DIETARY EFFECT OF BLACK SEED (*NIGELLA SATIVA*) AND METHI (FENUGREEK) AS SINGLE AND COMBINED DOSE ON LAYING HEN

A Thesis

By

PROTUYSH ROY
Registration No. 1405095
Semester: January -June, 2015
Session: 2014-2015

MASTER OF SCIENCE (M.S.)
IN
POULTRY SCIENCE

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

JUNE, 2016

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Department of Dairy and Poultry Science
Hajee Mohammad Danesh Science and Technology University
Dinajpur-5200

JUNE, 2016

DEDICATED
TO MY
BELOVED PARENTS
AND
FAMILY

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ABSTRACT

This study was carried out to assess, examine and quantify the effect of dietary black seed and methi supplementation on egg production and egg quality of laying hens at 20 to 36 week age from January to April, 2015. A total of fourty eight (48) Hisex Brown hens of 20 weeks old were allocated to 4 groups, each containing hens. The hens in individual cage were supplied fixed amount of feed (120gm/day) containing 18.5% CP and 2725 Kcal ME/kg diet. Hens were randomly alloted to four (4) different dietary treatments: T_0: (control), T_1: (black seed 2gm/kg), T_2: (Methi 2gm/kg) and T_3: (black seed 2gm/kg and methi 2gm/kg). Body weight change, egg production, feed conversion ratio, egg weight, egg size characteristics were recorded and compared. Shape index, yolk index, albumen index, Haugh unit, shell thickness, per cent shell, percent albumen and percent yolk parameters were also recorded. No. of egg production, total egg mass production and feed conversion ratio increased at increasing level of black seed and methi. But, there were no significant change of shell weight, shell percent, shell thickness, shape index, albumin index, yolk index, and percent yolk have not showed with increasing levels of black seed and methi ($p>0.05$).

CONTENTS

CONTENTS (Contd.)

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREBIATION

BCS : Black Cumin Seed

bw : Body Weight

BWS : Buckwheat Seed

CF : Crude Fiber

cm : Centimeter

CP : Crude Protein

DCP : Di Calcium Phosphate

EIMB : Eosin-Methylene-Blue

EMB : Eosin Methylene Blue

FCR : Feed Conversion Ratio

FE : Feed Efficiency

gm : Gram

HDL : High Density Lipoprotein

HU : Haugh Unit

IU : International Unit

kg : Kilogram

LDL : Low Density Lipoprotein

MC : MacConkey

mg : Milligram

MIU : Motility Indole Urea

MIU : Motility Indole Urea medium

mm : Millimeter

MR : Methyl Red

NA : Nutrient Agar

NB : Nutrient broth

NRC : National Research Council

PBS : Phosphate Buffered Saline

RBD : Randomized Block Design

SE : Standard Error

T : Treatment

TSI : Triple Sugar Iron

VP : Voges-proskauer

CHAPTER 1

INTRODUCTION

Bangladesh is a densely populated agriculture based country with 154 million people and positioned currently 8th among all the countries of the world (UNFPA, 2012). The human population growth in developed countries is stabilizing while that of developing countries including Bangladesh is increasing rapidly. The depth and severity of poverty is worse in rural areas, 80% of the total population lives in the rural areas that primarily depend on a poorly developed agriculture for livelihood (BBS, 2014). The domestic production of milk, meat and egg are 3.46, 2.33, and 7303 million tons in the 2011-2012 fiscal year against the demand of 13.50, 6.48, and 15392 million tons, respectively (Hossain and Hassan, 2013). There is a deficit of 80% milk; 82% meat and 63% eggs (FAO, 2008). Due to the severe poverty most of these people faces an acute deficiency of animal protein sources, like meat, milk, egg etc. Poultry meat and eggs contribute approximately 37% of total animal protein in the country (Ahmed and Islam, 1990). The poultry sub-sector is considered an important avenue to reduce poverty and malnutrition as well as unemployment problems of Bangladesh. There is a great possibility of growth and expansion of this sector. Currently the farmers of this country are more interested in layer farming. As a result, a huge amount of feed is required to sustain this growing industry. On the other hand, higher price, quality feed and non-availability of feed ingredients are major limitation for the growth and development of this enterprise, which has direct influence on production cost.

The layer industry in Bangladesh is developing at a rapid pace and its success depends on how rapidly attains a maximum marketable egg in a minimum period. The feed

accounts about 65-70 percent of the total cost of poultry production. Hence it is necessary to improve the efficiency of feed at a minimum cost.

Inclusion of antibiotics as a principal growth promoter in poultry feed often resulted in the incidence of cross resistance among pathogens and also a source of residues in animal body tissues (Schwarz *et al.,* 2001). Consequently, the European Union banned the use of antibiotics as a growth promoter in animal feeds in January, 2006 (Toghyani *et al.,* 2010) and the scientists searched for the alternative natural growth promoting substances, essential oils and medicinal plants, which are proving more beneficial because of their antimicrobial effects (Valero and Salmeron, 2003). Such medicinal plants also possess stimulating on the animal digestive system (Jang *et al.,* 2004).

Nigella sativa (black cumin) species belonging to the family Ranunculaceae is famous for its medicinal properties. Seeds of N. sativa contain alkaloids, volatile as well as fixed oils and a variety of pharmacologically active substances like thymoquinone, dithymoquinone, carvacrol, thymol, nigellicine-N-oxide, nigellidine and α-hedrin (Nasir *et al.,* 2005). Black cumin is also enriched with the fat content of 35.5% (Babyan *et al.,* 1978). The seeds of *N. sativa* contain volatile oil (0.5-1.6%), fixed oil (35.6-41.6%), protein and acids (22.7%) (AL-Gaby, 1998). So N. sativa seeds appear to be a multipurpose feed growth promoter and may be promising in improving broiler performance (AL-Beitawi *et al.,* 2009). Many researchers have found encouraging results regarding the use of *N. sativa* as an alternative to antibiotics and a source of nutrition in the poultry feeds and many of them found encouraging results.

Feed efficiency (FE) is the prime factor to assess feed quality. Research regarding the effect of N. sativa on FE is neutral as well as positive. Feed efficiency was improved by incorporating black seeds in the broiler rations (Halle *et al.,* 1999).

Black seeds contain mixture of essential fatty acids, particularly oleic, linoleic and linolenic acids that cannot be synthesized in the body. There are fifteen amino acids comprising the proteins of N. sativa out of which eight are essential (Takruri and Dameh, 1998). Addition of N. sativa in feed increased bile flow rate results in increased emulsification that activates the pancreatic lipases which then aid in fat digestion and absorption of fat soluble vitamins (Crossland, 1980).

Black seed oil and thymoquinone have hepatoprotective effects (Mahmoud *et al.,* 2002) so these seeds have been traditionally used in a wide range of gastrointestinal disorders. The increased performance might also be due to antimicrobial effects of the active ingredients of black seed. Antimicrobial activities of N. sativa inhibit Shigella dysenteriae, Vibrio cholera, Shigella sonne, Escherichia coli, Bacillus pumilus, Bacillus suptilus, Staphylococcus lutea, Shigella flrxneri Staphylococcus aureus and Pseudomonas aeruginos. The anthelmintic activity of black cumin was observed by Agarwal *et al.* (1978a) and antifungal activity against pathogenic yeast Candida albicans.

