Journal of
Animal Physiology and Animal Nutrition

DOI: 10.1111/jpn.12109

ORIGINAL ARTICLE

# Effect of different levels of black cumin (*Nigella sativa* L.) on performance, intestinal *Escherichia coli* colonization and jejunal morphology in laying hens

J. Boka, A. H. Mahdavi, A. H. Samie and R. Jahanian

Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

## Summary

This study was conducted to investigate the effects of different levels of black cumin seeds (*Nigella sativa* L.) on performance, intestinal *Escherichia coli* count and morphology of jejunal epithelial cells 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. The experimental period lasted for a total of 10 weeks, and egg quality indexes and laying hens' performance were measured as two 35-day trial periods. At the final day, two hens per replicate were slaughtered to investigate the influence of dietary treatments on intestinal *E. coli* colonization and morphology of jejunal cells. 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. In addition, dietary supplementation with black cumin improved ($p < 0.05$) eggshell quality and Haugh unit. The best feed conversion ratio was obtained when diets were supplemented with 2% black cumin. This improvement was due to the increase ($p < 0.05$) in egg mass and contemporaneous decrease ($p < 0.01$) in feed consumption. The present results indicated that regardless of supplementation level, dietary inclusion of black cumin decreased *E. coli* enumeration in ileal digesta and improved serum lipid profile and eggshell quality, whereas the best intestinal health indices and haying hens' performance were obtained by at least 2% black cumin seeds.

**Keywords** laying hens, black cumin, egg quality, intestinal morphology, *Escherichia coli* colonization

**Correspondence** Amir Hossein Mahdavi, Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran. Tel: +98 3113913505, +98 9133225740 (cell); Fax: +98 3113913501; E-mail: mahdavi@cc.iut.ac.ir

Received: 3 October 2012; accepted: 7 July 2013

## Introduction

Antibiotics have been used for years to promote the profitability of poultry production by reducing of pathogenic bacteria in the gut lumen, thereby improving weight gain, feed conversion ratio and flock uniformity (Wegener et al., 1998). There is increasing pressure to reduce or eliminate the application of antibiotic medications in poultry feed because of the negative issues related to human health especially antibiotic resistance in human populations. Additionally, the proposed ban of antimicrobial growth promoters in the European Union in 2006 and other countries has forced producers to use other safe natural additives in poultry feeds such as phytobiotics (Adibmordi et al., 2006; Windisch and Kroismayr, 2006). Phytobiotics are included a very wide range of substances and four subclasses in animal feeding and may classify to herbs (product from flowering, non-woody and non-persistent plants), botanicals (entire or processed parts of a plant such as root, leaves, bark), essential oils (hydro-distilled extracts of volatile plant compounds) and oleoresins (Windisch and Kroismayr, 2006).

Black cumin (*Nigella sativa* L.) is one of the best alternatives used as feed additive in poultry feeds.

Black cumin is also known as black seed and grows in Asian and Mediterranean countries (Aydin et al., 2008). It was demonstrated that black cumin seeds have considerable antioxidant (Guler et al., 2007), antibacterial (Hanafy and Hatem, 1991), digestive and appetite stimulant (Guler et al., 2006) and hepatoprotective (Mahmoud et al., 2002) and immunomodulative (Salem, 2005) properties, and the essential oils of black cumin could markedly decrease the growth of *Escherichia coli*, *Bacillus subtilis* and *Streptococcus faecalis* (Saxena and Vyas, 1986). Dietary supplementation with black cumin seeds has shown some positive effects on broiler performance (Guler et al., 2006; Abu-Dieyeh and Abu-Darwish, 2008), laying hens' performance and egg quality indexes (Akhtar et al., 2003; Aydin et al., 2008; Yalcin et al., 2009). Yalcin et al. (2009) noted that black cumin seeds are rich in unsaturated fatty acids such as linoleic and oleic acids, and dietary inclusion of black cumin seeds at the levels of 10 and 15 g/kg had beneficial effects on egg weight, feed efficiency, egg cholesterol content and egg yolk fatty acid composition; however, dietary black cumin did not significantly affect feed intake, egg production, egg quality characteristics and some of blood parameters such as serum concentrations of total protein, triglycerides, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Akhtar et al. (2003) reported that dietary inclusion of *Nigella sativa* seeds at the level of 1.5% in laying hen diets could improve egg production, egg weight, egg shell thickness, Haugh unit and feed conversion ratio, with suppressive effect on egg yolk cholesterol. Furthermore, Aydin et al. (2008) showed that although black cumin at the levels of 2% or 3% could positively influence egg production, egg weight and eggshell quality and decrease the concentration of egg yolk cholesterol, but had no effect on live weight, feed consumption, feed conversion ratio, organ weights and abdominal adipose tissue. Stimulatory effects of black cumin and thyme essential oil on enhancing intestinal weight and length and reduction in intestinal pH have been also demonstrated by Denli et al. (2004b).

Because there are limited published data concerning the effects of black cumin on intestinal histology and ileal bacterial populations of laying hens, the present study was designed to evaluate the efficacy of dietary supplementation of black cumin seeds on intestinal *Escherichia coli* enumeration, intestinal morphology, some blood biochemical parameters and production performance of Leghorn laying hens.

## Materials and methods

### Experimental design and dietary treatments

A total of 100 Hy-Line W-36 Leghorn laying hens of 49 weeks of age were randomly allocated into the five cage replicates with five hens in each according to a completely randomized design. Dietary treatments were consisted of different levels (0%, 1%, 2% and 3% of diet) of black cumin. All treatment groups received the normal isocaloric and isonitrogenous diets formulated to meet or exceed the nutrient requirements of laying hens as recommended by Hy-Line W36 manual (Table 1). Black cumin seeds

**Table 1** The composition and nutrient content of experimental diets (49–58 weeks of age)

| Item | Dietary treatments, black cumin seed supplementation (%) | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| Ingredient (%) | | | | |
| Yellow corn | 61.33 | 59.80 | 58.26 | 56.72 |
| Soybean meal | 21.78 | 21.63 | 21.47 | 21.31 |
| Soybean oil | 1.50 | 1.50 | 1.50 | 1.50 |
| Fat powder | 1.50 | 1.50 | 1.50 | 1.50 |
| Oyster shell | 5.00 | 5.00 | 5.00 | 5.00 |
| Monocalcium phosphate | 1.53 | 1.54 | 1.55 | 1.56 |
| Limestone | 5.66 | 5.66 | 5.65 | 5.65 |
| Common salt | 0.22 | 0.22 | 0.22 | 0.22 |
| L-Lysine HCL | 0.03 | 0.04 | 0.05 | 0.06 |
| DL-Methionine | 0.17 | 0.18 | 0.18 | 0.18 |
| Na bicarbonate | 0.77 | 0.78 | 0.81 | 0.83 |
| Vitamin–mineral premix* | 0.50 | 0.50 | 0.50 | 0.50 |
| Sand | – | 0.66 | 1.31 | 1.97 |
| Black cumin† | – | 1.00 | 2.00 | 3.00 |
| Calculated analysis | | | | |
| ME (kcal/kg) | 2790 | 2790 | 2790 | 2790 |
| Crude protein (%) | 14.90 | 14.90 | 14.90 | 14.90 |
| Methionine (%) | 0.42 | 0.42 | 0.42 | 0.42 |
| Methionine + cysteine (%) | 0.66 | 0.66 | 0.66 | 0.66 |
| Lysine (%) | 0.76 | 0.76 | 0.76 | 0.76 |
| Threonine (%) | 0.62 | 0.62 | 0.62 | 0.62 |
| Calcium (%) | 4.25 | 4.25 | 4.25 | 4.25 |
| Available phosphorus (%) | 0.45 | 0.45 | 0.45 | 0.45 |
| Electrolyte balance (mEq/kg) | 250 | 250 | 250 | 250 |

