THE DIETARY EFFECT OF GARLIC, ZINGER AND BLACK PEPPER ON EGG PRODUCTION, YOLK CHOLESTEROL AND FECAL MICROBIAL STATUS OF LAYER STRAIN IN BANGLADESH

A THESIS

BY

MD. REZAUL KARIM
Registration No.: 1405179
Session: 2014-2015
Semester: July-December, 2014

MASTER OF SCIENCE (M S)

IN

POULTRY SCIENCE

DEPARTMENT OF DAIRY AND POULTRY SCIENCE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Approved as to style and content by

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DEPARTMENT OF DAIRY AND POULTRY SCIENCE

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Dedicated to.....

My father who insisted me the value of my education, my mother whose unending love and sacrifices inspired and encouraged me and the Almighty Allah who blessed me with the ability and strength to accomplish it.

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The Author

ABSTRACT

The experiment was conducted from January-April 2015 at the poultry farm under the Dairy and poultry science department, Hajee Mohammad Danesh Science and Technology University, Dinajpur to investigate the effects of feeding three medicinal herbs extracts (black peper, garlic and ginger) to Hisex brown layer chicken on their cholesterol metabolism, antibacterial activity and productivity. In this feeding trial, total 4-month experiment period, 60 Hi-sex brown laying hens (age 20 weeks) were assigned to five dietary treatments with three replication of four (4) birds in each. Diets were supplied with T_0 (control), T_1 (Black pepper 0.5gm/kg, Ginger 1.00 gm/kg and Garlic 1.00 gm/kg), T_2 (Black pepper 1.00 gm/kg, Ginger 1.50 gm/kg and Garlic 1.50 gm/kg), T_3 (Black pepper 1.50 gm/kg, Ginger 2.00 gm/kg and Garlic 2.00 gm/kg) and T_4 (Black pepper 2.00 gm/kg, Ginger 2.50 gm/kg and Garlic 2.50 gm/kg) sun-dried Black pepper, Ginger and Garlic powder mixed meal. 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_4 (2.01) compared with other group. Egg production was more or less similar all of treatment group. Data obtained on egg weight expressed as maximum level in T_2 (65.80 gm) than the other feeds fed group but almost similar to diet. Egg mass were statistically similar in all groups. Shape index were found to be highest at diet with T_2 (81.01 %) but almost same to all other feed groups. Shell thicknesses were indifferent with diet at T_0 (0.40 mm), T_1 (0.41 mm), T_2 (0.43 mm) T_3 (0.41 mm) and T_4 (0.42 mm) in the diet. Data obtained on albumin index exhibited maximum level in diet with T_4 (8.44 percent) than the other feeds fed group but almost similar to diet with T_0 (8.36 percent), T_1 (8.40 percent) and T_2 (8.00 percent) and T_3 (8.42). 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 (13.8 mg/gm) and lower in diet at T_2 (8.99 mg/gm). In the present study, supplementation of Black pepper, Ginger and Garlic powder mix in the diet of laying hens significantly ($P<0.01$) decreased the population of harmful bacterium, *E. coli,* as well as total cultivable bacteria than those of control (T_0).

ABBREVIATION AND ACRONYMS

AGP	Antimicrobial Growth Promoters
CRD	Completely Randomized Design
DLS	Department of Livestock Services
FAO	Food and Agricultural Organization
FC	Feed Consumption
FCR	Feed Conversion Ratio
GDP	Gross Domestic Product
HDL	High Density Lipoprotein
Kcal	Kilocalorie
LDL	Low Density Lipoprotein
NRC	National Research Council
SPSS	Statistical Package for the Social Sciences
SID	Statistics and Informatics Division
%	Percentage

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INTRODUCTION

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INTRODUCTION

Poverty alleviation is one of the most important challenges of the twenty first century in Bangladesh. Agricultural development is the main key to alleviate poverty from the country. Livestock is the most important agricultural component which alone contributes about 17.3% GDP to agriculture (DLS, 2015). Livestock population in Bangladesh is currently estimated about 25.7 million cattle, 0.83 million buffaloes, 14.8 million goats, 1.9 million sheep, 118.7 million chicken and 34.1 million ducks. The density of livestock population per acre of cultivable land is 7.37 (Banglapedia, 2012). In spite of a high density of livestock population, the country suffers from an acute shortage of livestock products like milk, meat and eggs. The shortage accounts for 85.9%, 88.1% and 70.7% for milk, meat and eggs, respectively (Banglapedia, 2012). Poultry is one of the most important sectors of livestock that provides the cheapest animal protein (nutritious egg and meat) for human consumption within the shortest period of time. Plants are the oldest friends of mankind. They not only provide food and shelter but also serve humanity by preventing and curing different ailments. Herbs and spices have always been helpful to cure diseases. The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs having been used in the treatment of diseases and for revivitalising body system in almost all ancient civilizations, the Egyptian, the Chinese and even Greek and Roman civilizations (Aftab and Sial, 1999).

In modern animal feeding, they are forgotten because of use of antimicrobial growth promoters (AGP). But due to the prohibition of most of AGP, plant extracts have gained interest in animal feed strategies (Charis, 2000). Antibiotics have been supplemented to animal to improve growth performance and protect animals from the adverse effects of pathogenic and non-pathogenic enteric microorganisms (Dahiya *et al*., 2006). The risk of the presence of antibiotic residues in milk and meat and their harmful effects on human health have led to their prohibition for use in animal feed in the European Union (Cardozo *et al*., 2004). Many plants also produce secondary metabolites such as phenolic compounds, essential oils and sarasaponins (Chesson *et al*., 1982; Wallace *et al*., 1994; Kamel, 2001).

Medicinal plants and herbs have played a significant role in maintaining human health and improving the quality of human life for thousands of years. Medicinal plants and herbs contain a wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins and phthalides (Zlatanow, 1994 and Craig, 1999). These plants posses biological activities such as that of antioxidants (Farag and El-Khawas, 1998) and stimulate the function of animal digestive system to increase production of digestive enzymes through enhance liver functions (Hermandez, 2004) and therefore could be effectively utilized in poultry ration as feed additives. Use of antibiotics and other synthetic chemicals in poultry and dairy industries may remain as residue in their products which are not safe for human consumption. However, most microbes have not been cultured. However, the use of therapeutic antibiotics in animal feed is not approved due to chances of development of antibiotic resistant microbes. Therefore various studies have been conducted to use plants and herbs as alternative of synthetic antibiotics. Herbs are identified to enhance antimicrobial, antiviral, and antioxidative activities and to simulate the endocrine and immune system (Dahiya *et al*., 2006). In food industry, herbs or their extracts having antioxidative properties are frequently used to improve quality and shelf life of meat products (Vichi *et al*., 2001), turkey meat (Botsoglou *et al*., 2007), and egg yolk (Botsoglou *et al*., 2005). Ahn *et al*. (2007) also reported grape seed extract Grape seed extract contains chemicals known as polyphenols, (including the subclass of proanthocyanidins), which are recognized to be effective polyphenol antioxidants and pine bark extract contributed significantly to antimicrobial and antioxidant activities in cooked beef.

Chicken eggs are well established as an excellent source of all essential nutrients for persons of all ages. However, it is recommended that people should limit the consumption of eggs because of their high cholesterol (208 mg/egg) content (Kritchevsky and Kritchevsky, 2000). Thus, eggs are considered to be a high-cholesterol food. In terms of the safety and quality of raw materials and foodstuffs originating from animals, constant effort in the area of animal nutrition goes into preventing the contamination of feeds with substances that may put human health at risk. Egg colour is an important indicator that makes an egg attractive for consumers. From the consumer health point of view, the level of cholesterol in egg yolk is of a great importance. The quality of eggs including the evaluation of cholesterol levels in egg yolk was studied by

Tumova *et al*. (2004), Wang and Pan (2003) and Murata *et al*. (2003). There are only a few scientific papers on the metabolic profile of clinically healthy hens during a laying period, which is one of the major indicators of the hens' state of health. Therefore, in recent years poultry research has focused on reducing yolk cholesterol content to satisfy the health conscious consumer (Basmacioglu and Ergul, 2005). To achieve this goal, alteration of the dietary composition of hens has been one of the targeted factors. People are paying more attention to health and are thus lowering their consumption of high-cholesterol food. Therefore, low-cholesterol eggs would not only be beneficial to the public's health but also bear business advantage. So, it would be beneficial for health to be able to provide a low-cholesterol egg and meat, and research efforts should be directed toward this goal. So, research with lowering egg-yolk cholesterol has centered mostly on diet and pharmacological intervention. Previous attempts to alter cholesterol level in the egg-yolk have ranged from the manipulation of the normal dietary components to the inclusion of drugs and other agents (Noble, 1987; Hargis, 1988). Thus the challenging task for scientists is to reduce cholesterol in egg-yolk or in egg-yolk and blood serum of chickens. Recently, animal biotechnologists have done some research on supplementation of chromium (Uyanik *et al*., 2002), cupric sulphate pentahydrate (Pesti and Bakalli, 1998), garlic (Chowdhury *et al*., 2002), and tamarind (Chowdhury *et al*., 2005) to the laying hen diet. However, as far as information available, limited research work has yet been conducted to study the hypocholesterolemic effects of medicinal plant on laying hens. Thus the studies will be conducted to investigate the effect of some medicinal plant as well as unconventional feed ingredients on cholesterol and triglyceride concentration in serum and egg-yolk and meat of different poultry species.

Main advantage of using black pepper, Garlic and ginger over antibiotics is that they do not bear any risk regarding bacterial resistance or undesired residues in poultry products. Research work on medicinal plants and herbs as feed ingredient for poultry in Bangladesh is scanty as well as unknown and hence this study was designed to evaluate the impact of black peper, garlic and ginger on the performance and economics of production of poultry industry for reducing feed cost as well as healthy food for human consumption.

Therefore, present studies were undertaken with the following objectives:

i) To evaluate the effect of feeding Black pepper, Garlic and Ginger as a combine doses on egg production performances of laying hen.

ii) To determine cholesterol level in yolk after feeding black pepper, Garlic and ginger as a combine doses.

iii) To determine the egg quality characteristics for the birds after feeding black pepper, Garlic and ginger as a combine doses.

iv) To determine the in-vitro anti-bacterial properties of laying hen in supplementation of black pepper, Garlic and ginger as a combine doses.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Cholesterol and human health

High cholesterol levels in the diet have been linked with increased incidence of atherosclerosis (Friedman, 1968). Concern about the relationship between dietary fat and the development of atherosclerosis has led to publication of a number of reports encouraging changes in the human diet. These have included recommendations for the reduction in total fat content in the ratio of saturated to unsaturated fatty acids and in intake to total cholesterol to less than 300 mg/day (Brown, 1990; Cannon, 1990). The mysteries of the fatty protein, cholesterol, essential to the production of hormone and cell membranes, have become an obsession with many people today, especially those whose sedentary life styles make heart disease a distinct possibility. The role of High Density Lipoprotein (HDL) is to carry fat to the liver and that of Low Density Lipoprotein (LDL) is to move fat around the blood steram but can also build up a residue on the walls of blood vessels and eventually block HDL have a high risk of heart disease even if their combined cholesterol is low (Anonymous, 1994). The evidence correlating plasma cholesterol levels with coronary heart disease was established from early observations that cholesterol is a major component of the atherosclerotic plaque. In 1985, the recommendation of a consensus conference in America was "All American (except children under 2 years of age) be advised to adopt a diet that reduces total dietary fat intake from the current level of 40% of total calories to 30% of total calories, reduces saturated fat intake to less than 10% of total calories and reduces daily cholesterol intake to 200 to 250 mg or less" Naber (1976) and Noble (1987) stated that the reason for particular concern about eggs has a questionable scientific basis for the presence of substantial amount of cholesterol.

2.2 The problem due to cholesterol

2.2.1 Cholesterol and coronary heart disease

Cholesterol is a sterol required by the body for a number of functions including the maintenance of cell membrane flexibility and permeability and the production of sex hormones, cortical, vitamin-D and bile salts. The human body can synthesize cholesterol

but it is also found in some foods, particular in meats, poultry, dairy products and eggs. Dietary cholesterol can elevate levels of blood cholesterol, however, it is increasingly accepted that saturated fats and trans-fats can have a greater impact than dietary cholesterol in raising blood cholesterol levels generally and LDL cholesterol specifically. This is important because a high level of cholesterol (in particular LDL) in the blood is a major risk factor for CHD, which in turn can lead to a heart attack. As eggs are a rich source of dietary cholesterol and experimental evidence showed that dietary cholesterol increased serum cholesterol, the public were cautioned about eating eggs because of concerns about the associated risk of CHD. However, the correlation between dietary fat and cholesterol and plasma lipid concentrations had been the subject of many contradictory views and studies over the years. Epidemiology studies, such as the undertaken by Hu *et al* (1990) to examine the effect of egg consumption on the risk of cardiovascular disease, concluded that the consumption of up to one egg per day was unlikely to have a significant effect on the risk of CHD or stroke among healthy men or women. The authors did however note that there was a tendency for egg consumption to be associated with an increased risk of CHD if the participant was diabetic and suggested that this aspect should be considered further. In contrast, a meta-analysis conducted by Weggemans *et al*. (2001) on data covering the periol 1974-1999 led to the conclusion that dietary cholesterol did raise the ratio of total to HDL cholesterol and hence 'the advice to limit cholesterol intake by reducing the consumption of eggs and other cholesterol-rich foods may therefore still be valid. Similar findings, albeit only for women (there was no correlation for men), have been reported recently by Nakamura *et al* (2004). In contrast Song and Kever (2000) concluded that dietary consumers of more than four eggs/week had a significantly lower mean serum cholesterol concentration compared with those who reported eating less than or equal to one egg per week.

