

## *Nigella sativa* L. supplemented diet decreases egg cholesterol content and suppresses harmful intestinal bacteria in laying hens\*

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### ABSTRACT

The effects of supplementation of varying doses of *Nigella sativa* L. seeds in diet on feed intake, health, egg laying performance, serum and egg cholesterol contents, and population of intestinal bacteria in laying hens were investigated. One hundred 27-wk-old laying hens (Hisex Brown) were offered manually prepared diets supplemented with 0, 1.5, 3.5 or 4.5% seed powder for 10 weeks. *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.

**KEY WORDS:** laying hens, *Nigella sativa* seeds, hen performance, egg quality, egg cholesterol, intestinal bacteria

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## INTRODUCTION

Antibiotics as feed additives have been used for years to improve the profitability of poultry production by helping to control pathogenic bacteria in the gut mucosa, thereby improving weight gain, feed conversion ratio, and uniformity. However, the development of direct antibiotic resistance of pathogens in the species receiving the feed (Jang et al., 2007), as well as the indirect resistance to similar antibiotics used in human medicine as the result of food chain residues (Shea, 2003), led to the ban of all sub-therapeutic levels of growth promoting antibiotics by EU and many other countries including Bangladesh. Removal of antibiotics from the diet may negatively affect profitability of the poultry industry. Therefore, there is a great interest in developing natural alternatives to antibiotic growth promoters in order to maintain both bird performance and health (Cross et al., 2007). Plant extracts and their essential oils have a wide range of activities, including inhibitory action on pathogens, effects on physio-pathologies (e.g., anti-inflammatory, anti-diarrhoea, etc.) and activity in different body systems, e.g., endocrine and immune system (Gali-Muhtasib et al., 2006; Yazan et al., 2009).

One of the alternatives to synthetic antibiotic feed additives in poultry feed could be the seeds of a famous herbal medicinal plant, *Nigella sativa* L. (Padhya et al., 2008). The *N. sativa* is an annual herb belongs to the family of *Ranunculaceae*, which grows in countries bordering in Mediterranean seas as well as in many countries in Asia including Bangladesh (Cheikh-Rouhou et al., 2007). The black seeds of this useful herb have been used for centuries in the Middle East, Northern Africa, Far East and Asia for the treatment of asthma (El-Tahir et al., 1993) and as an antitumor agent (El-Daly, 1998). The seed has been reported to have many biological properties including antiparasitic (Mahmoud et al., 2002), antidiabetic (Al-Hader et al., 1993), anticancer (Padhya et al., 2008) and diuretic effects (Zaoui et al., 2000). Antibacterial activity of *N. sativa* seed extracts has also been reported (Nair et al., 2005).

Feeding of powdered *N. sativa* seeds has been shown to lower serum cholesterol, increase egg production, egg mass and eggshell thickness in laying hens (Akhtar et al., 2003). The yolk from a large egg contains about 213 mg of cholesterol (USDA, 1991). To avoid elevations in blood cholesterol and reduce the risk of coronary heart disease, consumption of no more than 300 mg of cholesterol per day has been recommended (Weggemans et al., 2001). Therefore, the poultry industry has continued to seek to reduce egg cholesterol contents so that an egg with reduced cholesterol will be available to the consumers who need to lower their dietary cholesterol intake.

Although medicinal properties of *N. sativa* seeds have been described in many papers, however, studies concerning the effects of *N. sativa* supplemented diets on feed intake, body weight, egg laying performance, egg parameters, cholesterol content in blood serum and egg yolk of the laying hens are scant (Akhtar et al., 2003). Intestinal bacteria play a vital role in digestion of feed and maintaining good health of poultry. However, no information is available on the effect of *N. sativa* seed supplementation in poultry diets on population of intestinal bacteria and performances of laying hens. The mode of action of the beneficial effects of *N. sativa* supplemented poultry feed is also poorly understood. To assess the beneficial effects and commercial potentials of *N. sativa* seeds as an alternative to antibiotic additives in poultry feed, a thorough investigation is needed. Therefore, the objectives of this study were to evaluate the effects of supplementation of varying doses of *N. sativa* seed powder into poultry diet on feed intake, body weight, egg laying performance, egg and blood cholesterol contents and population of intestinal bacteria in laying hens.

## MATERIAL AND METHODS

### *Preparation of N. sativa seed powder*

*N. sativa* L. seeds were obtained from a local market of Magura (Bangladesh). The seeds were coarsely powdered by a mechanical grinder and then directly mixed with manually prepared diets in appropriate doses (Table 1).

