Indian J. Anim. Res., **47** (6) : 515-520, 2013

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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EFFECTS OF DIETARY SUPPLEMENTATION OF ESSENTIAL OILS AND VITAMIN E ON PERFORMANCE, EGG QUALITY AND *ESCHERICHIA COLI* COUNT IN EXCRETA

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Received: 17-01-2012 Accepted: 15-09-2012

ABSTRACT

This experiment was conducted to investigate the effects of dietary essential oil combination (sage, thyme, and mentha extracts) (EOC) and vitamin E (Vit E) supplementation on laying hens' performance, egg quality, yolk Thiobarbituric Acid Reactive Substance (TBARS) values and *Escherichia coli* (*E. coli*)and Coli form bacterium counts in excreta. One-hundred and twenty Lohmann white layers (36 weeks of age) were randomly allocated to five treatment groups, each having six replicate cages as subgroups comprising of four hens. Experimental groups were fed the basal diet (control group), basal diet plus 150 ppm of EOC (EOC-1), 300 ppm of EOC (EOC-2), 150 ppm Vitamin E (Vit E-1),or300 ppm Vitamin E (Vit E-2).The performance parameters, except for egg production and feed consumption, were not affected by the essential oil combination and Vit E supplementation. Feeding 300 ppm EOC caused significant reduction in egg production as compared to other groups($P< 0.01$). Dietary groups consumed less feed than the control group ($P< 0.01$). According to the control group, supplemental EOC and Vit E increased shell stiffness (kg/cm^2) and shell weight ($P< 0.01$) but did not affect other egg quality parameters. The essential oil combination and Vit E supplementation reduced the values of TBARS in eggs refrigerated at 4^0C on days 0, 21, and 42 ($P< 0.01$). However, there was no effect on the content of *E. coli* and Coli form bacterium in excreta ($P> 0.05$). It may be concluded that Vit E can easily be replaced by essential oil combination at the level of 150 ppm for inhabitation of lipid oxidation, without having an adverse effect on performance of laying hens and quality of eggs.

Key words: Antioxidant effect, *E. coli*, Egg quality, Essential oil combination, Layer.

INTRODUCTION

Plant extracts and spices, as single compounds or as mixed preparations, can play a role in supporting both the performance and health status of animals (Çabuk *et al.*, 2006). Given the positive effects of plant extracts on feed efficiency and animal performance, these substances were also identified by several investigators (Bozkurt *et al.*, 2009). Moreover, scientists confirm the antioxidant (Bozkurt *et al.*, 2012a) and hypocholesterolemic (Craig, 1999) role of essential oils. Synthetic antioxidants (BHA and BHT) are quite effective but are suspected to possess mutagenic activity, emphasizing the need for naturally occurring antioxidants without side effects. Many plants, especially the Apiaceae and Lamiaceae families, show significant ant oxidative properties (Vichi *et al.*, 2001).The antioxidant capacity of some plant extracts are often reported to be higher than that of synthetic antioxidants (Pizzale *et al.*, 2002).Natural products isolated from spices and herbs can also act as antioxidants either solely or synergistically in mixtures with other natural and/or synthetic additives (Bandoniene *et al.*, 2002). In addition, some *in vitro* studies report antimicrobial actives of aromatic plant extracts (Denli *et al.*, 2004; Govaris *et al.*, 2005). The objective of the present study was to evaluate the effect of dietary supplementation of EOC and Vit E on layers performance, egg quality, lipid oxidation, and *E. coli* and Coli form bacteria density in excreta.