*Vitamin and mineral premix provided per kilogram of diet: vitamin A (from vitamin A acetate), 10 000 IU; vitamin D3, 2500 IU; vitamin E (from dl-α-tocopheryl acetate), 10 IU; vitamin $B_1$, 2.2 mg; vitamin $B_2$, 4 mg; pantothenic acid, 8 mg; vitamin $B_6$, 2 mg; niacin, 30 mg; vitamin $B_{12}$, .015 mg; folic acid, 0.5 mg; biotin, 0.15 mg; choline chloride, 200 mg; manganese, 80 mg; copper, 10 mg; iodine [from Ca (IO3)2·H2O], 0.8 mg; cobalt, 0.25 mg; selenium, 0.3 mg; zinc, 80 mg; iron, 80 mg.
†Proximate analysis of black cumin seed (g/kg DM basis): moisture, 52.5; ash, 45; CP, 208; ether extract, 389; crude fibre, 97; ME, 5,622 kcal (Takruri and Dameh, 1998 and Aydin et al., 2008).

crushed and then added to diets. Birds were housed in the cages in a windowless poultry house with a light regimen of 16L: 8D.

Hens had free access to water and to the experimental diets during 10 weeks experimental period, and body weights were recorded at the beginning and the end of experiment.

### Performance and egg quality measurements

During the present study, laid eggs were collected daily (at 9:00 am) and immediately weighed. These data (egg numbers and weights) were used to calculate egg mass. Feed consumption, egg production percentage, egg weight, egg mass and feed conversion ratio were measured as 35-day intervals and during entire trial period. Three eggs (15 eggs per each treatment group) were collected from each replicate at days 35 and 70, and egg quality indexes including egg shape index, yolk index, Haugh unit, eggshell weight, eggshell thickness and eggshell breaking strength were measured. The yolk and egg shape indices were calculated by yolk height/yolk diameter and egg width/egg length formulas respectively. The following formula was used to calculate Haugh unit: $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$. Yolk cholesterol of two eggs per replicate was extracted by the method described by Folch et al. (1956), with consideration of the modifications made by Washburn and Nix (1974) at the end of study and estimated by the colorimetric Leibermann–Burchard method.

### Intestinal *E. coli* enumeration

On day 70 of experiment, the total contents of ileal digesta from Meckel's diverticulum to the ileocecal junction were carefully collected from two birds per replicate. The contents were then mixed thoroughly, placed in the sterile tubes and kept in an ice-covered box and then immediately transferred to the laboratory. A volume containing 1 g of digesta was serially (1:10) diluted to $10^{-6}$ using 0.85% NaCl, and 0.1 ml of each dilution was plated in duplicate on eosin methylene blue agar plates (Merck, Darmstadt, Germany). The plates were incubated at 37 °C for 24 h, and *E. coli* colonies were confirmed using biochemical tests of indole, methyl red, Voges–Proskauer and citrate reactions. The number of colony-forming units per plate was counted by spread plate method to determine the total number of bacterial colony-forming units per gram of digesta, as described by Mahdavi et al. (2010).

### Jejunal histological alterations

At the end of trial, two randomly selected hens from each replicate were killed to determine the effect of dietary treatments on jejunal cell morphology as described by Mahdavi et al. (2010). Tissue samples were collected, and a 3-cm segment of jejunum anterior to Meckel's diverticulum was excised for light microscopic observations. The scraps of tissues were immediately immersed in a 10% phosphate-buffered formalin solution for fixation. Two portions per sample were cut, and cross sections (5 $\mu$m thick) of each intestinal fragment were processed in paraffin wax, according to the histopathological routine techniques and stained by haematoxylin and eosin (H&E). The slides were examined using a light microscope with the magnifications of 100× and 400×. The variables measured were included villus height, crypt depth, villus height to crypt depth ratio (VCR), goblet cell numbers (GCN) and lamina propria lymphatic follicles (LLF). In each intestinal slide, 10-well-oriented villus heights and crypt depths were measured. The average of 10 measurements per each sample was used for statistical analysis.

### Relative weights of liver and pancreas

At the final day of trial, two birds per replicate were selected randomly to evaluate the relative weights of liver and pancreas. Feed was removed 3 h before slaughtering. Each bird was exsanguinated by cutting the jugular vein and allowed to bleed for approximately 2 min, and the weights of pancreas and liver were immediately obtained. The relative weights of these organs were calculated as a percentage of live body weight, and mean data of two birds per cage were used for analysis of variance.

### Serum biochemical parameters

Blood samples of two hens per replicate were collected at the final trial day and centrifuged (3000 × $g$ for 15 min). Sera samples were harvested and stored (−20 °C) until further analysis for serum cholesterol, triglycerides and HDL concentrations using turbidimetric method (Pars Azmoon, Tehran, Iran).

### Statistical analysis

All data were analysed using the GLM procedure of SAS software (SAS Institute, 2001). The following model was assumed in the analysis of all studied traits: $Y_{ij} = \mu + A_i + \varepsilon_{ij}$, where $Y_{ij}$ = observed value for a particular character, $\mu$ = overall mean, $A_i$ = effect of the $i$th treatment and $\varepsilon_{ij}$ = random error associated with the $ij$th recording. Least significant difference test was used to identify the significant differences between groups at the 0.05 significance level.

## Results and discussion

### Intestinal *E. coli* enumeration

As shown in Table 2, dietary inclusion of black cumin caused a significant ($p < 0.05$) decrease in *E. coli* number in the ileal content of laying hens. In agreement, the initial reports showed that essential oil of black cumin included antibacterial activity against some gram-positive and gram-negative bacteria such as *E. coli, Staphylococcus aureus, Streptococcus faecalis, Pseudomonas aeruginosa, Shigella dysenteriae, S. sonnei, S. boydii, Vibrio cholerae* and *Bacillus subtilis* (Saxena and Vyas, 1986; Fedorus et al., 1992). Also, the *in vivo* study by Bölükbaşi et al. (2009) indicated that *Nigella sativa* oil had strong potential for reducing ileal *E. coli* enumeration in laying hens; however, Erener et al. (2010) found that dietary supplementation with black cumin seed or its extract had no significant effect on coli form count in broiler chicks.