Fenugreek (Trigonella foenum graecum) a multi-functional herb is known for its antifungal, antiviral, anticarcinogenic, antidiabetic, antileukemic, antipyretic and antimicrobial properties (Kaviarasan *et al.*, 2004). Some others authors analysed its anti-lithogenic properties (Acharya *et al.,* 2007a; Basu *et al.,* 2007; Reddy and Srinivasan, 2009a; Reddy and Srinivasan, 2009b). Many researchers (Abaza, 2007; Abdouli *et al.*, 2014; Criste *et al.*, 2013; Safaa, 2007) studied the use of fenugreek in poultry feeding, due to its antioxidant properties. The results suggested that the fenugreek extracts could act as an effective source of antioxidants, therefore the plant had been incorporated at different levels as feed additives in laying hen nutrition, with antagonistic results reported by the researchers. Productive performances were influenced by the fenugreek rate inclusion into laying hens diets. In this regard, Moustafa (2006) found that fenugreek

at the level of 0.05% improved feed conversion and (Abaza, 2007) obtained 2.23% increase of egg production and a significantly lower feed intake at 0.5% fenugreek but Criste *et al.* (2013) noticed unlike the previous observations that the use of fenugreek in layer diets in amounts of 1 and 2% had a negative influence on the egg production.

Fenugreek is an annual herbaceous plant with single flowers which are light yellowor violet white with Strong smell and bitter taste and are aromatic. This plant is oneyear, herbaceous, standing with 15 to 50 cm height that is planted in most of areas in Iran and of which flowering is in May, June and July (Qhraman, 1999). Fenugreek seed has 45 to 60% carbohydrate (mostly Galactomannanmucilage), 20 to 30% protein enriched by lysine, tryptophan, 5 to 10% lipid, considerable amounts of pyridine-like alkaloid (including 0.2 to 0.36% Trigonelline, 0.5%), flavonoids, free amino acids (arginine, histidine and lysine and 0.09% hydroxyisoleucine), calcium and iron, 0.6 to 1.7% saponins, glycosides, cholesterol and citostrol, vitamins (A, B_1, C) and 0.015% volatile oils (American Botanical Council, 2000).

Main advantage of using natural feed additives 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 medicinal plants on the performance and economics of production of poultry industry for reducing feed cost as well as to produce healthy food for human consumption. As dietary effect of black seed (*Nigella sativa)* and methi (Fenugreek) as single and combined dose on laying hen were not studied in Bangladesh so far, I have decided to work in this aspect with the following objectives:

i) To determine quality of egg after supplementation of black seed and methi as a single and combine doses.

ii) To evaluate production performance after feeding trial by black seed & methi as a single does & combined doses.

iii) To determine the cost effective feeding technique using black seed and methi.

CHAPTER 2

REVIEW OF LITERATURE

The review of literature is presented in order to collect the information related to the experiment by other researches in home and abroad in different times. It is a path of demonstration for advancement of the recent study. The purpose of this chapter is to provide a selective review of the previous research conducted so far in relation to the present study, the number of works directly related to the present study were scanty.

Black cumin (*Nigella sativa*):

Islam *et al.*, (2016) conducted a study about dietary effects of buckwheat (Fagopyrum esculentum) and black cumin (*Nigella sativa*) seed on growth performance, serum lipid profile and intestinal microflora of broiler chicks. The study was conducted to investigate the effects of different levels of buckwheat seed (BWS) with black cumin seed (BCS) supplementation on the performance, serum lipid profile and intestinal bacterial flora in broiler chicks. One hundred and twenty day-old Cobb-500 broiler chicks were randomly allotted equally to four experimental groups, designated T1 (untreated control, no BWS and BCS); T_2 (10% BWS + 1.5% BCS); T_3 (20% BWS + 2.5% BCS); and T_4 (30% BWS + 3.5% BCS), respectively. The results of the study showed that BWS and BCS significantly improved final bodyweight gain of group T_2 compared with the control group. Higher levels of buckwheat and black cumin did not improve growth performance of the chicks. Serum cholesterol and triglyceride concentrations significantly decreased with an elevation of HDL-cholesterol concentration as the level of BWS and BCS increased.

Siddiqui *et al.*, (2015) evaluated the effect of dietary supplementation of acetone extracts of *nigella sativa* L. seeds on serum cholesterol and pathogenic intestinal bacterial count in broilers. Feeds supplemented with 0, 1.5, 2.5, 3.0% seed powder or 0, 0.2, 0.4% acetone extracts of *N. sativa* seed for 4 weeks. N. sativa supplemented feed had no significant effects on feed intake, body weight and mortality rate of broiler. However, supplementation of either 3.0% seed powder or 0.4% extracts of N. sativa seeds significantly ($p<0.05$) decreased serum cholesterol and triglycerides contents in broiler. Furthermore, both N. sativa seed powder and extract supplemented feed also suppressed harmful bacterial (*Escherichia coli*) population in the feces.

Azeem *et al.,* (2014) observed the effect of *Nigella Sativa* on poultry health and production. *Nigella sativa* not only promoted bird's health and production performance, but also played a significant role as a natural antioxidant and immuno-stimulant. The polyunsaturated fatty acids share is almost double than mono-unsaturated fatty acids in oil content of black seed, so it reduced the total cholesterol content. The bioactive compounds in black cumin are anticancerous. The present review describes the natural beneficial effect of *Nigella sativa* on poultry health and production when used in poultry diet.

Hossain *et al.,* (2014) studied the evaluation of locally available herbs and spices on physical, biochemical and economical parameters on broiler production. Different herbs like- cumin, myrobalan, turmeric, garlic, ginger, mushroom, black cumin, coriander, cinnamon, chilli powder and neam leaves were applied on 390 broiler chicks. A basal diet was supplemented with 1g/L antibiotic (positive control), 0g antibiotic (negative control), 1% dose of concentration of cumin, myrobalan, turmeric, garlic, ginger, mushroom, black cumin, coriander, cinnamon, chilli powder and neam leaves. At the age of 28 days, the FCR value of chilli powder, cinnamon, antibiotics, and black cumin were

better (lower) than control. Significantly higher ($p<0.05$) dressing percentage was found in black cumin compared to the control. In this research, black cumin, cinnamon and antibiotics were showed significantly ($p<0.05$) lower blood glucose level than control. At 28 days of age, blood cholesterol was significantly lower ($p<0.05$) in neam leaves and black cumin than antibiotics and control. All treatments were significantly ($p<0.05$) higher antibody SP ratio for Gumboro disease compared to the control. Black cumin and cinnamon were significantly ($p<0.05$) higher antibody SP ratio for Newcastle disease compared to the antibiotics and control. All treatments were found significantly lower ($p<0.05$) E. coli population than the control. In this research cinnamon, antibiotics, black cumin and chilli were significantly ($p<0.05$) more profitable compared to the control. In conclusion, 1% black cumin, 1% cinnamon and 1% chilli powder were significantly ($p<0.05$) better for FCR, body growth, cholesterol level, sugar level in blood, immunity level, cecal microbial population, profit per bird, benefit cost ratio and can be used as good alternative of antibiotics in broiler diet.

Boka *et al.*, (2014) evaluated the effect of different levels of black cumin (*Nigella sativa L.*) on performance, intestinal *Escherichia coli* colonization and jejuna morphology in laying hens. A total of 100 Leghorn laying hens (Hy-Line W-36) of 49 weeks old were randomly distributed among five cage replicates of five birds each. Experimental diets consisted of different levels (0%, 1%, 2% and 3% of diet) of dietary black cumin inclusion. Although dietary black cumin in all supplementation levels decreased ($p < 0.05$) the enumeration of ileal *E. coli*, the morphological and histological alterations in small intestine such as enhancement of villus height to crypt depth ratio, increased goblet cell numbers and proliferation of lamina propria lymphatic follicles were observed after dietary supplementation with at least 2% black cumin. Dietary treatments decreased ($p < 0.05$) the concentration of serum cholesterol and triglycerides and increased ($p < 0.05$)

serum HDL concentration and relative weight of pancreas; however, the egg yolk cholesterol was not influenced by dietary treatments.

