Use of black cumin in layer diet as cholesterol lowering agents in egg yolk

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ABSTRACT

This study was conducted to investigate the effects of different levels of black cumin seeds *(Nigella sativa L.)* on egg production and cholesterol concentration in egg yolk of laying hens. A total of 60 commercial layer strain day old layer chicks were collected and divided into three groups treated with 1.0%, 1.5% and 2.0% black cumin inclusion. The concentrations of total lipids, total cholesterol, phospholipids and triacylglycerols in serum and egg yolk were measured. Feeding of the diets with 1%, 1.5% and 2% black cumin seeds during the laying period found egg yolk cholesterol by 11.12, 9.88 and 9.83 mg/g respectively. The results found that feed efficiency ratio, egg production, body weight, feed intake and egg weight were nonsignificant between the treatments. However, egg yolk cholesterol concentration was found that 1.5% and 2.0% black cumin in diet were reduced cholesterol concentration insignificance ($P<0.05$). So, dried black cumin supplementation in diets had no any adverse effect on egg production and egg weight. Furthermore, egg yolk cholesterol concentrations were decreased. Hence, it is concluded that black cumin (*Nigella sativa L.*) seeds and/or the active principle are of interest as potential egg-yolk cholesterol-lowering agents.

Key words: Laying hen, egg yolk, black cumin, cholesterol

INTRODUCTION

Egg yolks provide excellent functional properties to variety of food products such as mayonnaise, ice-cream, bakery items and other egg products. The functional properties of egg yolk are attributed primarily to the phospholipids and protein components. However, in addition to these desirable components, yolk from a large egg contains about 213 mg cholesterol. Concern regarding the cholesterol content of the human diet is growing, because of the probable importance of cholesterol and cholesterol oxide products in the development of atherosclerosis . Thus, egg processors are interested in finding technologies to reduce the cholesterol content of their products to meet consumer demands for healthier food products. The poultry industry has continued to seek to reduce egg cholesterol concentrations so that an egg with reduced cholesterol will be available to those consumers who need to lower their dietary cholesterol intake. The use of nutritional strategies to reduce egg cholesterol concentrations is an attractive alternative. Also researcher thinking decrease cholesterol in egg yolk from herbs and spices. Chowdhury et al. (2004) found that tamarind supplementation 2%, 4%, 6% and 8% can decrease cholesterol in egg yolk. Study to effect of 1% and 3% black cumin supplementation on diet can reduce cholesterol content in egg yolk (Aydin, 2008). The aim of this study is to reduce cholesterol level in egg yolk by using black cumin. Therefore, the purpose of the study was to investigate the effect of the productive performance of laying hen by using black cumin supplementation in layer diet and to find out the effects of black cumin supplementation in layer diet on cholesterol concentration in egg yolk.

METHODOLOGY

Statement of the experiment

The study was conducted at Sher-e-Bangla Agricultural University (SAU) Poultry farm, Dhaka, Bangladesh. About 60 commercial day old layer strain day old layer chicks were collected and divided into treatment groups 1 to 4. The chicks of each treatment group were further randomly divided in the form of 3 replications each having 5 birds. The chicks of treatment group 1 to 4 were mentioned in (table 1) treated with 1.0%, 1.5% and 2.0% respectively.

Table 1: Design of Experiment

Treatments	Doses
T1	1.0% Black cumin
T2	1.5% Black cumin
T3	2.0% Black cumin
T0	Control

Management Practices

Different aspects of the management of chicks, experimental events and management procedures are described below in details:

Preparation of layer house

The layer shed was an open sided natural house. Cross ventilation system was provided by using wire-net. It was a tin shed house with concrete floor. There was 1ft side wall around the shed with no ceiling. The floor was above 1ft. from the ground and the top of the roof was above 15ft from the floor. Polythene sheet was hanged around the side wall to protect the chicks from cold, storm, dusts and heavy rainfall. The house was properly cleaned, rubbed with bleaching powder and washed the floor by using tap water and then disinfected by diluted iosan solution before starting the experiment.

