reduced with 5 and 10 g/kg garlic powder supplementation. However, Reddy *et al.* (1991) found that 0.2 g/kg garlic oil in the diets of laying hens did not significantly reduce total plasma cholesterol. Sakine Yalçın *et al.* (2007) also reported that 5 and 10 g/kg garlic powder to

the laying quail diets reduced egg yolk cholesterol and blood serum cholesterol concentration significantly ($P < 0.01$).

Table 4.8 Effect of garlic mix supplementation on Egg yolk cholesterol (%) of laying quails

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4st week	15.34± 0.20	14.20± 0.41	14±0.50	14.33± 0.17	NS
12th week	14.46± 0.17[a]	12.20± 0.11[b]	12.26± 0.37[b]	11.36±0.55[c]	*

Diets were supplemented with garlic powder (%) was fed for 12 weeks; values are expressed as mean ± standard error of means. a, b, c Means within row with different superscripts are statistically different ($P < 0.05$, $P < 0.01$), NS= Not significant, *= Significant at 5% level of significance

From the above discussion, it is said that egg-yolk cholesterol was decreased significantly without affecting egg qualities with increased level of garlic powder meal supplementation. Garlic powder contains allicin that reduces cholesterol synthesis, inhibits fatty acid synthesis and platelet aggregation and prevents thrombosis. As a result, cholesterol of egg-yolk was reduced.

4.4 Microbial effect of garlic powder

This study showed (Table 4.13 and 4.14) that, microbial load in ceca of laying quail was decreased significantly by supplementation of dietary garlic powder at the level of 1.5, 3, 4.5 percent in quail ration. Microbial load was decreased with increased level of garlic powder. The lowest number of microbs found at 4.5 percent level of garlic powder mixed feed. This is in agreement with antibacterial activity of garlic as reported by Ankri and Mirelman (1999). The result also agrees with observations of Sarica *et al.* (2005) and Mahmoud *et al.* (2006) who respectively noted reduced concentrations of total aerobic bacteria and *Escherichia coli* in the small intestine of broiler chickens and a linear suppression of bacterial counts in egg contents by garlic and garlic juice.

Table 4.9 Effect of garlic powder mix supplementation on bacterial load in cloaca of laying quail (*E. coli*)

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4st week	10.34±0.01	10.28±.003	10.26±0.03	10.30±0.05	NS
8th week	$10.28±0.01^{a}$	$10.22±0.01^{ab}$	$10.21±0.013^{ab}$	$10.17±0.04^{b}$	*
12thweek	$10.24±0.01^{a}$	$10.10±0.013^{b}$	$10.07±0.02^{b}$	$9.83±0.02^{a}$	*

Diets were supplemented with garlic powder (%) was fed for 12 weeks; all values are converted into log10; values are expressed as mean ± standard error of means. a, b, c Means within row with different superscripts are statistically different (P <0.05), NS= Not significant, *= Significant at 5% level of significance

Table 4.10. Effect of garlic powder mix supplementation on bacterial load in cloaca of laying quail (*Salmonella* spp.)

Period	Treatment				Levels of Significance
	T_0 (Control)	T_1 (1.5% garlic powder)	T_2 (3% garlic powder)	T_3 (4.5% garlic powder)	
4stweek	10.31±0.02	10.29±0.02	10.25±.008	10.31±0.04	NS
8thweek	10.22±0.017	10.10±0.036	10.01±.03	10.00±0.01	NS
12thweek	$10.27±0.02^{a}$	$10.12±0.04^{b}$	$9.97±0.01^{c}$	$9.87±0.01^{d}$	*

Diets were supplemented with garlic powder (%) was fed for 12 weeks; all values are converted into log10; values are expressed as mean ± standard error of means. a, b, c Means within row with different superscripts are statistically different (P <0.05), NS= Not significant, *= Significant at 5% level of significance

CHAPTER V

SUMMARY AND CONCLUSIONS

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Garlic powder was collected from dealer of Pran Company, Uttara, Dhaka to observe its effect on reduction of egg yolk cholesterol, harmful bacterial load and any alteration of egg quality characteristics and production performance. The feeding value of Garlic powder for laying quail was evaluated at Hajee Mohammad Danesh Science and Technology University poultry farm, Dinajpur District. Ninety six quail (*Coturnix japonica)* of 70 days old were allocated to 4 groups, each containing 24 quail. The quails in individual cage were supplied feed (26 g/bird/day) containing 18.21% CP and 2762.21 MJ ME/kg diet. Quails were randomly allowed to 4 dietary treatments: $T_{0,}$ (control), $T_{1,}$ (1.5% garlic powder), T_2 (3% garlic powder), T_3 (4.5% garlic powder). In experimental diets, laying performance, external and internal quality characteristics of eggs in different dietary treatments were almost similar and the differences were statistically non-significant except egg yolk cholesterol, microbial load. Body weight change, egg production, feed conversion, egg weight and egg size were recorded and compared. Egg production, egg weight, egg yolk cholesterol were improved at increasing level of garlic powder up to 4.5%.

