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The Effects of Supplementation of Bergamot Oil (*Citrus bergamia*) on Egg Production, Egg Quality, Fatty Acid Composition of Egg yolk in Laying Hens

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This research was conducted to determine effects of dietary bergamot oil levels (0, 0.25, 0.50, 0.75 ml/kg) on performance, egg quality, blood metabolic profile and fatty acid composition of egg yolk in laying hens. Sixty four of 67 weeks old white Lohman LSL laying hens were randomly assigned to four groups equally ($n=16$). Each treatment was replicated four times

Dietary supplementation of bergamot oil had no significant effect on feed conversion ratio, egg weight, and egg production, shell thickness, ratio of albumen and shell. But, supplementation of bergamot oil decreased feed intake. The addition of 0.50 ml/kg bergamot oil to the laying hens feed led to a significant increase in the yolk ratio. It was also observed that egg shell ratio, serum cholesterol and calcium concentration reduced significantly with supplementation of bergamot oil in laying hens diets. The highest IgG concentration was obtained from hens fed 0.25 ml/kg bergamot oil. Addition of bergamot oil to feeds significantly increased eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and n-3 concentration and decreased n-6/n-3 ratio in egg yolk.

The results of this research indicated that the addition of bergamot oil to the laying hens feed led to a significant decrease in the feed intake and concentrations of serum cholesterol. It was also concluded that dietary supplementation of bergamot oil significantly increased egg shell strength and, the EPA, DHA and n-3 ratio of the egg yolk.

Key words: bergamot oil, cholesterol, egg production, fatty acid, laying hen

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Introduction

Growing concern about antibiotic growth promoters in animal nutrition has created tremendous efforts to develop and use different plant compounds as possible natural alternatives. Phytogenic feed additives or plant extracts also referred to as "Phytogenics"- are an extremely heterogeneous group of feed additives originating from leaves, roots, tubers or fruits of herbs, spices or other plants. They are either available in a solid, dried and ground form or as extracts or essential oils.(Steiner, 2009).

Essential oils have been known biological activity, including antibacterial, antivirucidal, antifungal, hypocholesterolemics and anti-inflammatory effects (Cowan, 1999; Craig, 1999; Faleiro *et al.,* 2003). Recoqillay (2006) reported that plant extracts and their essential oils have a wide range of activities, including inhibitory action on pathogens, effects on physio-pathologies (e.g. anti-inflammatory, anti-diarrhoea properties) and activity in different body systems, e.g. endocrine and immune system.

Some researchers reported that plant extracts stimulated the voluntary feed intake especially in young animals (Riebau *et al.,* 1997; Ultee *et al.,* 2002; Giannenas *et al.,* 2003; Kroismayr *et al.,* 2008).

Ramakrishna *et al.* (2003) and Williams and Losa (2001) assumed that phytogenics may stimulate the production of digestive enzymes such as lipase and amylase, thus having a beneficial effect on nutrient utilization in different categories of animals.

It has been reported several plant extracts had antimicrobial, anticoccidial, fungicidal or antioxidant properties *in vitro* (Cowan, 1999; Faleiro *et al.,* 2003). Some studies have shown that essential oils of rosemary (*Rosmarinus officinalis*), sage (*Salvia sclarea*), thyme (*Thymus vulgaris*) in this respect were the most active against strains of *E. coli* (Smith-Palmer *et al.,* 1998; Hammer *et al.,* 1999; Dorman and Deans, 2000). Jamroz *et al.* (2003) determined that plant extract (carvacrol, cinnamaldehyde and capsaicin) reduced the total *E. coli* and Clostridium perfringes numbers in the intestines of broiler chickens.

