Effect of feeding *Nigella sativa* oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and *Escherichia coli* count in feces

Einfluss eines Zusatzes von *Nigella sativa* zum Legehennenfutter auf die Leistung, den Gehalt an Cholesterol und einigen Proteinen im Dotter sowie auf die Anzahl von *Escherichia coli* im Kot

Ş. Canan Bölükbaşi, Özgür Kaynar, M. Kuddusi Erhan and Hilal Ürüştan


Introduction

The use of antibiotics as growth promoters in poultry diets was started around 60 years ago, (NASIR and GRASHORN, 2006). Since then antibiotics in sub-therapeutic levels are used as growth promoters in poultry diets. Meanwhile, the use of sub-therapeutic levels of antibiotics for growth promotion and disease prevention are suspected to increasing the risk of bacteria acquiring resistance to specific antibiotics (NASIR and GRASHORN, 2006). Therefore, usage of antibiotics as growth promoters has been prohibited in the European Union since 2006 (NASIR and GRASHORN, 2006). Therefore, many scientists have searched for alternatives to in feed antibiotics. Essential oils or extracts from herbs have received considerable attention as replacements for antibiotic growth promotants (DESCHEPPER et al., 2003).

Plant extracts and their essential oils have a wide range of activities, including inhibitory action on pathogens, effects on physio-pathologies and activity in different body systems, e.g. endocrine and immune system (FRANCOIS, 2006; NASIR and GRASHORN, 2006). One of the alternatives used as growth promotants is *Nigella sativa* (Black seed or Black cummin). *Nigella sativa* plant and its seed contain alkaloids, fixed and volatile oils and a variety of pharmacologically active substances (NASIR and GRASHORN, 2006). FERDOUS et al. (1992) reported that *Nigella sativa* had antibacterial activity against gram positive and gram negative bacteria as e.g. Staphylococcus aureus, Pseudomonas aeruginosa, Shigella dysentriae, S. sonnei, S. boydii, Vibrio cholerae and Escherichia coli. It was reported that essential oil of black seeds inhibited the growth of Escherichia coli, Bacillus subtilis and Streptococcus feacalis (SAXENA and VAS, 1986).

*Nigella sativa* and Thyme essential oil supplementation to quail feed significantly increased intestinal weight and length and decreased intestinal pH (DENLI et al., 2004a).

NASIR et al. (2005) demonstrated that *Nigella sativa* seeds supplementation in layer diets improved egg production, egg weight, egg shell thickness and Haugh unit value. EL-SHEIKH et al. (1998) recommended using *Nigella sativa* seeds in layer diets to improve performance. DENLI et al. (2004b) found that *Nigella sativa* extracts in diets of laying quails has improved animal performance, shell weight, shell thickness, albumin height and yolk index.

The *Nigella sativa* seeds have also been reported to reduce serum and yolk total cholesterol, LDL-cholesterol, triglycerides content and increased HDL-cholesterol (ASHTAR et al., 2003).

It was reported that the pure components of essential oils inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase activity which is a key regulatory enzyme in cholesterol synthesis (CROWELL, 1999). Also, it was reported that there was a correlation between HMG-CoA reductase activity and either total or LDL cholesterol in chicken, but not with HDL cholesterol (QURESHI et al., 1983). All lipids of egg yolk are associated with proteins to form lipoproteins, which are commonly classified in low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (ANTON, 1998).

There are very few published data on the effects of *Nigella sativa* oil components in laying hens. Therefore, the effect of dietary *Nigella sativa* oil supplementation on performance of laying hens was investigated in this study. Furthermore, effects on triglyceride and cholesterol ratios in serum and egg yolk, on albumin and conalbumin ratios in egg and on some proteins in egg yolk were determined, as well as feces samples were examined with respect to *E. coli* content.