2.2.2 Cholesterol, animal fat, and heart disease

Numerous types of heart diseases contribute to the death toll of few million persons in the world every year; among them, hypertension, cerebro-vascular disease (stroke), congestive heart failure and atherosclerosis are common. Much attention has been given to the role of animal fats in atherosclerosis, a type of disease wherein a build-up of soft, amorphous lipids and connective tissue develops on the walls of the arteries of the heart. When these deposits become sufficiently large, clots may form and subsequently decrease the diameter of the arterial lumen. In some cases blood flow is greatly impaired

resulting a heart attack. Research indicated that individuals with high serum cholesterol levels had a higher rate of atherosclerosis. Increased serum cholesterol levels can be induced in susceptible individuals when animal fats which are highly saturated and foods high in cholesterol as in eggs are consumed. Thus, the hypothesis that cholesterol levels can be induced in susceptible individuals when animal fats which are highly saturated and foods high in cholesterol as in eggs are consumed. Thus, the hypothesis that cholesterol is responsible for heart disease becomes accepted by many as fact. In recent years, research has clearly indicated that this position is entirely too simplistic. For example, studies have shown that certain African tribes whose diets consist almost entirely of animal products do not have elevated serum cholesterol levels. It is fact that dietary fat is implicated in atherosclerosis, but must be realized from, further research that it is not the sole cause, rather, a number of factors enter into the cause of heart disease, many of which are more important than cholesterol; among them, stress, heredity, hypertension, diabetes mellitus, smoking, lack of exercise, and obesity. When the heart disease is correlated with the consumption of animal products one must also consider the benefits against the hazards. Countries with the highest life expectancies (70 to 72 years) such as, Sweden, Norway, Denmark, Japan, Israel and Switzerland are noted for their high egg production and per capita egg consumption. The nutrients supplied by eggs and meat provide well balanced nutrition, hence poultry products must not be eliminated from the diet. Rather, a well-planned diet, along with exercise and a minimum of stress provides the best prevention against heart disease. Bangladesh is a developing country. A great majority of her people suffers from protein deficiencies and per capita consumption of egg is very low. When we compare egg consumption in between rural and urban people, the urban people consume more eggs and they are the consumes of most of the eggs produced in this country. In fact, a part of entire population is facing many health problems and such as blood pressure, heart disease etc. which may be related to dietary cholesterol intake. Even the people who do not have any vascular disease or heart problem are showing a tendency to cut down egg intake in fear of any future problem.

2.3 Egg yolk composition

Hen egg yolk is a complex mixture of different micro particles held in suspension. The solids content of yolk is about 50%. Proteins and lipids are the major constituents of yolk accounting 15.7-16.6% and 32-35%, respectively (Powrire and Nakai, 1985). The

yolk fraction contains approximately 66% triglycerol, 28% phospholipids, 5% cholesterol and minor amounts of other lipids. It was estimated that the composition of yolk phospholipids is 74% phophatidylcholine (PC), 2.5% shingomyelin, 2.1% lysophosphatidylethanolamine (LPE), 0.9% plasmalogen, and 0.6% inositol phospholipids. Egg yolk is a homogeneously emulsified fluid (Juneja, 1997). When diluted with water or saline it can be separated by centrifugation into plasma (the supernatant) and granule (the precipitate). The granule consists mainly of high density lipoprotein (HDL) and phosvitin. The major component of plasma is low-density lipoprotein (LDL) accounting for 65% of the total egg yolk protein and livetin, which accounts for 30% of the plasma protein. The livetin fraction consist of α-,β-, χ- livetins in egg yolk, HDL consists of α- and β-lipovitellins and exists as a complex with phosvitin (Li-Chan *et al.*, 1995). Phosvitin is a phosphor-protein containing about 10% phosphorus. About 80% of the phosphorus in yolk exists in phosvitin. It has been shown that LDL composes 7 major polypeptides ranging from 19-225 kDa and some minor polypeptides by SDS-PAGE analysis (Minc, 1998).

2.4 Cholesterol level in plasma and egg yolk

Harris and Wailcox (1963) found an apparent lack of association between yolk and serum cholesterol (correlation values of -0.03 and 0.08). Marion *et al.*, (1960) reported a significant negative correlation (-0.29) between serum, and yolk cholesterol. Washburn and Nix (1974) observed that low (0.13 and 0.14) phenotypic correlation between plasma and yolk cholesterol in two random-bred chicken selected for low plasma cholesterol also had lower yolk cholesterol. Weiss and Scott (1979) reported that the overian synthesis of cholesterol is responsible for maintaining the level of cholesterol in the egg and that plasma cholesterol levels have little effect on egg cholesterol levels.

Kundu and Singh (1991) reported that quail egg contained 53.68+4.50 mg cholesterol/egg. The Japanese quail had the lowest cholesterol concentration (18.08+0.79 mg/g yolk). The differences in different breeds of chicken were not statistically significant. Between indigenous breeds, Aseel eggs contained more cholesterol (1.2 mg/g of yolk) than did Kadaknth eggs. Broiler IR-3 eggs contained 20.87 mg/g yolk or 407 mg/yolk. Marks and Washburn, (1990) reported that yolk cholesterol derived from plasma cholesterol making it logical to assume that a relationship should exist between yolk and plasma cholesterol levels. Sutton and Vavich (1958) and Daghir and Balloum

(1961) found that serum cholesterol levels were related to the sex of bird; the male sex having the higher serum cholesterol values. Hollands *et al* (1980) reported that chickens selected for low plasma cholesterol also had lower yolk cholesterol.

Vargas and Naber (1984) reported that egg yolk cholesterol was positively but not significantly associated with energy balance. Yolk cholesterol was significantly and positively correlated with body weight change (r= 0.23) which in turn was significantly and positively correlated with energy balance (r=0.23). A significant negative (P<0.01) relationship was found between yolk cholesterol and egg production (r=0.45).

2.5 Genetic selection for reduced egg cholesterol content

Although breed, strain, and/or age of the hen can influence egg yolk cholesterol contents in chickens, differences attributed to these variables were, in absolute terms, fairly minijmal and not to practical significance. Thus, selective breeding programs were undertaken in the 1970s by several groups in an attempt to produce low cholesterol eggs. Based on significant sire effects on yolk cholesterol levels and heritability estimates ranging from 0.14 to 0.22, Wash Burn and Nix (1974) suggested that sufficient genetic variability existed to allow for the possible alteration of yolk cholesterol content by selection. However, five subsequent studies showed that only small changes (< 8%) in this variable could be elicited by divergent selection for up to five generations. Rather than breeding for lower egg cholesterol content *per se*. an alternate approach to cholesterol reduction would to select for a lower proportion of yolk in the egg. Miyoshi and Mitusumoto (1980) found that selection for a higher yolk: albumen ratio was more effective than selection for a lower yolk: albumen ratio. Similarly, in a one generation divergent, selection study, Hartmann *et al*., (2000) reported that yolk weight was increased, while egg weight was decreased, in the 'high-line'. In contrast, egg weight remained fairly constant, while yolk weight decreased slightly, in the 'low line'. Thus, as was the case with increases the proportion of yolk, but not to decrease it Hartmann and Wilhemoson, 2001; Hartmann *et al*., (2003) Taken together, the lack of progress in selection for either reduced egg cholesterol content or a smaller proportion of yolk suggests, as previously concluded by Hargis (1988) and Naber, (1990), that a low-cholesterol egg will not result from single trait selection studies. The literature refiewed indicate that selection is not significant suitable to reduce the yolk cholesterol content;

so, bio-technical or then intensive study is necessary to get much information (Sarker, 1995).

2.6 Effect of Piper nigrum (Black pepper) in poultry

Black pepper (*P. nigrum*) is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus congestion, fever, intermittent fever, cold extremities, colic, gastric ailments and diarrhea. It has been shown to have antimicrobial activity (Perez and Anesini, 1994; Dorman and Deans, 2000). Both aqueous and ethanol extracts of black pepper screened for antibacterial activity against a penicillin G resistant strain of *Staphylococcus aureus,* showed antibacterial activity, which was determined by the agar-well diffusion method, using cephazolin as a standard antibiotic (Perez and Anesini,1994). Piperine, [1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2,4, pentadienyl piperidine, a pungent alkaloid present in *P. nigrum,* enhanced the bioavailability of various structurally and therapeutically diverse drugs. A concise mechanism of its bioavailability enhancing action is poorly understood. However, data suggests that piperine is absorbed very fast across the intestinal barrier; it may form non-polar complexes with drugs and solutes thus increasing permeability across the barriers (Khajuria *et al*., 1998). Piperine exerted significant protection against tert-butyl hydroperoxide and carbon tetrachloride hepatotoxicity in mice. Silymarin, a known hepatoprotective drug, was also tested simultaneously for comparison. Piperine showed lower hepatoprotective potency than silymarin (Koul and Kapil, 1993). Platel *et al*. (2002) showed that the spice mix of coriander, turmeric, red chilli, black pepper and cumin favorably enhanced the pancreatic lipase, chymotrypsin and amylase activity when consumed via diet. In addition, these spice mix brought about a pronounced stimulation of bile flow and bile acid secretion. Activities of pancreatic lipase, amylase and chymotrypsin were elevated by 40, 16 and 77%, respectively. The higher secretion of bile, especially with an elevated level of bile acids, and a beneficial stimulation of pancreatic digestive enzymes, particularly lipase, could be two mechanisms by which these combinations of spices aid in digestion and increased performace.

Black pepper and white pepper are made from the Piper nigrum plant. Black pepper is ground from dried, whole unripe fruit. White pepper is ground from dried, ripe fruit that has had the outer layer removed. The black pepper and white pepper powder are used to make medicine.

People take black pepper for stomach upset, bronchitis, and cancer. They take white pepper for stomach upset, malaria, cholera, and cancer. Black pepper is sometimes applied directly to the skin for treating nerve pain (neuralgia) and a skin disease called scabies. Black pepper and white pepper are also used topically as a counterirritant for pain. In foods and beverages, black pepper, white pepper, and pepper oil (a product distilled from black pepper) are used as flavoring agents.

Black pepper (*Piper nigrum*) is a flowering vine extracted from the core of a pepper plant, and belongs to the family Piperaceae, genus Piper and species Piper nigrum. Black pepper has been shown to be rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase (Karthikeyan and Rani, 2003). The antioxidant and radical scavenging properties of black pepper seeds have been well documented (Gülcin, 2005). Khalaf *et al*. (2008) showed that piperine can increase the absorption of selenium, vitamin B complex, beta-carotene and curcumin as well as other nutrients. Furthermore, it is an active alkaloid modulate benzopyrene metabolism through cytochrome P450 which is essential for metabolism and transport of xenobiotics and metabolites (Reen *et al*., 1996), enhances thermogenesis of lipid (Malini *et al.,* 1999), and increases the flow of digestive juice (Moorthy *et al*., 2009).

Black pepper (*Piper nigrum*) bioactive compounds such as piperine have different characteristics like antimutagenic properties (El Hamss *et al.,* 2003). Piperine is an active alkaloid that modulates benzopyrene metabolism through cytochrome P450 enzyme (CYP), which is important for the metabolism and transport of xenobiotics and metabolites (Reen *et al*., 1996) and depresses aflatoxin B1 toxicity by suppressing CYP mediated bioactivation of the mycotoxin (Singh *et al.,* 1994; Reen *et al.,* 1997). Brenes and Roura (2010) indicated that botanicals interactions need to be investigated due to the complexity in terms of the number and the variability of bioactive compounds, and the interactions between essential oils, and feasible synergistic effects to maximize or minimize the concentrations required to achieve a particular impact of the botanicals. The objective of the current study was to evaluate the effect of supplementary TRP and BP and their interaction on serum components and performance of male broiler chickens.

Essential oils from aromatic spice plants have been shown to be good antioxidants using various antioxidant assay models (Politeo, *et al*. 2006; Nakatami, 1997 and Puertas-Mej, *et al*, 2002) and the antioxidant activities have been linked to the phenolic contents in

some of the oils (Kulisic, *et al*, 2004 and Amiri, 2012). Furthermore, some medicinal properties of essential oils from aromatic spice plants have been established, especially essential oils from black pepper (*Piper guineense*) seeds which have been shown to have antimicrobial, antihypertensive, anticonvulsive, and sedative activities (Amiri, 2012 ; Neuwinger, 2000 and and Udoh, 1999). Black pepper (*Piper guineense*) is a spicy plant whose essential oils from the seed and leaves are being extracted and sold in commercial quantities in many countries (Douglas, 2005). Monoterpenes, benzoids, and sesquiterpenes have been identified among the volatile compounds of the black pepper (Jirovetz, *et al*, 2001).

2.7 Effect of Garlic (*Allium sativum*) in poultry

Garlic (*Allium sativum*) and its products are known to have potential hypolipidemic/hypocholesterolemic, hypotensive, hypoglycemic, hypothrombotic and hypoatherogenic properties.1–4 Garlic contains a variety of organosulfur compounds such as allicin, ajoene, *S*-allylcysteine, diallyl disulfide, *S*-methylcysteine sulfoxide and *S*-allylcysteine sulfoxide.5 Despite the fact that the mechanisms primarily responsible for the hypocholesterolemic action of garlic are uncertain at present, the composition and quantity of

the sulfur components of different garlic preparations used in various studies could account in part for the inconsistent findings.6 Other contributing factors may include subject recruitment, duration of experiment, dietary control, lifestyle and methods of lipid analysis.3,4 *S*-Methylcysteine sulfoxide7,8 and *S*-allylcysteine sulfoxide8 had a potent antihypercholesterolemic effect on cholesterol-fed rats. However, previous studies with laying hens and broilers showed controversial results on the hypocholesterolemic effect of garlic.9–13 In experiments with diets containing garlic paste at 38 g kg−1 or solvent fractions or garlic oil equivalent to 38 g kg−1 garlic paste, the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase decreased by 50–69% in 12-week-old broilers and by 72–83% in 12-week-old Leghorn pullets, with concomitant decreases in serum cholesterol of 7–25 and 20–25%, respectively.9 Egg yolk cholesterol was reduced by the feeding of 10 and 30 g kg−1 garlic powder to laying hens for 3 weeks at the level of 5.45 and 4.10mgg−1 yolk, respectively.14 Birrenkott *et al*.,12 however, reported that 30 g kg−1 garlic powder did not have any significant effect on yolk and serum cholesterol concentrations when laying hens were fed diets for 8 months. Reddy *et*

*al.*10 reported that egg production, egg weight, feed efficiency, total plasma lipids, plasma cholesterol and yolk cholesterol were not affected during or at the end of 8weeks of feeding garlic oil to layers. Chowdhury *et al.*(2005) concluded that sun-dried dietary garlic paste at up to 80 g kg−1 can be used as a hypocholesterolemic agent in practical layer diets.

