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EFFECTS OF DIETARY OREGANO ESSENTIAL OIL ON PERFORMANCE, EGG QUALITY AND EGG SHELL BACTERIAL CONTAMINATION IN LAYING HENS HOUSED IN FREE-RANGE

Muzaffer DENLI[1*], Simten YESILMEN ALP[2], Aydin VURAL[3]

[1]Dicle University, Faculty of Agriculture, Department of Animal Science, 21280, Diyarbakır, Turkey
[2]Dicle University, Faculty of Veterinary Medicine, Department of Microbiology, 21280 Diyarbakir, Turkey
[3]Dicle University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, 21280 Diyarbakir, Turkey

Corresponding author email: muzaffer.denli@gmail.com

Abstract

This study was conducted to determine the effects of dietary oregano essential oils (OEO) on performance, egg quality and eggshell bacterial contamination in laying hens housed in free-range system. A totally of 300, 31 weeks of age laying hens (Atak-S) were allocated into two groups: negative control (NC; basal diet) and oregano essential oils (basal diets plus 150 mg/kg oregano essential oils) for 6 months. Totally 30 eggs were collected from each trial group on the last day of each week to determine the egg quality characteristics during the experiment. The highest egg weight and lowest feed conversion ratio were measured in OEO group compared to control (P<0.05). OEO significantly (P<0.05) reduced the number of eggshell contaminated with the contamination of Enterobacteria, Coliform and Escherichia coli. However, no significant differences on egg quality parameters were noticed between OEO and control groups throughout the experiment (P>0.05). In conclusion, the supplementation of OEO to diet may reduce the total bacteria contamination on the egg shell surface obtained laying hens housed in free range systems.

Key words*: Free-range, oregano essential oil, egg quality, egg shell bacterial contamination, laying hens.*

INTRODUCTION

Rearing system has been one of the most frequently addressed issues in poultry farming after European Directive 1999/74/EC requires the ban of conventional cages for housing laying from 2012. Since this request, some alternative rearing systems such as enriched cages, aviary and free-range systems have been proposed. Housing in the cage is the most common system for growing of laying hens (Denli et al., 2018).

Alternative housing systems have been evaluated in terms of production performance (Denli et al., 2018), egg quality (Guesdon et al., 2006) welfare (Abrahamson and Tauson, 1998) and bacterial contamination of eggshell (Mallet et al., 2006).

Ellen et al. (2000) reported that dust concentrations in the air were higher in percheries and aviaries systems than cage systems. Eggs produced in aviary and free-range systems may have higher aerobic bacteria on the shell than eggs from conventional and furnished cages systems (De Reu et al., 2005). De Buck et al (2004) reported that eggshell bacterial load could have an impact on shelf life and food safety and bacteria present on its surface may lead to actual contamination of the egg.

Rearing systems and feed ingredients have important effects on egg quality and hygiene (shell microbial contamination) (Hidalgo et al., 2008; Holt et al., 2010). Tactacan et al (2009) reported that the number of dirty eggs in conventional cages is lower than that in enriched cages. Contamination of *Salmonella enteritis* is the most common microbial contamination in eggs. Kinde et al. (1996) found that 0.3% of the eggs produced in a farms were exposed to *Salmonella enteritidis* contamination. In another study, it was determined that the total number of Gram negative bacteria found in the eggs of the hens raised in the mid-range system was higher than the cages (De Reu et al., 2005). Similarly,

Parisi et al. (2015) reported that eggs produced in free-range system was more polluted in terms of microbial contamination than those produced in cage systems. These microbial contamination causes disease in poultry and serious risks to human health (Havelaar et al., 2010; Arnold et al., 2014). Therefore, reducing this risk without using antibiotics in animal feeds has been one of the most researched subjects in our time.