### *Animals and design of experiment*

This study was conducted at the animal research farm of Hajee Mohammad Danesh Science and Technology University, Dinajpur (Bangladesh). One hundred 27-week-old laying chickens (Hisex Brown) were randomly assigned into 5 groups with 4 replications, each of which has 5 birds, i.e. 20 laying hens per group. Hens were kept in cages of length 46 cm, width 38 cm and height 38 cm and 16 h light have been maintained throughout the experiment. The birds were offered restricted diets (120 g/ bird) supplemented with 0 (no seed powder); ( $T_0$ ), 1.5 ( $T_1$ ), 3.0 ( $T_2$ ) or 4.5% ( $T_3$ ) *N. sativa* seed powder for 10 weeks. Water was available *ad libitum* to the birds during the trial. The composition of manually prepared experimental diet used in different treatments for the layers is presented in Table 1.

Table 1. Composition of experimental diets for the laying hens under different treatments

Item	Dietary level of <i>Nigella sativa</i> , %			
	T <sub>0</sub> , kg (0)	T <sub>1</sub> , kg (1.5)	T <sub>2</sub> , kg (3.0)	T <sub>3</sub> , kg (4.5)
<i>Feed ingredients, %</i>				
maize	53	53	53	52
soyabean meal	22.6	21.1	21.1	20.6
rice polish	11.5	11.5	10	10
meat-and-bone meal	4	4	4	4
<i>N. sativa</i> seed	0.0	1.5	3.0	4.5
oyster shell	7.8	7.8	7.8	7.8
dicalcium phosphate	0.75	0.75	0.75	0.75
salt	0.35	0.35	0.35	0.35
methionine	0.1	0.1	0.1	0.1
vitamin-mineral premix	0.25	0.25	0.25	0.25
<i>Calculated composition/kg feed</i>				
ME, kcal/kg	2747.2	2752.81	2753.73	2760.45
crude protein, %	17.77	17.40	17.53	17.52
crude fibre, %	3.28	3.25	3.25	3.31
Ca, %	3.51	3.53	3.55	3.58
P, %	0.45	0.52	0.59	0.66
methionine, %	0.33	0.281	0.31	0.29
lysine, %	0.95	0.912	0.920	0.931

### *Parameters measured*

Birds were randomly assigned to diets and fed daily. Body weight (BW), g, was measured every two weeks. Feed consumption (g) and egg production were recorded fortnightly and daily, respectively. Weight per egg (g), shape index (%), albumen index (%), yolk index (%), shell thickness (mm) and Haugh unit were determined weekly following standard procedures (Akhtar et al., 2003). Eggs were collected weekly from each replicate for cholesterol analysis beginning at week 2 and ending of the experiment at week 10 from starting of the feeding. Eggs were subsequently hard-boiled to separate the yolk. Cooked yolk weight was recorded individually followed by chemical extraction for cholesterol analysis. Excreta at week 2 and week 10 were collected and analysed for culturable bacterial counts using selective media.

### *Blood collection and serum lipid profile analyses*

Blood was collected from wing vein of each bird at the experimental farm in every two weeks beginning at the week 2 of feeding using sterilized syringes and needles. Each syringe with blood sample was kept at normal temperature in an inclined position. After 20 min, the blood serum was collected and centrifuged for 15 min at 2500 rpm. After centrifugation, the supernatant was carefully separated

by a micropipette and preserved in an eppendorf vial. The collected serum was stored at -15°C until determination of total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol and triglycerides using lipid profile kit (Crescent Diagnostics).

#### *Extraction of yolk, analysis of cholesterol and dietary nutrients*

One gram of yolk was placed into a centrifuge tube. Fifteen milliliters of solvent mixture, CHCl<sub>3</sub>:MeOH (2:1 v/v) were added, blended on a vortex mixture, and allowed to extraction for 12 h. Diet lipid was extracted by the same procedure using a 5 g sample with 40 ml of solvent mixture. Cholesterol contents in serum, extracted yolk and diet samples were determined by colorimetric method (Liebermann-Burchard reaction) as described by Kenny (1952). Diets were analysed for dry matter, crude protein, crude fibre and crude fat according to the AOAC (1980) methods.

#### *Culture media for enumeration of bacteria*

*Escherichia coli* was grown using specific media, Mac Conkey agar incubated for 24 h at 37°C, and for *Lactobacillus*, Rogosa agar media was used incubating 48 h at 34°C, in anaerobic conditions.

