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Research Article

EFFECT OF DIETARY SUPPLEMENTATION OF BLACK CUMIN *(Nigella Sativa* L.*)* AND CINNAMON *(Cinnamomum Zeylanicum* L.*)* ESSENTIAL OILS ON PERFORMANCE AND EGG QUALITY OF LAYING HENS

Samet YALÇIN*[1]** ***Muzaffer DENLİ*[1]

[1]Dicle University, Faculty of Agriculture, Department of Animal Science, 21280, Diyarbakir

* Corresponding author; muzaffer.denli@gmail.com

Abstract: *In this study, we aimed to determine the effects of dietary supplementation of black cumin (Nigella sativa* L.*) (BCEO) and cinnamon (Cinnamomum zeylanicum* L.*) essential oils (CEO) on yield performance, egg quality, and eggshell bacterial contamination of laying hens. A total of 315 Atak-S, 28-weeks-old of age were randomly assigned to three groups with 5 replicates of 21 hens each and fed diets supplemented with 0.5 ml/kg feed black cumin and cinnamon essential oil respectively for 11 weeks. During the experiment performance parameters, egg external and internal quality characteristics, and eggshell bacterial microbial contamination were measured weekly. At the end of the all experimental trial, CEO addition improved feed conversion rate and increased eggshell thickness (P<0.05). Dietary BCEO reduced eggshell Escherichia coli contamination (P<0.05). However, there was no significant statistical difference between the experimental groups in terms of feed intake, egg production, egg weight, and other egg quality characteristics (P>0.05). In conclusion, we found that the addition of CEO may improve the performance of laying hens while the addition of BCEO may reduce the eggshell escherichia coli bacteria contamination.*

Keywords*: Black cumin essential oil, cinnamon essential oil, egg quality, shell bacterial contamination, laying hens,*

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1. Introduction

Foods of animal origin have an important place in the healthy and regular nutrition of people. Consumption of animal-based foods with rich and high-quality protein content is also essential for human healthy life. The production of foods obtained from animals in the shortest time and economically is directly related to the health and productivity performance of animals. Nowadays, chicken breeding is an important animal husbandry activity in obtaining quality and economical animal products in a short time.

Antibiotics have been used as a growth promoter for many years for treatment purposes in animal husbandry and especially due to their positive effects on the microflora in the digestive system [1]. Up to now various antibiotics have been used in animals' feeds for a long time as a growth promoter. Antibiotics used in animal nutrition as growth agents were banned in European Union (EU) countries and the United States of America (USA) in 1999-2001, as pathogenic microorganisms in animals, gain

resistance to antibiotics and leave residues in products obtained from these animals and have negative effects on human health. In our country, the addition of antibiotics to feeds as a growth promoter was prohibited in 2006.

Following a ban on the prophylactic use of antibiotics in animal feed as growth-promoters some performance parameters (feed conversion rate, growth rate, egg production, feed intake) have been negatively affected, and that thus causing great economic damage in the poultry industry. These problems have increased the interest of researchers in alternatives to antibiotics and natural feed additives that do not have adverse health effects. For this purpose, the possibilities of using various alternative natural feed additives, especially in poultry, have been tested. In particular, they used various feed additives to protect animal health, increase feed utilization, and increase the quality and quantity of products obtained from animals [2]. Natural medicinal and aromatic herbal extracts without side effects, vegetable essential oils, organic acids, probiotics, prebiotics have been the most commonly tested antibiotic alternatives.

Components in the structure of plant essential oils cause them to show different effects *in vitro* and *in vivo* conditions. The different effects of many essential oil types have been revealed in studies conducted to date. Two different views have been presented on the functioning of essential oils. The first of these views is to protect the health of the animal by the regulation of microbial flora in the intestine, and the other is to improve the utilization of nutrients by increasing enzyme amount and activity as a result of the stimulation of endogenous enzymes [3]. Since essential oils consist of many complex structures, the chemical concentration and composition of each of these components vary. Therefore, the biological effects of essential oils also differ [4]. It has been reported that the mechanisms of action in plants are generally caused by bioactive compounds such as glucosinolate and flavonoid, which are isoprene derivatives, and these compounds have antioxidant and antibiotic activity. Herbal extracts and herbs mainly act on the animal's digestive system, where they act either by enabling better absorption and digestion of nutrients or by inhibiting microbial activity.