Experimental chicks and diets

The day-old layer chicks were collected from Kazi Hatchery, Gazipur, Bangladesh.The basal diet was prepared by using locally available feed ingredients. The proximate analysis of layer feed was done in Poultry Nutrition Laboratory at BLRI (Bangladesh Livestock Research Institute) and Animal Nutrition Laboratory at SAU. In the laboratory report, the calorie and protein were found almost similar as per recommended level of poultry scientists. In control group the birds were fed the basal diet with no feed supplement. The other groups were given basal diet with feed supplements (black cumin) according to treatments of experiments. The ingredients percentage of basal diet in different periods of experiments and their calculated chemical composition are shown in table 2.

Table 2: Different diets and chemical composition

Ingredients %	1-7 weeks	8-18 week	19-72 weeks
Yellow corn	58	65	69
Soybean meal (45%)	34	27	20
Fish meal	5	5	5
Methionine	0.15	0.15	0.15
Lysine	0.1	0.1	0.1
Vitamin-mineral premix*	0.25	0.25	0.25
Oyster shell	2	2	5
Salt	0.5	0.5	0.5
Calculated chemical composition of the diet*			
ME (Kcal/kg)	2800	2800	2850
Crude protein (%)	20	16	16
Calcium (%)	1	1	3.5
Lysine	1.00	0. 80	0.70
Methionine	0.45	0.32	0.30
Methionine+cystine	0.80	0.70	0.65

*Provided per kilogram of diet:
Vitamin A-12,500IU, Vitamin D3- 2,500IU, Vitamin E- 20mg, Vitamin K3- 4mg, Vitamin B1- 2.5mg, Vitamin B2- 5mg, Vitamin B6- 4mg, Nicotinic acid- 40mcg, Pantothenic acid- 12.5mg, Vitamin B12- 12mcg, Folic acid- 0.8mg, Biotin- 0.1mg ; Cobalt- 0.4 mg, Copper 10mg, Iron 40mg, Iodine- 0.4mg, Manganese- 60mg, Zinc- 50mg, Selenium- 0.15mg; Di-Calcium-Phosphate- 0.38gm; DL-Methionine- 100mg, L-Lysine- 60mg, Zinc-bacitracin- 4mg, Anti-Oxidant- 5mg, Carrier (lime stone)- 2.5g

Litter management

Fresh, clean and sun dried rice husk was used as shallow litter to absorb moisture from fecal discharge of layer chicken. At first litter was 5 cm in depth. About 250g calcium oxide powder was mixed with rice husk in every pen as disinfectant. At the end of each week the litter was harrowed to prevent accumulation of toxic gases and to reduce moisture and parasitic infection. Every week, droppings were cleaned from the surface level by removing a thin layer of litter and same amount new litter was placed in each pen.

Care and brooding of chicks

After receiving of chicks, vaccination (IB+ND, 0 day) was done and chicks were placed under the pre-heated brooder. Rice husk litter was used under the hover of the brooder**.** Just after arrival of day old chicks to the poultry house the initial weight of the chicks were recorded by a digital electronic balance, vaccination was done and distributed them under the hover for brooding. The chicks were supplied glucose water with vitamin-C to drink for the first 3 hours to overcome dehydration and transportation stress. Subsequently small feed particles were supplied on the newspapers to start feeding for the first 24 hours. Gas brooder was used to brood chicks. Partitioning brooding was done due to different experimental treatment. Each brooder had one hover and a round chick guard to protect chicks and four portioning chambers. Thereafter healthy baby chicks were randomly distributed to the pen according to the design of the experiment. The recommended brooding temperature was 35-21^{0}C from 1st to 4th weeks of age. Moreover, at that time the wall polythene sheet spread over the net-wire to protect the layer chicks from cold and wind. After 7 weeks of age, birds were transferred to the cage as per treatment groups.

Feeding and drinking

Mash feed was used as starter (0-7 wks.), grower (8-17 wks.) ration and layer (18-72 wks) ration. Black cumin were supplied to the birds with mash basal feed as per lay-out of the experiments. Fresh clean drinking water was also supplied *Ad libitum*. Feeds and water were supplied 2 times: morning, and evening daily. Left over feeds and water were recorded to calculate actual intake. Digital electronic balance and measuring plastic cylinder was used to take record of feed and water. Daily water consumption (ml) and weekly feed consumption (gm)/bird were calculated to find out weekly and total consumption of feed and water. All feeders and drinkers were washed and sun-dried before starting the trial. Feeder and drinker size were changed according to the age of the birds. Feeders were washed at the end of the week and drinkers once daily.