Bergamot (*Citrus bergamia*), an aromatic herb, is a

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member of the Rutaceae. The essential oil of bergamot oil is extracted from the peel of the fruit by a cold-pressing procedure or steam distillation. Bergamot essential oil is composed of various chemical constituents and includes α-pinene, β-pinene, myrcene, limonene (40%), α-bergaptene, β-bisabolene, linalool (8%), linalyl acetate (28%), nerol, neryl acetate, geraniol, geraniol acetate and a-terpineol (Cum *et al.,* 1991). Kırbaşlar *et al.* (2001) indicated that the chemical composition of the bergamot oil was stongly influenced by harvesting time. It was decleraded that bergamot, and its major components citral, limonene and linalool, have been generally recognised as safe (Food and Drug Administration, 2005).

It has been reported that bergamot oil exhibited analgesic, antidepressant, antifungal, antiseptic, antibiotic, antispasmodic, calmative (Fisher and Phillips, 2006; Sanguinetti *et al.,* 2007).

Fisher and Phillips (2006) and Deans and Ritchie (1987) shown that citrus essential oils (bergamot, lemon, orange etc) have potential bactericidal properties not only against yeast, moulds and spore forming bacteria but also against food-poisoning bacteria.

There are few published data on the effects of essential oils components in laying hens. Also, there is no study known about the effect of bergamot oil on the egg parameters and laying hens performance in the chickens. The objective of this study was to research the effects of bergamot oil on the laying performance, egg quality, egg yolk fatty acid composition and metabolic profile of laying hens.

Material and Methods

Experimental Design and Animals

A total of 64 "Lohman LSL" laying hens aged 67 weeks were assigned to 4 treatments in a completely randomized design. Four layers were housed in one cage (50×46×46 cm) with four animals. The dietart treatments were: 0 m*l*/kg bergamot oil (basal diet- Table 1), 0.25, 0.50 or 0.75 m*l*/kg bergamote oil. The bergamot oil contain, 33% limonene, 12% linalool, 24% linalyl acetate (bergamot oil was purchased from Ege Lokman San. Tic. Company, Manisa, Turkey). During the experiment (8 week) hens were provided feed and water *ad libitum*. Egg production and feed consumption were recorded daily. Egg weight was meusered biweekly. Feed conversion rate was expressed as kilogram feed intak eper kilogram egg production. Eggs used for evaluation of yolks and albumen were broken and the weights of yolks, albumen, shell, shell strength and shell thickness recorded ($n=8$). At the end of the experiment, blood samples were taken each treatment ($n=8$) in order to determine some blood metabolites. Also, sample of four eggs was randomly collected from each experimental group to asses fatyy acid composition of egg yolk at the end of experiment.

Collection of Blood Samples

Blood samples were taken from wing vena into additive-free blood tubes. Serum was obtained followinge centrifugation at 4000 xg for 10 minute at +20℃. AST, ALT, triglyceride, VLDL, total protein, albumin, phosphorus, cholesterol, calcium and IgG were determined in serum by spectrophotometric metod using commerical kits (Roche) in auto-analyzer (Cobas 6000, Japan).

Table 1. **Composition of diets offered to laying hens**

Ingredients	Composition (g/kg)
Corn	535.63
Soybean meal 44	257.44
Wheat	80.56
Limestone	90.80
Soybean oil	15.30
Dicalcium phosphate	11.54
Vit+Min premix[1]	2
Salt	5
DL-methionine 99%	1.23
L-Lysine	0.5
Total	1000
Calculated composition	
Crude Protein	16.2
ME, kcal/kg	2753
Methionine	0.34
Lysine	0.90
Met and Cys	0.62

[1] Supplied per kilogram of diet: 12 000 IU vitamin A; 2 500 IU vitamin D_3; 30 IU vitamin E; 4 mg vitamin K_3; 3 mg vitamin B_1; 6 mg vitamin B_2; 30 mg niacin; 10 mg calcium D-pantothenate; 5 mg vitamin B_6; 0.015 mg vitamin B_{12}; 1 mg folic acid; 0.050 mg D-biotin; 50 mg vitamin C; 300 mg choline chloride; 80 mg manganese; 60 mg iron; 60 mg zinc; 5 mg copper; 0.5 mg cobalt; 2 mg iodine; 0.15 mg selenium.