It has been stated that phenolic components are chiefly responsible for the antibacterial activities of essential oils (Cosentino et al., 1999). Of course, the antibacterial activity of black cumin has been attributed to the thymoquinone substances such as terpenes (Mahfouz and El-Dakhakhny, 1960; Bourgou et al., 2010). The antibacterial properties of black cumin could be associated with the ability of its essential oil, which can destabilize membranes including mitochondrial membranes and also can disturb cellular integrity of bacteria and eukaryotic cells and result in cell death via necrosis and apoptosis as described by Bakkali et al. (2007). Therefore, with considering these findings, it seems that dietary administration of black cumin at all supplementation level could provide a quantities of bioactive antimicrobial components, thereby significantly reducing *E. coli* proliferation by 1.06 log cfu/g of ileal content in laying hens.

**Table 2** Effect of different levels of black cumin on ileal *Escherichia coli* enumeration and histological changes in jejunum in laying hens

| Black cumin (%) | *E. coli* Count* ($\log_{10}$ cfu/g) | Villus height ($\mu$m) | Crypt depth ($\mu$m) | VCR | GCN | LLF |
|---|---|---|---|---|---|---|
| 0 | 4.93$^a$ | 779$^b$ | 133$^a$ | 5.76$^b$ | 1+† | 1+ |
| 1 | 3.87$^b$ | 772$^b$ | 124$^{ab}$ | 6.30$^b$ | 2+ | 1+ |
| 2 | 4.18$^b$ | 932$^a$ | 121$^b$ | 7.68$^a$ | 2+ | 3+ |
| 3 | 4.11$^b$ | 912$^a$ | 117$^b$ | 7.50$^a$ | 4+ | 3+ |
| SEM | 0.15 | 2.41 | 1.01 | 0.08 | – | – |
| p-Value | 0.04 | 0.01 | 0.05 | 0.01 | – | – |

VCR, villus height to crypt depth ratio; GCN, goblet cell numbers; LLF, lamina propria lymphatic follicles.

Means with no common superscript letters within each column are significantly ($p < 0.05$) different.

*The number of *E. coli* was calculated as $\log_{10}$ cfu per gram of ileal content.

†Number of +'s indicates severity of the histological changes such as cellular hypertrophy or hyperplasia.

### Measurement of jejunal histological alterations

As shown in Table 2, the VCR was increased significantly ($p < 0.01$) via dietary inclusion of 2% and/or 3% black cumin seed. This observation resulted from the increase ($p < 0.01$) in villus height and concomitant decrease ($p < 0.05$) in crypt depth. There are limited histological studies in laying hens fed diets supplemented with herbs or their active substances. Although it has been suggested that thinner intestinal epitheliums promote nutrient absorption and decline the metabolic demands of gastrointestinal system (Visek, 1987), a taller villus would increase the surface area for nutrient absorption. On the other hand, the crypt can be regarded as the villus cells producer, and a small crypt shows slower tissue turnover and so a lower need for new tissue regeneration (Yason et al., 1987).

The VCR is a histological index for digestive capacity of the small intestine. Enhancement in this ratio improves digestion and absorption processes (Montagne et al., 2003; Mahdavi et al., 2010). Recently, the positive effects of extracted essential oils, originated from aromatic plants, on the intestinal absorptive cells and local immunological responses have been reported by Lavinia et al. (2009). In agreement with the present findings, the previous studies demonstrated that some medicinal plant such as garlic powder could increase the villus height (Adibmordi et al., 2006), and administrating mint, sage and thyme markedly decreased crypt depth (Demir et al., 2008); however, Catala-Gregori et al. (2008) found that dietary supplementation with plant extracts had no remarkable effects on intestinal cell morphology. As explained previously, using all black cumin levels in diets could decrease the enumeration of intestinal *E. coli* as an avian pathogenic bacteria species. Therefore, it is possible that the improvement in VCR had been associated by antimicrobial properties of black cumin; because it has been suggested that declined microbial activity in the intestinal contents, at the level of brush border, would reduce both the damage of enterocytes and the demands for new cell proliferation in the gut (Hughes, 2003), resulting in taller villus and smaller crypts (Xu et al., 2003).

After inclusion of at least 2% black cumin in hens' diets, the increases in GCN and proliferation of LLF were motivated. These findings reinforce the previous observations that demonstrated some bioactive components of herbs and spice plants, such as cinnamaldehyde, carvacrol or capsaicin, have the ability to preserve microvillus as nutrients absorption site, by increasing intercellular antioxidant (Jamroz et al., 2006) and promoting mucin secretion (Best, 2000). These functions of intestinal mucosa layer are associated with their inherent antioxidative activity at both cell and tissue levels (Best, 2000; Tschirch, 2000). Goblet cells are accountable for the secretion of mucin that is used for the mucinous lining of the intestinal epithelium (Schneeman, 1982). It is possible that the active substances of black cumin such as nigella, thymoquinone and thymohydroquinone at high levels (Mahfouz and El-Dakhakhny, 1960) stimulate the proliferation of goblet cells to secret mucin, whereby it could protect the intestinal epithelium layer against pathogenic bacteria. The previous studies suggested that the creation of thicker mucinous layer by herbal bioactive substances could reduce the probability of adhesion of pathogenic microflora such as *E. coli* and *Clostridium perfringens* and fungi to the epithelial cells (Jamroz et al., 2006). This can explain the lower enumeration of ileal *E. coli* in laying hens fed black cumin seeds as described above. Furthermore, in the present study, it was demonstrated that supplementing 2% black cumin in addition to increase in goblet cells number motivated the LLF proliferation. It notes that the high levels of black cumin seeds may have the local inflammatory effects on gut-associated lymphoid tissue. Therefore, our results suggest that administration of black cumin could induce widespread structural and functional alterations in the intestine of haying hens and that it might result in the lower enumeration of pathogenic bacteria in the gastrointestinal tract.

### Liver and pancreas relative weights

As shown in Table 3, supplementation of different levels of black cumin had no effect on relative weight of liver as a percentage of live body weight; however, dietary inclusion of black cumin seeds increased ($p < 0.05$) the relative weight of pancreas. In agreement with the present findings, the ineffectual herbal inclusions on relative weight of liver have been reported when diets had been supplemented by black cumin (Al-Beitawi and El-Ghousein, 2008), rosemary (Ghazalah and Ali, 2008), oregano and garlic essential oils (Kirkpinar et al., 2011). Although Hernandez et al. (2004) and Kirkpinar et al. (2011) found that the relative weight of pancreas was not affected in broilers fed plant extracts, Platel and Srinivasan (1996) indicated that dietary supplementation of curcumin, capsaicin and piperine could promote the digestive enzyme activities of pancreas and intestinal mucosa and that it might be due to the antioxidant activity of these substances for stimulating of protein synthesis by enzymatic system (Osawa et al., 1995; AL-Sultan, 2003). On the other hand, many studies have demonstrated that *Nigella sativa* seeds, *Pimpinella anisum* seeds and *Thymus vulgaris* mixture have stimulatory effects on animal digestive function and, in turn, improve protein, fats and cellulose digestibilities (Jamroz and Kamel, 2002; Cabuk et al., 2003; Ramakrishna et al., 2003). These events may be occurred by the alteration in pancreas activities. Furthermore, black cumin has choleric properties producing a definite enhancement in bile flow (Mahfouz and El-Dakhakhny, 1960). Bile is identified as an emulsifying agent, activating the pancreatic lipase that helps in the digestion and absorption of fats and fat-soluble vitamins (Crossland, 1980; Hassan et al., 2004). Therefore, in the current study, it is possible that improved egg production and feed conversion ratio might be due to the enhancement of relative weight of pancreas and its activities as described above.