Abbas and Ahmed (2010) found poor Feed Efficiency (FE) was observed in broiler chicks fed diet supplemented with 1 and 2 % black seeds. Feed intake remained unaltered by feeding diet having 1, 2 and 3% black cumin seeds (Aydin *et al.,* 2008) and 1, 2 and 3 ml/kg N. sativa oil in 27 weeks old laying hens.

Bolukbasi *et al.* (2009) reported that in 27 weeks old laying hens, fed diets supplemented with 1, 2, and 3% black cumin seeds, had no significant effects on body weight and FCR.

Aydin *et al.* (2008) observed Black cumin (*nigella sativa l.*) supplementation into the diet of the laying hen positively influences egg yield parameters, shell quality and decreases egg cholesterol. In this study, eighty 27-wk-old laying hens (Hyline-5 White) were randomly assigned into 4 groups with 4 replicates of 5 birds each (20 laying hens per group) and fed diets supplemented with 1, 2, or 3% black cumin. Eggs were collected and weighed daily. Laying performance, egg quality, and feed conversion ratio were evaluated. Laying hens fed the diet supplemented with 3% black cumin had greater egg production than the control. Diets supplemented with 2 or 3% black cumin increased egg weight compared with other groups. Yolk weights of the eggs from hens fed diets containing 1, 2, and 3% black cumin were significantly greater than those from the control group. Shell thickness of the eggs from chickens fed 2 or 3% black cumin seed was significantly greater than those from chickens fed diets supplemented with 0 or 1% black cumin seed. Also, shell strength of the eggs from hens fed diets supplemented with 3% black cumin seed was significantly greater than the control.

Durrani *et al.* (2007) found that diets with 4% grounded black cumin resulted in less feed intake but better FE as compared to control diet.

El- Bagir *et al.* (2006) reported that supplementation of 1 and 3% black cumin in diet resulted in reduced egg production by approximately 9 and 16%, respectively without effecting egg length and width. The reduction in egg production might be due to the 10% increased final body weight of layers as energy from the black cumin oil extract was used to increase the weight gain rather than egg production.

Guler *et al.* (2006) reported no significant change in dietary intake of broiler by consuming feed containing black cumin and antibiotics. El-Bagir *et al.* (2006) found that dietary N. sativa at the level of 1 and 3% significantly increased final body weight of laying hens, so caused negative impact on egg production. The inclusion of black cumin seeds into the diet significantly decreased body weight of chickens (Akhtar *et al.,* 2003).

Different scientists documented contradictory results regarding the effect of N. sativa on egg production in layers. Egg production markedly increased by using 1.5% powdered black cumin (Akhtar *et al.,* 2003) and 3% black seeds (Bolukbasi *et al.,* 2009) in layer diet.

The dropped egg production might also be due to decrease in cholesterol (Akhtar *et al.,* 2003), because in a study by Elkin *et al.* (1993) it was observed that decrease in egg yolk cholesterol up to 30% by the addition of synthetic HMG-CoA reductase inhibitor in the diet resulted in reduced egg production by 20 % without effecting egg weight. So it can be inferred that cholesterol is needed for egg production and there may be a certain limit for cholesterol level, below which egg formation or production may be completely stopped.

Egg weight increased from 54 to 58g by supplementation of 1.5% black cumin in layers (Akhtar *et al.,* 2003) and 1% black cumin extract increased egg weight as well as egg shell weight and thickness in quails (Denli *et al.,* 2004).

Shell thickness and strength increased with 2 and 3% black seed in layer diets as compared to low levels i.e. 1% and without black cumin (Aydin *et al.,* 2008).

Bolukbasi *et al.* (2009) reported that dietary supplementation of N. sativa oil had no significant effect on egg weight, egg production, ratio of yolk, albumen and shell. The addition of 3 ml/kg N. sativa oil in layer diet decreased the Haugh unit of the egg. Diet containing 3% black cumin seeds decreased the egg-yolk total lipids, cholesterol, phospholipids as well as triacylglycerols by 34, 45, 11 and 20%, respectively. The decrease in egg yolk cholesterol is highly desirable as efforts are being made to decrease the total cholesterol consumption in human diets because of its damaging effects on the health. The mechanism by which black cumin decreases the egg yolk cholesterol is not fully understood. However speculations are made that the decrease in cholesterol can be related to the decreased in serum cholesterol by the black seeds. It is further assumed that seeds may inhibit the de-novo synthesis of cholesterol (EL-Bagir *et al.,* 2006). Albumin quality of eggs was improved by addition of black cumin in the diet (Akhtar *et al.,* 2003).

The cholesterol level of eggs was markedly decreased from 227 to 199 mg/egg yolk when diet supplemented with 1.5% black cumin (Akhtar *et al.,* 2003).

The study conducted by El-Bagir *et al.* (2006) indicated that addition of 1 or 3% black cumin in the diets of 68 weeks old layers resulted in a dose dependent decrease of serum phospholipids and cholesterol whereas a general decline in serum lipids was observed. The addition of 3% black cumin reduced the serum cholesterol and serum phospholipids by 23 and 30% respectively. The feeding of 3% crushed and non-crushed N. sativa seeds reduced plasma cholesterol, triglycerides concentration and increased the plasma High Density Lipoprotein (HDL) concentrations compared to 1.5, 2 and 2.5% crushed N. sativa seeds. The reduction in the triglycerides and cholesterol level might be due to the

active ingredients such as thymoquinone and compounds like monounsaturated fatty acids that lower the cholesterol synthesis by hepatocytes and decrease the fractional absorption of cholesterol from small intestine.

Black cumin bears an excellent potential as alternative to antibiotics and vaccines to improve immunity and to reduce mortality in poultry. Mortality was decreased from 16.67 to 4.17% by supplementation of layer diet with 1.5% black cumin and from 3.5% in the control group to 2% in the group fed diet containing 1% powdered N. sativa in broilers.

AL-Jabre *et al.* (2003) found that volatile oils in N. sativa exhibit 67 constituents capable of inducing beneficial and pharmacological effects against bacteria such as Staphylococcus and E. coli. Active components of black seed possessing antibacterial, antioxidant, and anti-inflammatory activities induced positive effects on the immunity and organs involved (Al-Saleh *et al.*, 2006).

The present commercial farming is becoming challenging for obtaining the desired weight without the use of antibiotics as growth promoters; therefore products capable of meeting the challenge are desired. Different studies on the effect of N. sativa seed on broiler performance have been carried out. Improved daily weight gain and better feed conversion ratio (FCR) in broilers was achieved with fed 1% N. sativa seed in broiler diet (AL-Beitawi *et al.*, 2008).

Methi (Fenugreek):

Zeweil *et al.* (2015) studied the effect of fenugreek and anise seeds as natural growth promoter on the performance, carcass, blood constituents and antioxidant status of growing rabbits. Group one fed basal control diet free of feed additives and served as a

control group. Group 2 and 3 givens basal diet supplemented with 0.6% of Fenugreek seed and anise seed powder, respectively. The different feed additives significantly ($P \leq 0.01$) improved final body weight, body weight gain, feed intake, feed conversion ratio and performance index as compared to the control group. Including different feed additives in the rabbit diets resulted in increasing absolute carcass weight. However, the results showed no significant differences were observed in hot or cold carcass weight percent and organs relative weight as compared to the control group, except testes percent.Serum glucose significantly ($P \leq 0.05$) decreased with inclusion of fenugreek in the diet.