Garlic (Allium sativum) is widely distributed and used in all parts of the world. Several clinical reports, including meta-analyses, have described the hypocholesterolemic effect of garlic in human (Warshafsky *et al*., Silagy and Neil, 1994). Animal studies suggest that garlic has potential hypolipidemic, hypoglyeemic, hypotensive and hypothrombotic properties (Bordia *et al*., 1975; Shoetan *et al*., 1984). Many studies indicated that allicin was the potentially active component of garlic which inhibits the growth of pathgenic bacteria (Samanta and Dey, 1991). Allicin is sulphur containing compound (thio-2-propene-1sulfinic acid S-allyl ester) and its production from an odorless precursor alliin, is catalyzed by an enzyme, alliinase or alliin ltase and responsible for the characteristic small of garlic (Yeh and Liu, 2001). Egg yolk cholesterol was reduced by feeding of 1% or 3% garlic powder to laying hen hens for 3 week. (Sharma *et al*., 1979). Sklan *et al*. (1992) reported decreased hepatic cholesterol concentration in chicken when 2% garlic was fed for 14 days. However, Birrenkott *et al*, (2000) reported that 3% garlic powder, did not have any significant effect on egg yolk and serum cholesterol concentrations when hens were fed diets for 8 months.

Some studies, however, suggested that commercial garlic oil, garlic powder and commercially available garlic extract may not be hypocholesterolemic (Issacsohn *et al*., 1998; Mccrindle *et al*., 1998). Although the reason for this is unknown, it likely relates to low level of garlic, preparation methods.

Garlic has acquired a reputation in the folklore of many cultures as a therapeutic agent (Amagase *et al*., 2001). According to folk medicine, garlic was used to treat cardiac disease (Essman, 1984). Several clinical reports, including meta-analyses, have described the hypocholesterolemic effect of garlic in humans (Silagy and Neil, 1994; Warshafsky *et al*., 1993). Some studies, however, suggested that commercial garlic oil, garlic powder, and commercially available garlic extract may not be hypocholesterolemic (Berthold *et al*., 1998; Isaacsohn *et al*., 1998; McCrindle *et al*., 1998). Although the reason for this is unknown, it likely relates to preparation methods, the stability of

chemical components, and the duration of the study (Amagase *et al*., 2001). Many studies indicated that allicin was the potentially active component of garlic; however, it was observed that allicin was unstable and poorly absorbed from the digestive tract (Lawson *et al*., 1992). Animal studies suggest that garlic has potential hypolipidemic, hypotensive, hypoglycemic, hypothrombotic, and hypoatherogenic properties (Bordia *et al*., 1975; Shoetan *et al*., 1984). Garlic paste (3.8%), solvent fractions, or garlic oil equivalent to this amount reduced serum cholesterol by 18 and 23% in broilers and 12-wk-old Leghorn pullets, respectively, when diets were fed for 4 wk (Qureshi *et al*., 1983b). Experiments in which garlic was fed to 5-wk-old male broilers for 3 wk and in vitro studies with chicken hepatocytes exposed to polar fractions of garlic powder (garlic equivalent to 1, 2, 4, 6 and 8% fresh garlic paste) showed a dose-dependent inhibition of hepatic β-hydroxy-β-methylglutaryl coenzyme A (HMGCoA) reductase, cholesterol 7α-hydroxylase, and fatty acid synthetase (Qureshi *et al*., 1983a). Egg yolk cholesterol was reduced by the feeding of 1 or 3% garlic powder to laying hens for 3 wk (Sharma *et al*., 1979). Sklan *et al*. (1992) observed depressed hepatic cholesterol concentrations in chickens when 2% garlic was fed for 14 d. Birrenkott *et al*. (2000), however, reported that 3% garlic powder did not have any significant effect on yolk and serum cholesterol concentrations when laying hens were fed diets for 8 mo. Garlic was also reported to enhance performance of laying hens and improve egg quality when supplemented in diet (Lim *et al.,* 2006; Yalcin *et al.,* 2006; Khan *et al.,* 2007). However, the reports on garlic influence on hen performance and egg quality are inconsistent (Yalcin *et al.,* 2006; Khan *et al.,* 2007) and its effects on gut bacterial load of laying birds have not been evaluated. Reddy *et al*. (1991) reported that egg production, egg mass, BW, feed intake, and feed efficiency were not affected during the 8 wk that 0.02% garlic oil was fed to the Babcock B-300 strain. Egg yolk cholesterol concentrations have been shown to vary depending on the genetic strain of the laying hens (Han and Lee, 1992).

Sarjaz, et. al., (2006) reported that addition of plant sterols to laying hen diet affects circulating cholesterol and may reduce egg cholesterol as well. Chowdhury, et. al., (2002) reported that after inclusion of garlic powder in different strain of laying died 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 ($P<0.05$). It was

concluded that garlic paste in the diets of laying hens reduced serum and yolk cholesterol concentrations. It was also concluded that dietary garlic paste had no adverse effects on layer performance. Azeke and Ekpo, (2009) conducted an experiment to evaluate the effect of garlic and tea on the performance, egg traits and laying parameters of laying hens. They found that all the garlic supplemented feeds resulted in significant reductions ($P < 0.05$) of total cholesterol, total triglyceride, LDL- and HDL-cholesterol. With exception of the 1% tea supplemented diet, the other tea supplemented diet resulted in significant reductions in the egg yolk concentration of the cholesterols tested. 1% tea supplementation had no significant effect on LDL-cholesterol concentration of egg yolk ($P > 0.05$). The combination of garlic and tea resulted in significant reductions of total- LDL- and HDL-cholesterol ($P < 0.05$) but not total triglycerides ($P > 0.05$). The control diets had in most cases non-significant effects on the lipid parameters tested. The results show that garlic and tea have great potential when low cholesterol egg is desired.

2.8 Effect of Ginger (*Zingiber officinale* Roscoe) in poultry

Aromatic, pungent and spicy, ginger adds a special flavor and zest to Asian stir fries and many fruit and vegetable dishes. Fresh ginger root is available year round in the produce section of your local market. Ginger is the underground rhizome of the ginger plant with a firm, striated texture. The flesh of the ginger rhizome can be yellow, white or red in color, depending upon the variety. It is covered with a brownish skin that may either be thick or thin, depending upon whether the plant was harvested when it was mature or young.

Historically, ginger has a long tradition of being very effective in alleviating symptoms of gastrointestinal distress. In herbal medicine, ginger is regarded as an excellent *carminative* (a substance which promotes the elimination of intestinal gas) and *intestinal spasmolytic* (a substance which relaxes and soothes the intestinal tract). Modern scientific research has revealed that ginger possesses numerous therapeutic properties including antioxidant effects, an ability to inhibit the formation of inflammatory compounds, and direct anti-inflammatory effects. A clue to ginger's success in eliminating gastrointestinal distress is offered by recent double-blind studies, which have demonstrated that ginger is very effective in preventing the symptoms of motion sickness, especially seasickness. In fact, in one study, ginger was shown to be far superior to Dramamine, a commonly used over-the-counter and prescription drug for motion sickness. Ginger reduces all symptoms associated with motion sickness including

dizziness, nausea, vomiting, and cold sweating.

Ginger (*Zingiber officinale* Roscoe, *Zingiberacerae*) rhizome (ginger root) is widely used as a spice and in practice of traditional Chinese herbal medicine (Masuda *et al*., 2004; Tapsell *et al*., 2006). Ginger and its main compounds have shown various pharmacological effects including immunomodulatory, antitumorigenic, antiinflammatory, antiapoptotic, antihyperglycemic, antilipidemic and antiemetic effects (Ali *et al*., 2008).

However, information is lacking on the effect of ginger as a feed additive on laying performance and antioxidant status of laying hens and on dietary oxidation stability. Ginger has been reported to enhance ani- mals' nutrient digestion and absorption because of the positive effects on the gastric secretion, enterokinesia, and digestive enzyme activities (Platel and Srinivasan, 2000). Furthermore, ginger contains several compounds such as gingerol, shogaols, gingerdiol, gingerdione, and some relating phenolic ketone derivatives (Kikuzaki and Nakatani, 1996; Fuhrman *et al*., 2000) that possess antioxidant activity.

Zinziber officinale (*Z. officinale*) has been shown to have antimicrobial activity (Habsah *et al*., 2000; Srinivasan *et al*., 2001). Ethanolic extract of the rhizomes of *Z. officinale* showed significant inhibition of growth of both certain gram-positive and gram-negative bacteria. It also displayed antiinflammatory, analgesic, antipyretic and antimicrobial activities. In rats, the extract reduced carrageenaninduced paw swelling and yeast-induced fever. The extract reduced blood glucose in rabbits (Mascolo, 1998). The essential oils of *Z. officinale* showed antimicrobial activity against gram-positive and gramnegative bacteria using the agar diffusion method (Martins *et al*., 2001). Toxicity studies conducted on *Z. officinale*, used as aphrodisiacs in Arab Medicine showed no toxicity during acute toxicity test. The percent lethality was insignificant as compared to the control (Qureshi *et al*., 1999). The safety and efficacy of herbal remedies is a concern for many people. Ginger, when subjected to clinical trials among pregnant women, was found clinically effective against chemotherapy-induced nausea and vomiting. While safety concerns exist in the literature for this herb with regards to its use by pregnant women, no clinical evidence of harm was observed (Westfall, 2004). Methanol extract of the dried powdered ginger rhizome and the isolated constituents, 6-, 8-,10-gingerol and 6-shogoal were tested against 19 strains of *H. pylori.* It inhibited growth of all 19 strains

in vitro with a minimum inhibitory concentration range of 6.25-50 µg/ml. The crude extract, containing gingerols, inhibited the growth of all strains of *H. pylori* with an MIC range of 0.78 to 12.5 µg /ml and with significant activity against the CagA+ strains (Mahady *et al*., 2002). The extracts of ginger exhibited antibacterial activity against the pathogens *S. aureus*, *S. pyogenes*, *S. pneumoniae* and *H. influenzae*. The MIC of extracts ranged from 0.0003 µg/ml to 0.7 µg/ml for ginger, while MBC ranged from 0.135 µg/ml to 2.04 µg/ml for ginger. Results indicated that extracts of ginger and *Garcinia kola* roots may contain compounds with therapeutic activity (Akoachere *et al*., 2002).

2.9 Effect of other medicinal plant in poultry

2.9.1 Antimicrobial activity

Earlier studies indicate that many plant extracts have antimicrobial activity. According to Almas (1999), the extracts of *Azadirachta indica* (neem plant) chewing sticks are effective against *Streptococcus mutans* and *Streptococcus faecalis.* Chewing sticks are recommended as oral hygiene tools for health promotion in developing countries. Hayat *et al.* (2004) studied the in vitro antimicrobial activity of *Zizyphus vulgaris* root extract against both gram positive and gram negative organisms using *Staphylococcus aureus* and *Escherichia coli*, respectively. Three different concentrations of the ethanolic extract of the roots were used and the activity compared with the standard antibiotics. All the concentrations showed excellent inhibitory effect on the growth of gram positive and gram negative microorganisms. It is evident, however, that in practice most individual herb or spice extracts must be included at a high concentration to observe effects comparable to those of antibiotics. This is only logical as many extracts contain a multitude of active substances. The *Origanum vulgare* is described as containing more than 30 antibacterial chemicals. Akilandeswari *et al.* (2003) tested aqueous neem extract prepared from the *Azadirachta indica* bark against the strain of bacteria *Proteus vulgaris* and fungi *Candida albicans*, to examine its efficacy as an antimicrobial agent. The growth inhibitory property of the aqueous extract was recorded in terms of zones of inhibition measured in 24 hours growth cultures using disc plate technique. The growth of *Proteus vulgaris* and *Candida albicans* was inhibited remarkably due to aqueous neem bark extract. Out of these two organisms tested in the experiment, the bacteria *Proteus vulgaris* showed more susceptibility to neem bark extracts in comparison with fungi *Candida albicans*.

2.9.2 Antioxidant properties

Oxygen is one of the most important element for life, growth and metabolism of living organisms. Autooxidation process results in the destruction of important molecules in diet formulations and also damages cellular tissues in living organisms. Therefore, autooxidation results in the formation of reactive oxygent species and causes different kinds of diseases. Flavonoids and phenoic acids are widely present in higher plants. These compounds are effective against the deleterious effect of reactive oxygent species. According to Middleton and Kandaswami (1993), some compounds found in Ocimum plant have been reported to possess strong antioxidant activity. Cinnamon has antioxidant characteristics (Middleton and Kandaswami, 1993). Cinnamon extracts show antioxidant activity which is comparable to synthetic antioxidants, beta hydroxy toulene.