Phytogenic feed additives are a recent class of alternatives to growth promoters, originating principally from herbs, spices and their products, which have gained wide attention in the feed industry in recent years (Wenk, 2003). Oregano *(Origanum vulgare L.)* essential oils, a phytogenic additive, are an aromatic plant that is indigenous to the Mediterranean region (He et al., 2017). Aromatic plants and essential oils (EO) extracted from these plants have become more important because of their antimicrobial actions, stimulating effects on the digestive systems of animals and antioxidant properties (Wenk, 2003).

Oregano essential oil is one of the more effective substance which has antimicrobial effects. Carvacrol, thymol, p-cymene and γ-terpenene are the major components of oregano essential oil (Kosar et al., 2003). Up to now, many beneficial effects of oregano essential oil on performance and health of animal have been reported (Lee et al., 2003). Dietary oregano essential oil decreased the effects of coccidiosis infection (Giannenas et al., 2003), increased egg production performance and hatchability in hens (Radwan et al., 2008).

In this study we aimed to determine the effects of dietary oregano essential oils (OEO) on performance, egg quality characteristics and bacterial egg shell contamination in laying hens housed in free-range systems.

MATERIALS AND METHODS

Totally three hundred 31 weeks-old-age of Atak-S laying hens were allocated into two groups in free-range systems (n= 300; 10 house pens; 15 hens per pen; floor space 200 cm^2/hen). Control group received the basal diet and oregano essential oils (basal diets plus 150 mg/kg oregano essential oils) for 24 weeks. Laying hens were fed the same diet formulated was based on National Research Council (NRC, 1994) containing 17.5% CP, 2750 ME/kg, 3.7% Ca and 0.45% available P. Throughout the experiment lights were on a 16L:8D schedule.

Feeders were filled manually every day and egg collection was conducted daily during the morning hours. Egg weight, feed intake and feed efficiency were determined weekly during the all experiment period. Egg production per group, per-cage-hen-day production and quality parameters were performed weekly. Totally 30 eggs were collected (in the morning) from each group for 2 consecutive days and stored at 4°C overnight and then broken onto a level surface. Percentage of cumulative mortality of laying hens were recorded during the laying periods. Egg height, width and shell thickness (mm) were measured by using micrometer screw from Mitutoya. The height of the albumen and yolk were measured by using tripod micrometer. The width of the albumen and yolk were measured by using a standard caliper. Yolk color was measured with a Roche yolk color fan scale (Roche scale). Totally 15 eggs were collected from each group (1 egg per hen) and pooled in sterile plastic bags singularly for eggshell bacterial contamination analysis. 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.

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

Housing system has an important influence on the egg quality parameters of laying hens (Vits et al., 2005). Effects of dietary inclusion OEO on egg production, feed consumption, feed conversion rate and mortality are presented in

Table 1. The supplementation of 150 mg/kg OEO significantly improved feed conversion ratio compared with the control group (P<0.01). Similar to our results, Radwan et al. (2008) observed the use of 1.0% OEO in the diet improved feed conversion rate in laying hens. The beneficial effects OEO on feed conversion rate may be due to the antimicrobial activity of its phenolic compounds (Bozin et al., 2006). These phenolic compounds (carvacrol and thymol) may be improve the feed conversion rate by increasing feed utilization (Lee et al., 2003). However, egg production, feed consumption and mortality were statistically not affected by OEO supplementation throughout the experimental period (P>0.05).

Shell and internal quality of egg is important for the economic success of a producer and also consumer demands (Singh et al., 2009). Egg quality may be influenced by several factors including housing regimen, hen strain and nutritional values. The effect of OEO supplementation on egg weight, egg shape index and eggshell thickness are presented in Table 2. Egg weight was significantly higher in group received the diet containing 150 mg/kg OEO than the control at 1 or 3 weeks and all the period (P<0.05). However, we no found significant difference between control and OEO group regarding the egg shape index, shell weight and shell thickness regarding appearance (P>0.05) for 1 to 6 months. Beneficial effects of the many kinds of herb essential oils alone or mixture on egg weight and egg quality characteristics were observed. In our study, the dietary inclusion of EOE significantly increased egg weight at 1, 3, 5 months and total (P<0.05). These results are similar to the findings that Bolukbasi et al., (2008) increases the egg weight when diets were supplemented with 200 mg/kg EO of thyme, sage or rosemary over a period of 12 weeks. These results may be due to the addition of OEO to the diet of laying hens may enhances intestinal digestive function by increasing the secretion of digestion enzymes.