Metin *et al.*, (2013) fed broiler chicks on diets containing (0, 5, 10, 20 and 40g) fenugreek seed powder per kg commercial broiler diet. Their results revealed decreased in body weight and breast weight in diets supplemented with fenugreek seed powder compared with untreated one, feed intake decreased after 5g Fenugreek seed, while 40g fenugreek treatment decreased feed efficiency. A 20g treatment enhanced blood glucose level and decreased triglyceride level compared to control.

Weerasingha and Atapattu (2013) observed the effects of Fenugreek (*Trigonella foenum-graecum L.)* Seed Powder on Growth Performance, Visceral Organ Weight, Serum Cholesterol Levels and the Nitrogen Retention of Broiler Chicken. In a Complete Randomized Design with six replicates, 108 chicks in 36 pens received one of the six experimental diets containing either 0, 1, 2, 3, 4, 5 % fenugreek powder from day 21-38. The weight gain of the birds that were fed with 4 and 5 % fenugreek was significantly lower ($p<0.05$) than that of the birds fed with 1 % fenugreek powder. One percent fenugreek powder improved the feed conversion ratio (FCR) by 13.8 %, compared to birds in the control group. Dietary fenugreek linearly increased the relative length of the

small intestine and the weight of the pancreas. The N retention and serum cholesterol level were not significantly ($p>0.05$) altered by dietary fenugreek.

Nadir *et al.* (2012) found that Fenugreek seeds supplementation to broiler chickens diets significantly affected live body weight, feed intake and feed conversion ratio, however, there is no significant difference for the slaughter parameters and mortality.

Nobakht *et al.* (2012) studied the effect of savory on growth yield carcass characteristics biochemical parameters of broiler chicken' blood at four levels of 0.5, 1, 1.5, 2%. Results confirmed the significance of savory on the growth and carcass characteristics of the broiler chickens. There was no significant difference among the treatments about immunological traits and biochemical parameters of blood. Therefore, savory can be confidently used in broiler chickens' diet to increase growth.

Ahmed (2011) evaluated the effect of graded levels (5, 10 and 15 gm/kg) fenugreek seeds addition to the Japanese quail males ration on semen quality and testis histological traits. Results showed a significant improvement in ejaculation volume, spermatozoa mortality, viability and semen concentration in comparison with control group. Also, the testis weight, seminiferous tubules diameter, germinal layer thickness and germinal layer area showed significant increase in fenugreek groups. However, fenugreek used as a supplement to poultry feeding to lowering plasma total lipids and total cholesterol in Hubbard broiler chicks (Azoua, 2001) and improve antioxidant status and production performance in laying hens (ALkatan, 2006). Fenugreek seeds improve the reproductive and physiological performance of broiler breeder males (Taha, 2008) and revealed positive significant results of semen trait in aged broiler breeder males (Abdul-Rahman *et al.,* 2010).

Bagyalashmi *et al.* (2011) investigated the effect of adding garlic, pepper, onion, ginger, fenugreek and curry on cholesterol concentration of egg yolk as invitro and using HPLC method. In this study, the egg yolk was extracted and then, 2 gr of each material was added to it and its cholesterol was extracted and measured after 10 minutes. Also, a part of the yolk impregnated by the mentioned materials was cooked and its amount of cholesterol was determined. Results for the cholesterol of raw yolk showed that, garlic and pepper had the highest influence by respectively 65.85 and 65.19% reduction in the amount of yolk cholesterol. About cooked yolk also, pepper and curry had the highest influence by respectively 61.68 and 47.00% reduction in the amount of yolk cholesterol. According to the mentioned results, the researchers suggested to use some pepper or curry while egg consumption.

Alloui *et al.* (2012) studied the effect of Fenugreek seeds at (3g Fenugreek seeds/ kg) as natural growth promoter for broiler chicken. They found that Fenugreek seeds supplementation significantly affected live body weight, feed intake and feed conversion ratio, however, there is no significant difference for the slaughters parameters and mortality.

Rabia (2010) studied the effect of fenugreek, parsely and sweet Basil seeds as natural feed additives on broiler performance. He observed that chicks fed basil diet had significantly heaviest body weight than those fed Fenugreek seeds. However, carcass characteristics had no significant differences.

Guo *et al.* (2004) reported that Chinese herbal medicine containing fenugreek and an antibiotic virginiamycin did not influence the fiber weight in broiler chicks. Ullah Khan *et al.* (2009) studied the effect of fenugreek essence on the internal organs of broiler chickens. In this study, treatments of 0.00 (control), 10, 20 and 30 ml/l of fenugreek

essence were applied. In the study on internal organs it was found that, liver weight was insignificantly higher in treatment of 20 and 30 ml/l compared to the treatments. For gizzardweight also there was no significant difference between fenugreek and control treatments. Intestinalweight was significantly higher in control treatment than fenugreek treatments. Totally, the results indicated that, fenugreek in broiler chickens' diet relatively causes to increase the size of some internal organs (except the intestines).

Al-Troudi and Hussein (2009) investigated the effect of fenugreek seed powder and blood parameters of broiler chickens. In this study, four treatments were used including: 1. control treatment (basal diet without additives), 2. Basal diet plus gentamicin antibiotic, 3, 4. Basal diet plus 1% and 2% of fenugreek seed powder respectively. Results showed that, adding fenugreek seed powder results in increase of body weight, growth speed, feed consumption improvementand FCR improvement. Red blood cells content andhemoglobin concentration also increased as a result of using fenugreek while, cholesterol and blood sugar concentration decreased, and these effects showed an increasing trend by increasing the amount of fenugreek from 0.00 to 2%.

Seleem *et al.* (2008) reported that supplementation of 0-3% fenugreek to rabbit diet showed a great role in enhancing the immune system, improved growth performance, blood metabolites and reproductive performance.

Jang *et al.* (2008) fed broiler chickens by 0.3 and 1% of a blend of three plants' extract and reported that, using these plants leads to improve the pectoral muscle' yield.

MottaqiTalab and Jamshidzehi (2008) in an experiment on 360 broiler chickens for four days with one of nine experimental diets without additives, basal diet plus 20 ppm virginiamycinantibiotic, basal diet plus 4, 2, 6 kg/ton of olive leaf powder and basal diet plus two types of olive leaf extract with levels of 200 and 400 gr/ton in a completely

randomized layout reported that virginiamycin antibiotic can be replaced effectively by olive leaf powder as an appropriate option.

Rahmani *et al.* (2008) studied the levels 0, 1, 1.5 and 2% green tea leaf and levels of 100 and 200 mg of vitamin E in broiler chickens' diet. They reported that, green tea leaf causes to reduce fat accumulationin the abdominal cavity of broiler chickens when slaughter.

Abaza (2007) studied the effect of using some medicinal plants (Fenugreek seeds, chamomile and radish) as feed additives on performance, egg quality, digestibility, blood constituents of laying hens, at the level of 0.5% for each the results showed that supplementation of diet with the medicinal plants increased numerically egg number than those fed control diet, at the same time significantly decreased feed consumption and improved feed conversion.