2.9.3 Anticarcinogenic activity

It is reported that leaves of *Ocimum tenuiflorum* possess anticancerous properties. Samresh *et al*. (2003) found that Ocimum suppressed benzo pyrine induced chromosomal aberrations in bone marrow and elevated glutathione (GSH) and glutathione-S-transferase (GST) activities in liver of mice. They also reported a suppressing effect of the plant on chemically induced hepatomas in rats and tumors in the fore-stomach of mice. Studies in mouse have also indicated the presence of flavonoids in Ocimum leaf extract. Flavonoidenriched diet has a preventive effect on cancer, coronary heart disease and strokes. Thus, Ocimum can play a definite role in developing a cancer preventive drug.

2.9.4 Analgesic and antipyretic activities

Godhwani and Godhwani (1987) conducted studies by using methanol extract and aqueous suspension of leaves of *Ocimum tenuiflorum* on albino rats. The methanol extract (in doses of 100, 250 and 500 mg/kg) showed analgesic activity in mice as evaluated by the mean time taken to withdraw tail when brought in contact with the hot plate. Methanol extract had more analgesic activity than the aqueous suspension. The analgesic activity was attributed to amino acids resembling creatine and isoleucine, which have been reported to be analgesic.

2.9.5 Insecticidal properties

Some herbs, especially neem, have strong insecticidal activity. The Meliaceae, especially *Azadirachta indica* (Indian neem tree) contains at least 35 biologically active principles (Mulla, 1999). Azadirachtin is the predominant insecticidal active ingredient in the seed, leaves and other parts of the neem tree. Azadirachtin and other compounds in neem products exhibit various modes of action against insects such as antifeedancy, growth regulation, fecundity suppression and sterilization, oviposition repellency or attractancy, changes in biological fitness and blocking fitness, and blocking development of vector-borne pathogens. Some of these bioactivity parameters of new products have been investigated at least in some species of insects of medical and veterinary importance, such as mosquitoes, flies, triatomines, cockroaches, fleas, bees and others. Neem works as a repellent by disrupting the appetite of insects and diminishing their urge to reproduce.

The greatest advantage to pest control with neem is the fact that it does not harm useful insects such as ladybirds, wasps and earwigs. Additionally, neem is benign to spiders and plant pollinators such as bees and wasps. Unlike most chemical pesticides that contain poisonous groups of nitrogen, chlorine, phosphorus and sulpher in their molecules, and are potentially hazardous, neem has been found to have little or no mammalian toxicity. Furthermore, in all scientific trials conducted to date, neem deters insects as effectively and economically as DDT and other synthetic pesticides

2.9.6 Anticoccidial activity

The herbs especially *Azadirahta indica, Hobrrhena antidysentrica, Barberis aristata, Embelia ribes, Acorus calamus* and *Artemisia annua* have strong anticocidial activity. Zycox, a herbal product of India containing *Hobrhena antidyseatria, Barberis aristata, Embelia ribes* and *Acorus calamus,* is used as a prophylactic measure against coccidiosis. Guha *et al.* (1991) observed that Zycox treated birds showed 3% mortality as compared to infected group.

According to Singh *et al.* (1991), Zycox at 0.3% in feed offers a convenient, effective and economical indigenous alternative for prophylactic medication against coccidial infection in chicken. It causes least interference to the natural development of immunity and is safe and not likely to induce resistance. Tipu *et al.* (2002) compared the

anticoccidial efficacy of salinomycin sodium and neem fruit in boilers. They concluded that the addition of 0.3% ground neem fruit in boiler feed has tremendous efficiency in combating coccidiosis as compared to salinomycin sodium (Table 2). They reported that neem fruit had compound margosate, responsible for the break down of Eimeria life cycle. Similarly, Allen *et al.* (1997) investigated the effect of feeding dried *Artemesia annua* leaves and its components to birds infected with Eimeria *acervulina*, *E. tennela* or *E. maxima.* When fed at a dose rate of 1% for 5 weeks prior to infection, significant protection was noted for both *E. tenella* and *E. acervulina*. Artemesia contains artemisinin which protected weight gains and reduced oocyst yields for both *E. tenella* and *E. acervulina*. According to Youn-Hee Jeong *et al.* (2001), the sophora flavescens extract was the most effective for survival rates, controlling bloody diarrhoea symptoms, lesion scores, body weight gains and oocyst excretion in the faeces

2.9.7 Weight gain and feed consumption

Previous literature shows that use of herbs in animal feed improved the weight gain of animals. These can be used simultaneously for treating parasitic diseases as well as increasing the weight gain and act as growth promoters. Kudke *et al*. (1999) fed calves on green fodder supplemented with or without powdered neem leaves (0, 5 or 10 gm daily) for 12 weeks. Faecal samples were examined fortnightly for coccidia, cestodes and nematodes. Significant differences in growth rate were observed between the treated and control groups. Daily rate of growth was 0.268, 0.346 and 0.400 Kg for groups treating with 0, 5 and 10 gm neem leaves daily, while daily dry matter intake was 2.09, 2.14 and 2.21 kg, respectively. Inclusion of neem leaves powder resulted in an increase in total feed intake by 5.7%. The control group was more prone to parasite infections compared with neem treated groups. Neem works as a growth promoter by killing parasites that hinder the growth of animal. The mature tree of *Azadirachta indica* (Neem) plant can produce 350 kg of leaves a year, which may be used for feeding cattle during famines. After the oil has been pressed out from the seeds of neem, the cake is used as fertilizer but it can also be used as feed. Kudke *et al.* (1999) concluded that upto 10% neem cake may be included in concentrates for cattle and upto 5% for poultry. Chemical composition and digestibility of neem is shown in Table 3. Hayat *et al.* (1996) studied comparative prophylactic effects of indigenous preparations of bakin (*Melia azadarach*) and kerala (*Momordica charntia*) in comparison with the salinomycin against coccidiosis in broiler chicks. Ninety day-old chicks were divided into five groups (salinomycin,

bakin, kerala, infected untreated and uninfected untreated), each comprising of 18 birds. The chicks were inoculated with mixed species of coccidia at the age of one month. The results revealed higher ($P<0.05$) weight gain in the birds using salinomycin and those of uninfected untreated groups. Addition of salinomycin, bakin and kerala in the ration markedly reduced the number of oocysts per gram of faeces from 50,000 to 1730, 3323 and 3669, respectively.

Mandal et *al.* (1992) studied the anticoccidial efficacy of Zycox at three different dose levels (0.3, 0.45 and 0.6%) in feed against *Eimeria necatrix* infection in broiler chicks. The performance index clearly depicted its efficacy at these dose levels. The efficacy was found to be higher in higher dose levels. The effect of medication on the development of immunity was also evaluated. The calculated immunity index coupled with survival (%), mean weight gain (%) and lesion score protection (%) conferred sufficient justification to conclude that the product had no interference with the development of immunity. The results showed that Zycox was effective against *E. necatrix* at all 3 dose levels.

Islam *et al*., (2011) conducted an experiment and reported that *N. sativa* supplemented diet had no significant effects on feed intake, body weight, egg laying performances, and physical properties of eggs of the hens, however, significantly ($P<0.05$) decreased both serum triglycerides (about 70%) and egg cholesterol (about 43%) contents (up to 3.0% supplementation). Interestingly, *N. sativa* supplementation also significantly suppressed (about 25%) the population of harmful intestinal bacteria such as *Escherichia coli*. Our results suggest that *N. sativa* seed might have potential as an alternative to synthetic feed additives to formulate low cost and environment-friendly diet for the laying hens for low cholesterol eggs.

Ashayerizadeh *et al*. (2009) reported that the lowest abdominal fat percent and serum cholesterol and triglycerides levels were recorded for broilers fed the diet supplemented with Biolex-MB and garlic powder, While, the highest High Density Lipoprotein (HDL) was recorded for birds fed diet supplemented with garlic powder. Ansari, *et al*. (2008) reported that medicinal plant especially Withania somnifera, Nigella sativa and Azodirachta indica can be used as growth promoters in the poultry diets with better production performance.

Tipu *et al*. (2006) reported that medicinal plants act as antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal, anticoccidial and growth promoters. These plant extracts compete with the synthetic drugs. Majority of medicinal plants do not have the residual effects. *Azadiracht indica, Zizyphus vulgaris, Ocimum gratissimum* and *Atlanta monophylla* have the strong antibacterial activity, whereas ocimum plant has strong antioxidant, anticarcinogenic, antifungal, analgesic and antipyretic properties. Leaves of *Azadirachta indica* are used for feeding and reducing the parasitic load of animals. The fruit of *Azadirachta indica* also has the anticoccidial activity for poultry.

Andallu (2001) have showed oral administration of mulberry leaves powder cause decrease in blood and urine glucose, TG, LDL-cholesterol and VLDL-cholesterol and Fatty acids in type-2 diabetic patients. Qureshi *et al*. (1983) reported that garlic paste (3.8%), solvent fractions, or garlic oil equivalent to this amount reduced serum cholesterol by 18 and 23% in broilers and 12-wk-old Leghorn pullets, respectively, when diets were fed for 4 wk. Narayana and Setty (1977a) studied that incorporation of shade-dried mulberry leaves in layers' mash to the extent of 6 percent showed an increase in egg production with desirable yolk colour without any adverse effect on body weight and egg quality.

2.9.8 Ancient use of medicinal herbs

Culinary herbs and their essential oils have been used extensively for many years in food products, perfumery, and dental and oral products due to their different medicinal properties (Suppakul *et al*., 2003). However, secondary plant metabolites are largely unexploited in 'conventional' animal production systems. In the past, plant metabolites were generally considered as a source of antinutritional factors. Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers (Greathead, 2003). In contrast to their regulated status in India, China, and other countries, herbal medicines are regarded as dietary supplements for humans in the US and are widely used. It is reported that approximately one quarter of adults used herbs to treat a medical illness within the past year in the US (Bent and Ko, 2004). Herbs contain some complicated mixtures of organic chemicals that may vary depending upon many factors related to the growth, production, and processing of the herbal product. Though herbs with antimicrobial properties are reported, their use in broiler diets has not been studied extensively.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

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3.1 Experimental site

This study was conducted from January-April, 2015 at the poultry shed under dairy and poultry science department, Hajee Mohammad Danesh Science and Technology University, Dinajpur to investigate the effects of feeding three medicinal herb extract (black peper, garlic and ginger) to layer hen on their cholesterol metabolism, antibacterial activity and productivity. In this feeding trial, total 4-month experiment period, 60 Hi-sex brown laying hens (age 20 weeks) were assigned to five dietary treatments with three replication of four (4) birds in each.

3.2 Collection and Preparation of Black pepper, Ginger and Garlic powder and test feed

Black pepper, Ginger and Garlic were collected from the local area of Dinajpur district. The Ginger and Garlic were initially cut into small pieces and then sun-dried for about fifteen (15) days. The sun-dried Black pepper, Ginger and Garlic were milled into a powder. The diets were formulated to as per recommendation of the National Research Council (NRC, 1994) to satisfy the nutrients requirement of the laying hens. Diets were supplied with T_0 (control), T_1 (Black pepper 0.5gm/kg, Ginger 1.00 gm/kg and Garlic 1.00 gm/kg), T_2 (Black pepper 1.00 gm/kg, Ginger 1.50 gm/kg and Garlic 1.50 gm/kg), T_3 (Black pepper 1.50 gm/kg, Ginger 2.00 gm/kg and Garlic 2.00 gm/kg) and T_4 (Black pepper 2.00 gm/kg, Ginger 2.50 gm/kg and Garlic 2.50 gm/kg) sun-dried Black pepper, Ginger and Garlic powder mixed meal.

3.3 Preparation of extracts

First the sample was dried and ground in a grinding machine without loss of active components. The sample was taken in separate non-metallic jar and was added one litre of hot water, kept it at room temperature overnight following the procedure mentioned by Liela (1977). This aqueous mixture was mixed in drinking water.

3.4 Preparation of bird

The Hajee Mohammad Danesh Science and Technology University Poultry farm was used for this study. The experimental poultry cages constructed with compartments for housing two birds in each cage. The cages and the poultry house were first of all disinfected. Two troughs were placed in the cages for feeding and drinking water respectively. Total sixty (60) Hi-sex brown laying birds were used for trial.

Fig. 3.1: Laying cage with experimental birds

3.5 Experimental period

The experiments were performed for four months of period from January to April 2015.

3.6 Experimental diets

The experimental diets in mash form and drinking water were provided *ad libitum.* All diets were formulated manually to meet nutrient requirements. The chemical composition of experimental diets is shown in the Table 3.1.

The experimental diets were designed as

T_0: Control

T_1: (Black pepper 0.5 gm/kg, Ginger 1.00 gm/kg and Garlic 1.00 gm/kg),

T_2: (Black pepper 1.00 gm/kg, Ginger 1.50 gm/kg and Garlic 1.50 gm/kg),

T_3: (Black pepper 1.50 gm/kg, Ginger 2.00 gm/kg and Garlic 2.00 gm/kg)

T_4: (Black pepper 2.00 gm/kg, Ginger 2.50 gm/kg and Garlic 2.50 gm/kg)

Table 3.1 Chemical composition of experimental diets

Feed ingredients	Dietary level of Black pepper, ginger and garlic				
	T_0	T_1	T_2	T_3	T_4
Maize (Kg)	53	53	53	53	53
Soybean meal (Kg)	22.6	22.6	22.6	22.6	22.6
Rice polish (Kg)	11.5	11.21	11.04	10.86	10.67
Meat & bone meal (Kg)	4.00	4.00	4.00	4.00	4.00
Oyster shell (Kg)	7.8	7.8	7.8	7.8	7.8
DCP (Kg)	0.75	0.75	0.75	0.75	0.75
Protein concentrate (Kg)	0.50	0.50	0.50	0.50	0.50
Black peppers (gm)	0.00	50gm	100gm	150gm	200gm
Garlic (gm)	0.00	100gm	150gm	200gm	250gm
Ginger (gm)	0.00	100gm	150gm	200gm	250gm
Salt (Kg)	0.350	0.350	0.350	0.350	0.350
Vitamin-mineral premix*	*	*	*	*	*
Calculated composition:					
ME (Kcal/Kg)	2766.8	2742.3	2745.6	2756.0	2752.5
CP (%)	17.8	17.4	18.0	17.9	18.2
CF (%)	3.32	3.44	3.27	3.50	3.23
Ca (%)	3.52	3.54	3.55	3.49	3.51
P (%)	0.45	0.47	0.54	0.52	0.56
Lysine (%)	0.94	0.96	0.92	0.98	0.88
Methionine (%)	0.34	0.31	0.33	0.29	0.30

*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.