**Table 3** Effect of dietary supplementation of black cumin on relative weights of liver and pancreas in laying hens

| | Relative organ weigh (g/kg live body weight) | |
|---|---|---|
| Black cumin (%) | Liver | Pancreas |
| 0 | 2.97 | $0.16^{b}$ |
| 1 | 2.61 | $0.23^{a}$ |
| 2 | 2.77 | $0.23^{a}$ |
| 3 | 2.66 | $0.21^{a}$ |
| SEM | 0.08 | 0.01 |
| p-Value | 0.1 | 0.04 |

Means with no common superscript letters within each column are significantly ($p < 0.05$) different.

### Serum biochemical variables

As noted in Table 4, dietary inclusion of black cumin decreased ($p < 0.05$) the concentration of serum cholesterol and triglycerides and increased ($p < 0.05$) serum HDL concentration. The lowest serum

**Table 4** Effect of different levels of black cumin seed on the serum concentration of cholesterol, triglycerides and HDL in laying hens

| Black cumin (%) | Cholesterol (mg/dl) | Triglycerides (mg/dl) | HDL (mg/dl) |
|---|---|---|---|
| 0 | $159^{a}$ | $2144^{a}$ | $25.2^{b}$ |
| 1 | $125^{b}$ | $1649^{ab}$ | $26.5^{ab}$ |
| 2 | $128^{ab}$ | $1314^{b}$ | $27.0^{a}$ |
| 3 | $139^{ab}$ | $1716^{ab}$ | $25.7^{ab}$ |
| SEM | 9.97 | 155.6 | 0.42 |
| p-Value | 0.05 | 0.02 | 0.04 |

Means with no common superscript letters within each column are significantly ($p < 0.05$) different.

cholesterol and triglyceride levels were observed when diets supplemented with 1% and 2% black cumin, respectively, and the highest level of serum HDL concentration was assigned to the birds on 2% black cumin diets. These findings are consistent with the results of Akhtar et al. (2003) that showed adding 1.5% black cumin to the diet of laying hens decreased plasma concentration of cholesterol and triglycerides and enhanced HDL content. Also, Al-Beitawi and El-Ghousein (2008) found the similar results after feeding 3% uncrushed *Nigella sativa* seed to the broiler chicks. These effects might be attributed to the hypocholesterolaemic activity of black cumin, as described by El-Dakhakhny et al. (2000). It has been suggested that decrease in serum and plasma cholesterol concentrations is related to the inhibitory effects of herbal extracts on hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, an allosteric enzyme that is necessary for cholesterol synthesis in liver (Crowell, 1999). Also, Brunton (1999) found that these improvements are due to the reduction in cholesterol reabsorption from the small intestine. Moreover, it has been demonstrated that thymoquinone substances of black cumin could either suppress de novo cholesterol synthesis or stimulate bile acid excretion (Badadry et al., 2000). All together, these effects would lead to the depression in serum cholesterol concentration (Beynen et al., 1987; Badadry et al., 2000). Consequently, our results suggest that black cumin with hypolipidaemic and hypocholesterolaemic properties had strong potential for improving the serum lipid profile.

## Egg quality

The egg quality traits were assessed two times in the present study, at days 35 and 70 of experiment (Table 5). Using the highest level of black cumin could improve ($p < 0.05$) albumin quality (Haugh unit) during the second experimental period. This finding is inconsistent with the reports of Akhtar et al. (2003) and Aydin et al. (2008) who showed that supplementation of black cumin up to 3% in laying hens' diet had no remarkable effect on albumin quality. Compared with these results, Bölükbaşi et al. (2009) found that feeding laying hens by 3 mg/kg *Nigella sativa* oil decreased Haugh unit markedly. The improvements in albumin quality might be due to the presence of alkaloid and saponin contents and also the antioxidant substances of black cumin such as thymoquinone and carvacrol (Denli et al., 2004a). Several studies have suggested that the bioactive components of medicinal plants have strong potential to protect magnum and uterus cells as well as promote the secretion of albumin in laying birds (Osawa et al., 1995; Denli et al., 2004a; Nadia et al., 2008).

Although feeding 2% black cumin decreased yolk index during the first ($p < 0.01$) 35-day experimental period, the egg shape index increased ($p < 0.05$) at the first 35 days by dietary black cumin supplementation. Furthermore, supplementation of diets with black cumin seeds for 70 days could significantly ($p < 0.05$) improve the eggshell thickness and strength. Aydin et al. (2008) reported that black

**Table 5** Effect of different levels of black cumin on egg quality traits of laying hens

| Black cumin (%) | Haugh unit | | Yolk index | | Shape index | | Eggshell weight (g) | | Eggshell thickness (mm) | | Eggshell hardness (kg/cm$^2$) | | Egg yolk cholesterol (mg/g egg yolk) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 35 | Day 70 | Day 35 | Day 70 | Day 35 | Day 70 | Day 35 | Day 70 | Day 35 | Day 70 | Day 35 | Day 70 | Day 70 |
| 0 | 73.50 | $75.94^{b}$ | $42.87^{a}$ | 44.13 | $72.30^{b}$ | 74.27 | 9.01 | 8.96 | 0.372 | $0.368^{b}$ | $2.08^{b}$ | $2.42^{b}$ | 14.71 |
| 1 | 74.17 | $76.57^{b}$ | $43.19^{a}$ | 45.55 | $72.39^{b}$ | 74.87 | 9.07 | 9.59 | 0.378 | $0.389^{ab}$ | $2.61^{a}$ | $2.71^{a}$ | 12.94 |
| 2 | 76.63 | $75.28^{b}$ | $39.03^{b}$ | 43.97 | $75.09^{a}$ | 74.58 | 9.02 | 9.34 | 0.369 | $0.390^{ab}$ | $2.54^{a}$ | $2.81^{a}$ | 17.19 |
| 3 | 74.94 | $79.14^{a}$ | $40.67^{ab}$ | 43.33 | $74.26^{ab}$ | 73.17 | 8.83 | 9.15 | 0.376 | $0.398^{a}$ | $2.87^{a}$ | $2.82^{a}$ | 15.22 |
| SEM | 0.96 | 0.54 | 0.79 | 0.63 | 0.72 | 0.47 | 0.24 | 0.28 | 0.002 | 0.007 | 0.15 | 0.93 | 2.22 |
| p-Value | 0.30 | 0.01 | 0.01 | 0.13 | 0.04 | 0.14 | 0.90 | 0.46 | 0.15 | 0.05 | 0.01 | 0.03 | 0.61 |