Lotfollahian *et al.,* (2007) reported that, using 0.5% greenherbal supplementof growth in the diet of broiler chickens is useful and appropriate. The cost of consumptive feed per kg of live weight increase in the group that used diet containing 0.5% greenherbal supplement of growth was less than control group and production index also in the same group was higher than the control group and other experimental diets. Masjedi *et al.* (2010) studied the effect of garlic extract on the amount of feed consumption and glucose level, cholesterol serumtriglycerides of diabetic rats'blood by streptozotocin. Results showed that, garlic extract decreased glucose level, cholesterol and triglycerides of diabetic rats' blood significantly; so that, glucose level of diabetic rats' blood that had received garlic extract, was same as the healthy rats. Also, the results showed that, garlic extract can be effective in adjustment of glucose, cholesterol and triglyceride levelin diabetic patients.

Saffa (2007) evaluated the variations in production efficiency, egg quality and cholesterol concentration of egg yolk of laying hens affected by adding fenugreek to their diet. In this experiment, laying hens were feed by a diet containing 2% for four weeks. Results showed that, there was no significant difference between fenugreek and control treatments about the body weight for the number of produced egg, egg weight, amount of feed consumption, FCR; but, the applied fenugreek increased the color of yolk. Also, concentration of total plasma cholesterol and yolk in response to fenugreek so that, HDL cholesterol was increased affected by the applied treatment but, LDL cholesterol was decreased. Results indicated that, the level 2% of fenugreek can have beneficial effects on the metabolism of cholesterol without having any adverse effect on the efficiency of laying hens.

Hernandes *et al.,* (2004) used 200 ppm of oregano, cinnamon, pepper extract and 5000 ppm of artemisia, thyme androsemary in diet of broiler chickens. Results showed that, the amount of growth in these treatments in the growth period of 14- 21 days was higher than the control group.

Afraz *et al.* (2002) studied experimental diets containing 1, 0, 2, 3, 4% alfalfa or clover powder, and the reported that, there is no significant difference among the diets' price economically but, by using the diet containing 1% clover, the final price perkilo of broiler live weight decreased by 7% compared to the control diet.In order to study the effect of various levels of Satureja Sahendica on broiler chickens' yield, Radar *et al.* (2005) used four treatments including control, 300, 200, 100 mg per kg of SaturejaSahendica. Results showed that, consumption of SaturejaSahendicaat level of 100 mg per kg decreases FCR when the poultries' growth is high.

Sayed and Hesham (2002) studied the feeding broiler chicks on diets containing various levels (1,1.5 and 2%) of local natural feed additives (hot pepper and Fenugreek seeds) at different levels of metabolizable energies (3200,3000 and 2800 Kcal/kg). Chicks fed fenugreek diet had significantly less body weight and higher feed intake and decrease abdominal fat percent.

Cross *et al.* (2002) reported that, there is no significant difference in increase of chickens fed by thyme and garlic extract compared to the control group. But, thyme oil causes to improve FCR in broiler chickens.

Shen *et al.* (1999) investigated the effect of seven types of medicinal plants including sage, fenugreekandfennel on lipid metabolism and production yield of laying hens. They reported significant results in the increase of egg production and significant reduction in the amount of yolk cholesterol.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site

Feeding trial of the study was conducted at poultry farm and nescessary laboratory analysis were performed at dairy and poultry science laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur to investigate the effects of feeding black cumin & methi seeds to layer chicken on egg quality & production performance. In this feeding trial, total 4-month experiment period, a total of fourty eight (48) Hi-sex brown laying hens (age 20 weeks) were allowed to four dietary treatments with three replication of four (4) birds in each for a period of 16 weeks.

3.2 Collection and Preparation of Black seed, Methi and test feed

Black seed and Methi were collected from the local area of Dinajpur district. The Black seed and Methi were sun-dried for one day. The sun-dried Black seed and Methi 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 seed 2gm/kg), T_2 (Methi 2gm/kg), T_3 (Black seed 2gm/kg and Methi 2.00gm/kg).

3.3 Preparation of bird

Birds were reared in the HSTU Poultry Farm. The experimental poultry cages constructed with compartments for housing two birds were the cages and the poultry shed were disinfected. Two troughs were placed in the cage compartment for feed and water, respectively. Total 48 laying birds were reared for egg production.

Fig. 3.1: Laying cage with experimental birds.

3.4 Experimental period

The experiment was conducted for a period of four months from January to April, 2015.

3.5 Experimental Diets

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

The experimental diets were designed as

Here,

T_0 (Control),

T_1 (Black seed 2 gm/kg),

T_2 (Methi 2gm/kg),

T_3 (Black seed 2gm/kg and Methi 2.00gm/kg).

3.6 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. Egg production recorded daily but external and internal quality characteristics of eggs were determined monthly.

Table 3.1 Chemical composition of experimental diets

Ingredients	T_0	T_1	T_2	T_3
Maize	53	53.5	54.8	56.5
Soyabean meal	22.6	21.9	22.2	22.1
Rice polish	11.5	11.3	11	11
Meat and bone meal	4	4	4	4
Limestone	7.8	7.8	7.8	7.8
DCP	0.75	0.75	0.75	0.75
Black seed	0	2	o	2
Methi	0	0	2	2
Salt	0.35	0.35	0.35	0.35

*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 k3: 800 mg; vitamin B1: 600 mg; vitamin B2: 2 mg; vitamin B3: 12 mg; vitamin B5: 3.2 mg; vitamin B6: 1.8 mg; vitamin B9: 2 mg; vitamin B12: 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 seed 2gm/kg), T_2 (Methi 2gm/kg), T_3 (Black seed 2gm/kg and Methi 2.00gm/kg).

3.7 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, albumin index, fresh albumin 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.

3.7.1 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-

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

3.7.2 Albumin index determination

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

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

3.7.3 Yolk index determination

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

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

3.7.4 Haugh unit determination

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

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

Where, HU = Haugh unit

H = Height of thick albumin

W = Egg Weight (gm)

3.7.5 Shell thickness

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

3.7.6 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 albumin with the help of a spatula and transferred to a previously weighed petridish and the raw yolk weight was taken. The albumin 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 albumin weight:

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

3. Shell dry weight:

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

3.8 Determination of cholesterol of egg yolk

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

3.9 Isolation of E. coli and salmonella from feces sample

3.9.1 Fecal sample collection, transportation and preparation

Fecal samples of chicken were collected from healthy broiler 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.

3.9.2 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 (EIMB. 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-Vogel Proskauer Broth (MR-VP Broth; HiMedia), Motility Indole Urea medium (MIU, HiMedia), Indole test.

3.9.3 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.

3.9.4 Sugars

- Dextrose
- Sucrose
- Lactose

3.9.5 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 45^0C 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.