Here, T_0: Control, T_1: (Black pepper0.5gm/kg, Ginger 1.oogm/kg and Garlic1.00gm/kg), T_2: (Black pepper 1.00 gm/kg, Ginger 1.50gm/kg and Garlic 1.50gm/kg), T_3: (Black pepper 1.50gm/kg, Ginger 2.oogm/kg and Garlic 2.00gm/kg), T_4: (Black pepper 2.00gm/kg, Ginger 2.5ogm/kg and Garlic 2.50gm/kg)

Fig. 3.2: Medicinal herbs extract (black peper, garlic and ginger)

3.7 Data Collection and Record Keeping

During the experimental period, eggs were collected and weighed daily. Data on feed intake were collected weekly. Initial and final body weights of birds were taken. The eggs used in the experiment were collected per hen on day zero (0) and after 28 days interval up to four months. Egg production recorded daily but external and internal quality characteristics of eggs were determined monthly.

3.8 Observation of internal and external egg qualities

Egg qualities were measured from those eggs laid by birds of different diets group. Measured egg qualities were egg weight, shape index, shell dry weight, shell thickness, albumen index, fresh albumen weight, yolk index, fresh yolk weight, and Haugh unit. 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 a slide calipers. The eggs were then carefully broken down on a glass plate (40 x 20 cm) to determine the internal egg qualities.

3.8.1 Egg shape index determination

The shape index calculated for each egg from the width and length of the eggs using the formula derived by Reddy *et al*. (1979). The formula used for calculating the shape index is given below-

$$\text{Egg shape index} = \frac{\text{Av.width of egg}}{\text{Av.length of egg}} \times 100$$

3.8.2 Albumen index determination

The albumen index was determined by dividing the height of thick albumen by the width of thick albumen (Heiman and Carver, 1936). The albumen index was then calculated by the following formula-

$$\text{Albumen index} = \frac{\text{Av.height of albumin}}{\text{Av.diameter of albumin}}$$

3.8.3 Yolk index determination

The yolk index was calculated as the ratio of yolk height to yolk width without removing the yolk from the albumen (Wesley and Staldelman, 1959). The yolk index was calculated by the following formula-

$$\text{Yolk index} = \frac{\text{Av.height of yolk}}{\text{Av.width of yolk}}$$

3.8.4 Haugh unit determination

The haugh unit was calculated for each egg from the weight and albumen height using the formula suggested by Haugh (1937).

$$HU = 100 \text{ Log } (H + 7.57 - 1.7 W^{0.37})$$

Where, HU = Haugh unit

H = Height of thick albumen

W = Egg Weight (gm)

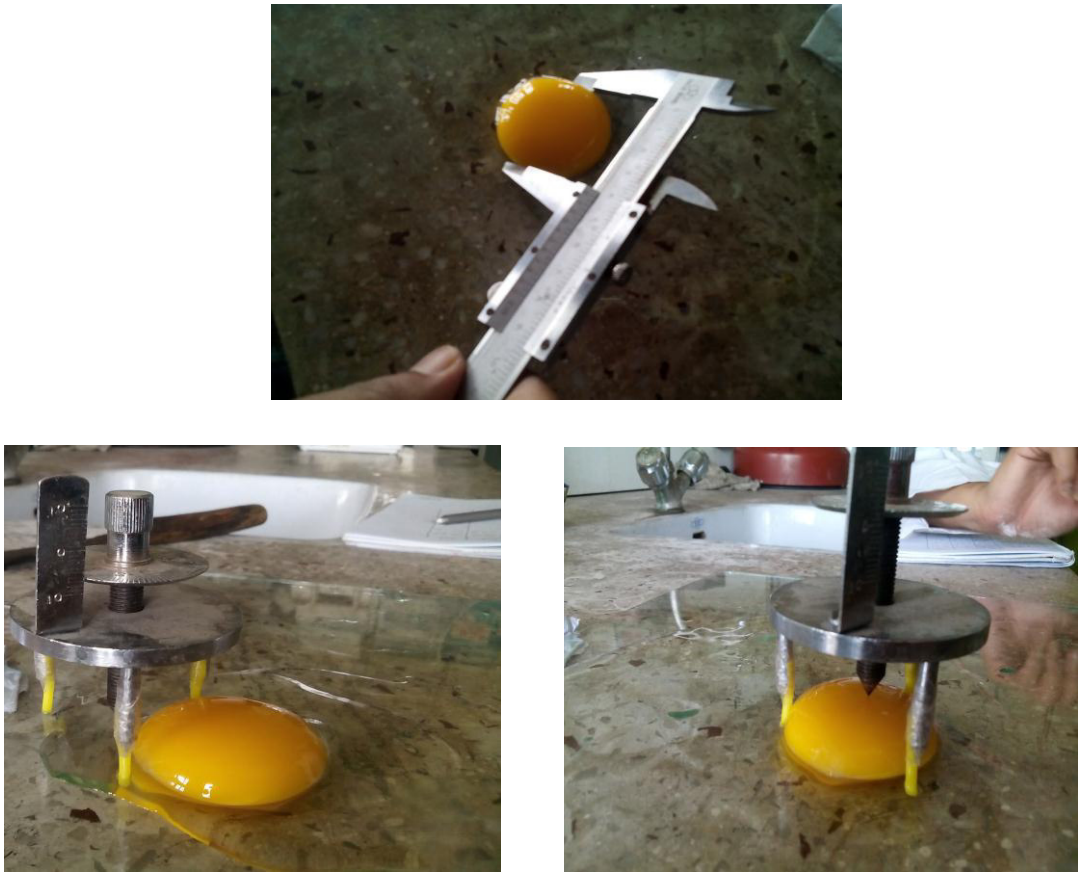

Fig. 3.3: Observation of internal and external egg quality

3.8.5 Shell thickness

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

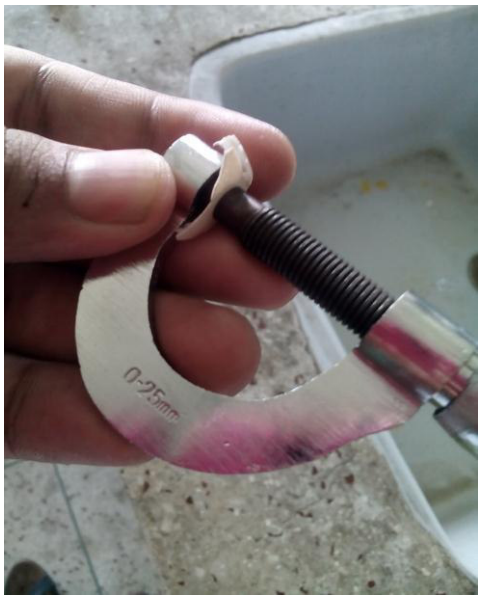

Fig. 3.4: Measurement of shell thickness

3.8.6 Weight of different egg components

The method outlined by Chowdhury (1988) was followed for partitioning different egg components. At first, egg was broken on glass plate. Then the yolk was separated carefully from albumen with the help of a spatula and transferred to a previously weighed petridish and the raw yolk weight was taken. The albumen was also transferred to a previously weighed petridish by a spatula and weighed. Precautions were taken at all stages to avoid rupture of yolk. The shells of the broken eggs were rinsed and washed thoroughly in tap water keeping the membranes intake. The washed shells with membranes were immersed in a beaker of water for removal of the shell membranes. The shell and shell membranes were oven dried separately at 105°C over night keeping them in a glass petridish. On the following day, oven dried shell and shell membranes were cooled in room temperature. Weight of shell and shell membranes were taken. Finally, the following calculations were made for different components suggested by Chowdhury (1988).

1. Fresh yolk weight:

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

2. Fresh albumen weight:

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

3. Shell dry weight:

(Weight of dried shell + weight of blotting paper) - Weight of blotting paper.

3.9 Chemicals/Kit

a) Kits (CRESCENT diagnostic)

b) Jeddah, K.S.A.

for the estimation of total cholesterol, HDL-cholesterol and Triglycerides.

3.10 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.

3.10.1 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.

3.10.2 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 (1gm) 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 (2:1v/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.3.2).

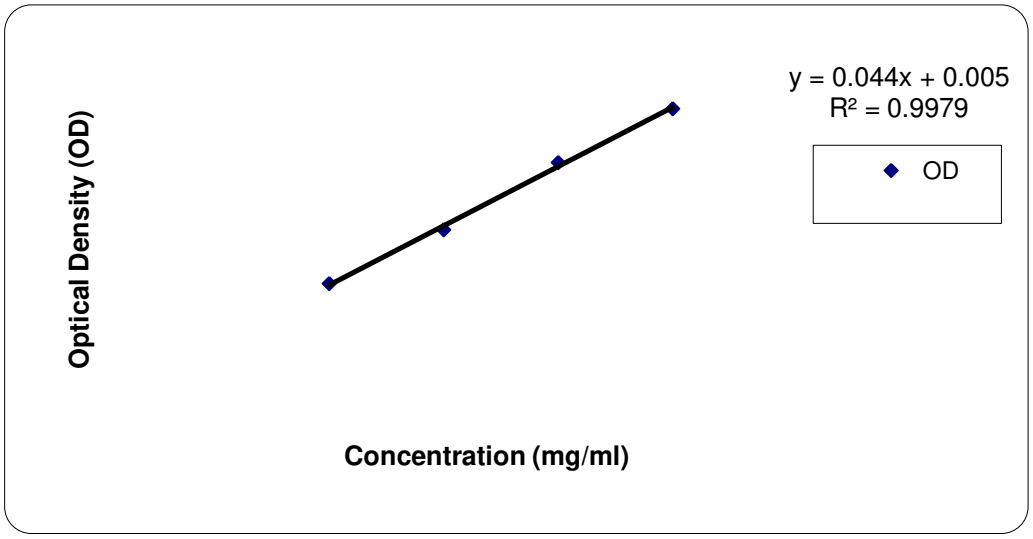

Fig. 3.5: Standard curve of Cholesterol

3.10.3 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.

3.11 Isolation of E. coli and salmonella from feces sample

3.11.1. Fecal sample collection, transportation and preparation

Fecal samples of chicken were collected from healthy layer at the last week of experiment. All samples were collected with the help of sterile cotton buds and transferring the buds immediately to sterile nutrient agar. All the samples were transferred carefully to appropriate container. These were kept in box, wrapped with ice and transferred to laboratory for subsequent bacteriological examination.

3.11.2 Bacteriological media

a. Cultural Media

Commercially available media were used during this study. The commercial media were prepared according to the direction of the manufacturer's. The composition and the

procedure for the preparation of media are presented in the Methods. The media used for bacteriological culture were Nutrient Agar (NA; HiMedia), Nutrient Broth (NB; HiMedia), Eosin-Methylene-Blue (EMB. Hi Media) Agar, MacConkey (MC; HiMedia) Agar.

b. Biochemical media

The following biochemical media were used for the bacteriological analysis: Triple Sugar Iron (TSI) medium, Methyl Red-Voges Proskauer Broth (MR-VP Broth; HiMedia), Motility Indole Urea medium (MIU, HiMedia), Indole test.

3.11.3 Bacteriological reagents

The reagents used were phenol red, phosphate buffered saline (PBS), mineral oil, normal physiological saline solution, peptone water, 3% tri sodium citrate solution and other common laboratory chemicals and reagents as and when required during the experiment.

3.11.4 Sugars

- Dextrose
- Sucrose
- Lactose

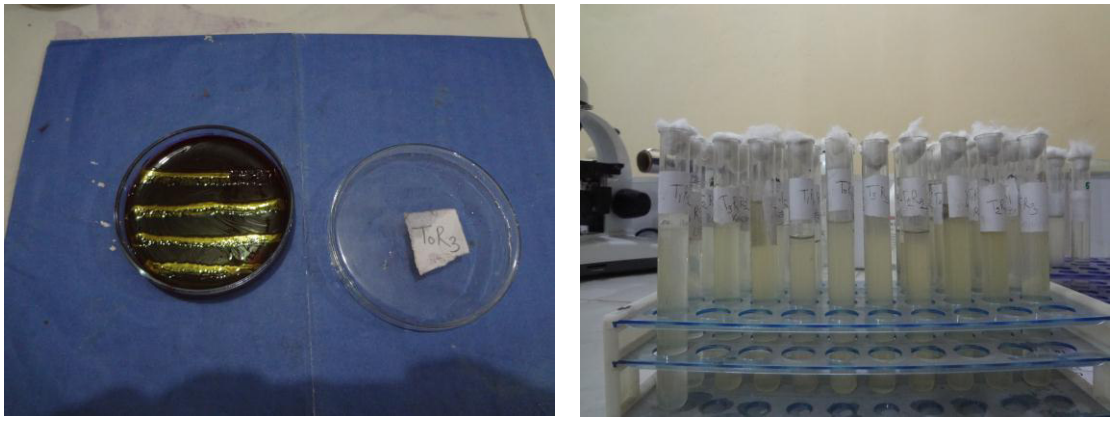

Fig. 3.6: Bacteriological media with organisms (Agar and broth)

3.11.5 Bacteriological media preparation

a) Nutrient broth (NB)

Nutrient broth was prepared by dissolving 13 grams of dehydrated nutrient broth (Himedia, India) into 1000 ml of distilled water and was sterilized by autoclaving, at 121°C tinder 15 Ib pressure per square inch for 15 minutes. Then the broth was dispensed into tubes (10 mUtube) and stored at 4°C in the refrigerator until used.

b) MacConkey (MC) agar media

51.50 grams powder of MC agar base (HITIVIedia, India) was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at I21°C maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45() C to decrease its temperature. After solidification of the medium in the petridishes, the petridishes were allowed for incubating at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

c) Eosin Methylene Blue (EMB) agar media

Thirty six (36) grams of EMB agar base (HiMedia, India) was added to 1000 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 12 FC maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45°C to decrease its, temperature. After solidification of the medium in the petridishes, the petridishes were allowed for incubation at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

d) Triple Sugar Iron (TSI) media.