#### *Statistical analyses*

Data were analysed by analysis of variance using the General Linear Models procedure of SAS for a factorial design (two factor analysis; diet and strain) (Kuehl, 1994; SAS, 2000). Differences among treatments, when significant, were also ordered using Tukey's test (Kuehl, 1994). Statements of statistical significance were based on P<0.05 or P<0.01.

## RESULTS

#### *Layer performance and egg parameters*

The effect of different level of *N. sativa* seed powder on body weight, egg production, egg weight, daily feed intake, eggshell thickness, yolk index, Haugh unit and albumen index of laying hens were given in Table 2. Statistical analyses revealed that all these parameters did not differed significantly (P<0.05) by varying doses of *N. sativa* seed supplementation in the diets.

Table 2. Effect of *Nigella sativa* L. seed supplementation on performances of laying hens and physical properties of eggs

Parameters	Dose of <i>Nigella sativa</i> seed powder incorporated in diets <sup>1</sup> , %			
	0	1.5	3.0	4.5
Body weight, g	1625 ± 1.3	1657 ± 1.0	1645 ± 1.2	1653 ± 1.8
Feed intake, g/hen/d	110.36 ± 2.0	112.63 ± 1.79	111.48 ± 2.60	111.45 ± 2.13
Egg production, %	89.35 ± 0.47	89.24 ± 0.44	88.99 ± 1.90	88.22 ± 0.59
Egg weight, g	63.83 ± 0.71	63.16 ± 0.55	63.13 ± 0.53	63.47 ± 0.42
Shape index, %	79.26 ± 5.13	80.43 ± 3.68	81.31 ± 3.66	81.44 ± 2.22
Albumen index, %	8.66 ± 0.38	8.50 ± 0.72	8.78 ± 0.66	8.64 ± 0.30
Yolk index, %	40.50 ± 0.70	42.63 ± 2.80	44.13 ± 1.65	43.25 ± 2.21
Shell thickness, mm	0.42 ± 0.00	0.41 ± 0.00	0.41 ± 0.01	0.42 ± 0.01
Haugh unit	88.03 ± 4.20	90.32 ± 1.90	88.88 ± 2.45	91.11 ± 1.50

<sup>1</sup> diets were supplemented with 0, 1.5, 3.0 or 4.5% *N. sativa* seed powder and were fed for 10 weeks; values are expressed as mean ± standard error of at least 4 replications each of which contains 5 birds; differences among the treatments of all the parameters were statistically not significant at P<0.05

### Serum and egg yolk cholesterol content

Effects of supplementation of *N. sativa* seed powder on the contents of serum cholesterol, HDL and triglycerides in laying hens at wk 10 are presented in Figure 1. It revealed that the *N. sativa* seed supplementation (1.5 to 4.5%) significantly decreased (P<0.01) the contents of triglycerides in blood serum of laying hens compared with control. Although, there was no significant difference among the doses of *N. sativa* seed supplementation, the lowest value (3.68 mmol/l) of triglycerides was recorded in hens those fed 4.5% seed supplemented

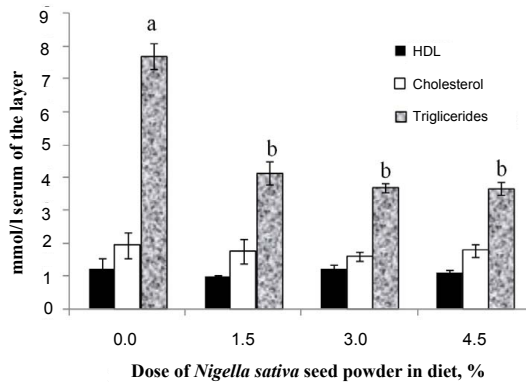


Figure 1. Effects of *Nigella sativa* seed powder supplemented diets on serum HDL, cholesterol and triglyceride in blood of laying hens at week 10 of feeding. The data are the averages ± standard error (error bars) of at least four replications each of which 5 birds for each dose of seed powder. Data points bearing different letters are significantly different at P<0.05

diets which was not significantly different from other two doses (1.5 and 3.0%) of seed powder (Figure 1). The reducing tendency of the contents of HDL and cholesterol were also observed in the supplemented diets compared with control but these differences were not statistically significant.

Cholesterol content per gram of yolk at week 10 are presented in Figure 2.