Means with no common superscript letters within each column are significantly ($p < 0.05$) different.

cumin had no considerable effects on yolk index, egg shape index and eggshell weight; however, addition of 2% and 3% black cumin to the diets of laying hens could increase eggshell thickness and shell breaking strength. Similarly, Akhtar et al. (2003) found that eggshell thickness and yolk index improved by feeding at least 0.5% black cumin. As described, the previous reports (Osawa et al., 1995; Denli et al., 2004a; Nadia et al., 2008) mainly have suggested that the antioxidant components of spices herbs are responsible for uterus health, as eggshell mineral deposition site and improving eggshell thickness. Nevertheless, it has been demonstrated that some herbal bioactive substances could stimulate the protein synthesis via avian enzymatic systems (AL-Sultan, 2003), and it might be another reason for enhancing eggshell hardness by dietary inclusion of black cumin in the present study.

Dietary supplementation with different levels of black cumin had no significant effect on egg yolk cholesterol concentration. This finding is consistent with the report of Chowdhury et al. (2005), who showed the inclusion of tamarind had no suppressive effect on egg yolk cholesterol content. However, Aydin et al. (2008) reported that dietary supplementation of black cumin decreased egg yolk cholesterol. Several studies mentioned that the efficiency of dietary manipulation for egg cholesterol depression is very low, because it seems that reducing the egg cholesterol content accompanies decreases in egg production and egg size (Elkin et al., 1993; Kim et al., 2004; Chowdhury et al., 2005; El-Bagir et al., 2006). Kim et al. (2004) observed that oral administration of pravastatin reduced the weights of egg and yolk; however, it decreased cholesterol content of egg yolk. Also, Elkin et al. (1993) reported that although dietary supplementation of a synthetic HMG-CoA reductase inhibitor caused remarkable decrease in egg cholesterol content (up to 30%), it redounded to egg production depression up to approximately 20%. On the other hand, our data indicated that the inclusion of black cumin at all supplementation levels decreased plasma cholesterol and triglycerides, whereas it had no significant effect on yolk cholesterol content. These findings are in well agreement with the results obtained by Marks and Washburn (1977) and Chowdhury et al. (2005). Overall, our results indicated that although dietary administration of black cumin had no suppressive effect on egg yolk cholesterol content, feeding at least 2% black cumin could improve albumin and eggshell quality.

### Laying performance

The effects of different levels of black cumin on laying hens performance were presented in Table 6. Although feed intake was not influenced by experimental diets during the first 35-day period, this parameter decreased ($p < 0.01$) by dietary supplementation of black cumin seeds in the second 35-day period as well as in the entire experimental period. Regarding the previous reports, the observed effects of herbs and spices on feed consumption are variable. For example, some researchers reported that this group of additives increased (Erener et al., 2010), reduced (Al-Beitawi et al., 2010) or not affected (Aydin et al., 2006) feed intake in birds. In the current study, the significant reduction in feed intake might be due to the bitter taste of black cumin seeds concerning the presence of phenolic terpenes, as described by previous reports (Cross et al., 2003; Tollba et al., 2010). Cross et al. (2003) suggested that the effect of essential oil on stimulating appetite may be dependent on its dietary level and birds age, as young growing chickens appear to find this compound unpalatable in diets during the early weeks; however, our results indicated that the feed intake depression could be occur in aged laying hens 58 weeks old.

Our data showed that the egg weight was not affected by different levels of black cumin seeds during the whole experimental period. In consistent,

**Table 6** Effect of dietary supplementation of black cumin on performance of laying hens

| | Feed intake (g/d per hen) | | | Egg weight (g) | | | Egg production (%) | | | Egg mass (g/d per hen) | | | Feed conversion ratio | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Black cumin (%) | 0–35 days | 35–70 days | 1–70 days | 0–35 days | 35–70 days | 1–70 days | 0–35 days | 35–70 days | 1–70 days | 0–35 days | 35–70 days | 1–70 days | 0–35 days | 35–70 days | 1–70 days |
| 0 | 112.8 | $113.3^{a}$ | $112.9^{a}$ | 63.26 | 64.52 | 64.21 | 75.43 | 80.14 | 77.78 | $46.78^{b}$ | 51.42 | $49.29^{b}$ | 2.46 | $2.15^{a}$ | 2.34 |
| 1 | 114.8 | $94.7^{b}$ | $106.8^{b}$ | 64.20 | 65.13 | 64.66 | 75.74 | 80.37 | 78.05 | $48.49^{ab}$ | 52.33 | $50.41^{ab}$ | 2.38 | $1.83^{b}$ | 2.16 |
| 2 | 114.7 | $99.1^{b}$ | $108.5^{ab}$ | 63.42 | 64.29 | 63.85 | 81.48 | 83.28 | 82.38 | $54.32^{a}$ | 55.39 | $54.86^{a}$ | 2.22 | $1.85^{b}$ | 2.07 |
| 3 | 111.4 | $91.6^{b}$ | $103.5^{b}$ | 64.44 | 64.78 | 64.61 | 72.80 | 74.05 | 73.42 | $46.90^{b}$ | 48.00 | $47.45^{b}$ | 2.41 | $1.91^{b}$ | 2.21 |
| SEM | 1.78 | 2.82 | 1.81 | 1.01 | 0.85 | 0.88 | 3.42 | 2.95 | 2.47 | 1.96 | 1.85 | 1.67 | 0.10 | 0.07 | 0.08 |
| p-Value | 0.49 | 0.01 | 0.01 | 0.80 | 0.90 | 0.87 | 0.36 | 0.20 | 0.12 | 0.05 | 0.10 | 0.05 | 0.43 | 0.02 | 0.17 |

Means with no common superscript letters within each column are significantly ($p < 0.05$) different.

Bölükbaşi et al. (2009) reported that administration of *Nigella sativa* oil up to 3 ml/kg of laying hens diet had no effect on egg weight. However, Aydin et al. (2008) and Yalcin et al. (2009) observed that dietary black cumin seed supplementation at the levels of 10–15 and 30 g/kg, respectively, increased egg weight.

Supplementation of 2% black cumin in different production periods caused a numerical ($p = 0.12$) increase in egg production percentage so that this parameter improved approximately 5% compared with control group in the entire experimental period. There are some reports that suggested the inclusion of black cumin seed at the levels of 0.05–1.0% (El-Sheikh et al., 1998) and 1.5% (Akhtar et al., 2003) of diet and also *Nigella sativa* extracts at the level of 1 g/kg of diet (Denli et al., 2004a) raised egg production. In contrast, Aydin et al. (2006), Bölükbaşi et al. (2009) and Yalcin et al. (2009) found ineffectual black cumin seed or oil inclusion on egg production. On the other hand, El-Bagir et al. (2006) reported that dietary black cumin inclusion (10 and 30 g/kg) resulted in significant decrease in egg production percentage.