A quantity of 65.0 gm of Bacto TSI medium (HiMedia) was dissolved in 1000 mlt1- of distilled water dispensed in 5 ml amount in each test tube and then the tubes were autoclaved at 121°C maintaining a pressure of 15 lb/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45°C to decrease its temperature. After solidification of the medium in the test tubes, the test tubes were allowed for incubation at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

e) Methyl-Red Voges-Proskauer (MR-VP) broth

A quantity of 17.0 gm of Bacto MR-VP medium (HiMedia) was dissolved in 250 ml of distilled water dispensed in 2 ml amount in each test tube and then the tubes were autoclaved at 121°C maintaining a pressure of 15 lb/sq. inch for 15 minutes. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

i) Motility Indole Urea (MIU) broth

18.00 grams powder of MIU agar base (HiMedia, India) was added to 950 ml of distilled water in a flask and heated until boiling to dissolve the medium completely. The medium was then sterilized by autoclaving at 121°C maintaining a pressure of 15 pounds/sq. inch for 15 minutes. After autoclaving, the medium was put into water bath of 45(' C to decrease its temperature. After this the medium in the test tubes were allowed for incubating at 37°C for overnight to check their sterility and then stored in a refrigerator for future use.

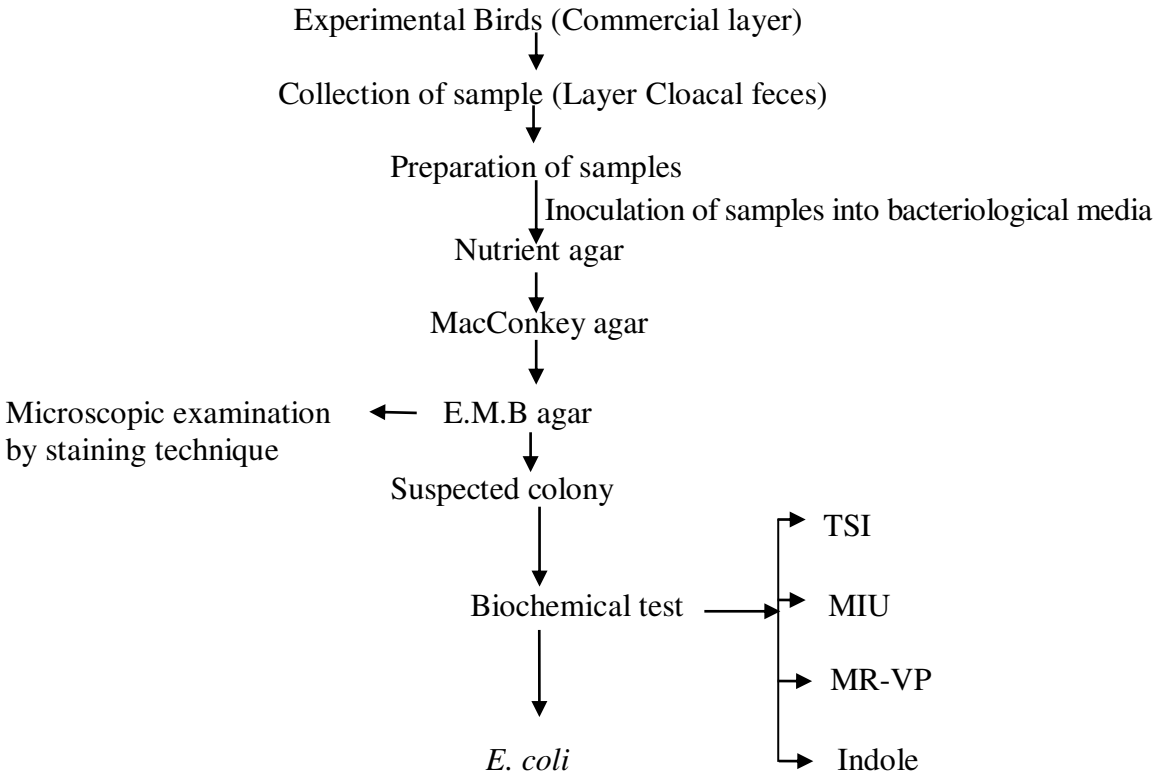

Fig. 3.7: Schematic Illustration of Experiment

3.12 Isolation of E. coli in pure culture

All samples were cultured primarily in nutrient agar at 37°C for 24 h, and then subcultured onto the MacConkey and EMB agar and S-S agar by streak plate method to observe the morphology. The organism showing, characteristic colony morphology of *E. coli* was repeatedly subcultured onto EMB agar until the pure culture with homogenous colonies were obtained.

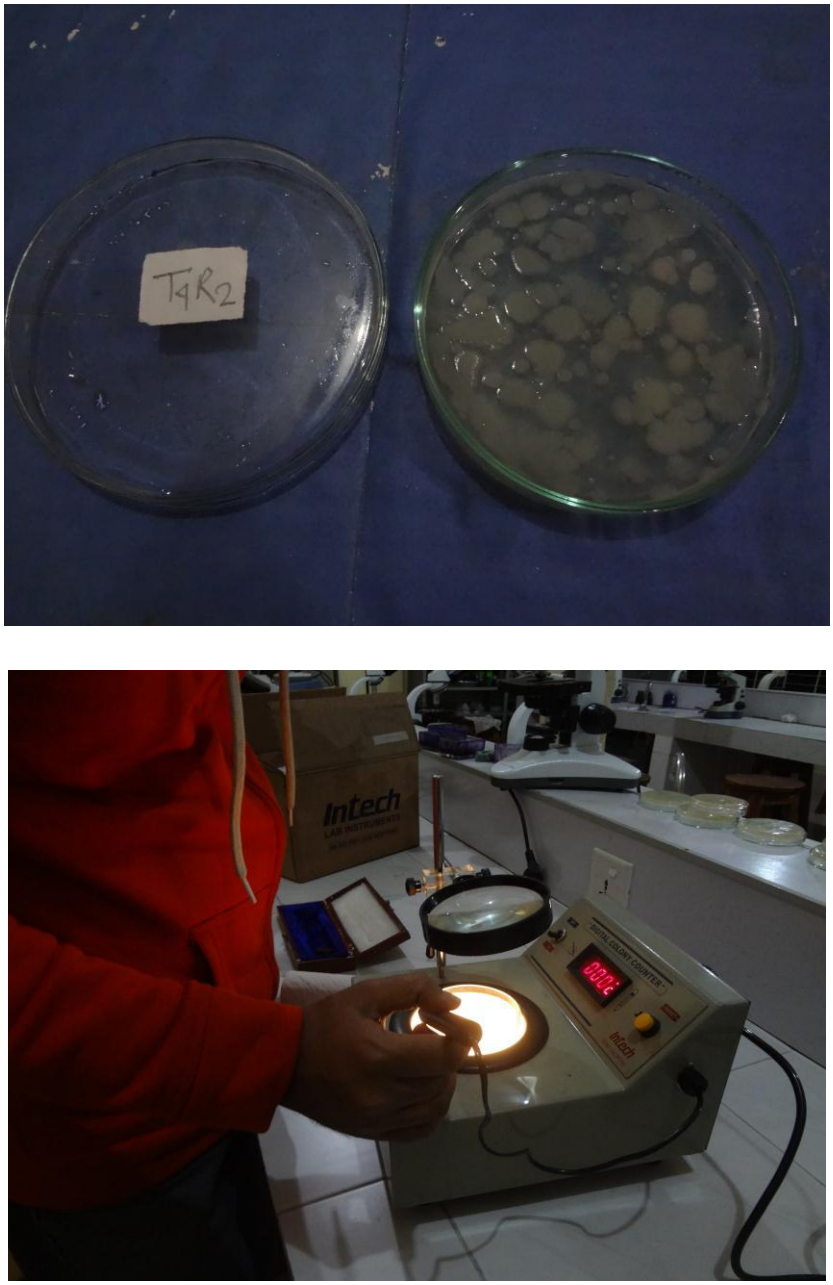

Fig. 3.8: Bacterial colony counting

3.13 Examination of Plates (Identification of the isolates)

a) Gross colony study

Morphological characteristics (shape. size, surface texture, edge, elevation, colour., opacity etc.) developed after 24 h of incubation were carefully studied as described by Marchant and Packer (1967) and recorded.

b) Microscopic study by staining method

Gram's staining method was done to study their morphology and staining character. Suspected colony from EMB agar were stained using Gram's stain as described by manual of Veterinary Investigation Laboratory Technique, 1984 (OIE, 2000).

The procedure was as follows:

A small colony was picked up with a bacteriological loop, smeared on a glass slide and fixed by gentle heating. Crystal violet solution was then applied on the smear to stain for two minutes and then washed with running water. Lugol's iodine was then added to act as mordant for one minute and then again washed with running water. Acetone alcohol was then added, which act as a decolourizer, for few seconds. After washing with water, safranine was added as counter stain and allowed to stain for two minutes. The slide was then washed with water, blotted and dried in air and then examined under microscope Is' with 10 X objectives and then with 100X objective using immersion oil. Gram negative rod shaped organisms were suspected for E. coll.

3.14 Biochemical test

The suspected isolated organism were subjected to different biochemical tests, such as sugar fermentation test for acid or acid and gas production, Indole production test, Methyl-red and Voges-proskauer (VP) test. Standard methods were followed for conducting these tests as described by Cowan (1985) during the experiment.

a) Sugar fermentation test

The sugar fermentation test was performed by inoculating a loop full of nutrient broth culture of the organisms into the tubes containing three basic sugars (dextrose, sucrose, and lactose) and incubated for 24 hours at 37°C to observe their sugar fermentation capability. Bacteria able to ferment all the five basic sugars were suspected for E. coll.

b) Indole production test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. 0.5 ml of Kovac's reagent was added, shaked well and examined after I minute. A red colour in the reagent laver indicated indole.

c) Voges-Proskauer (V-P) test

2 ml of sterile glucose phosphate peptone water was inoculated with the 5 ml of test organism. It was incubated at 35-37°C for 48 hours. A very small amount (knife point) of creative was added and mixed. 3 ml of the sodium hydroxide reagent was added and shacked well. The bottle cap was removed left for an hour at room temperature. It was looked for the slow development of a pink-red colour.

d) Methyl Red Test:

The test was performed by inoculating a colony of the test organism in 0.5 ml of sterile glucose phosphate broth (as used in the V-P test). After overnight incubation at 35-37°C, a drop of methyl red solution was added. A positive methyl red test was shown by the appearance of a bright red colour, indicating acidity.

Procedure for total viable and *E. coli* count:

Nutrient agar media was used for total viable count and Eosin Methylene Blue (EMB) agar media for *E. coli* count in this study. The procedure was as follows-

At first 10% suspension of the collected fecal sample was prepared in 0.1% peptone water. Then serial 10 fold dilution of the suspension was prepared in 10 sterile test tubes using 0.1% peptone water as diluent. Then 1 ml of diluted sample from each test tube was taken and poured into a sterile petri dish. Three different petridishes were used for each dilution. Then 10ml of melted Glucose tryptone yeast agar was poured into each petridish when the temperature was reduced at 45 C. Then the petridishes were rotated clockwise and anticlockwise gently to mix the sample with the culture media. Then the petridishes were allowed for solidification of the media. After solidification of the media the petridishes were marked and incubated at 30 C for 72 hours. Then the colonies of each petridish were counted. The petridishes containing 30 to 300 colonies were taken in consideration. Then average numbers of colonies were counted. Then the result was obtained by using the following formula:

The number of total viable organisms per ml of sample = Average number of colonies x dilution factor. Therefore, The number of total viable organisms per gm of fecal sample = Average number of colonies x dilution factor x 10.

3.15 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 Mstatc and SPSS Program.

CHAPTER IV

RESULTS AND DISCUSSION

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4.1 Laying performances

4.1.1 Egg production

The hen-day-egg production observed in different dietary treatments was almost similar and the differences were statistically non-significant (P> 0.01) at initial stage where it was nearly about 70% (Table 4.1). Result indicates that the feeding of Black pepper, Ginger and Garlic powder mixed meal in the diet of laying hen has significant effect on egg production from first to fourth month. Feeding of Black pepper, Ginger and Garlic powder mixed meal showed slightly higher egg production whereas the production was slightly increase (88% in 4th month) when the birds received T_2 (Black pepper 1.00gm/kg, Ginger 1.5 gm/kg and Garlic 1.50 gm/kg) mixed meal in the diet. These results are closed with the previous report of Lokaewmanee *et al.* (2009), however slightly differed from the observations of Ravindran *et al.* (1986), who found decreased egg production with the increased of increased of the herbs.

Table 4.1 Effect of Black pepper, Ginger and Garlic mix supplementation on Egg production (%) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	$68.1^{a} \pm 5.1$	$71.5^{a} \pm 7.4$	$78.7^{b} \pm 1.2$	$72.6^{a} \pm 6.1$	$71.8^{a} \pm 4.4$	**
2nd month	$71.8^{a} \pm 6.45$	$77.3^{b} \pm 2.7$	$84.8^{c} \pm 4.5$	$76.2^{b} \pm 5.5$	$78.18^{b} \pm 4.4$	**
3rd month	$75.7^{a} \pm 4.71$	$80.3^{b} \pm 3.64$	$86.77^{c} \pm 5.9$	$81.2^{b} \pm 5.4$	$79.18^{b} \pm 3.5$	**
4th month	$75.8^{a} \pm 3.70$	$81.53^{b} \pm 3.67$	$88.7^{c} \pm 5.59$	$82.3^{b} \pm 1.45$	$82.78^{a} \pm 1.54$	**

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, **= Significant at 1% level of significance.