Fatyy Acid Analysis

Fatty acid analyses were performed at the Biotechnology Application and Research Centre. After extracting (Folch *et al.,* 1957) yolk samples were methlated for gas chromatographic analysis (GC- Agilent 6980 Mass, a fused silica capillary column, and film thickness of 0.25 μm). Oven temperature was from 165℃ to 200℃ at 5℃/min. Detector temperature was 200℃; head pressure was 5 psi.

Differences between groups were analysed with one-way analysis of variance (ANOVA) by using the statistical package SPSS for Windows (1999), version 10.0. Treatment means were evaluated for statistically significant differences using Tukey test.

Results

Feed intake, feed conversion ratio, egg production and egg weight of the layers fed diet containing bergamot oil are presented in Table 2. Throughout the experimental period, the feed intake was significantly ($P<0.05$) decreased in layers fed diets containing bergamot oil supplementation compared to that of the control. The diet with 0.75 m*l*/kg bergamot oil had induced decreases in feed intake by 13.5%. There were no effects of the ex-

Table 2. **Effects of dietary bergamot oil on the productive performance of laying hens**

Groups	Feed intake (g)	Feed conversion ratio (g: g)	Egg production (%)	Egg Weight (g)
Control	$115.5^{a} \pm 2.78$	2.57 ± 0.05	67.2 ± 1.41	66.6 ± 1.80
Bergamot oil 0.25 ml/kg	$112.4^{ab} \pm 1.52$	2.44 ± 0.05	70.2 ± 1.67	63.7 ± 1.41
Bergamot oil 0.50 ml/kg	$102.4^{bc} \pm 1.78$	2.35 ± 0.06	67.7 ± 1.78	60.6 ± 2.10
Bergamot oil 0.75 ml/kg	$99.8^{c} \pm 3.00$	2.27 ± 0.04	68.7 ± 1.78	64.2 ± 1.90
P	*	NS	NS	NS

*: $P < 0.05$, NS: not significant, $^{a, b, c}$: Column means with no common superscript differ significantly.

Table 3. **Influence of dietary Bergamot oil on egg quality of laying hen**

Groups	Yolk (%)	Albumen (%)	Shell (%)	Haugh Unit	Shell thickness (μm)	Breaking strength kg mc^3
Control	$28.7^{b} \pm 0.60$	61.0 ± 0.50	$10.2^{a} \pm 0.42$	72.5 ± 3.20	391 ± 14.1	$1.06^{b} \pm 0.07$
Bergamot oil 0.25 ml/kg	$29.7^{b} \pm 0.44$	61.5 ± 0.45	$8.7^{b} \pm 0.32$	78.5 ± 2.30	368 ± 15.3	$1.00^{b} \pm 0.06$
Bergamot oil 0.50 ml/kg	$32.7^{a} \pm 0.46$	59.7 ± 0.49	$7.6^{b} \pm 0.20$	83.5 ± 2.30	436 ± 21.8	$0.97^{b} \pm 0.06$
Bergamot oil 0.75 ml/kg	$30.1^{b} \pm 0.49$	61.7 ± 0.48	$8.2^{b} \pm 0.26$	86.7 ± 2.20	409 ± 13.8	$1.26^{a} \pm 0.08$
P	*	NS	**	NS	NS	**

*: $P < 0.05$, **: $P < 0.01$, NS: not significant, $^{a, b, c}$: Column means with no common superscript differ significantly.