Egg mass was improved ($p < 0.05$) by black cumin supplementation especially at the level of 2% during the experimental period. This enhancement was largely due to the increase in egg production, as reported by previous studies (El-Sheikh et al., 1998; Akhtar et al., 2003; Denli et al., 2004a). The increase in egg mass in birds fed black cumin may be, in part, related to antioxidant property of black cumin. It is demonstrated that by enhancing free radicals, the plasma concentrations of VLDL and vitellogenin, which are egg yolk precursors, may be reduced due to the disturbance of hepatic cell functions (Bollenger-Lee et al., 1998). Therefore, it is possible that using black cumin might have increased the egg yolk precursor's secretion from liver, by preserving hepatocytes from oxidative damage, resulted in improvement in yolk formation and ovulation, as explained by Bollenger-Lee et al. (1998).

However, the feed conversion ratio was improved ($p < 0.05$) with dietary black cumin seed supplementation in the second 35-day period. The best feed conversion ratio in the entire experimental period was obtained when diets supplemented with black cumin at the level of 2%. Similar results have also been obtained by El-Sheikh et al. (1998), Denli et al. (2004a) and Yalcin et al. (2009). Improvement in feed conversion ratio was due to the increase in egg production ($p = 0.12$), reflected in egg mass ($p < 0.05$), and contemporaneous decrease ($p < 0.01$) in feed consumption. However, Aydin et al. (2006) and Bölükbaşi et al. (2009) showed that feed conversion ratio was not significantly affected by black cumin seed supplementation up to the level of 30 g/kg or *Nigella sativa* oil up to 3 ml/kg in laying hens respectively.

It seems that the beneficial effects of black cumin on inhibition of bacteria and development of intestinal morphology such as increase in absorptive surface area and slight stimulation of mucosal inflammation resulted in improvement in the health status of the gastrointestinal tract and subsequent production performance. As previously explained, using two higher levels of black cumin not only could increase the VCR, as an index for digestive capacity, but also enhanced the numbers of goblet cells and LLF. Therefore, it is probable that up-regulatory effects of black cumin on mucosal defensive mechanism and its antimicrobial properties could decrease the enumeration of faecal *E. coli*, as a pathogenic bacteria species. There is evidence that the control of gut micro-organisms can lead to decrease in competition for nutrients and decline in growth inhibitory metabolites (Catala-Gregori et al., 2008).

In confirmation with the present findings, Langhout (2000) and Williams and Losa (2001) reported that medicinal essential oils have positive effects on animal digestive systems. Many studies postulated that these effects could be, in part, due to the enhanced production of digestive enzymes such as pancreatic lipase and amylase (Langhout, 2000; Williams and Losa, 2001; Ramakrishna et al., 2003). As noted, the relative weight of pancreas increased by administration of black cumin in the diet, and it might have improved the nutrient digestibility that was appeared in feed conversion ratio.

Another factor that could be a reason for the favourable effects of some spices plants, such as black cumin, is the presence of a mixture of linolenic, linoleic and arachidonic acids (as essential fatty acids) that are necessary for proper production performance (Babayan et al., 1978). In this regard, Atta (2003) and Yalcin et al. (2009) reported that the dominant fatty acid of plant glycolipids fractions from black cumin seeds is linoleic acid (C 18:2 n-6), followed by oleic acid (C 18:1 n-9).

On the other hand, our findings showed that dietary supplementation of 2% black cumin improved egg production, egg mass and feed conversion ratio compared with the highest level (3%). These results are consistent with the findings of Al-Beitawi and El-Ghousein (2008) that noticed feeding low level (1.5%) of crushed black cumin could improve the performance of broiler chicks. According to these results, Mandour et al. (1998) reported that low doses of black cumin increased thyroxin concentration, which means that black cumin seeds had an effect on thyroid gland hormones known as the enhancers of metabolic rate. Based on these observations, we can speculate that the direct and indirect effects of black cumin, as an antimicrobial and antioxidant agent, on both local and systemic responses have been improved haying hens' performance.

In conclusion, the present results indicated that dietary supplementation with 1% black cumin decreased ileal *E. coli* enumeration and improved serum lipid profile and eggshell quality; however, the best intestinal health indices and haying hens' performance were obtained by feeding 2% black cumin seed in laying hens' diets.

## References


Abu-Dieyeh, Z. H. M.; Abu-Darwish, M. S., 2008: Effect of feeding powdered black cumin seeds (*Nigella sativa* L.) on growth performance of 4-8 week-old broilers. *Journal of Animal and Veterinary Advance* **7**, 286–290.

Adibmordi, M.; Navidshad, B.; Seifdavati, J.; Royan, M., 2006: Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. *Journal of Poultry Science* **43**, 378–383.

Akhtar, M. S.; Nasir, Z.; Abid, A. R., 2003: Effect of feeding powdered *Nigella Sativa* L. seeds on poultry egg production and their suitability for human consumption. *Veterinarski Arhiv* **73**, 181–190.

Al-Beitawi, N. A.; El-Ghousein, S. S., 2008: Effect of feeding different levels of *Nigella sativa* seeds (Black Cumin) on performance, blood constituents and carcass characteristics of broiler chicks. *International Journal of Poultry Science* **7**, 715–721.

Al-Beitawi, N. A.; El-Ghousein, S. S.; Athamneh, M. Z., 2010: Effect of adding crushed *Pimpinella anisum*, *Nigella sativa* seeds and *Thymus vulgaris* mixture to antibiotics-free rations of vaccinated and non-vaccinated male broilers on growth performance, antibody titer and haematological profile. *Italian Journal of Animal Science* **9**, 222–228.

AL-Sultan, S. I., 2003: The effect of *Curcuma longa* (Tumeric) on overall performance of broiler chickens. *International Journal of Poultry Science* **2**, 351–353.

Atta, M. B., 2003: Some characteristics of nigella (*Nigella sativa* L.) seed cultivated in Egypt and its lipid profile. *Food Chemistry* **83**, 63–68.

Aydin, R.; Bal, M. A.; Özugur, A. K.; Toprak, H. H. C.; Kamalak, A.; Karaman, M., 2006: Effects of black seed (*Nigella sativa* L.) supplementation on feed efficiency, egg yield parameters and shell quality in chickens. *Pakistan Journal of Biological Science* **9**, 243–247.

Aydin, R.; Karaman, M.; Cicek, T.; Yardibi, H., 2008: Black Cumin (*Nigella sativa* L.) supplementation into the diet of the laying hen positively influences egg yield parameter, shell quality, and decreases egg cholesterol. *Poultry Science* **87**, 2590–2595.