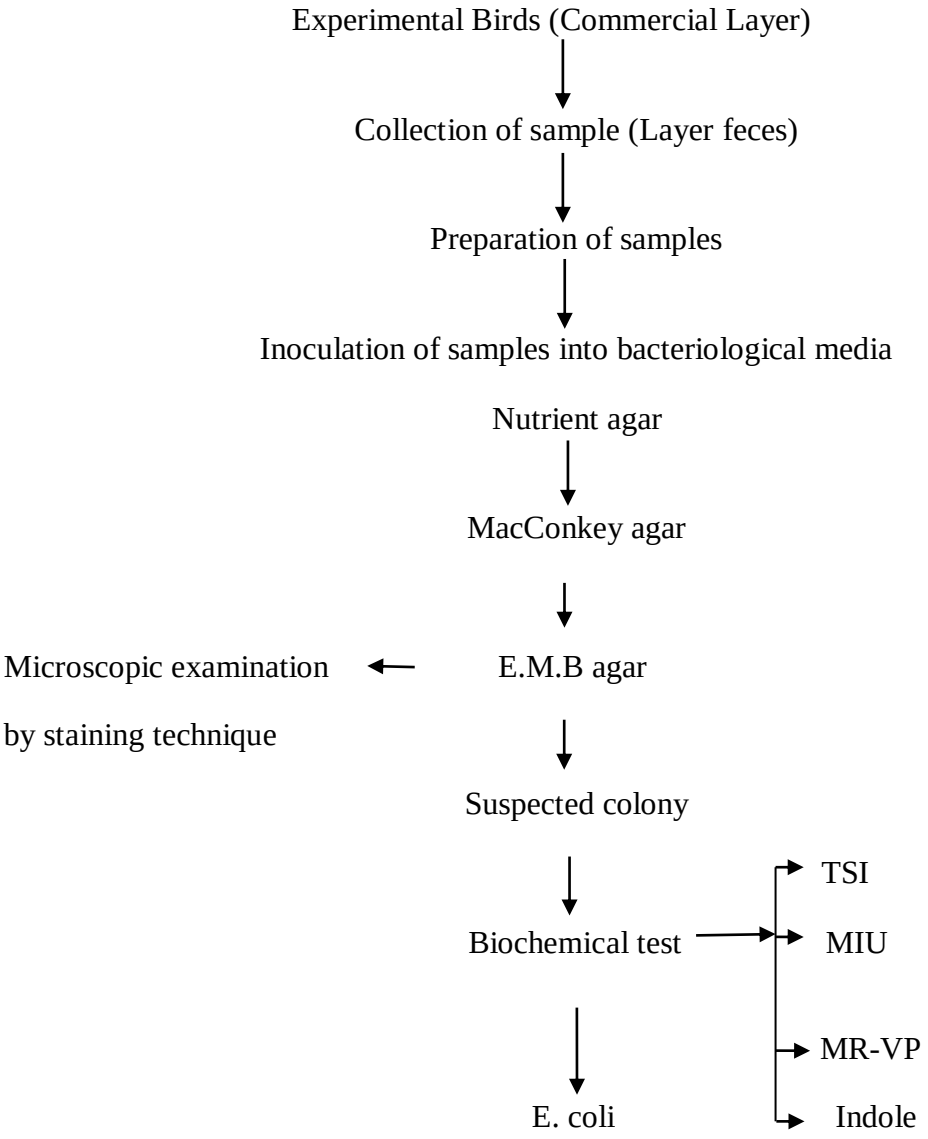

Fig. 3.2: Schematic Illustration of Experiment

3.10 Isolation of E. coli in pure culture

All the 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 was obtained.

3.11 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.

3.12 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.

- 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.

- 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.

- 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.

- 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:

We were used Nutrient agar media 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.

3.13 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 4

RESULTS AND DISCUSSION

This study was conducted to determine the dietary effect of black seed and methi on laying hen. The results of this study are discussed here under following headings.

4.1 Live weight

Table 4.1: Dietary effect of black seed and methi on live weight

Treatment	Average live weight of laying hen				
	Initial weight (kg) (Mean ± SE of mean)	1st month weight (kg) (Mean ± SE of mean)	2nd month weight (kg) (Mean ± SE of mean)	3rd month weight (kg) (Mean ± SE of mean)	4th month weight (kg) (Mean ± SE of mean)
T_0	$1.58^{a} \pm .03$	$1.70^{a} \pm .07$	$1.80^{a} \pm .07$	$1.97^{a} \pm .07$	$2.04^{a} \pm .12$
T_1	$1.56^{a} \pm .02$	$1.62^{a} \pm .03$	$1.79^{a} \pm .01$	$1.93^{a} \pm .03$	$2.07^{a} \pm .08$
T_2	$1.66^{a} \pm .05$	$1.76^{a} \pm .08$	$1.90^{a} \pm .05$	$1.93^{a} \pm .04$	$2.03^{a} \pm .04$
T_3	$1.63^{a} \pm .09$	$1.64^{a} \pm .06$	$1.80^{a} \pm .10$	$2.02^{a} \pm .09$	$2.14^{a} \pm .12$

Values with the different superscripts in the same column are statistically significant ($P<0.05$).

Data in Table 4.1 shows that final body weight of laying hens did not differ ($p>0.05$) against the dietary level of black seed and methi.

4.2 Egg production

Table 4.2: Dietary effect of black seed and methi on Egg production

Treatment	Egg production			
	1st month (Mean ± SE of mean)	2nd month (Mean ± SE of mean)	3rd month (Mean ± SE of mean)	4th month (Mean ± SE of mean)
T_0	$2.82^{a} \pm .03$	$3.24^{a} \pm .04$	$3.42^{a} \pm .04$	$3.34^{b} \pm .03$
T_1	$2.64^{a} \pm .09$	$2.93^{c} \pm .04$	$3.42^{a} \pm .12$	$3.55^{a} \pm .04$
T_2	$3.02^{a} \pm .08$	$3.18^{ab} \pm .09$	$3.33^{a} \pm .11$	$3.54^{a} \pm .08$
T_3	$2.90^{a} \pm .18$	$2.98^{bc} \pm .09$	$3.15^{a} \pm .09$	$3.49^{ab} \pm .06$

Values with the different superscripts in the same column are statistically significant ($P<0.05$).

Egg production of different groups of hens are presented in Table 4.2. The study reveled that egg production was the highest in four month, which was treated with black seed and methi. The combined treatment significantly ($p \leq .0.05$) affect the egg production. Egg production also increased indicating that the treatment were effective enough to promote the egg production.The present results are agreed with other results. Egg production markedly increased by using 1.5% powdered black cumin (Akhtar *et al.,* 2003) and 3% black seeds (Bolukbasi *et al.,* 2009) in layer diet.

Aydin *et al.* (2008) observed Black cumin (*Nigella sativa* L.) supplementation into the diet of the laying hen positively influences egg yield parameters, laying hens fed the diet supplemented with 3% black cumin had greater egg production than the control. Shen *et al.* (1999) reported significant results in the increase of egg production.

4.3 Egg Weight

Table 4.3: Dietary effect of black seed and methi on Egg Weight

Treatment	Average weight of egg			
	1st month (Mean ± SE of mean)	2nd month (Mean ± SE of mean)	3rd month (Mean ± SE of mean)	4th month (Mean ± SE of mean)
T_0	58.99 ^{b} ± .78	60.61 ^{b} ± 1.20	61.90 ^{b} ± 1.34	62.31 ^{a} ± .75
T_1	60.96 ^{ab} ± 1.04	61.96 ^{ab} ± 1.16	62.97 ^{ab} ± .78	62.18 ^{a} ± .49
T_2	64.27 ^{a} ± .97	64.12 ^{a} ± .56	64.93 ^{a} ± .20	63.60 ^{a} ± .31
T_3	61.29 ^{ab} ± 1.56	62.51 ^{ab} ± .73	64.34 ^{ab} ± .57	63.81 ^{a} ± .16

Values with the different superscripts in the same column are statistically significant ($P<0.05$)

Daily Egg Weight of different groups of hens are presented in Table 4.3. The study reveled that daily egg weight was increased after treatment with black seed and methi.

Saffa (2007) Results showed that, there was significant difference between fenugreek and control treatments about the body weight for the number of produced egg, egg weight. Abaza (2007) results showed that supplementation of diet with the black cumin increased numerically egg number than those fed control diet.