Material and Methods

Experimental design and animals

In this study, sixty four 26-wk-old Lohman LSL hybrid laying hens were divided into 4 groups, each of which comprised 4 cages (50 × 46 × 46 cm) with four animals. The four dietary treatments included a control (basal diet), basal diet + 1ml/kg *Nigella sativa* oil, basal diet + 2 ml/kg *Nigella sativa* oil, and basal diet + 3 ml/kg *Nigella sativa* oil. *Nigella sativa* oil (28–54% thymocine, 7–15% p-cymene, 5–11% carvacrol, 0.5–3% t-anethole, 2–7% 4-terpineol, and 3–5% linalool) was purchased from Ege Lokman San. Tic. Company (Manisa, Turkey). Composi-
tion of the experimental diets is presented in Table 1. During the experiment (10 wk) hens were fed and watered ad libitum. Feed intake and egg production were recorded, and feed conversion ratio was calculated daily. Egg quality characteristics including egg weight, Haugh units, and ratio of albumen, yolk and shell were measured biweekly using eight eggs from each dietary treatment.

At the end of the experiment, eight blood and egg samples were taken from each treatment in order to determine the ratio of triglyceride, egg yolk proteins and cholesterol and egg albumin. Feces samples were taken from each treatment in order to determine the ratio of triglyceride, egg yolk proteins and cholesterol and egg albumin. Feces samples were taken from each replicate in plastic bags to determine total Coliform and *E. coli* count at the end of the study. All samples were processed within the same day.

The triglyceride and cholesterol ratio in serum and yolk was determined by high performance thin layer chromatography (HPTLC) and the egg yolk proteins were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

**Collection of blood samples**

Blood samples were taken from wing vein and blood was collected in tubes containing cloth activator. Tubes were centrifuged at 4°C, 3000 × g for 5 minute and the supernatant was collected.

**Isolation and homogenization of hen-egg yolk**

Egg shells were broken manually, yolk were carefully separated from the white, washed gently with distilled water and rolled on Whatman 3 MM filter paper to remove any adherent egg white. The yolk membrane was then punctured with disposable pasteur pipette and egg-yolk transferred into pre-weighed falcon tubes. Two volumes of 20% SDS were added to each g of isolated egg-yolk in falcon tubes and homogenized at 1000 rpm for 2 min using an Ultraturrax homogenizer. The homogenate was aliquoted and used for SDS-PAGE and HPTLC analysis.

**Total Lipid Extraction**

Each volume of serum and egg-yolk lipids was shaken vigorously with 1 volume of a mixture of n-hexane/2-propanol (3/2) (Merck KgaA, Darmstadt/Germany). After, centrifugation of suspension at +4°C, 2000 × g for 10' upper phase was aspirated and used for HPTLC analysis (HARA and RADIN, 1978).

HPTLC: For separation and identification high performance thin-layer chromatography (HPTLC) plates (20 × 10 cm) (Merck KgaA, Darmstadt/Germany) were used. To the end five-μl portions of extracted lipids of egg-yolk and serum were spotted with a micropipette 2 cm from the bottom of HPTLC plates. The lipids were developed 6 cm from application point using a mobile phase of n-hexane: diethylether: formic acid (80:20:2 (v/v/v) (Merck KgaA, Darmstadt/Germany). To visualize lipid classes, the entire plate was sprayed with 10% CuSO4 (w/v) in 8% H3PO4 (v/v) (Merck KgaA, Darmstadt/Germany) and charred at 180°C (DAMYANOVA, 2002). Then lipid spots on HPTLC plates were evaluated by Phoretix 1D (TL120) software.

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SE 260 vertical electrophoresis unit and EPS 301 power supply, Amersham Biosciences Piscataway/USA) was carried out by using Laemmli 4% stacking and 12% resolving gel. The serum and of egg-yolk homogenate was diluted with 2 × sample buffer (Sigma-Aldrich Chemie GmbH, Germany) and applied each well. The electrophoresis was carried out according to LAAEMLLI (1970) at 20 mA/gel constant current for 90’. Then proteins were visualized and after drying evaluated by Phoretix 1D (TL120) software.