Feed consumption for the entire experimental period in different treatment groups was recorded and expressed as g/day. The rate of feed intake varied from day to day but in first month the feed intake was highest in T_3 (4.5% garlic powder) and in the last month was T_2 (3% garlic powder). Data obtained on final average body weight indicated that there was no positive correlation between body weight and food consumption. Feed conservation ratio (FCR) was the highest in T_3 (3.18) compared with other group. Data obtained on egg weight expressed as maximum level in T_3 group (10.39 gm) than the other feeds fed group but almost similar to diet. Egg mass were statistically non-significant in all groups. Shell thicknesses were indifferent with diet at T_0 (0.212 mm), T_1 (0.212 mm), T_2 (0.211 mm) and T_3 (0.206 mm) in the diet. Data obtained on albumen index exhibited maximum level in diet with T_2 (8.63 percent) than the other feeds fed group but almost similar to diet with T_0 (7.97 percent), T_1 (8.49 percent) and T_3 (8.49 percent). The yolk index, Haugh unit values were found to be almost the same with diet. Data obtained on egg yolk cholesterol exhibited a higher level in control group (14.466 mg/gm) and lower in diet at T_2 (11.36 mg/gm).

Intestinal bacteria play an important role in the health status of host animals including poultry. In general, intestinal bacteria may be divided into species that exert either harmful (pathogenic) or beneficial effects on host health. Therefore, a common approach to maintain host health is to increase the number of desirable bacteria (e.g., probiotics) in order to inhibit colonization of invading pathogens (Guo *et al.*, 2004). In the present study, supplementation garlic powder mix in the diet of laying quails significantly ($P < 0.01$) decreased the population of harmful bacterium (*E. coli* and *Salmonella spp.*). In conclusion, we found that garlic powder mix supplemented diet significantly decreased the levels egg yolk cholesterol of laying hens without affecting feed intake, body weight and physical parameters of the eggs. Furthermore, the growth of harmful bacterium, *E. coli,* in the excreta and *Salmonella spp.* of laying quails was significantly suppressed by garlic powder mix supplemented diets for 12 weeks. Taken together, our results suggest that supplementation of garlic powder mix in diets has high potential as commercial applications for the production of low-cholesterol eggs. Therefore, garlic powder can be considered as a feed additive and its contain cholesterol lowering agent and other active ingredient which are beneficial for human health.

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APPENDICES

APPENDICES

Appendix Table 1: Chemical composition of feed ingredients used for formulation of experimental diets

Ingredients	DM %	ME (Kcal/ kg)	CP %	EE %	CF %	Ca %	TP %	Lys. %	Meth. %	Cyst. %	Tryp. %
Maize	90.0	3400	10.0	3.50	2.00	0.02	0.35	0.24	0.12	0.18	0.07
Rice polish	88.1	3090[a]	16.4	14.8	10.5	0.27[a]	0.14[a]	0.57[a]	0.22[a]	0.21[a]	0.13[a]
Soybean meal	89.0	2426	42.0	3.50	6.50	0.25	0.20	2.70	0.60	0.62	0.58
Bone & Meat meal	93.0	2536	50.0	8.50	2.80	9.20	4.70	2.60	0.67	0.33	0.26
Oyster shell	99.0	-	-	-	-	35.0[d]	0.017[d]	-	-	-	-
Garlic powder	-	348	19.5	0.09	1.73	-	-	-	-	-	-
Soybean oil	-	8800	-	99	-	-	-	-	-	-	-

a = NRC, 1994

d = Singh and Panda, 1992

Appendix Table 2: Food intake trend over the experimental period

Diet	1st week (g)	2nd week (g)	3rd week (g)	4th week (g)	5th week (g)	6th week (g)	7th week (g)	8th week (g)	9th week (g)	10th week (g)	11th week (g)	12th week (g)
Control diet	182	183	185	190	194	198	201	204	208	209	209	210
Diet with 3% GP	183	180	178	177	188	193	200	198	184	186	200	204
Diet with 6% GP	178	177	175	183	185	188	189	200	201	194	180	182
Diet with 9% GP	180	178	176	182	187	185	200	204	206	198	197	190

Appendix Table 3: Chemicals and instruments used in egg-yolk cholesterol and microbial load determination

Chemicals	**Instruments**
Chloroform	Water bath
Methanol	Vortex Mixture
Potassium Hydro-oxide (KOH)	Incubator
Acetic Ahydride	Sonnicator
Conc. Sylphuric acid (H_2SO_4)	Hot air oven
Glacial acetic acid	Vacuum evaporator
Ethanol	Centrifuge machine
Distilled water	Spectrophotometer
Deionized water	Centrifuge tube
Petroleum ether	Routine laboratory articles
PBS (Phosphate Buffer Solution)	Pertidish, Test tube, pipette
SS agar	Autoclave
MacConcy agar	Conical flask, Sprit lamp

Composition of SS agar media

Ingredients	**Gms / Litre**
Beef extract	5.000
Peptic digest of animal tissue	5.000
Lactose	10.000
Bile salts mixture	5.500
Sodium citrate	10.000
Sodium thiosulphate	8.500
Ferric citrate	1.000
Brilliant green	0.00033
Neutral red	0.025
Agar	12.000
Final pH (at 25°C)	7.2±0.2

Composition MacConkey Agar media

Ingredients	**Gms / Litre**
Peptones (meat and casein)	3.000
Pancreatic digest of gelatin	17.000
Lactose monohydrate	10.000
Bile salts	1.500
Sodium chloride	5.000
Crystal violet	0.001
Neutral red	0.030
Agar	13.500
pH after sterilization(at 25°C)	7.1±0.2