4.4 External and Internal Egg Quality

Table 4.4: Dietary effect of black seed and methi on egg quality (1st month)

Parameter	T_0 (Mean ± SE of mean)	T_1 (Mean ± SE of mean)	T_2 (Mean ± SE of mean)	T_3 (Mean ± SE of mean)
Weight of Egg (gm)	$69.37^{a} \pm 1.88$	$66.52^{ab} \pm 1.97$	$65.57^{ab} \pm 2.33$	$61.95^{a} \pm 1.08$
Width of Egg (mm)	$45.78^{a} \pm .69$	$45.64^{a} \pm .58$	$44.70^{a} \pm .51$	$45.31^{a} \pm .34$
Length of Egg (mm)	$58.67^{a} \pm .37$	$58.15^{a} \pm .83$	$59.13^{a} \pm .97$	$58.11^{a} \pm 1.42$
Height of Thick Albumin (mm)	$8.18^{a} \pm .63$	$7.50^{a} \pm .08$	$7.67^{a} \pm .22$	$7.57^{a} \pm .72$
Diameter of Albumin (mm)	$83.09^{ab} \pm 3.98$	$80.27^{b} \pm .422$	$87.45^{a} \pm .75$	$80.69^{ab} \pm .56$
Height of Yolk (mm)	$16.26^{a} \pm .26$	$17.18^{a} \pm .58$	$16.43^{a} \pm .54$	$15.65^{a} \pm .53$
Width of Yolk (mm)	$40.62^{a} \pm 1.23$	$40.28^{a} \pm 1.54$	$42.48^{a} \pm 3.81$	$38.21^{a} \pm .67$
Shell thickness (mm)	$.40^{a} \pm .003$	$.40^{a} \pm .003$	$.40^{a} \pm .003$	$.39^{a} \pm .003$
Fresh yolk wt.(gm)	$18.48^{a} \pm 1.18$	$16.50^{ab} \pm .19$	$15.87^{ab} \pm .90$	$14.84^{b} \pm .62$
Fresh Albumin wt. (gm)	$43.86^{a} \pm 2.52$	$43.53^{a} \pm 2.10$	$41.74^{a} \pm 1.30$	$43.06^{a} \pm 2.20$
Shell dry wt. (gm)	$6.95^{a} \pm .19$	$6.49^{ab} \pm .08$	$6.40^{b} \pm .13$	$6.56^{ab} \pm .11$

Values with the different superscripts in the same column are statistically significant ($P<0.05$)

Table 4.5: Dietary effect of black seed and methi on egg quality (2nd month)

Parameter	T_0	T_1	T_2	T_3
Weight of Egg (gm)	$66.88^{a} \pm .54$	$67.59^{a} \pm 3.26$	$66.43^{a} \pm 1.98$	$70.37^{a} \pm .92$
Width of Egg (mm)	$45.34^{a} \pm .49$	$45.48^{a} \pm .81$	$44.45^{a} \pm .96$	$45.97^{a} \pm .42$
Length of Egg (mm)	$58.47^{a} \pm .253$	$58.71^{a} \pm .355$	$58.78^{a} \pm 1.15$	$59.91^{a} \pm .332$
Height of Thick Albumin (mm)	$8.92^{b} \pm .04$	$9.85^{c} \pm 1.87$	$9.21^{b} \pm .38$	$10.05^{a} \pm 1.11$
Diameter of Albumin (mm)	$81.91^{ab} \pm 1.07$	$76.93^{b} \pm .645$	$80.51^{a} \pm 1.14$	$86.67^{ab} \pm 1.02$
Height of Yolk (mm)	$17.03^{a} \pm .54$	$17.61^{a} \pm .57$	$22.86^{a} \pm 5.52$	$18.73^{a} \pm .78$
Width of Yolk (mm)	$41.11^{a} \pm 1.00$	$38.86^{a} \pm .64$	$30.78^{a} \pm 8.30$	$38.58^{a} \pm .90$
Shell thickness (mm)	$.39^{a} \pm .003$	$.39^{a} \pm .006$	$.38^{a} \pm .008$	$.38^{a} \pm .003$
Fresh yolk wt.(gm)	$16.80^{a} \pm .42$	$16.17^{a} \pm .40$	$16.02^{a} \pm .43$	$18.06^{a} \pm 1.37$
Fresh Albumin wt. (gm)	$41.86^{a} \pm .98$	$44.20^{a} \pm 2.0$	$43.21^{a} \pm 1.33$	$44.69^{a} \pm 1.75$
Shell dry wt. (gm)	$7.44^{a} \pm .69$	$7.20^{a} \pm .88$	$7.19^{a} \pm .24$	$7.48^{a} \pm .46$

Values with the different superscripts in the same column are statistically significant ($P<0.05$)

Table 4.6: Dietary effect of black seed and methi on egg quality (3rd month)

Parameter	T_0	T_1	T_2	T_3
Weight of Egg (gm)	68.27 [a] ± 1.12	68.48 [a] ± .97	68.26 [a] ± .77	68.40 [a] ± .34
Width of Egg (mm)	45.05 [a] ± .929	42.78 [b] ± .22	45.52 [a] ± .46	45.07 [a] ± .064
Length of Egg (mm)	58.55 [a] ± .63	57.86 [a] ± .04	58.75 [a] ± 1.04	59.04 [a] ± .28
Height of Thick Albumin (mm)	8.23 [a] ± .39	8.35 [a] ± .09	8.56 [a] ± .29	8.32 [a] ± .12
Diameter of Albumin (mm)	84.20 [a] ± 3.42	83.32 [a] ± 2.22	85.86 [a] ± 2.56	86.49 [a] ± 1.08
Height of Yolk (mm)	17.98 [a] ± .55	16.69 [b] ± .15	17.29 [ab] ± .25	17.84 [a] ± .12
Width of Yolk (mm)	39.79 [a] ± 2.41	37.65 [a] ± .13	41.02 [a] ± 1.73	41.62 [a] ± .36
Shell thickness (mm)	.40 [a] ± .01	.38 [a] ± .005	.39 [a] ± .003	.39 [a] ± .003
Fresh yolk wt.(gm)	17.50 [ab] ± .30	16.60 [b] ± .12	18.80 [a] ± 1.04	18.86 [a] ± .57
Fresh Albumin wt. (gm)	42.84 [a] ± 1.37	44.50 [a] ± 1.17	42.06 [a] ± .24	41.86 [a] ± .34
Shell dry wt. (gm)	8.00 [a] ± .12	7.38 [a] ± .18	7.40 [a] ± .21	7.66 [a] ± .20

Values with the different superscripts in the same column are statistically significant ($P<0.05$)

Table 4.7: Dietary effect of black seed and methi on egg quality (4th month)