**Bacteriology**

Fecal samples were blended in a stomacher (Stomacher 400; AJ Seward, London, England) for 2 min in 50 ml of 0.85% (w/v) salt water. A series of fermentation tubes containing Fluorocult Lauryl sulfat broth (Merck, Germany) were inoculated with water and incubated for 48 hours at 35°C. The fermentation tube contained an inverted tube to trap gases produced by the coliform bacteria. After 48 hours, the fermentation tube was examined for gas production. After that the tubes were examined under a 366-nm UV Lamp (Lampe UV, 4W/366nm; Merck, Germany) for *E. coli*. A table of most probable numbers was used to estimate the coliform content of dilutions that showed positive for coliform and *E. coli*. The results were reported as most probable number (MPN) of coliform and *E. coli* per g (ANONYMOUS, 1992).

**Statistical analysis**

Differences between groups were analysed by one-way analysis of variance (ANOVA), using the statistical package SPSS for Windows (1999; version 10.0). Significantly different means were subjected to a multiple comparison test (Duncan).
Results

The effects of dietary treatments on performance of laying hens are shown in Table 2. There was no significant (P > 0.05) difference in feed intake, feed conversion ratio, egg production and egg weight among the dietary treatments.

The addition of *Nigella sativa* oil to the diet had no impact on yolk, albumen and shell percentage of egg in this study. Haugh unit was significantly influenced by treatment. The diet with 3 ml/kg black seed oil had induced decreased Haugh unit by 6.5% compared to control group (Table 3).

In this study, the coliform count in the feces did not differ (P > 0.05) by any of the supplemental treatments (Table 4). The control group and 1ml/kg *Nigella sativa* oil group showed the highest average concentration of *E. coli* in the feces. The groups fed with the 2 ml and 3 ml/kg *Nigella sativa* oil had significantly (P < 0.01) lower *E. coli* count than the control group and 1ml/kg *Nigella sativa* oil group. The inhibitory effect of 3 ml/kg *Nigella sativa* oil on the proliferation on *E. coli* seemed to be stronger.

The effect of dietary supplementation of the experimental treatments on ratios of cholesterol and triglyceride as a percentage of total lipids in serum and egg yolk is presented in Table 5. The addition of 3 ml/kg *Nigella sativa* oil to the basal diet significantly reduced triglyceride ratio of egg yolk compared with the other groups. On the other hand, supplementing 3 ml/kg *Nigella sativa* oil to the basal diet increased (P < 0.01) cholesterol ratio of egg yolk above the control. The groups receiving 1 and 2 ml/kg *Nigella sativa* oil showed significantly (P < 0.01) lower egg yolk cholesterol ratio compared to the other groups. No significant differences (P > 0.05) were observed in the serum triglyceride ratio between dietary treatments. Supplementing *Nigella sativa* oil 2ml/kg to the basal diet decreased (P < 0.05) serum cholesterol ratio compared to the other groups.

The effect of dietary treatments on egg albumin and conalbumin is shown in Table 6. 2 and 3 ml/kg *Nigella sativa* oil treatment significantly (P < 0.05) reduced proportion of ovalbumin and conalbumin compared with the control.

The effects of *Nigella sativa* oil on apoproteins were tested in the present study. It was found that HDL apoproteins (Apo vitellin 3 + 4) and LDL apoproteins (γ-livetin + apovitellenin VI, α-livetin/apovitellenin) and phosvitin of egg yolk increased significantly with supplementation of 3 ml/kg *Nigella sativa* oil (Table 7). Apovitellin 7, β-livetin, Apovitellenin IV, Apovitellenin II and Apovitellenin III were not influenced by treatment groups.

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Table 2. Daily feed intake, feed conversion, egg production and egg weights (n = 16)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Daily Feed intake (g)</th>
<th>Feed conversion (g : g)</th>
<th>Egg production (%)</th>
<th>Egg Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>134</td>
<td>2.60</td>
<td>85.0</td>
<td>63.2</td>
</tr>
<tr>
<td>Nigella sativa 1 ml/kg</td>
<td>133</td>
<td>2.55</td>
<td>85.2</td>
<td>62.8</td>
</tr>
<tr>
<td>Nigella sativa 2 ml/kg</td>
<td>130</td>
<td>2.69</td>
<td>80.1</td>
<td>61.5</td>
</tr>
<tr>
<td>Nigella sativa 3 ml/kg</td>
<td>137</td>
<td>2.65</td>
<td>83.9</td>
<td>62.9</td>
</tr>
<tr>
<td>SEM</td>
<td>1.63</td>
<td>0.04</td>
<td>1.58</td>
<td>0.25</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: not significant