Table 4. **Influence of dietary Bergamot oil on metabolic profil of laying hen**

Parameter	Control	Bergamot oil 0.25 ml/kg	Bergamot oil 0.50 ml/kg	Bergamot oil 0.75 ml/kg	*P*
AST U/L	188.66 ± 6.70	194.33 ± 7.43	199.00 ± 6.50	184.33 ± 4.28	NS
ALT U/L	2.66 ± 0.33	3.33 ± 0.63	3.33 ± 0.33	2.33 ± 0.33	NS
Triglyceride mg/dl	1003.33 ± 24.5	983 ± 19.8	924.66 ± 23.9	914.00 ± 17.6	NS
Cholesterol mg/dl	$146.66^{a} \pm 12.0$	$111.66^{ab} \pm 4.3$	$86.66^{b} \pm 2.6$	$92.66^{b} \pm 5.3$	*
VLDL mg/dl	196.66 ± 11.2	196.66 ± 19.3	206.00 ± 12.5	176.66 ± 9.8	NS
Total protein g/dl	5.76 ± 0.12	5.56 ± 0.12	6.03 ± 0.23	5.56 ± 0.18	NS
Albumin g/dl	2.23 ± 0.08	2.23 ± 0.06	2.20 ± 0.10	2.20 ± 0.09	NS
Ca mg/dl	$22.76^{a} \pm 0.8$	$19.10^{b} \pm 0.5$	$17.06^{b} \pm 0.9$	$20.13^{ab} \pm 0.6$	*
P mg/dl	6.06 ± 0.61	5.20 ± 0.34	5.06 ± 0.8	5.20 ± 0.54	NS
IgG g/l	$1.49^{b} \pm 0.01$	$1.62^{a} \pm 0.04$	$1.54^{ab} \pm 0.08$	$1.50^{b} \pm 0.05$	*

VLDL = very low density lipoprotein.

perimental diets on feed conversion ratio, egg production and egg weight.

The effect of experimentl diets of on egg quality parameters were shown in Table 3. The addition of bergamot oil to the diet had no impact on albumen percentage of egg, eggshell thickness and Haugh unit in this study. The yolk percentage of egg was significantly ($P < 0.05$) influenced by treatment. The group fed with the 0.50 ml/kg bergamot oil showed the highest yolk percentage of egg. Shell percentage of egg was significantly influenced by treatment. The addition of bergamot oil to the basal diet significantly ($P < 0.01$) reduced shell percentage of egg compared to control group. Shell breaking strength increased dependent on increasing of dietary bergamot oil (0.75 ml/kg).

None of the dietary factors affected serum aspartate transaminase (AST) alanine transaminase (ALT), triglyceride, VLDL, total protein, albumin and phosphorus concentration. Serum cholesterol concentration for hens fed 0.5 and 0.75 ml/kg bergamot oil was lower than hens fed basal diet and 0.25 ml/kg bergamot oil. Bergamot oil supplementation decreased serum calcium (Ca) concentration ($P < 0.05$). The diet with 0.50 ml/kg bergamot oil had induced decreases in Ca concentration by 5.7 mg/dL compared to control group. IgG concentration was significantly ($P < 0.05$) influenced by treatment. There were significantly increase in IgG concentration with supplementation 0.25 ml/kg bergamot oil.

Table 5 shows the effect of dietary factors on egg yolk fatty acid composition. None of the dietary factors affected palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidonic, total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and n-6 concentrations in egg yolk in the present study.

The groups fed on the 0.5 and 0.75 ml/kg bergamot oil

Table 5. **Influence of dietary bergamot oil on fatty acid composition of egg yolk lipids of laying hen**

Fatty acids (%)	Control	Bergamot oil 0.25 ml/kg	Bergamot oil 0.50 ml/kg	Bergamot oil 0.75 ml/kg	*P*
Palmitic	22.77 ±0.29	21.79 ±0.60	25.09 ±0.46	22.21 ±0.58	NS
Palmitoleic	3.00 ±0.09	2.60 ±0.21	2.75 ±0.01	2.48 ±0.15	NS
Stearic	9.25 ±0.19	10.08 ±0.21	13.58 ±0.25	10.33 ±0.23	NS
Oleic	39.67 ±0.9	39.60 ±1.4	41,07 ±0.9	37.15 ±0.4	NS
Linoleic	18.49 ±1.3	18.07 ±0.6	15.63 ±1.3	17.64 ±0.8	NS
Linolenic	0.15 ±0.03	0.13 ±0.02	0.26 ±0.03	0.22 ±0.03	NS
Arachidonic	0.35 ±0.07	0.20 ±0.06	0.29 ±0.02	0.31 ±0.07	NS
EPA	2.20^{b}±0.09	2.44^{b} ±0.11	2.86^{a}±0.12	2.82^{a}±0.08	*
DHA	0.80^{b}±0.02	1.32^{a} ±0.06	1.38^{a}±0.13	1.16^{a}±0.14	**
SFA	32.03 ±0.87	31.87 ±0.98	30.60 ±0.73	32.54 ±0.14	NS
MUFA	42.67 ±0.87	42.20 ±1.58	43.34 ±0.55	39.64 ±1.10	NS
PUFA	22.00 ±1.07	22.17 ±1.03	20.44 ±0.68	22.16 ±	NS
n-3	3.15^{b}±0.14	3.89^{a} ±0.15	4.51^{a}±0.27	4.20^{a}±0.25	**
n-6	18.84 ±1.66	18.27 ±1.35	15.78 ±0.48	17.95 ±1.54	NS
n-6/n-3	5.97^{a}±0.49	4.69^{ab}±0.62	3.52^{b}±0.16	4.26^{b}±0.28	*