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MATERIALS AND METHODS

One hundred and twenty Lohman layers (36 weeks of age) were randomly assigned into five dietary treatments with six replicates of four hens each using battery type cages. Hens were fed the basal diet (control group), basal diet plus 150 ppm of EOC (EOC-1), 300 ppm of EOC (EOC-2), 150 ppm Vitamin E (Vit E-1),or 300 ppm Vitamin E (Vit E-2).Vit Experimentation lasted for eight weeks, after a two week trial period. Food and water were given ad-libutum and laying hens were subjected to a 17-hour-day lighting program during the experiment. Ingredients and chemical composition (as per AOAC, 1990) of basal feed used in the experiment are provided in Table 1. EOC was claimed to containing the same amount of thyme(75.97% 1.8 Cineole, 11.66% Camphor, and 7.44% Carvacrol), sage (77.75% Carvacrol, 6.10% Thymol, 1.76% Borneol, and 0.56% L-Linalool) and mentha(34.74% Pulegone, 7.38% Carvacrol, 3.75 % Isomenthone, and 1.75% Sabinen). The extracts were obtained from the EgeLokman Botanical Plant Industry, and commercially commercially available Vit E was used. Feed intake and egg production were recorded daily, egg weight on a biweekly basis, and body weights at the beginning and the end of the experiment. Feed conversion ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced. Samples of 12 eggs from each experimental group were randomly picked every month to evaluate egg quality parameters such as shape index, shell strength, shell thickness, albumen index, yolk index, yolk color (Yolk Colour Fan, the CIE standard colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and Hough unit were determined by formulas and methods given by Kaya *et al*. (2011).

Determination of TBARS in egg yolk: Lipid oxidation was assessed on the basis of the MDA formed during refrigerated storage. MDA is the compound used as an index of lipid per oxidation (Botsoglou *et al.*, 2005). TBARS values were determined in 18 eggs from each group at the end of the experiment (day 0) or after storage at 4 ^{0}C on day21 or 42 using the Kýlýç and Richards (2003) method.

Determination of *E. Coli* and Coliform bacterium concentration in excreta: At the end of experimental period, excreta samples were taken from each replicate cage for determination of total *E. coli* and Coli form bacterium concentration. These samples were blended in a stomacher (Stomacher 400; AJ Seward, London, England) for two minutes in 50 mL of 0.85% (w/v) salt water. A series of fermentation tubes containing Fuluorocult laurel sulphate broth were inoculated with the water sample and incubated for 48 hours at 35 ^{o}C. The fermentation tube contained an inverted tube to trap gases produced by the Coli form bacterium. After 48 hours, the fermentation tube was examined for gas production. Later, the tubes were examined under a 366-nm UV Lampe for *E. coli*. Dilutions indicated positive for Coli form bacterium and *E. coli*. Atable of the most probable numbers was used to estimate the Coli form bacterium content of the

TABLE 1: Ingredients and chemical composition of the basal layer diet (%).

Ingredients	(%)	Chemical Composition	(%)
Maize	53.06	Dry matter	87.9
Soybean meal	28.39	Crude protein	18.9
Full-fat soybean	9.06	Crude fibre	4.9
Ground limestone	6.94	Ether extract	3.1
Soybean oil	1.25	Crude ash	6.4
DCP	0.62	Non-free extractives	54.6
Salt	0.31		
Vit-Min. premix*	0.20		
L-Lysine	0.09		
D-L-Methionine	0.08	Metabolizable energy (kcal/kg) (ME)**	2660

* Supplied per two kilogram of diet: 12.000.000 IU Vitamin A, 2.500.000 IU Vitamin D_3, 30.000 mg Vitamin E, 34.000 mg Vitamin K, 3.000 mg Vitamin B_1, 6.000 mg Vitamin B_2, 30.000 mg NicotinAcid, 10.000 mg Cal.-D-Paln, 5.000 mg Vitamin B_6, 15 mg Vitamin B_{12}, 1.000 mg Folic Acid, 50 mg D-Biotin, 300.000 mg Cholin, 50.000 mg Vitamin C, 80.000 mg Manganese, 60.000 mg iron (Fe), 60.000 mg zinc (Zn), 5.000 mg copper (Cu), 2.000 mg iodine (I), 500 µg cobalt (Co), 150 µg selenium (Se), 10000 mg Antioxidan, 2500 mg contaxantin, 500 mg Apoester.

**Calculated.

samples. The results were reported as most probable number (MPN) of *E. coli* and Coli form bacterium per gram (Anonymous, 1992).