Table 3. Egg composition and Haugh units (n = 8)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Yolk (%)</th>
<th>Albumen (%)</th>
<th>Shell (%)</th>
<th>Haugh Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.4</td>
<td>62.7</td>
<td>11.9</td>
<td>79.5(^a)</td>
</tr>
<tr>
<td>Nigella sativa 1 ml/kg</td>
<td>26.1</td>
<td>62.3</td>
<td>11.6</td>
<td>76.6(^{ab})</td>
</tr>
<tr>
<td>Nigella sativa 2 ml/kg</td>
<td>25.4</td>
<td>63.8</td>
<td>10.8</td>
<td>76.6(^{ab})</td>
</tr>
<tr>
<td>Nigella sativa 3 ml/kg</td>
<td>25.9</td>
<td>62.9</td>
<td>11.1</td>
<td>74.3(^b)</td>
</tr>
<tr>
<td>SEM</td>
<td>0.26</td>
<td>0.29</td>
<td>0.13</td>
<td>1.46</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
</tr>
</tbody>
</table>

*: P < 0.05 NS: not significant \(^a,b,c\): Column means with no common superscript differ significantly

Table 4. Coliform and *E. coli* counts (MPN/g) in fecal samples (n = 8)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Coliform</th>
<th>E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110</td>
<td>110(^a)</td>
</tr>
<tr>
<td>Nigella sativa 1 ml/kg</td>
<td>110</td>
<td>110(^a)</td>
</tr>
<tr>
<td>Nigella sativa 2 ml/kg</td>
<td>110</td>
<td>78(^b)</td>
</tr>
<tr>
<td>Nigella sativa 3 ml/kg</td>
<td>110</td>
<td>5.2(^c)</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>**</td>
</tr>
</tbody>
</table>

\(^{**}\): P < 0.01 NS: not significant \(^a,b,c\): Column means with no common superscript differ significantly
Egg yolk (%)

Cholesterol

Serum (%)

Conalbumin %

Triglyceride

Cholesterol

EL-SHEIKH et al. (1998) reported that black cumin (10 and 30 g/kg) supplementation in layer diets significantly (P < 0.05%) decreased egg production. Similarly, CASE et al. (1995) and ELSON (1996) reported that inhibition of hepatic HMG-CoA reductase activity which is a key regulatory enzyme in cholesterol synthesis by essential oils is mediated by down-regulating the regulatory enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. CROWELL (1999) reported the inhibition of HMG-CoA reductase activity which is a key regulatory enzyme in cholesterol synthesis by essential oils. Similarly, CASE et al. (1995) reported that inhibition of HMG-CoA reductase lowered serum cholesterol by 2% in poultry. CROWELL (1999) and ELSON (1996) reported that thymol and carvacrol and beta-ionone might induce a putative regulatory non-sterol product. In accordance with the present findings, it has been shown that Nigella sativa seeds (1 and 1.5%) supplementation in layer diets significantly (P < 0.05%) reduced serum and yolk total cholesterol, LDL-cholesterol, triglycerides content and increased HDL-cholesterol (NASIR et al., 2005). Similarly, EL-BAGIR et al. (2006) reported that feeding of diets with 1 and 3% black cumin seeds for a period of three months reduced egg yolk total cholesterol by 34 and 42%, respectively.