*: $P<0.05$, **: $P<0.01$, NS: not significant, ^{a,b,c}: Column means with no common superscript differ significantly.

diet had the greatest the proportions of EPA than other groups. The concentrations of DHA and n-3 in egg yolk lipids was significantly ($P<0.05$) increased levels of dietary bergamot oil. Supplemental bergamot oil tended to decrease n-6/n-3 ratio in egg yolk of laying hens. The lowest proportions of n-6/n-3 in yolk lipids was obtained with the diet containing 0.5 and 0.75 ml/kg bergamot oil.

Discussion

It was reported that essential oils have a stimulating effect on animal digestive systems (Langhout, 2000; Williams and Losa, 2001; Ramakrishna *et al.,* 2003). They postulated that these effects could be due to the increased production of digestive enzymes and the improved utilization of digestive products through enhanced liver functions. The results of this experiment showed that supplementation of the diet with bergamot oil had no effect on feed conversion, egg production and egg weight compared with controls, but caused a significant decrease feed intake. As a result, addition of bergamot oil directly effected some of the performance indices such as reducing feed consumption but no significant differences in the others in this present study. Different essential oils acted differently to performance of laying hens the controversial results could be due to the active substances of these oils. However, Hertrampf (2001) reported that essential oils derived from spices and herbs could be successfully used as growth promoters, since they increased the feed intake due to their aromatic characteristics in chickens. In contrast to our results, some researchers reported that supplementation of the diet with essential oils has improved egg production, feed efficiency, egg weight (El-Sheikh *et al.,* 1998; Akhtar *et al.,* 2003; Denli *et al.,* 2004; Botsoglou *et al.,* 2005; Nasir *et al.,* 2005; Aydın *et al.,* 2006; Çabuk *et al.,* 2006; Bölükbaşı *et al.,* 2007, 2008). In accordance with the present findings, Bölükbaşı *et al.* (2009) showed that supplementation of dietary essential oil had no effect on feed efficiency, egg weight and egg production.

There was significantly increase in yolk percentage of egg with supplementation 0.50 ml/kg bergamot oil. Similarly, Denli *et al.* (2004) reported that addition of 1 g/kg *Nigella Sativa* extracts in diets of laying quails increased weight of yolk in quail egg. In contrast Bölükbaşı *et al.* (2008) reported that addition of essential oils(thyme, sage, rosemary) to diet significantly reduced proportion of yolk compared with the control. However, Bölükbaşı *et al.* (2009) found that *Nigella Sativa* oil (1,2 and 3 ml/kg) supplementation in layer diets had no impact on yolk percentage.

The addition of bergamot oil to the diet caused a significant decrease percentage of egg shell in this study. In contrast this study, Bölükbaşı *et al.* (2008) reported that supplementing thyme, sage and rosemary oil to diet of laying hen increased proportion of egg shell above the control. Similarly, Denli *et al.* (2004) found that addition of 1 g/kg black seed extract increased weight of egg shell in quail egg and they reported that black seed extract could be associated with the included components such as thymocinene and carvacrol. Egg shell strength for hens fed 0.75 ml/kg bergamot oil was highest than other groups.