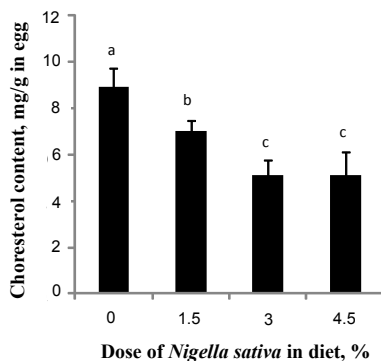


Figure 2. Influence of varying doses of *Nigella sativa* seed powder supplemented diets on egg yolk cholesterol content (mg/g) of laying hens at week 10 of feeding. The data are the averages  $\pm$  standard error (error bars) of at least four replications each of which 5 birds for each dose of seed powder. Data points bearing different letters are significantly different at  $P < 0.05$

Dietary *N. sativa* seed powder up to 3.0% significantly ( $P < 0.05$ ) reduced egg yolk cholesterol. However, there was no statistical difference between the content of egg yolk cholesterol at the doses of 3.0 and 4.5% seed powder supplementation to the diet of layers. The lowest value of cholesterol content was recorded (5.11 mg/g yolk) at both 3.0 and 4.5% seed supplemented diet, which was approximately 43% lower than that of control diet. Cholesterol content in hens fed control diet was recorded as 8.96 mg/g egg yolk.

#### *Bacterial counts in excreta*

Figure 3 shows the effect of varying doses of *N. sativa* seed supplementation in diets on excreta culturable bacterial counts. Supplementation of *N. sativa* seed powder in the diets significantly ( $P < 0.01$ ) decreased the population of harmful bacterium, *Escherichia coli*, and total culturable bacteria than those of control (no seed powder). Although the population of beneficial bacterium, *Lactobacillus* sp., was slightly increased in supplemented group, however, these increments were not significantly different from the control.

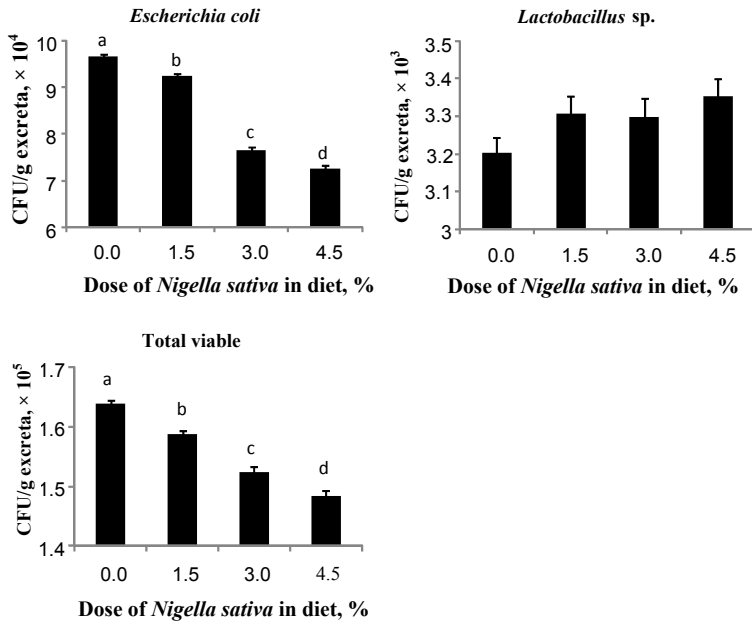


Figure 3. Effects of varying doses of *Nigella sativa* seed powder supplemented diets on culturable bacteria in the excreta of laying hens at 10 week of feeding. The data are the averages  $\pm$  standard error (error bars) of at least four replications each of which 5 birds for each dose of seed powder. Data points bearing different letters are significantly different at  $P < 0.001$  CFU, colony forming unit

## DISCUSSION

In this study, the effects of *N. sativa* seed supplemented diets on body weight, feed intake, egg laying performance, population of intestinal bacteria, physical properties of eggs and cholesterol contents in blood serum and eggs of laying hens (Hisex Brown) were investigated. Although seed supplemented diets had no effects on body weight, feed intake, egg laying performance and physical properties of the eggs, however, the triglycerides in blood serum and cholesterol content in egg yolks were significantly decreased by supplementation of *N. sativa* seed powder in manually prepared diets (Table 2; Figures 1 and 2). Furthermore, *N. sativa* seed supplementation in diets significantly suppressed the number of harmful bacteria such as *E. coli* in the excreta. Taken together, these results suggest that *N. sativa* seeds 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.