Babayan, V. K.; Koottungal, D.; Halaby, G. A., 1978: Proximate analysis, Fatty acid composition of *Nigella sativa* L. seeds. *Journal of Food Science* **43**, 1314–1315.

Badadry, O. A.; Abdel-Naim, A. B.; Abdel-Wahab, M. H.; Hamada, F. M., 2000: The influence of thymoquinone on doxorubicin-induced hyperlipemic nephropathy in rats. *Toxicology* **143**, 219–226.

Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M., 2007: Biological effects of essential oils. *Food Chemistry and Toxicology* **46**, 446–475.

Best, P., 2000: Health boosters from botany. *Feed International* **6**, 15–16.

Beynen, A. C.; Katan, M. B.; Zutphen, L. F. M. V., 1987: Hypo- and hyperresponders: individual differences in the response of serum cholesterol concentration to changes in diet. *Advances in Lipid Research* **22**, 115–171.

Bollenger-Lee, S.; Mitchell, M. A.; Utomo, D. B.; Williams, P. E. V.; Whitehead, C., 1998: Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. *British Poultry Science* **39**, 106–112.

Bölükbaşi, Ş. C.; Kaynar, Ö.; Erhan, M. K.; Ürüþan, H., 2009: Effect of feeding *Nigella sativa* oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and *Escherichia coli* count in feces. *Archive Geflügelkde* **73**, 167–172.

Bourgou, S.; Pichette, A.; Marzouk, B.; Legault, J., 2010: Bioactivities of black cumin essential oil and its main terpenes from Tunisia. *South African Journal of Botany* **76**, 210–216.

Brunton, L. L., 1999: Agents affecting gastrointestinal water flux and motility, digestants and bile acids. In: A. Goodman Gilman, T. W. Rall, A. S. Nies, P. Taylor (eds), *The Pharmacological Basis of Therapeutics*, 8th edn. Pergamon Press, Oxford, UK, pp. 914–932.

Cabuk, M. A.; Alcicek, A.; Bozkurt, M.; Imre, N., 2003: Antimicrobial properties of the essential oils isolated from aromatic plants and using possibility as alternative feed additives. II. National Animal Nutrition congress. 18-20 September, pp. 184–187.

Catala-Gregori, P.; Mallet, S.; Travel, A. L.; Orengo, J.; Lessire, M., 2008: Efficiency of a prebiotic and a plant extract alone or in combination on broiler performance and intestinal physiology. *Canadian Journal of Animal Science* **194**, 146–150.

Chowdhury, S. R.; Sarker, D. K.; Chowdhury, S. D.; Smith, T. K.; Roy, P. K.; Wahid, M. A., 2005: Effects of dietary tamarind on cholesterol metabolism in laying hens. *Poultry Science* **84**, 56–60.

Cosentino, S.; Tuberoso, C. I. G.; Pisano, B.; Satta, M.; Mascia, V.; Arzedi, E.; Palmas, F., 1999: *In vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Letter in Applied Microbiology* **29**, 130–135.

Cross, D. E.; Svoboda, K.; McDevitt, R. M.; Acamovic, T., 2003: The performance of chickens fed diets with and without thyme oil and enzymes. *British Poultry Science* **44**, 18–19.

Crossland, J., 1980: *Pharmacological Aspects of Allergy. Lewis Pharmacology*. Churchill Livingstone, New York, p. 393.

Crowell, P. L., 1999: Prevention and therapy of cancer by dietary monoterpenes. *Journal of Nutrition* **129**, 775–778.

Demir, E.; Kilinc, K.; Yildirim, Y.; Dincer, F.; Eseceli, H., 2008: Comparative effects of mint, sage, thyme and flavomycin in wheat-based broiler diets. *Archiva Zootechnica* **3**, 54–63.

Denli, M.; Okan, F.; Uluocak, A. N., 2004a: Effect of dietary black seed (*Nigella sativa* L.) extract supplementation on laying performance and egg quality of quail (Coturnix coturnix japonica). *Journal of Applied Animal Research* **26**, 73–76.

Denli, M.; Okan, F.; Uluocak, A. N., 2004b: Effect of dietary supplementation of herb essential oils on the growth performance, carcass and intestinal characteristics of quail (*Coturnix coturnix japonica*). *South African Journal of Animal Science* **34**, 174–179.

El-Bagir, N.; Hama, A. Y.; Hamed, R. M.; Rahim, A. G. A. E.; Beynen, A. C., 2006: Lipid composition of egg yolk and serum in laying hens fed diets containing black cumin (*Nigella sativa*). *Journal of Poultry Science* **5**, 574–578.

El-Dakhakhny, M.; Mady, N. I.; Halim, M. A., 2000: *Nigella sativa* induced hepatotoxicity and improves serum lipid profile in rats. *Arzneim Forsch Drug Research* **50**, 832–836.

Elkin, R. G.; Freed, M. B.; Kieft, K. A.; Newton, R. S., 1993: Alteration of egg yolk cholesterol content and plasma lipoprotein profiles following administration of a totally synthetic HMG-CoA reductase inhibitor to laying hens. *Journal of Agriculture and Food Chemistry* **41**, 1094–1101.

El-Sheikh, A. M. A.; Amin, A. E.; Khadiga, A. A., 1998: The effect of feeding different levels of *Nigella sativa* seeds on layers performance and egg quality characteristics. *The Sudan Journal of Veterinary Science and Animal Husbandry* **37**, 121–128.

Erener, G.; Altop, A.; Ocak, N.; Aksoy, H. M.; Cankaya, S.; Ozturk, E., 2010: Influence of black cumin seed (*Nigella Sativa*) and seed extract on broiler performance and total coliform bacteria count. *Asian Journal of Animal and Veterinary Advance* **5**, 128–135.

Fedorus, A. J.; Islam, S. N.; Ahasn, M.; Hasan, C. M.; Ahmad, Z. U., 1992: In-vitro antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug resistant isolates of Shigella species and isolates of *Vibrio cholerae* and *Escherichia coli*. *Phytotherapy Research* **6**, 137–140.

Folch, J.; Less, M.; Solane Stanley, G. H., 1956: A simple method for the isolation and purification of total lipid from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Ghazalah, A. A.; Ali, A. M., 2008: Rosemary leaves as a dietary supplement for growth in broiler chickens. *International Journal of Poultry Science* **7**, 234–239.

Guler, T.; Dalkdic, B.; Ertas, O. N.; Ciftci, M., 2006: The effect of dietary black cumin seeds (*Nigella sativa* L.) on the performance of broilers. *Asian Australian Journal of Animal Science* **19**, 425–430.

Guler, T.; Ertas, O. N.; Kizil, M.; Dalkylyc, B.; Ciftci, M., 2007: Effect of dietary supplemental black cumin seeds on antioxidant activity in broilers. *Medycyna Vetrrina* **63**, 1060–1063.

Hanafy, M. S. M.; Hatem, M. E., 1991: Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *Journal of Ethnopharmacology* **34**, 275–278.