Various studies have shown that black seed has antioxidant, antibacterial, immune system supportive, antidiabetic, and antitumoral activity [5]. On the other hand, the cinnamon-based essential oil obtained from the skins of cinnamon has a strong smell of cinnamon. Cinnamyl acetate, cinnamic aldehyde, cinnamyl, and cinnamaldehyde alcohol are included in the content of cinnamon. In an in vitro study, cinnamaldehyde extract obtained from cinnamon was found to inhibit *Bacteroides fragilis* and *Clostridium perfringens* strongly and to moderately inhibit *Lactobacillus acidophilus* and *Bifidobacterium longum* [6]. In another study, the effects of oregano essential oil and various antibiotics (neomycin, tetracycline and neomycin, metronidazole, and enrofloxacin) on egg shape index were compared, but no significant effect of oregano essential oil was found as a result of the study [7].

This study was conducted to determine the effects of the addition of black cumin and cinnamon essential oils on the yield performance, egg quality, and eggshell bacterial contamination in laying hens.

2. Materials and Methods

A total of 315 Atak-S laying hens at the age of 28 weeks were used in the experiment. The nutrient contents of the compound feeds used in the experiment were prepared in accordance with the nutrient requirements of laying hens reported in NRC, 1994 [8]. The composition (%) and nutrient contents of the basal diet are shown in Table 1. The study was carried out in the enriched cage system. The enriched cage system has 3 floors and 5 cage sections on each floor. Illumination of the experimental room was

provided by fluorescence and 8 hours of dark and 16 hours of light program was applied daily. Determination of nutrient contents of feeds (except crude cellulose) was performed according to the Weende analysis method and determination of crude cellulose according to the Lepper method. Black seed and cinnamon essential oils were supplied from a commercial company. Additives were added to the feeds in the form of pre-mixtures and at the last stage of feed production. Laying hens were divided into 3 groups with 5 replicates and 21 hens were placed in each repetition. During the trial, laying hens were fed *ad libitum* and they were provided with continuous access to water with nipple drinkers. Throughout the trial, while the control group was fed with the basal diet, 0.5 ml/kg feed black cumin and cinnamon essential oils were supplemented to the feeds of other groups respectively.

Table 1. Ingredients and chemical composition of experimental diets (as-fed basis)

Ingredients	**%**
Corn	45.00
Soybean Meal (44% CP)	10.00
Full Fat Soybean	17.00
Sunflower Meal (32% CP)	9.60
Wheat	7.50
Dicalcium Phosphate (DCP)[a]	1.77
Calcium Carbonate	8.80
NaCI	0.30
Vitamin+ Mineral Premix[b]	0.10
Dl-Methionine	0.15
Chemical Analysis	
Dry Matter	89.10
Crude Protein	18.10
Crude Oil	4.10
Crude Ash	11.39
Calculated values	
ME (kcal/kg)	2744
Calcium (%)	3.90
Available Phosphor (%)	0.40
Na (%)	0.18
L-lysine (%)	0.91
Methionine+Cysteine (%)	0.78
Treonin (%)	0.67
Tryptophane (%)	0.24
Linoleik asit (%)	2.00

[a] Premix supplied per 1 kg; Calcium 24,5%, Phosphor; 18%.

[b] Premix supplied per 1 kg: vitamin A; 12.000.000 IU; vitamin D3; 2.500.000, vitamin E; 30.000 mg, vitamin K3; 4.000 mg; vitamin B1; 3.000 mg, vitamin B2; 7.000 mg, vitamin B12; 5.000 mg, vitamin B6; 5.000 mg, vitamin C; 50.000 mg, Niacin; 30.000 mg, Cal-D-Pantothenate; 10.000 mg, Biotin; 45 mg, Folic acid; 1.000 mg, Choline Chloride; 200.000 mg, Xanthate;1.500 mg, Manganese; 80.000 mg, Iron; 60.000 mg, Zinc; 60.000 mg, Co; 5.000 mg, Iodine; 1.000 mg, Cobalt; 200 mg, Selenium;150 mg.

At the beginning of the experiment, all hens were weighed and were placed in cages according to similar live weight and egg production level. Throughout the trial the egg production, feed intake, and egg weight of the animals were measured on a weekly basis and the feed conversion rate was calculated using the data obtained. Feed Conversion Rate (FCR) = Feed Intake (g)/ Egg Weight (g). Internal and external quality analyzes were performed on 15 eggs collected weekly from each group on the same

day. Egg weight was determined by weighing with precision balance (0.01g) daily. Egg Shape Index (ESI): The width and length of the egg were measured by digital caliper and calculated using the formula ESI = (Width of egg / Length of egg) x 100. Egg Specific Gravity was measured with a density analyzer consisting of precision balance, beaker, and apparatus. For this purpose, the weight of the eggs which were kept at room temperature for 24 hours was first weighed in the air and then the weight in the water at an average temperature of 20-22 ^{o}C was calculated to determine the specific gravity of the egg.