Lighting and ventilation

The lighting was maintained as per following schedule (Table 3) the layer shed was south facing and open-sided. Due to wire-net cross ventilation, it was easy to remove polluted gases from the farm. Besides, on the basis of necessity ventilation was regulated by folding polythene screen.

Table 3: Lighting schedule during the experimental period

Age (week)	Lighting Hours
1	22
2-16	Natural day light
17	14
18	15
19	15
20-72	16

Bio security, medication and sanitation

To keep disease away from the layer farm the following vaccination (table 4), medication and sanitation program was undertaken. The layer chicks were fed antibiotic drug against bacterial diseases. Vitamin-B complex, vitamin-A, D3, E and sinacal-D were also used against deficiency diseases. Electromin and vitamin-C also used to save the birds from heat stress. Proper hygienic measures were maintained throughout the experimental period. Cleaning and washing of layer shed and its premises were under a routine sanitation work. Flies and insects were controlled by spraying Phenol and Lysol to the surroundings of the layer shed. The attendants used farm dress and shoe. There was a provision of Foot Bath at the entry gate of the layer shed to prevent any probable contamination of diseases.

Table 4*:* Vaccination schedule

Age	Name of Disease
0 day	Infectious Bronchitis + Newcastle Disease (IB+ND)
18 days	Gumboro (IBD)
24 days	Gumboro, Newcastle, Bronchitis
30 days	Gumboro
6 weeks	Newcastle, Bronchitis
10 weeks	Newcastle, Bronchitis
13 weeks	Pox, Newcastle, Bronchitis, Salmonella
15 weeks	Newcastle, Bronchitis

Data collection

The feed intake, live weight gain, feed conversion ratio and cholesterol level of eggs of different experimental birds were calculated. Detail of each data collection procedure are given below:

Feed intake (FI)

Daily feed consumption record of each replication was kept to get weekly and total feed consumption record per bird. Total feed intake a replication was divided by number of live birds in each replication to get average feed intake per bird.

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

Body weight gain (BWG)

The initial day-old live weight and weekly live weight of each replication was recorded to get final live weight per bird. The average body weight gain of each replication was calculated by deducting initial body weight from the final body weight of the birds.

Feed Conversion ratio (FCR)

Total feed consumption per bird was divided by wt. of total production.

$$FCR = \frac{Feed\ intake\ (g)}{Egg\ weight\ (g)}$$

Total cholesterol in egg yolk

Eggs were collected from each treatment and 5% of total eggs were selected for total cholesterol (mg/g) analysis. Analysis was done in Central Disease Investigation Laboratory (CDIL), Kazi Alauddin Road, Dhaka.

Statistical Analysis

Total data were complied, tabulated and analyzed in accordance with the objectives of the study. Excel Program was practiced for preliminary data calculation. Data were analyzed in factorial experiment with Randomized Completely Block Design (RCBD) for ANOVA table. MSTAT-C computer package program (Russel, 2004) was used for data analysis. Duncan Multiple Range Tests (DMRT) was done at 5% level of significance.

