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THE INFLUENCE OF OREGANO ESSENTIAL OIL ON EGG QUALITY AND EGG SHELL CONTAMINATION OF LAYING HENS KEPT IN FURNISHED CAGES

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Abstract

The aim of this study was to determine the effects of adding oregano essential oils (OEO) to diets on egg quality and egg shell contamination of laying hens kept in furnished cages. For this aim 300 Atak-S at 31 weeks old laying hens were randomly divided into two groups negative control (NC; basal diet) and oregano essential oils (OEO; basal diets plus 150 mg/kg oregano essential oils) for 6 months. Dietary inclusion of OEO significantly decreased E.coli and Coliform contamination on egg shell ($P<0.05$). In addition, including diet with OEO improved means of egg weight comparing to the control ($P<0.05$). However, no significant differences in the internal quality characteristics of eggs were observed between two groups during the all experimental period ($P>0.05$). Results of the study indicated that the adding OEO to diets plays an important role in decreasing egg shell bacterial contamination.

Key words*: furnished cage, egg shell contamination, egg quality, oregano essential oil, laying hens.*

INTRODUCTION

The housing system is an external factor that influences both the performance of hens and the egg quality characteristics. Conventional cages have been banned in the European Union since 2012. The free range system for laying hens is the most widely accepted alternative (cages and non-cages) to animal welfare. In this rearing system, hens can access the outdoor range area at any time during the day. In the free range system tend to require more feed and land to produce eggs or meat. Other benefits of free-range farming are greater comfort for the hens resulting in quality products with lower possibility of egg shell contamination of pathogens (Hammershøj and Steenfeldt, 2015; Pesavento et al., 2017). In addition, the environmental impacts of this system can be higher than that of cage system production. Injury and mortality rates were found to be higher in the free range system than cage system (Michel and Huonnic, 2003).

De Vylder et al. (2009) reported that eggs obtained from chickens housed in welfare-friendly housing systems do not pose more risks of layers being colonized with Salmonella compared to conventional battery cage. On the other hand there are many scientific studies showing that the total bacterial levels is higher in the shells surface of the eggs obtained from the hens housed in the free range system. *Escherichia coli* were the most frequently isolated *Enterobacteriaceae* species (Singh et al., 2009; Jones and Anderson, 2013).

Many kinds of herbs, plant extracts, spices and essential oils, have received great attention being used as feed supplements in poultry feeds for improving their performance and also egg quality (Akyildiz and Denli, 2016). In addition, many synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) have been used widely in the food industry for prevention the deteriorative changes (Al-Hijazeen et al., 2006). However the most of them have not been received consumer concern due to their negative effects on human health. Therefore, the use of natural organic plant

extracts and some essential oils derived from them received attention.

Oregano *(Origanum vulgare L.)* essential oils, a phytogenic additive, are an aromaticplant that is indigenous to the Mediterranean region (He et al., 2017). Essential oils of thyme is derived from thyme (*Thymus vulgaris*), a medicinal plant with several therapeutic properties. Ghasemi et al. (2010) reported that dietary inclusion of garlic and thyme can have beneficial effects on performance of laying hens in terms of improving egg weight and yolk color. Bolukbasi (2008) reported that supplementation with 200 mg/kg EO of sage, thyme or rosemary reduced total coliform and *E. coli* counts in feces of 24-week old laying hens after a 12-week administration period. However, there is limited published data concerning with using thyme essential oil to improve egg quality and reduce the bacterial contamination on egg shell surface obtained in free-range systems.

MATERIALS AND METHODS

A total number of 300, 31 week old Atak-S laying hens with an average body weight of 1650±20 g were housed in furnished cages (750 cm^2/hen, 10 hens per cage) to 54 week of age. Hens were fed the same diet formulated was based on National Research Council (NRC) (1994). The composition of basal diet is shown in Table 1. Water and feed were provided as *ad libitum*. The daily photoperiod consisted of 16 h of light and 8 h of darkness (16L: 8D) throughout the experimental period. Temperature was maintained at 21 ± 1°C during the experimental period. Feeders were filled manually every day and eggs were collected daily during the morning hours. Laying hens were randomly divided into two groups; negative control (NC; basal diet) and oregano essential oils (OEO; basal diets plus 150 mg/kg oregano essential oils) from weeks to 54 weeks of age. Eggs were collected using sterile shears then placed in sterile bags and transported to the laboratory for eggshell bacterial contamination analysis. Egg samples were stored at 4°C overnight before analysis. Thirteen eggs were collected from each group approximately every 4 week from 31 to 54 week of hen age (6 sampling periods). Total aerobic populations were determined by duplicate spread plating 100 µL 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.

Table 1. Composition of the basal hen diet

Components	**(%)**
Maize	45.0
Soybean meal (44%, CP)	10.0
Full-fat soybean meal	16.5
Sunflower meal (32%, CP)	9.50
Wheat	7.50
Limestone	8.80
Dicalcium phosphate[a]	1.85
Vitamin-mineral premix[b]	0.25
NaCl	0.30
DL-methionine	0.15
Calculated analysis	
Dry Matter	89.1
Crude Protein	18.0
Metabolic energy (kcal/kg feed)	2750
Ca	3.6
Available P	0.45
Methionine + Cysteine	0.63

[a] Composition (for each kg premix): Ca 24.5%; P 18%

[b] Composition (for each kg premix): vitamin A 12,000,000 IU; vitamin D_3 2,500,000 IU; vitamin E 30,000 mg, vitamin K_3 4,000 mg; Vitamin B_1 3,000 mg; Vitamin B_2 7,000 mg; Vitamin B_{12} 5,000 mg; Vitamin B_6 5,000 mg; Vitamin C 50,000 mg; Niacine 30,000 mg; Cal-D-Pantotenat 10.000 mg; Biotine 45 mg; Folicasit 1,000 mg; Cholincloride 200,000 mg; Canstatin 1,500 mg; Mn 80,000 mg; Fe 60,000 mg; Zn 60,000 mg; Cu 5,000 mg; I 1,000 mg; Co 200 mg; Se150 mg

Statistical analysis was performed using the mixed model and t-test procedure of SPSS 18.0. Tukey's test was used to separate group means. A significant difference was at $P<0.05$

RESULTS AND DISCUSSIONS

There are two basic food safety concerns that are microbiological safety and chemical contamination. Contamination of egg and egg products with pathogenic microorganisms (*Salmonella, Coliforms*, *Escherichia coli* etc.) can turn the egg into a poisonous food.