Supplementing Nigella sativa oil 1 and 2ml/kg to the basal diet decreased egg yolk cholesterol in this study. Serum cholesterol levels were reduced by the supplementation of 2ml/kg Nigella sativa oils. The hypocholesterolemic effects of essential oil are mediated by down-regulating the regulatory enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (FARAG et al., 1989a, b). The presence of functional groups and aromaticity are responsible for the antibacterial activity (FARAG et al., 1992; HANAFY and HATEM, 1991). Similarly, SAXENA and VYAS (1986) reported that essential oil of black seeds inhibited the growth of Escherichia coli. DENLI et al. (2004a) reported that Nigella sativa oil had no effect on feed intake, feed conversion, egg production and weight compared with controls. In contrast to our results, DENLI et al. (2004b) reported that addition of 1 g/kg Nigella sativa extracts in diets of laying quails increased (P < 0.05%) weight of yolk and shell in quail egg. Supplementation of Nigella sativa oil reduced concentration of E. coli in the feces in this study. Essential oils basically consist of two classes of compounds, the terpenes and phenylpropanoids (LEE et al., 2004). HELANDER et al. (1998) reported that terpenoids and phenylpropanoids can penetrate the membrane of the bacteria and reach the inner part of the cell because of their lipophilic. But, it was also reported that structural properties, such as the presence of functional groups and aromaticity are responsible for the antibacterial activity. FERDOUS et al., 1992; HANAFY and HATEM, 1991. Similarly, SAXENA and VYAS (1986) reported that essential oil of black seeds inhibited the growth of Escherichia coli. DENLI et al. (2004a) found that Nigella sativa essential oil supplementation to quail feed significantly increased intestinal weight and length and decreased intestinal pH.
complex mixtures of compounds and their chemical compositions (Lee et al., 2004). It is thought any of them may cause reduction in ovalbumin and conalbumin proportion.

It is well known that all lipids of egg yolk are associated with proteins to form lipoproteins, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (Anton, 1998). It was also shown that the HDL fraction of egg yolk (lipovitellin) consists of two forms, α- and β-lipoprotein (apovitellin) (Bernardi and Cook, 1960). It was determined that HDL apoproteins (Apovitellin 3 + 4) and LDL apoproteins (γ-livetin + apovitellenin VI, α-livetin/apovitellenin) and phosvitin of egg yolk were increased with supplementation of 3 ml/kg Nigella sativa oil in this study. Similarly, Bölükbası et al. (2008) found that some HDL apoproteins (Apovitellin 3 + 4, Apolipoprotein CII) and LDL apoproteins (apovitellenin Va, α-livetin/apovitellenin) of egg yolk were increased with supplementation of essential oils in laying hens. It is thought that HMG CoA reductase may also have changed protein ratios of egg yolk.

As to our knowledge, no publication was found on this topic in literature, so no comparison could be made with other publications. Further work is needed in order to give more detailed information on this topic in chickens.

In conclusion, the beneficial effects of dietary Nigella sativa oil supplementation on laying hen performance were not evident in this study. The addition of 3 ml/kg Nigella sativa oil to the laying hens feed led to a significant decrease in the Haugh unit of the egg. It was also observed that E. coli concentration of feces samples were reduced significantly (P < 0.05) with supplementation of Nigella sativa oil to laying hen diets. Supplementation of 3 ml/kg Nigella sativa oil exhibited higher antimicrobial activity than for the other groups. The lowest cholesterol ratio of egg yolk was obtained from hens fed 1 and 2 ml/kg Nigella sativa oil. Although, supplementation of 3 ml/kg Nigella sativa oil decreased ratio of egg triglyceride, it resulted in the highest egg yolk cholesterol. The addition of 2 and 3 ml/kg Nigella sativa oil to laying hens feed led to a significant decrease in egg albumin and conalbumin ratio.

Key words
Layer, Nigella sativa oil, E. coli, cholesterol, egg yolk protein.

Zusammenfassung
Einfluss eines Zusatzes von Nigella sativa zum Legehennenfutter auf die Leistung, den Gehalt an Cholesterol und einigen Proteinen im Dotter sowie auf die Anzahl von Escherichia coli im Kot

Ziel der Untersuchung war die Bestimmung der Effekte eines Zusatzes von Nigella sativa Öl (0, 1, 2, 3 ml/kg) zum Legehennenfutter auf die Leistung, die Eiqualität, den Gehalt an Triglyceriden, Cholesterol und einiger Proteine im Blutserum und im Dotter sowie auf die Anzahl an E. coli im Kot. Für die Untersuchung wurden 64 Legehennen (26 Wochen alt) der Herkunft Lohmann LSL verwendet und zufällig auf vier Behandlungsgruppen (n = 16) verteilt. Jede Behandlung umfasste 4 Wiederholungen.