Statistical Analysis: Data from the experiment was statistically analyzed using the GLM procedure of SPSS software(1996) and significant differences between means were determined by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Performance parameters(Table 2), except for egg production and daily feed intake were not affected (P> 0.05) by supplementation of EOC and Vit E into the control diet. Supplementation of 300 ppm EOC decreased (P< 0.01) egg production. Botsoglou *et al.* (2005) and Florou-Paneri *et al.* (2005) reported that supplementation of thyme, sage, and Vit E did not affect egg production. Feed intake of hens on diet EOC 1, EOC 2, or Vit E 2werelower (P< 0.01) than in the control diet. Similar to present findings, Çetingül *et al.* (2008) reported reduced daily feed consumption by supplementation of peppermint extract. However, some report showed increased feed intake with supplementation of Vit E in quails and layers' diets respectively (Bölükbasi *et al.*, 2007). Inclusions of EOC and Vit E at different levels significantly(P < 0.01) improved shell strength and weight as compared to the control group remaining egg quality parameters were comparable (Table 3).Positive effects associated with supplementation of aromatic plant extracts on metabolism may be speeding up of the secretion of digestive enzymes and improvement in the digestibility of nutrients (Bozkurt *et al.*, 2009). In contrast with the current findings in some studies, inclusion of plant extracts (Özek *et al.*, 2012) and essential oil mixture (Bozkurt*et al.*, 2012b) at different levels did not report any effect on shell strength. Supplementation of EOC and Vit E reduced (P< 0.01)TBARS values of eggs throughout the course of storage(day 0, 21, and 42)compared with the control group (Table 4). The mean antioxidant activity of the different treatment groups remain incomparable among themselves. The higher TBARS values on different storage days (i.e.day 21 and 42), as compared with the day 0 value in different groups, indicated a chronic oxidative disruption in eggs. The ant oxidative effect of different treatment groups may be due to free radical scavenging activity, transition metal-chelating activity, or singlet oxygen-quenching capacity (Pizzale *et al.*, 2002).The phenolic compounds found in oregano EO,*i.e.* carvacrol, and thymol are responsible for the antimicrobial and antioxidant properties of different oregano species; these compounds make up over 78% of total volatile oils. The effectiveness of the main anti oxidant constituent of sage oil (carnosic acid) was markedly higher than in traditional chemical antioxidants (Bozkurt *et al.*, 2012a). Similar to present findings, Bozkurt *et al.* (2012a) reported that supplementation of EOC lower edyolk MDA concentration during refrigerated storage on days 3, 6, 9, 12 and 15, while in the control group, the MDA values increased with time. Bölükbaþý *et al.* (2007) reported that dietary supplementation of Vit E at different levels (45, 65, and 85 IU/kg) reduced egg yolk TBARS values on day 42. Similarly, Cherian *et al.* (1996) also reported that dietary use of á-tocopherol in laying hens increased oxidative stability of fresh eggs. Kang *et al.* (2001) reported that supplementation of tocopherol into diets rich in polyunsaturated fatty acids inhibited lipid oxidation in laying hens' muscle and eggs. Furthermore, Botsoglou *et al.* (2005) reported decreased lipid

TABLE 2: Effect of feeding EOC and Vit E on laying performance parameters.

Groups	Egg Production (%)	Egg Weight (g)	Feed Intake (g)	FCR[1]	Cracked egg yield (%)	Initial Weight (g)	Final Weight (g)	Weight Change (g)
Control	88.41[a]	66.99	130.63[a]	2.21	0.61	1636	1670	34
EOC -1	88.45[a]	68.69	122.52[bc]	2.02	0.67	1665	1697	32
EOC -2	80.01[b]	67.70	120.69[c]	2.26	0.96	1656	1695	40
Vit E-1	90.24[a]	67.74	129.13[ab]	2.12	0.25	1636	1757	121
Vit E-2	87.22[a]	67.93	123.10[bc]	2.09	0.72	1659	1707	48
SEM	1.82	1.09	2.17	0.06	0.34	15	24	24
P	0.005	0.867	0.011	0.089	0.680	0.55	0.17	0.08

[1]FCR= feed conversion ratio (kg feed consumed per kg egg produced)

a,b,c: Means within columns with different superscripts differ at P < 0.05

TABLE 3: Effect of feeding EOC and Vit E on egg quality parameters.