Dust samples were found to more readily detect the presence of *Salmonella* than fecal samples (EFSA, 2006).

The housing system has significant effects on the total count of bacteria on the egg surface and the microbial contamination of *Enterococcus* and *Escherichia coli* (Englmaierová et al., 2014). It has been reported that the rate of dirty and cracked eggs was increased in the eggs obtained from chickens grown in furnished cages (Tauson, 2002). Microbial contamination is generally higher in eggs or dirty eggs obtained from sick animals.

On the other hand, oregano essential oils have been used in poultry feeds as alternative antibiotics due to its antimicrobial, antioxidant, antiseptic and antiparasitic activities.

Eggs collected from alternative housing systems may have higher bacterial contamination probability of eggs due to contact with faeces or bedding material (Englmaierová et al., 2014). Similarly, Singh et al. (2009) reported that eggs from cages had lower *Escherichia coli* and coliform contamination than those from nest-boxes and the floor. In a recent review, DeReu et al. (2008), in observed that the aerobic bacterial counts on the egg shells were lower than the flocks (cage and ground) with cages (bird and cage), and the difference was that the eggs placed outside the nest boxes in the cage were very pronounced.

Effects of dietary supplementation of OEO on the percentage of shells positive for bacterial contamination by enrichment are presented in Table 2.

He et al, (2017) found that the addition of oregano essential oils decreased the number of intestinal *Escherichia coli*. Furthermore, Roofchaee et al. (2011) discovered that feed supplementation with OEO displayed potent antibacterial effects against cecal *Escherichia coli.*

In our study, the percentage of eggshells positive for *Enterobacteri, Coliform* and *Escherichia coli* were significantly decreased by dietary supplementation of OEO ($P<0.05$). This decrease of the percentage of the bacterial contamination on eggshells in was probably caused by the antimicrobial effects of OEO phenolic compounds such as thymol and carvacrol.

The results of eggshell bacterial contamination analysis are summarized in Table 3. Eggs from OEO treatment group had significantly ($P<0.05$) lower values of egg shell contamination for both the average of total *Coliform* and *Escherichia coli*; had 2.35 and 1.96 log CFU/egg, respectively, and control group had 2.35 and 3.02 log CFU/egg, respectively. These results are in agreement with Turcu et al. (2014), who observed the inclusion of OEO in to broiler diets significantly reduced *Enterobacteriaceae*, *E. coli* and staphylococci in the intestinal microflora compared to the control group. In addition, Criste et al. (2017) reported a significant decrease of *Escherichia coli* colony in the intestinal microflora of broilers reared under heat stress (32^{o}C) and fed with diets that included 2% oregano powder ($P\leq0.05$).These results may be due to the antimicrobial effect of thyme essential oils in the intestinal system of poultry.

Table 2. Effects of dietary inclusion OEO on contaminated eggshell ratio in laying hens housed in free-range system for 6 months

Period (months)	*Enterobacteria* (positive/total, %)		*Coliform* (positive/total, %)		*E.coli* (positive/total, %)	
	Control	OEO	Control	OEO	Control	OEO
1	60	10	30	ND	10	ND
2	ND	ND	ND	ND	ND	ND
3	50	20	50	20	50	20
4	70	50	70	50	40	ND
5	20	30	20	20	20	10
6	70	70	70	20	ND	20
Periods Average (1 to 6)	45	30	40	18	20	8

Table 3. Effects of dietary inclusion OEO on average of bacterial colony count of eggshell in laying hens housed in free-range system for 6 months

Period (months)	Average of total aerobes (log CFU/mL)		Average of total coliforms (log CFU/mL)		Average of total *E. coli* (log CFU/mL)	
	Control	OEO	Control	OEO	Control	OEO
1	3.38±0.14	3.57±0.11	4.32	ND	2.48	ND
2	2.03±0.18	1.99±0.01	ND	ND	ND	ND
3	3.01±0.11	2.60±0.21	2.07	2.15	1.95	1.93
4	3.71±0.22	4.13±0.17	3.65	2.24	3.23	ND
5	3.41±0.33	3.64±0.13	2.23	4.31	3.54	ND
6	6.10±0.19	5.52±0.15	4.38	3.30	3.88	ND
Periods Average (1 to 6)	3.60±0.18	3.35±0.15	2.96^{a}±0.2	2.35^{b}±0.2	3.02^{a}±03	1.93^{b}±0.0

Our results are in agreement with the previous reports who found that oregano had antibacterial activity against *Escherichia coli.* The antimicrobial effects of oregano essential oils may be attributed to its phenolic compounds (Bozin et al., 2006).

CONCLUSIONS

Results of our study indicated that dietary inclusion oregano essential oils play an important role in decreasing egg shell bacterial contamination. In addition, no adverse effect were observed on any parameters checked in this study, therefore this kind of feed additive would be beneficial in egg safety in furnished cages system.

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