Other dietary effects of OEO on egg internal quality parameters including albumen height, albumen width and yolk height and yolk width are presented in Table 3. None of these parameters was significantly affected with the dietary OEO treatment (P>0.05). These results are in agreement with previous reports (Florou-Paneri et al., 2005) showing no significant differences in egg internal quality when laying hens fed a diets supplemented 50 and 100 mg/kg oregano essential oils. Contrary to our results, He et al. (2017) observed increases of the percentage of yolk ratio and egg shape index in laying hens fed diet supplemented at 50 mg/kg oregano essential oil. The differences between the studies may be due to the OEO levels supplemented.

Table 1. Effects of dietary inclusion OEO on egg production, feed consumption, feed conversion rate and mortality in laying hens housed in free-range system for 6 months

Period (months)	Egg Production (%)		Feed Consumption (FC, g/hen/day)		Feed Conversion Rate (FCR)		Mortality (%)	
	Control	OEO	Control	OEO	Control	OEO	Control	OEO
1	84.3±1.22	86.6±0.54	106.4±1.38	108.2±1.44	2.26^{a}±0.02	2.16^{b}±0.02	0.26±0.10	0.20±0.10
2	85.9±0.42	86.3±0.42	111.0±1.89	113.7±2.26	2.36^{a}±0.04	2.27^{b}±0.04	0.74±0.10	0.36±0.10
3	86.5±+0.42	86.2±0.70	114.7±1.84	115.3±1.65	2.44^{a}±0.04	2.30^{b}±0.03	1.04±0.10	1.11±0.10
4	83.5±1.24	83.4±0.65	117.3±1.47	118.2±1.44	2.49^{a}±0.03	2.36^{b}±0.03	0.93±0.10	0.47±0.10
5	81.7±0.76	81.5±0.52	119.5±1.48	118.7±1.38	2.54^{a}±0.03	2.36^{b}±0.03	0.94±0.10	1.40±0.10
6	81.4±0.42	85.5±0.79	120.7±2.04	121.5±1.41	2.56^{a}±0.04	2.42^{b}±0.03	0.67±0.10	0.69±0.10
Periods Average (1 to 6)	83.7±0.74	84.9±0.60	114.9±0.56	115.9±0.67	2.44^{a}±0.01	2.31^{b}±0.01	0.76±0.10	0.70±0.10

^{a,b}Means± SE within each period with different superscript letters are significantly different (P<0.05), OEO: Oregano Essential Oil

Table 2. Effects of dietary inclusion OEO on weight, shape index and shell thickness of eggs of laying hens housed in free-range system 6 months

Period (months)	Egg weight (g)		Egg Shape Index		Shell Thickness (mm)	
	Control	OEO	Control	OEO	Control	OEO
1	55.8[b]±0.15	57.9[a]±0.12	75.0±0.91	75.3±0.99	0.40±0.01	0.39±0.01
2	58.6±0.13	60.5±0.17	74.1±0.49	75.3±0.80	0.36±0.01	0.35±0.007
3	60.3[b]±0.19	61.6[a]±0.07	74.6±0.92	74.9±1.11	0.33±0.01	0.33±0.005
4	60.6±0.14	61.7±0.10	74.5±0.65	74.2±0.95	0.34±0.006	0.33±0.004
5	61.3±0.09	61.5±0.09	73.8±0.51	74.9±0.61	0.34±0.007	0.33±0.006
6	61.6±0.08	62.6±0.09	73.9±0.63	73.1±0.57	0.35±0.006	0.35±0.006
Periods Average (1 to 6)	59.7[b]±0.16	62.8[a]±0.11	74.3±0.28	74.6±0.31	0.35±0.003	0.34±0.004