The shells taken from the middle parts of the broken eggshell under laboratory conditions were measured by digital micrometer after drying and separating the membranes. The shell rate was determined by the ratio of the value of the eggshells obtained with the precision balance after the membrane was removed and dried to the egg weight. Egg yolk color was determined by a digital colorimeter (Minolta CR-300) in *L**, *a**, and *b**. The height of white was measured with digital foot micrometer and calculated with the formula AI = [albumen height (mm) / ((albumen length (mm) + albumen width (mm)) / 2)] x100. Yolk Index (YI): The diameter of the egg yolk was measured by digital caliper and the height was measured by digital foot micrometer and it was determined by the formula; YI = [(Yolk height / Yolk diameter) x 100]. Haugh Unit was calculated by using the egg weight and albumen height and by using the formula; Haugh Unit = 100 Log (H + 7.57-1.7G 0.37). H: Albumen height (mm), G: Egg weight (g). 15 eggs per week were collected from each group (1 egg per pen) and pooled in sterile plastic bags singularly for eggshell bacterial contamination analysis. Total aerobic populations were determined by duplicate spread plating 100 uL of the serial dilutions made from the rinse solution on to plate count agar. Plates were incubated at 35°C for 48 h before enumeration. Coliforms were enumerated by dispensing 1 mL of appropriate dilutions from shell emulsions into violet red bile agar pour plates with overlay. Duplicate plates per sample were incubated at 37°C for 18 to 20 h before typical colonies were counted. Statistical analysis of the data obtained at the end of the experiment was performed using SPSS 18.0 package program [9]. The analysis of variance of the averages was performed with General Linear Model (GLM) ANOVA. Tukey's multiple comparison test was used to compare differences between means.

3. Results and Discussion

Results of the performance data are given in Table 2. During the trial, there was no statistical difference between the groups in terms of feed intake, egg weight, and egg production (P> 0.05). Our findings regarding feed intake are similar to the research results obtained by Islam et al [10] in which they added different levels of black cumin seeds to the diets of laying hens. In another study [11], researchers added a mixture of cinnamon and rosemary essential oils to quail feeds and obtained similar results to the findings in our study. They determined that BCEO and CEO additives to basal diet did not have a significant effect on egg weight during the experiment (P> 0.05). Many researchers [12,13,14,15,16,17] found that the addition of BCEO to basal diets improved egg weight [11], and some researchers [12] reported that the addition of essential oil negatively affected egg weight. It is assumed that the differences between the results of the research may be due to the difference in the level of black seed essential oil added to feeds. As of the end of the trial, the best feed conversion rate was obtained in the group with CEO added to their feed with 2.40, followed by the control group with 2.43 and the BCEO group with 2.52 (P <0.05). Similar to our study, different researchers [15,17,18] reported that dietary black cumin seed supplementation had no shown effect on feed conversion rate in laying hens.

Table 2. Effects of dietary supplementation of BCEO and CEO on the performance in laying hens

Parameters	Groups		
	Control	BCEO (0.5 ml/kg feed)	CEO (0.5 ml/kg feed)
Feed intake, g/day	114.2±0.9	115.1±1.2	112.8±1.1
Feed conservation ratio	2.43^{ab}±0.03	2.52^{a}±0.02	2.40^{b}±0.03
Egg production, %	82.3±0.9	80.2±0.8	82.9±0.9
Egg yield, egg/hen/week	5.8^{ab}±0.1	5.6^{b}±0.1	5.8^{a}±0.1
Egg weight, g	56.8±0.2	56.9±0.2	57.0±0.2

[a,b]Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
BCEO: Black Cumin Essential Oil, CEO: Cinnamenon Essential Oil

The effects of dietary supplementation of BCEO and CEO in the basal diet of laying hens on the egg quality characteristics are given in Table 3.