In the present experiment AST, ALT, triglyceride, VLDL, total protein, albumin and phosphorus for concentration were not affected by supplementation of bergamot oil. Serum cholesterol concentration reduce by the supplementation of bergamot oil. This also confirms earlier findings of Bölükbaşı *et al.* (2007, 2008, 2009), Case *et al.* (1995) and Elson (1996). The hypocholesterolemic effects of essential oil are mediated by down-regulating the regulatory enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. It was reported the inhibition of

hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity which is a key regulatory enzyme in cholesterol synthesis by essential oils (Crowell, 1999). In accordance with the present findings, Case *et al.* (1995) reported that inhibition of HMC-CoA reductase lowered serum cholesterol by 2% in poultry. It has been reported (Qureshi *et al.,* 1988) that when cockerels are fed dietary limonene at levels of 25–100 ppm for 26 days, hepatic HMG-CoA reductase activity and serum cholesterol show a dose-dependent decrease whereas hepatic fatty acid synthetase activity was unaffected.

The diet with bergamot oil had induced decreases in Ca concentration compared to control group. This situation might be depend on the reduction in feed consumption. But, supplementation of 0.75 m*l*/kg bergamot oil in basal diet did not affect eggshell strength. Occhiuto and Circosta (1996, 1997) explaned that bergamottin, another important component of the non-volatile fraction of bergamot esential oil, might be endowed with Ca^{2+} antagonist properties *in vitro.* Also it is reported that bergamottine has a ability to inhibit calcium overload (Occhiuto and Circosta, 1996).

It is reported that plant extracts and their essential oils have a wide range of activities, including include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion and the activation of immune responses and antioxidant actions (Baratta *et al.,* 1998; Jamroz *et al.,* 2003, 2005; Botsoglou *et al.,* 2005). In this study, 0.25 m*l*/kg bergamot oil diet had a significantly higher IgG concentration of serum than that of the other groups. It is known that many essential oils have a remarkable ability to both support the immune system and increase one's rate of healing (Recoqillay, 2006).

The addition of bergamot oil to feed significantly increased the DHA, EPA and n-3 concentration and decreased n-6/n-3 ratio in the egg yolk in this research. Deans *et al.* (1993) and Recsan *et al.* (1997) reported that dietary supplementation with plant essential oils protects and maintains levels of PUFA in cell membranes. Morever, Youdim and Deans (2000) found that addition of thyme oil and thymol in diets of rat changed in fatty acid composition in the brain phospholipid fraction. And they reported that thyme oil and thymol provided beneficial effects on the antioxidant status of the rat brain, which may in turn have influenced the concentrations of PUFA, especially DHA. Therefore the findings of the present study may also show that bergamot oil provided beneficial effects on the antioxidant status of the egg yolk, resulting in concentrations of PUFA, especially DHA and EPA. In contrast our study, it was reported that rosemary extract supplementation in layer diets had no effect on fatty acid composition of egg yolk of laying hen (Galobart *et al.,* 2001). In a study conducted in broilers, it was shown that thyme essential oil caused a lower concentrations of SFA and PUFA in the leg and breast tissues, whereas it caused a higher MUFA concentrations (Bölükbaşı *et al.,* 2006).

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

In conclusion, supplementation of different levels of bergamot oil directly effected some of the performance indices such as reducing feed consumption but no significant differences in the others. It was also determined that cholesterol concentrations of serum of laying hens were significantly reduced by diet including bergamot oil. The addition of 0.25 m*l*/kg bergamot oil to the laying hens feed led to a significant increase in the IgG concentration in the serum. DHA, EPA and n-3 ratio of the egg yolk increased whereas the proportions of n-6/n-3 of the egg yolk decreased dependent on increasing of dietary bergamot oil levels. It suggested that it should be use 0.75 m*l*/kg bergamot oil level to increase egg shell strength and EPA, DHA and n-3 concentrations in egg yolk in during late laying period.

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