It has been reported that 1-3% of *N. sativa* seed supplemented diets improved physical properties such as shell thickness, shell weight, Haugh unit, etc., of the eggs of hens (Akhtar et al., 2003). However, in the present study *N. sativa* supplemented diets had no significant effect on shell thickness, albumen index, yolk index and Haugh unit. The average mean albumen index found in current study was slightly higher than those of Subramanian et al. (2001), who observed lower albumen index (7.3) in peahen. The higher value of albumen index suggests that the egg is broader and rounder. The data of other parameters of the eggs were in agreement with findings of earlier investigations (Akhtar et al., 2003).

In current study, we observed that *N. sativa* seed supplemented diets significantly decreased only blood serum triglycerides but not HDL and total cholesterol contents compared with control. In an earlier study, Akhtar et al. (2003) demonstrated that *N. sativa* (1.5%) supplemented diets significantly reduce serum total cholesterol content of the hens. Although mechanism is not known, the volatile oils of *N. sativa* seeds contain quinines including thymoquinone (TQ) and dithymoquinone, which might be involved in sharp decrease in serum triglyceride content in hens fed *Nigella*-supplemented diet (Swamy and Tan, 2000). Serum triglyceride lowering effect of TQ in rats has recently been reported (Baosa et al., 2009). The decrease of serum cholesterol and triglycerides by supplementation of *N. sativa* in diets might be associated with the choleric activity of the seed powder as shown by El-Dhakhny et al. (2000). The choleric function may be due to either by reducing synthesis of triglycerides and cholesterol by hepatocytes or by decreasing its fractional reabsorption from the small intestine (Brunton, 1999). A further study is warranted to elucidate the mechanisms of serum triglyceride decreasing function of *N. sativa* seed in laying hens.

In the present study, supplementation of dietary *N. sativa* seed powder reduced egg yolk cholesterol content as low as 43% (Figure 2) compared with control. Although egg cholesterol lowering effect of *N. sativa* seeds have been described in several reports (Akhtar et al., 2003; Aydin et al., 2008), our results showed the highest cut (about 43%) of egg cholesterol at 3% seed supplementation in the diets. The mechanism by which *N. sativa* decreases yolk cholesterol is difficult to precisely clarify from our current knowledge, however, the contents of secondary metabolites in seeds such as TQ might be involved (Swamy and Tan, 2000). TQ has been shown to exert hypocholesterolaemic, anti-inflammatory, anti-oxidant, anti-cancer and anti-neoplastic effects both *in vitro* and *in vivo* (Gali-Muhtasib et al., 2006; Yazan et al., 2009). Further bioassay-guided fractionation and purification

of active principle(s) is needed for better understanding of egg yolk cholesterol lowering effects of the *N. sativa* seeds. Cholesterol is primarily biosynthesized in the liver of laying hens and incorporated into vitellogenin and very low density lipoprotein particles, which are secreted into the bloodstream and subsequently taken up by growing oocytes *via* receptor-mediated endocytosis (Elkin, 2006). Therefore, the decrease in the egg yolk cholesterol by *Nigella* seeds might be associated with the decrease in cholesterol synthesis in the liver possibly by the action of secondary metabolites contents in the seeds.

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 *N. sativa* powder in the diet of laying hens significantly ( $P < 0.01$ ) decreased the population of harmful bacterium, *E. coli*, as well as total culturable bacteria but did not significantly affect the population of beneficial bacterium, *Lactobacillus* sp., than those of control (without *N. sativa*) (Figure 3). In correspondence to our findings, the number of coliform bacteria in broilers has been reported to decrease by *Nigella*-supplemented diets (Abu-Dieyh 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). We also found that the crude acetone extract of *N. sativa* seeds suppressed the growth of *Staphylococcus aureus* and *Bacillus subtilis* as low as 10 µg/disk in a disk diffusion bioassay (data not shown).

## CONCLUSIONS

In conclusion, we found that *N. sativa* supplemented diet significantly decreased the levels of serum triglycerides and 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 *N. sativa* seed supplemented diets for 10 weeks. Taken together, our results suggest that supplementation of *N. sativa* seed powder in diets has high potential as commercial applications for the production of low-cholesterol eggs. Therefore, *N. sativa* seed powder can be considered as feed additives and an environment safe alternative to the banned and hazardous synthetic antibiotics. A further study is needed to understand the active principle(s) of cholesterol lowering and other beneficial effects of *N. sativa* seed powder shown in this experiment before its practical use as poultry feed additive.

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