4.1.2 Egg weight

The egg weights in different dietary treatments during experimental periods were statistically insignificant (P> 0.01) at the initial stage and gradually increased in T_2 (65g in fourth month) treatment group (Table 4.2). These results indicate that inclusion of Black pepper, Ginger and Garlic powder mixed meal in the diet of laying hens has effect on egg size. The results are consistent with the report of Tateno *et al.* (1999) and Sudo *et al*. (2000). Both of these researchers found non-significant difference in egg size after the birds exposed to 15 percent mulberry leaf meal in the diet. Addition of 1.5 % hot pepper in the diet resulted in a significant ($p \leq 0.01$) improvement in egg weight by 3.7 % and feed conversion ratio by 7.9 %. A slight numerical improvement was observed in egg production by 3.3 % and egg mass by 7.2 % as compared to the control hens.

Table 4.2 Effect of Black pepper, Ginger and Garlic mix supplementation on Egg weight (gm/egg) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	56.1 ± 11.7	57.65 ± 1.81	57.7 ± 6.2	56.8 ± 1.8	57.9 ± 7.2	NS
2nd month	56.2^{a} ± 2.23	57.41^{a}± 1.51	61.56^{b}± 1.1	57.8^{a}± 0.85	56.5^{a}± 0.85	**
3rd month	57.0^{a}± 1.3	59.11^{a}± 0.11	64.4^{b}± 2.4	58.40^{a} ± 3.1	59.5^{a}± 0.6	**
4th month	59.8^{a}± 0.53	61.3^{a}± 0.51	65.8^{a}± 0.41	61.1^{a}± 0.31	60.0^{a}± 0.55	**

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, **= Significant at 1% level of significance

4.1.3 Egg mass output

The results of the present study showed that the egg mass output (gm/hen/day) in different dietary treatments during experimental periods were statistically insignificant (P>0.01) from initial stage to end of fourth month (Table 4.6). The results are agreement with the report of Tateno *et al.* (1999) and Sudo *et al.* (2000).

Table 4.3 and 4.4 Effect of Black pepper, Ginger and Garlic mix supplementation on Body weight (g) and feed intake (g) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
Body weight (g)	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	
Initial	1468.67±11.6	1563.33±16.1	1516.67 ±8.8	1596.67±10.1	1590± 16.2	NS
1st month	1590.33± 23.5	1689.67± 18.3	1666.67±14.7	1706 ± 9.4	1720.67±17.1	NS
2nd month	1766±33.8	1843.67± 22.8	1666.67 ± 17.3	1829 ± 31.4	2496.67± 15.9	NS
3rd month	1830.67± 33.1	1893.33± 16.5	1899.22 ± 11.9	1877.33 ± 25.2	1917± 9.7	NS
4th month	2208.33± 16.7	2186.67± 22.8	2227 ± 17.2	2195 ± 16.3	2126.67± 9.9	NS
Feed intake (g)	**T_0**	**T_1**	**T_2**	**T_3**	**T_4**	
Initial	110.9± 0.71	111.6 ± 0.64	110.2± 1.40	111.2± 2.21	113± 2.41	NS
1st month	111.2^{a}± 0.32	113.1^{a}± 1.61	106.5^{b}± 4.6	113^{a}± 0.34	114.1^{a}± 2.30	**
2nd month	115.22^{a}± 0.53	114.1^{a}± 4.32	108.2^{b}± 3.2	115.1^{a}± 5.43	115.6^{a}± 5.3	**
3rd month	118.21^{a}± 1.4	119.1^{a}± 2.41	111.3^{b}± 3.3	117.3^{a}± 0.7	118.7^{a}± 6.6	**
4th month	121.1^{a}± 2.6	120.5^{a}± 1.90	114.9^{a}± 3.6	120.1^{a}± 3.36	119.8^{a}± 3.6	**

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, **= Significant at 1% level of significance

4.1.4 Body weight

Body weight in different dietary treatments during experimental periods was almost similar and the differences were not significant (P> 0.01) (Table 4.3). These results indicate that inclusion up to T_4 (Black pepper 2.00 gm/kg, Ginger 2.5 gm/kg and Garlic 2.50gm/kg) mixed meal had no adverse effect on body weight. However, the body weight slightly improved in the dietary treatment at T_3 (Black pepper 1.50 gm/kg, Ginger 2.0 gm/kg and Garlic 2.00 gm/kg) mixed meal in comparison to T_0 (control). This is in agreement with the results of Machii (2000) who observed there was no adverse effect of

mulberry leaf meal on body weight when mulberry leaves were given as part of the diet to domestic fowl. The results of (Mei Ling and Margaret, 1980; Winterhoff and Egen, 1991 and Elnagar *et al.,* 2005) reported that garlic increased the levels of serum T3, T4 and insulin hormones, these hormones are responsible for increasing the metabolic rate in the body, which may be due to egg production and egg weight improvement. Conversely, Samanta and Dey (1991) and Khan *et al*. (2007) reported significant weight gain and increased egg production without effects on feed consumption and efficiency when GP was included in hen diet. Marshall and Kokoete (2008) similarly noted a 10% increase in BW in layers with 1% dietary GP. In other farm animals, Cullen *et al*. (2005) reported reduced feed intake and improved feed efficiency in grower-finisher pigs fed garlic. Botsoglou *et al*. (2005) suggested that environmental standards might play a role in the response of layers to performance-promoting supplements. They explained that well-nourished, healthy hens may not respond to a performance-promoting supplement when they are housed under clean, disinfected conditions and moderate stocking density.

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> 0.01) from initial stage to fourth month (Table 4.4). So, the result clearly showed that T_4 (Black pepper 2.00 gm/kg, Ginger 2.5 gm/kg and Garlic 2.50 gm/kg) mixed meal dietary level had no detrimental effect on feed consumption. Similar results have been observed by Lokaewmanee *et al.* (2009) who found that there was no adverse effect in feed intake compared to control. But, Ravindran *et al.* (1986), Limcangco-Lopez (1989), Udedibie and Opara (1998), Odunsi (2003) and Akande *et al.* (2007) reported a reduction in feed intake with increased dietary leaf meals in the diets for broilers and laying hens. A decrease in feed intake for increased levels of mulberry leaf may be due to bulkiness and unpalatable taste which may affect the appetite of the birds. Khalil *et al*.(2007) showed that feed conversion ratio was significantly better in growing Japanese quail birds fed 1.6 % dried garlic than the control group. Also, Elnagar *et al.* (2005) found better feed conversion ratio by given tablets containing alliin in Pekin ducklings. This result is partly in agreement with the reports of 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.

4.2 External and Internal egg quality

It was observed that the shape index (Table 4.8), shell thickness (Table 4.9), albumen weight (Table 4.10), albumen index (Table 4.11), yolk weight (Table 4.12), yolk index (Table 4.13) and Haugh of the eggs (Table 4.15) laid by hens fed different diets were almost similar during experimental periods and the differences were non-significant (P> 0.01). These results indicate that feeding of Black pepper, Ginger and Garlic powder mixed meal up to T_4 (Black pepper 2.00 gm/kg, Ginger 2.5 gm/kg and Garlic 2.50 gm/kg) mixed meal had no adverse effect on external and internal qualities of eggs. However, egg shell weight (gm) decreased slightly after supplementation of T_2 (Black pepper 1.00 gm/kg, Ginger 1.50 gm/kg and Garlic 1.50 gm/kg), T_3 (Black pepper 1.50 gm/kg, Ginger 2.0 gm/kg and Garlic 2.00 gm/kg) and T_4 (Black pepper 2.00 gm/kg, Ginger 2.5 gm/kg and Garlic 2.50 gm/kg) mixed meal.

Table 4.5 Effect of Black pepper, Ginger and Garlic mix supplementation on FCR (gm feed /gm egg) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	1.9 ± 0.11	1.93 ± 0.24	1.92 ± 0.21	1.92 ± 0.34	1.98 ± 0.12	NS
2nd month	1.94 ± 0.54	1.89 ± 0.5	1.94 ± 0.11	1.90 ± 0.15	1.89 ± 0.61	NS
3rd month	1.89 ± 0.7	1.90 ± 0.55	1.91 ± 0.23	1.94 ± 0.34	1.90 ± 0.17	NS
4th month	1.98 ± 0.07	1.97 ± 0.05	1.95 ± 0.04	1.96 ± 0.08	2.01 ± 0.08	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant

Table 4.6 Effect of Black pepper, Ginger and Garlic mix supplementation on Egg mass (egg/hen/day) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	50.11 ± 1.4	52.9 ± 0.80	52.86 ± 0.5	52.1 ± 1.2	52.1 ± 1.33	NS
2nd month	52.6 ± 1.6	54.2 ± 3.0	55.6 ± 0.1	54.9 ± 0.2	55.41 ± 1.2	NS
3rd month	54.4 ± 0.7	55.42 ± 2.10	56.11±0.16	55.11 ± 0.9	55.1 ± 0.70	NS
4th month	56.84 ± 1.2	56.92 ± 2.1	57.86 ± 1.6	56.31 ± 1.9	56.31 ± 1.6	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant

Garlic1.1.50gm/kg) mixed meal. Albumen weight decreased in the dietary treatments T_2 (Black pepper 1.00 gm/kg, Ginger 1.50gm/kg and Garlic1.1.50gm/kg), T_3 (Black pepper 1.50gm/kg, Ginger 2.00gm/kg and Garlic 2.00gm/kg) and T_4 (Black pepper 2.00gm/kg, Ginger 2.5ogm/kg and Garlic2.50gm/kg)mixed meal but a little bit increased in dietary treatment T4 from those of control groups. Moreover, Yolk weight and yolk index slightly decreased after the inclusion of T_2 (Black pepper 1.00 gm/kg, Ginger 1.50gm/kg and Garlic1.1.50gm/kg), T_3 (Black pepper 1.50 gm/kg, Ginger 2.0 gm/kg and Garlic 2.00 gm/kg) and T_4 (Black pepper 2.00gm/kg, Ginger 2.5 gm/kg and Garlic 2.50 gm/kg) mixed meal but yolk index slightly improved at T_3 (Black pepper 1.50 gm/kg, Ginger 2.0 gm/kg and Garlic 2.00gm/kg) mixed meal. Similar results have been obtained by Tateno *et al.* (1999) and Sudo *et al.* (2000) who did not found any significant differences in the external and internal qualities of eggs up to 9 percent level of mulberry leaf meal.

4.3 Egg-yolk cholesterol

This study showed that egg-yolk cholesterol was decreased significantly by supplementation of sun-dried Black pepper, Ginger and Garlic powder mixed meal in layer-ration ($P< 0.01$). It is evident from Table 4.14 that a tendency of reduced egg yolk cholesterol was observed in the dietary treatments with inclusion of T_2 (Black pepper 1.0

gm/kg, Ginger 1.50 gm/kg and Garlic 1.50 gm/kg) and T_4 (Black pepper 2.00 gm/kg, Ginger 2.5 gm/kg and Garlic 2.50 gm/kg) sun-dried Black pepper, Ginger and Garlic powder mixed meal. However, the highest level of cholesterol was 13.8 mg/gm at T_0 group and lowest level was 8.9 mg/gm of egg-yolk at T_2 sun-dried Black pepper, Ginger and Garlic powder mixed 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 sun-dried Black pepper, Ginger and Garlic powder mixed meal at T_2 dietary level had beneficial effect in reduction of egg yolk cholesterol. The similar results obtained from (Machii, 1990) who found reduced egg-yolk cholesterol at 2% level of herbs. Liver is the organ that regulates the deposition of lipids and phospholipids in egg-yolk (Bell and Freeman, 1971). Since liver and serum cholesterol are decreased by supplementation of mulberry leaf meal which may leads decreased egg-yolk cholesterol. Thus, the decrease in egg-yolk cholesterol by dietary herb meal supplementation may be due to a lesser deposition of cholesterol by liver in egg-yolk during yolk synthesis. Zeweil *et al.* (2006) reported that Japanese quail laying hens fed on diet supplemented with 1 or 2 g thyme flowers / Kg diet did not affect egg quality trait. These results were in agreement, more or less, with those of Zeweil *et al.,* (2006) who reported that inclusion of 1 or 2 g thyme flowers / Kg diet decreased egg yolk cholesterol of Japanese quail laying hens at 24 weeks of age. Dias *et al* (2006) reported that the total serum cholesterol levels were significantly decreased by dietary supplementation of 1% ginger extract meal in Wistar rats ($P<0.05$). They stated that ginger treatment can reduce total serum cholesterol by enhancing the activity of liver cholesterol-7-a-hydrolase or inhibition of hydroxyl-methyl-glutarylcoenzyme- A (HMG-CoA) reductase, either by bile-acid conversion or fecal excretion of cholesterol.

From the above discussion, it is said that egg-yolk cholesterol was decreased significantly without affecting egg qualities with T_2 diet supplementation. These herbs contain phytosterol that is responsible for lower absorption of cholesterol from the intestine resulting lower deposition of cholesterol in egg-yolk. As a result, cholesterol of egg-yolk was reduced. The addition of dried garlic and hot pepper did not demonstrate any significantly effect on feed consumption. It is of interest to note that egg weight did not decline by the increase of egg production in dried garlic-fed hens during the experimental period. There was no mortality in the groups given the experimental diets during the experimental period. The reduction was more pronounced in the plasma of

birds fed dried garlic. Results obtained are in agreement with the previous findings of Mottaghitalab and Taraz (2004) who showed that garlic had a significant effect on lowering the level of plasma total lipids and cholesterol of birds.