Group	Shape index (%)	Shell Strength (kg/cm^2)	Shell thickness ($mmx10^{-2}$)	Shell weight (g)	Yolk colour	Yolk index (%)	Albumen index (%)	Haugh unit
Control	74.21	1.09^{b}	0.37	7.08^{b}	6.50	38.45	7.78	76.99
EOC-1	75.71	2.14^{a}	0.41	8.21^{a}	6.50	37.65	7.92	79.04
EOC-2	74.54	1.88^{a}	0.38	7.99^{a}	6.42	38.17	7.70	78.46
Vit E-1	75.29	1.82^{a}	0.38	7.89^{a}	6.83	37.65	7.37	75.64
Vit E-2	76.45	1.82^{a}	0.38	8.00^{a}	6.92	38.21	8.44	80.93
SEM	0.78	0.18	0.01	0.21	0.18	0.62	0.34	1.70
P	0.279	0.007	0.299	0.008	0.205	0.846	0.292	0.261

a, b: Means within columns with different superscripts differ at $P < 0.05$

TABLE 4: Effect of feeding EOC and Vit E on TBARS values of egg samples stored for 21 and 42 days (MDA,ng/g).

Groups	0 Day	21Day	42 Day
Control	2.90	17.32^{a}	21.07^{a}
EOC-1	2.62	10.85^{b}	12.65^{b}
EOC-2	4.57	7.41^{c}	11.77^{b}
Vit E-1	3.39	5.78^{cd}	12.21^{b}
Vit E-2	3.73	4.44^{d}	12.19^{b}
SEM	1.41	0.68	1.86
P	0.874	0.000	0.023

OVERALL EFFECTS

Group Effect	Control	EOC-1	EOC-2	Vit E-1	Vit E-2	SEM	P
	13.76^{A}	8.71^{B}	7.92^{B}	7.13^{B}	6.79^{B}	0.809	0.01
Time Effect	0 Day	21 Day		42 Day			
	3.44^{C}	9.16^{B}		13.98^{A}		0.627	0.00
Group x Time	0.001						

a, b, c, d: Means within columns with different superscripts differ at $P < 0.05$
A, B, C: Means within row with different superscripts differ at $P < 0.05$

oxidation of egg yolks from laying hens that were fed rosemary, thyme, saffron, and á-tocopherol acetate supplemented diets. The addition of EOC at 300 ppm decreased E. coli and Coli form bacterium intensity infeces(Table 5), but statistically remain incomparable among groups ($P > 0.05$). In contrast to the present findings, Bölükbaþý et al. (2008) observed that supplementation of sage, thyme, and rosemary oil into laying hens' diets decreased concentrations of fecal E. coli and Coli form bacterium; among them, sage had less antimicrobial effects than other oils. While in another study, Jamroz et al. (2003) also reported that plant extracts (thymol, cinnamaldhyde, and capsaicin) reduced total E. coli and Clostridium perfringens in the intestines of broiler chickens. Mitsch *et al.* (2004) also found that supplementation of specific blends of essential oils with thymol and carvacrolas the main components in broiler chicken diets blocked fecal and intestinal colonization of Clostridium perfringens. It may be concluded that EOC at 150 ppm level has potential to be used as an alternative to antioxidant vitamin E for the purpose of inhibiting lipid oxidation without any adverse effects on performance and egg quality traits of layer hens.

TABLE 5: Concentrations of E. coli and Coliformbacterium (MPN/g) in fecal samples of laying hens fed with EOC and Vit E

Groups	E.COLI	COLIFORM
Control	110.00	110.00
EOC-1	84.83	83.03
EOC-2	110.00	110.00
Vit E-1	110.00	110.00
Vit E-2	110.00	110.00
SEM	11.26	12.06
P	0.438	0.438

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