Der Zusatz von Nigella sativa Öl zum Futter hatte keinen Einfluss auf die Futteraufnahme, die Futterverwertung, das Eigewicht, die Legeleistung sowie auf die Anteile an Dotter, Eiklar und Schale. Der Zusatz von 3 ml/kg Nigella sativa Öl führte zu einer signifikanten Verminderung der Haugh-Einheiten. Es wurde ferner festgestellt, dass der Zusatz von Nigella sativa Öl die Gehalte an E. coli im Kot signifikant reduzierte. Generell zeigte der Zusatz von 3 ml/kg Nigella sativa Öl eine höhere antimikrobielle Aktivität als in den anderen Behandlungsgruppen. Der geringste Dottercholesterol-

Table 7. Relative contents of some egg yolk proteins (n = 8)

<table>
<thead>
<tr>
<th>Names of yolk proteins</th>
<th>Control</th>
<th>Nigella sativa oil 1 ml/kg</th>
<th>Nigella sativa oil 2 ml/kg</th>
<th>Nigella sativa oil 3 ml/kg</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-livetin + apovitellenin VI²</td>
<td>10.09b</td>
<td>10.16b</td>
<td>10.39b</td>
<td>11.74a</td>
<td>0.20</td>
<td>*</td>
</tr>
<tr>
<td>Apovitellin 3 + 4²</td>
<td>22.04b</td>
<td>22.61b</td>
<td>22.46b</td>
<td>25.93a</td>
<td>0.41</td>
<td>**</td>
</tr>
<tr>
<td>Phosvitin</td>
<td>5.75b</td>
<td>5.62b</td>
<td>5.53b</td>
<td>6.30b</td>
<td>0.18</td>
<td>*</td>
</tr>
<tr>
<td>α-livetin/apovitellenin III²</td>
<td>4.21b</td>
<td>4.06b</td>
<td>4.32b</td>
<td>5.62b</td>
<td>0.16</td>
<td>*</td>
</tr>
<tr>
<td>Apovitellin 7²</td>
<td>15.10</td>
<td>14.90</td>
<td>14.28</td>
<td>15.66</td>
<td>0.55</td>
<td>NS</td>
</tr>
<tr>
<td>β-livetin</td>
<td>12.36</td>
<td>12.13</td>
<td>12.10</td>
<td>13.32</td>
<td>0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Apovitellenin IV²</td>
<td>19.71</td>
<td>20.45</td>
<td>19.52</td>
<td>19.03</td>
<td>0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Apovitellenin II²</td>
<td>6.65</td>
<td>8.08</td>
<td>8.51</td>
<td>8.68</td>
<td>0.56</td>
<td>NS</td>
</tr>
<tr>
<td>Apovitellenin I²</td>
<td>3.70</td>
<td>3.37</td>
<td>3.15</td>
<td>3.84</td>
<td>0.17</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹: LDL apoproteins; ²: HDL apoproteins; *: P < 0.05; **: P < 0.01; a,b,c: Means within the same row with no common superscript differ significantly.
ceived was bei den Hennen, die mit einem Zusatz von 1 oder 2 ml/kg *Nigella sativa* Öl gefüttert wurden, beobachtet. Allerdings führte der Zusatz von 3 ml/kg *Nigella sativa* Öl zu geringeren Triglyzeridgewichten und somit zu den höchsten Dottercholesteroleinheiten. Der Zusatz von 2 oder 3 ml/kg *Nigella sativa* Öl bewirkte eine signifikante Verminderung der Albumin- und Conalbumingewichte im Eiklar.

**Stichworte**

Legehenne, *Nigella sativa* Öl, E. coli, Cholesterol, Dotterproteine

**References**


SSPS, 1999: SPSS for Windows Release 1.0.0, SPSS Inc.


Correspondence: Dr. Ş.Canan Bölükbaşı, Atatürk University, Agricultural Faculty, Animal Science Department, 25240 Erzurum, Turkey, E-mail: cananbolukbas@hotmail.com or canang@atauni.edu.tr