Table 4.7 Effect of Black pepper, Ginger and Garlic mix supplementation on Egg Shell weight (g/egg) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	6.34 ± 3.03	6.82 ± 1.17	6.21 ± 0.52	6.35 ± 3.02	7.03 ± 3.16	NS
2nd month	6.45 ±5.81	6.58 ± 4.21	6.54 ± 2.1	6.71 ± 2.7	7.4 ± 7.07	NS
3rd month	7.04 ± 2.34	6.61 ± 4.75	6.68 ±5.51	6.58 ± 4.62	6.98±7.1	NS
4th month	6.56 ± 0.09	6.42 ± 0.11	6.34 ± 0.12	6.33 ± 0.08	6.37 ± 0.06	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments of all the parameters were statistically non-significant at $P<0.05$

Table 4.8 Effect of Black pepper, Ginger and Garlic mix supplementation on Shape index (%) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	78.33±7.42	76.3 ± 2.6	72.88±0.33	75.41±5.1	76.68±2.7	NS
2nd month	77.21 ±2.55	73.56 ±3.44	74.65±3.43	72.66±2.22	72.21±6.33	NS
3rd month	75.03± 1.2	77.13 ± 0.56	76.33±5.22	78.31±4.31	76.38±3.57	NS
4th month	80.04 ± 0.67	80.41 ± 0.63	81.01±0.39	80.09 ± 0.91	80.78 ± 0.82	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments of all the parameters were statistically non-significant at $P<0.05$

Table 4.9 Effect of Black pepper, Ginger and Garlic mix supplementation on Shell thickness (mm) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	0.45 ± 0.2	0.46 ± 0.54	0.46 ± 0.2	0.45 ± 0.73	0.45 ± 0.6	NS
2nd month	0.43 ± 0.54	0.45 ± 0.66	0.44 ± 0.43	0.44 ± 0.70	0.45± 0.87	NS
3rd month	0.42 ± 0.21	0.43 ± 0.42	0.44 ± 0.62	0.43 ± 0.67	0.42 ± 0.71	NS
4th month	0.40 ± 0.82	0.40 ± 0.88	0.41 ± 0.40	0.40 ± 0.72	0.40 ± 0.61	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments of all the parameters were statistically non-significant at $P<0.05$

Table 4.10 Effect of Black pepper, Ginger and Garlic mix supplementation on Albumen weight (gm/egg) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	40.55± 6.2	44.43 ± 1.66	42.2 ±2.5	43.4 ± 5.8	46.6 ± 7.2	NS
2nd month	36.44±4.81	41.6±2.60	45.6 ±3.11	47.2 ± 4.38	48.31 ± 4.66	NS
3rd month	40.41± 5.54	40.5 ± 6.32	40.33 ±6.6	42.1±7.1	41.05 ± 7.21	NS
4th month	38.06 ± 3.66	38.41 ± 4.7	37.4 ±5.32	37.30 ± 3.3	38.88 ± 7.7	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments of all the parameters were statistically non-significant at $P<0.05$

Table 4.11 Effect of Black pepper, Ginger and Garlic mix supplementation on Albumen index (%) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	9.02 ±6.35	9.72 ± 5.4	9.66±5.34	9.8 ±2.32	9.5 ± 7.6	NS
2nd month	9.11 ±5.55	10.02 ±2.13	9.80 ± 5.5	10.22 ± 8.2	9.9±6.65	NS
3rd month	9.02 ±6.35	9.72 ± 5.4	9.66±5.34	9.8 ±2.32	9.5 ± 7.6	NS
4th month	8.36 ± 5.18	8.40 ± 4.21	8.00 ± 3.31	8.42 ± 7.1	8.44 ± 3.82	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments of all the parameters were statistically non-significant at $P<0.05$

Table 4.12 Effect of Black pepper, Ginger and Garlic mix supplementation on Yolk weight (gm/egg) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	16.28 ± 2.02	16.86 ± 3.44	16.38 ± 6.4	15.88 ± 8.65	16.49 ± 5.77	NS
2nd month	16.77 ± 7.66	16.02 ± 7.32	17.11±6.44	16.22 ± 5.11	16.41 ± 6.29	NS
3rd month	16.75 ± 7.58	16.27 ± 6.91	16.83±4.73	17.04 ± 5.74	17.06 ± 6.38	NS
4th month	17.29 ± 6.71	17.18 ± 5.82	16.29±7.72	16.06 ± 9.29	16.99 ± 5.86	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments of all the parameters were statistically non-significant at $P<0.05$

Table 4.13 Effect of Black pepper, Ginger and Garlic mix supplementation on Yolk index (%) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	39.42± 3.28	39.91 ± 5.93	40.2 ± 4.22	39.71± 8.28	38.6 ± 6.18	NS
2nd month	40.09± 4.4	41.1 ± 6.15	41.5 ± 3.43	41.07 ± 9.15	41.54 ± 8.66	NS
3rd month	41.6± 4.59	42.28± 5.32	42.71±6.17	43.02±10.31	42.4 ± 4.56	NS
4th month	43.7 1± 7.33	42.73 ± 8.16	41.91±7.16	43.94 ± 8.34	42.10 ± 6.48	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments of all the parameters were statistically non-significant at $P<0.05$

Table 4.14 Effect of Black pepper, Ginger and Garlic mix supplementation on yolk cholesterol of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	13.4± 1.82	12.53 ± 3.79	11.9 ± 4.11	12.98± 4.21	13.07 ± 2.12	NS
2nd month	12.42^{a} 2.9	12.67^{a}± 4.27	9.54^{b}± 1.16	12.01^{a}± 4.11	12.96^{a}± 2.61	**
3rd month	13.3^{a}± 1.8	12.94^{a}± 0.33	9.12^{b}± 2.21	12.86^{a}± 1.04	12.55^{a}± 1.6	**
4th month	13.8^{a} ± 0.28	12.5^{a} ±0.16	8.99^{a}±0.2	11.12^{a}±0.1	10.02^{a}±0.13	**

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, **= Significant at 1% level of significance

Table 4.15 Effect of Black pepper, Ginger and Garlic mix supplementation on Haugh unit (%) of laying hens

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
1st month	85.7 ± 4.2	85.11 ± 7.5	86.28 ± 3.9	84.14 ± 8.71	84.04 ± 7.52	NS
2nd month	86.33 ± 8.43	85.09 ± 5.9	86.41±7.71	85.42 ± 4.32	84.5 ± 3.16	NS
3rd month	87.3 ± 6.48	87.9 ± 10.3	87.34±7.43	87.46 ± 8.42	87.38 ± 8.2	NS
4th month	89.70 ± 2.11	88.70 ± 1.92	88.79± .11	86.32 ± 2.31	89.99 ± 2.40	NS

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments of all the parameters were statistically non-significant at $P<0.05$

Also, Tollba *et al.* (2007) reported that total lipids and cholesterol in the plasma of broilers or laying hens fed diet supplemented with hot pepper were significantly ($P \leq 0.01$) Egg Meat lower than those of the control groups. The mechanism of this reduction is due to the garlic content of sulfur compounds that responsible for inhibiting biosynthesis of cholesterol and lipids (Chi *et al*., 1982). One suggestion is that garlic blocks hydroxymethylgutaryl- CoA (HMG-CoA), which reacts with a reductase to yield mevalonate and is rate-limiting step in cholesterol biosynthesis (Mathews and Van Holde, 1990).

4.4 Bacterial colony count

Table 4.16 shows the effect of varying doses of Black pepper, Ginger and Garlic {T_1 (Black pepper 0.5gm/kg, Ginger 1.00gm/kg and Garlic1.00gm/kg), T_2 (Black pepper 1.00 gm/kg, Ginger 1.50gm/kg and Garlic1.1.50gm/kg), T_3 (Black pepper 1.50gm/kg, Ginger 2.oogm/kg and Garlic 2.00gm/kg) and T_4 (Black pepper 2.00gm/kg, Ginger 2.5ogm/kg and Garlic2.50gm/kg)} supplementation in diets on excreta cultivable bacterial colony counts. Supplementation of Black pepper, Ginger and Garlic powder in the diets significantly ($P<0.01$) decreased the population of harmful bacterium, *Escherichia coli,* and total culturable bacteria than those of control. The highest colony count was found in control group T_0 (300) and lowest found in T_2 (151) treatment group. This may partly explain the variation in experimental results. A non-significant decrease

was found in log colony-forming units of bacterial count in feces with increasing GP in a dose-dependent manner. 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. Furthermore, Black pepper, Ginger and Garlic mix supplementation in diets significantly suppressed the number of harmful bacteria such as *E. coli* in the excreta. Taken together, these results suggest that Black pepper, Ginger and Garlic mix supplementation could be a potential natural additive in poultry diets for environment safe and low cost commercial feed formulation for low cholesterol eggs. Our results are in agreement with the findings of Aydin *et al*. (2008), but slightly differed from the observations of Akhtar *et al*. (2003). In the latter case, *N. sativa* seed supplemented diets were found to significantly increase egg production and egg weight of Hy Line White hens. In correspondence to our findings, the number of coliform bacteria in layer has been reported to decrease by *Nigella*-supplemented diets (Abu-Dieyeh and Abu-Darwish, 2008). Our finding and previously described results suggest that herbal feed additives might be an effective alternative to synthetic antibiotics for the promotion of health and performances of poultry (Cross *et al*., 2007).

Table 4.16 Effect of Black pepper, Ginger and Garlic mix supplementation on bacterial load in faeces of laying hen (E. coli)

Parameters	Dietary levels of Black pepper, Ginger and Garlic (gm/kg)					Levels of Significance
	T_0	T_1	T_2	T_3	T_4	
Initial	246.67± 6.93	230.67±2.9	241.33 ± 2.96	236.67 ± 4.98	244.0± 7.00	NS
1st month	267.51^{a}±19.4	198.87^{b}±13.6	171.34^{c}± 15.12	200.24^{b}± 7.18	216.41^{a}± 9.16	*
2nd month	251.4^{a}± 11.5	177.62^{b}± 21.4	169.31^{c}± 9.51	180.32^{b}± 6.25	181.2^{b}± 6.44	*
3rd month	261.42^{a}±14.7	175.4^{b}± 9.31	156.53^{c}± 16.89	177.32^{b}± 13.2	170.31^{b}± 17.2	*
4th month	300.46^{a}±11.6	181.23^{b}± 13.4	151.38^{c}± 8.42	176.44^{b}± 18.46	171.24^{b}± 15.41	*

Diets were supplemented with Black pepper, Ginger and Garlic (gm/kg) powder and were fed for 16 weeks; values are expressed as mean ± standard error of at least 3 replications each of which contains 4 birds; differences among the treatments most of the parameters were expressed as NS= Not significant, **= Significant at 1% level of significance

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Black pepper, Ginger and Garlic were collected from local market of Dinajpur district 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 Black pepper, Ginger and Garlic mix for laying hen (Hi-sex brown) was evaluated at Hajee Mohammad Danesh Science and Technology University poultry farm, Dinajpur district. In feeding trial, five (5) diets were prepared including T_0 (control), T_1 (Black pepper 0.5gm/kg, Ginger 1.0 gm/kg and Garlic 1.00 gm/kg), T_2 (Black pepper 1.00 gm/kg, Ginger 1.50 gm/kg and Garlic 1.50 gm/kg), T_3 (Black pepper 1.50 gm/kg, Ginger 2.0 gm/kg and Garlic 2.00 gm/kg) and T_4 (Black pepper 2.00 gm/kg, Ginger 2.5 gm/kg and Garlic 2.50gm/kg). 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. Egg yolk cholesterol decreased with increased level of Black pepper, Ginger and Garlic up to T_2 treatment group. Feed consumption for the entire experimental period in different treatment groups was recorded and expressed as g/day. In all test groups feed consumption was almost similar to control group. 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_4 (2.01) compared with other group. Egg production was more or less similar all of treatment group. Data obtained on egg weight expressed as maximum level in T_2l group (65.80 gm) than the other feeds fed group but almost similar to diet. Egg mass were statistically similar in all groups. Shape index were found to be highest at diet with T_2 (81.01 %) but almost same to all other feed groups. Shell thicknesses were indifferent with diet at T_0 (0.40 mm), T_1 (0.41 mm), T_2 (0.43 mm) T_3 (0.41 mm) and T_4 (0.42 mm) in the diet. Data obtained on albumen index exhibited maximum level in diet with T_4 (8.44 percent) than the other feeds fed group but almost similar to diet with T_0 (8.36 percent), T_1 (8.40 percent) and T_2 (8.00 percent) and T_3 (8.42). 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 (13.8 mg/gm) and lower in diet at T_2 (8.99 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 of Black pepper, Ginger and Garlic powder mix in the diet of laying hens significantly ($P<0.01$) decreased the population of harmful bacterium, *E. coli,* as well as total cultivable bacteria than those of control (T_0) (Table 4.16). In conclusion, we found that Black pepper, Ginger and 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 of laying hens was significantly suppressed by Black pepper, Ginger and Garlic powder mix supplemented diets for 16 weeks. Taken together, our results suggest that supplementation of Black pepper, Ginger and Garlic powder mix in diets has high potential as commercial applications for the production of low-cholesterol eggs. Therefore, Black pepper, Ginger and Garlic mix powder can be considered as feed additives and an environment safe alternative to the banned and hazardous synthetic antibiotics. But a further study is to be needed to realize the active principle of cholesterol lowering and other beneficial effects of Black pepper, Ginger and Garlic mix powder. However, it is necessary to further investigate the *in vivo* antibacterial activities of the extracts in chicks.

CHAPTER VI

REFERENCES

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