RESULTS AND DISCUSSION

In this experiment, 1%, 1.5%, 2.0% and 0% of black cumin in diets has no effect on body weight, feed intake and egg weight of laying hen. However egg production, feed conversion ratio (FCR) and cholesterol level in egg yolk has significant effect on different level of black cumin in laying hen diets as shown in Table 5. Data regarding the effects of different level dietary black cumin on BW has no significant effect *(p>0.05)*, however 1.5% black cumin in diet has little bit higher body weight than other treatments though statistically insignificant. In a study conducted in chickens, Bagir *et al.* (2006) showed that dietary black cumin at the level of 1% or 3% significantly (P<0.01) increased the final BW of laying hens. On the other studies showed that addition of black cumin seeds into the diet significantly decreased BW of the chickens (Steel *et al.,* 1980)**.** Because increase in body mass of laying hens was negatively correlated with egg production, reduction of body mass in layers fed diets supplemented with black cumin can be considered a favorable factor in increasing egg production (El-Bagir *et al.,* 2006)**.** Conversely, the results of the present study was showed that supplementation of black cumin in diet did not negatively influence final BW of the laying chickens. No significant different was found in discussing feed intake (kg/bird) of bird up to 72 weeks with different level of black cumin in diet of laying bird. In our present study inclusion of black cumin in the layer improves FCR significantly in comparison with no black cumin used in the diet to 1.5% black cumin used in the diet (2.48 to 2.28) that is feed cost of the farm reduced and increased profitability. El-Bagir et al. (2006) showed that inclusion of black cumin in the diets of the laying hens improved FCR per dozen eggs from 1.97 to 1.50 and FCR per kilogram of egg mass from 2.90 to 2.22. Egg production expressed as percentage was not significantly influenced within the diet treated with 1.0%, 1.5% and 2.0% black cumin but all diet significantly influenced with control group. However, group-mean egg production was much higher in the birds fed diet T2 than in their counterparts fed one of the other diets. Aydin *et al*. (2008) used diets with 0, 1, 2 or 3% black cumin seed and reported egg production values of 77, 81, 78 and 84%, respectively. Yalcin *et al*. (2009) fed laying hens on diets containing 0, 0.5, 1 or 1.5% black cumin seed and measured egg yields 91, 91, 92 and 92%, respectively. El-Sheikh *et al*. (1998) used diets with 0, 0.5, 1 or 2% black cumin seed and found egg production values of 69, 79, 79 and 77%, respectively. The outcomes of the present study and that of Yalcin *et al*. (2009) are comparable with regard to the egg production level by the control group and the lack of effect of dietary black cumin seed. In the studies of El-Sheikh *et al*. (1998), El-Bagir *et al*. (2006) and Aydin *et al*. (2008), baseline egg production was similar but the feeding of black cumin had inconsistent effects.

Table 5: Effect of black cumin on performance of laying hen

Parameters	Treatments			
	T1	T2	T3	T0
Body weight(kg)	1.92 [a]	1.93 [a]	1.98 [a]	1.82 [a]
Feed Intake(kg/bird) up to 72 weeks	44.12 [a]	43.98 [a]	43.81 [a]	45.01 [a]
Egg Production (%)	79.11 [a]	80.01 [a]	79.89 [a]	75.23 [b]
Egg weight (g)	60.11 [a]	60.23 [a]	60.40 [a]	60.31 [a]
FCR	2.30 [a]	2.28 [a]	2.31 [a]	2.48 [b]
Cholesterol in yolk (mg/g)	11.12[b]	9.88 [a]	9.83 [a]	12.38[c]

Table 5 shows that egg weights and other parameters of egg such as haugh unit, color score, albumen index, yolk index and shell thickness were no significant different between treatments ($p>0.05$). Cholesterol concentration in egg yolk were reduced markedly in 1.0% of black cumin (11.12 mg/g), 1.5% black cumin (9.88 mg/g) and 2.0% black cumin (9.83 mg/g) when compared with control (12.38 mg/g) group. Karaman *et al.* (2008) shows the effect of black cumin supplementation on the egg yolk cholesterol and egg yolk cholesterol was decreased significantly ($P < 0.05$) by supplementation of black cumin in the diet of the layer. Leskanish and Noble (1997) found that feeding black cumin seeds caused significant reductions in the concentrations of egg yolk triacylglycerols and phosphorlipids. The seeds were also found to lower the serum concentrations of triacylglycerols and phospholipids. It could thus be suggested that the decrease in serum lipids had caused a decrease in egg yolk lipids. It is not known how black cumin seeds lower the contents of triacylglycerols and phospholipids in serum, it is tempting to speculate that a component of the seeds inhibits in the liver the flux of acetyl-CoA into the lipogenic pathway and such an effect would also dampen cholesterol synthesis.

CONCLUSION

The results found that feed efficiency ratio, egg production, body weight, feed intake and egg weight were no significant between treatments. However, egg yolk cholesterol concentration was founded that 1.5% and 2.0% black cumin in diet were reduced cholesterol concentration with statistical significance ($P<0.05$). So that dried black cumin supplementation in diets were no any effect on egg production and egg weight. Furthermore, egg yolk cholesterol concentrations were decreased. Hence, supplementation of dried black cumin in diets could benefit to consumer health.

ACKNOWLEDGEMENT

Authors are grateful to Ministry of Science and Technology, Dhaka, Bangladesh for giving fund to conduct the research program.

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