Table 3. Effects of dietary supplementation of BCEO and CEO on the external and internal egg quality in laying hens

Measurements	Groups		
	Control	BCEO (0.5 ml/kg feed)	CEO (0.5 ml/kg feed)
Shell rate, %	11.4±0.1	11.5±0.1	11.5±0.1
Shell thickness, mm	0.35±0.002	0.34±0.002	0.35±0.002
Specific gravity, g/cm^3	1.076±0.001	1.076±0.001	1.077±0.001
Shape index	76.9±0.30	76.5±0.28	76.5±0.24
Yolk index	43.9±0.28	43.7±0.30	43.4±0.28
Albumen index	5.04±0.12	5.17±0.12	4.86±0.10
Haugh unit	79.6±0.86	80.8±0.78	78.7±0.75
L* value	59.1±0.5	58.9±0.5	57.8±0.3
a* value	25.2^{ab}±0.7	25.4^{a}±0.7	23.0^{b}±0.6
b* value	36.8^{a}±0.6	36.8^{a}±0.5	35.0^{b}±0.4

[a,b]Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).
BCEO: Black Cumin Essential Oil, CEO: Cinnamenon Essential Oil
L (+) Partial white, ΔL (-) Siyah (L=0 Black, L=100 White
a (+) Partial red expansion, Δa (–) Partial green expansion
b (+) Partial yellow expansion, Δb (–) Partial blue expansion

The inclusion of BCEO and CEO in the diet of laying hens had no significant effect on the values of the external (shape index, haugh unit, specific gravity, shell thickness, and weight) and internal quality (white and yellow index) characteristics of the egg (P> 0.05). In agreement with the present study some researchers [10,12]. Egg yolk color was examined as L, a, b values , and values were measured as (59.1, 58.9, and 57.8), (25.2, 25.4 and 23), and (36.8, 36.8 and 35), respectively. While there was no statistically significant difference between the groups in terms of egg yolk L value (P> 0.05), the differences between the groups in terms of egg yolk color (a and b) were found to be statistically significant (P <0.05). These results obtained from the research on egg yolk color are in accordance with the findings of some researchers [19,20].

The effects of dietary inclusion BCEO and CEO on eggshell bacterial contamination in laying hens are presented in Table 4. The positive number of eggshells contaminated by *Escherichia coli* was

significantly decreased for laying hens fed the diet supplemented with 0.5 ml/kg of BCEO versus another group. However, there was no effect of BCEO on the eggshell Enterococcus population. These results are in agreement with those by Saxena and Vyas [21], who reported that the essential oil of black seeds inhibited the growth of *escherichia coli*. According to Dorman and Deans [22], essential oils could control the common intestinal pathogen growth of poultry. On the other hand, no effect of CEO on eggshell contamination of *escherichia coli* and *enterococcus* populations was observed at the level of ECO (0.5 ml/kg feed) in laying hens diet.

Table 4. Effects of dietary supplementation of BCEO and CEO on eggshell bacterial contamination in laying hens

Period (week)	*Escherichia Coli* (positive/total, %)			*Enterococcus* (positive/total, %)		
	Control	BCEO (0.5 ml/kg feed)	CEO (0.5 ml/kg feed)	Control	BCEO (0.5 ml/kg feed)	CEO (0.5 ml/kg feed)
29	3/5	2/5	3/5	ND	ND	ND
30	2/5	2/5	2/5	ND	4/5	2/5
31	2/5	2/5	2/5	ND	1/5	2/5
32	2/5	ND	2/5	1/5	ND	2/5
33	2/5	1/5	2/5	3/5	3/5	3/5
34	1/5	1/5	2/5	2/5	2/5	3/5
35	1/5	ND	2/5	3/5	ND	1/5
36	2/5	2/5	1/5	1/5	1/5	3/5
Periods Average (29 to 36)	15/40 (37.5)	10/40 (25.0)	16/40 (40.0)	10/40 (25.0)	11/40 (27.5)	16/40 (40.0)

BCEO: Black Cumin Essential Oil, CEO: Cinnamenon Essential Oil, ND: Not detected

It can be concluded that the addition of CEO may improve the performance of laying hens while the addition of BCEO may reduce the egg shell ***Escherichia Coli*** bacteria contamination.

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The compliance to Research and Publication Ethics: This work was carried out by obeying research and ethics rules.

The Declaration of Ethics Committee Approval

The author declares that this document does not require an ethics committee approval or any special permission. Our study does not cause any harm to the environment.

The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the author.

Authors' Contributions

S.Y: Conceptualization, Methodology, Resources, Formal analysis, Writing

M.D: Methodology, Formal analysis, Writing- Original draft preparation

All authors read and approved the final manuscript.

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