Hassan, I. I.; Askar, A. A.; Gehan, A.; El-Shourbagy, A., 2004: Influence of some medicinal plants on performances; physiological and meat quality traits of broiler chicks. *Egyptian Poultry Science* **24**, 247–266.

Hernandez, F.; Madrid, J.; Garcia, V.; Orengo, J.; Megias, M., 2004: Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poultry Science* **83**, 169–174.

Hughes, R. J., 2003: Energy metabolism of chickens physiological limitations. A report for the rural industries research and development corporation. *RIRDC Publication* **2**, 151.

Jamroz, D.; Kamel, C., 2002: Plant extracts enhance broiler performance. In non ruminant nutrition: antimicrobial agents and plant extracts on immunity, health and performance. *Journal of Animal Science* **80**, 41.

Jamroz, D.; Wertelecki, T.; Houszka, M.; Kamel, C., 2006: Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical characteristics of the stomach and jejunum walls in chicken. *Journal of Animal Physiology and Animal Nutrition* **90**, 255–268.

Kim, J. H.; Hong, S. T.; Lee, H. S.; Kim, H. J., 2004: Oral administration of pravastatin reduces egg cholesterol but not plasma cholesterol in laying hens. *Poultry Science* **83**, 1539–1543.

Kirkpinar, F.; Bora Ünlü, H.; Özdemir, G.; Baaliouamer, A., 2011: Effects of oregano and garlic essential oils on performance, carcase, organ and blood characteristics and intestinal microflora of broilers. *Livestock Science* **137**, 219–225.

Langhout, P., 2000: New additives for broiler chickens. *World Poultry* **16**, 22–27.

Lavinia, S.; GabiI, D.; Drinceanu, D.; Stef, D.; Daniela, M.; Julean, C.; Ramona, T.; Corcionivoschi, N., 2009: The effect of medicinal plants and plant extracted oils on broiler duodenum morphology and immunological profile. *Rome Biotechnology Letter* **14**, 4606–4614.

Mahdavi, A. H.; Rahmani, H. R.; Nili, N.; Samie, A. H.; Soleimanian-Zad, S.; Jahanian, R., 2010: Effects of dietary egg yolk antibody powder on growth performance, intestinal *Escherichia coli* colonization, and immunocompetence of challenged broiler chicks. *Poultry Science* **89**, 484–494.

Mahfouz, M.; El-Dakhakhny, M., 1960: Chemical and pharmacological properties of the new anti-asthmatic drug, Nigellone. *Egyptian Pharmacology Bulletin* **24**, 411–424.

Mahmoud, M. R.; El-Ahbar, H. S.; Saleh, S., 2002: The effect of *Nigella sativa* oil against the liver damage induced by Schistosoma mansoni infection in mice. *Journal of Ethnopharmacology* **79**, 1–11.

Mandour, A. A.; Ashry, K. M.; Hedaya, S. A., 1998: Biochemical profile of serum constituents of broiler chickens supplemented with different levels of *Niglla sativa* with special references to its effect on hormonal and mineral concentrations. *Egyptian Poultry Science* **18**, 429–439.

Marks, H. L.; Washburn, K. W., 1977: Divergent selection for yolk cholesterol in laying hens. *British Poultry Science* **18**, 179–188.

Montagne, L.; Pluske, J. R.; Hampson, D. J., 2003: A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology* **108**, 95–117.

Nadia, L. R.; Hassan, R. A.; Qota, E. M.; Fayek, H. M., 2008: Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. *International Journal of Poultry Science* **7**, 134–150.

Osawa, T.; Sugiyama, Y.; Inayoshi, M.; Kawakishi, S., 1995: Antioxidative activity of tetrahydrocurcuminoids. *Bioscience, Biotechnology and Biochemistry* **9**, 1609–1612.

Platel, K.; Srinivasan, K., 1996: Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. *International Journal of Food Science* **47**, 55–59.

Ramakrishna, R. R.; Platel, K.; Srinivasan, K., 2003: *In vitro* influence of spices and spice-active principles on digestive enzymes of rat pancreas and small intestine. *Nahrung* **47**, 408–412.

Salem, M. L., 2005: Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *International Immunopharmacology* **5**, 1749–1770.

SAS Institute, 2001: *SAS User's Guide*. Version 8.02 ed. SAS Institute, Cary, NC.

Saxena, A. P.; Vyas, K. M., 1986: Antibacterial activity of seeds of some ethnomedical plants. *Journal of Ecology and Taxonomy* **8**, 291–299.

Schneeman, B. D., 1982: Pancreatic and digestive function. In: G. V. Vahoung, D. Kritchevsky (eds), *Dietary Fibre in Health and Disease*. Plenum Press, New York, pp. 73–83.

Takruri, H. R. H.; Dameh, M. A. F., 1998: Study of the nutritional value of black cumin seeds (*Nigella sativa* L.). *Journal of the Science of Food and Agriculture* **76**, 404–410.

Tollba, A. A. H.; Shabaan, S. A. M.; Abdel-Mageed, M. A. A., 2010: Effects of using aromatic herbal extract and blended with organic acids on productive and physiological performance of poultry the growth during cold winter stress. *Egyptian Poultry Science* **30**, 229–248.

Tschirch, H., 2000: The use of natural plant extracts as production enhancers in modern animal rearing practices. *Zeszyty Naukowe AR Wroclaw, Zootechnika XXV* **376**, 25–39.

Visek, W. J., 1987: The mode of growth promotion by antibiotics. *Journal of Animal Science* **46**, 1447–1469.

Washburn, K. W.; Nix, D. F., 1974: A rapid technique for extraction of yolk cholesterol. *Poultry Science* **53**, 1118–1122.

Wegener, H. C.; Aarestrup, F. M.; Jensen, L. B.; Hammerum, A. M.; Bager, F., 1998: The association between the use of antimicrobial growth promoters and development of resistance in pathogenic bacteria towards growth promoting and therapeutic antimicrobials. *Journal of Animal and Feed Science* **7**, 7–14.

Williams, P.; Losa, R., 2001: The use of essential oils and their compounds in poultry nutrition. *World Poultry* **17**, 14–15.

Windisch, W.; Kroismayr, A., 2006: The effects of phytobiotics on performance and gut function in monogastrics. *World Nutrition Forum: The Future of Animal Nutrition*, 85–90.

Xu, Z. R.; Hu, C. H.; Xia, M. S.; Zhan, X. A.; Wang, M. Q., 2003: Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science* **82**, 648–654.

Yalcin, S.; Yalcin, S.; Erol, H.; Bugdayc, K.; Ozsoy, B.; Cakir, S., 2009: Effects of dietary black cumin seed (*Nigella sativa* L.) on performance, egg traits, egg cholesterol content and egg yolk fatty acid composition in laying hens. *Journal of Science and Food Agriculture* **89**, 1737–1742.

Yason, C. V.; Summers, B. A.; Schat, K. A., 1987: Pathogenesis of rotavirus infection in various age groups of chickens and turkeys. *American Journal of Veterinary Research* **48**, 927–938.