Parameter	T_0	T_1	T_2	T_3
Weight of Egg (gm)	$68.73^{a} \pm 1.03$	$68.21^{a} \pm .44$	$68.18^{a} \pm .95$	$67.55^{a} \pm .48$
Width of Egg (mm)	$45.76^{a} \pm .83$	$45.04^{a} \pm .11$	$44.83^{a} \pm .10$	$44.82^{a} \pm .05$
Length of Egg (mm)	$58.59^{a} \pm .31$	$57.92^{a} \pm .196$	$57.96^{a} \pm .04$	$58.42^{a} \pm .20$
Height of Thick Albumin (mm)	$8.22^{a} \pm .07$	$8.28^{a} \pm .09$	$8.26^{a} \pm .17$	$8.27^{a} \pm .26$
Diameter of Albumin (mm)	$87.50^{a} \pm .85$	$87.58^{a} \pm .45$	$87.76^{a} \pm .34$	$86.76^{a} \pm .81$
Height of Yolk (mm)	$18.01^{a} \pm .05$	$18.12^{a} \pm .08$	$17.94^{a} \pm .18$	$17.58^{a} \pm .24$
Width of Yolk (mm)	$39.95^{b} \pm .171$	$40.76^{ab} \pm .069$	$40.32^{ab} \pm .385$	$41.76^{a} \pm .88$
Shell thickness (mm)	$.40^{a} \pm .003$	$.39^{a} \pm .003$	$.40^{a} \pm .003$	$.40^{a} \pm .005$
Fresh yolk wt.(gm)	$17.71^{ab} \pm .24$	$17.05^{b} \pm .08$	$17.31^{ab} \pm .84$	$18.95^{a} \pm .59$
Fresh Albumin wt. (gm)	$43.48^{a} \pm .97$	$43.86^{a} \pm .56$	$43.35^{a} \pm .92$	$41.63^{a} \pm .69$
Shell dry wt. (gm)	$7.53^{a} \pm .20$	$7.30^{a} \pm .30$	$7.21^{a} \pm .29$	$6.96^{a} \pm .12$

Values with the different superscripts in the same column are statistically significant ($P<0.05$)

Egg quality parameter of different groups of hens are presented in Table – 4.4, 4.5, 4.6 and 4.7. The study reveled that egg quality parameter were improved after treatment with black seed and methi. In this study, demonstrate that there exist a significant ($P<0.05$) difference among the mean values like Weight of the egg (gm), Diameter of the albumin (mm), Height of the yolk (mm), Fresh albumin wt (gm) corresponding to the different level of black seed and methi treatment .But no significant ($p>0.05$) difference among the mean values like Width of the egg (mm), Length of the egg (mm, Height of the thick albumin (mm), Width of the yolk (mm), Shell thickness (mm), Fresh yolk wt (gm), Shell dry wt (gm) corresponding to the different level of black seed and methi treatment. These

results indicate that combined treatment had no adverse effect on external and internal qualities of eggs. The present results are agreed with other results. Saffa (2007) evaluated the variations in production efficiency, egg quality and cholesterol concentration of egg yolk of laying hens affected by adding fenugreek to their diet.

Table 4.8: Dietary effect of black seed and methi on Feed intake weekly

Week	T_0 (Mean ± SE of mean)	T_1 (Mean ± SE of mean)	T_2 (Mean ± SE of mean)	T_3 (Mean ± SE of mean)
1st	$2.677^{ab} \pm .149$	$2.370^{b} \pm .10$	$2.790^{a} \pm .13$	$2.743^{ab} \pm .08$
2nd	$2.977^{a} \pm .073$	$2.730^{a} \pm .14$	$2.757^{a} \pm .079$	$2.893^{a} \pm .045$
3rd	$3.093^{a} \pm .033$	$3.087^{a} \pm .069$	$2.843^{b} \pm .081$	$2.917^{ab} \pm .037$
4th	$3.040^{a} \pm .037$	$2.870^{a} \pm .217$	$2.847^{a} \pm .035$	$3.010^{a} \pm .058$
5th	$3.070^{a} \pm .071$	$3.043^{a} \pm .092$	$4.00^{a} \pm 1.05$	$2.880^{a} \pm .025$
6th	$3.043^{a} \pm .038$	$2.983^{a} \pm .077$	$3.043^{a} \pm .038$	$2.957^{a} \pm .033$
7th	$3.117^{a} \pm .004$	$3.113^{a} \pm .004$	$3.160^{a} \pm .025$	$3.103^{a} \pm .055$
8th	$3.173^{a} \pm .039$	$3.200^{a} \pm .011$	$3.240^{a} \pm .021$	$3.233^{a} \pm .031$
9th	$3.227^{a} \pm .012$	$3.180^{a} \pm .029$	$3.230^{a} \pm .035$	$3.240^{a} \pm .011$
10th	$3.100^{b} \pm .051$	$3.317^{a} \pm .033$	$3.317^{a} \pm .020$	$3.317^{a} \pm .020$
11th	$3.150^{a} \pm .034$	$3.213^{a} \pm .018$	$3.170^{a} \pm .055$	$3.210^{a} \pm .009$
12th	$3.150^{a} \pm .025$	$3.183^{a} \pm .026$	$3.213^{a} \pm .049$	$3.220^{a} \pm .055$
13th	$3.077^{b} \pm .038$	$3.160^{ab} \pm .031$	$3.16^{ab} \pm .048$	$3.210^{a} \pm .010$
14th	$3.14^{a} \pm .061$	$3.233^{a} \pm .033$	$3.140^{a} \pm .005$	$3.163^{a} \pm .044$
15th	$3.117^{a} \pm .008$	$3.147^{a} \pm .026$	$3.190^{a} \pm .030$	$3.133^{a} \pm .081$
16th	$3.053^{b} \pm .049$	$3.180^{a} \pm .021$	$3.230^{a} \pm .035$	$3.177^{a} \pm .024$

Values with the different superscripts in the same column are statistically significant ($P<0.05$)

Feed intake of different groups of hens are presented in Table 4.8. The study reveled that feed intake of hen were improved after treatment with black seed and methi. The present results are agreed with other results. Sayed and Hesham (2002) showed that, fenugreek diet had significantly higher feed intake. Alloui *et al.*, (2012) found that Fenugreek seeds supplementation significantly affected feed intake and feed conversion ratio.

4.5 Bacterial colony count

Table 4.9: Dietary effect of black seed and methi on Bacterial colony count

Treatments	Number of bacterial colony
T_0	$244.17^{c} \pm 0.29$
T_1	$189.83^{b} \pm 6.30$
T_2	$160.33^{ab} \pm 7.39$
T_3	$156.50^{a} \pm 9.31$

Values with the different superscripts in the same column are statistically significant ($P<0.05$)

Bacterial colony counts of different groups of hens are presented in Table 4.9. The study reveled that bacterial colony count was gradually decreased after treatment with black seed and methi.

CHAPTER 5

SUMMARY AND CONCLUSIONS

This study was carried out to assess, examine and quantify the effect of dietary black seed and methi supplementation on egg production and egg quality of laying hens at 20 to 36 week age. Total 48 Hisex Brown hens of 20 weeks old were allocated to 4 groups, each containing 12 hens. The hens in individual cage were supplied fixed amount of feed (120 gm/day) containing 18.5% CP and 2725 Kcal/kg diet. Hens were randomly alloted to 4 dietary treatments: T_0: (control), T_1: (black seed 2gm/kg), T_2: (Methi 2gm/kg) and T_3: (black seed 2gm/kg and methi 2gm/kg). Body weight change, egg production, feed conversion, egg weight, egg size characteristics were recorded and compared. Shape index, yolk index, albumen index, Haugh unit, shell thickness, per cent shell, per cent albumen and per cent yolk were also recorded. Increased egg production, egg mass production and feed conversion increased at increasing level of black seed and methi. Shell weight, shell percent, shell thickness, shape index, Albumin index, yolk index, and percent yolk did not affected with increasing levels of black seed and methi ($p>0.05$).

Considering the above facts, following conclusions were drawn. Combined treatment of black seed and methi supplementation at different level in laying hen's diet improved persistence of lay and feed conversion during the period of 20-36 week of age.

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