[a,b]Means± SE within each period with different superscript letters are significantly different (P<0.05), OEO: Oregano Essential Oil

Table 3. Effects of dietary inclusion OEO on height and width of albumen and yolk of eggs of laying hens housed in free-range system 6 months

Period (months)	Albumen height (mm)		Albumen width (mm)		Yolk height (mm)		Yolk width (mm)	
	Control	OEO	Control	OEO	Control	OEO	Control	OEO
1	8.3±0.48	8.5±0.35	40.1±0.43	40.1±0.43	16.5±1.04	18.4±0.44	40.1±0.43	41.5±0.50
2	7.6±0.29	7.8±0.23	40.7±0.31	40.7±0.31	17.6±0.40	18.8±0.31	40.7±0.31	40.9±0.41
3	6.4±0.17	6.3±0.20	41.1±0.25	41.1±0.25	17.5±0.30	17.0±0.26	41.1±0.25	41.8±0.35
4	6.8±0.17	6.9±0.20	40.9±0.61	40.9±0.61	18.0±0.26	18.2±0.34	40.9±0.61	40.2±0.45
5	6.0±0.24	6.1±0.25	40.2±0.24	40.2±0.24	17.8±0.22	18.0±0.31	40.2±0.24	40.6±0.18
6	7.1±0.12	6.6±0.17	41.6±0.43	41.6±0.43	17.8±0.71	17.1±0.27	41.6±0.43	41.8±0.40
Periods Average (1 to 6)	7.2±0.10	6.4±0.17	40.9±0.18	40.9±0.18	17.5±0.25	17.8±0.15	40.9±0.18	41.4±0.19

[a,b]Means± SE within each period with different superscript letters are significantly different (P<0.05), OEO: Oregano Essential Oil

Table 4. Effects of dietary inclusion OEO on egg shell bacterial contamination in laying hens housed in free-range system 6 months

Period (months)	Enterobacteria (positive/total, %)		Coliform (positive/total, %)		*E. coli* (positive/total, %)	
	Control	OEO	Control	OEO	Control	OEO
1	6/10 (60)	1/10 (10)	3/10 (30)	ND	1/10 (10)	ND
2	ND	ND	ND	ND	ND	ND
3	5/10 (50)	2/10 (20)	5/10 (50)	2/10 (20)	5/10 (50)	2/10 (20)
4	7/10 (70)	5/10 (50)	7/10 (70)	5/10 (50)	4/10 (40)	ND
5	9/10 (90)	2/10 (20)	2/10 (20)	2/10 (20)	2/10 (20)	1/10 (10)
6	7/10 (70)	7/10 (70)	7/10 (70)	7/10 (70)	1/10 (10)	2/10 (20)
Periods Average (1 to 6)	68/60 (56.6)	17/60 (28.3)	24/60 (40)	16/60 (26.6)	16/60 (21.7)	4/60 (6.7)

[a,b]Means± SE within each period with different superscript letters are significantly different (*P*<0.05), OEO: Oregano Essential Oil

Many researchers indicated that oregano had antibacterial activity against *E. coli* and Salmonella (Ouwehand et al., 2010; Mathlouthi et al., 2012). This antimicrobial activity may be due to their major active components such as thymol and carvacrol (He et al., 2017). The results of eggshell bacterial contamination analysis are summarized in Table 4. The positive number of eggshells contaminated by Enterobacteria, Coliform and *Escherichia coli* were significantly decreased for laying hens fed diet supplemented with 150 mg/kg of OEO versus another group.

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 ($P \leq 0.05$). 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 ($P \leq 0.05$).

CONCLUSIONS

The supplementation of OEO increased average of egg weight, improved feed conversion rate and reduced the total bacteria contamination on the egg shell surface obtained laying hens housed in free range systems.

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